SHIMADZU
Excellence in Science

Food Safety Solutions

Application Data Book

Foreword

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Dear Valued Customers,

Shimadzu believes in total customer satisfaction. Every Shimadzu instrument sold is backed up by our strong after-sales support team. Be it service support, or application support, we are always striving to meet the demands of every customer. For without the customer, there will be no Shimadzu.

Our customer support centre primarily provides application support and continuous training for our valued customers. Our team of experienced scientists are dedicated to solving customers' application problems.

This year 2015, Shimadzu Corporation celebrates 140th anniversary and on this occasion we wish to release comprehensive anlysis book called as “Food Safety Solutions” pertaining to all our customer's food analysis needs. For customer's satisfaction we keep challenging to provide cutting-edge technologies and solutions with application support and technical services that can help customers to understand Shimadzu and grow with us. By this way, Shimadzu can contribute to the society through our customer's success.

Our customer support will continue to improve to meet the requirements of customers. We trust that this book will be helpful to you.

Yours Sincerely,
Yoshiyuki Fujino
Managing Director

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SHIMADZU TECHNOLOGY

With an immense background in instrumentation technology, and it's pionerring R&D, Shimadzu realizes the importance of MS in various application fields. This paved the way in successful development of UltraFast Mass Spectrometers [UFMSseries].

Realising the importance of ensuring safety of food, consumed by the people, and to satisfactorily meet norms laid by various regulatory authorities, Shimadzu has invested time, money, and labour in coming up with latest technological advances in the analytical field. New mass spectrometers especially triple quadrupole mass spectrometers have been launched alongwith gas chromatographic and liquid chromatographic systems that meet the growing demands of the food safety markets in terms of sensitivity, reliability and throughput capacities.

LCMS-8030

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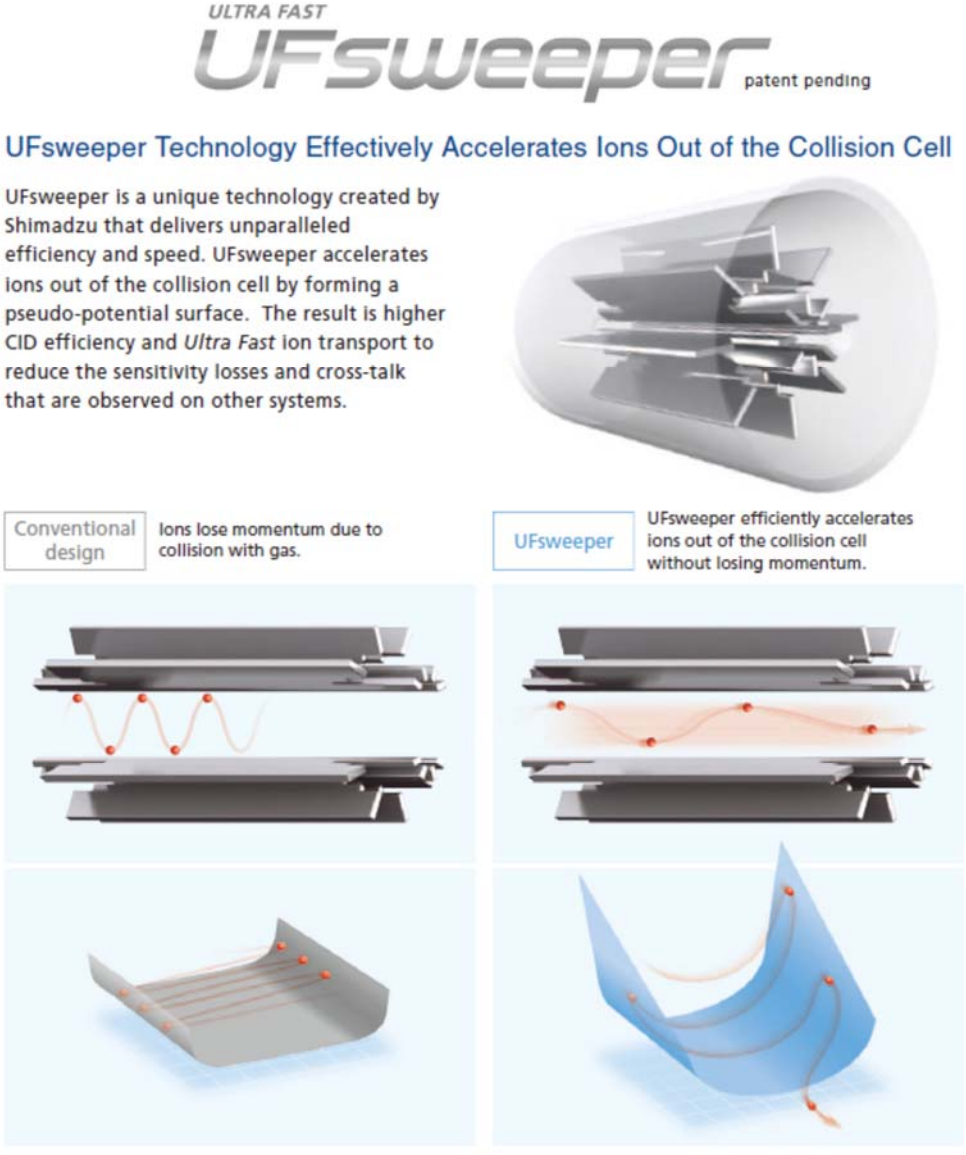
LCMS-8040

LCMS-8050

GCMS-TQ8040

To provide best-in-class performance for Ultra Fast data acquisition rates in MS/MS detection, Shimadzu has developed UFsweeper technology. This technology efficiently accelerates ions out of the collision cell, dramatically minimizing cross talk and shortening MRM analysis time to the lowest possible level.



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LCMS-8030

The LCMS-8030 achieves best-in-class performance for *ultra fast* mass spectrometry detection and opens new opportunities for all application areas. The LCMS-8030 ion source works with the most challenging samples, delivering robust, high-sensitivity detection using ESI, APCI or our dual probe ionization interface. System maintenance for the ion source is simplicity itself. Cleaning the heated desolvation capillary is quick and maintains system vacuum to provide greater uptime and usability.

Equipping a true high-speed pulse-counting detector and conversion dynode system has resulted in astonishing data acquisition rates even with polarity switching. The development of a unique semi-floating high-voltage power supply realizes ultra-high speed polarity switching (15 msec).

In addition, the combined performance of Nexera and LCMS-8030 Reduces Analytical Cycle Times.

Features of LCMS-8030:

- Mass range is from m/z 10 to 2000 with Scan speed - Max 15,000 u/sec
- 1 msec dwell time and pause time.
- 15 msec polarity switching speed.
- No ion intensity loss even at high-speed measurement due to UFsweeper technology.
- Applicable flow rate for ESI - 1 uL/min to 2 mL/min
- MRM transition speed - Max 500 channels / sec

LCMS-8040

Ultra-High Sensitivity

By incorporating newly improved ion optics and collision cell technology, the LCMS-8040 provides higher multiple reaction monitoring (MRM) sensitivity. A five-fold increase in sensitivity (reserpine, S/N ratio) has been achieved by improving ion focusing and minimizing ion losses between multi-pole lenses. This higher sensitivity expands the potential range of LC/MS/MS applications.

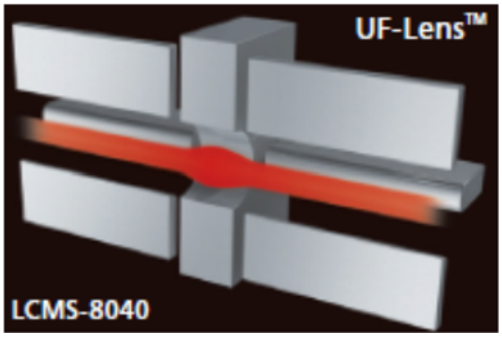
Ultra-High Speed

The LCMS-8040 was designed to provide significantly higher sensitivity while maintaining the high speed offered by the LCMS-8030. Ultrafast MRM transition speeds, up to 555 MRMs per second, are achieved by Shimadzu's UFsweeper™ collision cell technology, proprietary high-precision quadrupole machining capabilities, and unique high voltage power supply technology.

Ultra-High Reliability

MRM optimization in Shimadzu's LCMS systems is based on a rapid series of automated flow injection analyses, requiring only minutes to perform. Multiple compounds can be optimized in an unattended sequence, freeing the analyst from tedious work. MRM parameters optimized for the LCMS-8030 can be transferred to the LCMS-8040, making it possible to transfer methods between systems. The LCMS-8040 offers the same ease of maintenance benefits as the LCMS-8030, and all consumables, such as desolvation lines (DL) and ESI capillaries, are interchangeable as well.

Ion losses between segments are minimized by utilizing quadrupole ion guides.



Higher CID Efficiency with Improved Collision Cell

The UFsweeper II is a high-sensitivity, high-speed collision cell that features improved ion focusing by using high-speed ion transport technology. This yields better product ion transmission in the collision cell, maintaining signal intensity and suppressing crosstalk, even for high-speed or simultaneous multi-component analysis. The capability for high-throughput analysis is thus maintained at lower levels of detection.

Features of LCMS-8040:

- Mass range is m/z 10 to 2000 with a scan speed of 15000 u/sec and polarity switching of 15 mesc.
- MRM transition speed is 555 channels/sec with a cross talk less than 0.003%.
- Quadrupole type ultra-fast collision cell (UFsweeper™ II collision cell)
- Minimum dwell time of 0.8 msec and pause time of 1 msec is given by the system.
- Interface options include ESI / APCI and Dual Ion Source (DUIS)
- LC flow rate applicable ranges between 1µL/min to 2 mL/min.

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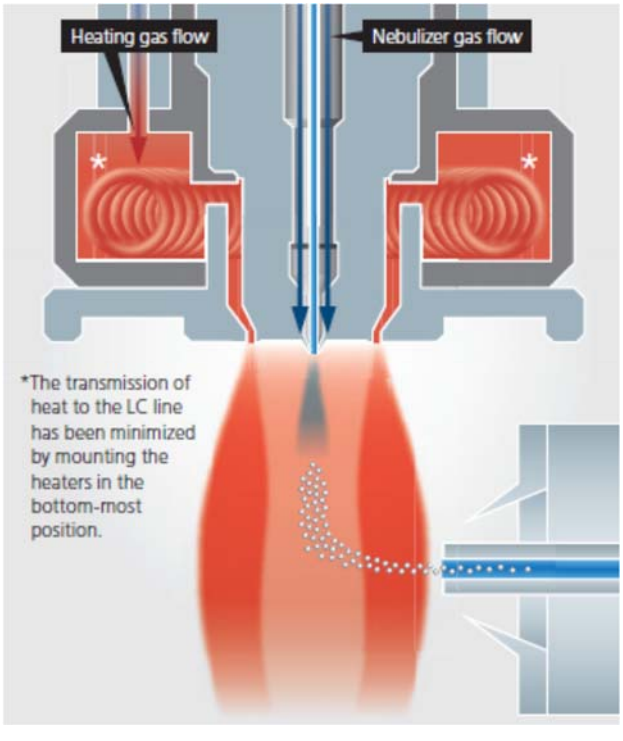
LCMS-8050

Continuing the evolution of Shimadzu's UF technology, Shimadzu introduced the LCMS-8050 triple quadrupole mass spectrometer, offering unparalleled measurement speeds and high-sensitivity performance.

A newly designed heated ESI probe and a new high-efficiency CID cell, the UFsweeper III meets the demands of very trace level quantitation. These technological improvements combined with Shimadzu’s patented ion optics system deliver durable high-sensitivity performance.

The LCMS-8050 uses unique high-voltage power supply technology to achieve an ultra-high-speed positive/negative ionization switching time of just 5 msec. The LCMS-8050 is also the only instrument of its type to maintain ion intensity even when performing polarity
switching at ultra-high speed, yielding consistent, reproducible data.



In order to improve desolvation efficiency, the newly developed heated ESI probe combines a high-temperature gas with the nebulizer spray, assisting in the desolvation of large droplets and facilitating ionization. This development allows for high-sensitivity analysis of a wide range of target compounds.

Employing ultra-high-speed scan technology [UFscanning], the LCMS-8050 maintains spectrum quality at any scan speed. Perform quantitative and qualitative analysis simultaneously with a high-speed scan rate of 30,000 u/sec. By controlling the voltage applied to the quadrupoles according to scan speed and *m/z*, the LCMS-8050 achieves superior ion transmission at any scan speed. Since Shimadzu maintains data collection at 0.1 u intervals, high-quality mass spectra are obtained without loss of sensitivity or mass accuracy.

UFsweeper III high-speed ion transport technology minimizes ion loss even at a dwell time of 0.8 sec. High-speed MRM transitions up to 555 ch/sec accelerate laboratory throughput
for simultaneous multi-component analyses. A high-sensitivity, high-speed collision cell, the proprietary UFsweeper III accelerates ions out of the collision cell without loss of momentum. By achieving fast sweeping on successive scans, it offers twice the CID efficiency of UFsweeper II, maintains signal intensity, and suppresses crosstalk, even for high-speed or simultaneous multi-component analysis.

The LCMS-8050 has been developed with advanced UF technology, and is the flagship model of the Shimadzu UFMS series, which features world-leading speed and sensitivity.
The LCMS-8050 is the ideal triple quadrupole LC/MS/MS system for high-sensitivity, high-throughput, and simultaneous quantitative and qualitative analysis. Utilizing the same user-friendly interface as HPLC/UHPLC and GC modules, LabSolutions MS workstation software provides intuitive functionality for efficient data processing and an easier, more productive analytical workflow.

Features of LCMS-8050:

- Mass range is m/z 2 to 2000 with a scan speed of 30,000 u/sec (in all modes of scanning ; 0.1 u step; 3,00,00 data points/sec) and polarity switching of 5 msec.

- MRM transition speed is 555 channels/sec with a cross talk less than 0.003%.
- Tapered multipole type ultra-high-speed collision cell (UFsweeper™ III collision cell)
- Minimum dwell time of 0.8 msec and pause time of 1 msec is given by the system.
- Interface options include ESI / APCI and Dual Ion Source (DUIS)
- LC flow rate applicable ranges between 1 µL/min to 2 mL/min.

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GCMS-TQ8040

The GCMS-TQ8040 Triple Quadrupole Gas Chromatograph Mass Spectrometer from Shimadzu Corporation, Japan, provides the speed, accuracy, and easy operation which are the need of Testing Labs today.

Shimadzu GCMS-TQ8040 Gas Chromatograph Mass Spectrometer

With enhanced sensitivity and selectivity, triple quadrupole GC/MS/MS is the analytical technique of choice for a wide range of applications, from food safety and environmental monitoring to clinical research and forensics. Components that cannot be analyzed by conventional scan or SIM modes are easily identified and quantified at trace levels in the presence of complex matrices using the Multiple Reaction Monitoring (MRM) mode (The fast Scan/MRM analysis mode expands this capability to include simultaneous acquisition of a full scan spectrum for identification or confirmation of unknowns along with the targeted compounds. As the only GC/MS/MS capable of running all triple quad operational modes and combination of modes without sacrificing sensitivity or accuracy, Shimadzu's GCMS-TQ8040 elevates this technique to a new level of performance.

High Sensitivity and Enhanced Selectivity:

The Single quadrupole GC/MS systems are able to identify individual components using retention time and mass spectra; they are useful for detecting trace-level contaminants in a variety of sample types. However, unambiguous identification can be difficult in the presence of a complex or problematic matrix. MRM) in Triple Quadrupole GC/MS/MS system separates analyte masses from matrix interference in two stages, making the instrument significantly more selective than a single quadrupole system. As a result, even components that cannot be analyzed by conventional scan or SIM modes can be easily identified and quantified in the presence of complex matrices using MRM; enabling over ten times higher sensitivity than conventional GC-MS systems, particularly when analyzing samples containing many contaminants. For example, MRM can be a particularly

effective measurement tool for analyzing residual pesticides in food matrix.

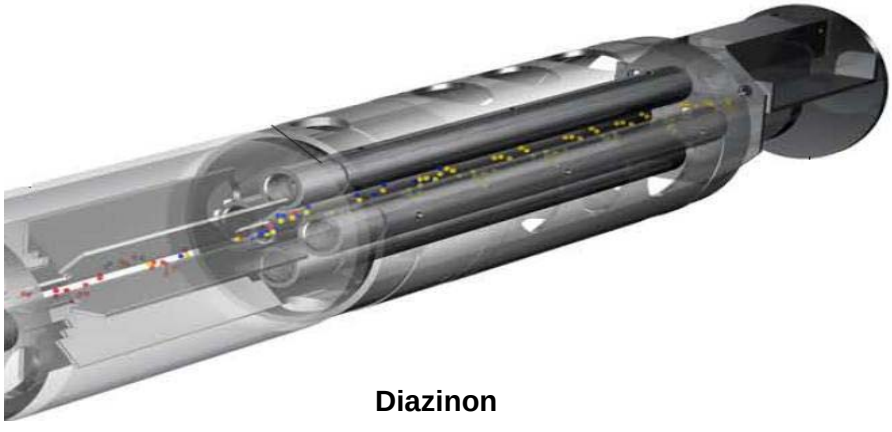
Identification/Confirmation:

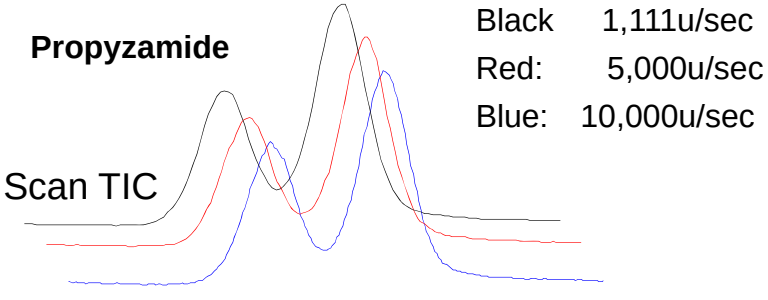
It is very essential to identify the presence of “the toxic compound” at this low level. The simultaneous Fast **Scan/SIM** mode and **Scan/MRM** of analysis without compromising the sensitivity gives confidence to report the analysis results unambiguously.

Fast Scanning Analysis with ASSP™

The GCMS-TQ8040 is equipped with an internal firmware protocol that optimizes the ion transmission parameters during the scan acquisition process. This technology, called Advanced Scanning Speed Protocol (ASSP™), is the key to this instrument’s ability to acquire high-quality spectra at scan spee ds of up to 20,000 u/sec.

ASSP™ enables high-speed scanning at 20,000 u/s





Shimadzu's GCMS-TQ8040 is the ultimate platform for the most challenging applications in Food Safety.

Features of GCMS-TQ8040:

- High-speed scan and data acquisition for accurate quantitation at 20,000 u/sec
- Capable of performing simultaneous Scan/MRM
- Ufsweeper® technology efficiently sweeps residual ions from the collision cell for fast, efficient ion transport and no cross-talk
- Fast MRM transitions at the speed of 800 MRMs/sec
- Two overdrive lenses reduce random noise from helium, high-speed electrons, and other factors to give the highest sensitivity (Highest S/N ratios) in both SIM mode and MRM mode
- Flexible platform with EI, CI, and NCI ionization techniques
- Full complement of acquisition modes including MRM, Scan/MRM, Neutral Loss Scan, and more.
- The Easy to operate common software platform.
- Solution package for easier start-up the analysis and Quick DB for Quantitative Screening with Built in calibration.

Shimadzu is able to provide an excellent solution for analysis of food safety, environmental monitoring to clinical research and forensics with speed, sensitivity and accuracy required in both the areas of GC/MS/MS and LC/MS/MS.

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1. Chemical Contamination

PREFACE

Pesticide residues in food continue to be the target of studies due to the uncertainty concerning adverse effects that those residues may have on human health after a lengthy exposure at low levels. More than 1000 active ingredients have been utilised and are formulated in thousands of different commercial products. They include a variety of compounds, mainly insecticides, herbicides and fungicides, as well as their metabolites, with very different physico-chemical characteristics and large differences in polarity, volatility and persistence. Consequently, in order to ensure food safety for consumers and to facilitate international trade, regulatory bodies around the world have established maximum residue levels (MRLs) for pesticide residues in food commodities; that is, the maximum amount of pesticide residue and its toxic metabolites that might be expected on a commodity if good agricultural practice was adhered to during the use of the pesticide.

In the European Union regulation 396/2005/EC was implemented in 2008 harmonising pesticide MRLs in all member states for 435 pesticide active substances in 378 commodities. This EU regulation covers pesticides both currently and formerly used in agriculture in or outside the EU. For pesticide and food commodity combinations not listed in the regulation a default MRL of 0.01 mg kg-1 applies (Art 18(1b) of European Union Regulation No 396/2005). In general, MRLs in the European Food regulation are in the range 0.01 - 10 mg/kg depending on the pesticide-commodity combination, with the lowest levels set for banned pesticides. For vegetables, fruits and cereals intended for the production of baby foods, Directive 2006/141/EC requires that baby food contains no detectable levels of pesticide residues defined as < 0.01 mg/kg and prohibits the use of certain very toxic pesticides in the production of infant foods and establishes even lower MRLs for a few other very toxic pesticides. Regulatory bodies around the world, as in the EU, have produced similar guidelines. In the US, tolerances for more than 450 pesticides and other ingredients are stated in the electronic Code of

Federal Regulations (US Environmental Protection Agency Office of Pesticide Programs) and are enforced by the US FDA. Japan's positive list system for agricultural chemical residues in foods, introduced in 2006, contains MRLs for over 400 pesticides in various commodities. China published national standard GB 2763-2005 in 2005 and more recently GB 28260-2011 which was introduced in 2012 and specifies 181 MRLS for 85 pesticides in food.

Consequently, pesticide analysis laboratories are under increasing pressure to expand the list of targeted pesticides, detect analytes at lower levels and with greater precision, reduce analysis turnaround times and reduce usage of hazardous solvents while maintaining or reducing costs. Pesticide residues were traditionally analysed mainly by GC-based multi-residue methods often with MS detection. However, many modern (semi) polar compounds and/or ionic compounds could not be analysed in this way due to poor thermal stability or volatility without the need for derivatisation. Recent improvements in liquid chromatography - tandem mass spectrometry, combined with the discussed pitfalls of GCMS, have meant LC/MS/MS has become a vital technique. LC-triple quadruple mass spectrometry enables highly selective and sensitive analysis and is well suited to the multi-class analysis of large numbers of pesticides at trace levels.

C-1 Determination of Ethyl Carbamate in wine by Multidimensional Gas Chromatography with Mass spectrometric Detection

INTRODUCTION

A novel, reliable and robust analytical method employing a multidimensional gas chromatography coupled to mass spectrometry（MDGC/GC-MS）technique was successfully developed for ethyl carbamate（EC）in wine. The EC was quantitatively analyzed and confirmed by heart-cutting multidimensional gas chromatography and mass selective detection in selected ion scan (SIM) mode. The average recovery was above 82% with a relative standard deviation of 2.29%. The Limit of Detection （LOD）is 1.0 µg/L. The proposed method show good accuracy and precision while minimizing erroneous result due to reduction of the effect of matrix.

Ethyl carbamate (EC), also known as urethane, is a chemical contaminant naturally formed in fermented foods during fermentation process or during storage. Measurable levels of EC have been found in foods such as bread, soy sauce and yogurt, and in alcoholic beverages such as spirits, wine and beer. It occurs at low levels, from ng/L to mg/L. EC is genotoxic and carcinogenic for a number of species such as mice, rats, hamsters and monkeys. It has been classified as a group 2B carcinogen, ‘probably carcinogenic to humans’, by the World Health Organization’s International Agency for Research on Cancer (IARC) in 2007.

There are many methods for determining EC in various samples. These methods include high performance liquid chromatography (HPLC), gas chromatography (GC), gas chromatography mass spectrometry (GC-MS), etc. Among the mentioned methods, GC-MS is the most frequently used methodologies.

However, the limitation of conventional GC-MS is that it is performed using a single column where co-elution of peaks is likely to occur, making identification of EC difficult or uncertain in wine samples. There is the possibility to make an incorrect identification if a trace EC is masked by a large peak. Heart-cutting multidimensional gas chromatography coupled to mass spectrometry（MDGC/GC-MS）can provide a good solution for identifying EC from complex samples owing to its enhanced separation capability.

The purpose of this study was to develop a novel, reliable and robust methodology based on solid phase extraction (SPE) and multidimensional gas chromatography coupled to mass spectrometry (SPE-MDGC/GC-MS) to quantify EC in wine. Chromatographic elements and parameters, such as column combination, temperature and pressure, were optimized in order to achieve a high resolution and good repeatability.

EXPERIMENTAL

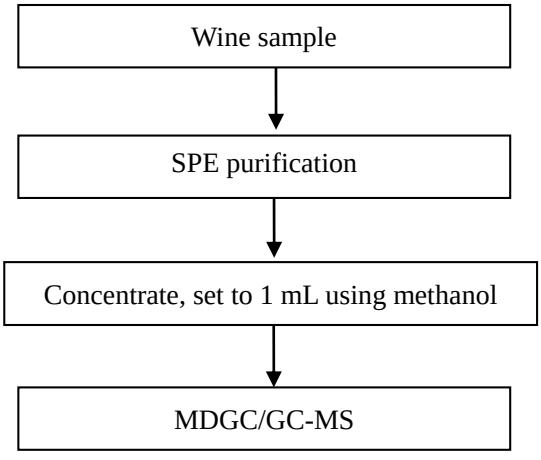
Instrument

Multidimensional gas chromatography-mass spectrometry (MDGC/GC-MS, Shimadzu Corporation）

Analysis Conditions

1D chromatography	:GC-2010 Plus model equipped with flame ionization detection (FID)
Analytical column	: Rtx-wax, 30m × 0.25mm id × 0.25μm
Inlet temp	: 250℃
Col oven temp program	: 60℃(1min)→@5℃/min→150℃→@10℃/min →240℃(5min)
Control mode	: Constant pressure (179 kPa)
Injection mode	: Splitless mode (1 min)
FID temperature	: 250℃
2D chromatography	: GCMS-QP2010 Ultra
Analytical column	: Rxi-5 ms, 30m × 0.25mm id × 0.25μm
Col oven temp program	:60℃(2min)→@10℃/min→230℃(2min) →@5℃/min→250℃(5min)
Ionization mode	: EI
Ion source temperature	: 230℃
IF temperature	: 250℃
Acquisition mode	: SIM (62, 74, 89)
Cut pressure	: 120 kPa
Cut program	: 20.7min-22.5min

Sample pretreatment



RESULTS AND DISCUSSION

SIM chromatogram

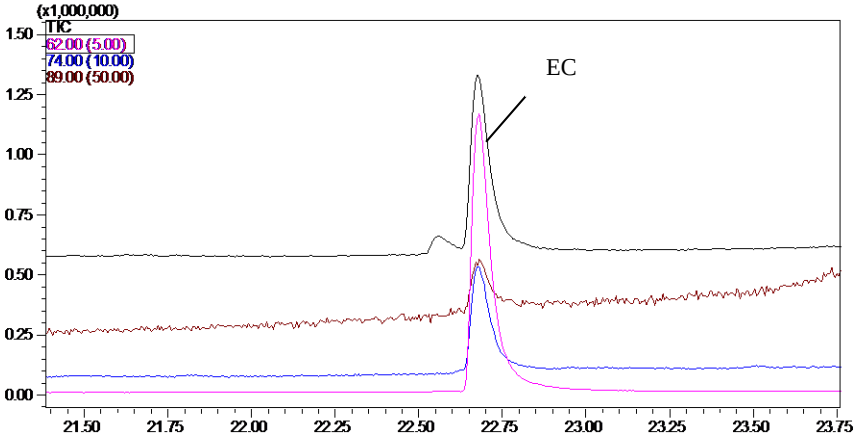


Fig.1 The SIM chromatogram of EC（1000 μg/L）

Calibration curve of EC

The standard sample was diluted by methanol to the content of 0, 10, 25, 50, 100, 200, 400, 1000 μg/L, the calibration curve and the Relative coefficients (R^2) were showed in Fig.2.The Limit of Detection (LOD) is 1.0 μg/L, calculated by the 3 S/N(Peak to Peak).

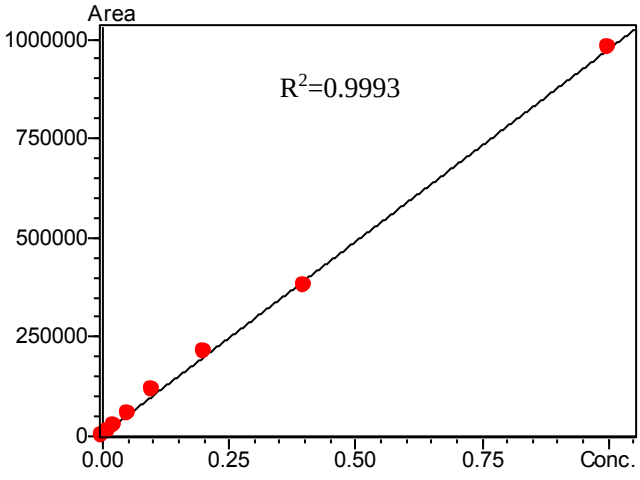


Fig.2 The calibration curve of EC

Repeatability test

The EC standard sample of 100μg/L was injected 6 times, contiuously, the relative standard deviations (RSD%) of area is 2.10%, this shows the good repetition results in table 1.

Table 1 the repeatability results of EC

	1	2	3	4	5	6	RSD%
Area	103011	109193	105759	104228	103973	104228	2.10
R.T	22.724	22.729	22.717	22.702	22.691	22.686	0.08

Recovery test

Add the amount of 40 μg/L EC standard samples in the wine. This was analyzed under the above conditions. Three replicates was analyzed for the spiked level. Table 2 lists the recoveries and precision values obtained in the validation portion of the study. The recovery was excellent, an average of 85% with a relative standard deviation of 2.29% (n=3).

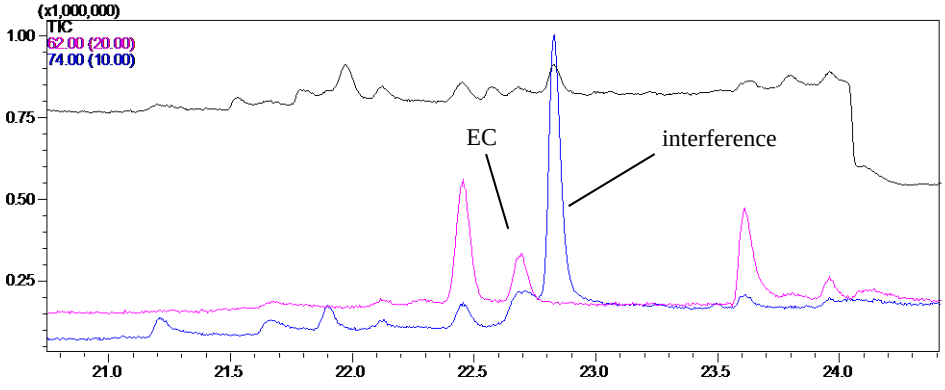


Fig. 3 The spectra of spiked sample

Table 2 The recovery results of EC

Spiking level (μg/L)	Recovery (%)			Average (%)	RSD (%)
	1	2	3		
40	82.97	85.45	86.82	85.08	2.29

CONCLUSION

A novel, reliable and robust analytical method based on SPE and multidimensional gas chromatography coupled to MS detector for determining the amount of EC in wine was developed. The EC could be quantified by using a heart-cutting technique in MDGC/GC-MS system with a combination of a polar (Rtx-wax) column and a low polar (Rxi-5 ms) column and monitoring in SIM mode. This SPE-MDGC/GC-MS method yields excellent precision, sensitivity and respectable selectivity for EC.

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C-2 Determination of Pesticide Residues in Hotpot Seasoning using Gas Chromatograph-Triple Quadrupole Mass Spectrometer

INTRODUCTION

A convenient, reliable and robust analytical method employing a gas chromatograph-triple quadrupole mass spectrometer (GC-MS/MS) was successfully developed for determination of pesticide residues in hotpot seasoning. The pesticides were confirmed and analyzed quantitatively by GC-MS/MS in MRM mode. The method demonstrate the average recovery in range of 61.5%-119.6%, the relative standard deviation of 5 μg/L standard pesticides sample in range of 1.32% to 7.05% and the limit of detection (LOD) less than 1.0 μg/kg.

Pesticide residues in the food have been an increasing concern for consumers worldwide. Monitoring programs based on analytical methodologies are established to ensure that the pesticide level in food is in compliance with national and international laws. However, the diversity of pesticides and complexity of food sample matrix present ongoing challenges for analytical chemists to meet the increasingly stringent requirement for sensitivity and precision.
Hotpot seasoning is a kind of condiments used widely. The raw materials, such as chili, pepper, garlic etc, may take pesticides into hotpot seasoning. In addition, there is a variety of plant or animal oils and pigments in hotpot seasoning. The sample matrix is complex. At present, the main technologies for pesticide analysis is gas chromatograph - single quadrupole mass spectrometer (GC-MS), GC-MS analysis is difficult, uncertain and less selective due to matrix interference. GC-MS/MS operated in multiple reaction monitoring (MRM) mode is an ideal technique for multi-residue analysis in complex matrix samples.
This application note describes GC-MS/MS method for detection of 40 pesticides in hotpot seasoning after extraction using the QuEChERS method. It shows good accuracy and precision due to reducing the effect of matrix

EXPERIMENTAL

Instrument

Gas chromatograph - Triple quadrupole mass spectrometer (GCMS-TQ8040, Shimadzu Corporation)

Analysis Conditions

GC conditions

Analytical column : Rtx-5MS, 30m × 0.25mm id × 0.25µm
Inlet temp : 250℃
Col oven temp program : 50℃(1min)→@25℃/min→150℃ →@10℃/min →300℃(15min)
Control mode : linear velocity (47.6 cm/sec)
Injection mode : Splitless mode (1min)
High pressure injection : 250 kPa

MS conditions

Ionization mode : EI
Ion source temperature : 230℃
IF temperature : 250℃
Detector voltage : Relative to the tuning result+0.4KV
Acquisition mode : MRM (Table.1)

Table.1 Quantifier and Qualifier MRM Transitions for 40 Pesticides

ID	Compound	R.T.	*m/z*	CE	Ref.Ions	CE
1	Methamidophos	5.580	141.00>95.00	8	141.00>126.00	4
2	Dichlorvos	5.705	185.00>93.00	16	185.00>109.00	16
3	alpha-HCH	9.665	219.00>183.00	10	219.00>147.00	22
4	Hexachlorobenzene	9.760	284.00>214.00	32	284.00>249.00	22
5	Dimethoate	9.855	125.00>79.00	8	125.00>47.00	14
6	beta-HCH	10.090	219.00>183.00	10	219.00>147.00	22
7	gamma-HCH	10.280	219.00>183.00	8	219.00>145.00	22
8	delta-HCH	10.760	219.00>183.00	10	219.00>147.00	22
9	Parathion-methyl	11.450	263.00>109.00	16	263.00>136.00	8
10	Heptachlor	11.660	272.00>237.00	20	272.00>117.00	32
11	Fenitrothion	11.920	277.00>260.00	6	277.00>109.00	14
12	Malathion	12.080	173.00>99.00	16	173.00>127.00	8
13	Chlorpyrifos	12.230	314.00>258.00	18	314.00>286.00	8
14	Aldrin	12.345	263.00>193.00	30	263.00>203.00	26
15	Isocarbophos	12.440	289.00>136.00	16	289.00>113.00	4
16	Isofenphos-methyl	12.745	199.00>121.00	12	241.00>121.00	24

17	Isodrin	12.890	193.00>157.00	22	193.00>123.00	30
18	Heptachlor-endo-epoxide	13.065	353.00>263.00	22	353.00>217.00	38
19	Quinalphos	13.160	157.00>129.00	16	157.00>102.00	24
20	Methidathion	13.430	145.00>85.00	8	145.00>58.00	16
21	o,p'-DDE	13.540	246.00>176.00	30	246.00>211.00	24
22	Profenofos	14.050	337.00>267.00	16	337.00>309.00	6
23	p,p'-DDE	14.145	246.00>176.00	30	246.00>211.00	22
24	Dieldrin	14.266	277.00>241.00	8	277.00>170.00	36
25	o,p'-DDD	14.270	235.00>165.00	28	235.00>199.00	22
26	Endrin	14.665	263.00>191.00	30	263.00>193.00	28
27	p,p'-DDD	14.925	235.00>165.00	26	235.00>199.00	20
28	o,p'-DDT	14.980	235.00>165.00	30	235.00>199.00	16
29	Triazophos	15.170	257.00>162.00	8	257.00>134.00	22
30	p,p'-DDT	15.630	235.00>165.00	30	235.00>199.00	18
31	Bifenthrin	16.490	181.00>165.00	28	181.00>153.00	8
32	Methoxychlor	16.640	227.00>169.00	26	227.00>141.00	30
33	Fenpropathrin	16.665	181.00>152.00	22	265.00>210.00	10
34	phosalone	17.135	182.00>111.00	16	182.00>75.00	28
35	Cyhalothrin	17.460	197.00>141.00	12	197.00>161.00	8
36	Mirex	17.615	272.00>237.00	22	274.00>239.00	18
37	Cyfluthrin-1	18.800	226.00>206.00	14	226.00>199.00	6
38	Cyfluthrin-2	18.895	226.00>206.00	14	226.00>199.00	6
39	Cyfluthrin-3	18.960	226.00>206.00	14	226.00>199.00	6
40	Cyfluthrin-4	19.000	226.00>206.00	14	226.00>199.00	6
41	Cypermethrin-1	19.125	181.00>152.00	22	181.00>127.00	22
42	Cypermethrin-2	19.225	181.00>152.00	22	181.00>127.00	22
43	Cypermethrin-3	19.285	181.00>152.00	22	181.00>127.00	22
44	Cypermethrin-4	19.325	181.00>152.00	22	181.00>127.00	22
45	Fenvalerate-1	20.020	419.00>225.00	6	419.00>167.00	12
46	Fenvalerate-2	20.225	419.00>225.00	6	419.00>167.00	12
47	Deltamethrin	20.815	253.00>93.00	18	253.00>172.00	8

Sample pretreatment

Extraction:

The hotpot seasoning sample (2g) mixed with acetonitrile (20mL), MgSO4 (6g) CH3COONa (1.5g) in 50 mL centrifuge tube was shaken for 5 minutes, and then centrifuged for 2 minutes at 5000 rpm. The supernatant was transferred to a new

centrifuge tube. Repeated the same extraction, and mixed two parts of acetonitrile extract.

Purification:

The mix of two extracts in N2 evaporation was evaporated to 3 mL. This portion transferred to a new centrifuge tube, mixed with PSA powder (50 mg), C18 powder (50 mg), MgSO4 (150 mg) was shaken for 5 minutes, and then centrifuged for 2 minutes at 5000 rpm. The sample was evaporated to nearly dryness, and diluted to 1mL with n-hexane. The sample (1 mL) was immediately transferred into a GC vial for GC-MS/MS analysis.

RESULTS AND DISCUSSION

Chromatogram

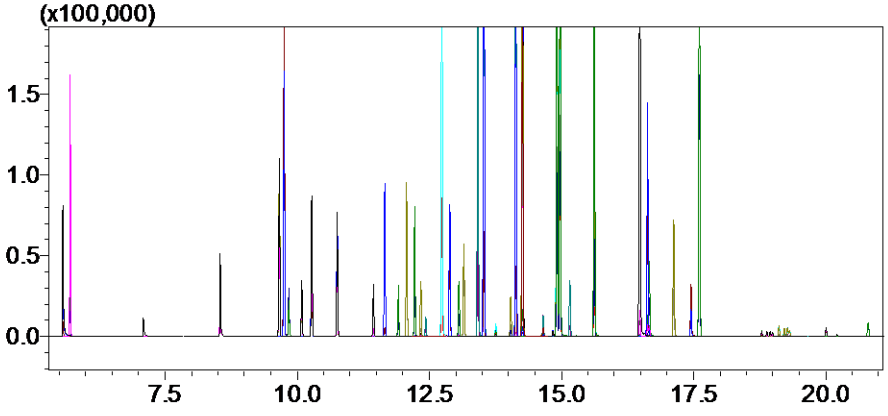
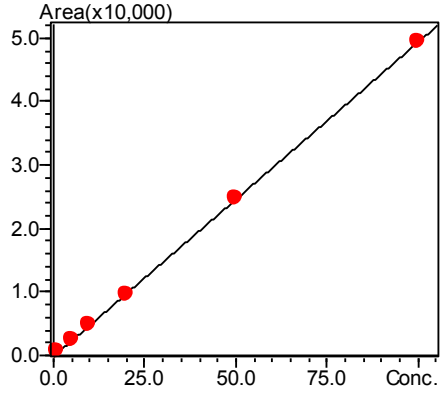


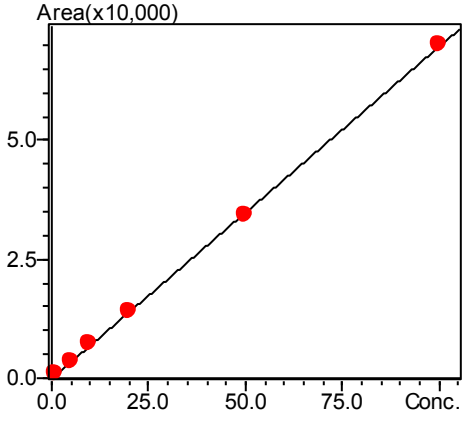
Fig.1 The MRM Chromatogram of Pesticides（100 μg/L）

Calibration Curve

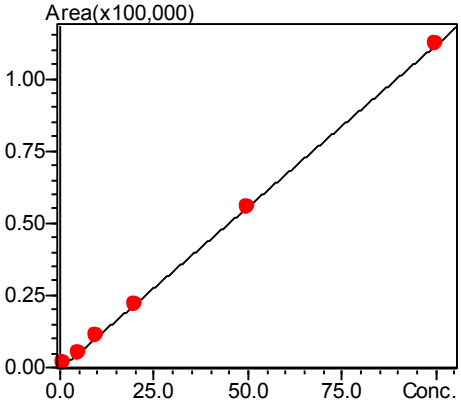
The organic chlorine pesticide and organic phosphorus pesticide was diluted by n-hexane to the concentration of 1, 5, 10, 20, 50, 100 μg/L. The pyrethroid pesticide was diluted by n-hexane to the concentration of 5, 10, 20, 50, 100 μg/L. The calibration curve, the relative coefficients (R^2) and the Limit of Detection (LOD)were showed in Fig.2. LOD was calculated by the 3 S/N(Peak to Peak).



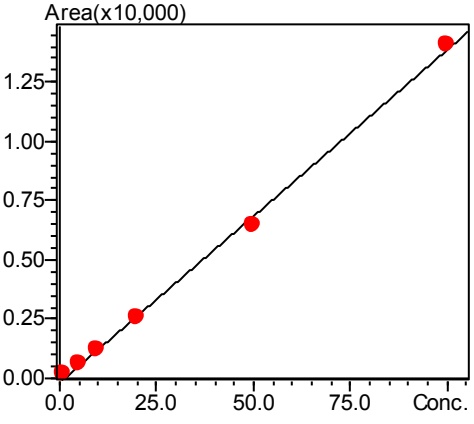
Dichlorvos



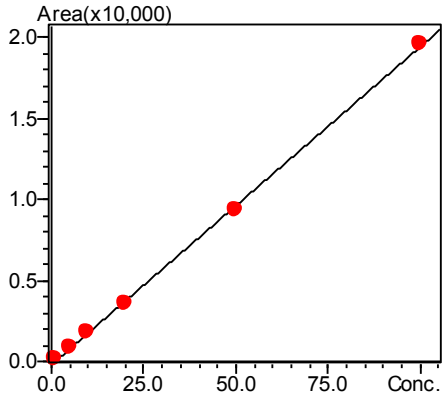
alpha-HCH



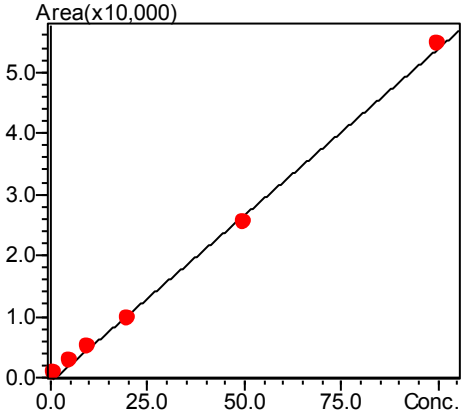
Hexachlorobenzene



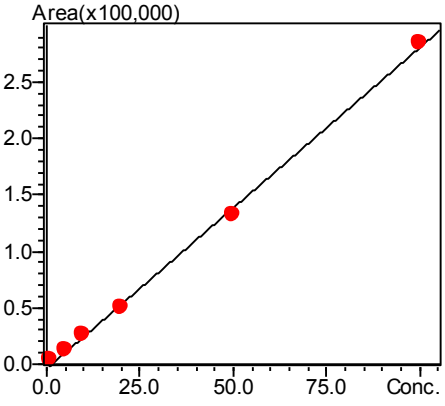
Dimethoate



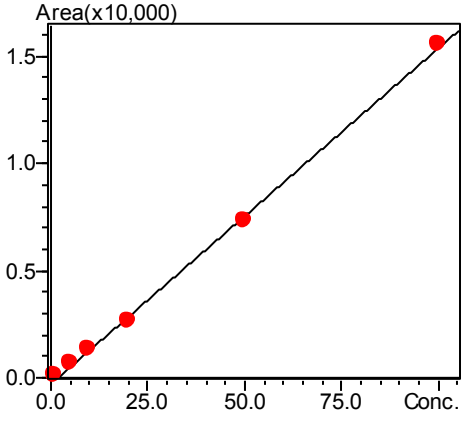
Fenitrothion



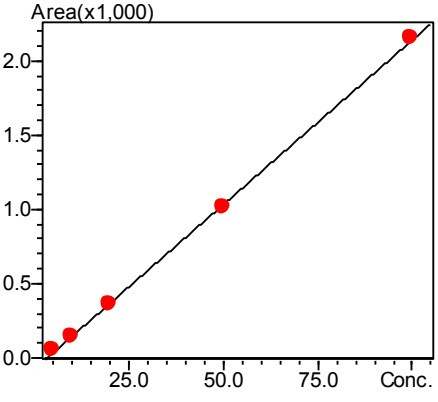
Malathion



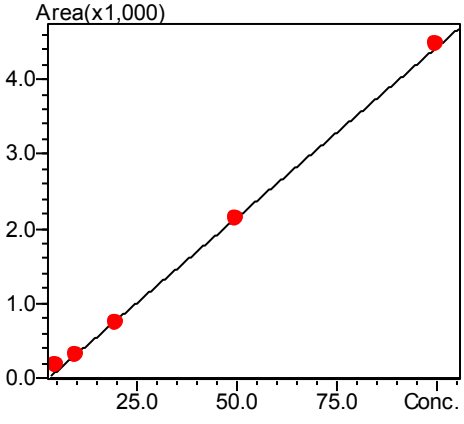
o,p'-DDE



Profenofos



Cyfluthrin



Cypermethrin

Fig.2 The Calibration Curve of some pesticides

Table.2 The Relative Coefficients and LOD

No.	Compound	R^2	LOD (μg/kg)	No.	Compound	R^2	LOD (μg/kg)
1	Methamidophos	0.9950	0.23	25	o,p'-DDD	0.9991	0.06
2	Dichlorvos	0.9999	0.08	26	Endrin	0.9985	0.40
3	alpha-HCH	0.9998	0.11	27	p,p'-DDD	0.9985	0.06
4	Hexachloro benzene	0.9999	0.06	28	o,p'-DDT	0.9997	0.09
5	Dimethoate	0.9991	0.60	29	Triazophos	0.9982	0.70
6	beta-HCH	0.9997	0.33	30	p,p'-DDT	0.9994	0.06
7	gamma-HCH	0.9995	0.11	31	Bifenthrin	0.9989	0.05
8	delta-HCH	0.9996	0.20	32	Methoxychlor	0.9983	0.10
9	Parathion-methyl	0.9999	0.31	33	Fenpropathrin	0.9997	0.43
10	Heptachlor	0.9998	0.14	34	phosalone	0.9990	0.19
11	Fenitrothion	0.9996	0.56	35	Cyhalothrin	0.9986	0.63
12	Malathion	0.9991	0.28	36	Mirex	0.9992	0.06
13	Chlorpyrifos	0.9990	0.28	37	Cyfluthrin-1	0.9998	0.11
14	Aldrin	0.9995	0.43	38	Cyfluthrin-2	0.9995	0.10
15	Isocarbophos	0.9990	0.35	39	Cyfluthrin-3	0.9992	0.26
16	Isofenphos-methyl	0.9996	0.06	40	Cyfluthrin-4	0.9993	0.22
17	Isodrin	0.9995	0.26	41	Cypermethrin-1	0.9996	0.26
18	Heptachlor-endo-epoxide	0.9991	0.13	42	Cypermethrin-2	0.9993	0.35
19	Quinalphos	0.9985	0.40	43	Cypermethrin-3	0.9997	0.50
20	Methidathion	0.9982	0.08	44	Cypermethrin-4	0.9982	0.35
21	o,p'-DDE	0.9992	0.04	45	Fenvalerate-1	0.9997	0.55
22	Profenofos	0.9993	0.40	46	Fenvalerate-2	0.9987	1.72
23	p,p'-DDE	0.9993	0.06	47	Deltamethrin	0.9995	0.71
24	Dieldrin	0.9993	0.06				

Repeatability test

The residues (5 μg/L) was injected 6 times contiuously, the relative standard deviations (RSD%) of area is shown in Table 3.

Table.3 The Repeatability Results

No.	Compound	RSD%	No.	Compound	RSD%
1	Methamidophos	2.99	25	o,p'-DDD	2.38
2	Dichlorvos	2.17	26	Endrin	4.61
3	alpha-HCH	4.70	27	p,p'-DDD	3.89
4	Hexachloro benzene	3.73	28	o,p'-DDT	3.46
5	Dimethoate	4.77	29	Triazophos	4.66
6	beta-HCH	5.98	30	p,p'-DDT	4.04
7	gamma-HCH	1.32	31	Bifenthrin	2.42
8	delta-HCH	1.28	32	Methoxychlor	4.67
9	Parathion-methyl	5.59	33	Fenpropathrin	2.71
10	Heptachlor	6.67	34	phosalone	2.73
11	Fenitrothion	6.60	35	Cyhalothrin	3.47
12	Malathion	4.83	36	Mirex	3.31
13	Chlorpyrifos	6.75	37	Cyfluthrin-1	7.00
14	Aldrin	4.86	38	Cyfluthrin-2	5.81
15	Isocarbophos	7.56	39	Cyfluthrin-3	6.79
16	Isofenphos-methyl	4.49	40	Cyfluthrin-4	6.21
17	Isodrin	5.81	41	Cypermethrin-1	4.44
18	Heptachlor-endo-epoxide	5.21	42	Cypermethrin-2	4.69
19	Quinalphos	3.08	43	Cypermethrin-3	6.87
20	Methidathion	3.41	44	Cypermethrin-4	5.25
21	o,p'-DDE	3.78	45	Fenvalerate-1	3.54
22	Profenofos	7.05	46	Fenvalerate-2	5.13
23	p,p'-DDE	4.11	47	Deltamethrin	6.02
24	Dieldrin	5.94			

Recovery test

Add the amount of 50 µg/kg residues in the hotpot seasoning. The spiked samples were analyzed under the same conditions. Three replicates were analyzed for the spiked level. Table 4 lists the recoveries and the validation portion of the study.

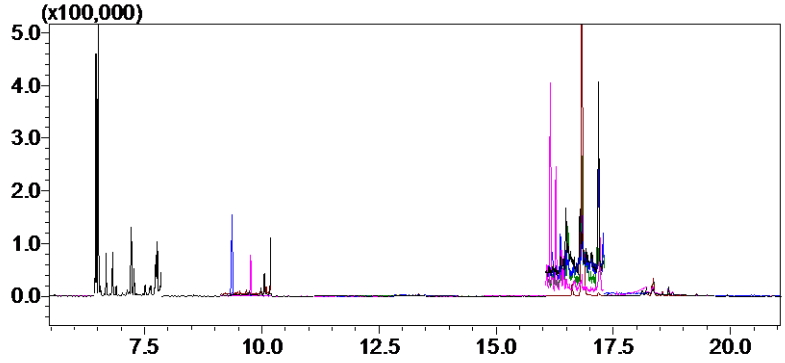


Fig.3 The MRM Chromatogram of Actual Sample

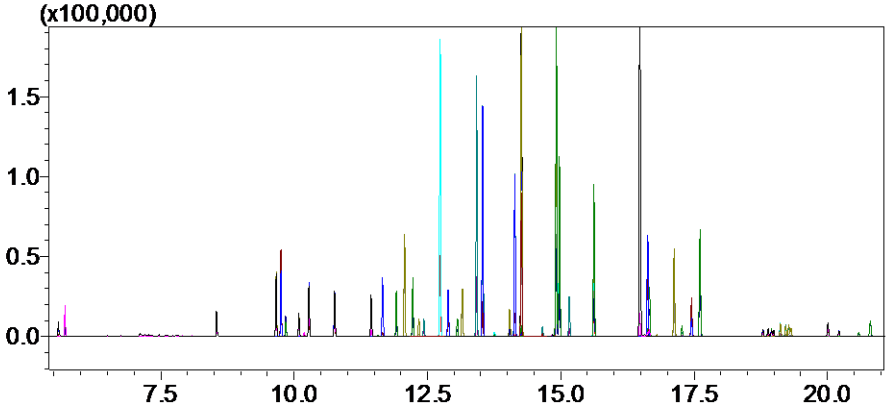


Fig.4 The MRM Chromatogram of Spiked Sample

Table.4 The Recovery Results of Pesticides

No.	Compound	Concentration (μg/kg)	Spiking level (μg/kg)	Recovery (%)	RSD%
1	Methamidophos	8.90	50	73.0	5.94
2	Dichlorvos	N.D	50	119.6	12.2
3	alpha-HCH	N.D	50	90.8	6.02
4	Hexachlorobenzene	N.D	50	77.9	3.70
5	Dimethoate	N.D	50	76.9	3.95
6	beta-HCH	N.D	50	87.4	1.62
7	gamma-HCH	N.D	50	83.2	2.12
8	delta-HCH	N.D	50	86.6	2.93
9	Parathion-methyl	N.D	50	107.6	3.34
10	Heptachlor	N.D	50	84.8	1.85
11	Fenitrothion	N.D	50	69.3	3.12
12	Malathion	N.D	50	69.4	3.51

13	Chlorpyrifos	N.D	50	90.1	2.91
14	Aldrin	N.D	50	78.8	5.28
15	Isocarbophos	N.D	50	72.3	4.09
16	Isofenphos-methyl	N.D	50	72.8	2.85
17	Isodrin	N.D	50	77.8	5.17
18	Heptachlor-endo-	N.D	50	82.8	4.85
19	Quinalphos	N.D	50	66.8	3.02
20	Methidathion	N.D	50	61.5	3.55
21	o,p'-DDE	N.D	50	76.4	3.54
22	Profenofos	N.D	50	65.3	4.22
23	p,p'-DDE	N.D	50	80.6	3.83
24	Dieldrin	N.D	50	84.7	3.89
25	o,p'-DDD	N.D	50	83.3	3.68
26	Endrin	N.D	50	88.1	4.25
27	p,p'-DDD	N.D	50	87.8	3.43
28	o,p'-DDT	N.D	50	87.3	4.02
29	Triazophos	N.D	50	72.0	3.13
30	p,p'-DDT	N.D	50	74.9	5.90
31	Bifenthrin	2.47	50	74.0	2.82
32	Methoxychlor	N.D	50	80.5	3.82
33	Fenpropathrin	N.D	50	74.5	3.06
34	phosalone	N.D	50	80.8	2.71
35	Cyhalothrin	N.D	50	65.6	4.80
36	Mirex	N.D	50	67.3	3.25
37	Cyfluthrin-1	N.D	50	83.6	1.74
38	Cyfluthrin-2	N.D	50	73.1	2.48
39	Cyfluthrin-3	N.D	50	76.2	3.54
40	Cyfluthrin-4	N.D	50	72.7	4.70
41	Cypermethrin-1	N.D	50	80.9	2.99
42	Cypermethrin-2	N.D	50	76.8	2.83
43	Cypermethrin-3	N.D	50	75.7	4.23
44	Cypermethrin-4	N.D	50	73.0	6.00
45	Fenvalerate-1	N.D	50	87.4	4.50
46	Fenvalerate-2	N.D	50	93.6	5.06
47	Deltamethrin	N.D	50	88.1	3.27

CONCLUSION

A convenient, reliable analytical method based on gas chromatograph - triple quadrupole mass spectrometer GCMS-TQ8040 for determining pesticide residues in hotpot seasoning was developed. Sample pretreatment process simplified to remove the matrix interference, eliminate false positives and to ensure the accuracy of quantitation by multiple reaction monitoring (MRM) mode. The method shows acceptable sensitivity for residues. The LOD of pesticides were less than 1 μg/kg. The average recoveries were in the range of 61.5% to 119.6%.

C-3 A method for simultaneous analysis of 174 pesticides in grape using GC-MS/MS

INTRODUCTION

A method was developed for the simultaneous analysis of 174 pesticides in grape using gas chromatography-tandem mass spectrometry (GC-MS/MS) by multiple reaction monitoring (MRM) acquisition mode. The sample was prepared using the QuEChERS method wherein 174 pesticides were added to the sample extract, with the concentration adjusted to 10 ng/mL. The prepared sample was then subjected to MRM analysis for 174 compounds using the analytical conditions registered in Smart Pesticides Database. The area repeatability of 174 compounds (10 ng/mL) is below 10% (n=6). The results shows that this method can be used to simultaneous analysis of 174 residual pesticide compounds in grape.

With the increasing volume of worldwide trade in agricultural products in recent years, analysts are paying increasing attention to the study of techniques for analysis of pesticide residues in agricultural products. The number of relevant pesticides grows yearly, and has reached hundreds of compounds. Multi-residue simultaneous analysis of hundreds of pesticides in agricultural products is always a challenge. As required detection limits for many pesticides fall to 10µg/kg (10ppb), more sophisticated analytical tools are demanded.

Due to its excellent sensitivity and selectivity, GC-MS/MS with MRM acquisition mode is utilized for the analysis of residual pesticides in foods. Two transitions were used for each compound. The MRM transitions and collision energies for every compound were acquired from the pesticide MRM database provided by Shimadzu Corporation which contains 479 pesticides.

In this report, the grape sample was extracted using the QuEChERS method wherein 174 pesticides were spiked in the blank sample extract (10 ng/mL). Spiked samples were analyzed in MRM mode using a gas chromatograph coupled with a triple quadrupole mass spectrometer (GCMS-TQ8040, Shimadzu Corporation, Japan). The result shows that the MRM chromatographic peaks of 174 pesticides

in spiked sample (10 ng/mL) is with high S/N. The area repeatability of 174 compounds is below 10% (n=6). The established method was sensitive, repeatable and reliable for simultaneous analysis of the 174 pesticides in grape samples.

EXPERIMENTAL

Instrument

GCMS-TQ8040

Analysis Conditions

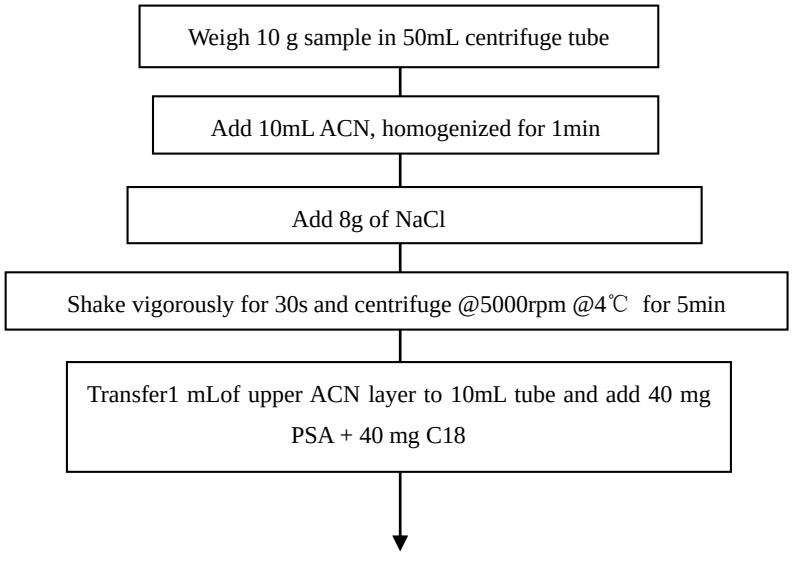
GC Conditions:

Analytical column	: Rxi-5MS sil, 30m × 0.25mm id × 0.25µm
Inlet temp	: 250℃
Col oven temp program	: 50℃(1min)→@(25℃/min)→150℃→ @(10℃/min)→300℃(15 min)
Control mode	: Constant Linear Velocity (47.2cm/sec)
Injection mode	: Splitless mode (1.5 min)
High Pressure Injection	: 250 kPa(1.5 min)
Injection Volume	: 1 µL

MS Conditions:

Ionization mode	: EI
Ion source temperature	: 200℃
IF temperature	: 250℃
Acquisition mode	: MRM (parameter showed in Table 1)
Solvent Cut Time	: 4 min

Sample pretreatment



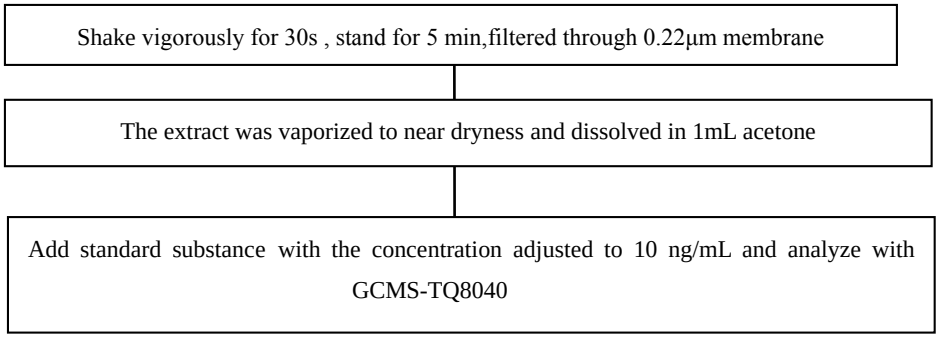


Fig.1 Sample pretreatment flow

Table 1 MRM parameter for174 pesticides

No.	Compound	CAS Number	R.T.	Quantitative ion	CE	Qualitative ion	CE
1	Allidochlor	93-71-0	6.259	138.1>96.0	6	138.1>110.1	6
2	Dichlormid	37764-25-3	6.912	172.0>108.1	6	172.0>136.1	6
3	Biphenyl	92-52-4	7.287	154.1>128.1	22	154.1>115.1	24
4	Butylate	2008-41-5	7.686	174.1>146.1	6	174.1>75.0	4
5	Etridiazole	2593-15-9	7.933	210.9>182.9	10	210.9>139.9	22
6	propham	122-42-9	8.004	179.0>137.0	5	120.0>77.0	15
7	Methacrifos	62610-77-9	8.410	240.0>208.0	4	240.0>180.0	10
8	Chloroneb	2675-77-6	8.519	206.0>141.0	20	206.0>113.0	24
9	2-Phenylphenol	90-43-7	8.691	170.1>141.1	24	170.1>115.1	28
10	heptenophos	23560-59-0	9.204	124.0>89.0	15	215.0>200.0	10
11	Tecnazene	117-18-0	9.454	260.9>202.9	14	260.9>230.9	8
12	Diphenylamine	122-39-4	9.832	169.1>66.0	24	169.1>77.0	28
13	Ethoprophos	13194-48-4	9.909	200.0>158.0	6	200.0>114.0	14
14	Ethalfluralin	55283-68-6	10.039	316.1>276.0	10	316.1>202.0	24
15	Chlorpropham	101-21-3	10.142	213.1>171.1	6	213.1>127.1	14
16	Dicrotophos	141-66-2	10.207	127.1>109.0	12	127.1>95.0	18
17	Trifluralin	1582-09-8	10.209	306.1>264.1	8	306.1>206.1	14
18	Benfluralin	1861-40-1	10.265	292.1>264.0	8	292.1>206.0	12
19	Dioxabenzofos (Salithion)	3811-49-2	10.293	216.0>201.0	10	216.0>183.0	10
20	Sulfotep	3689-24-5	10.308	322.0>202.0	10	322.0>294.0	4
21	Cadusafos	95465-99-9	10.452	158.9>130.9	8	158.9>97.0	18
22	Phorate	298-02-2	10.532	260.0>75.0	8	260.0>231.0	4
23	alpha-HCH	319-84-6	10.636	218.9>182.9	8	218.9>144.9	20
24	Hexachlorobenzene	118-74-1	10.706	283.8>248.8	24	283.8>213.8	28

25	Thiometon	640-15-3	10.779	125.0>47.0	14	125.0>79.0	10
26	pentachloroanisole	1825-21-4	10.823	265.0>237.0	15	280.0>265.0	10
27	Dicloran	99-30-9	10.892	206.0>176.0	10	206.0>160.0	16
28	monolinuron	1746-81-2	11.133	126.0>99.0	15	214.0>61.0	10
29	beta-HCH	319-85-7	11.135	218.9>182.9	8	218.9>144.9	20
30	Dimethipin	55290-64-7	11.138	118.0>58.0	6	118.0>73.0	2
31	Clomazone	81777-89-1	11.196	204.1>107.0	20	204.1>78.0	26
32	Quintozene	82-68-8	11.215	294.8>236.8	16	294.8>264.8	12
33	gamma-HCH (Lindane)	58-89-9	11.316	218.9>182.9	8	218.9>144.9	20
34	Terbufos	13071-79-9	11.398	231.0>174.9	14	231.0>128.9	26
35	Cyanophos	2636-26-2	11.399	243.0>109.0	14	243.0>116.0	6
36	Fonofos	944-22-9	11.476	246.0>109.1	18	246.0>137.1	6
37	Propyzamide	23950-58-5	11.478	172.9>144.9	16	172.9>74.0	28
38	Diazinon	333-41-5	11.533	304.1>179.1	10	304.1>162.1	8
39	Phosphamidon-1	13171-21-6	11.616	264.1>127.1	14	264.1>193.1	8
40	Chlorothalonil	1897-45-6	11.639	265.9>230.8	14	265.9>168.0	22
41	Disulfoton	298-04-4	11.744	186.0>97.0	16	186.0>153.0	6
42	Isazophos	42509-80-8	11.774	161.0>119.0	15	161.0>146.1	6
43	Terbacil	5902-51-2	11.774	161.0>144.0	14	161.0>118.0	14
44	Tefluthrin	79538-32-2	11.817	177.0>127.1	16	177.0>137.1	16
45	Etrimfos	38260-54-7	11.862	292.1>181.1	8	292.1>153.1	20
46	delta-HCH	319-86-8	11.864	218.9>182.9	10	218.9>144.9	20
47	Tri-allate	2303-17-5	11.902	268.1>226.0	14	268.1>184.0	20
48	Formothion	2540-82-1	12.160	224.0>125.0	18	224.0>155.0	12
49	Phosphamidon-2	13171-21-6	12.311	264.1>127.1	14	264.1>193.1	8
50	Benfuresate	68505-69-1	12.329	163.1>121.1	6	163.1>135.1	6
51	Dichlofenthion	97-17-6	12.351	279.0>222.9	14	279.0>250.9	8
52	Dimethenamid-P	87674-68-8	12.369	230.0>154.1	10	230.0>137.1	20
53	Propanil	709-98-8	12.388	160.9>99.0	24	160.9>90.0	22
54	Chlorpyrifos-methyl	5598-13-0	12.473	285.9>93.0	22	285.9>270.9	14
55	Metribuzin	21087-64-9	12.475	198.1>82.0	14	198.1>110.1	10
56	Vinclozolin	50471-44-8	12.548	285.0>212.0	12	285.0>178.0	14
57	Parathion-methyl	298-00-0	12.596	263.0>109.0	14	263.0>136.0	8
58	Tolclofos-methyl	57018-04-9	12.618	264.9>249.9	14	264.9>93.0	24
59	Heptachlor	76-44-8	12.768	271.8>236.9	20	271.8>117.0	32
60	Ametryn	834-12-8	12.769	227.1>170.1	14	227.1>185.1	6
61	Fenchlorphos	299-84-3	12.811	284.9>269.9	16	284.9>93.0	24

62	Prometryn	7287-19-6	12.825	241.2>199.1	6	241.2>58.0	14
63	Dithiopyr	97886-45-8	12.875	354.1>306.1	8	354.1>286.1	14
64	S421	127-90-2	12.953	130.0>95.0	20	181.0>85.0	10
65	Pirimiphos-methyl	29232-93-7	13.043	305.1>180.1	8	305.1>290.1	12
66	Fenitrothion	122-14-5	13.092	277.0>260.0	6	277.0>109.1	14
67	Ethofumesate	26225-79-6	13.135	286.1>207.1	8	286.1>161.1	18
68	methyl pentachlorophenyl sufide	1825-19-0	13.183	296.0>263.0	15	246.0>211.0	20
69	Dichlofluanid	1085-98-9	13.231	223.9>123.1	8	223.9>77.0	28
70	Phorate sulfoxide	2588-03-6	13.287	125.0>97.00	9	125.0>115.0	3
71	Chlorpyrifos	2921-88-2	13.405	313.9>257.9	14	313.9>285.9	8
72	Phorate sulfone	2588-04-7	13.415	153.0>97.00	15	153.0>125.0	6
73	(E)-Dimethylvinphos	2274-67-1	13.439	294.9>109.0	14	294.9>279.9	16
74	(Z)-Dimethylvinphos	2274-67-1	13.439	294.9>109.0	14	294.9>279.9	16
75	Aldrin	309-00-2	13.476	262.9>193.0	28	262.9>203.0	26
76	Fenthion	55-38-9	13.484	278.0>109.0	20	278.0>125.0	20
77	Chlorthal-dimethyl	1861-32-1	13.499	300.9>222.9	26	300.9>272.9	14
78	Parathion	56-38-2	13.552	291.1>109.0	14	291.1>137.0	6
79	Triadimefon	43121-43-3	13.615	208.1>181.0	10	208.1>127.0	14
80	Tetraconazole	112281-77-3	13.615	336.0>218.0	14	336.0>204.0	28
81	Isocarbophos	24353-61-5	13.634	289.1>136.0	14	289.1>113.0	6
82	Dicofol deg. (DCBP)	0-00-0	13.699	250.0>139.0	14	250.0>215.0	8
83	Nitrothal-isopropyl	10552-74-6	13.705	254.1>212.0	10	254.1>165.0	22
84	Fluorochloridone	61213-25-0	13.711	311.0>174.1	15	311.0>103.1	18
85	pirimiphos-ethyl	23505-41-1	13.828	304.0>168.0	10	318.0>166.0	15
86	Bromophos	2104-96-3	13.829	330.9>315.9	14	330.9>285.9	28
87	Diphenamid	957-51-7	13.846	167.1>152.1	20	167.1>128.1	26
88	Fosthiazate-1	98886-44-3	13.909	283.0>195.0	8	283.0>103.0	18
89	Isofenphos-methyl	99675-03-3	13.944	199.0>121.0	14	241.1>121.1	22
90	Pendimethalin	40487-42-1	14.044	252.1>162.1	10	252.1>191.1	8
91	Isodrin	465-73-6	14.045	193.0>123.0	30	193.0>157.0	21
92	(E)-Chlorfenvinphos	470-90-6	14.048	323.0>267.0	16	323.0>295.0	6
93	Cyprodinil	121552-61-2	14.065	224.1>208.1	16	224.1>197.1	22
94	Fipronil	120068-37-3	14.132	366.9>212.9	30	366.9>254.9	22
95	Penconazole	66246-88-6	14.168	248.1>192.1	14	248.1>157.1	26
96	Heptachlor-exo-epoxide	1024-57-3	14.196	352.8>262.9	14	352.8>281.9	12

97	Chlozolinate	84332-86-5	14.221	330.9>258.9	6	330.9>186.0	20
98	Isofenphos	25311-71-1	14.222	213.1>121.1	14	213.1>185.1	6
99	(Z)-Chlorfenvinphos	470-90-6	14.252	323.0>267.0	16	323.0>295.0	6
100	Heptachlor-endo-epoxide	28044-83-9	14.253	352.8>289.0	6	352.8>253.0	26
101	Ethychlozate	27512-72-7	14.293	238.1>165.0	12	238.1>138.0	28
102	Mecarbam	2595-54-2	14.321	329.0>131.1	18	329.0>159.1	4
103	Phenthoate	2597-03-7	14.350	273.9>125.0	20	273.9>246.0	6
104	Quinalphos	13593-03-8	14.370	157.1>129.0	14	146.1>118.0	10
105	furalaxyl	57646-30-7	14.394	242.0>95.0	15	301.0>224.0	20
106	Captan	133-06-2	14.405	149.1>105.1	4	149.1>79.1	14
107	Procymidone	32809-16-8	14.432	283.0>96.0	10	283.0>255.0	12
108	Bromophos-ethyl	4824-78-6	14.634	358.9>302.9	16	358.9>330.9	10
109	trans-Chlordane	5103-74-2	14.671	372.8>336.8	10	372.8>263.9	28
110	Chlorbenside	103-17-3	14.683	125.0>89.0	16	125.0>99.0	18
111	Propaphos	7292-16-2	14.688	304.1>140.1	26	304.1>220.1	14
112	o,p'-DDE	3424-82-6	14.733	246.0>176.0	30	246.0>211.0	22
113	Tetrachlorvinphos	22248-79-9	14.758	328.9>109.0	20	328.9>313.9	18
114	Paclobutrazol	76738-62-0	14.790	236.1>125.0	14	236.1>167.0	10
115	Butachlor	23184-66-9	14.820	188.1>160.1	12	188.1>146.1	14
116	cis-Chlordane	5103-71-9	14.936	372.8>336.8	10	372.8>263.9	28
117	alpha-Endosulfan	959-98-8	14.943	338.9>160.0	18	338.9>266.9	8
118	Fenamiphos	22224-92-6	15.042	303.1>195.1	8	303.1>154.1	18
119	Napropamide	15299-99-7	15.074	128.1>72.0	6	128.1>57.0	12
120	Flutolanil	66332-96-5	15.108	173.0>145.0	14	173.0>95.0	26
121	Prothiofos	34643-46-4	15.184	309.0>238.9	14	309.0>280.9	10
122	Profenofos	41198-08-7	15.266	336.9>266.9	14	336.9>308.9	6
123	p,p'-DDE	72-55-9	15.348	246.0>176.0	30	246.0>211.0	22
124	Tribufos	78-48-8	15.392	258.0>202.0	4	258.0>147.0	10
125	Myclobutanil	88671-89-0	15.418	179.1>125.0	14	179.1>152.0	8
126	Dieldrin	60-57-1	15.459	276.9>241.0	8	276.9>170.0	38
127	Flusilazole	85509-19-9	15.459	233.1>165.1	14	233.1>152.1	14
128	o,p'-DDD	53-19-0	15.475	235.0>165.0	24	235.0>199.0	14
129	Diclobutrazol	75736-33-3	15.538	270.0>159.0	14	270.0>201.0	8
130	Chlorfenapyr	122453-73-0	15.648	247.1>227.0	16	247.1>200.0	24
131	Cyproconazole-1	94361-06-5	15.740	222.1>125.1	24	222.1>82.0	12
132	Cyproconazole-2	94361 - 06 - 5	15.756	222.1>125.1	24	222.1>82.0	12

133	Endrin	72-20-8	15.855	262.9>191.0	30	262.9>193.0	28
134	Chlorobenzilate	510-15-6	15.969	251.0>139.0	14	251.0>111.0	28
135	Fensulfothion	115-90-2	16.003	293.0>153.0	8	293.0>125.0	14
136	beta-Endosulfan	33213-65-9	16.056	338.9>160.0	18	338.9>266.9	8
137	Ethion	563-12-2	16.132	230.9>174.9	14	230.9>184.9	12
138	p,p'-DDD	72-54-8	16.139	235.0>165.0	24	235.0>199.0	14
139	o,p'-DDT	789-02-6	16.189	235.0>165.0	24	235.0>199.0	16
140	Fluacrypyrim	229977-93-9	16.272	352.1>188.1	10	352.1>320.1	6
141	Mepronil	55814-41-0	16.389	269.1>119.1	14	269.1>227.1	6
142	Triazophos	24017-47-8	16.416	257.0>162.0	8	257.0>134.0	22
143	Benalaxyl	71626-11-4	16.579	148.1>105.1	16	148.1>133.1	14
144	Carbophenothion	786-19-6	16.632	341.9>157.0	14	341.9>143.0	18
145	Edifenphos	17109-49-8	16.691	310.0>173.0	14	310.0>109.0	26
146	Propiconazole-1	60207-90-1	16.703	259.0>69.0	14	259.0>191.0	8
147	(Z)-Pyriminobac-methyl	136191-64-5	16.752	302.1>256.1	18	302.1>230.1	18
148	(E)-Pyriminobac-methyl	136191-64-5	16.752	302.1>256.1	18	302.1>230.1	18
149	Endosulfan sulfate	1031-07-8	16.773	386.8>288.8	10	386.8>252.9	16
150	Propiconazole-2	60207-90-1	16.814	259.0>69.0	14	259.0>191.0	8
151	p,p'-DDT	50-29-3	16.843	235.0>165.0	24	235.0>199.0	16
152	Hexazinone	51235-04-2	16.935	171.1>71.0	16	171.1>85.0	16
153	Diclofop-methyl	51338-27-3	17.111	340.0>253.0	14	340.0>281.0	10
154	Piperonyl butoxide	51-03-6	17.237	176.1>131.1	12	176.1>117.1	20
155	Resmethrin-2 (Bioresmethrin)	10453-86-8	17.289	171.1>128.1	14	171.1>143.1	6
156	Tetramethrin-1	7696-12-0	17.628	164.1>107.1	14	164.1>135.1	8
157	Bifenthrin	82657-04-3	17.725	181.1>166.1	12	181.1>153.1	8
158	EPN	2104-64-5	17.748	169.1>140.9	8	169.1>77.0	22
159	Bromopropylate	18181-80-1	17.775	340.9>182.9	18	340.9>184.9	20
160	Tetramethrin-2	7696-12-0	17.783	164.1>107.1	14	164.1>135.1	8
161	cloquintocet-1-methylhexyl ester	99607-70-2	17.849	192.0>162.0	25	192.0>126.0	25
162	Methoxychlor	72-43-5	17.869	227.1>169.1	24	227.1>212.1	14
163	Fenpropathrin	39515-41-8	17.908	265.1>210.1	12	265.1>172.1	14
164	Phenothrin-1	26002-80-2	18.171	183.1>168.1	14	183.1>153.1	14
165	Tetradifon	116-29-0	18.256	355.9>228.9	12	355.9>159.0	18
166	Phenothrin-2	26002-80-2	18.285	183.1>168.1	14	183.1>153.1	14

167	Pentoxazone	110956-75-7	18.339	285.0>70.0	14	285.0>187.0	10
168	Pyriproxyfen	95737-68-1	18.522	136.1>78.0	20	136.1>96.0	14
169	Cyhalothrin-1	68085-85-8	18.534	197.0>161.0	8	197.0>141.0	12
170	Cyhalofop-butyl	122008-85-9	18.567	357.1>256.1	10	357.1>229.1	14
171	Acrinathrin-1	101007-06-1	18.661	289.1>93.0	14	289.1>77.0	26
172	Cyhalothrin-2	68085-85-8	18.699	197.0>161.0	8	197.0>141.0	12
173	Mirex	2385-85-5	18.812	272.0>237.0	15	270.0>235.0	5
174	Pyrazophos	13457-18-6	18.812	221.1>193.1	12	221.1>149.1	14
175	Acrinathrin-2	101007-06-1	18.883	289.1>93.0	14	289.1>77.0	26
176	Fenarimol	60168-88-9	18.908	251.0>139.0	14	251.0>111.0	26
177	Azinphos-ethyl	2642-71-9	19.031	160.1>132.1	4	160.1>77.0	18
178	Permethrin-1	52645-53-1	19.501	183.1>168.1	14	183.1>165.1	14
179	Permethrin-2	52645-53-1	19.628	183.1>168.1	14	183.1>165.1	14
180	Fluquinconazole	136426-54-5	19.637	340.0>298.0	20	340.0>313.0	14
181	Dioxathion	78-34-2	19.679	271.0>153.0	8	271.0>125.0	14
182	Cyfluthrin-1	68359-37-5	20.033	226.1>206.1	14	226.1>199.1	6
183	Cyfluthrin-2	68359-37-5	20.144	226.1>206.1	14	226.1>199.1	6
184	Cyfluthrin-3	68359-37-5	20.201	226.1>206.1	14	226.1>199.1	6
185	Cyfluthrin-4	68359-37-5	20.241	226.1>206.1	14	226.1>199.1	6
186	Cypermethrin-1	52315-07-8	20.363	181.1>152.1	22	181.1>127.1	22
187	Cypermethrin-2	52315-07-8	20.464	181.1>152.1	22	181.1>127.1	22
188	Cypermethrin-3	52315-07-8	20.520	181.1>152.1	22	181.1>127.1	22
189	Flucythrinate-1	70124-77-5	20.526	199.1>157.1	10	199.1>107.1	22
190	Cypermethrin-4	52315-07-8	20.562	181.1>152.1	22	181.1>127.1	22
191	Etofenprox	80844-07-1	20.683	163.1>135.1	10	163.1>107.1	18
192	Flucythrinate-2	70124-77-5	20.720	199.1>157.1	10	199.1>107.1	22
193	Silafluofen	105024-66-6	20.822	286.1>258.1	14	286.1>207.1	14
194	Fluridone	59756-60-4	20.897	328.1>259.0	24	328.1>313.0	22
195	Fenvalerate-1	51630-58-1	21.243	419.1>225.1	6	419.1>167.1	12
196	Fluvalinate-1	102851-06-9	21.356	250.1>55.0	20	250.1>200.0	20
197	Fluvalinate-2	102851-06-9	21.421	250.1>55.0	20	250.1>200.0	20
198	Fenvalerate-2	51630-58-1	21.444	419.1>225.1	6	419.1>167.1	12
199	Deltamethrin-1	52918-63-5	21.798	252.9>93.0	20	252.9>171.9	8
200	Deltamethrin-2	52918-63-5	22.002	252.9>93.0	20	252.9>171.9	8
201	Famoxadone	131807-57-3	22.394	330.1>224.1	10	330.1>196.1	22

ANALYSIS AND RESULTS

The grape sample was extracted using the QuEChERS method wherein 174 pesticides were spiked in the blank sample extract (10 ng/mL). Spiked samples were analyzed in MRM mode using GC-MS/MS. Two transitions were used for each compound. The MRM transitions and collision energies for each compound were acquired from the pesticide MRM database provided by Shimadzu Corporation contains 479 pesticides. Fig.2 shows representative mass chromatograms for Dichlormid, Dichlormid, Propanil, Propanil, Tetramethrin and Deltamethrin. Table 2 shows the area repeatability values for 174 compounds (n = 6).

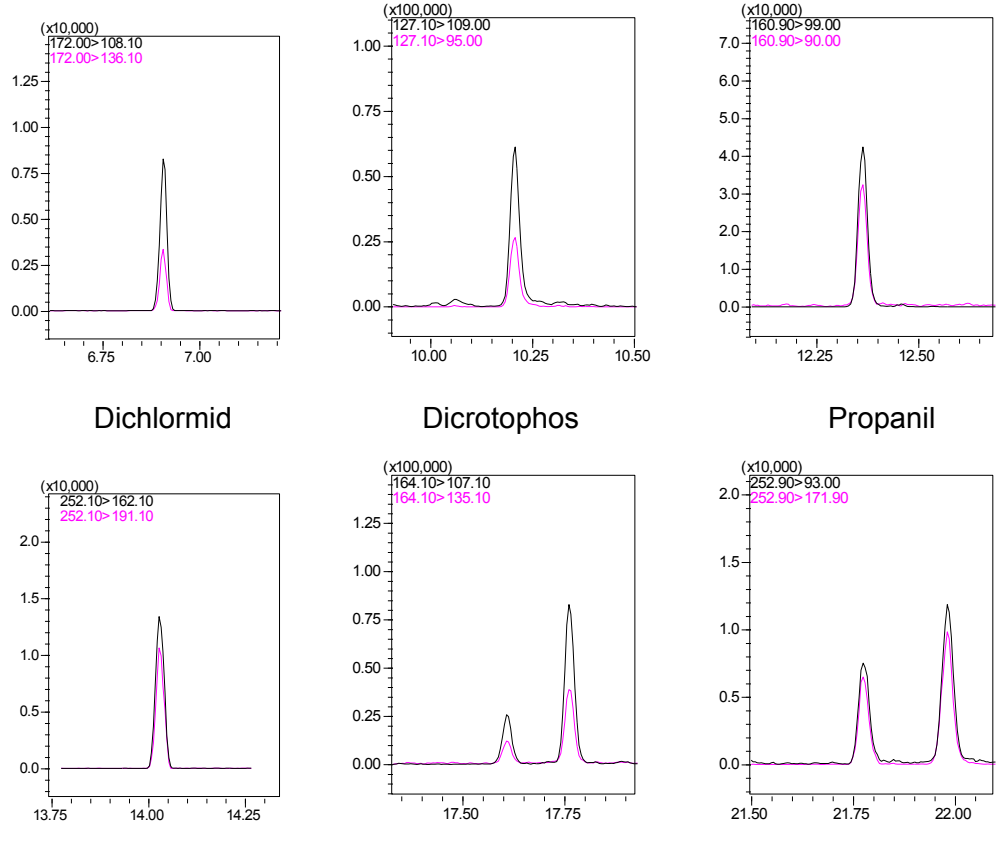


Fig. 2 Mass chromatograms for few pesticides

Table 2 Area repeatability values for 174 compounds (n = 6).

Compound	RSD %	Compound	RSD %	Compound	RSD%
Allidochlor	1.37	Fenchlorphos	2.60	o,p'-DDD	0.82
Dichlormid	3.91	Prometryn	5.77	Diclobutrazol	2.03

Biphenyl	1.33	Dithiopyr	2.61	Chlorfenapyr	8.33
Butylate	2.24	S421	3.65	Endrin	2.15
Etridiazole	2.87	Pirimiphos-methyl	4.05	Chlorobenzilate	1.56
propham	1.09	Fenitrothion	5.13	Fensulfothion	8.90
Methacrifos	1.14	Ethofumesate	3.31	Ethion	1.65
Chloroneb	2.02	methyl entachlorophenyl sufide	1.23	p,p'-DDD	1.52
2-Phenylphenol	1.20	Dichlofluanid	1.75	o,p'-DDT	3.34
heptenophos	1.56	Phorate sulfoxide	1.92	Fluacrypyrim	7.77
Tecnazene	2.91	Chlorpyrifos	3.57	Mepronil	3.43
Diphenylamine	1.33	Phorate sulfone	2.32	Triazophos	2.64
Ethoprophos	2.20	Aldrin	3.83	Benalaxyl	3.35
Ethalfluralin	3.76	Fenthion	4.74	Carbophenothion	2.63
Chlorpropham	4.04	Chlorthal-dimethyl	1.76	Edifenphos	2.18
Dicrotophos	4.56	Parathion	4.08	Endosulfan sulfate	9.01
Trifluralin	2.74	Triadimefon	2.36	p,p'-DDT	3.03
Benfluralin	1.94	Tetraconazole	3.71	Hexazinone	1.56
Dioxabenzofos	0.71	Isocarbophos	4.09	Diclofop-methyl	1.78
Sulfotep	3.20	Dicofol deg. (DCBP)	2.35	Piperonyl butoxide	1.31
Cadusafos	2.74	Nitrothal-isopropyl	9.05	Bioresmethrin	5.82
Phorate	9.75	Fluorochloridone	1.75	Bifenthrin	0.89
alpha-HCH	2.09	pirimiphos-ethyl	3.97	EPN	2.58
Hexachlorobenzene	2.65	Bromophos	2.29	Bromopropylate	2.54
Thiometon	1.36	Diphenamid	1.42	cloquintocet-1-methylhexyl ester	4.92
pentachloroanisole	1.17	Isofenphos-methyl	2.82	Methoxychlor	3.45
Dicloran	2.07	Pendimethalin	4.15	Fenpropathrin	2.76
monolinuron	4.43	Isodrin	2.82	Tetradifon	3.76
beta-HCH	2.58	Cyprodinil	1.37	Pentoxazone	1.66
Dimethipin	2.84	Fipronil	5.99	Pyriproxyfen	2.49
Clomazone	2.03	Penconazole	3.79	Cyhalofop-butyl	2.03
Quintozene	2.84	Heptachlor-exo-epoxide	4.50	Mirex	1.97
gamma-HCH	2.93	Chlozolinate	5.71	Pyrazophos	2.59

Terbufos	2.63	Isofenphos	1.91	Fenarimol	3.51
Cyanophos	2.05	Ethychlozate	2.98	Azinphos-ethyl	3.69
Fonofos	1.80	Mecarbam	8.04	Fluquinconazole	1.86
Propyzamide	1.44	Phenthoate	3.37	Dioxathion	3.63
Diazinon	3.40	Quinalphos	4.40	Halfenprox	1.66
Chlorothalonil	2.18	furalaxyl	2.85	Silafluofen	1.89
Disulfoton	7.32	Captan	6.45	Fluridone	6.54
Isazophos	3.08	Procymidone	1.39	Famoxadone	4.02
Terbacil	2.94	Bromophos-ethyl	0.78	Chlordane	5.86
Tefluthrin	1.68	Chlorbenside	2.80	Endosulfan	5.55
Etrimfos	6.52	Propaphos	1.91	Phosphamidon	3.99
delta-HCH	1.96	o,p'-DDE	2.28	Chlorfenvinphos	5.26
Tri-allate	2.40	Tetrachlorvinphos	1.93	Cyproconazole	2.47
Formothion	4.81	Paclobutrazol	2.19	Propiconazole	0.68
Benfuresate	2.05	Butachlor	4.49	Tetramethrin	1.96
Dichlofenthion	0.37	Fenamiphos	4.99	Phenothrin	4.60
Dimethenamid	1.54	Napropamide	1.21	Cyhalothrin	3.81
Propanil	2.71	Flutolanil	1.03	Acrinathrin	3.64
Chlorpyrifos-methyl	2.94	Prothiofos	1.44	Permethrin	2.52
Metribuzin	3.24	Profenofos	3.13	Cyfluthrin	1.91
Vinclozolin	5.94	p,p'-DDE	1.87	Cypermethrin	1.95
Parathion-methyl	2.27	Tribufos	6.42	Flucythrinate	2.45
Tolclofos-methyl	2.45	Myclobutanil	2.24	Fenvalerate	4.43
Heptachlor	3.63	Dieldrin	3.61	Fluvalinate	3.72
Ametryn	3.28	Flusilazole	1.89	Deltamethrin	2.30

CONCLUSION

A method was developed for the simultaneous analysis of 174 pesticides in grape using GC-MS/MS in multiple reaction monitoring. The result showed that the MRM chromatographic peak of 174 pesticides in spiked sample (10 ng/mL) is clean with high S/N. The area repeatability of 174 compounds is below 10% (n=6). The established method was sensitive, repeatable and reliable for simultaneous analysis of the 174 pesticides in grape samples.

C-4 A Multi-residue Analysis of Organochlorine Pesticides in Milk Powder Using GPC-GC-MS/MS

INTRODUCTION

Organochlorine pesticides are widely used in recent years, which are also considered as permanent organic pollutants (POPs) because they are difficult to degrade. GPC system served as on-line clean up system to remove most part of fat in milk and MRM acquisition mode of GC-triple quadrupole MS/MS can greatly reduce the matrix interference. A very quick, easy, effective, reliable multi-pesticides residues analysis method in milk powder based on GPC-GC-MS/MS was developed and evaluated with milk powder samples spiked with standard pesticides.

EXPERIMENTAL

Instrument and chemical

Shimadzu GPC-GC-MS/MS system, HPLC grade acetone, cyclohexane, organochlorine pesticide standards were used.

Analytical conditions

GPC parameters

Column	: Shodex CLNpak EV-200 (2.1 mm x 150 mm)
Mobile phase	: acetone:cyclohexane (3:7, V/V)
Flow rate	: 0.1mL/min
Column oven	: 40 ºC

GC/MS/MS parameters

Column	: Inert empty column : 5 m x 0.53 mm
Pre-column	: Rtx-5 MS, 5 m x 0.25 mm x 0.25 µm
Analysis column	: Rtx-5 MS, 25 m x 0.25 mm x 0.25 µm
Injection port temperature program	: 120 ºC (5 min)→@100 ºC/min→250 ºC (30.6 min)
Column oven temperature program	: 82ºC (5 min)→@20 ºC/min→220 ºC (1 min) →@5 ºC/min→300 ºC (8 min)
Carrier gas	: He

Pressure program : 120 kPa (0 min)→@100 kPa/min→180 kPa (4.4 min)→@49.8 kPa/min→120 kPa (30.7 min)

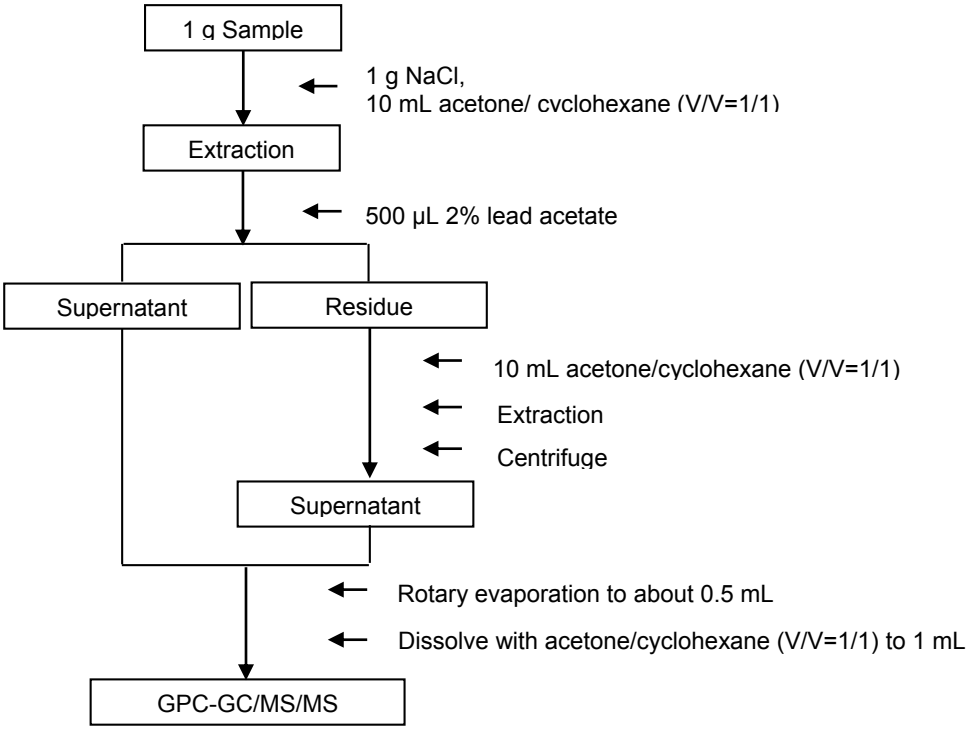
Injection mode : splitless, 7 min

Interface temperature : 300 ºC

Ion source temperature : 200 ºC

Sample pretreatment

Samples were prepared by the following procedure.



RESULTS

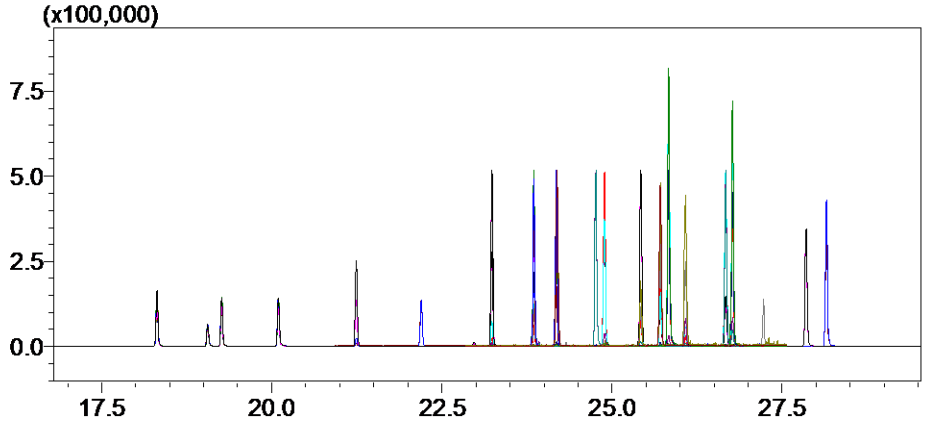


Fig.1 MRM chromatograms of organochlorine pesticides (10 µg/L each)

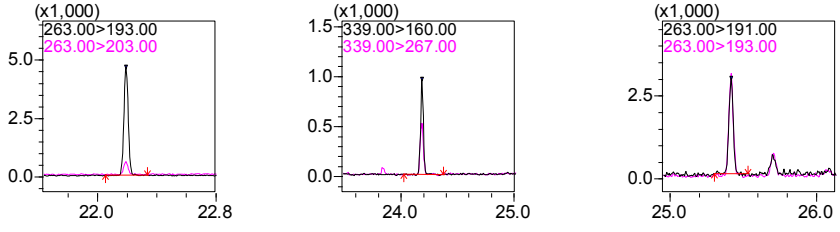


Fig.2 MRM chromatograms of aldrin, α-endosulfan and endrin (1 µg/L)

Calibration curves, detection limits, repeatability and recovery

1, 5, 10, 20 and 50 µg/L standard solutions with acetone/cyclohexane (v/v=3/7) were prepared. The calibration curves are shown in Fig. 3.

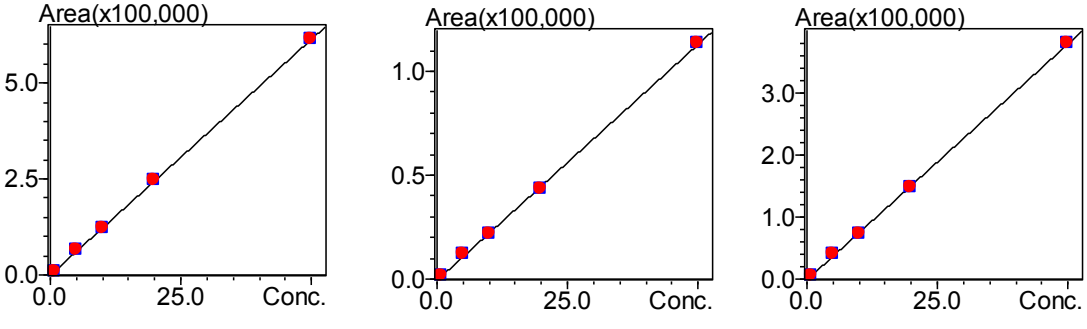


Fig. 3 Calibration curves of aldrin, α-endosulfan and endrin

For 1 µg/L standard solution, the LODs were calculated by GCMSsolution software (S/N=3), limits of detection (LODs) are shown in Table 1.

Mixed standard solution was spiked into blank samples. The final concentration of organochlorine pesticides was 2.5 μg/kg. The relative standard deviations (%RSD, n=5) were better than 5.5%. The average recoveries are shown in Table 1.

Table 1 Retention times, linear correlation coefficients, LODs, %RSDs and the average recoveries for organochlorine pesticides

No.	t_R (min)	Pesticide	Quantitative transition (CE)	Qualitative transition (CE)	r	LOD (μg/L)	%RSD	Recovery%
1	18.296	α-BHC	219>183(8)	219>145(18)	0.9999	0.002	2.67	74.09
2	19.037	β-BHC	219>183(8)	219>147(20)	0.9999	0.005	3.59	93.07
3	19.247	γ-BHC	219>183(8)	219>147(20)	0.9999	0.002	4.10	79.24
4	20.077	δ-BHC	219>183(10)	219>145(22)	0.9999	0.003	2.25	83.70
5	21.224	heptachlor	272>237(20)	272>117(32)	0.9999	0.001	3.13	75.96
6	22.178	aldrine	263>193(28)	263>203(26)	0.9999	0.014	3.01	74.70
7	23.216	heptachlor epoxide	353>263(14)	353>282(12)	0.9999	0.008	4.77	76.58
8	23.832	trans-chlordan	373>337(10)	373>143(26)	0.9999	0.010	4.90	74.49
9	24.162	cis-chlordan	373>266(22)	373>337(6)	0.9999	0.003	3.68	73.57
10	24.171	α-endosulfan	339>160(18)	339>267(4)	0.9997	0.026	5.46	72.71
11	24.742	p,p'-DDE	246>176(28)	246>211(22)	0.9999	0.002	2.14	78.74
12	24.869	dieldrin	277>241(8)	277>170(38)	0.9999	0.010	3.90	72.69
13	25.403	endrin	263>191(30)	263>193(28)	0.9998	0.106	3.72	78.29
14	25.691	β-endosulfan	339>267(8)	339>160(16)	0.9999	0.072	3.98	75.00
15	25.812	p,p'-DDD	235>165(24)	235>199(14)	0.9999	0.003	3.26	81.77

16	26.057	endrin aldehyde	345>317(10)	347>319(10)	0.9998	0.098	4.37	37.24
17	26.65	endosulfan sulfate	387>289(10)	387>253(16)	0.9999	0.020	3.29	75.61
18	26.751	p,p'-DDT	235>165(22)	235>199(14)	0.9998	0.004	3.40	71.91
19	27.832	endrin ketone	315>279(10)	317>281(10)	0.9999	0.009	2.44	75.09
20	28.132	methoxychlor	227>169(24)	227>212(14)	0.9998	0.004	3.99	73.22

CONCLUSION

The method is simple, rapid and characterized with acceptable sensitivity and accuracy to meet the requirements for the analysis of organochlorine pesticides in milk powder products.

C-5 Multiresidue Pesticides Analysis in Spinach by Modified QuEChERS with PTV-GC-MS/MS

INTRODUCTION

Over last years, evolution of GC/MS pesticide residues analysis in vegetables has been incredible. There have been clearly two different streams, the main effort have been done either in sample preparation or detection. In this report, a novel approach for the determination of multiple pesticide residues in spinach by modified QuEChERS method with temperature-programmed vaporizer-gas chromatography tandem mass spectrometry (PTV-GC-MS/MS) has been developed. The established method was sensitive, repeatable and reliable for detecting the 54 pesticides in spinach.

EXPERIMENTAL

Sample pretreatment

Samples were prepared by the modified QuEChERS method.

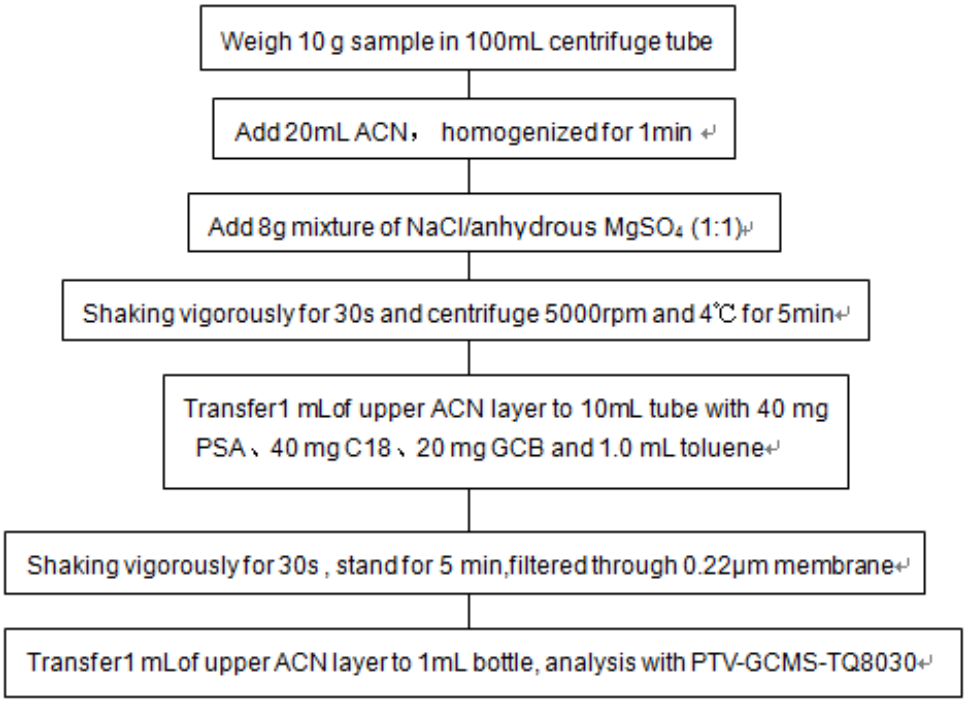


Figure 1 Sample pretreatment step

GC/MS/MS Analysis

Treated samples were analyzed in MRM mode using temperature-programmed vaporizer-gas chromatography tandem mass spectrometry (GCMS-TQ8030, Shimadzu Corporation, Japan). The MRM transitions and collision energies for all compounds were acquired from the pesticide MRM database provided by Shimadzu Corporation which contains 440 pesticides.

Analytical Conditions

GC

Column : Rxi-5 Sil ms, 30 m x 0.25 mm, 0.25 µm

Injection mode:

Injection volumn : 2 µL

Carrier gas : He

Linear velocity : 36.2 cm/sec

Injection temperature : 65 ºC (1 min)→@(200 ºC/min)→250 ºC (15 min)

Temperature program : 40 ºC for 4 min, programmed to125 ºC at 25 ºC/min, then to 300 ºC (5 min) at 10 ºC/min. The total run time was 30 minutes.

MS/MS

Ionization : EI

Collision gas : Argon

Solvent cutting time : 7 min

Ion source temperature : 200 ºC

Interface temperature : 250 ºC

Detector voltage : Tuning voltage + 0.6 kV

Monitoring mode : MRM (see Table 1)

RESULTS

Modified QuEChERS method

For the pigmented vegetables, the addition of GCB in the dispersive SPE tube can greatly remove pigments and phytosterols from the matrix. However, GCB also retains pesticides with planar structures resulting in poor recovery and precision. In the modified QuEChERS, an aliquot (1 mL) of toluene was added for improving the extraction efficiency of the planar pesticides.

Table 1 List of pesticides

NAME	CAS No	RT(min)	Ion for Quantitation	CE	Ion for Qualification	CE
Methamidophos	10265 - 92 - 6	9.667	141.0>95.0	8	141.0>126.0	4
Dichlorvos	62 - 73 - 7	9.853	185.0>93.0	14	185.0>109.0	14
Omethoate	1113 - 02 - 6	13.686	156.0>110.0	8	156.0>141.0	4
Ethoprophos	13194 - 48 - 4	14.099	200.0>158.0	6	200.0>114.0	14
Monocrotophos	6923 - 22 - 4	14.593	127.1>109.0	12	127.1>95.0	16
Sulfotep	3689 - 24 - 5	14.627	322.0>202.0	10	322.0>294.0	4
Phorate	298 - 02 - 2	14.776	260.0>75.0	8	260.0>231.0	4
alpha-HCH	319 - 84 - 6	14.948	218.9>182.9	8	218.9>144.9	20
Dimethoate	60 - 51 - 5	15.18	125.0>79.0	8	125.0>47.0	14
beta-HCH	319 - 85 - 7	15.5	218.9>182.9	8	218.9>144.9	20
gamma-HCH	58 - 89 - 9	15.635	218.9>182.9	8	218.9>144.9	20
Terbufos	13071 - 79 - 9	15.645	231.0>174.9	14	231.0>128.9	26
Quintozene	82 - 68 - 8	15.73	294.8>236.8	16	294.8>264.8	12
Fonofos	944 - 22 - 9	15.762	246.0>109.1	18	246.0>137.1	6
Pyrimethanil	53112 - 28 - 0	15.83	198.1>183.1	14	198.1>158.1	18
Diazinon	333 - 41 - 5	15.836	304.1>179.1	10	304.1>162.1	8
Phosphamidon-1	13171 - 21 - 6	15.909	264.1>127.1	14	264.1>193.1	8
delta-HCH	319 - 86 - 8	16.115	218.9>182.9	10	218.9>144.9	20
Phosphamidon-2	13171 - 21 - 6	16.651	264.1>127.1	14	264.1>193.1	8
Vinclozolin	50471 - 44 - 8	16.841	285.0>212.0	12	285.0>178.0	14
Parathion-methyl	298 - 00 - 0	16.874	263.0>109.0	14	263.0>136.0	8
Fenitrothion	122 - 14 - 5	17.405	277.0>260.0	6	277.0>109.1	14
Malathion	121 - 75 - 5	17.56	173.1>99.0	14	173.1>127.0	6
Fenthion	55 - 38 - 9	17.775	278.0>109.0	20	278.0>125.0	20
Chlorpyrifos	2921 - 88 - 2	17.806	313.9>257.9	14	313.9>285.9	8
Parathion	56 - 38 - 2	17.829	291.1>109.0	14	291.1>137.0	6
Dicofol deg.	0 - 00 - 0	17.869	250.0>139.0	14	250.0>215.0	8
Triadimefon	43121 - 43 - 3	17.906	208.1>181.0	10	208.1>127.0	14
Isocarbophos	24353 - 61 - 5	17.952	289.1>136.0	14	289.1>113.0	6

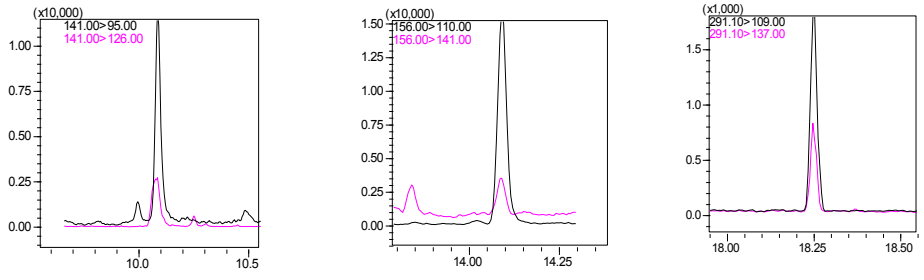
Isofenphos-methyl	83733-82-8	18.301	199.0>121.0	14	241.1>121.1	22
Fipronil	120068 - 37 - 3	18.578	366.9>212.9	30	366.9>254.9	22
Phosfolan	947-02-4	18.579	255.0>227.0	6	255.0>140.0	22
Phenthoate	2597-03-7	18.673	273.9>125.0	20	273.9>246.0	6
Quinalphos	13593 - 03 - 8	18.674	157.1>129.0	14	157.1>93.0	10
Procymidone	32809 - 16 - 8	18.805	283.0>96.0	10	283.0>255.0	12
Methidathion	950 - 37 - 8	18.984	145.0>85.0	8	145.0>58.0	14
alpha-Endosulfan	959 - 98 - 8	19.268	338.9>160.0	18	338.9>266.9	8
Profenofos	41198 - 08 - 7	19.543	336.9>266.9	14	336.9>308.9	6
beta-Endosulfan	33213 - 65 - 9	20.362	338.9>160.0	18	338.9>266.9	8
Triazophos	24017 - 47 - 8	20.71	257.0>162.0	8	257.0>134.0	22
Iprodione	36734 - 19 - 7	21.797	314.0>245.0	12	314.0>56.0	22
Bifenthrin	82657 - 04 - 3	21.969	181.1>166.1	12	181.1>153.1	8
Phosmet	732 - 11 - 6	22.041	160.0>133.0	14	160.0>77.0	24
Fenpropathrin	39515 - 41 - 8	22.121	265.1>210.1	12	265.1>172.1	14
Phosalone	2310 - 17 - 0	22.711	182.0>111.0	14	182.0>138.0	8
Cyhalothrin-1	68085 - 85 - 8	22.747	197.0>161.0	8	197.0>141.0	12
Cyhalothrin-2	68085 - 85 - 8	22.941	197.0>161.0	8	197.0>141.0	12
Permethrin-1	52645 - 53 - 1	23.716	183.1>168.1	14	183.1>165.1	14
Permethrin-2	52645 - 53 - 1	23.834	183.1>168.1	14	183.1>165.1	14
Pyridaben	96489 - 71 - 3	23.895	147.1>117.1	22	147.1>132.1	14
Coumaphos	56-72-4	24	362.0>109.0	16	362.0>226.0	14
Cyfluthrin-1	68359 - 37 - 5	24.276	226.1>206.1	14	226.1>199.1	6
Cyfluthrin-2	68359 - 37 - 5	24.356	226.1>206.1	14	226.1>199.1	6
Cyfluthrin-3,4	68359 - 37 - 5	24.47	226.1>206.1	14	226.1>199.1	6
Cypermethrin-1	52315 - 07 - 8	24.597	163.1>127.1	6	163.1>91.0	14
Cypermethrin-2	52315 - 07 - 8	24.685	163.1>127.1	6	163.1>91.0	14
Cypermethrin-3,4	52315 - 07 - 8	24.78	163.1>127.1	6	163.1>91.0	14
Flucythrinate-1	70124 - 77 - 5	24.794	199.1>157.1	10	199.1>107.1	22
Flucythrinate-2	70124 - 77 - 5	24.987	199.1>157.1	10	199.1>107.1	22
Fenvalerate-1	51630 - 58 - 1	25.622	419.1>225.1	6	419.1>167.1	12
Fluvalinate-1	69409 - 94 - 5	25.795	250.1>55.0	20	250.1>200.0	20
Fenvalerate-2	51630 - 58 - 1	25.854	419.1>225.1	6	419.1>167.1	12
Fluvalinate-2	69409 - 94 - 5	25.86	250.1>55.0	20	250.1>200.0	20
Difenoconazole-1	119446 - 68 - 3	26.189	323.0>265.0	14	323.0>202.0	28
Difenoconazole-2	119446 - 68 - 3	26.276	323.0>265.0	14	323.0>202.0	28
Deltamethrin-1	52918 - 63 - 5	26.289	252.9>93.0	20	252.9>171.9	8
Deltamethrin-2	52918 - 63 - 5	26.57	252.9>93.0	20	252.9>171.9	8

Choice of PTV injection

For the analysis of pesticides in spinach by modified QuEChERS with GC-MS/MS method, PTV technique has advantage over the traditional split/splitless injection. With PTV injection, acetonitrile was vented via the split vent during solvent elimination step which reduces column bleed and extends the life of the column. To guarantee the repeatability and reliability of the results, the parameters of PTV were optimized.

Validation of the method

Based on the modified QuEChERS method with PTV-GC-MS/MS established above, seven different concentrations of pesticides (0.5, 1, 2, 5, 10, 50, 100 µg/L) were analyzed by PTV-GC-MS/MS in multiple reaction monitoring (MRM) mode. The linear relation between peak area and concentration of target were good from 0.5 µg/L to 100 µg/L (r^2>0.99). The limits of quantitation (LOQs) of all the pesticides studied were 2.0-10.0 µg/kg. The average recoveries were 70%-120% of target compounds and the relative standard deviation (%RSD, n=5) were 0.3%-19.6% in three spiked levels at 10.0 µg/kg, 20.0 µg/kg and 100.0 µg/kg. Fig. 2 shows MRM chromatograms of representative pesticides spiked in spinach (1.0 µg/L).



Methamidophos 1.0 µg/L, spiked **Omethoate 1.0 µg/L, spiked** **Parathion 1.0 µg/L, spiked**

Fig. 2 MRM chromatogram of some toxic pesticides

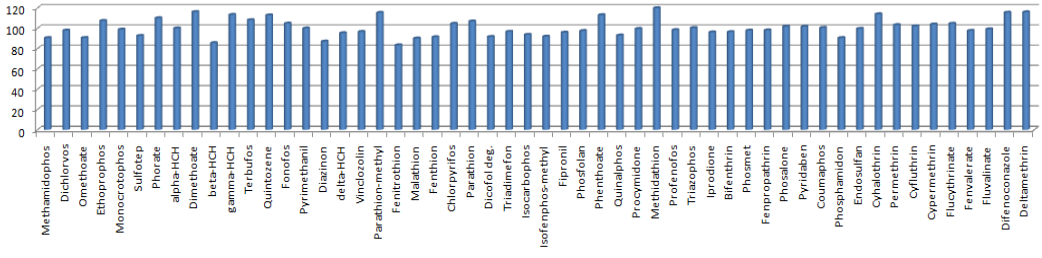


Fig. 3 Recovery of pesticides at 10.0 µg/kg spiked in spinach sample

CONCLUSIONS

- The developed method is sensitive, rapid, precise, and widely linear, therefore it is suitable for the determination of pesticides in spinach.
- The recoveries and LOQs were acceptable for multi-residue pesticides determination in spinach.

C-6 Determination of Residual Pesticides in Complex Matrix (Ginger, Leaf Lettuce) with GC-MS/MS

INTRODUCTION

In this paper, an analytical method was developed for detecting more than 50 residual pesticides in complex matrix (ginger, leaf lettuce) simultaneously with a triple quadrupole GC-MS/MS. In order to minimize matrix effect on the analysis of pesticide residues, blank matrix was used to prepare calibration curves. The proposed method provided satisfactory linearity for all of the assessed pesticides in the concentration range of 1~100μg/L with correlation coefficients greater than 0.997. The %RSD of peak area for all 6 consecutive injections of 1 μg/L standard solution was less than 8.0%. The limits of detection (LODs) of most pesticides were below 1.0 μg/kg. The proposed method provided a spike recovery between 70.0 and 120.0% for most pesticides at concentrations of 1 μg/kg and 5 μg/kg, meeting the requirements of routine detection on the analysis of pesticide residues.

Pesticides are extensively used in agricultural production to increase yield, as a result, the hazards of pesticide residues have become more and more serious day by day. Many advanced countries have established MRLs for pesticides in vegetable successively, such as those in EU Directive 91/414/EEC and The Food Quality Protection Act (FQPA) of the United States. In China, the research on analytical methods for determination of residual pesticides began in 1990s and a series of national standards on analytical methods of pesticides have been issued in response to the rapid development of residual pesticide inspection in the country.

As vegetable samples usually contains complex compositions, it is necessary to extract and clean up the samples to minimize the influence of matrix before the direct determination of pesticide residues in the samples. However, ordinary clean up may fail to meet the analytical requirements of samples with much complex matrix, such as ginger and scallion, and traditional detection methods including ECD, FPD, NPD and MS all have their limitation. As a result, the qualitative

analysis may not be accurate. Tandem mass spectrometry is used for pesticide analysis simply because it is more accurate, reliable and sensitive.

In this experiment, an analytical method was developed for the satisfactory determination of more than 50 pesticide residues in vegetables simultaneously with GC-MS/MS based on the detection items of pesticide residues in food of EU, Japan and the United States. More than 50 pesticides on the annual assessment list issued by the Ministry of Agriculture (China) were assessed using the proposed method. Since they covered organophosphorus, organochlorine and pyrethroid pesticides, they are quite representative. The proposed method uses matrix-matched calibration curves and multiple reaction monitoring (MRM) mode which can increase specificity, thus effectively reducing background interference and improving sensitivity. For the pesticides to be detected, the LODs reached in the range of 0.003~3.061 μg/kg. Therefore, the method is suitable for routine detection of trace pesticide residues.

EXPERIMENTS

Apparatus:

GC-MS/MS: GCMS-TQ 8030 (Shimadzu)

1.1 Analytical conditions

GC/MS/MS

Column	: Rxi-5 ms, 30 m×0.25 mm×0.25 μm
Injector temperature	: 250 °C
Column temperature program	: 50 °C(1min)→@(25 °C/min)→150°C →@(10°C/min)→300 °C(5 min)
CLV mode	: 47.6 cm/sec
Injection mode	: Splitless injection
Splitless time	: 1 min
High pressure injection	: 250 kPa(1 min)
Ion source	: 200 °C
Transmission line	: 230 °C
Detector voltage	: Relative tuning voltage+0.3kV
Solvent cut time	: 1.5 min

See Table 1 for MRM conditions

Sample Preparation

Preparation of standard solution:

The 200 µg/L working standard solution of 72 pesticides was prepared by accurate weighing and further diluting with n-hexane.

Sample pretreatment:

Pretreatment of samples was carried out as shown in Fig. 1 below.

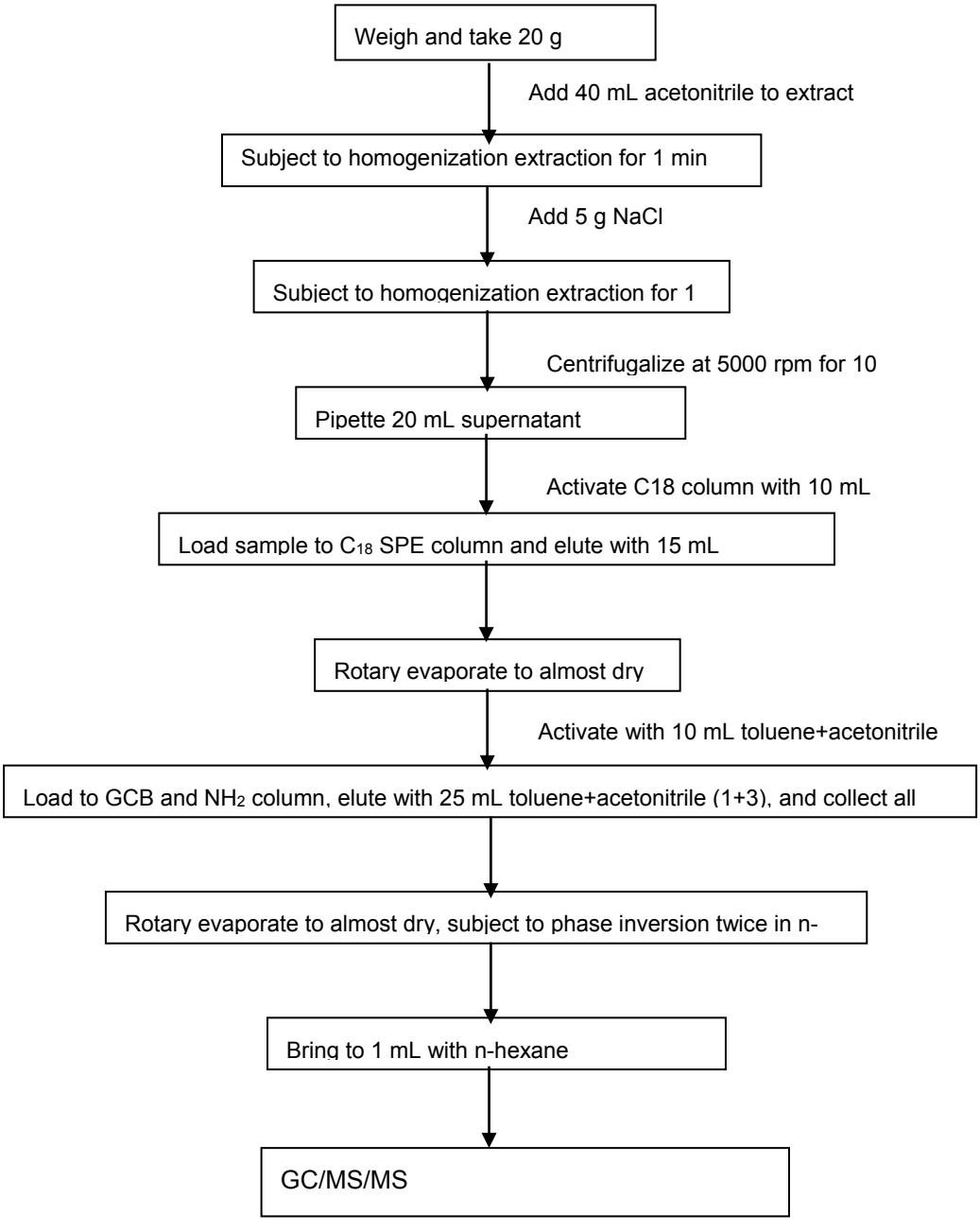


Table 1 Retention time and MRM parameters of pesticide components

No.:	Retention Time	Names of Pesticide	Quantitative Ion (CE)	Qualitative Ion (CE)
1	5.753	Methamidophos	141>95（8）	141>126（4）
2	5.950	Dichlorvos	185>93（14）	185>109（14）
3	5.950	Trichlofon	185>93（14）	185>109（14）
4	9.551	Omethoate	156>110（8）	156>141（4）
5	10.050	Ethoprophos	200>158（6）	200>114（14）
6	10.444	Sulfotep	322>294（4）	322>202（10）
7	10.495	Monocrotophos	127>109（12）	127>95（16）
8	10.678	Phorate	260>75（8）	260>231（4）
9	10.787	α-BHC	219>183（8）	219>145（18）
10	11.018	Dimethoate	125>79（8）	125>47（14）
11	11.286	β-BHC	219>183（8）	219>147（20）
12	11.378	Quintozene	295>237（16）	295>265（12）
13	11.468	γ-BHC	219>183（8）	219>147（20）
14	11.546	Terbufos	231>175（14）	231>129（26）
15	11.632	Fonofos	246>109（18）	246>137（6）
16	11.678	Diazinone	304>179（10）	304>162（8）
17	11.684	Phosphamidon-1	264>127（12）	264>193（8）
18	11.769	Pyrimethanil	198>183（14）	198>158（18）
19	12.018	δ-BHC	219>183（10）	219>145（22）
20	12.444	Phosphamidon-2	264>127（14）	264>193（8）
21	12.702	Vinclozoline	285>212（12）	285>178（14）
22	12.741	Parathion-methyl	263>109（14）	263>136（8）
23	13.239	Fenitrothion	277>260（6）	277>109（14）
24	13.412	Malathion	173>127（6）	173>99（14）

25	13.560	Chlorpyrifos	314>258（14）	314>286（8）
26	13.704	Parathion	291>109（14）	291>137（6）
27	13.768	Triadimefon	208>181（10）	208>127（14）
28	13.780	Isocarbophos	289>136（14）	289>113（6）
29	13.859	Dicofol	251>216（8）	251>139（16）
30	14.093	Isofenphos methyl	241>199（8）	241>121（22）
31	14.283	Fipronil	367>213（30）	367>255（22）
32	14.390	Phosfolan	196>140（12）	196>168（6）
33	14.406	Heptachlor epoxide	353>263（14）	353>282（12）
34	14.498	Phenthoate	274>125（20）	274>246（6）
35	14.519	Quinalphos	157>129（14）	157>93（10）
36	14.590	Procymidone	283>96（10）	283>255（12）
37	14.789	Methidathion	145>85（8）	145>58（14）
38	15.124	α-Endosulfan	339>160（18）	339>267（4）
39	15.427	Profenofos	337>267（14）	337>309（6）
40	15.520	p,p'-DDE	246>176（28）	246>211（22）
41	16.309	p,p'-DDD	235>165（24）	235>199（14）
42	16.365	o,p'-DDT	235>165（24）	235>199（16）
43	16.564	Triazophos	257>162（8）	257>134（22）
44	17.022	p,p'-DDT	235>165（22）	235>199（14）
45	17.729	Iprodione	314>245（12）	314>56（22）
46	17.859	Phosmet	160>133（14）	160>77（24）
47	17.893	Bifenthrin	181>166（12）	181>153（8）
48	18.074	Fenpropathrin	265>210（12）	265>172（14）
49	18.691	Cyhalothrin-1	197>161（8）	197>141（12）
50	18.868	Cyhalothrin-2	197>161（8）	197>141（12）
51	19.678	Permethrin-1	183>168（14）	183>165（12）

52	19.803	Coumaphos	362>109（14）	362>226（12）
53	19.806	Permethrin-2	183>168（14）	183>165（14）
54	19.655	Pyridaben	147>117（22）	147>132（14）
55	20.212	Cyfluthrin-1	226>206（14）	226>199（6）
56	20.309	Cyfluthrin-2	226>206（14）	226>199（6）
57	20.370	Cyfluthrin-3	226>206（14）	226>199（6）
58	20.411	Cyfluthrin-4	226>206（14）	226>199（6）
59	20.537	Cypermethrin-1	181>152（22）	181>127（22）
60	20.638	Cypermethrin-2	181>152（22）	181>127（22）
61	20.699	Cypermethrin-3	181>152（22）	181>127（22）
62	20.703	Flucythrinate-1	199>157（10）	199>107（22）
63	20.895	Cypermethrin-4	181>152（22）	181>127（22）
64	20.895	Flucythrinate-2	199>157（8）	199>107（22）
65	21.430	Fenvalerate-1	419>225（6）	419>167（12）
66	21.544	Fluvalinate-1	250>55（18）	250>200（20）
67	21.609	Fluvalinate-2	250>55（20）	250>200（20）
68	21.637	Fenvalerate-2	419>225（6）	419>167（12）
69	21.890	Difenoconazole-1	323>265（14）	323>202（28）
70	21.959	Difenoconazole-2	323>265（14）	323>202（28）
71	21.998	deltamethrin-1	253>93（18）	253>172（4）
72	22.216	deltamethrin-2	253>93（20）	253>172（8）

RESULT

TIC of working standard sample

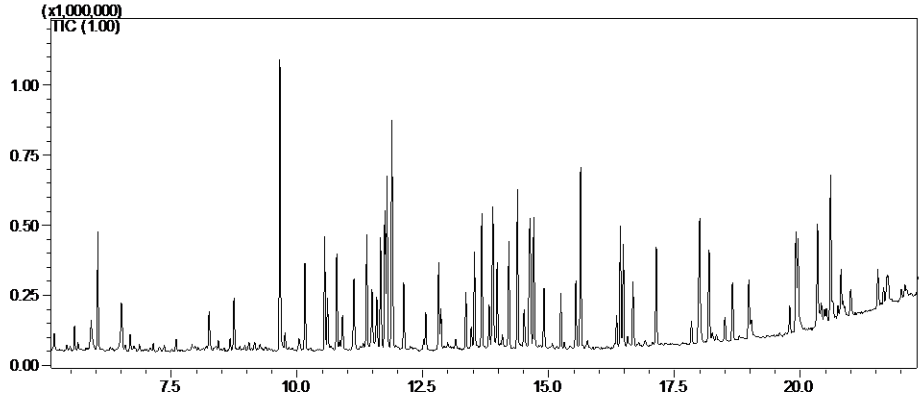
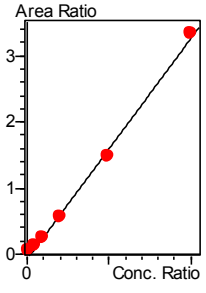


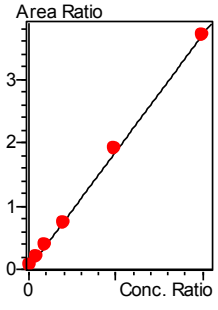
Fig. 2 TIC (100 ppb)

2.2 Calibration curve

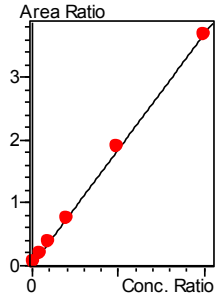
Mixed pesticides standard solutions of 1, 5, 10, 20, 50, 100 μg/L were prepared respectively with blank matrix solution, using heptachlor epoxide as internal standard (28 μg/L). As shown in the following figures, calibration curves were plotted with the concentration as abscissa and the peak area as ordinate; the correlation coefficients and LODs are as shown in Table 2.



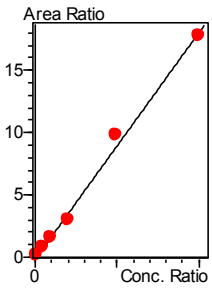
Methamidophos



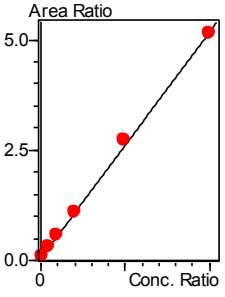
Dichlorvos



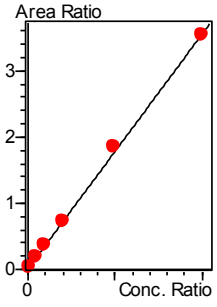
Trichlofon



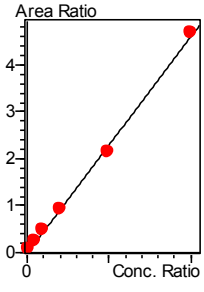
Omethoate



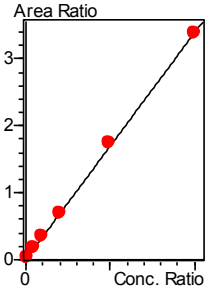
Ethoprophos



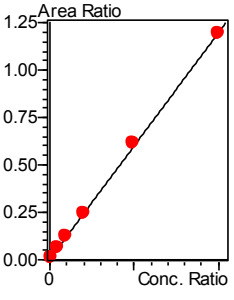
Sulfotep



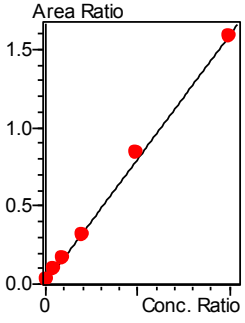
Monocrotophos



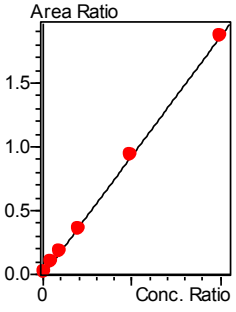
Phorate



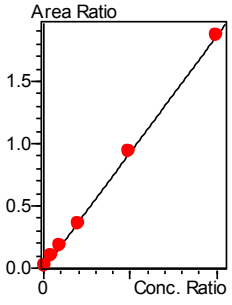
α-BHC



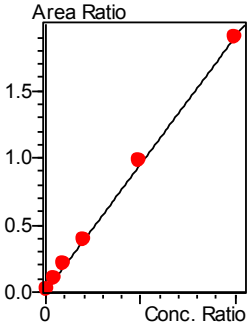
Dimethoate



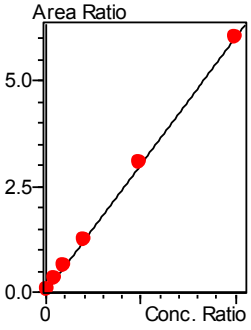
β-BHC



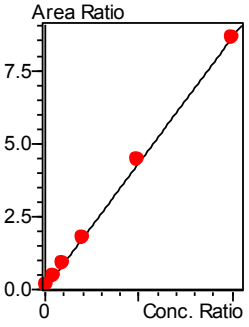
Quintozene



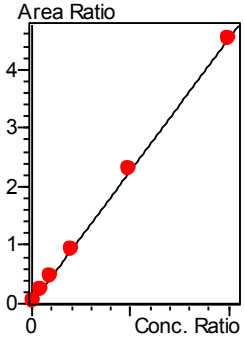
γ-BHC



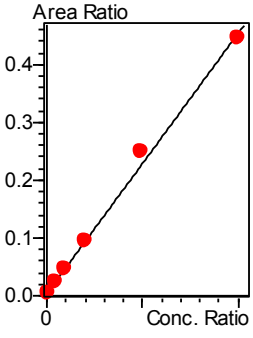
Terbufos



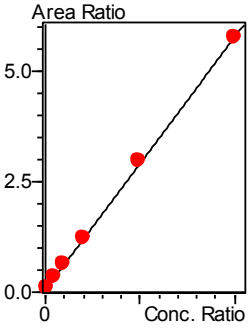
Fonofos



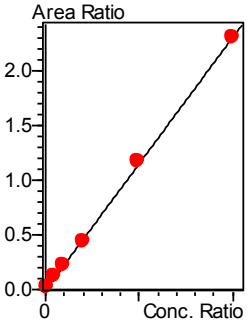
Diazinone



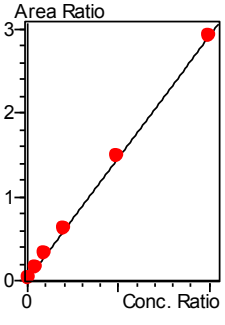
Phosphamidon



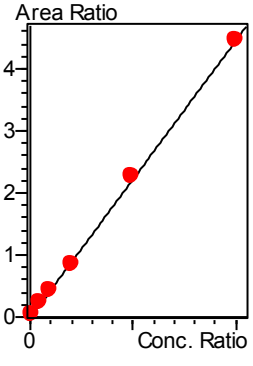
Pyrimethanil



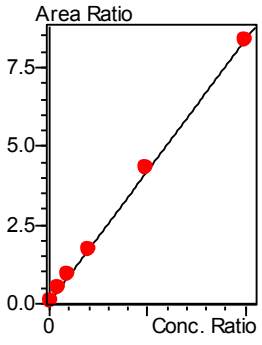
δ-BHC



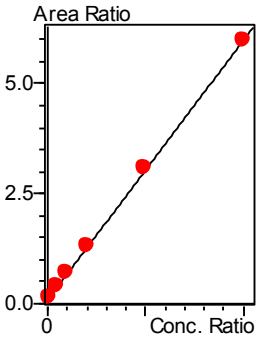
Vinclozoline



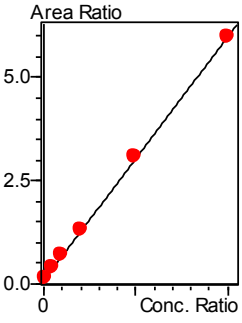
Parathion-methyl



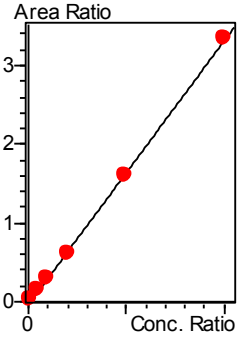
Fenitrothion



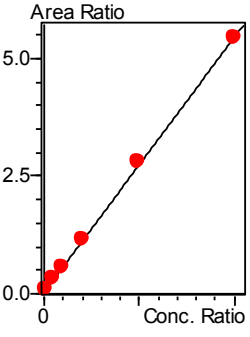
Malathion



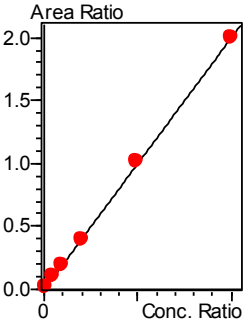
Chlorpyrifos



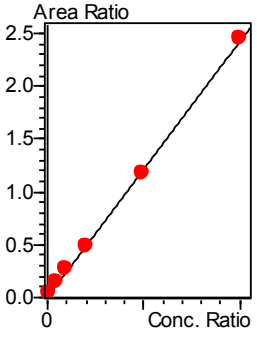
Parathion



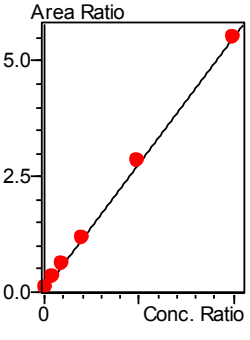
Triadimefon



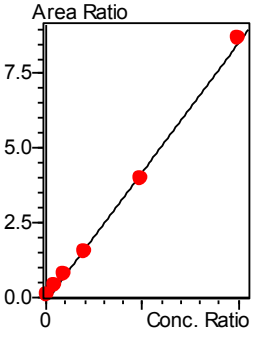
Isocarbophos



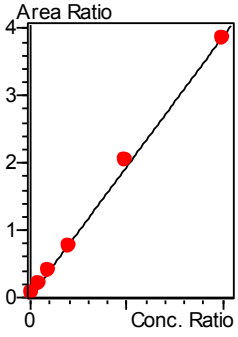
Dicofol



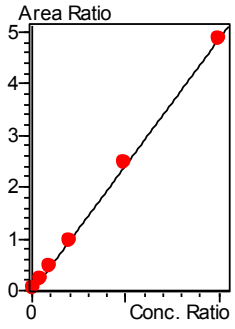
Isofenphos methyl



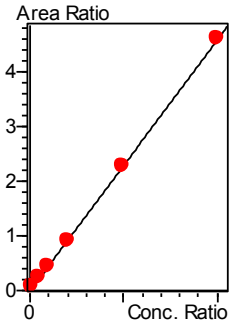
Fipronil



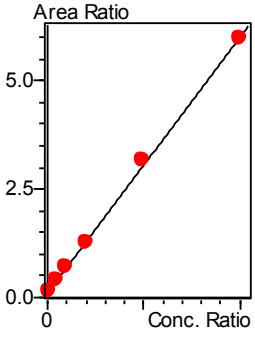
Phosfolan



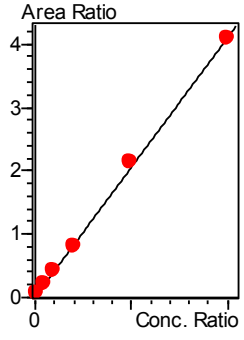
Phenthoate



Quinalphos



Procymidone



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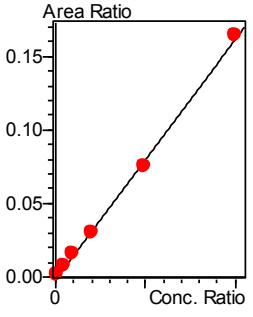
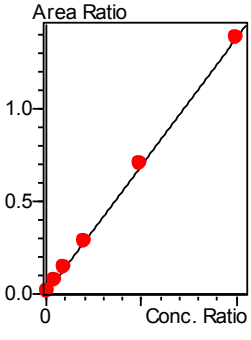


alpha-Endosulfan



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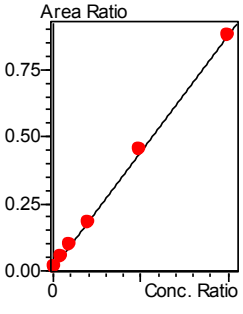

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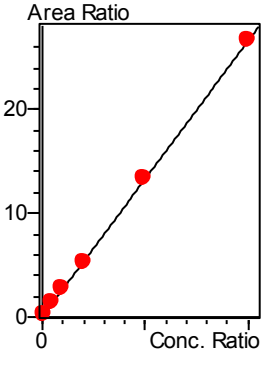

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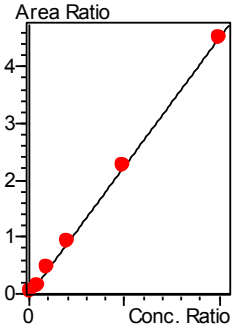

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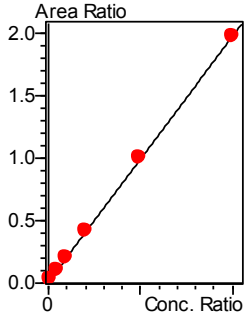

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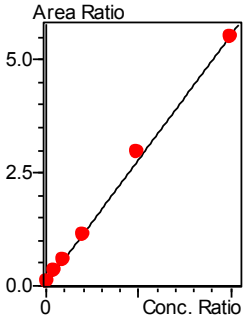

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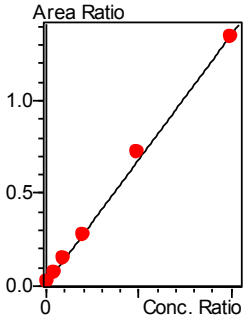

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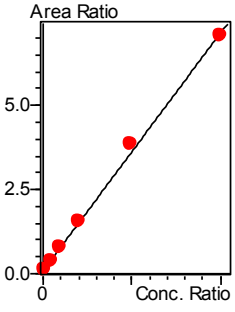

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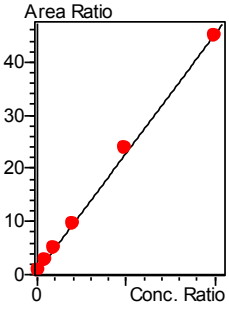

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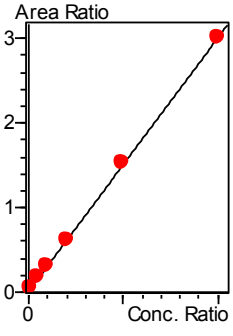

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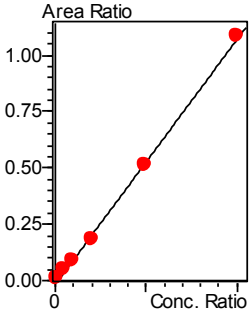

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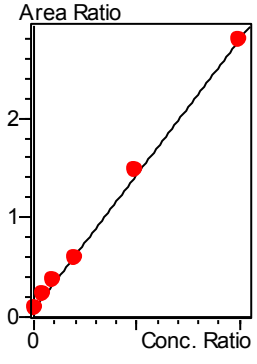

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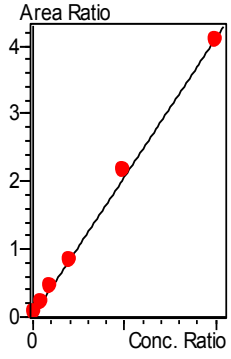

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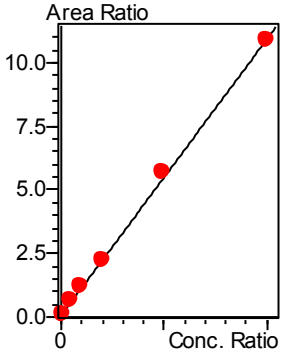

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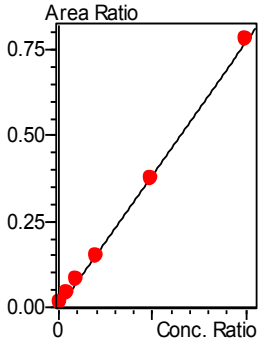

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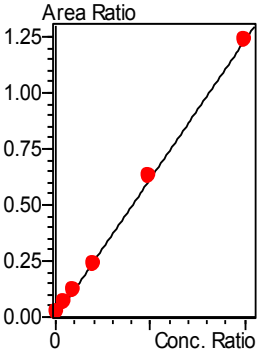

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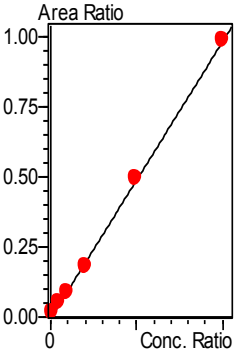

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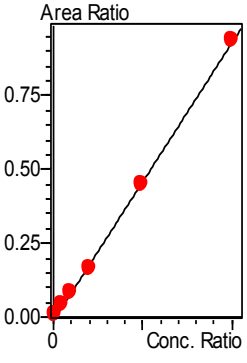

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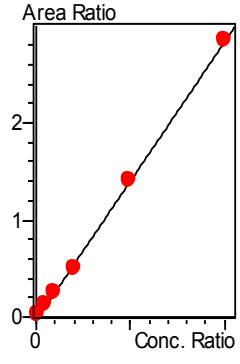

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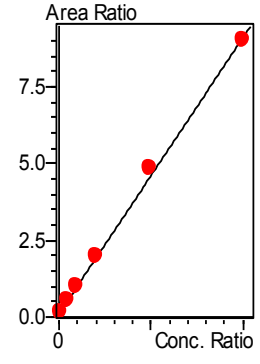
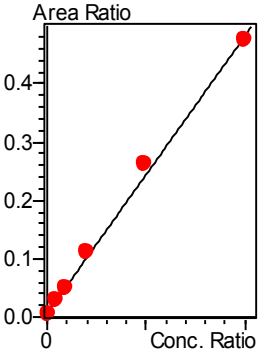


Difenoconazole



Deltamethrin

Table 2 Correlation coefficients and limits of detection (LODs) of pesticides

No.	Compound Name	Correlation Coefficient (R^2)	LOD (µg/kg)
1	Methamidophos	0.9983	3.061
2	Dichlorvos	0.9999	0.030
3	Trichlofon	0.9999	0.036
4	Omethoate	0.9978	0.112
5	Ethoprophos	0.9997	0.250
6	Sulfotep	0.9998	0.010
7	Monocrotophos	0.9990	0.224
8	Phorate	0.9999	0.012
9	α-BHC	0.9998	0.051
10	Dimethoate	0.9995	1.327
11	β-BHC	0.9999	0.023
12	Quintozene	0.9999	0.026
13	γ-BHC	0.9999	0.044
14	Terbufos	0.9999	0.138
15	Fonofos	0.9999	0.060
16	Diazinone	0.9999	0.010
17	Phosphamidon	0.9986	0.088
18	Pyrimethanil	0.9999	0.334
19	δ-BHC	0.9999	0.036
20	Vinclozoline	0.9999	0.022
21	Parathion-methyl	0.9999	0.289
22	Fenitrothion	0.9999	0.057
23	Malathion	0.9998	0.069
24	Chlorpyrifos	0.9999	0.006
25	Parathion	0.9997	0.233

26	Triadimefon	0.9999	0.140
27	Isocarbophos	0.9999	0.180
28	Dicofol	0.9997	0.021
29	Isofenphos methyl	0.9999	0.075
30	Fipronil	0.9991	0.003
31	Phosfolan	0.9995	0.183
32	Phenthoate	0.9999	0.027
33	Quinalphos	0.9999	1.422
34	Procymidone	0.9997	0.012
35	Methidathion	0.9998	0.068
36	alpha-Endosulfan	0.9991	0.122
37	Profenofos	0.9999	0.030
38	ppDDE	0.9999	0.579
39	ppDDD	0.9999	0.016
40	opDDT	0.9997	0.250
41	Triazophos	0.9999	0.139
42	ppDDT	0.9995	0.093
43	Iprodione	0.9993	0.101
44	Phosmet	0.9991	0.606
45	Bifenthrin	0.9996	0.047
46	Fenpropathrin	0.9999	0.124
47	Cyhalothrin	0.9999	0.241
48	Permethrin	0.9998	0.428
49	Coumaphos	0.9996	0.125
50	Pyridaben	0.9998	0.461
51	Cyfluthrin	0.9999	0.564
52	Cypermethrin	0.9997	0.918

53	Flucythrinate	0.9998	0.410
54	Fenvalerate	0.9999	0.110
55	Fluvalinate	0.9998	0.146
56	Difenoconazole	0.9994	0.029
57	Deltamethrin	0.9997	0.452

Repeatability test

6 replicate injections were performed using 1 μg/L mixed standard solution prepared with blank matrix, and the repeatability results are as shown in Table 3.

Table 3 Repeatability of peak area

No.	Compound Name	1	2	3	4	5	6	%RSD
1	Methamidophos	3986	4423	4209	4283	4391	4793	6.17
2	Dichlorvos	6263	6103	5979	5957	6285	6486	3.30
3	Trichlofon	5962	6081	6290	6235	6428	6395	2.91
4	Omethoate	29246	31275	32701	31797	35320	32683	6.21
5	Ethoprophos	8729	8502	8925	8999	9193	9162	2.97
6	Sulfotep	4761	5020	5298	5162	4412	4779	6.53
7	Monocrotophos	10846	11179	10885	10774	11493	10372	3.48
8	Phorate	4255	4657	4343	4483	4867	4649	4.98
9	α-BHC	1513	1645	1591	1584	1653	1385	6.42
10	Dimethoate	2233	2456	2111	2433	2131	2169	6.76
11	β-BHC	4442	5134	4594	4329	4978	4899	6.79
12	Quintozene	2280	2327	2484	2271	2663	2427	6.24
13	γ-BHC	2253	2693	2416	2684	2354	2474	7.18
14	Terbufos	9343	9317	8952	9772	9168	9624	3.17
15	Fonofos	13227	12887	12032	13423	14234	14926	7.56
16	Diazinone	6115	6109	6230	5810	7067	6256	6.79
17	Phosphamidon-1	3478	3653	3721	4013	3570	3623	5.01

18	Pyrimethanil	13059	12552	12700	11401	11137	12068	6.24
19	δ-BHC	2758	2780	2906	3084	2828	2974	4.33
20	Vinclozoline	4240	3593	4116	4219	4271	3898	6.52
21	Parathion-methyl	6430	6669	6837	6850	6660	6171	3.95
22	Fenitrothion	6750	6033	6668	6029	6489	6145	5.11
23	Malathion	12391	12736	12389	12630	13166	13508	3.50
24	Chlorpyrifos	16422	16784	16182	16227	16658	16553	1.45
25	Parathion	5323	5632	5686	5365	5595	5928	3.98
26	Triadimefon	9409	10193	10975	9168	10657	9973	6.94
27	Isocarbophos	3243	2802	3223	3278	3291	3220	5.84
28	Dicofol	3723	3843	3908	4232	3562	4204	6.77
29	Isofenphos methyl	11499	11815	10740	11689	12647	11998	5.33
30	Fipronil	10579	10258	11025	10055	10325	10500	3.39
31	Phosfolan	6869	6965	7135	7554	6741	6831	4.22
32	Phenthoate	5920	5736	5717	5693	5961	5314	4.00
33	Quinalphos	7382	6987	8209	7830	8069	8621	7.51
34	Procymidone	18592	18510	18031	19449	20208	19209	4.11
35	Methidathion	6633	6594	6953	6841	7163	7135	3.52
36	alpha-Endosulfan	147	162	163	161	156	163	3.96
37	Profenofos	2508	2142	2299	2073	2214	2382	7.06
38	p,p'-DDE	1822	1532	1562	1755	1859	1784	8.03
39	p,p'-DDD	42629	41691	43952	42502	43373	44236	2.24
40	o,p'-DDT	3363	3078	3164	3065	3090	3320	4.10
41	Triazophos	3133	3153	3587	2897	3326	3267	7.12
42	p,p'-DDT	9448	8781	9183	8353	8814	9252	4.44
43	Iprodione	3137	3048	3233	3332	3261	3276	3.23
44	Phosmet	36431	38090	36724	37678	35833	36758	2.24
45	Bifenthrin	80493	84853	81571	88821	86168	85534	3.63

46	Fenpropathrin	5824	5708	5768	5121	5154	5549	5.63
47	Cyhalothrin	3392	4063	3479	3716	3825	4127	7.94
48	Permethrin	17453	19802	19112	19181	18371	17339	5.38
49	Coumaphos	7175	7797	7494	7423	7393	7577	2.77
50	Pyridaben	8175	9368	9685	9560	9874	9958	6.93
51	Cyfluthrin	5892	6043	6149	7025	6119	6128	6.47
52	Cypermethrin	8547	8829	9039	9953	9842	9523	6.14
53	Flucythrinate	10135	11507	10500	11675	11085	12333	7.18
54	Fenvalerate	1887	1808	2063	1953	2090	1888	5.64
55	Fluvalinate	5038	5594	5617	5956	5743	5754	5.45
56	Difenoconazole	45544	45726	46755	48316	48938	48005	3.00
57	Deltamethrin	2534	2689	2594	2783	2724	2507	4.18

Recovery test

Mixed pesticides standard solution was added to blank ginger and leaf lettuce matrices at the concentration of 1 µg/kg and 5 µg/kg, respectively. 3 parallel samples were processed and the spike recoveries (average recovery and %RSD of the 3 parallel samples) of the two matrices are shown in Table 4.

Table 4 Spike recovery

No.	Compound Name	Spiked Concentration 1 µg/kg				Spiked Concentration 5 µg/kg			
		Leaf Lettuce Recovery	%RSD	Ginger Recovery	%RSD	Leaf Lettuce Recovery	%RSD	Ginger Recovery	%RSD
1	Methamidophos	114.4	2.69	32.7	12.46	69.0	4.34	66.6	11.90
2	Dichlorvos	75.7	2.82	70.9	2.16	74.6	4.78	87.7	5.67
3	Trichlofon	75.8	1.93	70.5	2.21	74.6	4.53	88.0	6.06
4	Omethoate	72.3	7.84	74.6	4.58	80.1	4.10	103.0	2.31
5	Ethoprophos	88.1	6.90	---	---	81.4	2.41	133.6	8.89
6	Sulfotep	88.6	1.72	56.4	2.13	76.7	2.20	37.7	7.55
7	Monocrotophos	86.6	3.94	70.1	3.09	81.7	5.51	54.5	5.30
8	Phorate	82.4	0.85	57.7	2.74	80.5	2.44	69.2	1.78
9	α-BHC	86.5	6.64	47.2	11.07	84.5	2.85	50.6	8.86
10	Dimethoate	82.3	6.43	136.9	18.32	93.2	2.93	101.6	12.00
11	β-BHC	90.9	0.52	106.5	2.41	87.3	1.13	100.7	1.61
12	Quintozene	88.5	4.80	78.0	7.8	82.8	0.99	102.8	3.68
13	γ-BHC	89.6	5.26	74.4	5.09	75.1	3.48	107.2	2.46
14	Terbufos	88.5	4.79	73.9	2.73	68.3	8.85	96.4	2.00
15	Fonofos	91.1	2.32	73.8	2.14	74.4	4.50	94.8	2.30
16	Diazinone	80.5	3.14	74.4	1.42	86.5	2.29	94.8	1.95
17	Phosphamidon-1	99.5	3.31	46.3	6.66	107.4	4.90	95.3	4.45
18	Pyrimethanil	92.7	5.04	112.4	3.61	84.6	5.28	106.0	5.48
20	δ-BHC	96.2	3.26	160.7	4.66	86.6	2.31	149.2	2.62
21	Vinclozoline	90.5	1.43	75.1	0.82	87.4	5.08	96.8	2.58
22	Parathion-methyl	99.6	2.80	83.5	1.3	92.9	4.63	113.4	4.52
23	Fenitrothion	97.5	1.42	84.6	1.75	92.7	2.89	112.9	2.66
24	Malathion	103.7	2.42	79.4	4.3	93.3	1.93	108.3	1.05

25	Chlorpyrifos	108.4	4.52	81.6	3.31	90.1	4.19	105.0	2.35
26	Parathion	104.4	6.09	97.6	3.77	89.7	1.90	122.9	2.46
27	Triadimefon	94.4	3.45	70.6	2.47	87.5	1.58	92.1	1.38
28	Isocarbophos	91.5	1.25	95.8	3.45	90.3	6.28	116.8	0.86
29	Dicofol	97.0	3.42	78.9	2.82	88.1	3.57	92.3	6.30
30	Isofenphos methyl	95.9	3.74	65.6	1.68	90.5	4.00	80.1	1.07
31	Fipronil	86.0	5.65	59.0	6.07	78.3	5.98	87.8	2.97
32	Phosfolan	98.5	4.65	---	---	104.5	2.69	---	---
34	Phenthoate	94.1	0.20	81.9	0.92	81.0	4.20	75.7	8.67
35	Quinalphos	108.6	3.13	55.2	16.27	79.0	3.65	87.2	3.69
36	Procymidone	110.3	7.95	80.2	1.24	93.1	3.23	96.2	1.70
37	Methidathion	98.0	1.11	69.5	1.38	85.6	3.56	92.6	1.36
38	alpha-Endosulfan	98.7	3.38	72.8	4.98	78.2	2.08	96.4	4.86
39	Profenofos	98.3	1.15	76.8	2.97	95.7	3.68	96.7	1.83
40	p,p'-DDE	105.9	2.01	33.1	19.1	76.6	6.07	76.3	12.80
42	p,p'-DDD	92.0	0.64	81.3	2.5	77.8	3.10	102.7	3.97
43	o,p'-DDT	88.7	0.88	77.2	5.36	85.5	5.84	108.2	4.93
44	Triazophos	101.7	2.76	68.3	2.49	99.4	1.90	66.5	1.00
45	p,p'-DDT	113.5	3.05	99.8	6.54	91.0	5.73	107.0	4.70
46	Iprodione	89.8	8.54	60.8	2.11	93.8	4.98	66.8	2.70
47	Phosmet	88.5	5.76	47.9	3.84	96.0	4.62	80.3	12.00
48	Bifenthrin	93.7	1.06	77.5	3.62	91.1	1.61	98.2	2.12
49	Fenpropathrin	91.4	2.73	98.2	5.39	87.5	2.36	113.9	1.43
51	Cyhalothrin	92.0	6.95	96.3	7.92	83.0	5.07	99.7	1.13
52	Permethrin	113.6	5.25	65.6	9.86	77.6	2.11	70.6	1.78
53	Coumaphos	96.8	0.95	90.9	3.96	89.2	2.14	105.0	2.55
54	Pyridaben	93.7	5.57	29.6	9.27	93.9	3.95	57.1	8.29

55	Cyfluthrin	115.9	1.79	139.6	6.38	100.6	2.76	96.7	5.92
56	Cypermethrin	117.4	2.86	122.8	0.81	101.1	2.74	125.4	3.17
57	Flucythrinate	117.6	2.59	136.8	2.66	96.5	2.08	102.9	2.09
58	Fenvalerate	117.1	1.57	129.7	3.94	102.1	3.51	104.7	7.70
59	Fluvalinate	111.9	1.71	154.4	7.15	102.5	4.06	75.1	6.18
60	Difenoconazole	113.4	3.46	71.2	4.95	100.5	4.34	81.4	2.50
61	Deltamethrin	116.7	2.36	148.9	4.56	109.5	4.10	105.5	6.22

Note: In the table, "---" indicates that the results cannot be calculated because of matrix interference of the sample.

CONCLUSION

A method was developed for the qualitative and quantitative analysis of more than 50 pesticide residues in vegetable using a Shimadzu GCMS-TQ8030. The method offered simplicity, satisfactory repeatability and sensitivity. For most pesticides, the method can achieve a recovery between 70.0% and 120.0% at the spiked concentration of 1 µg/kg and 5 µg/kg. Therefore, it can be used for routine analysis of trace pesticide residues satisfactorily. The experiment showed that tandem mass spectrometry could avoid the interference of matrix components, especially when analyzing complex samples, thereby effectively reducing false positive detection with improved selectivity and sensitivity, simplified sample pretreatment process, and reduced analysis cost.

C-7 Determination of Residual Pesticides in Onion and Chinese Chive with GC-MS/MS

INTRODUCTION

In this paper, an analytical method was developed for the determination of more than 50 pesticide residues in onion and Chinese chive by GC/tandem mass spectrometry (GC/MS/MS). The samples were, after being extracted with acetonitrile and added salt, centrifuged to separate the organic layer, which was purified and concentrated with SPE column. Then qualitative and quantitative analysis of multi-residues was carried out with a GC-MS/MS, using heptachlor-endo-epoxide as internal standard. The experiment results showed that the proposed method provided correlation coefficients (R^2) greater than 0.999 for all pesticides in the range between 1 and 100 µg/L. Repeatability test was conducted on standard solutions (1.0 µg/L) prepared with onion matrix. The result showed that the relative standard deviation (%RSD) of peak area was below 7.0% (n=6), and the limits of detection (LODs) of more than 50 pesticides were all below 1.0 µg/kg. The spike recoveries of most pesticides were between 60.0~120.0% at the spiked concentration of 1.0 µg/kg and 5.0 µg/kg, showing that the proposed method can meet the requirements of routine detection on pesticide residues analysis satisfactorily.

Pesticide residues have become a global issue of concern that is attracting more and more attention. In 2006, Japan proposed a positive list system in which the maximum residue limits (MRLs) of hundreds of agricultural chemicals in food were stipulated to be 10 µg/L. In 2008, EU enforced Commission Regulation No. 396/2005/EC which harmonized the regulations on pesticide MRLs of EU member countries and set 0.01mg/kg as the default MRL of pesticides.

At present, the mainstream technology for pesticide analysis is GC/MS, which is of poor selectivity with regard to the analysis of complex matrices, such as onion and Chinese chive, because single quadrupole mass spectrometry is vulnerable to matrix interference, and consequently the analysis results are subject to significant uncertainty. In this paper, a method was proposed for the determination of more than 50 residual pesticides in onion and Chinese chive with Shimadzu's new GCMS-TQ8030 in multiple reaction monitoring (MRM) mode. The method can effectively reduce matrix interference and improve sensitivity. It

is capable of accurate qualitative analysis and meeting the requirements of MRLs in quantitation satisfactorily.

Experiments

GC-MS/MS	: GCMS-TQ 8030

Conditions of Analysis

GC conditions

Column	: Rxi-5 Sil ms (30 m × 0.25 mm × 0.25 µm)
Injector temperature	: 250 °C
Column temperature program	: 50 °C(1min)→@(25 °C/min)→150°C →@(10°C/min)→300 °C(15 min)
CLV mode	: 47.6 cm/sec
Injection mode	: Splitless injection
Splitless time	: 1 min
High pressure injection	: 250 kPa (1min)

MS conditions

Temperature of ion source	: 230 °C
Determination mode	: MRM (See Table 1)
Interface temperature	: 250 °C
Detector voltage	: Tuning voltage+0.4kv

Table 1 Retention time and MRM parameters of pesticide components

ID	Compound Name	Retention Time	Quantitative Ion	CE	Qualitative Ion	CE
1	Methamidophos	5.913	141.00>95.00	8	141.00>126.00	4
2	Dichlorvos	6.033	185.00>93.00	14	185.00>109.00	14
3	Trichlorfon	6.033	185.00>93.00	14	185.00>109.00	14
4	Omethoate	9.686	156.00>110.00	8	156.00>141.00	4
5	Ethoprophos	10.168	158.00>97.00	15	158.00>114.00	5
6	Sulfotep	10.559	322.00>294.00	4	322.00>202.00	10
7	Monocrotophos	10.635	127.00>109.00	12	127.00>95.00	16
8	Phorate	10.799	260.00>75.00	8	260.00>231.00	4
9	alpha-HCH	10.916	219.00>183.00	8	219.00>145.00	18
10	Dimethoate	11.157	125.00>79.00	8	125.00>47.00	14
11	beta-HCH	11.447	219.00>183.00	8	219.00>147.00	20
12	Quintozene	11.502	295.00>237.00	16	295.00>265.00	12
13	gamma-HCH	11.598	219.00>183.00	8	219.00>147.00	20
14	Terbufos	11.672	231.00>129.00	26	231.00>175.00	14
15	Fonofos	11.761	246.00>109.00	18	246.00>137.00	6
16	Diazinon	11.797	304.00>179.00	10	304.00>162.00	8
17	Phosphamidon-1	11.814	264.00>127.00	14	264.00>193.00	8
18	Pyrimethanil	11.908	198.00>183.00	14	198.00>158.00	18
19	delta-HCH	12.171	219.00>183.00	10	219.00>145.00	22
20	Phosphamidon-2	12.572	264.00>127.00	14	264.00>193.00	8
21	Vinclozolin	12.828	285.00>212.00	12	285.00>178.00	14
22	Parathion-methyl	12.869	263.00>109.00	14	263.00>136.00	8
23	Fenitrothion	13.365	277.00>260.00	6	277.00>109.00	14
24	Malathion	13.535	173.00>127.00	6	173.00>99.00	14
25	Chlorpyrifos	13.683	314.00>258.00	14	314.00>286.00	8

26	Parathion	13.828	291.00>109.00	14	291.00>137.00	6
27	Triadimefon	13.895	208.00>181.00	10	208.00>127.00	14
28	Isocarbophos	13.909	289.00>136.00	14	289.00>113.00	6
29	dicofol	13.988	251.00>216.00	8	251.00>139.00	16
30	Isofenphos-methyl	14.217	241.00>199.00	8	241.00>121.00	22
31	Fipronil	14.414	367.00>213.00	30	367.00>255.00	22
32	Phosfolan	14.524	196.00>140.00	12	196.00>168.00	6
33	Heptachlor-endo-epoxide	14.536	353.00>289.00	6	353.00>253.00	26
34	Phenthoate	14.62	274.00>125.00	20	274.00>246.00	6
35	Quinalphos	14.643	157.00>129.00	14	157.00>93.00	10
36	Procymidone	14.714	283.00>96.00	10	283.00>255.00	12
37	Methidathion	14.915	145.00>85.00	8	145.00>58.00	14
38	a-Endosulfan	15.249	195.00>160.00	10	195.00>125.00	25
39	Profenofos	15.549	337.00>267.00	14	337.00>309.00	6
40	p,p'-DDE	15.643	246.00>176.00	28	246.00>211.00	22
41	p,p'-DDD	16.436	235.00>165.00	24	235.00>199.00	14
42	o,p'-DDT	16.491	235.00>165.00	24	235.00>199.00	16
43	Triazophos	16.685	257.00>162.00	8	257.00>134.00	22
44	p,p'-DDT	17.146	235.00>165.00	22	235.00>199.00	14
45	Iprodione	17.853	314.00>245.00	12	314.00>56.00	22
46	Phosmet	17.989	160.00>133.00	14	160.00>77.00	24
47	Bifenthrin	18.007	181.00>166.00	12	181.00>153.00	8
48	Fenpropathrin	18.192	265.00>210.00	12	265.00>172.00	14
49	Cyhalothrin-1	18.982	197.00>161.00	8	197.00>141.00	12
50	Permethrin-1	19.8	183.00>168.00	14	183.00>165.00	12
51	Permethrin-2	19.929	183.00>168.00	14	183.00>165.00	14

52	coumaphos	19.929	362.00>109.00	14	362.00>226.00	12
53	Pyridaben	19.969	147.00>117.00	22	147.00>132.00	14
54	Cyfluthrin-1	20.331	226.00>206.00	14	226.00>199.00	6
55	Cyfluthrin-2	20.428	226.00>206.00	12	226.00>199.00	6
56	Cyfluthrin-3	20.488	226.00>206.00	14	226.00>199.00	6
57	Cyfluthrin-4	20.529	226.00>206.00	14	226.00>199.00	6
58	Cypermethrin-1	20.655	163.00>127.00	5	181.00>152.00	22
59	Cypermethrin-2	20.756	163.00>127.00	5	181.00>152.00	22
60	Flucythrinate-1	20.815	199.00>157.00	10	199.00>107.00	22
61	Cypermethrin-3	20.813	163.00>127.00	5	181.00>152.00	22
62	Cypermethrin-4	20.854	163.00>127.00	5	181.00>152.00	22
63	Flucythrinate-2	21.007	199.00>157.00	8	199.00>107.00	22
64	Fenvalerate-1	21.546	419.00>225.00	6	419.00>167.00	12
65	Fluvalinate-1	21.66	250.00>200.00	20	250.00>55.00	18
66	Fluvalinate-2	21.724	250.00>200.00	20	250.00>55.00	20
67	Fenvalerate-2	21.755	419.00>225.00	6	419.00>167.00	12
68	Difenoconazole-1	22.02	323.00>265.00	14	323.00>202.00	28
69	Difenoconazole-2	22.089	323.00>265.00	14	323.00>202.00	28
70	Deltamethrin-1	22.126	253.00>172.00	4	253.00>93.00	20
71	Deltamethrin-2	22.345	253.00>172.00	4	253.00>93.00	20

Pretreatment of samples

Refer to GB/T 19648-2006 for pre-treatment of samples, as shown in Fig. 1 below.

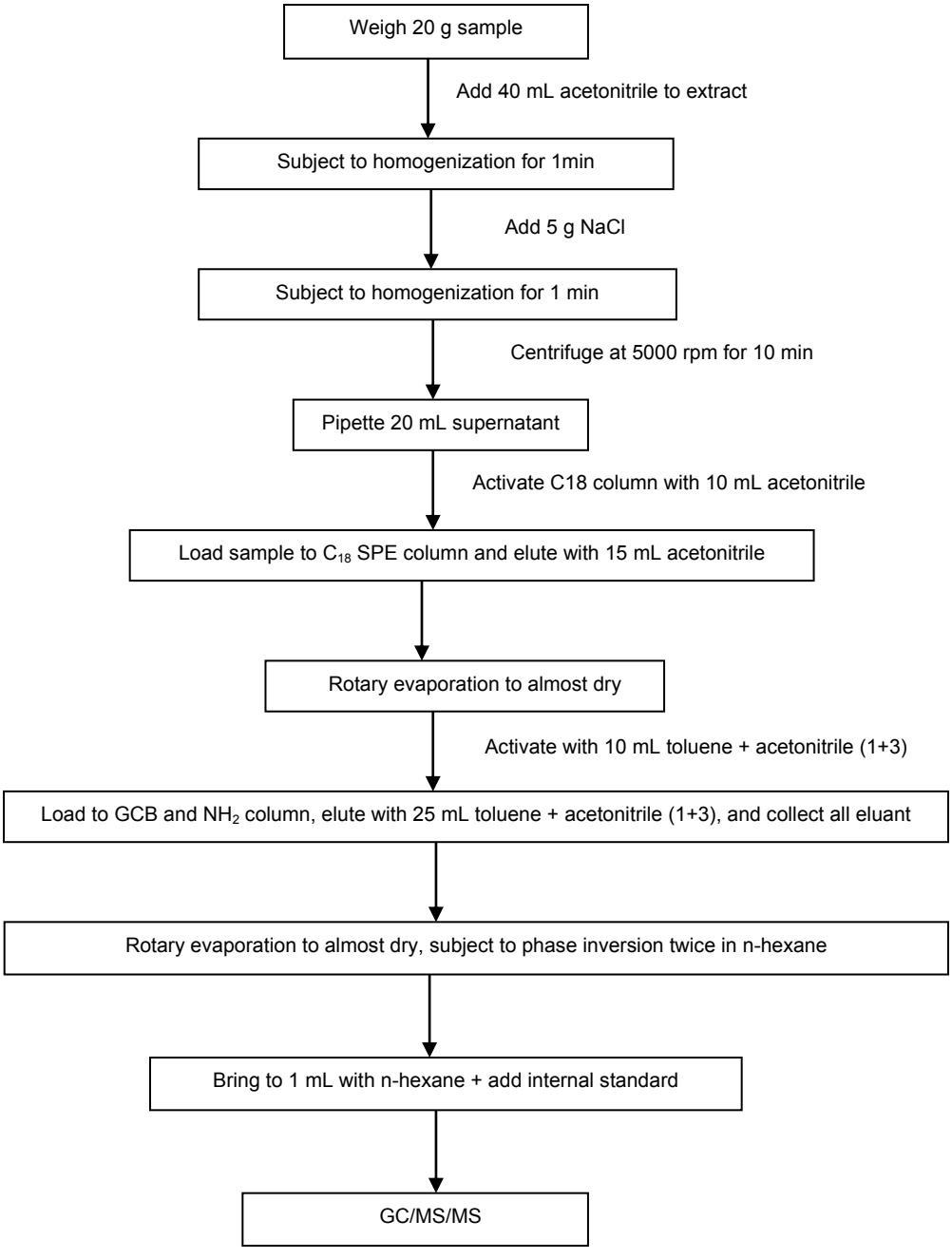


Fig. 1 Sample pretreatment

RESULTS AND DISCUSSION

MRM chromatogram of standard sample

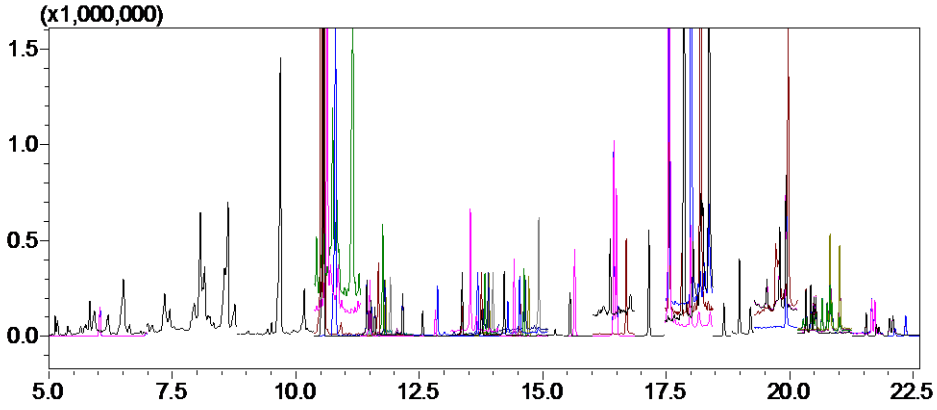
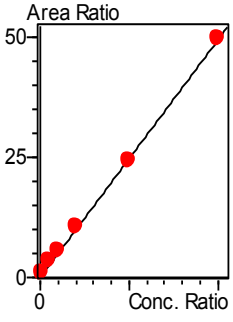


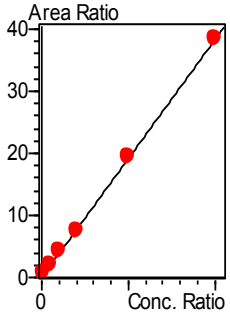
Fig. 2 MRM chromatogram of 20 µg/L mixed standard solution

Calibration curve and LOD

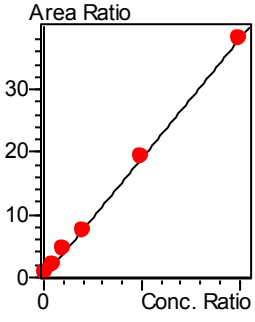
Mixed pesticide standard solutions of 1, 5, 10, 20, 50, 100 µg/L concentrations were prepared respectively with blank matrix - samples that contained none of the above-mentioned pesticides and pre-treated in the same way as test samples. Internal standard (28 µg/L heptachlor epoxide) was spiked into the matrix for preparation of calibration curves which take the concentration ratio of pesticide compositions to internal standard concentration as abscissa and the peak area ratio as ordinate. The calibration curves plotted were as shown in Fig. 3; the correlation coefficients and LODs were as shown in Table 2.



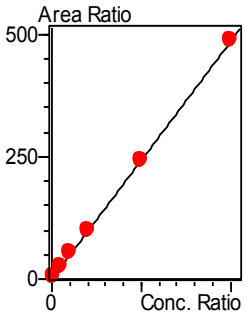
Methamidophos



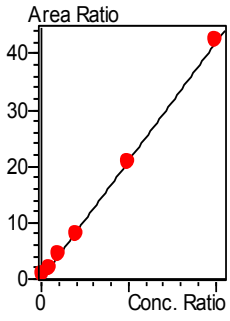
Dichlorvos



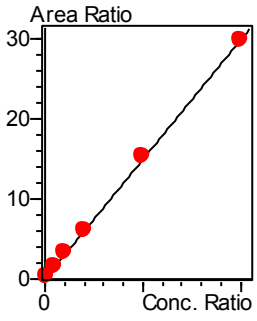
Trichlofon



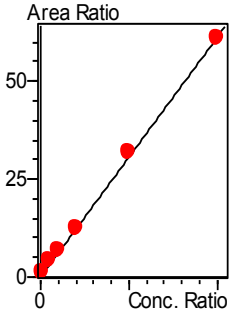
Omethoate



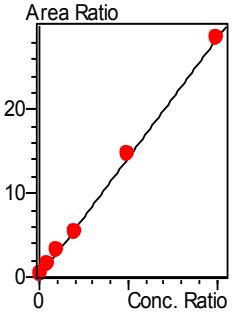
Ethoprophos



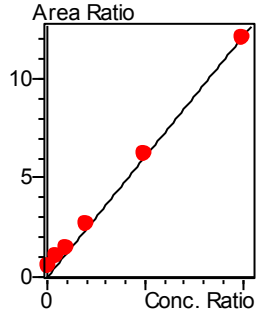
Sulfotep



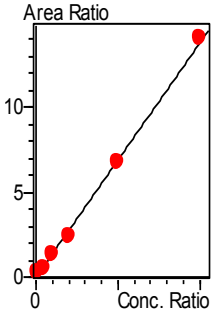
Monocrotophos



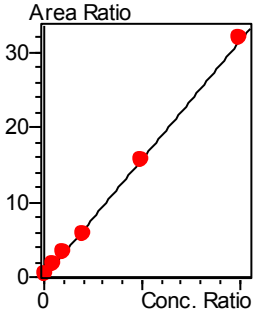
Phorate



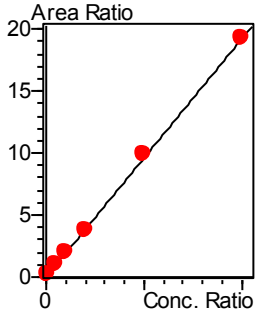
alpha-HCH



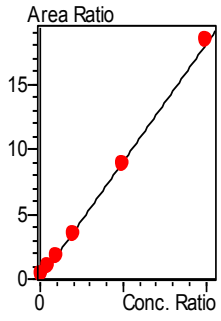
Dimethoate



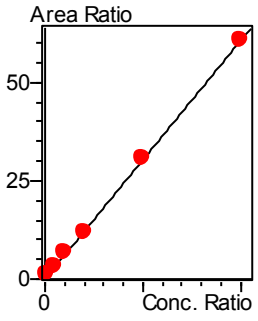
beta-HCH



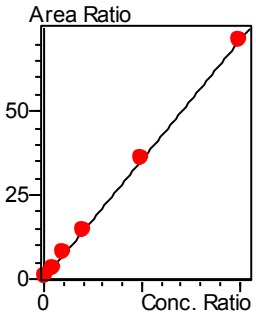
Quintozene



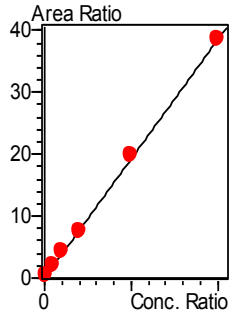
gamma-HCH



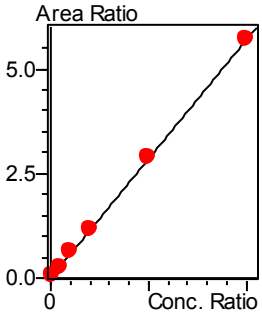
Terbufos



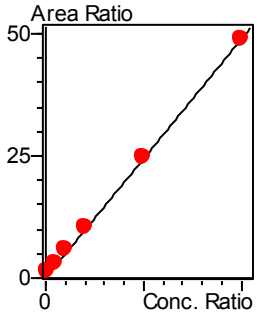
Fonofos



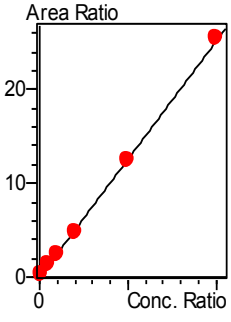
Diazinon



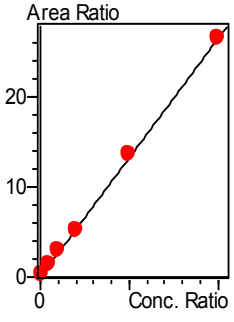
Phosphamidon



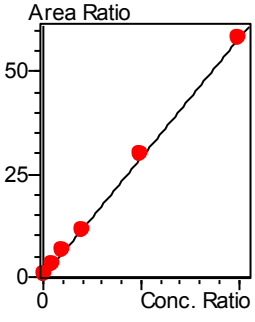
Pyrimethanil



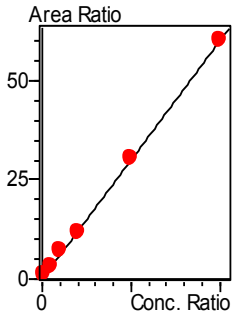
delta-HCH



Vinclozolin



Parathion-methyl



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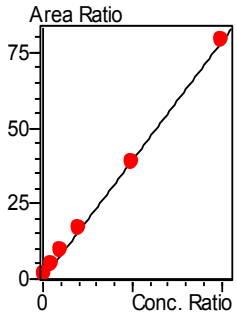

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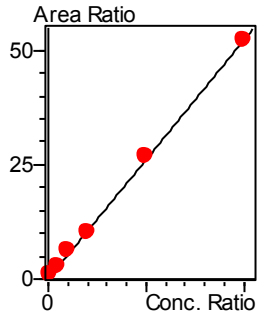

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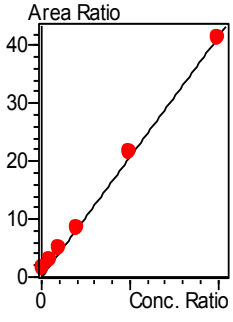

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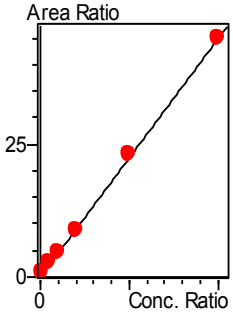

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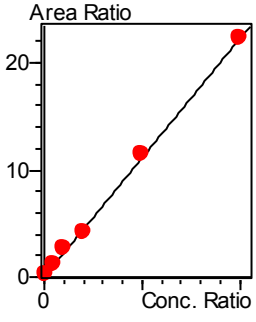
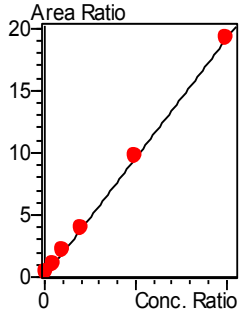


Isocarbophos



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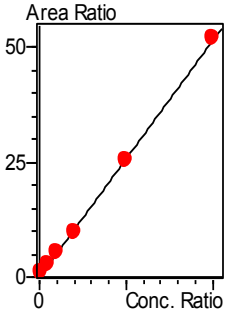
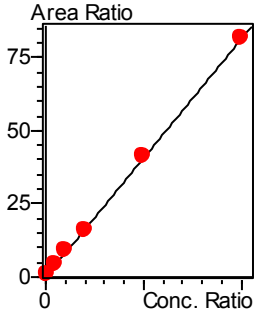
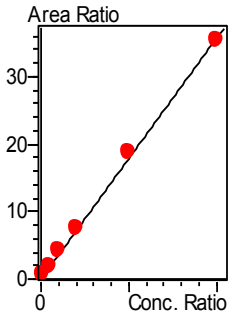


Isofenphos-methyl



Fipronil



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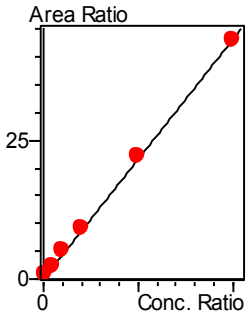

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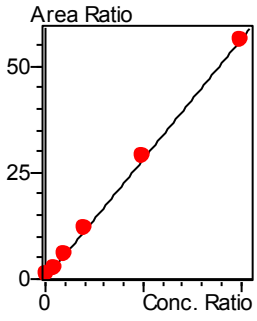

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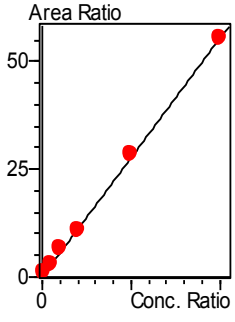

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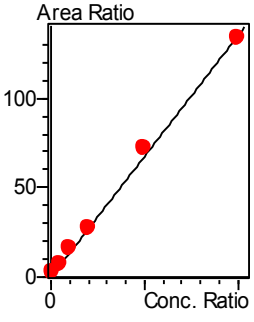

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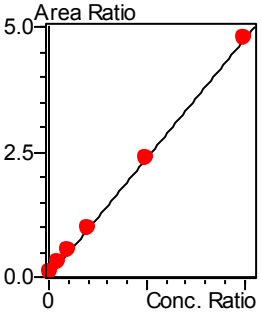
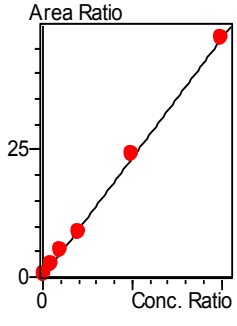


Endosulfan



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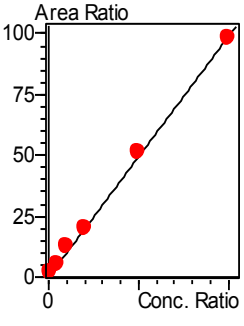

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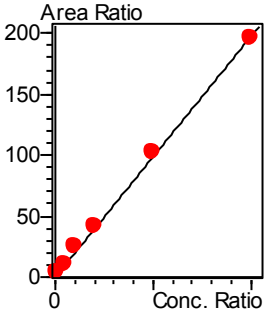

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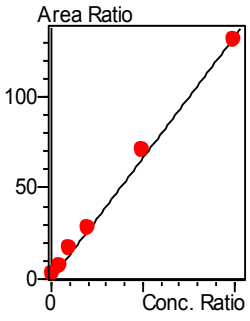
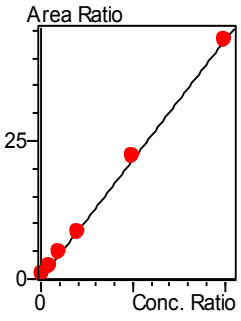
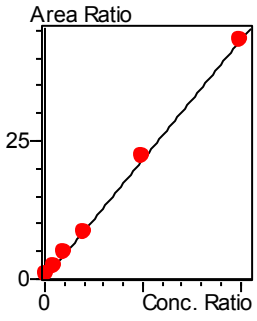


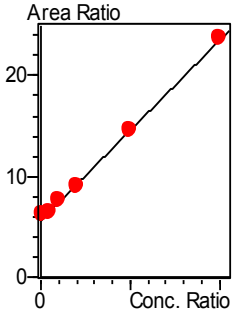
o,p'-DDT



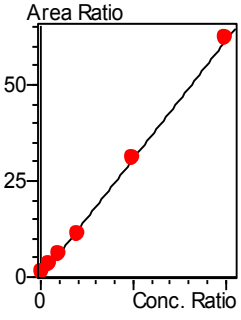
Triazophos



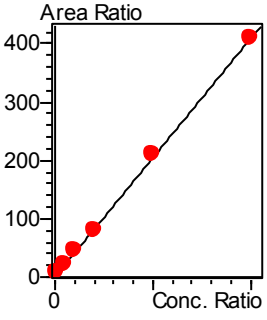
p,p'-DDT



Iprodione



Phosmet



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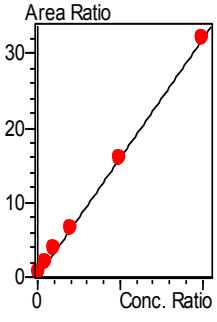

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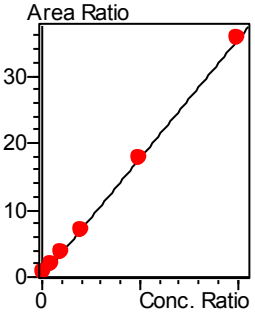
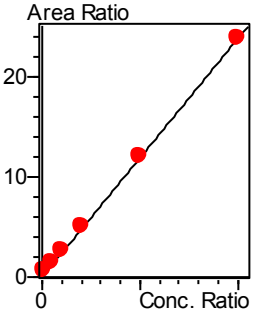


Cyhalothrin



Permethrin

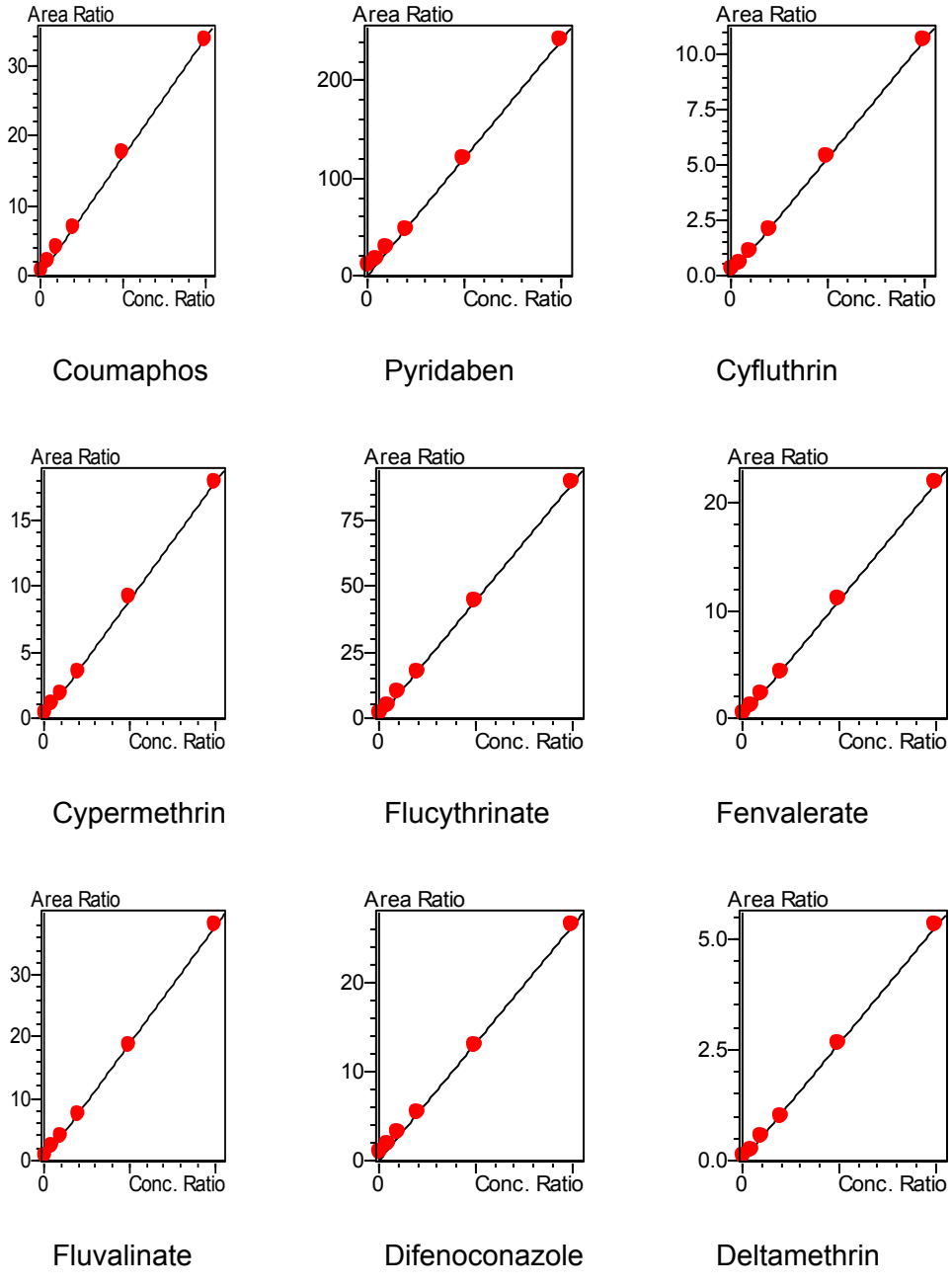


Fig. 3 Calibration curves of pesticide components

Table 2 Linearity coefficients and LODs of pesticide components

No.	Compound Name	Linearity Coefficient	LOD (μg/kg)	No.	Compound Name	Linearity Coefficient	LOD (μg/kg)
1	Methamidophos	0.9998	0.92	30	Fipronil	0.9999	0.01
2	Dichlorvos	0.9999	0.02	31	Phosfolan	0.9996	0.34
3	Trichlofon	0.9998	0.02	32	Phenthoate	0.9998	0.06
4	Omethoate	0.9999	0.06	33	Quinalphos	0.9998	0.52
5	Ethoprophos	0.9998	0.23	34	Procymidone	0.9997	0.06
6	Sulfotep	0.9999	0.01	35	Methidathion	0.9993	0.11
7	Monocrotophos	0.9998	0.40	36	Endosulfan	0.9999	0.17
8	Phorate	0.9998	0.01	37	Profenofos	0.9997	0.01
9	alpha-HCH	0.9999	0.04	38	p,p'-DDE	0.9996	0.03
10	Dimethoate	0.9994	0.86	39	p,p'-DDD	0.9996	0.02
11	bata-HCH	0.9996	0.05	40	o,p'-DDT	0.9992	0.05
12	Quintozene	0.9999	0.01	41	Triazophos	0.9998	0.16
13	gamma-HCH	0.9996	0.04	42	p,p'-DDT	0.9999	0.03
14	Terbufos	0.9999	0.14	43	Iprodione	0.9992	0.10
15	Fonofos	0.9999	0.05	44	Phosmet	0.9997	0.26
16	Diazinon	0.9998	0.01	45	Bifenthrin	0.9999	0.05
17	Phosphamidon	0.9999	0.04	46	Fenpropathrin	0.9999	0.16
18	Pyrimethanil	0.9999	0.60	47	Cyhalothrin	0.9999	0.35
19	delta-HCH	0.9938	0.03	48	Permethrin	0.9999	0.50
20	Vinclozolin	0.9998	0.03	49	Coumaphos	0.9997	0.35
21	Parathion-methyl	0.9999	0.14	50	Pyridaben	0.9996	0.16
22	Fenitrothion	0.9999	0.08	51	Cyfluthrin	0.9998	0.86
23	Malathion	0.9997	0.13	52	Cypermethrin	0.9998	0.71
24	Chlorpyrifos	0.9998	0.01	53	Flucythrinate	0.9999	0.47

25	Parathion	0.9998	0.28	54	Fenvalerate	0.9999	0.30
26	Triadimefon	0.9998	0.61	55	Fluvalinate	0.9998	0.26
27	Isocarbophos	0.9996	0.22	56	Difenoconazole	0.9999	0.20
28	Dicofol	0.9999	0.04	57	Deltamethrin	0.9999	0.59
29	Isofenphos methyl	0.9998	0.23				

Repeatability test

Six replicate injections were performed using 1 μg/L mixed standard solution prepared with onion blank matrix. The repeatability results were as shown in Table 3.

Table 3 Repeatability of peak area

	Compound	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	%RSD
1	Methamidophos	50886	59804	55334	59410	59380	60465	6.49
2	Dichlorvos	16036	16243	16667	16891	16704	16520	1.92
3	Trichlofon	16154	16404	16430	16282	16565	16620	1.06
4	Omethoate	145660	144810	148321	148127	145264	140748	1.89
5	Ethoprophos	64505	68674	72262	69572	67252	68677	3.74
6	Sulfotep	9989	9910	9320	9894	9618	10136	3.00
7	Monocrotophos	21236	22187	20635	22020	22165	20709	3.37
8	Phorate	9341	9104	9104	9431	9400	9288	1.55
9	alpha-HCH	20653	22088	21246	22206	22752	23243	4.32
10	bata-HCH	14866	14852	15339	15408	14112	13026	6.16
11	Quintozene	6543	6247	7010	6658	6139	6439	4.79
12	gamma-HCH	5689	5472	6447	5994	5918	5456	6.43
13	Terbufos	23264	21408	22937	21857	24902	24683	6.16
14	Fonofos	25679	25809	24818	24796	25206	26828	3.00
15	Diazinon	12515	12468	11935	11381	12827	12431	4.22

16	Phosphamidon	8396.00	8451.00	8016.00	8590.00	8417.00	8613.00	2.33
17	Pyrimethanil	37853	35724	34335	36705	34943	34919	3.69
18	delta-HCH	8780	8966	8534	8788	8882	8660	1.76
19	Vinclozolin	8559	8352	8963	8802	9255	8762	3.57
20	Parathion-methyl	21022	20549	20047	21139	21591	20631	2.58
21	Fenitrothion	22829	22575	22686	22069	22727	22423	1.22
22	Malathion	45792	46059	49107	44363	44830	45576	3.63
23	Chlorpyrifos	21425	21182	21017	21684	22171	21943	2.06
24	Fenthion	21225	23327	21986	21466	22927	23867	4.75
25	Parathion	35346	35474	35918	33655	35290	34814	2.23
26	Triadimefon	26622	26443	28230	26953	24891	28435	4.83
27	Isocarbophos	8583	8963	8416	7847	8010	8899	5.40
28	Dicofol	8897	8442	7488	8434	7785	7935	6.34
29	Isofenphos methyl	20359	22581	20837	23289	21747	20860	5.28
30	Fipronil	29070	28127	30325	29641	28667	29262	2.62
31	Phosfolan	13239	13386	12884	12566	12369	13289	3.23
32	Phenthoate	20473	21024	21484	20817	19940	20775	2.51
33	Quinalphos	14511	14096	14856	14000	14224	13736	2.78
34	Procymidone	27079	27194	28285	28078	28503	28410	2.25
35	Methidathion	55049	55959	57123	53944	58631	56520	2.91
36	Endosulfan	5728.0	5636.0	5731	5883	5789	5838	1.40
37	Profenofos	16294	16097	16131	16447	16442	16488	1.04
38	p,p'-DDE	36435	37372	38907	38323	39886	38257	3.13
39	p,p'-DDD	73928	72803	75345	77548	74924	76887	2.36
40	o,p'-DDT	49670	54561	51548	50595	50812	49340	3.68
41	Triazophos	19132	18459	17557	18979	20229	18982	4.63

42	p,p'-DDT	51237	48250	49238	50764	48369	48302	2.69
43	Iprodione	184242	184601	184942	184627	186831	187606	0.75
44	Phosmet	30849	30739	29168	30273	30592	28913	2.79
45	Bifenthrin	178626	174725	176445	169014	172934	172781	1.91
46	Fenpropathrin	14279	14422	15082	14986	14382	14605	2.29
47	Cyhalothrin	15005	14816	15194	15861	14656	14962	2.80
48	Permethrin	42308.0	43015.0	42327.0	40797.0	42382.0	41375.0	1.70
49	Coumaphos	15241	15732	16139	15549	15703	15095	2.41
50	Pyridaben	284522	287468	293466	290853	303958	293012	2.29
51	Cyfluthrin	25932.0	25504.0	25246	25202	26576	25255	1.93
52	Cypermethrin	40297.0	39349.0	37882.0	40210.0	39997.0	39278.0	2.08
53	Flucythrinate	86237.0	91747.0	94501	87275	91195	89674	3.08
54	Fenvalerate	9802	9718	9759	9916	9483	9576	1.61
55	Fluvalinate	41243.0	42079.0	41508	40710	43916	42072	2.41
56	Difenoconazole	61048	62647	58346	60725	60556	61327	2.10
57	Deltamethrin	8361	7494	7616	7723	8314	8204	4.40

Recovery test

Pesticide mixtures were spiked into onion and Chinese chive samples at concentrations of 1 µg/kg and 5 µg/kg respectively. Three parallel samples were processed and the spike recoveries of the two samples were as shown in Table 4.

Table 4 Spike recovery

No.	Compound Name	Spiked Amount 1 µg/kg				Spiked Amount 5 µg/kg			
		Onion Recovery	RSD%	Chinese Chive Recovery	RSD%	Onion Recovery	RSD%	Chinese Chive Recovery	RSD%
1	Methamidophos	90.5	6.8	64.9	7.8	90.8	6.8	71.8	4.1
2	Dichlorvos	92.2	6.2	68.8	7.7	92.1	5.9	77.7	4.8
3	Trichlofon	92.1	6.2	69.1	7.6	92.6	5.9	77.6	4.8
4	Omethoate	57.9	8.3	77.7	8.0	58.5	5.8	77.6	8.3
5	Ethoprophos	97.5	7.1	95.4	8.9	114.9	3.3	109.5	8.6
6	Sulfotep	116.3	8.8	110.8	4.4	115.8	4.7	119.7	8.7
7	Monocrotophos	83.2	4.7	89.4	4.3	75.5	6.8	107.0	4.8
8	Phorate	86.6	1.4	92.4	7.2	99.2	6.1	91.1	6.2
9	alpha-HCH	106.0	6.6	95.8	7.3	100.2	7.1	86.1	4.1
10	bata-HCH	92.9	8.5	99.2	4.5	98.1	6.4	91.1	5.1
11	Quintozene	92.4	4.8	94.7	3.5	101.0	7.0	92.3	9.6
12	gamma-HCH	99.6	8.0	91.8	2.4	94.0	4.3	86.7	8.8
13	Terbufos	123.2	7.3	114.8	4.6	94.2	5.4	117.4	4.9
14	Fonofos	120.7	7.6	110.6	5.1	89.0	9.7	120.5	2.8
15	Diazinon	91.2	0.7	93.3	6.7	100.0	2.0	92.9	5.1
16	Phosphamidon	95.53	3.2	109.5	6.7	116.0	1.2	117.3	7.0
17	Pyrimethanil	109.4	4.7	107.9	3.7	104.2	5.0	103.9	6.0
18	delta-HCH	97.4	4.9	82.2	5.5	95.2	4.7	77.3	8.1
19	Vinclozolin	86.2	4.6	91.6	4.2	98.6	8.6	89.7	8.0
20	Parathion-methyl	85.5	4.1	99.5	8.4	100.6	3.1	95.2	4.0
21	Fenitrothion	89.6	2.4	97.2	6.2	104.7	6.3	95.4	3.5
22	Malathion	122.2	9.4	108.9	7.9	110.5	7.1	98.3	4.2
23	Chlorpyrifos	91.4	5.4	135.6	3.2	96.3	6.3	89.5	8.4

24	Fenthion	91.8	7.4	98.4	6.8	104.5	4.6	94.4	3.3
25	Parathion	105.8	6.1	117.7	2.5	114.9	7.4	100.9	5.5
26	Triadimefon	105.4	2.4	101.7	5.7	108.3	7.5	113.4	6.3
27	Isocarbophos	92.8	4.4	90.2	9.6	100.9	6.6	92.0	4.8
28	Dicofol	105.5	6.7	96.1	4.9	106.6	4.9	92.9	6.9
29	Isofenphos methyl	109.3	6.1	110.2	4.5	110.7	7.6	98.9	3.1
30	Fipronil	90.5	6.2	83.1	8.1	96.4	5.5	85.5	6.9
31	Phosfolan	105.5	5.5	108.3	5.4	102.0	8.0	119.4	4.2
32	Phenthoate	114.7	3.5	114.1	6.8	101.3	7.7	121.8	8.2
33	Quinalphos	113.7	2.4	108.9	5.4	105.3	3.8	123.8	7.7
34	Procymidone	104.9	2.8	106.2	2.5	105.0	8.7	94.5	9.7
35	Methidathion	111.8	7.3	111.4	6.4	102.6	6.7	115.2	9.3
36	Endosulfan	121.5	2.1	107.9	6.0	104.8	6.4	116.9	5.8
37	Profenofos	84.0	2.0	84.7	6.2	93.1	6.8	84.0	4.2
38	p,p'-DDE	123.3	1.7	105.6	6.5	100.3	4.7	118.0	6.9
39	p,p'-DDD	127.4	3.8	122.4	5.4	107.7	3.6	132.5	3.8
40	o,p'-DDT	105.2	6.3	73.6	5.2	83.3	2.9	69.3	4.7
41	Triazophos	100.6	9.0	112.6	3.1	110.7	8.1	103.0	3.8
42	p,p'-DDT	82.2	6.8	80.0	8.0	79.3	2.2	54.0	6.7
43	Iprodione	91.6	4.4	64.1	1.2	76.6	8.7	93.9	3.8
44	Phosmet	60.9	8.3	90.9	4.1	58.7	4.3	82.0	9.7
45	Bifenthrin	101.3	7.4	106.7	4.4	112.6	7.2	98.5	8.1
46	Fenpropathrin	101.2	7.4	111.2	7.8	94.9	5.7	96.2	6.3
47	Cyhalothrin	104.7	7.6	76.1	8.3	106.2	6.9	79.1	8.2
48	Permethrin	124.8	8.8	108.8	3.8	103.1	9.3	103.8	8.3
49	Coumaphos	112.2	3.7	116.5	6.4	93.3	7.1	129.1	4.6
50	Pyridaben	113.6	6.5	103.7	6.9	110.4	3.0	110.0	4.1

51	*Cyfluthrin*	*116.1*	*4.5*	*103.9*	*9.3*	*113.9*	*4.2*	*95.8*	*7.5*
52	*Cypermethrin*	*115.8*	*5.9*	*119.9*	*4.7*	*116.6*	*8.8*	*99.1*	*7.1*
53	*Flucythrinate*	*113.1*	*6.6*	*109.4*	*5.6*	*117.6*	*8.7*	*102.1*	*6.2*
54	*Fenvalerate*	*108.6*	*7.1*	*108.0*	*3.8*	*117.4*	*6.3*	*100.9*	*5.9*
55	*Fluvalinate*	*105.5*	*9.9*	*90.0*	*8.6*	*120.3*	*8.8*	*91.4*	*7.1*
56	*Difenoconazole*	*110.5*	*6.6*	*81.2*	*8.0*	*78.9*	*8.2*	*83.0*	*4.1*
57	*Deltamethrin*	*112.4*	*4.2*	*91.4*	*7.2*	*97.5*	*5.7*	*83.3*	*6.4*

CONCLUSION

A method was proposed for the determination of more than 50 pesticide residues in onion and Chinese chive with GCMS-TQ8030 (Shimadzu's Triple Quadrupole GC-MS). The matrix can be effectively eliminated and false positive results excluded by means of multiple reaction monitoring (MRM) mode and as a result the accuracy of quantitation was further guaranteed. This method demonstrated satisfactory sensitivity. Its LODs for most pesticides in a complex matrix were well below 1 µg/kg and its spike recovery ranged between 60% and 120% and could therefore meet the detection requirements set by international regulations.

C-8 Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Edible Oil with GCMS-TQ8030

INTRODUCTION

In this paper, a method was developed for analysis of polycyclic aromatic hydrocarbons (PAHs) in edible oil with GCMS-TQ8030, Shimadzu's GC-MS/MS. The method shows good linearity in the concentration range of 1~100 µg/L. The proposed method provides a recovery between 63% and 104% at the spiked concentrations of 1 µg/kg, with LODs ranging between 0.001~0.067 µg/kg.

Polycyclic aromatic hydrocarbons (PAHs) refer to a category of environment pollutants which, featuring a fused ring consisting of 2 or more benzene rings, are with strong carcinogenic, teratogenic, and mutagenic effects.

Edible oil is widely used in daily food which may, for reasons of PAHs containing raw materials, poor production & processing technology, and exposure to environmental pollution during the transportation and storage processes, contain PAHs. EU commission regulation (EC) NO 1881/2006 has stipulated a MRL of 2 µg/kg for benzo[α]pyrene in edible oil. Spain and Italy also stipulated that the total content of 8 PAHs shall not exceed 5 µg/kg and the content of an individual PAH shall not exceed 2 µg/kg. In China, it is stipulated in national standard GB 2762-2005 maximum levels of contaminants in foods that the MRL of benzo[α]pyrene is 10 µg/kg.

PAHs have strong liposolubility, which makes them easily to be enriched in vegetable oil and hard to be analyzed and purified. Moreover, the content of PAHs in vegetable oil is rather low and analysis of PAHs is apt to be interfered by other matrices in oil. Therefore, it is very hard to detect and analyze the PAHs in edible oil at low levels.

In this paper, PAHs in vegetable oil were extracted with DMF: water (9:1) and then enriched and purified with C_{18} solid-phase extraction column. The method demonstrated satisfactory repeatability and its spike recovery at 1 µg/kg

remained between 63% and 104% with an LOD between 0.001~0.067 μg/kg. The results showed that the application of MRM for acquisition could effectively eliminate matrix interference and improve sensitivity.

Apparatus

GCMS-TQ8030

Conditions of Analysis

Column	: Rxi-5Sil MS, 30 m×0.25 mm×0.25 μm
Injector temperature	: 280 °C
Injection mode	: Splitless injection
Column temperature program	: 60 °C(1 min)→@20 °C/min→200 °C(1 min)→ @10 °C/min→310 °C(10 min)
CLV	: 40cm/sec
Injection volume	: 2 μL
Ionization mode	: EI
Temperature of ion source	: 230 °C
Temperature of MS interface	: 280 °C
Solvent cut time	: 4 min
Collection mode	: Selected MRM were as listed in Table 1

Sample Preparation

Take 2 g edible oil, accurately weighed, add 100 μL deuterated internal standard of 200 μg/L concentration, then add 10 mL n-hexane to dissolve. Extract with 20 mL DMF:water (9:1, v/v) twice, combine the extract. Add certain amount of distilled water to the extract, adjust the volume ratio of DMF to water to 1:1.

Activate the C_{18} solid-phase extraction column with 10 mL methanol and 10 mL DMF/water (1:1, v/v), respectively. Load the above-mentioned extract to the C_{18} extraction column, rinse with 10 mL DMF:water (1:1, v/v) and 10 mL distilled water, respectively; then dry under vacuum.

Elute the C_{18} extraction column with 10 mL n-hexane, collect the eluant and concentrate it to 1 mL before transferring it to a vial for analysis on the instrument.

RESULTS AND DISCUSSION

Chromatogram

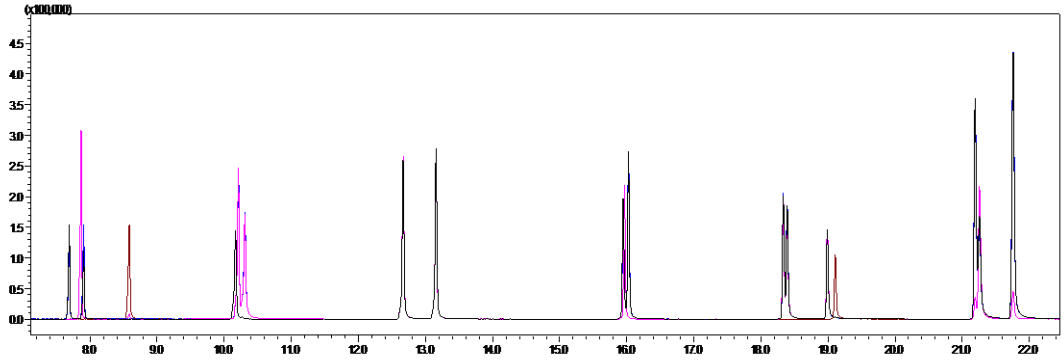


Fig. 1 TIC of standard solutions of PAHs (50 μg/L)

Table 1 Names, retention times and monitor ions of compounds

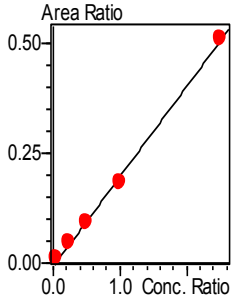
NO.	Compound Name	Retention Time (min)	Quantitative Ion Precursor>Product	CE	Reference Ion Precursor>Product	CE
1	Acenaphthene-d10	7.875	162.0>160.0	25	162.0>134.0	27
2	Acenaphthene	7.917	153.0>127.0	30	153.0>77.0	31
3	Fluorene	8.592	166.0>139.0	40	166.0>115.0	35
4	Phenanthrene-d10	10.175	188.0>160.0	21	188.0>158.0	33
5	Phenanthrene	10.217	178.0>176.0	29	178.0>152.0	20
6	Anthracene	10.317	178.0>176.0	29	178.0>152.0	20
7	Fluoranthene	12.675	202.0>200.0	30	202.0>152.0	25
8	Pyrene	13.167	202.0>200.0	30	202.0>152.0	25
9	Benzo[α]anthracene	15.958	228.0>226.0	34	228.0>202.0	20.
10	Chrysene-d12	15.975	240.0>236.0	34	240.0>212.0	26

11	Chrysene	16.033	228.0>226.0	34	228.0>202.0	20
12	Benzo[b]fluoranthene	18.342	252.0>250.0	34	252.0>226.0	20
13	Benzo[k]fluoranthene	18.392	252.0>250.0	34.	252.0>226.0	20
14	Benzo[α]pyrene	18.992	252.0>250.0	34	252.0>226.0	20
15	Perylene-d12	19.108	264.0>260.0	40	264.0>236.0	25
16	Indeno[1,2,3-cd]pyrene	21.200	276.0>274.0	40	276.0>275.0	25
17	Dibenzo[a,h]anthracene	21.267	278.0>276.0	42	278.0>252.0	25
18	Benzo[g,h,i]perylene	21.767	276.0>274.0	40	276.0>275.0	25

Linear range and LOD

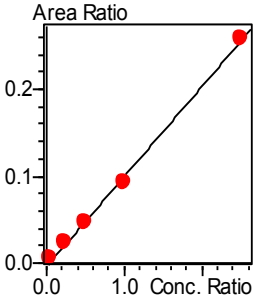
100 mg/L PAHs mixed standard solution was diluted and PAHs standard solutions of concentrations of 1, 10, 20, 50, 100 µg/L were prepared. MRM mode was used for data acquisition. The calibration curves and correlation coefficients (R) are shown below.

LODs were calculated and the results were as shown in Table 2.



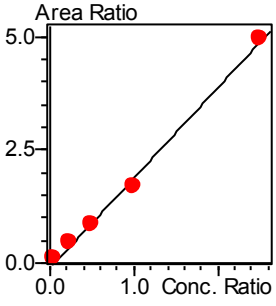
Acenaphthene

R = 0.9997



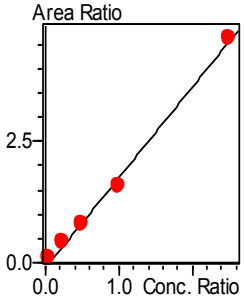
Fluorene

R = 0.9996



Phenanthrene

R = 0.9996



Anthracene

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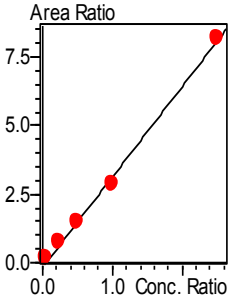

Fluoranthene

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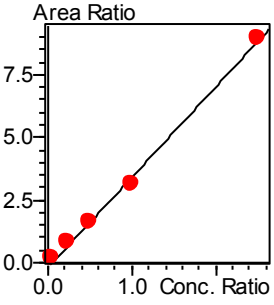

Pyrene

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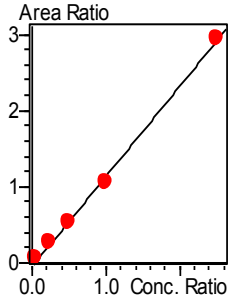
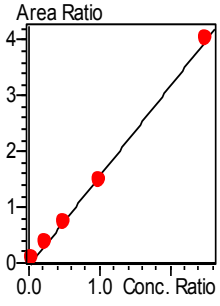


Benzo[α]anthene

R = 0.9993



Chrysene

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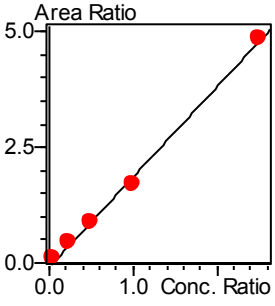

Benzo[b]fluoranthene

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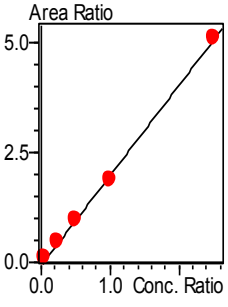

Benzo[k]fluoranthene

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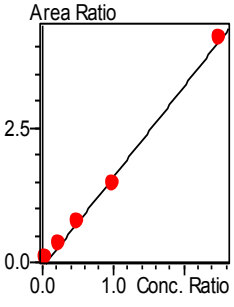
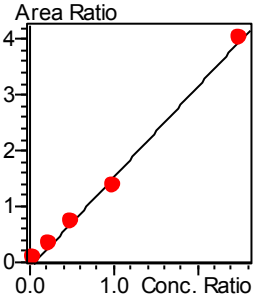


Benzo[α]pyrene

R = 0.9999



Indeno[1,2,3-cd]pyrene

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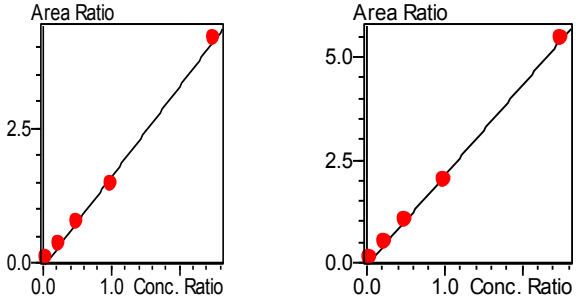


Dibenzo[a,h]anthracene R = 0.9999

Benzo[g,h,i]perylene R = 0.9999

Fig. 2 Calibration curves of PAHs

Recovery and repeatability test

100 μL PAHs mixed standard solution of concentration of 20 μg/L (spiked concentration of 1 μg/kg) was added to the blank olive oil samples. 3 samples were prepared. The samples were subjected to pretreatment according to the above-mentioned steps. The concentrations of PAHs were determined. Then relative standard deviations and recoveries were calculated. The results were shown in Table 2.

Table 2 Recoveries and LODs of PAHs

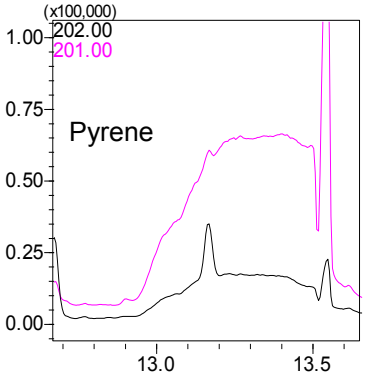
Compound Name	Recovery (%)			Mean (%)	RSD (%)	LOD (μg/kg)
	1	2	3			
Acenaphthene	80.36	73.86	87.95	80.72	8.74	0.023
Fluorene	82.80	77.97	80.00	80.25	3.02	0.067
Phenanthrene	80.98	68.98	69.68	73.22	9.20	0.010
Anthracene	66.20	63.72	63.24	64.38	2.47	0.009
Fluoranthene	77.77	67.22	66.67	70.56	8.87	0.002
Pyrene	84.07	71.51	69.17	74.92	10.69	0.001
Benzo[α]anthracene	89.27	71.31	72.11	77.56	13.08	0.008
Chrysene	97.75	104.03	103.11	101.63	3.34	0.006

Benzo[b]fluoranthene	87.34	93.44	88.96	89.91	3.52	0.003
Benzo[k]fluoranthene	63.47	75.16	72.97	70.53	8.81	0.007
Benzo[α]pyrene	93.62	86.64	87.44	89.23	4.28	0.009
Indeno[1,2,3-cd]pyrene	103.98	98.66	96.72	99.79	3.76	0.006
Dibenzo[a,h]anthracene	69.85	78.39	76.32	74.85	5.95	0.004
Benzo[g,h,i]perylene	83.47	99.56	94.16	92.40	8.86	0.007

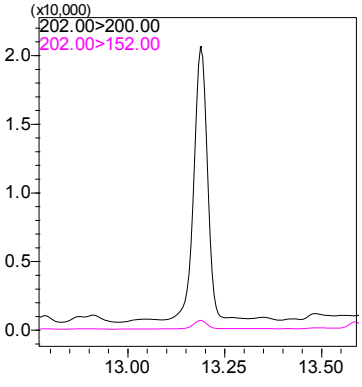
Comparison with SIM collection mode

Spiked 1 μg/kg olive oil samples were analyzed in SIM and in MRM respectively. As vegetable oil contains much fatty acid, tocopherol, sterol and other substances, if SIM is adopted, the analysis may be interfered by these matrices and as a result the sensitivity may not be good. The application of MRM can minimize the matrix interference in vegetable oil and increase signal-to-noise ratio, thereby yielding the best quantitative analysis results of target compound.

SIM



MRM



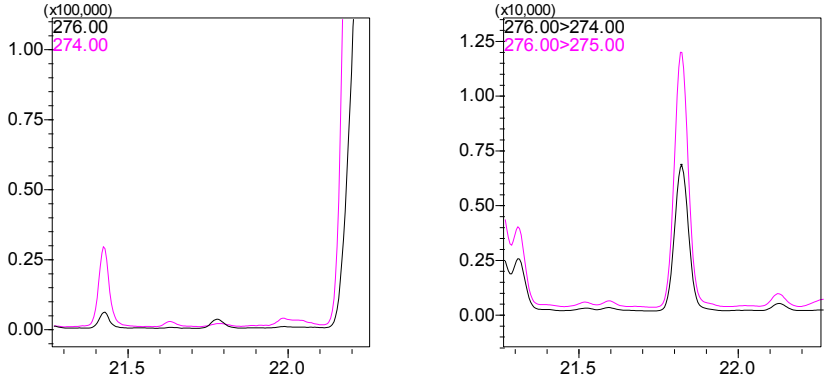


Fig. 2 Comparison between SIM and MRM (spiked olive oil sample (1 µg/kg))

CONCLUSION

A method was developed for the analysis of PAHs in vegetable oil using GCMS-TQ8030. The method was easy to operate and with satisfactory repeatability. Its recovery remained between 63% and 104% at the spiked concentration of 1 µg/kg. The use of MRM mode for analysis could eliminate matrix interference and increase selectivity and sensitivity.

C-9 Determination of Residual Pesticides in Tea with GC-MS/MS (QuEChERS Method)

INTRODUCTION

In this paper, an analytical method is described determining 61 pesticide residues in tea simultaneously with GC-MS/MS. The correlation coefficients of all assayed pesticides were greater than 0.999 in the concentration range of 2~200 µg/L. The %RSDs of peak areas of 6 consecutive injections of 50 µg/L standard solution were less than 3.2%. When individual sample weight was 5g, the proposed method demonstrated a LOD less than 5.0 µg/kg for most pesticides. The proposed method provided a spike recovery between 70.0 and 110.0% for most of the pesticides at spiked concentrations of 10 µg/kg and 100 µg/kg, meeting the requirements of routine analysis of pesticide residues in tea.

Some pesticides may be used during growing of tea to prevent and reduce the damages caused by pests. As a result there might be possibility of presence of pesticide residues in tea which are harmful to human health. In countries like EU and Japan, strict requirements on MRLs of pesticides in tea have been stipulated. In China, Announcement No. 199 of the Ministry of Agriculture issued in 2002, clearly prohibits the use of 39 pesticides, including Dicofol, Fenvalerate, Parathion-methyl, and Monocrotophos, in tea plantation. The announcement also stipulates MRLs of pesticides in tea.

China's national standard GB/T 23204-2008 provides a GC/MS method for detecting the content of 519 pesticides and related chemicals in tea. The method covers almost all regulated pesticides in tea plantation, aiming to meet the detection requirements of pesticide residues in tea. However, the complex matrices of tea may, even after two-stage purification with SPE column, interfere with the detection of pesticides in tea. In this paper, a modified QuEChERS pretreatment method was used in conjunction with GC/MS/MS technique for the analysis of 61 pesticide residues in tea. The method not only simplified the pretreatment of samples but also eliminates possibility of false positive and negative detection using GC/MS arising due to matrix interference of tea, thereby improving the sensitivity.

EXPERIMENTS

GC-MS/MS : GCMS-TQ8030 (Shimadzu)

Analytical Conditions

GC-MS/MS conditions

Column	: Rxi-5 ms, 30 m×0.25 mm×0.25 μm
Injector temperature	: 250 °C
Column temperature program	: 50°C(1min)→@(25°C/min)→150°C →@(10°C/min)→300°C(5min)
CLV mode	: 47.6 cm/sec
Injection mode	: Splitless injection
Splitless time	: 1 min
High pressure injection	: 250 kPa (1 min)
Ion source	: 200 °C
MRM collection conditions	: Given in Table 1

Sample Preparation

Pretreatment of samples was as shown in Fig. 1 below

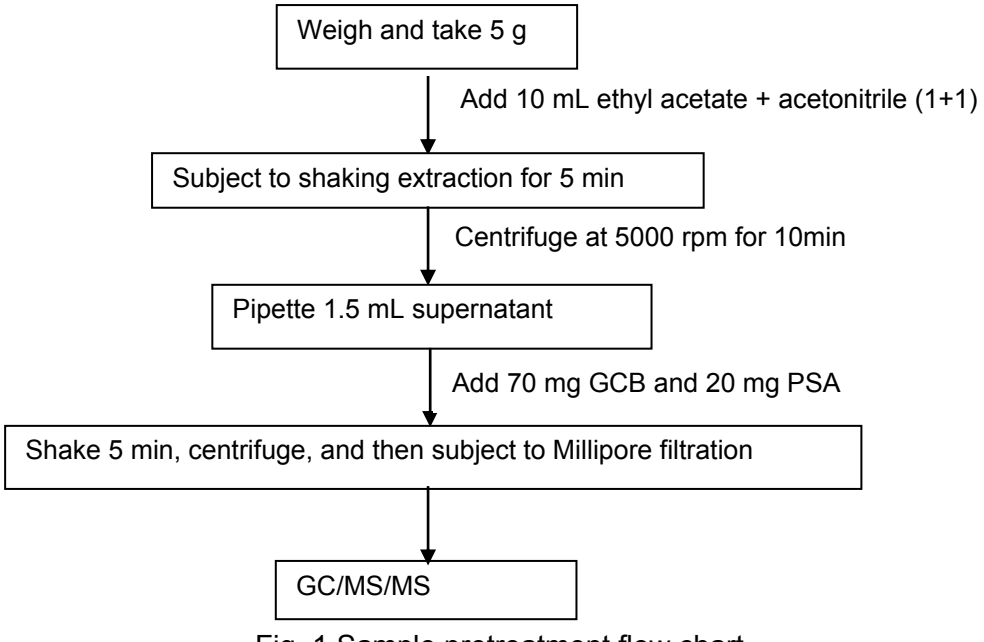


Fig. 1 Sample pretreatment flow chart

Table 1 Retention time and MRM parameters of pesticide components

No. :	Retention Time	Names of Pesticide	Quantitative Ion (CE)	Qualitative Ion (CE)
1	5.230	O,O,O-triethylphosphorothioate	198>114 (15)	198>121 (15)
2	5.995	Dichlorvos	185>93 (14)	185>109 (14)
3	7.800	Mevinphos	192>164 (4)	192>127 (12)
4	9.025	Isoprocarb	136>121 (10)	136>103 (22)
5	9.305	TEPP	179>99 (16)	179>81 (30)
6	9.750	Thionazine	143>79 (15)	143>52 (25)
7	9.785	Fenobucarb	150>121 (10)	150>103 (24)
8	10.105	Ethoprophos	200>158 (6)	200>114 (14)
9	10.490	Sulfotep	322>294 (4)	322>202 (10)
10	10.570	Monocrotophos	127>109 (12)	127>95 (16)
11	10.730	Phorate	260>75 (8)	260>231 (4)
12	10.855	Alpha-HCH	219>183 (8)	219>145 (18)
13	11.080	Demton S	142>112 (6)	142>79 (14)
14	11.195	Carbofuran	164>149 (8)	164>131 (18)
15	11.240	Simazine	201>173 (6)	201>186 (6)
16	11.335	Atrazine	215>58 (14)	215>200 (6)
17	11.370	Beta-HCH	219>183 (8)	219>147 (20)
18	11.410	Propazine	229>187 (14)	229>58 (14)
19	11.540	Gamma-HCH	219>183 (8)	219>147 (20)
20	11.725	Diazinon	304>179 (10)	304>162 (8)
21	11.865	Chlorothalonil	266>231 (14)	266>168 (22)
22	11.950	Disulfoton	186>97 (16)	186>153 (6)
23	12.095	Delta-HCH	219>183 (10)	219>145 (22)

24	12.810	Methyl parathion	263>109 (14)	263>136 (8)
25	12.940	Carbaryl	144>116 (12)	144>89 (38)
26	13.005	Heptachlor	272>237 (20)	272>117 (32)
27	13.035	Fenchlorphos	285>270 (16)	285>93 (24)
28	13.465	Malathion	173>127 (6)	173>99 (14)
29	13.615	Chlorpyrifos	314>258 (14)	314>286 (8)
30	13.690	Fenthion	278>109 (20)	278>125 (20)
31	13.715	Aldrin	263>193 (28)	263>203 (26)
32	13.760	Parathion	291>109 (14)	291>137 (6)
33	13.920	Dicofol	251>216 (8)	251>139 (16)
34	14.465	Heptachlor-exoepoxide	353>263 (14)	353>282 (12)
35	14.920	tans-Chlordane	373>337 (10)	373>143 (26)
36	14.975	Tetrachlorvinphos	329>109 (20)	329>314 (18)
37	15.175	cis-Chlordane	373>266 (22)	373>337 (6)
38	15.405	Prothiofos	339>160 (18)	339>267 (4)
39	15.575	p,p'-DDE	246>176 (28)	246>211 (22)
40	15.695	Dieldrin	277>206 (15)	277>241 (15)
41	16.095	Endrin	263>191 (30)	263>193 (28)
42	16.210	Fensulfothion	293>153 (8)	293>125 (14)
43	16.290	Beta-Endosulfan	339>160 (18)	339>267 (4)
44	16.370	p,p'-DDD	235>165 (24)	235>199 (14)
45	16.495	Endrin aldehydel	281>209 (30)	281>246 (20)
46	16.645	Sulprofos	322>156 (8)	322>97 (24)
47	16.755	Famphur	218>109 (16)	218>79 (24)
48	17.010	Endosulfan sulfate	387>289 (10)	387>253 (16)
49	17.070	p,p'-DDT	235>165 (22)	235>199 (14)
50	17.895	Endrin ketone	317>101 (20)	317>147 (15)

51	17.925	Bifenthrin	181>166 (12)	181>153 (15)
52	17.965	EPN	169>141 (8)	169>77 (22)
53	18.080	Methoxychlor	227>169 (24)	227>212 (14)
54	18.115	Fenpropathrin	265>210 (12)	265>172 (14)
55	18.665	Azinphos-methyl	160>132 (6)	160>77 (20)
56	18.725	Cyhalothrin-1	197>161 (8)	197>141 (12)
57	18.895	Cyhalothrin-2	197>161 (8)	197>141 (12)
58	19.840	Coumaphos	362>109 (14)	362>226 (12)
59	20.245	Cyflurthrin-1	226>206 (14)	226>199 (6)
60	20.335	Cyflurthrin-2	226>206 (14)	226>199 (6)
61	20.395	Cyflurthrin-3	226>206 (14)	226>199 (6)
62	20.440	Cyflurthrin-4	226>206 (14)	226>199 (6)
63	20.570	Cypermethrin-1	181>152 (22)	181>127 (22)
64	20.660	Cypermethrin-2	181>152 (22)	181>127 (22)
65	20.725	Cypermethrin-3	181>152 (22)	181>127 (22)
66	20.765	Cypermethrin-4	181>152 (22)	181>127 (22)
67	21.455	Fenvalerate-1	419>225 (6)	419>167 (12)
68	21.660	Fenvalerate-2	419>225 (6)	419>167 (12)
69	22.020	Deltamethrin-1	253>93 (18)	253>172 (4)
70	22.250	Deltamethrin-2	253>93 (20)	253>172 (8)

RESULT

Chromatogram of standard sample

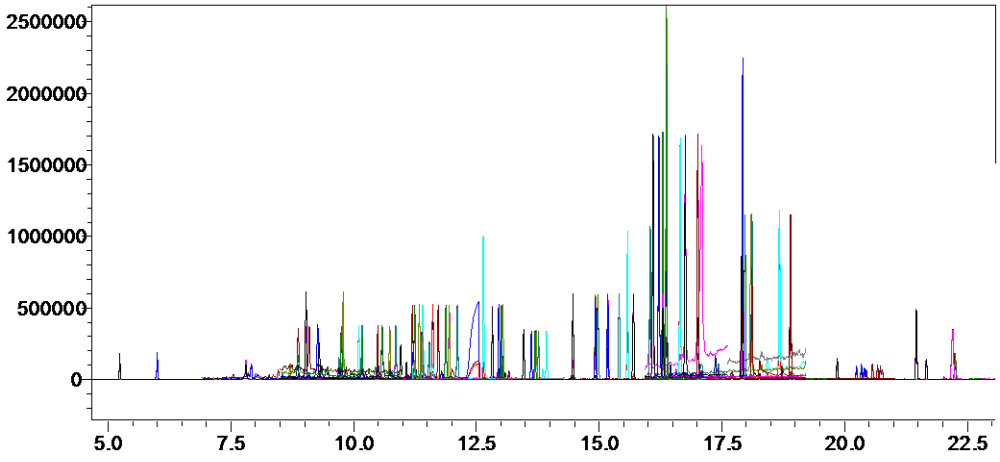
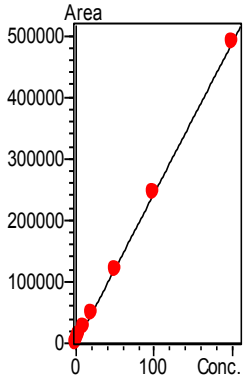


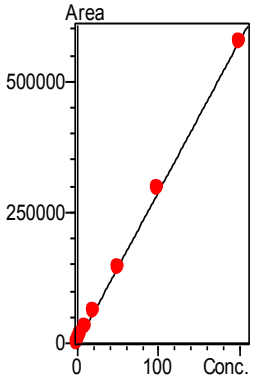
Fig. 2 MRM chromatograms of standard pesticides (100 µg/L)

Calibration curve

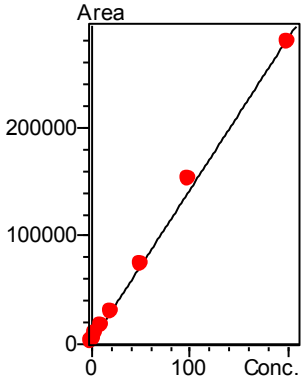
Mixed pesticide standard solutions of concentrations of 2, 5, 10, 20, 50, 100, 200 µg/L were prepared respectively using tea matrix solution. Calibration curves were plotted as shown in the figure below, using the concentration as abscissa and the peak area as ordinate. LODs were calculated as 3 times of S/N ratio (peak to peak). To assess the repeatability of peak area, 50 µg/L standard samples were injected 6 times in succession and the %RSD was calculated. The correlation coefficients of calibration curves, LODs and %RSD of peak areas were as shown in Table 2.



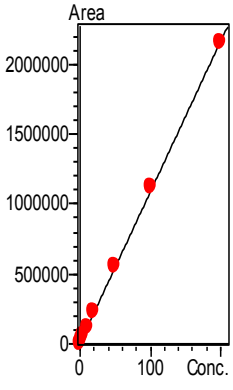
O,O,O-triethylphosphorothioate



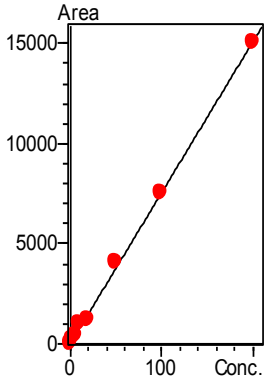
Dichlorvos



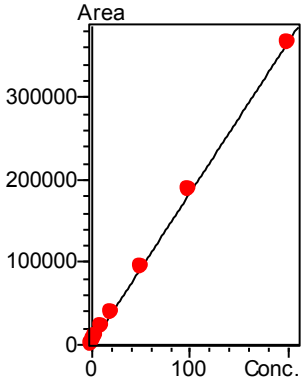
Mevinphos



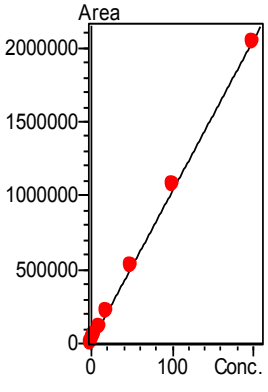
Isoprocarb



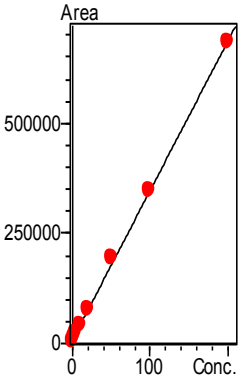
TEPP



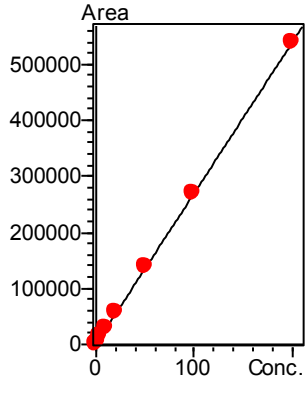
Thionazine



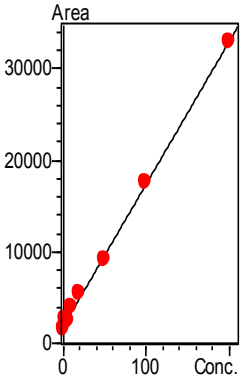
Fenobucarb



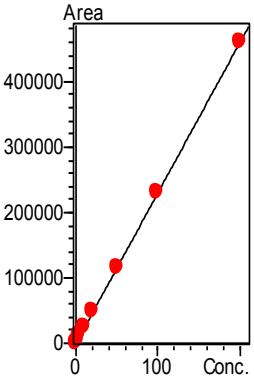
Ethoprophos



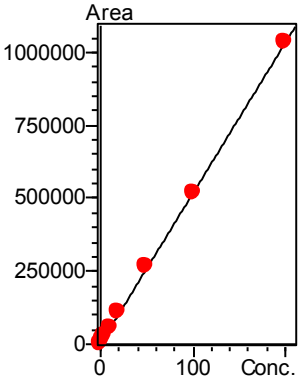
Sulfotep



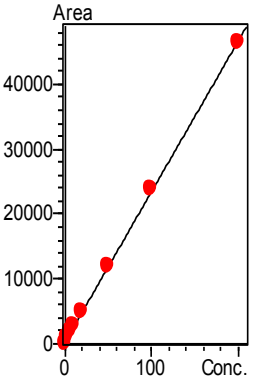
Monocrotophos



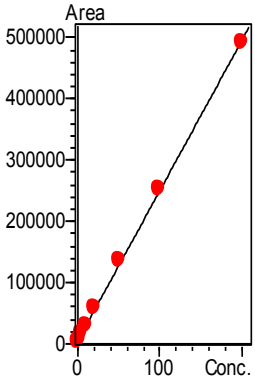
Phorate



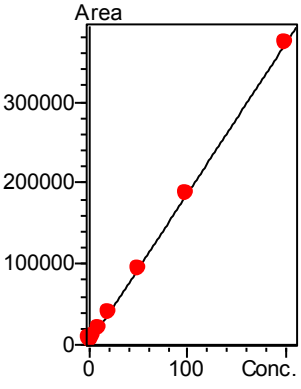
Alpha-HCH



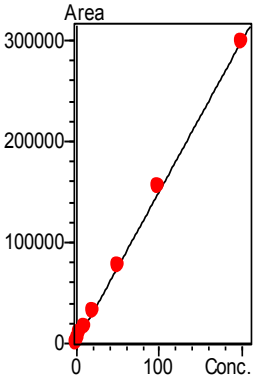
Demton S



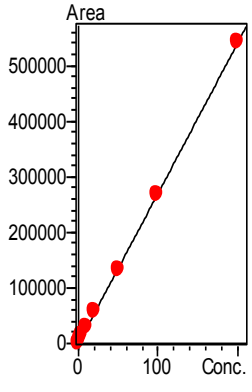
Carbofuran



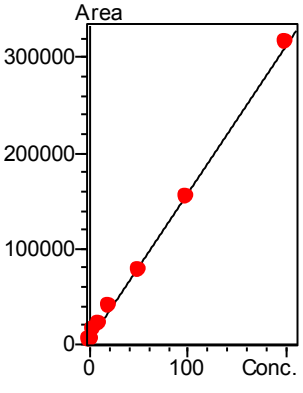
Simazine



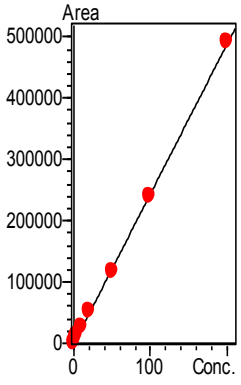
Atrazine



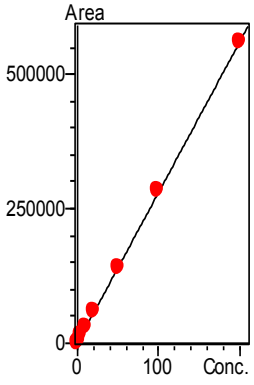
Beta-HCH



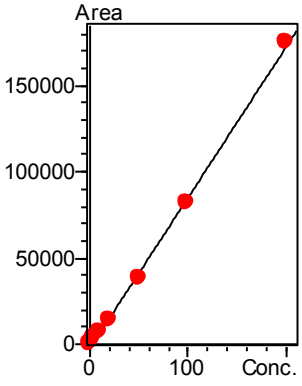
Propazine



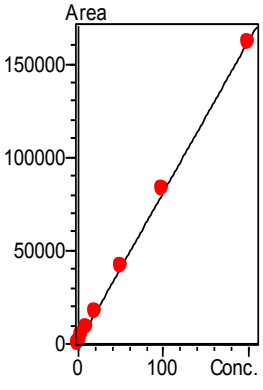
Gamma-HCH



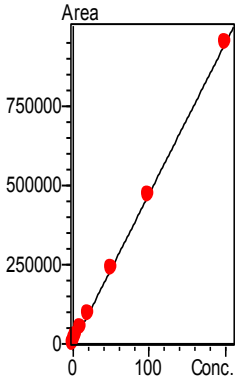
Diazinon



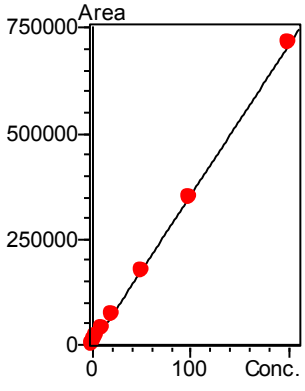
Chlorothalonil



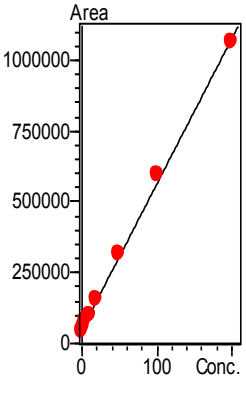
Disulfoton



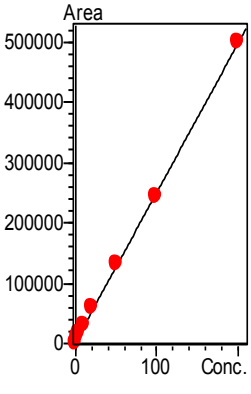
Delta-HCH



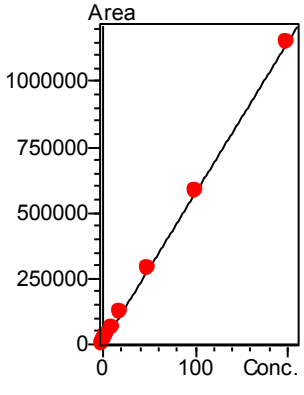
Methyl parathion



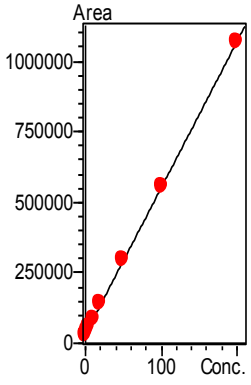
Carbaryl



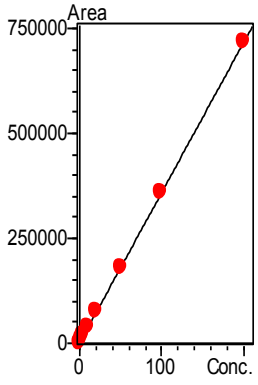
Heptachlor



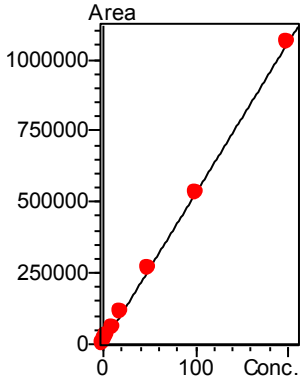
Fenchlorphos



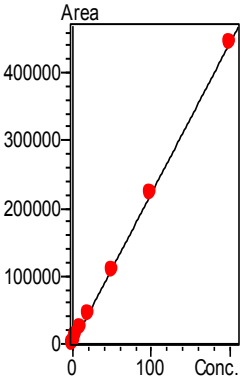
Malathion



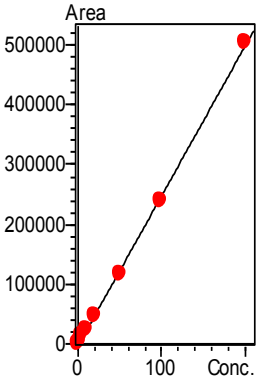
Chlorpyrifos



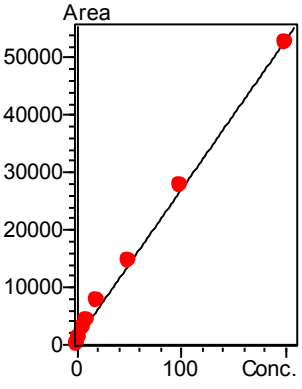
Fenthion



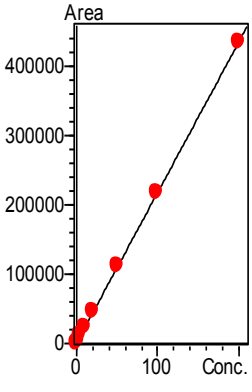
Aldrin



Parathion



Dicofol



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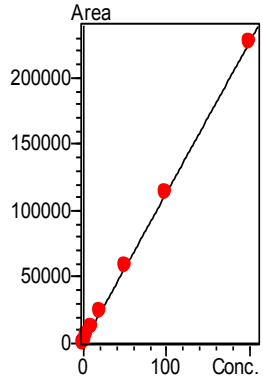

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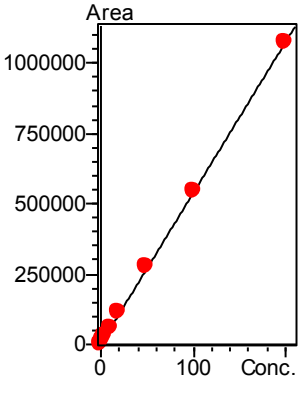

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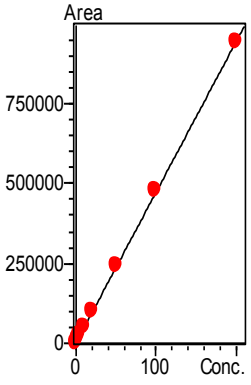

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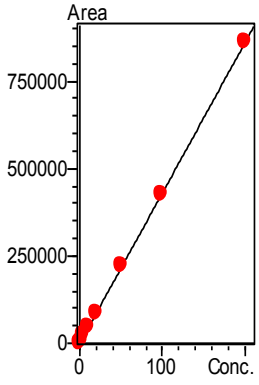

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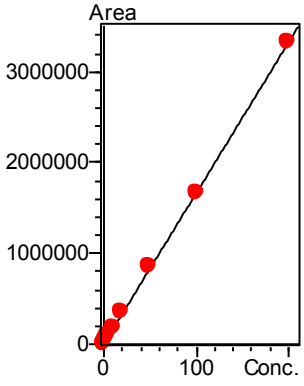

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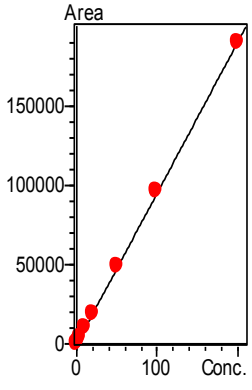
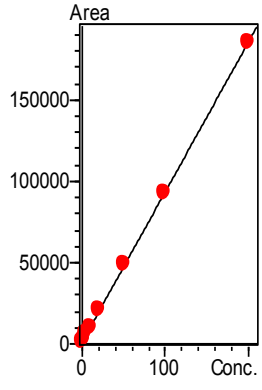


Dieldrin



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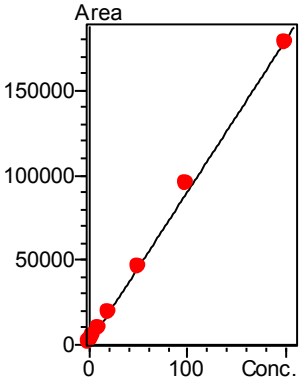

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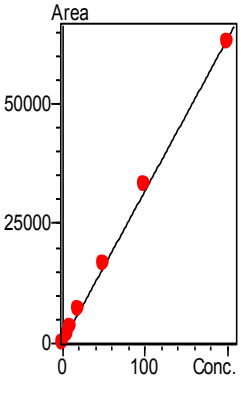
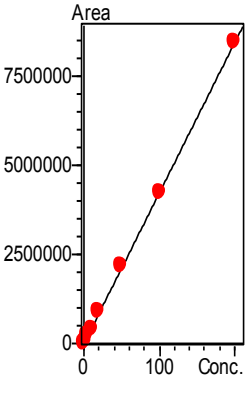
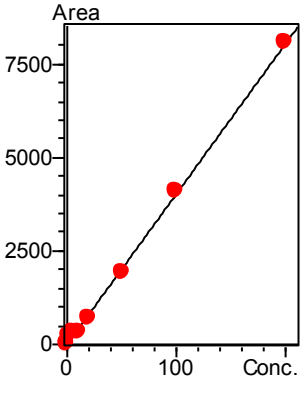


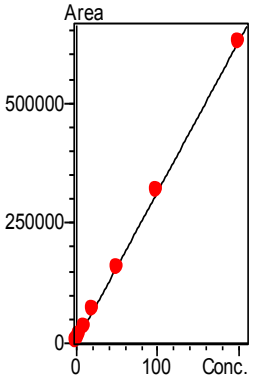
Beta-Endosulfan



p,p'-DDD



Endrin aldehydel



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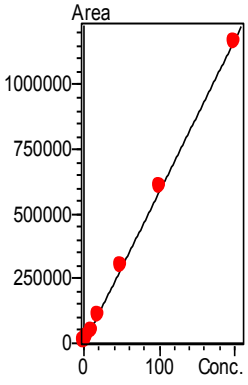

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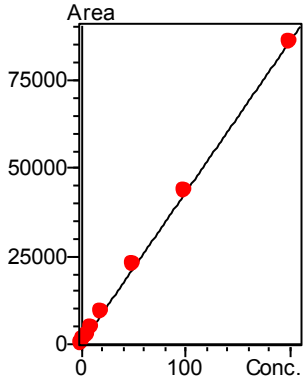
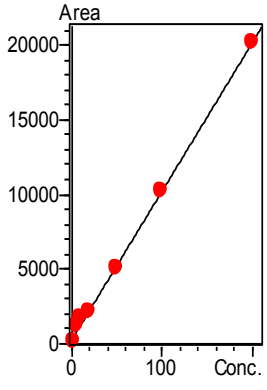


Endosulfan sulfate



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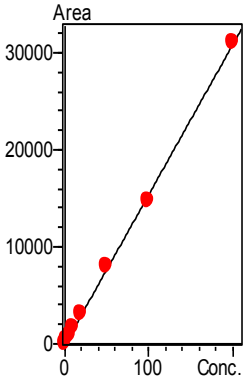
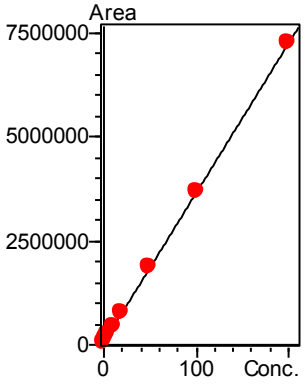


Endrin ketone



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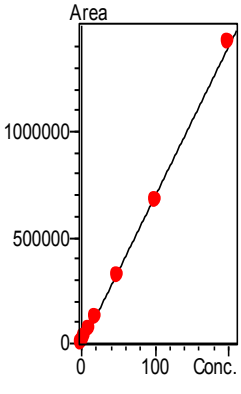

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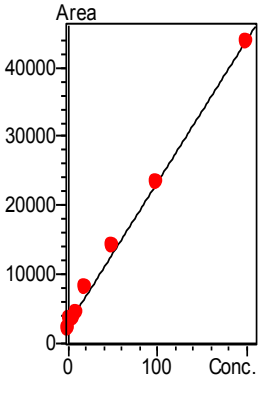
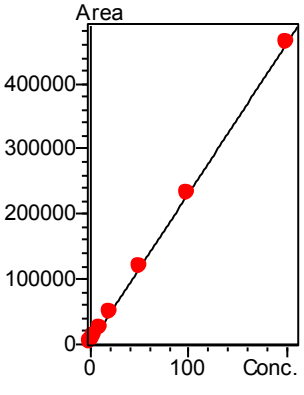
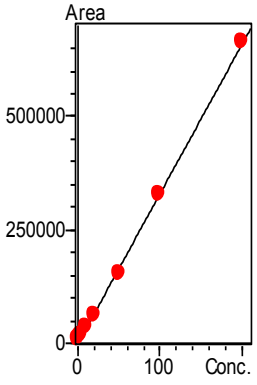


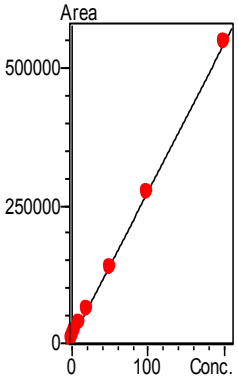
Methoxychlor



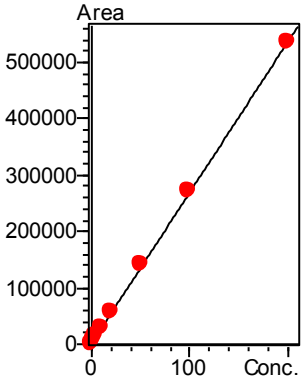
Fenpropathrin



Azinphos-methyl



Cyhalothrin



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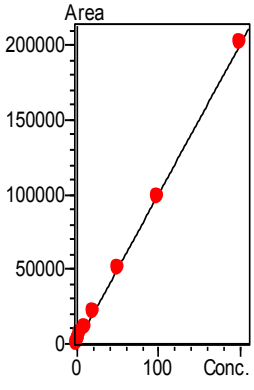
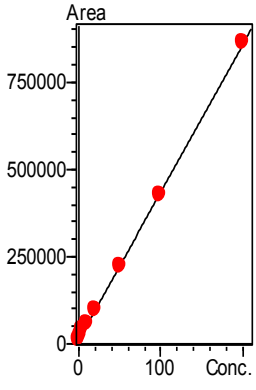
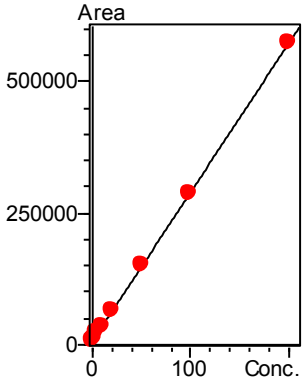


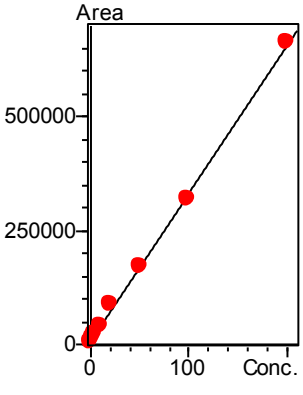
Cyflurthrin



Cypermethrin



Fenvalerate



Deltamethrin

Table 2. Correlation coefficients of calibration curves, LODs and RSD (%) of peak areas of pesticides

No.	Compound Name	Correlation Coefficient (R^2)	LOD (μg/kg)	%RSD (n=6)	No.	Compound Name	Correlation Coefficient (R^2)	LOD (μg/kg)	%RSD (n=6)
1	O,O,O-triethylphosphorothioate	0.9999	0.04	1.08	32	Parathion	0.9997	0.37	1.37
2	Dichlorvos	0.9999	0.08	1.98	33	Dicofol	0.9992	0.18	2.66
3	Mevinphos	0.9992	2.78	2.6	34	Heptachlor-exoepoxide	0.9999	0.04	1.94
4	Isoprocarb	0.9998	2.32	1.17	35	Trans-Chlordane	0.9999	0.07	1.28
5	TEPP	0.9994	4.29	2.28	36	Tetrachlorvinphos	0.9999	0.02	1.01
6	Thionazine	0.9999	0.41	1.57	37	Cis-Chlordane	0.9999	0.04	1.09
7	Fenobucarb	0.9998	1.29	0.98	38	Prothiofos	0.9999	0.06	1.2
8	Ethoprophos	0.9997	1.7	1.52	39	p,p'-DDE	0.9999	0.03	0.71
9	Sulfotep	0.9999	0.04	1.62	40	Dieldrin	0.9999	0.84	1.19
10	Monocrotophos	0.9993	12.77	3.14	41	Endrin	0.9999	1.72	2.5
11	Phorate	0.9999	0.05	0.83	42	Fensulfothion	0.9996	2.62	2.26
12	□Alpha-HCH	0.9999	0.02	1.46	43	Beta-Endosulfan	0.9996	1.91	2.56
13	Demton S	0.9999	2.52	2.26	44	p,p'-DDD	0.9999	0.03	2.1
14	Carbofuran	0.9997	0.97	2.22	45	Endrin aldehydel	0.9994	11.49	1.69
15	Simazine	0.9998	3.01	2.28	46	Sulprofos	0.9999	0.45	2.03
16	Atrazine	0.9998	0.69	1.46	47	Famphur	0.9997	2.23	2.05
17	□Beta-HCH	0.9999	0.07	2.02	48	Endosulfan sulfate	0.9999	0.24	2.68

18	Propazine	0.9995	1.47	2.42	49	p,p'-DDT	0.999	3.20	2.69
19	Gamma-HCH	0.9998	0.06	1.75	50	Endrin ketone	0.9996	0.57	1.37
20	Diazinone	0.9999	0.04	1.76	51	Bifenthrin	0.9999	0.15	2.36
21	Chlorothalonil	0.9992	0.18	2.21	52	EPN	0.9994	0.78	2.17
22	Disulfoton	0.9999	0.42	2.30	53	Methoxychlor	0.999	1.95	1.58
23	Delta-HCH	0.9999	0.04	1.95	54	Fenpropathrin	0.9999	0.35	1.04
24	Parathion-methyl	0.9999	0.62	2.20	55	Azinphos-methyl	0.9996	3.72	2.59
25	Carbaryl	0.9993	0.50	1.46	56	Cyhalothrin	0.9999	0.73	1.64
26	Heptachlor	0.9997	0.06	3.18	57	Coumaphos	0.9999	0.51	1.65
27	Fenchlorphos	0.9999	0.14	0.97	58	Cyfluthrin	0.9999	3,16	2.55
28	Malathion	0.9999	0.11	0.98	59	Cypermethrin	0.9999	2.97	0.98
29	Chlorpyrifos	0.9999	0.05	0.63	60	Fenvalerate	0.9999	0.30	1.35
30	Fenthion	0.9999	0.16	1.00	61	Deltamethrin	0.9993	2.61	1.30
31	Aldrin	0.9999	0.29	0.61					

Recovery test

Pesticide mixtures were spiked at concentrations of 10 µg/kg and 100 µg/kg respectively to tea matrix and 5 parallel samples were processed for each case. The resulted spike recoveries of tea matrices (average recoveries and %RSDs of 5 parallel samples) were as shown in Table 3.

Table 3 Spike recoveries of tea

No.	Compound Name	Spiked Amount 10 µg/kg		Spiked Amount 100 µg/kg	
		Average recovery (%)	RSD (%)	Average recovery (%)	RSD (%)
1	O,O,O-triethylphosphorothioate	106.06	2.75	92.37	2.68
2	Dichlorvos	84.94	5.63	88.52	4.00
3	Mevinphos	94.62	7.56	86.81	3.51
4	Isoprocarb	116.87	8.23	91.08	2.20
5	TEPP	54.23	9.14	79.85	4.78
6	Thionazine	100.35	7.18	88.09	2.16
7	Fenobucarb	107.60	8.25	88.62	1.36
8	Ethoprophos	78.72	6.45	91.32	5.47
9	Sulfotep	89.28	6.13	89.21	1.82
10	Monocrotophos	58.34	8.46	75.43	6.12
11	Phorate	105.53	5.89	88.35	1.47
12	Alpha-HCH	100.16	8.63	87.98	4.94
13	Demton S	97.95	8.03	86.01	3.54
14	Carbofuran	110.23	8.91	84.33	7.41
15	Simazine	77.22	8.75	84.18	2.24
16	Atrazine	94.14	9.62	85.97	1.46
17	Beta-HCH	76.21	6.15	86.80	4.12
18	Propazine	69,05	8.26	83.04	1.47
19	Gamma-HCH	78.23	4.12	80.58	3.11
20	Diazinone	102.40	5.82	88.13	1.77
21	Chlorothalonil	83.24	6.36	85.64	3.60
22	Disulfoton	100.87	8.10	88.49	2.28

23	Delta-HCH	105.50	7.15	88.25	2.32
24	Parathion-methyl	88.15	6.75	88.51	1.84
25	Carbaryl	59.74	9.18	81.82	2.68
26	Heptachlor	81.24	7.98	89.23	4.98
27	Fenchlorphos	103.14	4.77	86.89	1.70
28	Malathion	125.32	9.27	88.70	3.94
29	Chlorpyrifos	104.94	6.76	87.59	1.68
30	Fenthion	101.70	6.72	87.80	1.85
31	Aldrin	102.49	6.76	88.81	1.90
32	Parathion	100.86	6.96	88.96	2.38
33	Dicofol	73.12	7.26	86.44	4.17
34	Heptachlor-exoepoxide	97.40	8.95	87.92	2.26
35	Trans-Chlordane	104.40	6.04	91.72	3.42
36	Tetrachlorvinphos	105.06	6.83	91.42	3.76
37	Cis-Chlordane	87.20	8.23	91.26	6.60
38	Prothiofos	86.92	8.29	89.70	4.01
39	p,p'-DDE	84.57	9.18	90.22	4.74
40	Dieldrin	83.28	7.13	90.01	3.85
41	Endrin	74.89	8,96	84.47	4.90
42	Fensulfothion	87.23	7.16	80.75	2.38
43	β- Endosulfan	72.38	6.28	83.25	3.90
44	p,p'-DDD	82.34	5.78	86.60	3.08
45	Endrin aldehydel	68.43	8.26	89.12	3.49
46	Sulprofos	88.13	7.24	86.78	2.19
47	Famphur	122.04	8.93	90.62	2.86
48	Endosulfan sulfate	71.59	8.34	83.43	5.01
49	p,p'-DDT	70.98	7.82	81.95	4.32

50	Endrin ketone	74.23	9.75	87.24	7.21
51	Bifenthrin	95.36	9.88	87.28	1.62
52	EPN	103.56	7.76	88.22	2.55
53	Methoxychlor	72.57	6.93	86.80	2.47
54	Fenpropathrin	84.96	8.17	88.45	2.10
55	Azinphos-methyl	70.14	8.97	100.38	7.24
56	Cyhalothrin	86.12	7.34	90.23	6.96
57	Coumaphos	108.65	9.27	87.12	2.24
58	Cyfluthrin	107.63	7.28	86.73	2.74
59	Cypermethrin	108.76	8.15	91.52	4.77
60	Fenvalerate	102.66	7.28	88.52	2.96
61	Deltamethrin	81.52	9.36	91.57	3.76

CONCLUSION

A method was developed for the qualitative and quantitative analysis of 61 pesticides in tea using GCMS-TQ8030 and QuEChERS pretreatment method. The method has the advantages of simple pretreatment operation, satisfactory repeatability and high sensitivity. For most pesticides, the method can achieve a recovery between 70.0% and 110.0% at the spiked concentration of 10 µg/kg and 100 µg/kg. Therefore, it can be used for routine analysis of trace pesticide residues satisfactorily. The experiment demonstrated that tandem mass spectrometry could avoid the interference of matrix and reducing false positive detection rate with improved selectivity and detection sensitivity. Simplified sample pretreatment process, and reduced analysis cost, especially when analyzing complex samples like tea gives additional advantage.

C-10 Determination of Benzopyrene in Instant Noodle with GCMS-TQ8030

INTRODUCTION

In this paper, an analytical method was established for the determination of benzopyrene in instant noodle with GC-MS/MS. The proposed method is of good linearity in the concentration range of 1~100 μg/L with a correlation coefficient of 0.9999 for benzo[*a*]pyrene. The %RSD of peak area for 10 successive injections of 1 μg/L standard solution was 2.85%.It provided a spike recovery between 95% - 119% at the spike concentrations of 1 μg/kg and 10 μg/kg with an LOD of 0.04 μg/kg, fully meeting the requirements for daily detection of benzopyrene in instant noodle.

Benzopyrene, also referred to as benzo[*a*]pyrene(B[*a*]P), is a carcinogen, teratogen and mutagen that irritates the eyes and the skin. Studies have shown that benzopyrene is the most poisonous carcinogen among polycylic aromatic hydrocarbons (PAHs) that is capable of inducing pulmonary carcinoma, hepatic carcinoma, and gastro-intestinal cancers. Therefore, it is classified as a Category 1 carcinogen.

In China, it is stipulated in national standard GB 2762-2005 *Maximum levels of contaminants in foods* that the MRLs of benzo[*a*]pyrene are 5 μg/kg in grains, 10 μg/kg in edible oil, and 5 μg/kg in smoked meat. In EU (EC) No. 1881/2006, the MRLs stipulated for benzo[*a*]pyrene are 2 μg/kg in edible oil and 5 μg/kg in bacon and bacon products.

The fat, cholesterol, protein, and carbohydrates in foods may undergo pyrolysis when we smoke, bake and fry foods, yielding pyrolytic products which, after cyclization reaction and polymerization, may be converted to benzopyrene and other PAHs. It has provoked much concern in the society that benzopyrene was detected in products of a well-known instant noodle brand recently.

In this paper, benzopyrene in instant noodle was extracted with n-hexane, enriched, and purified in a C_{18} solid-phase extraction column. The proposed method is of good repeatability and can provide a spike recovery between 95%-119% at spike concentrations of 1 μg/kg and 10 μg/kg. The experiment results

showed that the MRM feature of tandem mass spectrometer can effectively reduce matrix interference and improve detection sensitivity.

Apparatus

GC-MS/MS: GCMS-TQ8030

Conditions of Analysis

Column	: Rxi-5Sil MS, 30 m × 0.25 mm × 0.25 μm
Injector temperature	: 280 °C
Injection mode	: Splitless injection
Column temperature program	: 60 °C (1 min)→@20 °C/min→200 °C (1 min)→ @10°C/min →310 °C (10 min)
CLV	: 40 cm/sec
Injection volume	: 1 μL
Ionization mode	: EI
Temperature of ion source	: 230 °C
Temperature of GC-MS interface	: 280 °C
Solvent dwell time	: 4 min
Collection mode	: MRM are listed in Table 1

Sample Preparation

Instant noodle cakes were pulverized with a grinder, sampled and accurately weighed 2 g, added 40 mL n-hexane, mixed for 2 min in a homogenizer, then subject to ultrasonic extraction for 30 min and high speed centrifugation (5000 rpm) for 10 min; after this, 20 mL supernatant was transferred with a graduated cylinder for purification.

The C_{18} solid-phase extraction column was activated with 10 mL n-hexane. The above-mentioned extract liquor was loaded to the C_{18} column, and eluted with 20 mL n-hexane. The eluent was collected and concentrated to 1 mL, then transferred to a injection vial, added internal standard (perylene-d12), and then loaded for analysis.

RESULTS AND DISCUSSION

Chromatogram

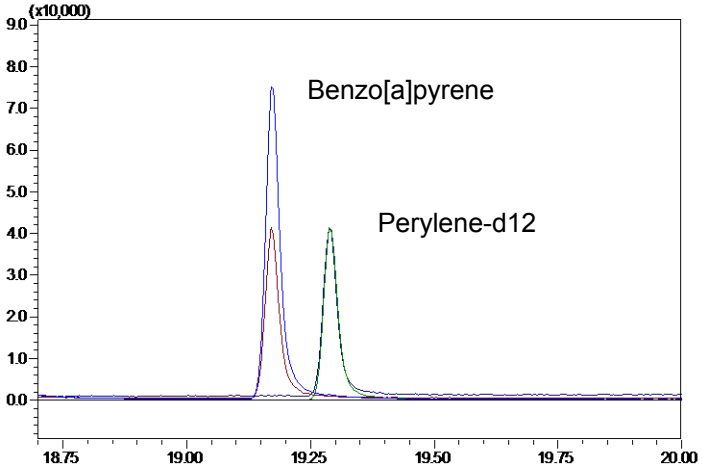


Fig. 1 MRM chromatograms of standard solutions of benzo[*a*]pyrene (50 µg/L)

Table 1 Retention times, names and monitor ions

No.	Retention Time	Compound Name	Quantitative Ion (CE)	Qualitative Ion (CE)
1	19.170	Benzo[*a*]pyrene	252>250 (34)	252>226 (20)
2	19.290	Perylene-d12	264>260 (40)	264>236 (25)

Linear range

Standard solutions of benzo[*a*]pyrene were prepared at concentrations of 1, 5, 10, 20, 50, 100 µg/L with a solution containing 100 µg/L perylene-d12 internal standard. MRM mode was used for data acquisition. The calibration curve was as shown in Fig. 2, with a correlation coefficient R^2=0.9999.

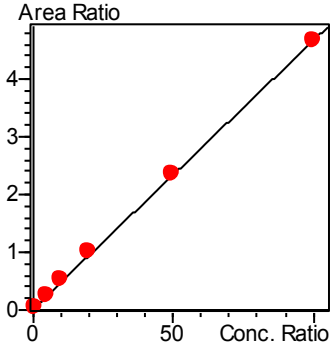


Fig. 2 Calibration curve of benzo[*a*]pyrene

Repeatability test

Ten replicate injections were performed using 1 µg/L benzo[*a*]pyrene standard solution. The results, as shown in Table 2, indicated that the proposed method is of good repeatability.

Table 2 Repeatability test of benzo[*a*]pyrene

	1	2	3	4	5	6	7	8	9	10	%RSD
Area Ratio	0.0453	0.0448	0.0442	0.0430	0.0417	0.0439	0.0416	0.0437	0.0429	0.0424	2.854
Retention Time	19.178	19.183	19.177	19.182	19.178	19.182	19.178	19.174	19.176	19.177	0.015

Recovery test

Benzopyrene standard solution was spiked into 2 g samples at concentrations of 1 μg/kg and 10 μg/kg, respectively. 3 parallel samples were processed and obtained recoveries are between 95% - 119% as shown in Table 3. The proposed method's LOD calculated was 0.04 μg/kg.

Table 3 Spike recovery and repeatability (n=3)

Spiked Amount (μg/kg)	Recovery (%)			Mean (%)	RSD (%)
	1	2	3		
1	95.07	95.89	99.85	96.94	2.64
10	110.50	118.57	115.52	114.86	3.55

Sample analysis

Samples of instant noodle products of a certain brand were analyzed and the results were as shown in Table 4.

Table 4 Assay results of samples (BDL: Below Detection Limit)

Compound	Assay results (μg/kg)		Mean (μg/kg)
	1	2	
Benzo-pyrene	BDL	BDL	BDL

CONCLUSION

The method proposed in this paper for analysis of benzopyrene in instant noodle with Shimadzu GCMS-TQ8030 is easy to operate and of satisfactory repeatability. It provides a spike recovery between 95% - 119% at spike concentrations of 1 μg/kg and 10 μg/kg with an LOD of 0.04 μg/kg. The application of tandem mass spectrometer and MRM mode can effectively reduce matrix interference and improve sensitivity of the method and reliability of detection results.

C-11 Determination of Pesticide Residues in Drinking Water with GC-MS/MS

INTRODUCTION

In this paper, a method was proposed for determination of 19 organophosphorus/organochlorine pesticides in drinking water by means of dichloromethane extraction and gas chromatography-triple quadrupole mass spectrometry. Calibration curves of good linearity in concentration range of 1~50 µg/L were plotted, all with a correlation coefficient greater than 0.996. The %RSDs of peak areas in 5 successive injections were better than 6.5% and the LODs were 0.02~1.67 µg/L. Recoveries of spiked samples are in the range of 70% ~ 100% at the two spiked levels of 1 µg/L and 5 µg/L. This method is suitable and reliable for fast determination of organophosphorus and organochlorine pesticides in drinking water.

Water is the essential part of life therefore the issue of drinking water safety is of vital health parameter. Organophosphorus and organochlorine pesticides have been widely used in agricultural production because of their great variety and extensive applications. But in the meantime, their residues have also become a major source of contaminants in water, especially in drinking water through ground water streams. Therefore, the detection of pesticide residues in drinking water is of considerable significance in assessment of drinking water quality.

The proposed method in this paper used dichlormethane to extract organophosphorus and organochlorine pesticides from drinking water. The method has, by adopting multiple reaction monitoring (MRM) mode to effectively reduce background interference, which is commonly seen in quantitative analysis with single quadrupole instrument, thereby enhancing analysis sensitivity. For most pesticides, the method provides LODs lower than 0.09 µg/L with good

reproducibility and recoveries greater than 70% for all spiked samples were observed. The results demonstrated that the method meets the regulatory requirements for monitoring pesticide residues in drinking water.

EXPERIMENTS

Apparatus

GC-MS/MS: GCMS-TQ8030

Conditions of Analysis

GC-MS/MS parameters:

Column	: Rxi-5Sil MS, 30 m × 0.25 mm × 0.2 5µm
Injector temperature	: 250 °C
Injection mode	: Splitless injection
Carrier gas control mode	: Constant Linear Velocity
Column flow	: 1.69 mL/min
Column temperature program	: 50 °C (1min)→@25 °C/min→125 °C→ @10 °C/min→300 °C(8 min)
Interface temperature	: 250 °C
Temperature of ion source	: 200 °C
Detector voltage	: Tuning voltage+0.3 kV
Solvent cut time	: 2 min
MRM conditions	: see Table 1

Sample Preparation

Pretreatment of samples was as shown in Fig. 1 below.

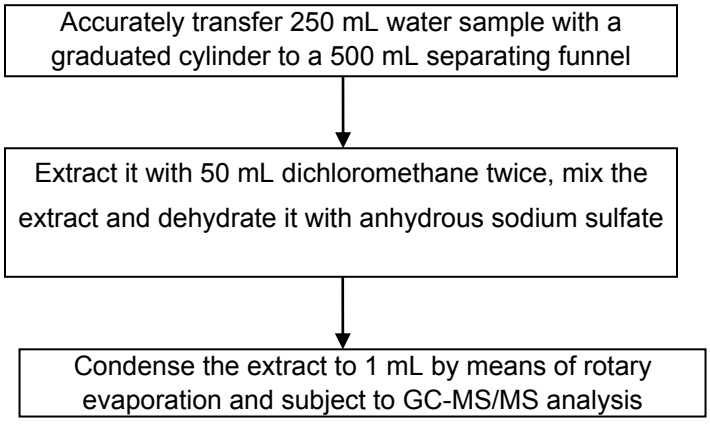


Fig. 1 Sample pretreatment flow chart

Table 1 Retention time and MRM parameters of pesticides

No.	Retention Time	Names of Pesticide	Quantitative Ion (CE)	Qualitative Ion (CE)
1	5.955	Dichlorvos	185>93 (14)	185>109 (14)
2	10.814	alpha-HCH	219>183 (8)	219>145 (18)
3	10.908	Hexachlorobenzene	284>249 (24)	284>214 (28)
4	11.031	Dimethoate	125>79 (8)	125>47 (14)
5	11.332	beta-HCH	219>183 (8)	219>147 (20)
6	11.496	Lindane	219>183 (8)	219>147 (20)
7	12.056	delta-HCH	219>183 (10)	219>145 (22)
8	11.860	Chlorothalonil	266>231 (14)	266>168 (22)
9	12.764	Parathion-methyl	263>109 (14)	263>136 (8)
10	12.962	Heptachlor	272>237 (20)	272>117 (32)
11	13.422	Malathion	173>127 (6)	173>99 (14)

12	13.583	Chlorpyrifos	314>258 (14)	314>286 (8)
13	13.820	Parathion	291>109 (14)	291>137 (6)
14	14.919	o,p'-DDE	246>176 (30)	246>211 (22)
15	15.538	p,p'-DDE	246>176 (28)	246>211 (22)
16	15.664	p,p'-DDD	235>165 (24)	235>199 (14)
17	16.330	o,p'-DDT	235>165 (24)	235>199 (16)
18	17.036	p,p'-DDT	235>165 (22)	235>199 (14)
19	21.945, 22.223	Deltamethrin	253>93 (18)	253>172 (4)

RESULTS

Chromatogram of standard sample

MRM chromatograms of 19 pesticide standard are shown in Fig. 2.

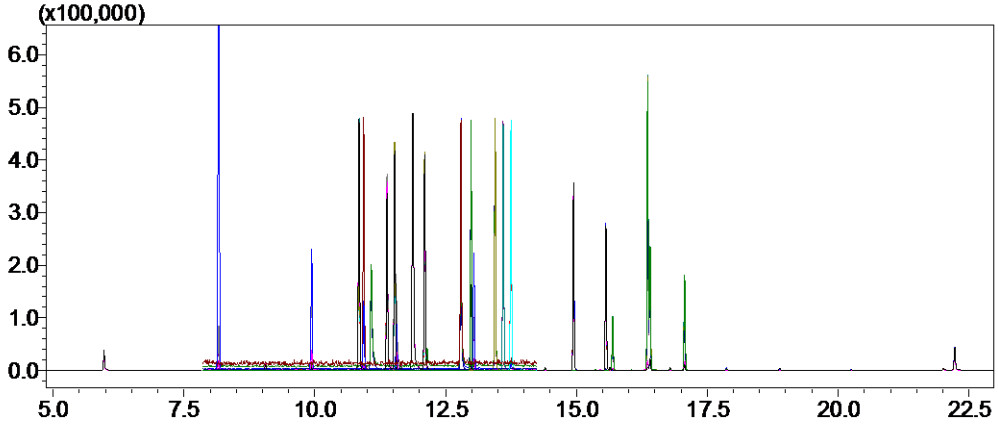
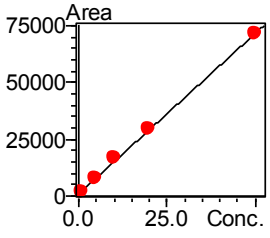


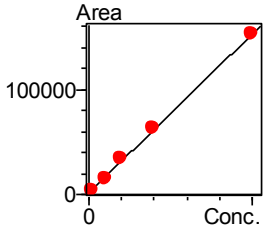
Fig. 2. MRM chromatogram of standard pesticides (50 µg/L)

Calibration curve

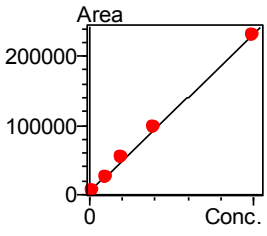
A series of solutions of mixed pesticide standard substances was prepared at concentrations of 1, 5, 10, 20, 50 µg/L using n-hexane as solvent. Calibration curves of the pesticides are as shown below. Correlation coefficients and LODs are listed in Table 2.



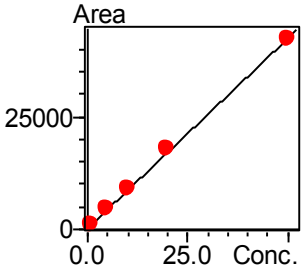
Dichlorvos



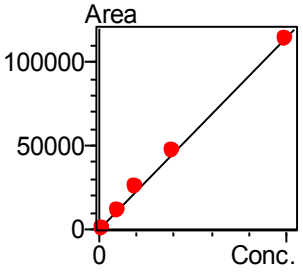
alpha-HCH



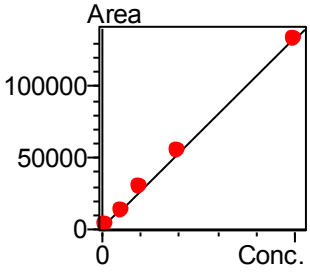
Hexachlorobenzene



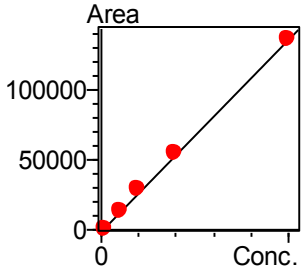
Dimethoate



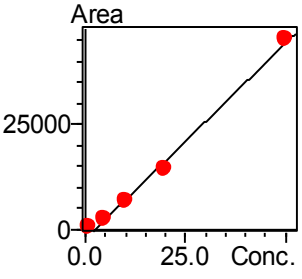
beta-HCH



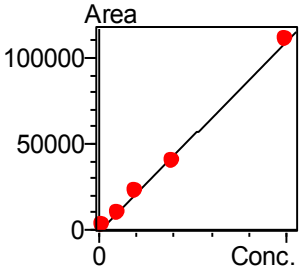
Lindane



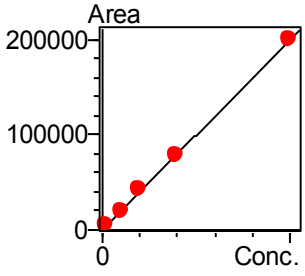
delta-HCH



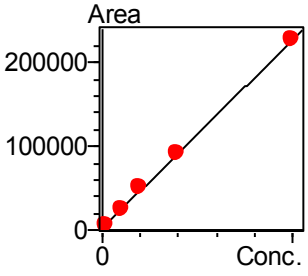
Chlorothalonil



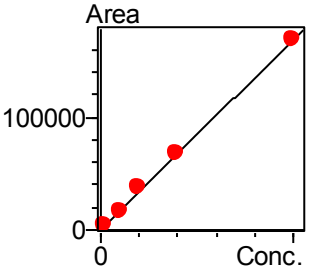
Parathion-methyl



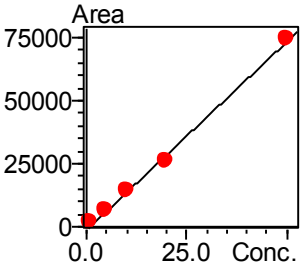
Heptachlor



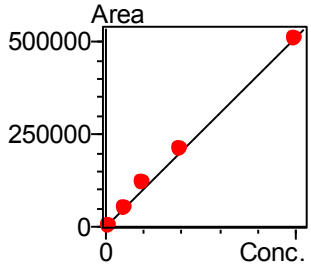
Malathion



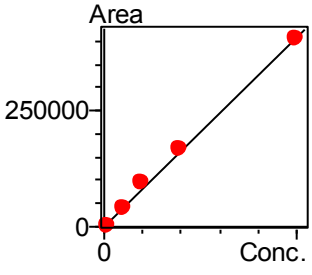
Chlorpyrifos



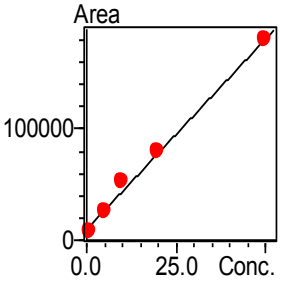
Parathion



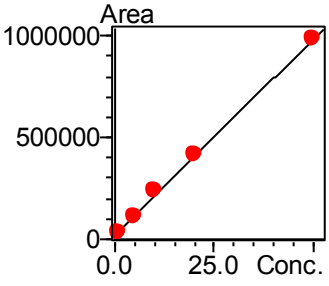
o,p'-DDE



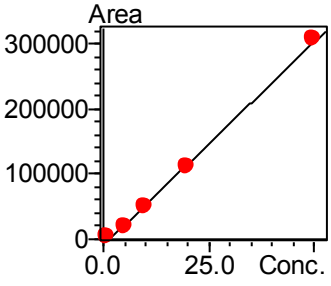
p,p'-DDE



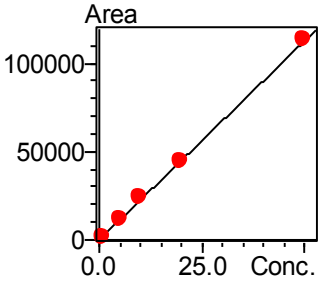
p,p'-DDD



o,p'-DDT



p,p'-DDT



Deltamethrin

LOD and repeatability

Repeatability was assessed by 5 replicate injections of standard mixture (1 µg/L). The %RSDs of peak areas in 5 successive injections were less than 6.5% and LODs were calculated based on the data obtained in the experiment.The %RSDs of peak areas and LODs of pesticides are shown in Table 2.

Table 2. Calibration curve, LOD and repeatability

No.	Compound Name	Correlation Coefficient	LOD (µg/L)	% RSD	No.	Compound Name	Correlation Coefficient	LOD (µg/L)	% RSD
1	Dichlorvos	0.9995	0.06	3.20	11	Malathion	0.9996	0.06	2.94
2	alpha-HCH	0.9996	0.02	3.80	12	Chlorpyrifos	0.9997	0.04	5.72
3	Hexachlorobenzene	0.9993	0.15	3.29	13	Parathion	0.9985	0.12	5.62
4	Dimethoate	0.9997	1.67	6.45	14	o,p'-DDE	0.9995	0.02	1.26
5	beta-HCH	0.9992	0.02	3.42	15	p,p'-DDE	0.9990	0.01	2.12
6	Lindane	0.9996	0.02	3.45	16	p,p'-DDD	0.9970	0.04	2.49
7	delta-HCH	0.9965	0.09	4.05	17	o,p'-DDT	0.9994	0.02	5.63
8	Chlorothalonil	0.9972	0.21	5.64	18	p,p'-DDT	0.9992	0.09	4.44
9	Parathion-methyl	0.9990	0.49	3.25	19	Deltamethrin	0.9998	0.45	3.56
10	Heptachlor	0.9996	0.02	2.43					

Recovery test

Pesticide mixture was spiked to two water samples, which were prepared in accordance with the sample pretreatment procedures, at spike concentrations of 1 µg/L and 5 µg/L, respectively. Three spiked samples were prepared in parallel for each concentration. Results of the recovery test are shown in Table 3.

Table 3 Recoveries of spiked samples

No.	Compound Name	Spiked sample (1 µg/L)		Spiked sample (5 µg/L)	
		Recovery (%)	%RSD	Recovery	%RSD
1	Dichlorvos	94.73	5.40	92.32	5.03
2	alpha-HCH	90.41	6.68	91.03	5.94
3	Hexachlorobenzene	95.00	4.07	90.60	6.26
4	Dimethoate	77.11	6.48	72.97	6.63
5	beta-HCH	70.36	7.91	86.95	8.54
6	Lindane	92.36	5.82	90.86	6.71
7	delta-HCH	73.17	6.78	93.04	5.75
8	Chlorothalonil	87.22	7.01	86.28	7.97
9	Parathion-methyl	90.61	4.93	78.51	8.65
10	Heptachlor	92.76	3.75	90.02	6.01
11	Malathion	95.65	2.99	91.03	5.03
12	Chlorpyrifos	90.81	8.60	92.16	6.88
13	Parathion	90.88	4.81	95.11	5.67
14	o,p'-DDE	97.18	1.45	89.18	6.16
15	p,p'-DDE	94.54	2.86	91.17	5.93
16	p,p'-DDD	95.82	2.12	90.53	1.72
17	o,p'-DDT	95.19	2.68	88.83	5.22
18	p,p'-DDT	92.34	7.73	86.63	3.84
19	Deltamethrin	86.00	6.13	96.45	4.83

CONCLUSION

A method was evaluated for the analysis of organophosphorus and organochlorine pesticide residues in drinking water using Shimadzu GCMS-TQ8030. The method is easy to operate and of good linearity with correlation coefficients greater than 0.996 for most pesticides within the concentration range of 1~50 µg/L. For most pesticides spiked at concentrations of 1 and 5 µg/L, the method's recoveries of spiked samples are in the range of 70~100%,

suggesting that it can meet the requirements for detection of organophosphorus and organochlorine pesticide residues in drinking water.

SHIMADZU
Excellence in Science

C-12 Higher Sensitivity Analysis of 2-Methoxy-3-Isobutylpyrazine (MIBP) in Wine Using GC-MS/MS(PCI)

INTRODUCTION

2-Methoxy-3- isobutylpyrazine (MIBP) is an aromatic substance with the fragrance of bell peppers. It is found in sauvignon blanc (a type of grape used for white wine) and cabernet sauvignon (a type of grape used for red wine), and gives the wines a favorable aroma. MIBP, which has a significant impact on the flavor of wine, has an extremely low threshold value in sensory tests, on the order of a few ng/L. Since wine contains many components, concentration and selective separation & detection are essential to analysis.

The trace amounts of MIBP in wine were selectively detected by utilizing the MonoTrap® silica monolithic absorbent for collection and concentration, and a GC/MS/MS (GCMS-TQ8030) in positive chemical ionization (PCI) mode. The MRM acquisition mode was used to monitor specific transitions for the compound of interest.

EXPERIMENTAL

A standard MIBP solution was added to commercially-available sauvignon blanc (produced in Chile in 2012) and cabernet sauvignon (produced in Chile in 2012) at different concentrations (0 ng/L, 1 ng/L, 5 ng/L, 10 ng/L, and 20 ng/L). The samples were heated for 1 hour at 50 °C, and then the gaseous phase MIBP was collected using MonoTrap® RGPS TDNote) (GL Sciences, P/N: 1050-74202). After collection, the MonoTrap® RGPS TD was measured with the analysis conditions shown in Table 1.

Note: MonoTrap® RGPS TD: This is formed by applying a polydimethylsiloxane coating to a silica substrate, and applying end caps, with graphite carbon added to the absorbent.

Table 1: Analysis Conditions

Autosampler:	AOC-5000 Plus
OPTIC Liner Auto Exchange System:	CDC + LINEX
Multipurpose Injection Port:	OPTIC-4
GC-MS:	GCMS-TQ8030
Column:	InertCap 17MS (Length: 30 m, 0.25 mm I.D., df=0.25 μm), (GL Sciences, P/N: 1010-20142)

[OPTIC-4]	
Initial Temp.:	35 °C
Ramp Rate:	10 °C/sec
Hold Temp.:	250 °C
Hold Time:	300 sec
Column Flow1:	5 mL/min
Column Flow Time:	320 sec
Column Flow2:	1.5 mL/min
Split Flow1:	5 mL/min
Split Flow Time:	320 sec
Split Flow2:	50 mL/min

[GC]	
Column Oven Temp.:	40 °C (5 min) → (10 °C/min) → 280 °C (5 min)

[MS]	
Ion Source Temp.:	200 °C
Interface Temp.:	250 °C
Ionization Method:	Positive chemical ionization (PCI)
Reagent Gas:	Isobutane (60 kPa)
Acquisition Mode:	MRM
Event Time:	0.3 sec
Monitor Ion and Collision Energy (CE):	*m/z* 167.1 > 124.1 (20 V)
	m/z 167.1 > 135.1 (15 V)

RESULTS

Fig. 1 shows the calibrations curves for sauvignon blanc and cabernet sauvignon via the standard addition method. Favorable results were obtained for the correlation coefficient (R) between area and concentration for each MIBP-spiked sample, with 0.9999 for the sauvignon blanc and 0.9998 for the cabernet sauvignon.
Fig. 2 shows the MRM chromatograms for the MIBP in the wines. Table 2 shows the results of quantitatively analyzing each wine 3 times via the standard addition method and the repeatability. The respective concentrations of MIBP in the wines were 5.4 ng/L for the sauvignon blanc and 12.1 ng/L for the cabernet sauvignon. In addition, favorable results of 3 % RSD were obtained for the repeatability.

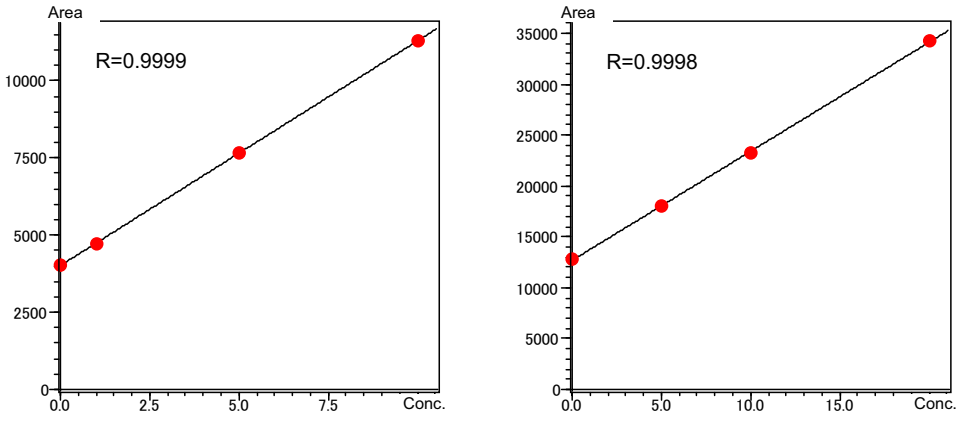


Fig. 1: Calibration Curves for the Wines via the Standard Addition Method

Left: Sauvignon Blanc (Concentrations of 0 ng/L, 1 ng/L, 5 ng/L, and 10 ng/L)
Right: Cabernet Sauvignon (Concentrations of 0 ng/L, 5 ng/L, 10 ng/L, and 20 ng/L)

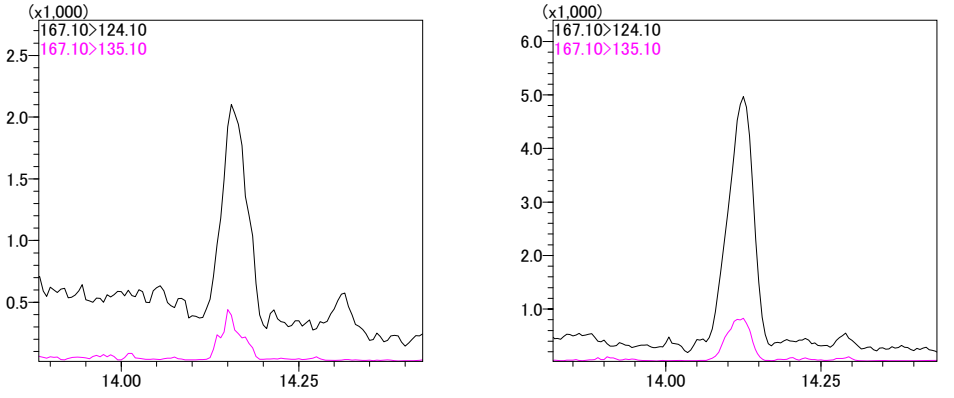


Fig. 2: MRM Chromatograms for the MIBP in the Wines (Left: Sauvignon Blanc, Right: Cabernet Sauvignon)

Table 2: Quantitative Results for the MIBP in the Wines via the Standard Addition Method (Concentration Units: ng/L), and the Repeatability (n=3)

Wine Type	1	2	3	Average	Standard Deviation	C.V. (%)
Sauvignon Blanc	5.5	5.3	5.3	5.4	0.1	2.47
Cabernet Sauvignon	11.8	12.3	12.1	12.1	0.2	1.91

CONCLUSION

The trace quantities of MIBP in the wines were collected and concentrated by the MonoTrap® RGPS TD, and then selectively detected by utilizing the GC-MS/MS in PCI mode with MRM acquisition mode. It was thus possible to detect MIBP at the ng/L level with high sensitivity.

C-13 Determination of 20 Phthalates in Beverage with GC-MS/MS

INTRODUCTION

This paper describes a method for determination of 20 phthalates in beverage with Shimadzu GCMS-TQ8030. Target compounds were DMP, DEP, DIPP, DALP DPRP, DIBP, DBP, DMOEP DMPP DEOEP, DAP, DHXP, BBP, DBOEP DCHP, DEHP, DPP, DNOP, DINP, DIDP. In the method, samples were extracted with n-hexane and then subjected to analysis with GC-MS/MS. The method is simple, convenient and capable of fast and accurate qualitative and quantitative analysis of phthalates with high sensitivity. For DINP and DIDP, the LOD was 10.0 µg/L; for the rest compounds, the LOD were all below 1.0 µg/L.

Phthalates (PAEs) are a group of plastic modifiers widely used in plastics, pesticides, coating materials, cosmetics, food packages, and other products. Now a days, PAEs have already become a major environmental pollutants.

Methods available for detection of PAEs in recent years mainly include HPLC, GC/MS, and LC/MS. However, seldom reported are GC-MS/MS based methods which, when used in MRM mode, can effectively eliminate matrix interference and improve instrument selectivity and sensitivity.

In this paper, a method was proposed in reference with a China standard, GB/T 21911-2008 *Determination of phthalate esters in foods*. In the method, samples were extracted with appropriate amount of n-hexane and the extract was subjected to centrifugation and allowed to settle, then the supernatant was removed and subjected to analysis with GC-MS/MS for determination of 20 phthalates in beverage.

EXPERIMENTAL

Apparatus

GC-MS/MS: GCMS-TQ8030

Conditions of Analysis

Column	: InterCap 5MS/NP, 30 m × 0.25 mm × 0.25 µm
Column temperature program	: 90 °C (1 min)→@15 °C/min→210 °C (2 min) →@5 °C/min→240 °C (5 min)→@5 °C/min →250 °C(5 min)→@25 °C/min→300 °C (4 min)
CID gas	: Argon
Injector temperature	: 250 °C
Injection volume	: 1 µL
Injection mode	: Splitless, 1 min
Control mode	: CLV
Linear velocity	: 37 cm/sec
Detector voltage	: Tuning voltage+0.3 kV
Interface temperature	: 280 °C
Temperature of ion source	: 230 °C
Operation mode	: MRM

Sample Preparation

Evenly mixed liquid beverage was precisely pipetted 5 mL and transferred to a centrifuge tube, added 2 mL n-hexane with a transfer pipette. The centrifuge tube was then capped and subjected to agitation for 2 min in a vortex mixer followed by centrifugalization and stratification. The supernatant then transferred to a sampler vial and subjected to GC-MS/MS analysis.

RESULTS AND DISCUSSION

MRM Chromatogram

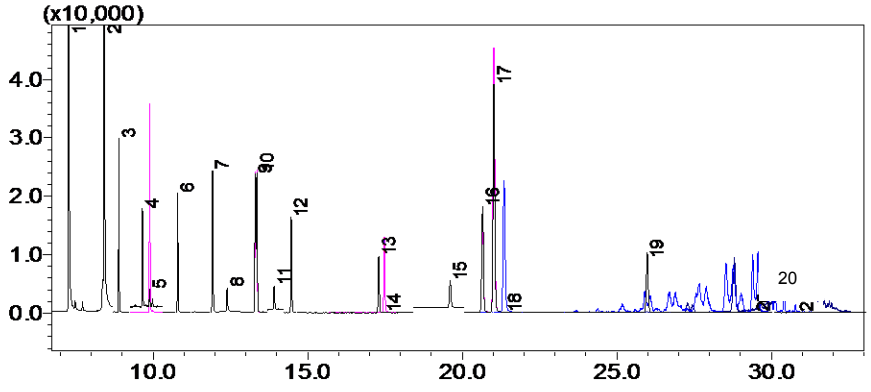


Fig. 2 MRM chromatograms of multi-standard solution of phthalates (10 µg/L)

Table 1 Retention time and MRM parameters of phthalates

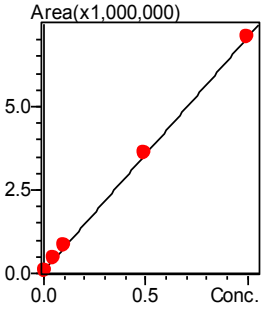
ID	Name	Retention Time	Quantitative Ion	CE	Qualitative Ion	CE
1	DMP	7.266	163>77	23	194>163	7
2	DEP	8.412	177>149	10	222>149	15
3	DIPP	8.900	209>149	10	167>149	10
4	DALP	9.648	132>104	7	189>105	17
5	DPRP	9.891	209>149	8	191>149	5
6	DIBP	10.802	223>149	10	205>149	5
7	DBP	11.924	223>149	10	205>149	5
8	DMOEP	12.388	207>59	5	176>149	10
9	DMPP1	13.332	167>149	10	251>149	20
10	DMPP2	13.338	167>149	10	251>149	20
11	DEOEP	13.920	176>149	10	176>104	25
12	DAP	14.470	237>149	10	219>149	5
13	DHXP	17.290	251>149	15	233>149	5

14	BBP	17.476	206>149	10	238>104	20
15	DBOEP	19.613	193>149	15	176>149	10
16	DCHP	20.652	167>149	10	249>149	15
17	DEHP	21.003	167>149	10	279>149	15
18	DPP	21.336	225>77	25	225>141	20
19	DNOP	25.985	279>149	12	279>71	17
20	DINP	29.413	293>149	10	293>167	5
					293>71	10
21	DIDP	31.371	307>149	20	307>167	5
					307>71	20

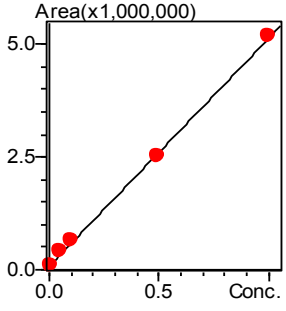
Calibration curve and LOD

A series of multi-standard solutions of phthalates were prepared at the 5 concentrations of 0.01, 0.05, 0.1, 0.5, 1.0 mg/L (for DINP and DIDP, the concentrations were 0.1, 0.5, 1.0, 5.0, and 10.0 mg/L), respectively. Calibration curves were plotted as shown in Fig. 3 with concentration as abscissa and the peak area of quantitative ions as ordinate.

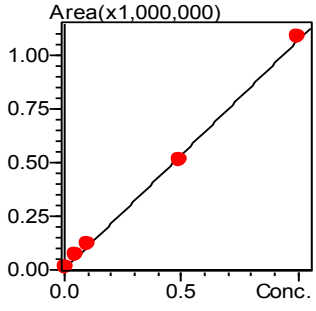
Based on the data obtained from 0.01 mg/L standard samples, LODs were calculated for the compounds and listed in Table 2.



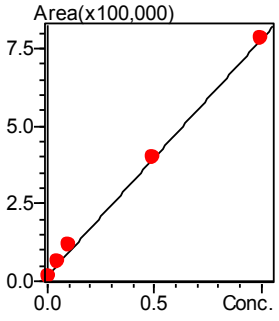
DMP



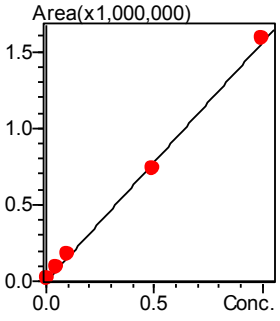
DEP



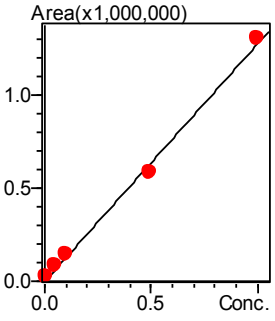
DIPP



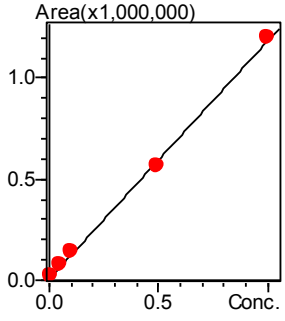
DALP



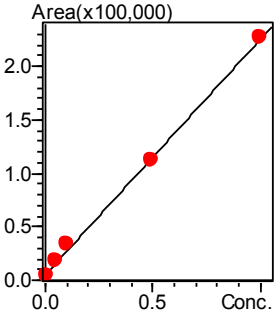
DPRP



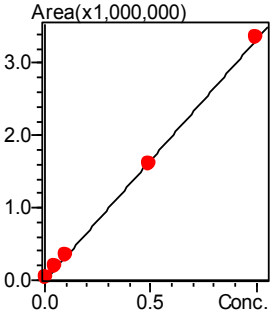
DIBP



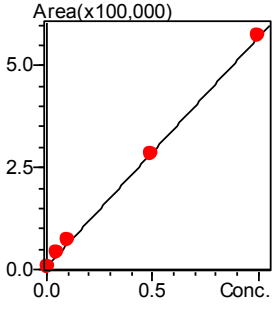
DBP



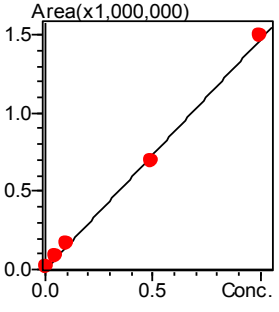
DMOEP



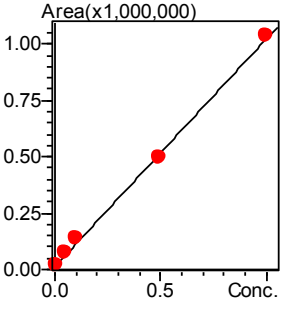
DMPP



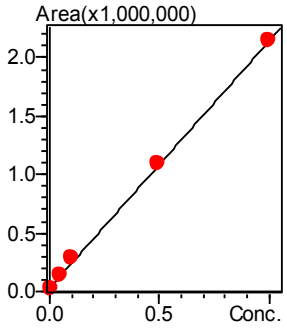
DEOEP



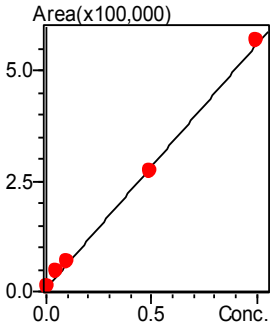
DAP



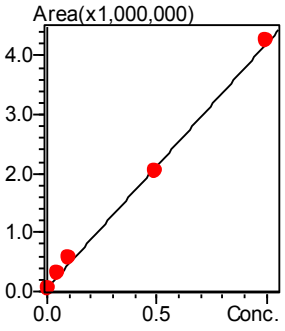
DHXP



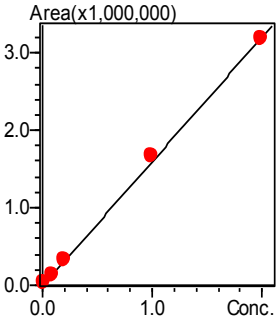
BBP



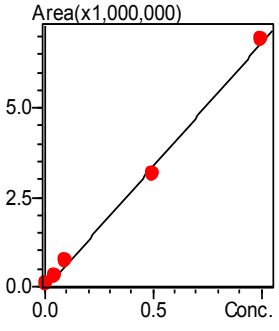
DBOEP



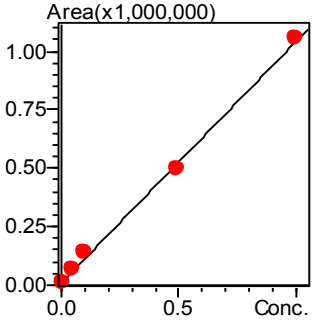
DCHP



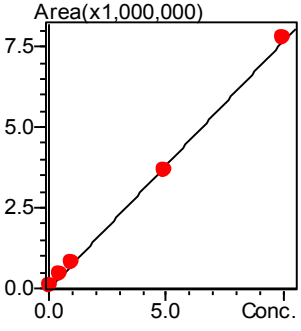
DEHP



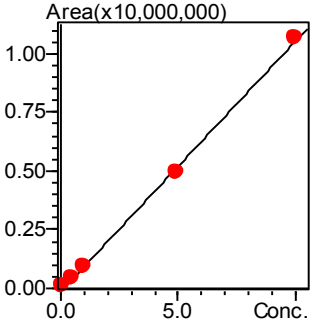
DPP



DNOP



DINP



DIDP

Fig. 3 Calibration curves of 20 phthalate compositions

Table 2. Correlation coefficients of calibration curves and LODs of 20 phthalates

No.	Compound Name	R^2	LOD (μg/L)	No.	Compound Name	R^2	LOD (μg/L)
1	DMP	0.999	1.0	11	DAP	0.997	1.0
2	DEP	0.996	1.0	12	DHXP	0.999	1.0
3	DIPP	0.998	1.0	13	BBP	0.999	1.0
4	DALP	0.998	1.0	14	DBOEP	0.999	1.0
5	DPRP	0.999	1.0	15	DCHP	0.997	1.0
6	DIBP	0.999	1.0	16	DEHP	0.999	1.0
7	DBP	0.999	1.0	17	DPP	0.999	1.0
8	DMOEP	0.999	1.0	18	DNOP	0.996	1.0
9	DMPP	0.998	1.0	19	DINP	0.998	10.0
10	DEOEP	0.998	1.0	20	DIDP	0.998	10.0

Recovery

5 mL of beverage sample was taken and added known amount of multi-standard solution of phthalates to achieve a spike concentration of 0.1 mg/kg (for DINP and DIDP, the concentrations were 1 mg/kg), subjected to pretreatment process in accordance with the above-mentioned method and 3 parallel assays. The recovery results and the reproducibility results of the 3 parallel assays were as shown in Table 3.

Table 3 Recovery and reproducibility results

No	Compound Name	Recovery				
		1	2	3	Mean	%RSD
1	DMP	77.43	84.48	83.31	81.74	4.62
2	DEP	69.07	78.46	70.37	72.63	7.00
3	DIPP	88.07	92.49	88.75	89.77	2.65
4	DALP	84.83	89.78	84.93	86.51	3.27
5	DPRP	92.84	89.40	87.52	89.92	3.00

6	DIBP	93.29	89.60	86.82	89.90	3.61
7	DBP	89.06	90.17	86.16	88.46	2.34
8	DMOEP	88.92	85.24	84.12	86.09	2.92
9	DMPP	85.35	83.61	82.99	83.98	1.46
10	DEOEP	86.62	83.60	85.17	85.13	1.77
11	DAP	77.31	87.60	80.39	81.77	6.46
12	DHXP	86.56	85.20	82.67	84.81	2.33
13	BBP	79.97	84.43	81.09	81.83	2.84
14	DBOEP	82.22	84.10	72.13	79.48	8.10
15	DCHP	82.04	84.69	78.33	81.69	3.91
16	DEHP	81.40	84.37	79.01	81.59	3.29
17	DPP	77.04	84.14	70.65	77.28	8.73
18	DNOP	94.48	84.77	96.77	92.01	6.92
19	DINP	80.95	81.92	78.08	80.32	2.49
20	DIDP	110.48	105.22	117.89	111.20	5.72

Conclusion

Phthalates in beverage were analyzed with Shimadzu GCMS-TQ8030. The proposed method was easy to operate and of good linearity in the concentration range of determination on the calibration curves and capable of accurate determination of phthalates in beverage with good recoveries.

C-14 Determination of 54 Pesticide Residues in Rice by GC/MS/MS in Conjunction with QuEChERS Method

INTRODUCTION

An analytical method is proposed in this paper for determination of 54 pesticide residues in rice with GC/MS/MS. The correlation coefficients of the pesticides were greater than 0.999 in the concentration range of 1~100 µg/L. The %RSDs of peak areas of 6 consecutive injections of 5 µg/L standard solution were less than 6.0%. When individual sample weight was 10 g, the proposed method demonstrated a LOD lower than 1.0 µg/kg for most of the pesticides. The proposed method provided a spike recovery between 70.0 and 120.0% for most of the pesticides at the two spike concentrations of 0.005 mg/kg and 0.02 mg/kg, meeting the requirements for routine detection of pesticide residues in rice.

India is a major rice production country and rice is one of the staple foods for Indians. The growth of paddy is susceptible to production drop caused by plant diseases, insect, pests, and weeds. A great number of pesticides are used during the cultivation of paddy in order to safeguard the crop harvest. These pesticides not only pollute the environment but also have direct adverse impact on food safety.

In consideration of this, various countries throughout the world have established strict maximum residue limits (MRLs) and maximum daily permissible intakes (MDPIs) for foods, including rice.

The assay of pesticides in rice requires complicated purification process because of the various starch, protein and fat contents in rice. In this paper, samples were subjected to simple but effective pretreatment by QuEChERS method and subsequent assay by GC/MS/MS in MRM mode, which not only reduced the matrix interference in pesticide determination but also lowered LODs. The proposed method can serve as reference for the establishment of high performance technology for multi-residue determination of pesticides in rice. It can also be used to improve the efficiency of daily food safety supervision.

EXPERIMENTAL

Apparatus

GC-MS/MS: GCMS-TQ8030 (Shimadzu)

Conditions of Analysis

GC/MS/MS conditions

Column	: Rxi-5 ms, 30 m × 0.25 mm × 0.25 µm
Injector temperature	: 250 °C
Column temperature program	: 50 °C (1 min)→@25 °C/min→150 °C →@ 10 °C/min→300 °C (5 min)
CLV mode	: 47.6 cm/sec
Injection mode	: Splitless injection
Splitless time	: 1 min
High pressure injection	: 250 kPa (1 min)
Ion source	: 200 °C
Interface	: 230 °C
MRM conditions	: see Table 1

Sample Preparation

Pretreatment of samples is shown in Fig. 1 below.

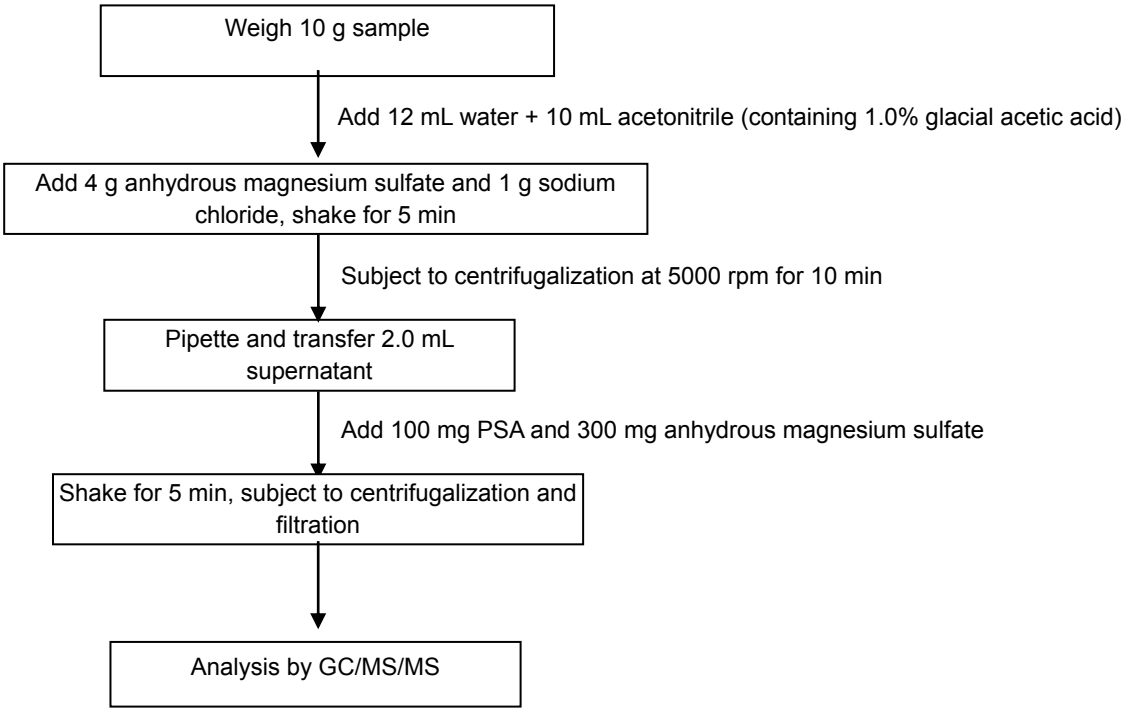


Fig. 1 Sample pretreatment flow chart

Table 1 Retention time and MRM parameters of pesticides

No.	Retention Time (min)	Name of Pesticide	Quantitative Ion (CE)	Qualitative Ion (CE)
1	5.110	O,O,O-triethylphosphorothioate	198>114（15）	198>121（15）
2	5.860	Dichlorvos	185>93（14）	185>109（14）
3	7.650	Mevinphos	192>164（4）	192>127（12）
4	8.850	Isoprocarb	136>121（10）	136>103（22）
5	8.955	Molinate	126>55（14）	126>83（6）
6	9.580	Thionazine	143>79（15）	143>52（25）
7	9.930	Ethoprophos	200>158（6）	200>114（14）
8	10.325	Sulfotep	322>294（4）	322>202（10）
9	10.400	Monocrotophos	127>109（12）	127>95（16）
10	10.555	Phorate	260>75（8）	260>231（4）
11	10.665	Alpha-HCH	219>183（8）	219>145（18）
12	10.745	Hexachlorobenzene	185>93（14）	185>109（16）
13	10.905	Demton	142>112（6）	142>79（14）
14	10.905	Dimethoate	125>79（8）	125>47（14）
15	11.055	Simazine	201>173（6）	201>186（6）
16	11.150	Atrazine	215>58（14）	215>200（6）
17	11.165	Beta-HCH	219>183（8）	219>147（20）
18	11.345	Gamma-HCH	219>183（8）	219>147（20）
19	11.555	Diazinon	304>179（10）	304>162（8）
20	11.665	Chlorothalonil	266>231（14）	266>168（22）
21	11.770	Disulfoton	186>97（16）	186>153（6）
22	11.900	Delta-HCH	219>183（10）	219>145（22）
23	12.620	Methyl parathion	263>109（14）	263>136（8）
24	12.810	Heptachlor	272>237（20）	272>117（32）
25	12.840	Fenchlorphos	285>270（16）	285>93（24）
26	13.070	Pirimiphos-methyl	305>180（8）	305>290（12）
27	13.290	Malathion	173>127（6）	173>99（14）
28	13.435	Chlorpyrifos	314>258（14）	314>286（8）
29	13.510	Fenthion	278>109（20）	278>125（20）
30	13.580	Parathion	291>109（14）	291>137（6）
31	13.770	EPN	297>269（14）	297>223（26）
32	14.290	Heptachlor-epoxide (I.S.)	353>263（14）	353>282（12）
33	14.330	Tributyl phosphorotrithioite	298>190（8）	290>156（8）
34	14.390	Quinalphos	157>129（14）	157>93（10）
35	14.770	o,p'-DDE	246>176（30）	246>211（22）
36	14.785	Tetrachlorvinphos	329>109（20）	329>314（18）
37	15.215	Prothiofos	339>160（18）	339>267（4）
38	15.250	Terbuchlor	262>202（10）	262>174（18）

39	15.390	p,p'-DDE	246>176（28）	246>211（22）
40	16.030	Fensulfothion	293>153（8）	293>125（14）
41	16.175	o,p'-DDD	235>165（24）	235>199（14）
42	16.230	o,p'-DDT	235>165（24）	235>199（16）
43	16.470	Sulprofos	322>156（8）	322>97（24）
44	16.575	Famphur	218>109（16）	218>79（24）
45	16.890	p,p'-DDT	235>165（24）	235>199（16）
46	17.755	Bifenthrin	181>166（12）	181>153（15）
47	17.780	EPN	169>141（8）	169>77（22）
48	17.935	Fenpropathrin	265>210（12）	265>172（14）
49	18.475	Azinphos-methyl	160>132（6）	160>77（20）
50	18.555	Cyhalothrin-1	197>161（8）	197>141（12）
51	18.730	Cyhalothrin-2	197>161（8）	197>141（12）
52	19.665	Coumaphos	362>109（14）	362>226（12）
53	20.070	Cyflurthrin-1	226>206（14）	226>199（6）
54	20.170	Cyflurthrin-2	226>206（14）	226>199（6）
55	20.220	Cyflurthrin-3	226>206（14）	226>199（6）
56	20.265	Cyflurthrin-4	226>206（14）	226>199（6）
57	20.400	Cypermethrin-1	181>152（22）	181>127（22）
58	20.495	Cypermethrin-2	181>152（22）	181>127（22）
59	20.555	Cypermethrin-3	181>152（22）	181>127（22）
60	20.600	Cypermethrin-4	181>152（22）	181>127（22）
61	21.285	Fenvalerate-1	419>225（6）	419>167（12）
62	21.480	Fenvalerate-2	419>225（6）	419>167（12）
63	21.845	Deltamethrin-1	253>93（18）	253>172（4）
64	22.050	Deltamethrin-2	253>93（20）	253>172（8）

RESULTS

MRM chromatograms of standard sample

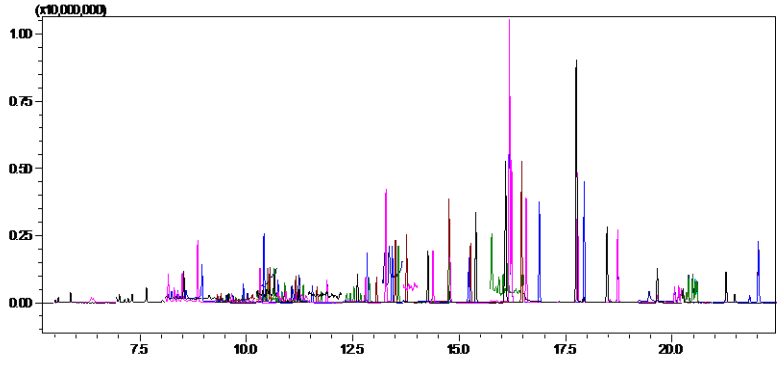


Fig. 2 MRM chromatograms of standard sample (100 µg/L each)

Calibration curve

A series of multi-standard solutions of pesticides were prepared at concentrations of 1, 2, 5, 10, 20, 50, and 100 µg/L, respectively, using rice matrix solution as diluent. A known amount of heptachlor epoxide was added into the solution as internal standard. Calibration curves were plotted with concentration ratio as abscissa and peak area ratio as ordinate. LODs were calculated as 3 times of S/N (peak-to-peak). The repeatability of peak area was assessed by 6 replicate injections of 5 µg/L standard sample and the %RSDs were calculated. Three representative pesticide calibration curves and MRM chromatograms are listed here as shown in Fig. 3. The correlation coefficients of calibration curves, LODs and %RSDs of peak areas are shown in Table 2.

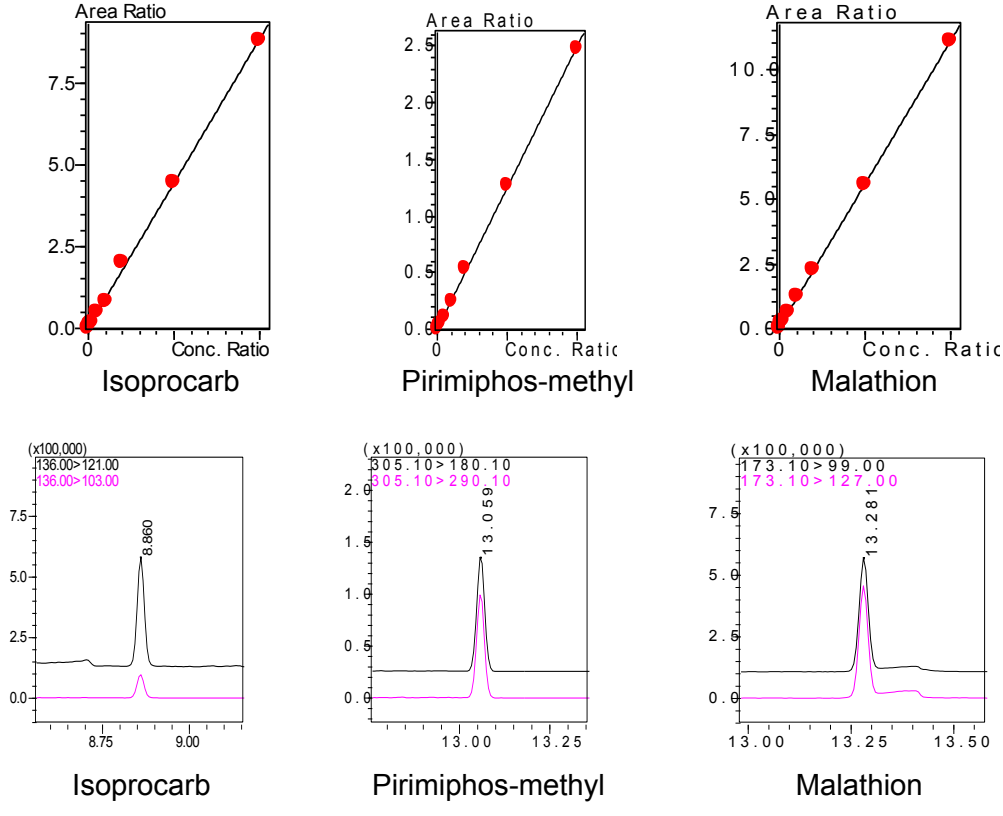


Fig. 3 Calibration curves and mass chromatogram of 3 pesticides

Table 2 Correlation coefficients of calibration curves, LODs and RSD(%) of peak areas of the pesticides

No.	Compound Name	Correlation Coefficient (R^2)	LOD (μg/kg)	RSD(%) (n=6)	No.	Compound Name	Correlation Coefficient (R^2)	LOD (μg/kg)	RSD(%) (n=6)
1	Thiosulfate	0.9993	0.055	1.75	28	Chlorpyrifos	0.9997	0.001	3.27
2	Dichlorvos	0.9993	0.125	2.93	29	Fenthion	0.9997	0.112	5.73
3	Mevinphos	0.9990	0.543	2.87	30	Parathion	0.9999	0.002	2.46
4	Isoprocarb	0.9994	0.333	3.50	31	EPN	0.9998	0.027	1.94
5	Molinate	0.9996	0.693	5.73	32	Merphos	0.9999	0.011	4.78
6	Thionazine	0.9990	0.062	5.35	33	Quinalphos	0.9999	0.481	5.64
7	Ethoprophos	0.9992	0.366	5.48	34	o,p'-DDE	0.9999	0.044	3.47
8	Sulfotep	0.9996	0.007	3.55	35	Tetrachlorvinphos	0.9998	0.003	5.09
9	Monocrotophos	0.9992	0.109	3.16	36	Prothiofos	0.9995	0.003	4.22
10	Phorate	0.9995	0.007	4.34	37	Terbuchlor	0.9999	0.113	2.70
11	□-HCH	0.9998	0.662	4.23	38	p,p'-DDE	0.9999	0.016	3.47
12	Hexachlorobenzene	0.9997	0.013	5.34	39	Fensulfothion	0.9990	0.019	5.40
13	Demton S	0.9992	0.068	4.94	40	o,p'-DDD	0.9999	0.016	4.25
14	Dimethoate	0.9996	2.708	4.85	41	o,p'-DDT	0.9992	0.036	5.96
15	Simazine	0.9996	0.459	4.84	42	Sulprofos	0.9998	0.003	4.49
16	Atrazine	0.9997	0.287	5.33	43	Famphur	0.9996	0.028	4.26
17	□-HCH	0.9993	0.002	3.48	44	p,p'-DDT	0.9995	0.029	6.79
18	□-HCH	0.9996	0.012	5.89	45	Bifenthrin	0.9995	0.024	4.32
19	Diazinone	0.9995	0.005	5.43	46	EPN	0.9991	0.190	3.93
20	Chlorothalonil	0.9994	0.066	5.78	47	Fenpropathrin	0.9997	0.106	2.71
21	Disulfoton	0.9991	1.398	3.75	48	Azinphos-methyl	0.9992	0.320	5.61
22	□-HCH	0.9992	0.002	4.48	49	Cyhalothrin	0.9994	0.184	5.73
23	Parathion-methyl	0.9997	0.440	4.66	50	Coumaphos	0.9998	0.004	5.14
24	Heptachlor	0.9999	0.031	3.30	51	Cyfluthrin	0.9992	0.241	4.94
25	Fenchlorphos	0.9999	0.142	2.33	52	Cypermethrin	0.9995	0.016	3.15
26	Pirimiphos-methyl	0.9996	0.004	3.65	53	Fenvalerate	0.9995	0.014	2.67
27	Malathion	0.9999	0.001	3.40	54	Deltamethrin	0.9993	0.774	2.59

Recovery test

Multi-standard solutions of pesticides were spiked at concentrations of 0.005 mg/kg and 0.02 mg/kg respectively into rice matrix and 5 parallel samples were processed for each case. The resulted spike recoveries of pesticides at various concentrations (average recoveries and %RSDs of 5 parallel samples) are shown in Table 3.

Table 3 Recoveries of pesticides in rice

No.	Compound Name	Spiked Level 0.005 mg/kg		Spiked Level 0.02 mg/kg	
		Average recovery (%)	RSD (%)	Average recovery (%)	RSD (%)
1	Thiosulfate	98.07	1.05	101.58	2.77
2	Dichlorvos	99.29	0.91	96.80	3.17
3	Mevinphos	116.26	1.34	112.66	3.34
4	Isoprocarb	123.99	1.03	114.86	3.74
5	Molinate	80.83	1.39	107.96	1.81
6	Thionazine	108.03	1.11	100.49	3.17
7	Ethoprophos	116.08	0.63	114.54	1.90
8	Sulfotep	126.52	0.74	116.77	2.49
9	Monocrotophos	72.53	0.28	86.95	0.50
10	Phorate	113.92	0.70	112.01	2.24
11	□-HCH	119.48	0.78	106.65	5.52
12	Hexachlorobenzene	95.75	0.91	98.98	2.62
13	Demton S	113.46	1.10	99.72	1.22
14	Dimethoate	111.44	0.54	104.01	1.58
15	Simazine	82.61	0.21	86.41	0.37
16	Atrazine	107.41	0.18	108.47	0.35
17	□-HCH	115.63	0.60	103.83	2.44
18	□-HCH	108.30	0.75	97.81	3.15
19	Diazinone	119.83	0.51	106.61	1.51
20	Chlorothalonil	99.12	0.37	97.33	1.25
21	Disulfoton	106.09	1.26	107.08	0.69
22	□-HCH	115.72	0.34	107.10	0.91
23	Parathion-methyl	113.93	0.32	112.92	1.19
24	Heptachlor	114.81	0.50	99.18	1.22
25	Fenchlorphos	119.96	0.26	110.20	0.82
26	Pirimiphos-methyl	113.43	0.23	104.50	0.46
27	Malathion	120.07	0.23	114.71	0.31
28	Chlorpyrifos	109.57	0.36	98.48	0.33
29	Fenthion	111.51	0.16	104.46	0.37
30	Parathion	116.48	0.17	112.76	0.48
31	EPN	114.71	0.16	111.49	0.48
32	Merphos	119.04	0.22	102.26	0.79
33	Quinalphos	121.42	0.25	110.26	0.54

34	o,p'-DDE	107.54	0.12	106.09	0.59
35	Tetrachlorvinphos	110.32	0.16	109.30	0.35
36	Prothiofos	85.21	0.31	95.90	3.39
37	Terbuchlor	119.69	0.16	108.55	0.90
38	p,p'-DDE	105.04	0.14	104.33	0.57
39	Fensulfothion	122.58	0.28	116.85	1.48
40	o,p'-DDD	105.07	0.27	103.50	1.64
41	o,p'-DDT	106.43	0.37	98.23	1.19
42	Sulprofos	108.70	0.17	103.17	0.87
43	Famphur	109.54	0.13	106.76	0.91
44	p,p'-DDT	111.69	0.51	88.66	1.90
45	Bifenthrin	104.04	0.23	98.61	1.31
46	EPN	106.86	0.16	100.98	1.04
47	Fenpropathrin	105.13	0.14	105.98	0.93
48	Azinphos-methyl	103.01	0.38	91.23	0.72
49	Cyhalothrin	119.25	0.17	100.84	0.86
50	Coumaphos	110.15	0.17	106.49	0.72
51	Cyfluthrin	122.79	0.33	103.01	1.02
52	Cypermethrin	112.11	0.45	101.26	0.83
53	Fenvalerate	118.50	0.21	106.39	1.10
54	Deltamethrin	111.69	0.28	100.76	0.97

Assay of real rice samples

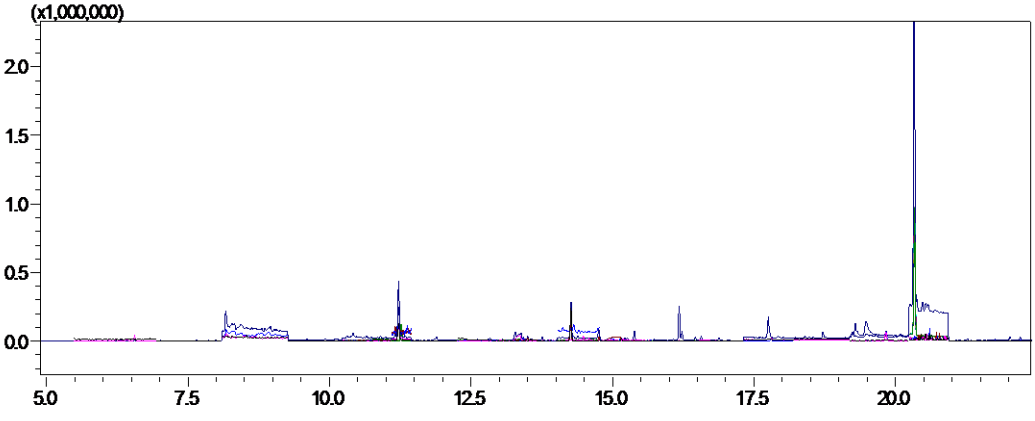


Fig. 4 MRM chromatograms of rice sample

Table 4 Results of rice sample assay

	Pesticide name	Content (μg/kg)
Sample 1	Isoprocarb	0.047
	Merphos	4.865
Sample 2	Isoprocarb	0.303
	Chlorpyrifos	1.487
	Fenthion	0.200

CONCLUSION

A method was developed for the qualitative and quantitative analysis of 54 pesticide residues in rice using GCMS-TQ8030, Shimadzu's triple quadrupole GC-MS/MS, and QuEChERS pretreatment method. The method has the merits of simple pretreatment, satisfactory repeatability, and high sensitivity. For most pesticides spiked at concentrations of 0.005 mg/kg and 0.02 mg/kg, the method can achieve good recoveries between 70.0% and 120.0%. Therefore, it can be used for routine analysis of trace pesticide residues satisfactorily. The experiment demonstrated that tandem mass spectrometry can effectively eliminate matrix interference, thereby reducing the possibility of false positive detection and improve selectivity and sensitivity. Simplified sample pretreatment process reduces analysis cost.

C-15 Determination of Pesticide Residues in Soybean Oil by GPC-GC-MS/MS

INTRODUCTION

A method for determination of pesticide residues in soybean oil with online gel permeation chromatograph coupled with gas chromatograph-triple quadrupole mass spectrometer (GPC-GC-MS/MS) was established. The correlation coefficients of the pesticides were all greater than 0.998 in the concentration range of 1~50 μg/L. The %RSDs of peak areas of 5 consecutive injections of standard solution (10 μg/L) was less than 5.0%. The proposed method provided spike recoveries between 70.0% and 120.0% for most of the pesticides at spike concentration of 0.05 mg/kg, meeting the requirements for routine analysis of pesticide residues in soybean oil.

Soybean oil having the highest production in the world, is a good edible oil of high nutrition value that contains rich linoleic acid, lecithin, and multiple vitamins. Due to the frequent application of pesticides during its cultivation, soybean and its product soybean oil may contain pesticide residues.

A number of food safety standards and regulations, including China's national standard GB2763-2012 *Maximum residue limits for pesticides in food*, Codex Alimentarius Commission(CAC) standard, and Japan's positive list system, have set maximum residue limits (MRLs) for pesticides in soybean oil. As stipulated in GB2763-2012 *Maximum residue limits for pesticides in food*, the MRL for chlorpyrifos and parathion in oil-bearing material soybean and its products is 0.1 mg/kg, and the MRL for treflan, phorate and other pesticides is 0.05 mg/kg. At present, Japan's positive list system has stipulated a general MRL of 0.01 mg/kg for pesticides in soybean oil.

The analysis of pesticide residues in soybean oil requires complicated extraction process because of the interference of the high lipid content in the oil. A method was evaluated which could effectively simplify the pretreatment process of soybean oil samples by means of extraction with online gel permeation chromatography. The method, when used in conjunction with GC-MS/MS technique in MRM mode, could reduce the impact of matrix interference on pesticides determination and simultaneously improve. It can be used as a simple, fast, and reliable analytical method for detection of pesticide residues in soybean oil.

EXPERIMENTS

Apparatus

Shimadzu online gel permeation chromatograph- gas chromatograph-triple quadrupole mass spectrometer (GPC-GC-MS/MS)

Conditions of Analysis

GPC conditions:

Column : Shodex CLNpak EV-200 (2.1 mm × 150 mm)
Mobile phase : Acetone/cyclohexane (3/7, V/V)
Flow rate : 0.1 mL/min
Column temperature : 40 °C
Injection volume : 10 µL

GC-MS/MS conditions:

Column : Inertia quartz tube: 5 m × 0.53 mm
Pre-column : Rtx-5 MS, 5 m × 0.25 mm × 0.25 µm
Analytical column : Rtx-5 MS, 25 m × 0.25 mm × 0.25 µm
Column temperature program : 82 °C (5 min)→@8 °C/min→300 °C (7.75 min)
PTV injector temperature program: 120°C (5 min)→@100°C/min
→250°C (33.7 min)
Injector pressure program : 120 kPa (0 min)→@100 kPa/min
→180 kPa (4.4 min)→@(-49.8 kPa/min)
→120 kPa (33.8 min)
Septum purge program :5.0mL/min→@(-10mL/min)→0mL/min(6min)
→10 mL/min→5 mL/min (5 min)
Splitless injection time : 7 min
Solvent cut time : 9.7 min
Interface temperature : 300 °C Temperature of ion source: 200 °C
MRM mode : MRM conditions were listed in Table 1

Sample Preparation

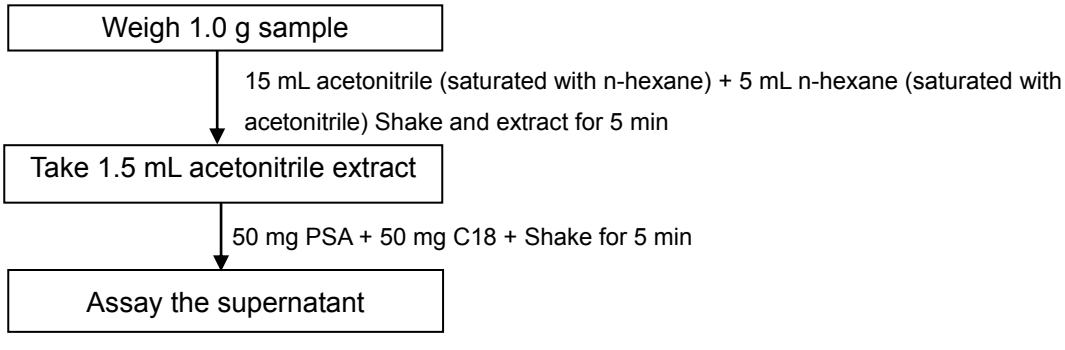


Fig. 1 Sample pre-treatment flow chart

Table 1 Retention time and MRM parameters of pesticides

No.	Retention Time	Name of Pesticide	CAS	Quantitative Ion (CE)	Qualitative Ion (CE)
1	11.075	Dichlorvos	62-73-7	185>93（14）	185>109（14）
2	17.750	Trifluralin	1582-09-8	306>264（8）	306>206（14）
3	18.167	Phorate	298-02-2	260>75（8）	260>231（4）
4	18.250	Alpha-HCH	319-84-6	219>183（8）	219>145（18）
5	19.000	Beta-HCH	319-85-7	219>183（8）	219>147（20）
6	19.200	Gamma-HCH	58-89-9	219>183（8）	219>147（20）
7	19.617	Chlorothalonil	1897-45-6	266>231（14）	266>168（22）
8	20.008	Delta-HCH	319-86-8	219>183（10）	219>145（22）
9	20.217	Pirimicarb	23103-98-2	238>166（12）	238>96（28）
10	20.775	Chlorpyrifos-methyl	5598-13-0	286>93（22）	286>271（14）
11	20.842	Metribuzin	21087-64-9	198>153（8）	198>110（10）
12	20.967	Methyl parathion	298-00-0	263>109（14）	263>136（8）
13	21.142	Heptachlor	76-44-8	272>237（20）	272>117（32）
14	21.633	Fenitrothion	122-14-5	277>260（6）	277>109（14）
15	21.900	Malathion	121-75-5	173>127（6）	173>99（14）
16	22.033	Chlorpyrifos	2921-88-2	314>258（14）	314>286（8）
17	22.083	Aldrin	309-00-2	277>206（15）	277>241（15）
18	22.258	Parathion	56-38-2	291>109（14）	291>137（6）
19	22.342	Triadimefon	43121-43-3	208>181（10）	208>127（14）
20	22.892	Pendimethalin	40487-42-1	252>162（10）	252>191（8）
21	23.117	Heptachlor-exoepoxide	1024-57-3	353>263（14）	353>282（12）
22	23.700	Methidathion	950-37-8	145>85（8）	145>58（14）
23	23.725	Trans-Chlordane	5103-74-2	373>337（10）	373>143（26）
24	24.058	Cis-Chlordane	5103-71-9	373>266（22）	373>337（6）
25	24.642	p,p'-DDE	72-55-9	246>176（28）	246>211（22）
26	24.758	Dieldrin	60-57-1	277>206（15）	277>241（15）
27	25.292	Endrin	72-20-8	263>191（30）	263>193（28）
28	25.717	p,p'-DDD	72-54 - 8	235>165（24）	235>199（14）
29	25.757	Beta-Endosulfan	33213-65-9	339>160（18）	339>267（4）
30	25.950	Endrin aldehydel	7421-93-4	281>209（30）	281>246（20）
31	26.542	Endosulfan sulfate	1031-07-8	387>289（10）	387>253（16）
32	26.650	p,p'-DDT	50-29-3	235>165（22）	235>199（14）
33	27.725	Endrin ketone	53494-70-5	317>101（20）	317>147（15）
34	28.042	Methoxychlor	72-43-5	227>169（24）	227>212（14）
35	28.083	Fenpropathrin	39515-41-8	265>210（12）	265>172（14）
36	28.900	Cyhalothrin-1	68085-85-8	197>161（8）	197>141（12）

37	29.142	Cyhalothrin-2	68085-85-8	197>161（8）	197>141（12）
38	30.442	Pyridaben	96489-71-3	147>117（22）	147>132（14）
39	31.367	Cypermethrin-1	52315-07-8	181>152（22）	181>127（22）
40	31.508	Cypermethrin-2	52315-07-8	181>152（22）	181>127（22）
41	31.575	Cypermethrin-3	52315-07-8	181>152（22）	181>127（22）
42	31.633	Cypermethrin-4	52315-07-8	181>152（22）	181>127（22）
43	32.550	Flumioxazin	103361-09-7	354>326（8）	354>176（20）
44	32.575	Fenvalerate-1	51630-58-1	419>225（6）	419>167（12）
45	32.850	Fenvalerate-2	51630-58-1	419>225（6）	419>167（12）
46	33.225	Difenoconazole-1	119446-68-3	323>265（14）	323>202（28）
47	33.325	Difenoconazole-2	119446-68-3	323>265（14）	323>202（28）
48	33.375	Deltamethrin-1	52918-63-5	253>93（18）	253>172（4）
49	33.683	Deltamethrin-2	52918-63-5	253>93（20）	253>172（8）

Results

MRM chromatograms of standard mixture

The MRM chromatograms of a standard sample are shown in Fig. 2.

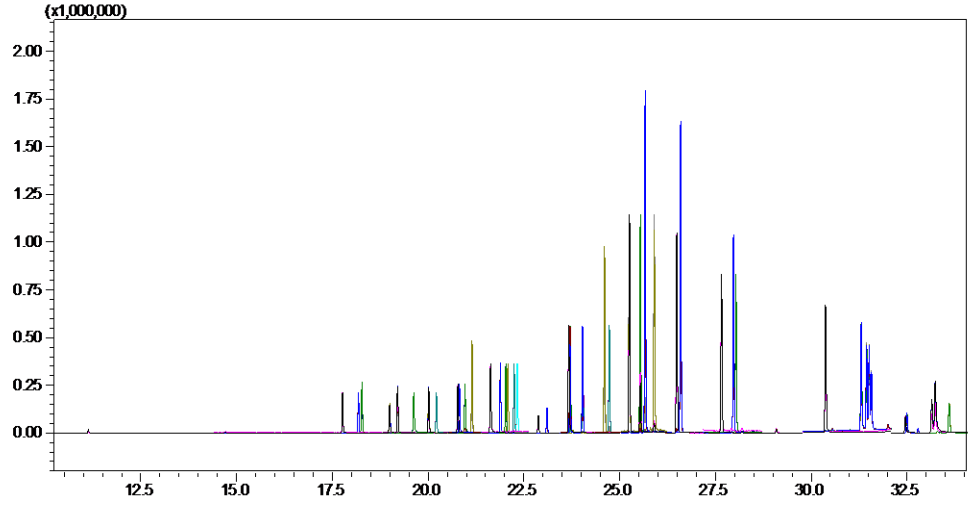


Fig. 2. MRM chromatograms of standard mixture (50 µg/L each)

Calibration curve and reproducibility

A series of multi-standard solutions of pesticides were prepared at concentrations of 1, 5, 10, 20, 50 µg/L using acetone/n-hexane (3/7, v/v) as solvent. All calibration curves of the pesticides had a correlation coefficient greater than 0.998 (Table 2). Representative calibration curves of some pesticides are shown in Fig. 3. To

assess the reproducibility of peak area, 10 µg/L standard samples were injected 5 times in succession and the %RSDs were calculated. Based on the data of 1 µg/L standard samples, the LODs of the pesticides were calculated. The results are shown in Table 2.

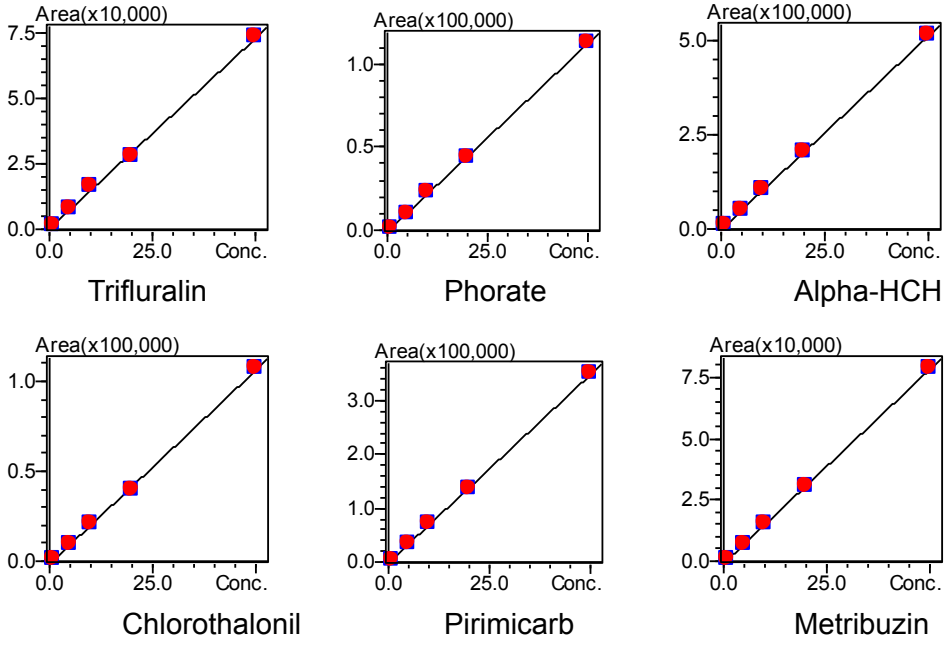


Fig. 3 Representative calibration curves of pesticides

Table 2 Correlation coefficients of calibration curves, peak area %RSDs (n=5) and LODs of pesticides

No.	Compound Name	Correlation Coefficient	%RSD	LOD (µg/kg)
1	Dichlorvos	0.9990	4.74	1.07
2	Trifluralin	0.9991	3.98	0.35
3	Phorate	0.9999	1.67	0.36
4	Alpha-HCH	0.9999	2.77	0.04
5	Beta-HCH	0.9998	2.11	0.15
6	Gamma-HCH	0.9998	3.69	0.07
7	Chlorothalonil	0.9997	2.90	0.69
8	Delta-HCH	0.9999	2.38	0.15
9	Pirimicarb	0.9997	3.77	0.19
10	Chlorpyrifos-methyl	0.9999	2.69	0.22
11	Metribuzin	0.9999	3.50	1.10
12	Methyl parathion	0.9991	2.42	0.94

13	Heptachlor	0.9999	2.49	0.04
14	Fenitrothion	0.9993	2.56	0.92
15	Malathion	0.9995	1.74	0.12
16	Chlorpyrifos	0.9999	2.24	0.25
17	Aldrin	0.9998	2.64	0.31
18	Parathion	0.998	2.58	0.67
19	Triadimefon	0.9997	1.89	0.86
20	Pendimethalin	0.9993	3.04	0.37
21	Heptachlor epoxide	0.9999	2.25	0.14
22	Methidathion	0.9998	2.50	0.07
23	Trans-Chlordane	0.9998	1.69	0.19
24	Cis-Chlordane	0.9999	1.26	0.07
25	p,p'-DDE	0.9999	2.07	0.02
26	Dieldrin	0.9999	2.03	0.45
27	Endrin	0.9999	2.62	0.71
28	p,p'-DDD	0.9999	1.34	0.02
29	β- Endosulfan	0.9999	2.23	0.85
30	Endrin aldehydel	0.9999	0.73	2.22
31	Endosulfan sulphate	0.9999	1.09	0.40
32	p,p'-DDT	0.9998	1.82	0.03
33	Endrin ketone	0.9999	2.38	0.24
34	Methoxychlor	0.9998	2.41	0.07
35	Fenpropathrin	0.9996	3.29	0.90
36	Cyhalothrin	0.9992	4.85	1.26
37	Pyridaben	0.9997	2.41	0.46
38	Cypermethrin	0.9992	2.10	2.74
39	Flumioxazin	0.998	3.12	2.01
40	Fenvalerate	0.9993	2.48	0.28
41	Difenoconazole	0.9999	1.97	0.53
42	Deltamethrin	0.998	1.84	0.62

Recovery test

Multi-standard solutions of pesticides were spiked into soybean oil samples at spiked concentration of 0.05 mg/kg. Three samples in duplicate were processed and subjected to the above-mentioned pre-treatment procedures. The samples were analyzed to determine the concentrations of the pesticides,

recoveries and %RSDs were calculated. The results are listed in Table 3:

Table 3 Recoveries of spiked samples

No.	Compound Name	Recovery (%)			Mean (%)	%RSD
		1	2	3		
1	Dichlorvos	103.10	104.29	107.99	105.13	2.43
2	Treflan	82.69	90.50	94.16	89.12	6.57
3	Phorate	94.31	96.24	98.72	96.43	2.29
4	□Alpha -HCH	92.45	94.46	96.01	94.31	1.89
5	Beta-HCH	95.27	98.83	101.00	98.36	2.94
6	Gamma-HCH	93.10	97.98	98.58	96.55	3.11
7	Chlorothalonil	72.34	72.47	75.92	73.58	2.76
8	Delta-HCH	95.23	98.02	99.56	97.60	2.25
9	Aphox	102.85	106.03	107.46	105.45	2.24
10	Chlorpyrifos-methyl	95.08	98.24	98.57	97.29	1.98
11	Metribuzin	105.37	105.10	108.77	106.41	1.92
12	Parathion-methyl	103.43	109.24	111.79	108.16	3.96
13	Heptachlor	74.44	70.24	71.98	72.22	2.92
14	Fenitrothion	103.33	109.17	110.89	107.80	3.68
15	Malathion	111.56	117.63	121.97	117.06	4.46
16	Chlorpyrifos	92.81	95.83	94.33	94.33	1.60
17	Aldrin	73.21	70.84	79.85	74.63	6.26
18	Parathion	100.41	104.81	107.84	104.35	3.58
19	Triadimefon	97.98	103.25	106.95	102.72	4.39
20	Pendimethalin	88.80	92.89	93.96	91.88	2.97
21	Heptachlor epoxide	87.46	89.22	90.86	89.18	1.90
22	Methidathion	103.90	107.73	101.28	104.30	3.11
23	Trans-Chlordane	72.64	75.66	75.97	74.76	2.46
24	Cis-Chlordane	78.14	79.66	80.87	79.56	1.72
25	p,p'-DDE	70.80	74.96	70.68	72.15	3.37
26	Dieldrin	80.69	83.41	80.82	81.64	1.88
27	Endrin	80.17	83.08	83.22	82.16	2.09
28	p,p'-DDD	91.51	93.67	94.80	93.33	1.79
29	β- Endosulfan	93.57	95.15	96.44	95.05	1.51
30	Endrin aldehydel	72.94	75.25	71.36	73.19	2.67
31	Endosulfan sulphate	98.76	101.88	104.31	101.65	2.73
32	p,p'-DDT	73.94	75.70	77.03	75.56	2.05
33	Endrin ketone	99.21	100.20	102.68	100.69	1.77

34	Methoxychlor	96.67	98.46	100.79	98.64	2.10
35	Fenpropathrin	89.39	94.27	94.01	92.56	2.96
36	Cyhalothrin	90.39	96.69	105.32	97.47	7.69
37	Pyridaben	84.45	88.83	89.60	87.63	3.17
38	Cypermethrin	92.59	98.88	101.16	97.55	4.55
39	Flumioxazin	100.74	101.69	109.91	104.11	4.84
40	Fenvalerate	93.78	98.64	104.81	99.08	5.58
41	Difenoconazole	124.74	126.30	104.80	118.61	10.11
42	Deltamethrin	83.83	89.92	97.01	90.25	7.31

CONCLUSION

A method for qualitative and quantitative analysis of pesticide residues in soybean oil with online gel permeation chromatograph-gas chromatograph-triple quadrupole mass spectrometer (GPC-GC-MS/MS) was evaluated. The method has the merits of simple pretreatment operation, satisfactory repeatability, and high sensitivity. For most of the pesticides spiked at concentrations of 0.05 mg/kg, the method can achieve a recovery between 70.0% and 120.0%. Therefore, it can be used for routine analysis of trace pesticide residues satisfactorily. The experiment showed that the combination of online GPC and tandem mass spectrometry as a detection method can effectively simplify sample pretreatment process and at the same time eliminates matrix interference, thereby improving selectivity and sensitivity of analysis.

C-16 Determination of 16 PAHs in drinking water by GC/MS/MS

INTRODUCTION

In this paper, an analytical method is proposed for determination of 16 PAHs in domestic drinking water with gas chromatograph-triple quadrupole mass spectrometer (GC-MS/MS). The proposed method demonstrated linear correlation coefficients greater than 0.999 for the 16 PAHs for the concentration range of 0.5~50 µg/L. The %RSDs of peak areas of the PAHs in 6 successive injections of 5 µg/L standard solution were all below 5%. At the spiked level of 0.02 µg/L, the proposed method showed spiked recovery range of 75~118%. LODs were calculated as 3 times of S/N (peak to peak) and the LODs below 0.011 ng/L for all 16 PAHs, meeting the requirements for routine analysis of PAHs in domestic drinking water satisfactorily. It can be used to safeguard the quality and safety of domestic drinking water.

Polycyclic aromatic hydrocarbons (PAHs) is a category of typical persistent organic pollutants that are extensively present in environment water. PAH are fused rings consisting of 2 or more benzene rings and demonstrate strong carcinogenic, teratogenic, and mutagenic effects. Therefore, they have become an important test item for environmental monitoring of water.

So far there are more than 200 kinds of PAHs, most of which are carcinogenic. In 2002, the Scientific Committee for Food (SCF) of EU listed 15 PAHs including benzo[α]anthracene, chrysene, benzo[b]fluoranthracene, benzo[k]fluoranthracene, and benzo[a]pyrene as priority PAHs; EPA also included 16 parent PAHs in its list of priority pollutants. China has listed benzo[a]pyrene (BaP) as a priority pollutant and stipulated in GB/T 5749-2006 *Standard for Drinking Water Quality* that the concentration of BaP shall not exceed 0.010 µg/L and the concentration of total PAHs shall not exceed 0.002 mg/L.

Currently available analytical methods for detecting PAHs include GC, GC/MS, and HPLC-FLD. In this paper, liquid-liquid extraction (LLE) was used in conjunction with GC/MS/MS in multiple reaction monitoring (MRM) mode for the assay of PAHs in order to effectively eliminate matrix interference and improve instrumental sensitivity. The proposed method can be used for daily monitoring of drinking water quality to safeguard the quality and safety of domestic drinking water.

Experiments

Apparatus

GC-MS/MS: GCMS-TQ8030

Conditions of Analysis

Column	: Rxi-5Sil MS, 30 m × 0.25 mm ×0.25 µm
Injection port temperature	: 280 °C
Injection mode	: Splitless injection
Column temperature program	: 60 °C(1 min)→@20 °C/min→200 °C(1 min) →@10 °C/min→310 °C(10 min)
CLV	: 40 cm/sec
Injection volume	: 2 µL
Ionization mode	: EI
Temperature of ion source	: 230 °C
Temperature of GC-MS interface	: 280 °C
Solvent delay time	: 4 min
Acquisition mode	: MRM are listed in Table 1

Sample Preparation

1 L of water sample was measured and transferred into a separating funnel. 20 g of NaCl was added and after shaking 50mL of dichloromethane (CH_2Cl_2) was added. The whole mixture was shaken for 30min. The solvent was kept for equilibrium and the organic phase was taken. Extraction was repeated. Organic phase from each extraction was combined and dehydrated with anhydrous sodium sulfate. The extract was transferred to a rotary evaporator for evaporation to almost dry. The residue was reconstituted and brought to the volume of 1 mL with dichloromethane for analysis by GC/MS/MS.

Table 1. MRM parameters of 16 PAHs

No.	Retention Time	Compound	CAS No.	Quantitative Ion(CE)	Qualitative Ion(CE)
1	5.908	Naphthalene	91-20-3	128>102 (20)	128>127 (15)
2	7.883	Acenaphthylene	208-96-8	152>151 (20)	152>126 (25)
3	8.100	Acenaphthene	83-32-9	153>152 (20)	153>127 (25)
4	8.817	Fluorene	86-73-7	165>139 (25)	165>115 (25)
5	10.483	Phenanthrene	1985-1-8	178>152 (20)	178>176 (25)
6	10.583	Anthracene	120-12-7	178>152 (20)	178>176 (25)
7	12.967	Fluoranthene	206-44-0	202>200 (30)	202>152 (30)
8	13.458	Pyrene	129-00-0	202>201 (25)	202>176 (25)

9	16.258	Benz[a]anthracene	56-55-3	228>226 (25)	228>202 (25)
10	16.333	Chrysene	218-01-9	228>226 (30)	228>202 (25)
11	18.658	Benzo[b]fluoranthene	205-99-2	252>250 (30)	252>226 (25)
12	18.717	Benzo[k]fluoranthene	207-08-9	252>250 (25)	252>226 (25)
13	19.317	Benzo[a]pyrene	50-32-8	252>250 (30)	252>226 (25)
14	21.617	Indeno[1,2,3-cd]pyrene	193-39-5	276>275 (30)	276>274 (30)
15	21.700	Dibenz[a,h]anthracene	53-70 -3	278>276 (30)	278>277 (25)
16	22.233	Benzo[ghi]perylene	191-24-2	276>274 (30)	276>275 (30)

RESULT AND DISCUSSION

MRM chromatogram of standard mixture

Representative MRM chromatograms are shown in Fig. 1.

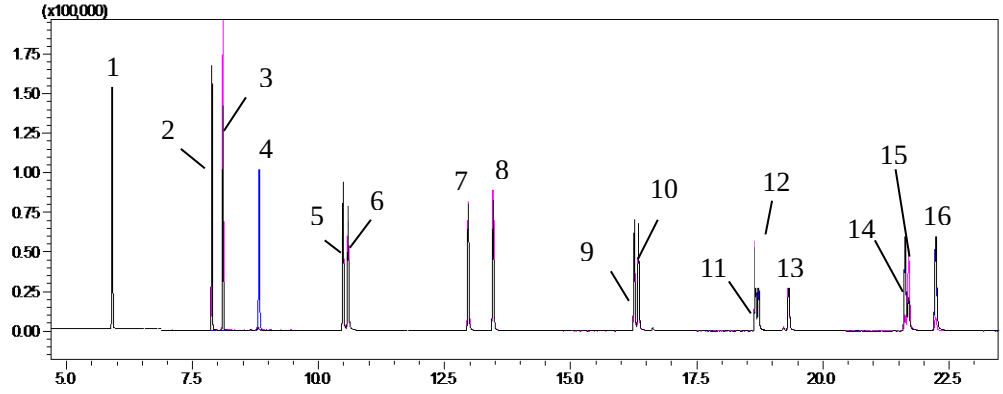


Fig.1 MRM chromatograms of the multi-standard solutions of 16 PAHs (10 µg/L each)

Linear range, repeatability and LOD

Multi-standard solutions of the 16 PAHs were prepared at concentrations of 0.5, 1, 5, 10, and 50 µg/L and subjected to MS/MS analysis in MRM mode. Representative calibration curves are plotted as shown in Fig. 2, using concentration as abscissa and peak area as ordinate. LODs were calculated as 3 times of S/N (peak to peak). To assess the repeatability of peak area, 5 µg/L standard samples were injected 6 times in succession and the %RSDs were calculated. The correlation coefficients of calibration curves, LODs and %RSDs of peak areas are shown in Table 2.

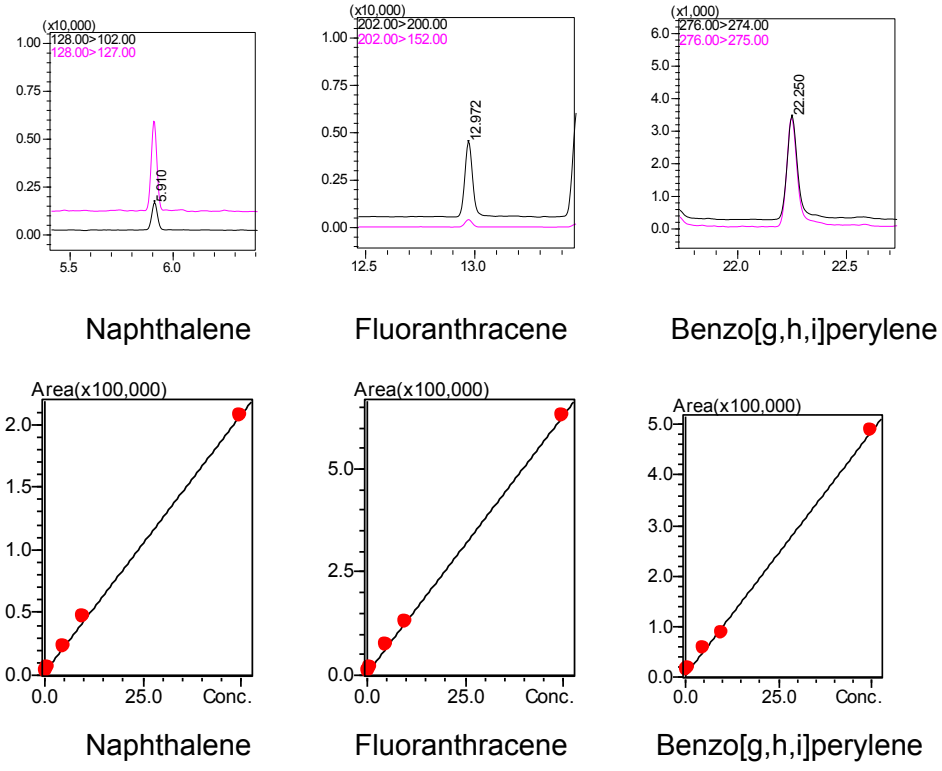


Fig.2 MRM chromatograms (5 µg/L) and calibration curves of 3 PAHs

Table 2 Correlation coefficients of calibration curves, LODs and %RSDs of peak areas of the PAHs

No.	Compound	Correlation Coefficient (R^2)	LOD (ng/L)	%RSD (n=6)
1	Naphthalene	0.9998	0.007	4.21
2	Acenaphthylene	0.9997	0.004	4.76
3	Acenaphthene	0.9998	0.003	4.33
4	Fluorene	0.9998	0.010	3.98
5	Phenanthrene	0.9999	0.005	4.25
6	Anthracene	0.9997	0.011	4.06
7	Fluoranthracene	0.9999	0.003	3.76
8	Pyrene	0.9999	0.004	4.93
9	Benzo[α]anthracene	0.9998	0.005	4.32
10	Chrysene	0.9999	0.004	4.01
11	Benzo[b]fluoranthracene	0.9996	0.004	4.82
12	Benzo[k]fluoranthracene	0.9997	0.004	2.98

13	Benzo[α]pyrene	0.9995	0.004	4.19
14	Indeno[1,2,3-cd]pyrene	0.9994	0.003	4.43
15	Dibenzo[a,h]anthracene	0.9992	0.007	4.41
16	Benzo[g,h,i]perylene	0.9993	0.002	4.60

Recovery

1 L of sample was spiked with known amount of multi-standard solution of PAHs at spiked level of 0.02 μg/L. The recovery results are in the range of 75% -118% as shown in Table 3.

Table 3 Spiked recovery of samples

No.	Compound	Determined value (μg/L)	Recovery (%)	No.	Compound	Determined value (μg/L)	Recovery (%)
1	Naphthalene	0.018	87.80	9	Benzo[α]anthracene	0.021	102.90
2	Acenaphthylene	0.015	76.55	10	Chrysene	0.023	113.65
3	Acenaphthene	0.018	91.55	11	Benzo[b]fluoranthracene	0.023	117.35
4	Fluorene	0.022	112.10	12	Benzo[k]fluoranthracene	0.022	111.15
5	Phenanthrene	0.017	86.00	13	Benzo[α]pyrene	0.015	75.25
6	Anthracene	0.017	85.15	14	Indeno[1,2,3-cd]pyrene	0.021	104.00
7	Fluoranthracene	0.019	97.40	15	Dibenzo[a,h]anthracene	0.022	108.25
8	Pyrene	0.020	100.65	16	Benzo[g,h,i]perylene	0.020	97.75

Sample analysis

The domestic drinking water from a place was subjected to the afore-mentioned pretreatment procedures and assay. The MRM chromatograms are shown in Fig. 3 and the assay results are listed in Table 4.

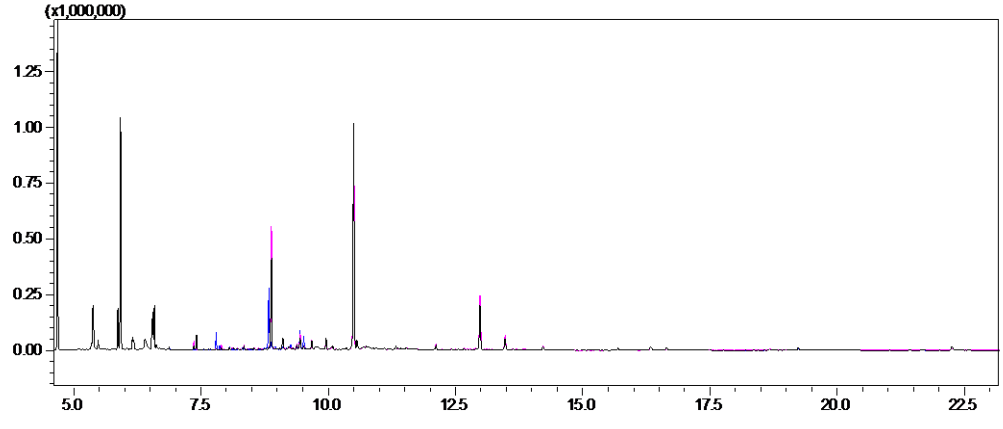


Fig. 3 MRM Chromatogram

Table 4. Assay results of drinking water sample

No.	Compound	Assay results (μg/L)	No.	Compound	Assay results (μg/L)
1	Naphthalene	0.122	9	Benzo[α]anthracene	0.006
2	Acenaphthylene	N.D.	10	Chrysene	N.D.
3	Acenaphthene	N.D.	11	Benzo[b]fluoranthracene	0.003
4	Fluorene	0.036	12	Benzo[k]fluoranthracene	N.D.
5	Phenanthrene	0.223	13	Benzo[α]pyrene	N.D.
6	Anthracene	0.016	14	Indeno[1,2,3-cd]pyrene	0.002
7	Fluoranthracene	0.049	15	Dibenzo[a,h]anthracene	N.D.
8	Pyrene	0.011	16	Benzo[g,h,i]perylene	0.005

N.D.: not detected

CONCLUSION

The proposed method for analysis of 16 PAHs in domestic drinking water with Shimadzu GCMS-TQ8030 gas chromatograph-triple quadrupole mass spectrometer is easy to operate and of satisfactory repeatability. It demonstrated spiked recoveries in the range of 75%~118% and LODs below 0.011ng/L for the 16 PAHs at spike level of 0.02μg/L. The application of tandem mass spectrometry in MRM mode for analysis can effectively reduce matrix interference (Noise) and improve detection sensitivity and assay results reliability. The proposed method meets the requirements for determination of PAHs in drinking water and can be used for monitoring and quality control of domestic drinking water to safeguard the quality and safety of drinking water.

C-17 Simultaneous determination of 19 phthalate compounds in milk powder by GC/MS/MS

INTRODUCTION

A method is proposed in this paper for simultaneous determination of 19 phthalates in milk powder with Shimadzu GCMS-TQ8030 gas chromatograph-triple quadrupole mass spectrometer. The method is simple and convenient and capable of fast and accurately performing qualitative and quantitative analysis of phthalates with high sensitivity. The calibration curves of this method are having good linearity for the concentration range of 0.05~2.00 mg/L. The method's LODs for the phthalates found to be in the range of 0.09~96.90 µg/L and the average recovery of spiked samples are within the range of 93%~125%. The %RSDs of peak areas in 5 consecutive injections were all less than 6.0 %.

Phthalates (PAEs) are frequently used in plastic industry as a plasticizer or softener to improve the plasticity and tenacity of plastic. At present, an effective way to preserve dairy products is to pack them in packages, including plastic packages. However, since packing material comes into direct contact with dairy products, PAEs may migrate into the dairy products and become contaminant source. Furthermore, another issue of concern is that PAEs may also contaminate dairy products during the mechanical milking and manufacturing processes because of the use of PVC hose. Therefore, dairy products may be contaminated with PAEs during their manufacturing, processing and/or packing process, which gives rise to food safety concerns of dairy products.

PAEs are a group of environmental hormone with estrogen-like action. Studies showed that they could have adverse effects on the endocrine system of human being and, in serious cases, give rise to cell mutation which may eventually lead to dysmorphosis and cancers. The determination method specified in GB/T 21911-2008 *Determination of Phthalate Esters in Food* has an LOD of 0.05 mg/kg for phthalates in oil-free samples.

An analytical method was proposed in this paper for determination of 19 PAEs in milk powder with gas chromatograph-tandem mass spectrometer. The method is of high sensitivity, low LOD, and wide applicability. Moreover, it is easy to operate and capable of determining the content of PAEs in milk powder with high accuracy.

EXPERIMENTS

Apparatus

GC-MS/MS: GCMS-TQ8030

Conditions of Analysis

Column	: Rxi-5Sil MS, 30 m × 0.25 mm × 0.25 µm
Column temperature program	: 60 °C (1min)→@20 °C/min→220 °C (1min) →@5 °C/min→280 °C(5min)
CID gas	: Argon
Injection port temperature	: 250 °C
Injection volume	: 1 µL
Injection mode	: Splitless, 1 min
Control mode	: CLV
Linear velocity of carrier gas	: 36.5 cm/sec
Interface temperature	: 280 °C
Temperature of ion source	: 230 °C
Acquisition mode	: MRM

Sample Preparation

5.0 g of milk powder sample was taken and 10 mL of pure water was added, subjected to vortex mixing until the milk powder was completely dissolved. 10 mL of n-hexane was added, subjected to ultrasonic extraction for 110 min. The sample was shaken for extraction for 20 min followed by centrifugation at 3000 rpm for 5 min. Extraction was repeated again, the supernatant was combined and subjected to rotary evaporation (at 40°C) until it dried. The residue was reconstituted with 2.0 mL of n-hexane (and dissolved using ultra sonication). The solution was filtered with 0.2 µm membrane for analysis.

RESULT AND DISCUSSION

MRM chromatogram

MRM chromatograms are shown in Fig. 1.

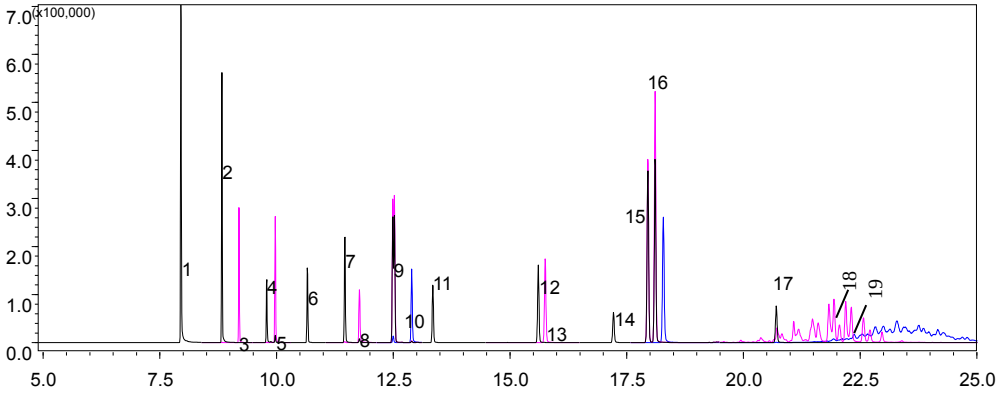


Fig. 2 MRM chromatogram of multi-standard solutions of phthalates (1.0 mg/L)

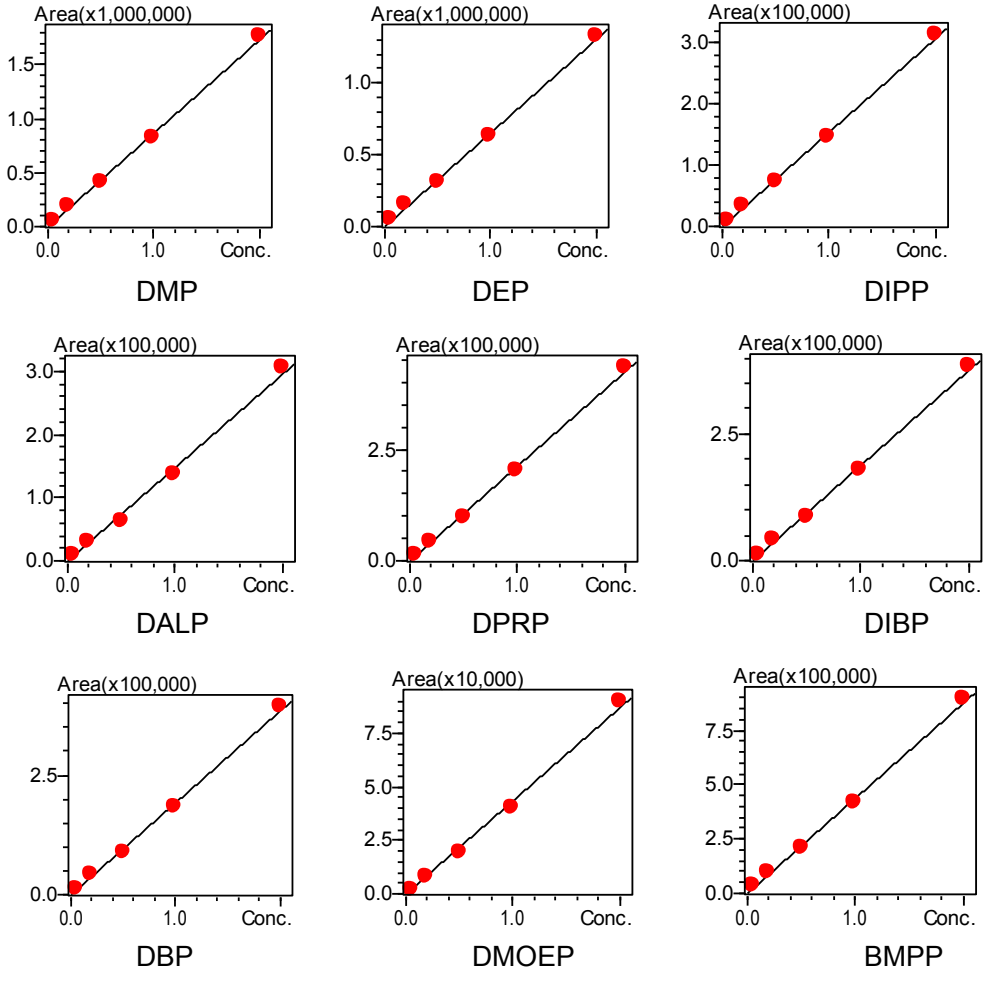
Table 1 Retention time and MRM parameters of phthalates

No.	Compound	CAS#	Retention Time	Quantitative Transition	CE	Qualitative Transition	CE
1	Dimethylphthalate (DMP)	131-11-3	7.951	163>77	23	194>163	7
2	Diethyl phthalate (DEP)	84-66-2	8.828	177>149	10	222>149	15
3	Di-iso-propyl-phthalate (DIPP)	605-50-5	9.195	209>149	10	167>149	10
4	Di-allyl phthalate (DALP)	131-17-9	9.788	132>104	7	189>105	17
5	Di-n-propyl phthalate (DPRP)	131-16-8	9.971	209>149	8	191>149	5
6	Diisobutyl phthalate (DIBP)	84-69-5	10.661	223>149	10	205>149	5
7	Dibutylphthalate (DBP)	84-74-2	11.463	223>149	10	205>149	5
8	Bis(methylglycol) phthalate (DMOEP)	117-82-8	11.775	207>59	5	176>149	10
9	Bis(4-methyl-2-pentyl)phthalate (BMPP1)	146-50-9	12.489	167>149	10	251>149	20
10	Bis(2-ethoxyethyl)phthalate (DEOEP)	605-54-9	12.894	176>149	10	176>104	25
11	Dioctyl phthalate (DAP)	131-18-0	13.348	237>149	10	219>149	5
12	Dihexylphthalate (DHXP)	84-75-3	15.606	251>149	15	233>149	5
13	Benzyl butyl phthalate (BBP)	85-68-7	15.754	206>149	10	238>104	20
14	Bis(2-n-butoxyethyl)phthalate (DBOEP)	117-83-9	17.217	193>149	15	176>149	10
15	Dicyclohexyl phthalate (DCHP)	84-61-7	17.954	167>149	10	249>149	15
16	Diphenyl phthalate (DPP)	84-62-8	18.284	225>77	25	225>141	20
17	Di-n-octyl phthalate (DNOP)	117-84-0	20.704	279>149	12	279>71	17
18	Diisononyl phthalate (DINP)	68515-48-0	21.939	293>149	10	293>167	5
						293>71	10
19	Di-iso-decyl phthalate (DIDP)	26761-40-0	23.288	307>149	20	307>167	5
						307>71	20

Calibration curve and LOD

A series of multi-standard solutions of phthalates were prepared at the 5 concentrations of 0.05, 0.2, 0.5, 1.0, and 2.0 mg/L (for DINP and DIDP, the concentrations were 0.5, 2.0, 5.0, 10.0, and 20.0 mg/L), respectively. Calibration curves were plotted as shown in Fig. 3 with concentration as abscissa and the peak area of quantitative ions as ordinate.

Based on the data obtained from 0.05 mg/L standard samples, LODs were calculated for the compounds and listed in Table 2.



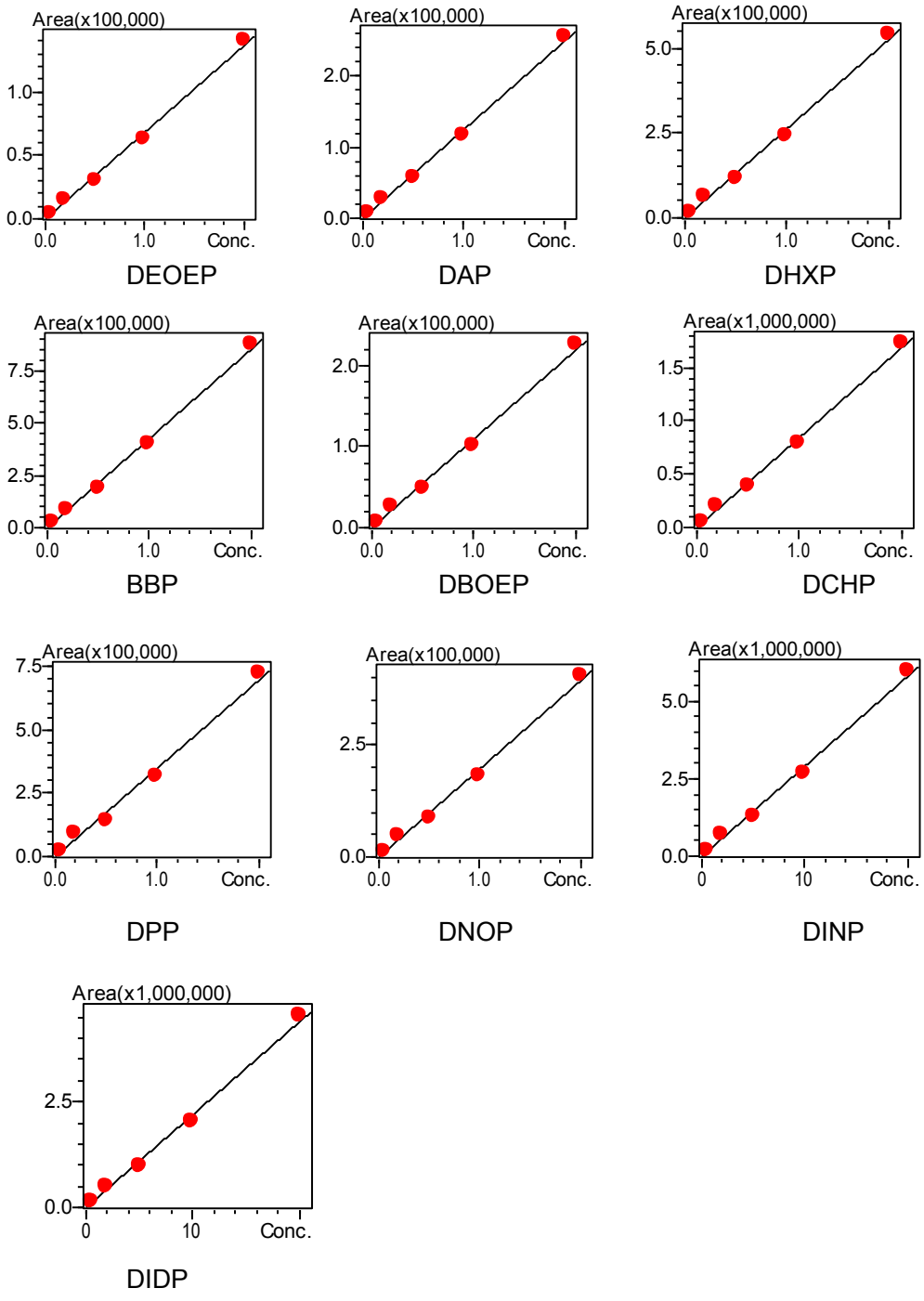


Fig. 3 Calibration curves of 19 PAEs

Table 2 Correlation coefficients of calibration curves and LODs of 19 PAEs

No.	Compound	Correlation Coefficient	LOD (μg/L)	No.	Compound	Correlation Coefficient	LOD (μg/L)
1	DMP	0.999	0.09	11	DAP	0.998	2.30
2	DEP	0.999	0.82	12	DHXP	0.998	1.11
3	DIPP	0.999	0.91	13	BBP	0.998	9.69
4	DALP	0.997	22.56	14	DBOEP	0.997	6.11
5	DPRP	0.999	1.44	15	DCHP	0.998	1.17
6	DIBP	0.999	3.22	16	DPP	0.995	2.50
7	DBP	0.999	4.53	17	DNOP	0.998	3.49
8	DMOEP	0.999	7.23	18	DINP	0.998	10.49
9	BMPP	0.998	1.49	19	DIDP	0.998	96.90
10	DEOEP	0.999	2.15				

Recovery

One of the market off-the-shelf milk powder product was selected to perform recovery test with the spiked level of PAEs at 120 μg/kg. Three replicate samples were prepared according to the afore-mentioned pretreatment procedures and processed for GCMSMS analysis. The results are as shown in Table 3.

Table 3 Recovery and reproducibility

No.	Compound	Recovery 1 (%)	Recovery 2 (%)	Recovery 3(%)	Average recovery (%)	%RSD
1	DMP	104.62	109.36	105.86	106.61	2.31
2	DEP	112.10	118.43	116.73	115.75	2.83
3	DIPP	97.48	102.28	99.92	99.89	2.40
4	DALP	94.91	100.20	101.74	98.95	3.62
5	DPRP	94.09	98.52	96.43	96.35	2.30
6	DIBP	122.52	127.78	125.38	125.23	2.11
7	DBP	111.81	117.14	115.06	114.67	2.34
8	DMOEP	91.12	94.88	94.18	93.39	2.14
9	BMPP	108.32	111.80	106.48	108.87	2.48
10	DEOEP	99.99	93.93	97.48	97.13	3.13
11	DAP	107.52	111.91	111.50	110.31	2.20
12	DHXP	111.34	116.40	115.55	114.43	2.37
13	BBP	101.44	105.88	104.83	104.05	2.23
14	DBOEP	107.03	110.26	112.75	110.01	2.61
15	DCHP	115.33	119.96	118.27	117.85	1.99

16	DPP	114.48	120.72	119.82	118.34	2.85
17	DNOP	114.41	120.48	118.70	117.86	2.65
18	DINP	109.93	115.45	111.61	112.33	2.52
19	DIDP	118.22	116.13	115.06	116.47	1.38

Repeatability test results

Five successive injections were performed using 0.2 mg/L standard samples, and the repeatability of peak area in the 5 assays is shown in Table 4.

Table 4 Repeatability of peak area

No.	Compound	1	2	3	4	5	RSD (%)
1	DMP	203283	195996	203049	201295	200028	0.75
2	DEP	158473	152674	159272	158140	156554	0.86
3	DIPP	33776	32642	34189	33752	33414	1.15
4	DALP	30875	30678	30994	30523	30116	1.44
5	DPRP	44948	43630	45337	45115	44595	0.85
6	DIBP	42150	40829	42363	41836	41400	1.15
7	DBP	43815	42228	43632	43369	42978	0.76
8	DMOEP	8540	8206	8442	8428	8205	1.59
9	BMPP	107088	104122	113537	111264	111619	1.09
10	DEOEP	15301	14706	15017	14780	14636	1.30
11	DAP	28301	27304	28057	27853	27523	0.97
12	DHXP	63541	61817	63606	62802	62135	1.17
13	BBP	91845	88408	91335	90651	89398	1.09
14	DBOEP	26015	25344	26320	26133	25982	0.65
15	DCHP	207140	199729	206985	204798	203283	0.91
16	DPP	93220	90353	94339	94211	93272	0.62
17	DNOP	48926	47179	49546	49362	49434	0.19
18	DINP	659585	672157	678200	650707	668354	2.09
19	DIDP	473955	471925	444650	447010	401968	5.88

CONCLUSION

The proposed method using Shimadzu GCMS-TQ8030 is easy to operate for analyzing 19 PAEs in milk powder. The calibration curves of this method shows good linearity for the concentration range of 0.05~2.00 mg/L. The method's LODs for the PAEs found to be in the range of 0.09~96.90 µg/L and the average recovery of spiked samples are within the range of 93%~125%.

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C-18

GC-MS
Gas Chromatograph Mass Spectrometer

Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) in Foods by GC-MS/MS

INTRODUCTION

In recent years, there has been a trend toward viewing high concentrations of polycyclic aromatic hydrocarbons (PAHs) in foods (particularly smoked products) as a problem. In 2009, the Codex Alimentarius Commission issued standards for reducing the PAH contamination level in smoked and directly dried foods. In addition, Europe, Canada, South Korea, China, and others established food standards for benzo[a]pyrene. This Application Data Sheet describes using a GC-MS/MS system to analyze the PAHs in "katsuobushi" (dried, smoked, and fermented skipjack tuna, which is very popular in Japanese cuisine).

EXPERIMENTAL

An extract solution from pretreated katsuobushi was injected into the GC-MS/MS system. The analysis conditions are shown in Table 1. MS/MS measurement program was created by Smart MRM in GCMSsolution automatically.

Table 1 Analysis Conditions

GC-MS:	GCMS-TQ8040
Column:	Rxi-PAH (60 m long, 0.25 mm I.D., df = 0.1 μm) (RESTEK, P/N: 49317)
Glass Insert:	Splitless insert with wool (Shimadzu, P/N: 221-48876-03)
[GC]	
Injection Volume:	2 μL
Injection Unit Temp.:	330 °C
Column Oven Temp.:	180 °C (2 min) → (5 °C /min) → 260 °C → (15 °C /min) → 350 °C (12 min)
Carrier Gas Control:	Constant linear velocity (40.0 cm/sec)
Injection Mode:	Splitless
Sampling Time:	2 min
Carrier Gas:	Helium

[MS]	
Interface Temp.:	330 °C
Ion Source Temp.:	230 °C
Measurement Mode:	MRM
Loop Time:	0.3 sec

MRM monitoring m/z:

Compound Name	Quantitative Transition Precursor>Product	CE(V)	Qualitative Transition Precursor>Product	CE(V)
Benzo[c]fluorene	216.10>215.10	22	216.10>189.10	30
Benzo[a]anthracene	228.10>226.10	28	228.10>202.10	26
Cyclopenta[c,d]pyrene	226.10>224.10	38	226.10>200.10	30
Chrysene	228.10>226.10	28	228.10>202.10	26
5-Methylchrysene	242.10>239.10	32	242.10>215.10	22
Benzo[b]fluoranthene	252.10>250.10	28	252.10>226.10	30
Benzo[k]fluoranthene	252.10>250.10	30	252.10>226.10	30
Benzo[j]fluoranthene	252.10>250.10	30	252.10>226.10	30
Benzo[a]pyrene	252.10>250.10	30	252.10>226.10	24
Dibenzo[a,h]anthracene	278.10>276.10	30	278.10>252.10	30
Indeno[1,2,3-cd]pyrene	276.10>274.10	34	276.10>250.10	30
Benzo[g,h,i]perylene	276.10>274.10	32	276.10>275.10	28
Dibenzo[a,l]pyrene	302.10>300.10	36	302.10>298.10	60
Dibenzo[a,e]pyrene	302.10>300.10	36	302.10>276.10	28
Dibenzo[a,i]pyrene	302.10>300.10	36	302.10>276.10	28
Dibenzo[a,h]pyrene	302.10>300.10	36	302.10>276.10	28
D12-Benzo[a]anthracene	240.20>236.20	28		
D12-Chrysene	240.20>236.20	28		
D12-Benzo[b]fluoranthene	264.20>260.20	32		
D12-Benzo[k]fluoranthene	264.20>260.20	32		
D12-Benzo[a]pyrene	264.20>260.20	32		
D14-Dibenzo[a,h]anthracene	292.20>288.20	34		
D12-Indeno[1,2,3-cd]pyrene	288.20>284.20	38		
D12-Benzo[g,h,i]perylene	288.20>284.20	38		
D14-Dibenzo[a,i]pyrene	316.20>312.20	40		

RESULTS

A standard sample was measured to calculate the lower limit of detection (see Table 2). For comparison purposes, the lower limit of detection values for a GC/MS (SIM) system are also indicated to the left. MRM allows achieving the lower limit of detection lower than SIM.
Fig. 1 shows chromatograms obtained from analyzing the katsuobushi extract. These results demonstrate that for actual samples containing contaminants, MRM, which offers superior separation, can provide better peak detection.

Table 2 Detection Limits

Compound Name	Detection Limit (concentration: pg/µl)	
	GC/MS(SIM)	GC/MS/MS(MRM)
Benzo[c]fluorene	0.028	0.036
Benz[a]anthracene	0.137	0.134
Cyclopenta[cd]pyrene	0.109	0.105
Chrysene	0.209	0.068
5-Methylchrysene	0.323	0.098
Benzo[b]fluoranthene	0.143	0.058
Benzo[k]fluoranthene	0.158	0.080
Benzo[j]fluoranthene	0.172	0.075
Benzo[a]pyrene	0.159	0.029
Indeno[1,2,3-cd]pyrene	0.075	0.011
Dibenzo[a,h]anthracene	0.063	0.032
Benzo[ghi]perylene	0.086	0.050
Dibenzo[a,l]pyrene	0.271	0.035
Dibenzo[a,e]pyrene	0.017	0.017
Dibenzo[a,i]pyrene	0.178	0.086
Dibenzo[a,h]pyrene	0.076	0.035

Note: Detection limits (3.3 σ) were calculated based on the standard deviation from consecutive analyses (n = 3) of 0.5 pg/µL standard samples.

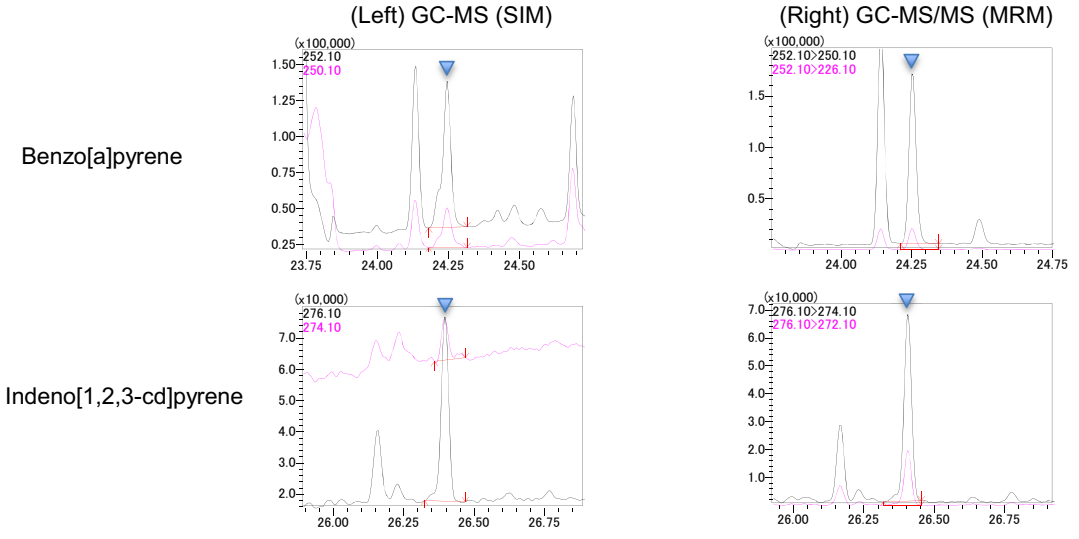


Fig. 1 SIM and MRM Chromatograms of PAHs in Katsuobushi Extract

CONCLUSION

This Application Data Sheet describes using a GC-MS/MS system to analyze polycyclic aromatics in food. The results showed that the GC-MS/MS system provided better sensitivity and peak separation than conventional GCMS methods.

C-19 Determination of 20 phthalate plasticizers in chewing gum by GC/MS/MS

INTRODUCTION

In this paper, a method is proposed for determination of 20 phthalate plasticizers in chewing gum with Shimadzu GCMS-TQ8030 gas chromatography-triple quadrupole mass spectrometer. Chewing gum was cut into small pieces and soaked in orthodichlorobenzene four hours for extraction. The extract was then filtered and used for analysis. The results showed that the method is having good linearity in the concentration range of 0.50~10.00 µg/mL with a correlation coefficient (R^2) of 0.999. The LODs of the phthalates, calculated as 3 times of signal-to-noise ratio, were in the range of 0.22~13.64 µg/L. The %RSDs of peak areas (n=5) of the phthalates were all less than 5%. The recoveries of samples spiked with standards were in the range of 87%~118%. The proposed method has merits such as simple pretreatment and fast analysis speed, making it suitable for the determination of 20 phthalate plasticizers in chewing gum.

Chewing gums, a type of gum-base candy, are chewing candies made of water insoluble vegetable gum and sweetener and flavors. Gum base is an important ingredient of chewing gum. With the expansion of production and market size, natural gum falls short of needs and is basically replaced by synthetic resin. In spite of its tenacity and elasticity, synthetic resin requires softener or plasticizer to mimic the plasticity and chewability of natural gum. Unfortunately, some plasticizers (DEHP in particular) are environment hormones for their endocrine interfering actions. According to available toxicological studies carried out by many countries on DEHP, the substance has a variety of toxicity, mainly reproductive toxicity, developmental toxicity, mutagenicity, carcinogenicity, endocrine toxicity, and immunotoxicity. Therefore, prolonged chewing of chewing gum may pose elevated safety risks to consumers, especially pregnant women or children, because of the intake of excessive plasticizer.
So far, few reports are publicly available on the determination of plasticizers in chewing gum. In addition, the lack of methodology and MRL standards also contributes to the inconvenience in regulating the industry, enforcing laws, and protecting consumer rights. Furthermore, a lot of plasticizers are of similar structure and hard to be quantitatively determined by ordinary GC or single-stage mass spectrometry because of the matrix interference of chewing gum. Based on the above consideration, a method is proposed for the determination of 20 phthalate plasticizers in chewing gum with Shimadzu GCMS-TQ8030 triple quadrupole mass spectrometer. The method is highly sensitive and easy to operate.

EXPERIMENTAL

Apparatus

GC-MS/MS: GCMS-TQ8030

Conditions of Analysis

Carrier gas	: Helium
CID gas	: Argon
Injection port temperature	: 250 °C
Injection mode	: Splitless (1 min)
Carrier gas control mode	: CLV(36.5 cm/sec)
Column	: Rxi-5Sil MS, 30 m × 0.25 mm × 0.25 µm
Column temperature program	: 60 °C(1 min)→@20 °C/min→220 °C(1 min) →@5 °C/min→280 °C(5 min)
Interface temperature	: 280 °C
Temperature of ion source	: 230 °C
Acquisition mode	: MRM

Pretreatment of samples

Chewing gum was cut into small pieces and mixed evenly. 0.30 g of the cut chewing gum was accurately weighed and transferred to a 10 mL stopper test tube, into which 2 mL of orthodichlorobenzene was added. The system was subjected to ultrasonic extraction for 5 min, allowed to rest for 4h, then filtered and used for analysis. Samples with high concentration of phthalates would be further diluted before analysis.

RESULTS AND DISCUSSION

MRM chromatogram

The MRM chromatograms of multi-standard solutions of PAEs are shown in Fig. 1, and the MRM parameters are listed in Table1.

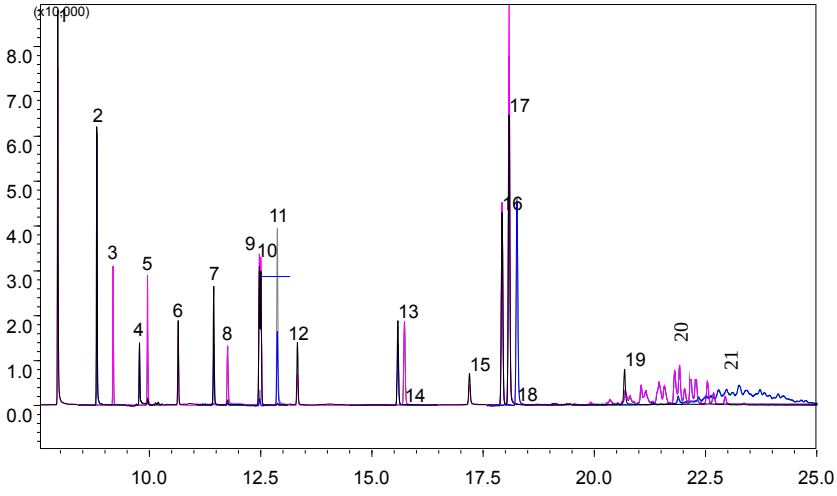


Fig.1 MRM chromatograms of PAEs (0.10 μg/mL)

Table 1 MRM parameters of PAEs

No.	Compound	Abbr.	CAS No.	Quantitative transition	CE	Qualitative transition	CE
1	Dimethylphthalate	DMP	131-11-3	163.0>77.0	23	194.0>163.0	7
2	Diethyl phthalate	DEP	84-66-2	177.0>149.0	10	222.0>149.0	15
3	Di-iso-propyl-phthalate	DIPP	605-45-8	209.0>149.0	10	167.0>149.0	15
4	Di-allyl phthalate	DALP	131-17-9	132.0>104.0	10	189.0>105.0	10
5	Di-n-propyl phthalate	DPRP	131-16-8	209.0>149.0	7	191.0>149.0	17
6	Diisobutyl phthalate	DIBP	84-69-5	223.0>149.0	8	205.0>149.0	5
7	Dibutylphthalate	DBP	84-74-2	223.0>149.0	10	205.0>149.0	5
8	Bis(methylglycol) phthalate	DMOEP	117-82-8	207.0>59.0	10	176.0>149.0	5
9	Bis(4-methyl-2-pentyl)phthalate	DMPP1	146-50-9	167.0>149.0	5	251.0>149.0	10
10	Bis(4-methyl-2-pentyl)phthalate	DMPP2	146-50-9	167.0>149.0	10	251.0>149.0	20
11	Bis(2-Ethoxyethyl) phthalate	DEOEP	605-54-9	176.0>149.0	10	176.0>149.0	20
12	Diisoamyl phthalate	DAP	605-50-5	237.0>149.0	25	219.0>149.0	10
13	Dihexylphthalate	DHXP	84-75-3	251.0>149.0	10	233.0>149.0	5
14	Butyl benzyl phthalate	BBP	85-68-7	206.0>149.0	15	238.0>104.0	5
15	Bis(2-n-butoxyethyl)phthalate	DBOEP	117-83-9	193.0>149.0	10	176.0>149.0	20
16	Dicyclohexyl phthalate	DCHP	84-61-7	167.0>149.0	15	249.0>149.0	10
17	Diethylhexyl phthalate	DEHP	117-81-7	167.0>149.0	10	279.0>149.0	15
18	Dioctyl phthalate	DPP	131-18-0	225.0>77.0	10	225.0>141.0	15
19	Di-n-octyl phthalate	DNOP	117-84-0	279.0>149.0	25	279.0>71.0	20
20	Dinonyl Phthalate	DINP	84-76-4	293.0>149.0	12	293.0>167.0	17
21	Di-iso-decyl phthalate	DIDP	26761-40-0	307.0>149.0	10	307.0>167.0	5

Calibration curve

Multi-standard working solutions of PAEs were prepared at concentrations of 0.5, 1.0, 2.0, 4.0, 6.0, and 10.0 μg/mL, respectively. Linearity was performed with concentration as abscissa and peak area as ordinate. Representative calibration curve of DEHP is shown in Fig. 2.

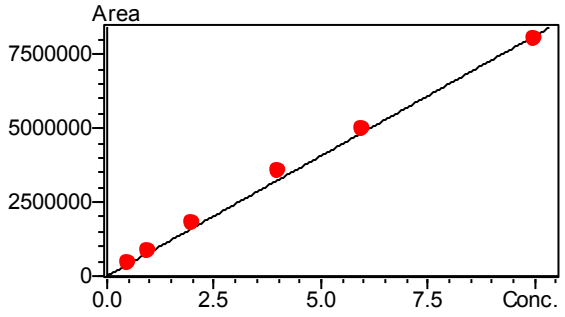


Fig. 2 Calibration curve of DEHP

Correlation coefficients, LODs, and repeatability

Based on the data of 0.01 μg/mL standard solution, the method's LODs were calculated (as 3 times of signal-to-noise ratio). Repeatability test was performed by 5 successive injections for analysis. The results were as listed in Table 2.

Table 2 Correlation coefficients and LODs of the compositions (μg/L)

No.	Compound Name	Correlation Coefficient	LOD (μg/L)	RSD (%)	No.	Compound Name	Correlation Coefficient	LOD (μg/L)	RSD (%)
1	DMP	0.999	0.27	0.70	12	DAP	0.999	0.57	1.12
2	DEP	0.999	0.32	0.86	13	DHXP	0.999	0.83	1.23
3	DIPP	0.999	0.40	0.55	14	BBP	0.999	0.91	1.31
4	DALP	0.999	6.99	2.44	15	DBOEP	0.999	1.42	1.01
5	DPRP	0.999	0.36	0.85	16	DCHP	0.999	0.68	0.41
6	DIBP	0.999	0.55	1.18	17	DEHP	0.999	0.29	0.07
7	DBP	0.999	0.41	1.76	18	DPP	0.999	0.40	0.86
8	DMOEP	0.999	2.53	1.95	19	DNOP	0.999	1.43	1.27
9	DMPP1	0.999	0.29	1.69	20	DINP	0.999	11.11	2.40
10	DMPP2	0.999	0.22	1.36	21	DIDP	0.999	13.64	3.56
11	DEOEP	0.999	1.96	0.97					

Recovery test

An off-the-shelf chewing gum product was selected for the recovery test. The chewing gum was subjected to the afore-mentioned pretreatment procedures. The spiked samples at spiked level of 1.50 mg/kg were analyzed for recovery using same method. The results are shown in Table3.

Table 3 Recovery assay results (%)

No.	Compound	Recovery	No.	Compound	Recovery
1	DMP	81.36	12	DAP	97.00
2	DEP	88.48	13	DHXP	92.75
3	DIPP	87.58	14	BBP	103.63
4	DALP	80.99	15	DBOEP	96.07
5	DPRP	105.12	16	DCHP	90.84
6	DIBP	114.71	17	DEHP	109.38
7	DBP	102.87	18	DPP	118.17
8	DMOEP	93.90	19	DNOP	105.38
9	DMPP1	87.05	20	DINP	97.22
10	DMPP2	90.77	21	DIDP	89.67
11	DEOEP	95.59			

Sample assay results

Two off-the-shelf chewing gum products were selected, subjected to the afore-mentioned pretreatment procedures, and then injected for analysis, yielding the following quantification results (Table 4).

Table 4 Quantification results of samples (mg/kg)

No.	Compound	1#	2#	No.	Compound Name	1#	2#
1	DMP	0.30	N.D.	12	DAP	N.D.	0.03
2	DEP	0.36	0.05	13	DHXP	N.D.	0.05
3	DIPP	N.D.	N.D.	14	BBP	N.D.	N.D.
4	DALP	N.D.	N.D.	15	DBOEP	N.D.	0.21
5	DPRP	N.D.	N.D.	16	DCHP	N.D.	0.11
6	DIBP	0.49	0.15	17	DEHP	329.08	1.05
7	DBP	0.38	0.20	18	DPP	N.D.	0.10
8	DMOEP	N.D.	N.D.	19	DNOP	N.D.	N.D.
9	DMPP1	N.D.	0.04	20	DINP	N.D.	0.17
10	DMPP2	N.D.	0.04	21	DIDP	N.D.	0.11
11	DEOEP	N.D.	N.D.				

N.D.: not detected

CONCLUSION

A method is proposed for determination of phthalate plasticizers in chewing gum with Shimadzu GCMS-TQ8030 gas chromatography-triple quadrupole mass spectrometer. The method is easy to operate and demonstrated good linearity in the concentration range of 0.50~10.00 μg/mL with a correlation coefficient (R^2) of

0.999. The method's LODs (calculated as 3 times of signal-to-noise ratio) is in the range of 0.22~13.64 µg/L. The recoveries of samples spiked with standards were in the range of 87%~118%. It is suitable for risk monitoring of the 20 phthalate plasticizers in chewing gum.

C-20 Fast analysis of 54 pesticide residues in vegetable by PTV-GC-MS/MS

INTRODUCTION

A method is proposed for fast analysis of 54 pesticide residues in vegetable by PTV-GC-MS/MS using QuEChERS extraction. The results demonstrated satisfactory linearity for the 54 pesticide residues in 0.5 µg/L to 100 µg/L concentration range with a correlation coefficient (R^2) greater than 0.991. The recoveries of 3 different sample matrix spiked with pesticide standards at concentrations of 10 µg/kg, 20 µg/kg, and 100 µg/kg were all in the range of 70%~130%. The %RSDs were found to be between 0.3%~19.6% and LODs were within the range of 2.0~10.0 µg/kg. The results showed that the method was simple, fast, sensitive, accurate, and rugged. It is suitable for fast screening of pesticide multi-residues in batch samples.

QuEChERS method has become a most commonly used pretreatment method in determination of pesticide residues in vegetable because it is quick, easy, cheap, rugged, and safe.

In this paper, an analytical method is proposed for fast quantitative analysis of 54 pesticide residues in vegetable by PTV-GC-MS/MS with QuEChERS pretreatment. The splitless injection mode of PTV injection port can remove acetonitrile at the time of injection at low temperature, thereby improving the life of chromatographic column. Furthermore, compared with single quadrupole mass spectrometry, triple quadrupole mass spectrometry is has merits of removing matrix interference and high ion transport efficiency in MRM mode. Thus MSMS enables accurate identification of target compounds even in complex matrix background. The method is simple, fast, sensitive, reliable and rugged which meets regulatory requirements for determination of pesticide residues in vegetable.

EXPERIMENTS

Apparatus

Triple Quadrupole GC-MS:
GCMS-TQ8030 (equipped with PTV injection port)

Conditions of Analysis

Column : Rxi-5 Sil ms (30 m×0.25 mm×0.25 μm)
Injection temperature program : 65 °C(1 min)→@(200 °C/min)
→250 °C(15 min)

Split valve program:

0-0.9 min	Split 20:1
0.9-3.5 min	Splitless
3.5 min	Split 20:1

Column temperature program : 40 °C(4 min)→@(25 °C/min)→125 °C→
@(10 °C/min)→300 °C(5 min)
CLV mode : 36.2 cm/sec
Sample size : 2 μL
Interface temperature : 280 °C
Temperature of ion source : 200 °C
Detector voltage : Tuning voltage +0.6 kV
Solvent delay time : 7 min
Acquisition mode : MRM listed in Table

Pretreatment of samples

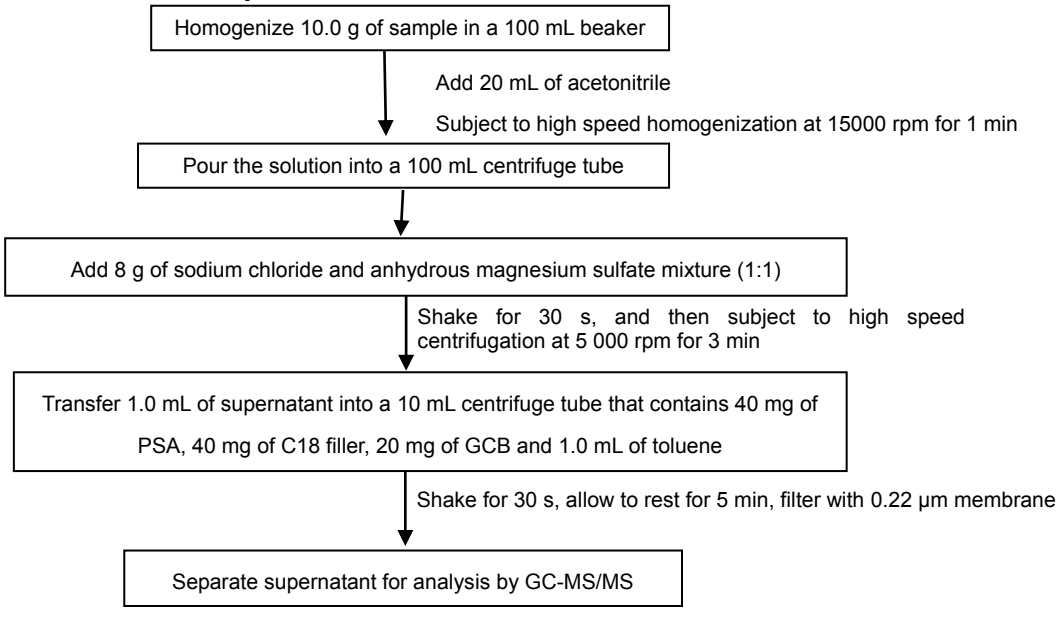


Fig.1 Sample pretreatment

Table 1 Retention time and MRM parameters of pesticides

No.	Compound	CAS No.	Retention Time	Quantitative transition	CE	Qualitative transition	CE
1	Methamidophos	10265 - 92 - 6	9.667	141.0>95.0	8	141.0>126.0	4
2	Dichlorvos	62 - 73 - 7	9.853	185.0>93.0	14	185.0>109.0	14
3	Omethoate	1113 - 02 - 6	13.686	156.0>110.0	8	156.0>141.0	4
4	Ethoprophos	13194 - 48 - 4	14.099	200.0>158.0	6	200.0>114.0	14
5	Monocrotophos	6923 - 22 - 4	14.593	127.1>109.0	12	127.1>95.0	16
6	Sulfotep	3689 - 24 - 5	14.627	322.0>202.0	10	322.0>294.0	4
7	Phorate	298 - 02 - 2	14.776	260.0>75.0	8	260.0>231.0	4
8	alpha-HCH	319 - 84 - 6	14.948	218.9>182.9	8	218.9>144.9	20
9	Dimethoate	60 - 51 - 5	15.180	125.0>79.0	8	125.0>47.0	14
10	beta-HCH	319 - 85 - 7	15.500	218.9>182.9	8	218.9>144.9	20
11	gamma-HCH	58 - 89 - 9	15.635	218.9>182.9	8	218.9>144.9	20
12	Terbufos	13071 - 79 - 9	15.645	231.0>174.9	14	231.0>128.9	26
13	Quintozene	82 - 68 - 8	15.730	294.8>236.8	16	294.8>264.8	12
14	Fonofos	944 - 22 - 9	15.762	246.0>109.1	18	246.0>137.1	6
15	Pyrimethanil	53112 - 28 - 0	15.830	198.1>183.1	14	198.1>158.1	18
16	Diazinon	333 - 41 - 5	15.836	304.1>179.1	10	304.1>162.1	8
17	Phosphamidon-1	13171 - 21 - 6	15.909	264.1>127.1	14	264.1>193.1	8
18	delta-HCH	319 - 86 - 8	16.115	218.9>182.9	10	218.9>144.9	20
19	Phosphamidon-2	13171 - 21 - 6	16.651	264.1>127.1	14	264.1>193.1	8
20	Vinclozolin	50471 - 44 - 8	16.841	285.0>212.0	12	285.0>178.0	14
21	Parathion-methyl	298 - 00 - 0	16.874	263.0>109.0	14	263.0>136.0	8
22	Fenitrothion	122 - 14 - 5	17.405	277.0>260.0	6	277.0>109.1	14
23	Malathion	121 - 75 - 5	17.560	173.1>99.0	14	173.1>127.0	6
24	Fenthion	55 - 38 - 9	17.775	278.0>109.0	20	278.0>125.0	20
25	Chlorpyrifos	2921 - 88 - 2	17.806	313.9>257.9	14	313.9>285.9	8
26	Parathion	56 - 38 - 2	17.829	291.1>109.0	14	291.1>137.0	6
27	Dicofol deg.	0 - 00 - 0	17.869	250.0>139.0	14	250.0>215.0	8
28	Triadimefon	43121 - 43 - 3	17.906	208.1>181.0	10	208.1>127.0	14
29	Isocarbophos	24353 - 61 - 5	17.952	289.1>136.0	14	289.1>113.0	6
30	Isofenphos-methyl	83733-82-8	18.301	199.0>121.0	14	241.1>121.1	22
31	Fipronil	120068 - 37 - 3	18.578	366.9>212.9	30	366.9>254.9	22
32	Phosfolan	947-02-4	18.579	255.0>227.0	6	255.0>140.0	22
33	Phenthoate	2597 - 03 - 7	18.673	273.9>125.0	20	273.9>246.0	6
34	Quinalphos	13593 - 03 - 8	18.674	157.1>129.0	14	157.1>93.0	10
35	Procymidone	32809 - 16 - 8	18.805	283.0>96.0	10	283.0>255.0	12

36	Methidathion	950 - 37 - 8	18.984	145.0>85.0	8	145.0>58.0	14
37	alpha-Endosulfan	959 - 98 - 8	19.268	338.9>160.0	18	338.9>266.9	8
38	Profenofos	41198 - 08 - 7	19.543	336.9>266.9	14	336.9>308.9	6
39	beta-Endosulfan	33213 - 65 - 9	20.362	338.9>160.0	18	338.9>266.9	8
40	Triazophos	24017 - 47 - 8	20.710	257.0>162.0	8	257.0>134.0	22
41	Iprodione	36734 - 19 - 7	21.797	314.0>245.0	12	314.0>56.0	22
42	Bifenthrin	82657 - 04 - 3	21.969	181.1>166.1	12	181.1>153.1	8
43	Phosmet	732 - 11 - 6	22.041	160.0>133.0	14	160.0>77.0	24
44	Fenpropathrin	39515 - 41 - 8	22.121	265.1>210.1	12	265.1>172.1	14
45	Phosalone	2310 - 17 - 0	22.711	182.0>111.0	14	182.0>138.0	8
46	Cyhalothrin-1	68085 - 85 - 8	22.747	197.0>161.0	8	197.0>141.0	12
47	Cyhalothrin-2	68085 - 85 - 8	22.941	197.0>161.0	8	197.0>141.0	12
48	Permethrin-1	52645 - 53 - 1	23.716	183.1>168.1	14	183.1>165.1	14
49	Permethrin-2	52645 - 53 - 1	23.834	183.1>168.1	14	183.1>165.1	14
50	Pyridaben	96489 - 71 - 3	23.895	147.1>117.1	22	147.1>132.1	14
51	Coumaphos	56-72-4	24.000	362.0>109.0	16	362.0>226.0	14
52	Cyfluthrin-1	68359 - 37 - 5	24.276	226.1>206.1	14	226.1>199.1	6
53	Cyfluthrin-2	68359 - 37 - 5	24.356	226.1>206.1	14	226.1>199.1	6
54	Cyfluthrin-3,4	68359 - 37 - 5	24.470	226.1>206.1	14	226.1>199.1	6
55	Cypermethrin-1	52315 - 07 - 8	24.597	163.1>127.1	6	163.1>91.0	14
56	Cypermethrin-2	52315 - 07 - 8	24.685	163.1>127.1	6	163.1>91.0	14
57	Cypermethrin-3,4	52315 - 07 - 8	24.780	163.1>127.1	6	163.1>91.0	14
58	Flucythrinate-1	70124 - 77 - 5	24.794	199.1>157.1	10	199.1>107.1	22
59	Flucythrinate-2	70124 - 77 - 5	24.987	199.1>157.1	10	199.1>107.1	22
60	Fenvalerate-1	51630 - 58 - 1	25.622	419.1>225.1	6	419.1>167.1	12
61	Fluvalinate-1	69409 - 94 - 5	25.795	250.1>55.0	20	250.1>200.0	20
62	Fenvalerate-2	51630 - 58 - 1	25.854	419.1>225.1	6	419.1>167.1	12
63	Fluvalinate-2	69409 - 94 - 5	25.860	250.1>55.0	20	250.1>200.0	20
64	Difenoconazole-1	119446 - 68 - 3	26.189	323.0>265.0	14	323.0>202.0	28
65	Difenoconazole-2	119446 - 68 - 3	26.276	323.0>265.0	14	323.0>202.0	28
66	Deltamethrin-1	52918 - 63 - 5	26.289	252.9>93.0	20	252.9>171.9	8
67	Deltamethrin-2	52918 - 63 - 5	26.570	252.9>93.0	20	252.9>171.9	8

RESULT AND DISCUSSION

Calibration curve

A series of multi-standard solutions of 54 pesticides were prepared at concentrations of 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, and 100.0 µg/L with blank spinach extract (subjected to the pretreatment procedures as described in 1.3), respectively. Regression curves were plotted with peak area (Y) vs. concentration (X, µg/L), yielding regression equations Y=aX+b and linear correlation coefficients for the 54 pesticides as shown in Table 2. The calibration curves of some pesticide residues are shown in Fig.2 below.

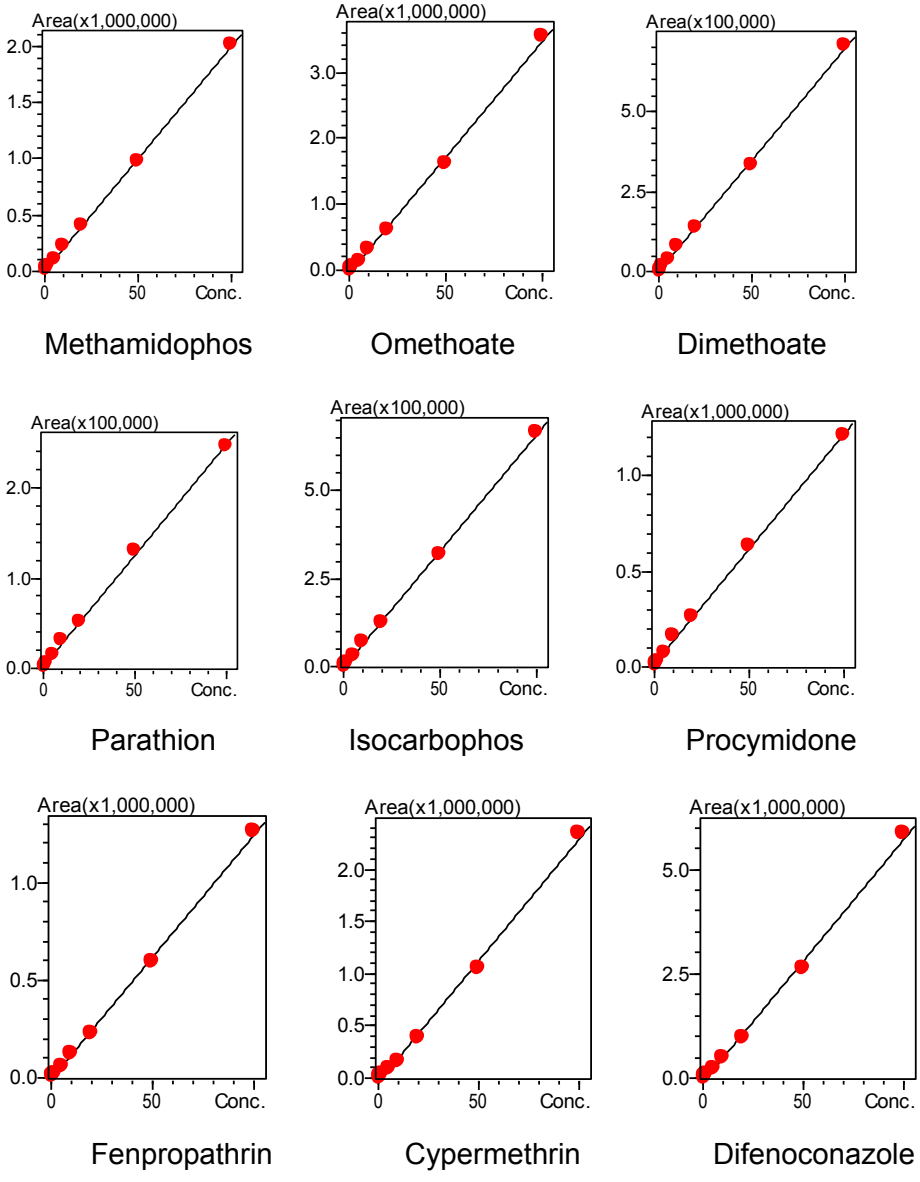


Fig.2 Calibration curves of some of the pesticides

Table 2 Linear correlation coefficients of the compositions

No.	Compound	Correlation Coefficient (R^2)	No.	Compound	Correlation Coefficient (R^2)	No.	Compound	Correlation Coefficient (R^2)
1	Methamidophos	0.999	19	Parathion-methyl	0.997	37	Iprodione	0.999
2	Dichlorvos	0.999	20	Fenitrothion	0.998	38	Bifenthrin	0.994
3	Omethoate	0.998	21	Malathion	0.995	39	Phosmet	0.998
4	Ethoprophos	0.986	22	Fenthion	0.99	40	Fenpropath	0.999
5	Monocrotophos	0.998	23	Chlorpyrifos	0.995	41	Phosalone	0.998
6	Sulfotep	0.999	24	Parathion	0.999	42	Pyridaben	0.998
7	Phorate	0.999	25	Triadimefon	0.999	43	Coumapho	0.998
8	α-BHC	0.999	26	Dicofol	0.998	44	Phosphami	0.999
9	Dimethoate	0.999	27	Isocarbophos	0.999	45	Endosulfan	0.999
10	β-BHC	0.998	28	Isofenphos-meth	0.996	46	Cyhalothrin	0.996
11	γ-BHC	0.999	29	Fipronil	0.998	47	Permethrin	0.999
12	Terbufos	0.999	30	Phosfolan	0.998	48	Cyfluthrin	0.997
13	Quintozene	0.998	31	Phenthoate	0.996	49	Cypermethr	0.997
14	Fonofos	0.999	32	Quinalphos	0.998	50	Flucythrinat	0.996
15	Pyrimethanil	0.991	33	Procymidone	0.999	51	Fenvalerate	0.996
16	Diazinone	0.998	34	Methidathion	0.999	52	Fluvalinate	0.993
17	δ-BHC	0.996	35	Profenofos	0.999	53	Difenocona	0.997
18	Vinclozoline	0.999	36	Triazophos	0.999	54	Deltamethri	0.993

The method's LOQs, spiked recoveries and precision

Quantification of pesticides in sample matrix was carried out by means of external standard method. Three blank matrixes, i.e. spinach, cowpea, and Chinese chive, were subjected to the sample pretreatment procedures as described and spiked with 54 pesticides at spiked levels of 10, 20, and 100 μg/kg for recovery test. Five replicate assays were carried out for each spiked level. The recoveries of all 54 pesticides were in the range of 70%~130%, with %RSDs falling in the range of 0.3%~19.6%. Table 2 shows the average recoveries and %RSDs of spinach, cowpea, and Chinese chive matrix at the 3 spiked levels. Matrix spike tests were conducted in the experiment by adding multiple low level multi-standard solutions of pesticides to spinach blank matrix. The solutions were subjected to analysis by GC-MS/MS and the concentration of individual pesticide at which the S/N ratio was closest to 10 was taken as the pesticide's LOQ (n=3). The results are shown in Table 2. The method demonstrated LOQs in the range of 2.0~10.0 μg/kg, meeting the China's regulatory requirements on pesticide MRLs.

Table 3 Average recoveries and %RSDs of pesticides in spinach, cowpea, and Chinese chive matrixes at 3 spiked levels

No.	Compound	LOQ (μg/kg)	Recovery of spinach matrix (RSD/%)			Recovery of cowpea matrix (RSD/%)			Recovery of Chinese chive matrix (RSD/%)		
			10 μg/kg	20 μg/kg	100 μg/kg	10 μg/kg	20 μg/kg	100 μg/kg	10 μg/kg	20 μg/kg	100 μg/kg
1	Methamidophos	4.0	90.5 (6.9)	101.5 (7.4)	86.5 (5.7)	83.1 (8.2)	111.0 (4.8)	86.3 (2.8)	88.6 (1.2)	101.6 (5.4)	91.5 (6.4)
2	Dichlorvos	4.0	97.9 (2.9)	98.5 (3.9)	89.0 (6.4)	99.6 (7.9)	120.8 (2.1)	95.7 (3.8)	83.2 (3.6)	93.8 (5.6)	98.5 (4.2)
3	Omethoate	2.0	90.6 (2.2)	103.9 (4.1)	84.8 (6.6)	100.1 (4.4)	111.0 (2.8)	96.7 (1.2)	98.6 (6.0)	96.1 (5.2)	93.1 (6.5)
4	Ethoprophos	2.0	107.4 (6.0)	105.9 (6.4)	91.7 (6.9)	88.3 (3.1)	112.4 (5.5)	96.7 (3.1)	99.5 (4.2)	92.3 (4.4)	90.9 (11.2)
5	Monocrotophos	2.0	98.8 (3.4)	103.5 (7.2)	86.8 (6.6)	92.4 (8.7)	105.8 (4.0)	97.9 (1.3)	100.9 (6.2)	91.6 (4.2)	91.2 (7.9)
6	Sulfotep	8.0	92.6 (9.2)	102.4 (8.5)	94.3 (8.2)	87.2 (8.5)	107.3 (9.6)	93.5 (4.3)	90.6 (11.7)	92.8 (7.2)	88.1 (13.2)
7	Phorate	8.0	110.1 (7.5)	99.8 (12.4)	93.5 (7.9)	89.8 (4.2)	109.7 (7.6)	98.6 (5.6)	88.8 (5.4)	104.7 (9.9)	92.4 (8.7)
8	α-BHC	8.0	100. (6.2)	114.5 (3.4)	94.9 (7.7)	94.4 (5.5)	112.0 (4.8)	94.7 (1.9)	104.7 (4.0)	91.3 (4.2)	95.6 (6.9)
9	Dimethoate	8.0	116.2 (7.2)	112.7 (7.1)	93.9 (3.3)	94.4 (6.2)	112.2 (4.0)	97.1 (2.8)	98.6 (2.6)	90.6 (8.4)	92.5 (3.3)
10	β-BHC	8.0	85.6 (6.9)	103.4 (6.5)	93.5 (6.9)	78.3 (10.7)	120.9 (3.4)	95.1 (4.5)	105.0 (8.0)	88.5 (8.7)	96.6 (12.1)
11	γ-BHC	8.0	113.4 (8.6)	103.9 (7.8)	92.8 (7.5)	87.8 (7.7)	114.9 (8.0)	97.6 (1.7)	90.0 (15.5)	97.9 (9.7)	98.0 (5.3)
12	Terbufos	8.0	108.2 (6.8)	110.1 (8.5)	93.8 (6.8)	95.2 (8.7)	114.0 (8.7)	95.2 (3.5)	91.7 (6.4)	95.7 (3.2)	88.3 (8.5)
13	Quintozene	10.0	112.9 (17.1)	93.2 (9.6)	92.7 (5.6)	75.7 (9.4)	115.5 (7.0)	95.7 (6.9)	85.3 (19.6)	93.2 (11.3)	91.1 (9.1)
14	Fonofos	2.0	104.8 (3.2)	105.9 (5.9)	94.2 (8.2)	91.0 (5.9)	115.0 (7.6)	97.4 (3.4)	106.5 (2.6)	93.6 (6.6)	87.2 (6.9)
15	Pyrimethanil	4.0	100.0 (8.2)	109.2 (4.7)	95.5 (6.0)	83.3 (3.7)	104.3 (3.9)	97.3 (3.2)	106.6 (5.4)	85.5 (2.6)	94.5 (8.2)
16	Diazinone	10.0	87.0 (3.4)	121.4 (11.2)	94.3 (7.1)	87.9 (10.4)	98.6 (6.2)	92.0 (2.7)	82.3 (11.4)	84.5 (4.5)	88.8 (18.7)
17	δ-BHC	4.0	95.3 (6.9)	107.4 (3.2)	93.3 (6.4)	71.7 (2.2)	111.2 (2.3)	99.1 (2.6)	105.2 (5.4)	84.3 (7.8)	99.1 (11.5)
18	Vinclozoline	4.0	96.6 (5.5)	104.6 (6.1)	93.3 (6.8)	74.6 (3.2)	108.9 (1.2)	99.4 (3.9)	105.1 (4.5)	89.1 (8.3)	95.2 (13.0)

19	Parathion-methyl	2.0	115.4	101.3	87.9	82.4	112.6	94.9	102.8	90.9	86.2
			(3.7)	(3.5)	(6.2)	(4.5)	(2.7)	(3.8)	(4.0)	(6.1)	(14.5)
20	Fenitrothion	2.0	83.4	104.3	87.4	77.0	116.2	98.1	90.8	92.5	91.9
			(3.8)	(5.1)	(5.6)	(6.2)	(2.0)	(0.8)	(6.2)	(7.6)	(13.8)
21	Malathion	2.0	90.1	108.8	90.5	80.1	112.6	99.2	98.8	85.5	93.3
			(7.2)	(1.7)	(5.7)	(3.9)	(3.4)	(2.7)	(5.0)	(6.5)	(12.7)
22	Fenthion	2.0	91.4	102.6	87.6	82.3	101.2	98.7	100.1	94.0	95.1
			(17.1)	(6.0)	(5.2)	(5.9)	(6.9)	(2.0)	(5.0)	(7.0)	(10.5)
23	Chlorpyrifos	4.0	104.6	107.0	90.5	72.5	113.0	97.5	98.1	92.3	96.9
			(5.1)	(5.7)	(5.7)	(5.7)	(3.3)	(3.8)	(4.8)	(6.3)	(13.4)
24	Parathion	2.0	106.9	104.3	87.9	85.4	106.0	96.1	99.9	100.9	88.7
			(11.8)	(2.5)	(8.1)	(5.3)	(3.8)	(2.1)	(4.0)	(5.0)	(17.4)
25	Triadimefon	2.0	91.6	108.5	90.8	85.9	111.4	98.1	95.4	88.0	97.5
			(4.3)	(2.5)	(5.0)	(6.0)	(2.5)	(1.4)	(4.1)	(5.6)	(7.7)
26	Dicofol	2.0	96.7	106.4	88.8	91.9	109.1	99.2	104.9	96.1	97.3
			(5.1)	(2.7)	(5.0)	(7.0)	(2.2)	(1.8)	(4.0)	(3.8)	(7.1)
27	Isocarbophos	4.0	93.7	110.7	91.2	83.3	99.1	97.7	101.2	88.3	89.6
			(6.3)	(1.8)	(6.1)	(4.9)	(3.9)	(1.8)	(5.2)	(6.7)	(7.3)
28	Isofenphos-methyl	2.0	91.9	107.4	92.0	81.3	105.1	98.8	99.8	87.6	97.8
			(3.1)	(3.3)	(6.3)	(6.4)	(8.2)	(2.6)	(4.9)	(5.6)	(11.0)
29	Phosfolan	4.0	95.9	87.9	88.3	108.2	94.4	100.2	83.7	105.4	88.0
			(7.7)	(14.5)	(6.1)	(8.0)	(3.8)	(1.1)	(5.0)	(8.1)	(11.3)
30	Fipronil	2.0	97.5	104.2	85.3	101.9	102.4	99.0	106.5	91.6	90.0
			(2.7)	(4.8)	(4.4)	(6.2)	(3.1)	(1.1)	(0.7)	(5.7)	(5.8)
31	Quinalphos	10.0	113.1	106.6	93.9	95.7	108.7	97.9	103.0	93.8	96.7
			(4.6)	(1.2)	(5.3)	(10.9)	(1.7)	(2.4)	(1.7)	(3.2)	(6.2)
32	Phenthoate	2.0	93.0	105.7	93.8	80.8	104.9	98.2	101.2	90.6	98.1
			(5.3)	(3.6)	(6.2)	(5.2)	(3.2)	(2.9)	(4.4)	(6.0)	(12.9)
33	Procymidone	2.0	99.5	101.6	89.1	90.8	104.6	96.9	94.7	90.3	95.5
			(5.7)	(3.0)	(3.4)	(4.8)	(3.3)	(1.9)	(4.2)	(4.6)	(6.1)
34	Methidathion	2.0	123.4	110.6	92.2	99.7	104.0	97.3	106.6	92.4	96.4
			(3.1)	(3.1)	(4.0)	(5.3)	(2.1)	(1.5)	(5.1)	(8.1)	(4.3)
35	Profenofos	2.0	98.4	103.7	88.8	99.5	102.8	99.2	102.0	93.0	95.0
			(2.7)	(2.7)	(4.7)	(4.9)	(1.4)	(1.2)	(1.9)	(4.6)	(4.6)
36	Triazophos	2.0	100.4	102.4	91.2	100.0	97.9	99.4	100.4	92.1	97.3
			(4.3)	(2.5)	(3.5)	(5.2)	(3.7)	(0.8)	(0.8)	(3.2)	(5.0)
37	Iprodione	2.0	96.1	107.6	85.6	98.2	102.3	102.3	94.3	90.0	91.9
			(6.8)	(3.9)	(4.0)	(6.6)	(6.1)	(2.3)	(2.8)	(2.0)	(5.8)
38	Bifenthrin	2.0	96.4	104.2	93.0	106.5	104.4	98.8	98.8	90.2	98.1
			(3.7)	(3.2)	(2.9)	(6.2)	(4.6)	(1.6)	(2.3)	(2.0)	(4.1)
39	Phosmet	2.0	97.8	102.4	91.1	104.5	106.2	99.5	88.3	81.6	97.7
			(2.6)	(4.7)	(3.7)	(12.0)	(8.0)	(1.4)	(3.2)	(5.5)	(5.8)

40	Fenpropathrin	2.0	98.1	101.5	92.0	99.7	101.5	98.7	99.9	89.6	96.4
			(2.0)	(4.3)	(2.6)	(3.0)	(3.6)	(1.3)	(2.6)	(4.1)	(4.1)
41	Phosalone	2.0	101.7	101.5	91.9	100.9	99.3	98.5	101.0	89.8	96.6
			(2.3)	(1.8)	(3.0)	(5.9)	(4.5)	(1.4)	(2.4)	(1.2)	(4.3)
42	Pyridaben	2.0	101.7	105.4	92.9	104.9	101.1	98.1	102.3	91.5	97.1
			(2.7)	(3.0)	(2.4)	(5.2)	(3.9)	(1.1)	(1.9)	(1.7)	(4.1)
43	Coumaphos	2.0	100.5	101.8	90.4	107.1	98.7	99.5	92.2	94.5	94.5
			(2.2)	(4.3)	(4.5)	(7.0)	(5.1)	(0.3)	(5.6)	(3.2)	(3.7)
44	Phosphamidon	4.0	90.5	108.0	87.8	93.9	107.3	97.8	94.9	82.7	91.1
			(2.6)	(2.1)	(5.2)	(6.1)	(2.8)	(2.5)	(5.8)	(8.2)	(6.6)
45	Endosulfan	10.0	99.6	93.6	98.1	96.6	111.6	100.9	100.4	78.7	96.7
			(8.9)	(7.7)	(6.9)	(3.8)	(3.1)	(4.4)	(13.8)	(9.6)	(11.1)
46	Cyhalothrin	2.0	114.0	105.5	94.1	102.7	99.4	98.4	101.7	89.3	93.1
			(1.5)	(3.1)	(3.2)	(5.2)	(4.5)	(0.9)	(1.4)	(8.3)	(4.1)
47	Permethrin	8.0	103.3	102.9	92.9	91.1	88.8	97.7	92.9	97.3	98.5
			(9.1)	(6.5)	(3.0)	(9.5)	(4.3)	(1.2)	(13.3)	(14.8)	(5.7)
48	Cyfluthrin	8.0	102.1	93.8	90.4	100.1	97.3	98.9	98.5	101.6	93.2
			(4.8)	(7.3)	(4.1)	(4.2)	(9.6)	(2.2)	(5.6)	(4.9)	(4.9)
49	Cypermethrin	2.0	103.9	97.9	93.3	103.0	99.3	97.2	94.7	89.4	91.0
			(1.0)	(3.7)	(2.9)	(5.2)	(3.5)	(2.9)	(13.5)	(5.6)	(4.4)
50	Flucythrinate	2.0	104.6	100.0	93.2	101.3	107.3	97.9	100.6	91.0	92.4
			(2.7)	(3.8)	(4.5)	(2.5)	(7.1)	(1.3)	(7.6)	(5.0)	(4.8)
51	Fenvalerate	2.0	97.6	102.6	90.9	97.7	99.3	99.6	94.5	90.0	93.7
			(3.7)	(2.0)	(5.6)	(3.7)	(3.4)	(4.8)	(6.3)	(3.4)	(8.0)
52	Fluvalinate	2.0	99.2	96.4	91.5	98.9	103.0	95.9	97.9	92.9	87.8
			(3.3)	(5.4)	(5.1)	(4.8)	(4.0)	(3.0)	(3.7)	(1.9)	(8.7)
53	Difenoconazole	2.0	115.5	102.7	92.3	95.8	103.7	95.9	96.8	94.6	87.6
			(1.6)	(4.6)	(4.4)	(3.7)	(6.0)	(3.7)	(3.5)	(2.9)	(9.5)
54	Deltamethrin	2.0	116.0	92.9	91.9	92.5	110.2	90.8	102.7	95.1	82.7
			(4.7)	(5.3)	(7.1)	(7.9)	(7.3)	(6.8)	(7.9)	(5.6)	(8.0)

CONCLUSION

In this paper, a method is proposed for fast determination of 54 pesticide residues in vegetable. Simple, fast, sensitive, accurate, and rugged. The method is suitable for highly convenient, efficient, and reliable screening of pesticide multi-residues in various vegetable samples.

C-21 Determination of illegally added Sudan dyes in chili oil with GC-MS/MS

INTRODUCTION

In this paper, an analytical method is proposed for the determination of illegally added Sudan I and Sudan II in chili oil with a gas chromatograph-tandem mass spectrometer (GC-MS/MS). The proposed method demonstrated linear correlation coefficients greater than 0.999 for the two Sudan dyes in the concentration range of 1~120 µg/L. The RSDs of peak areas of the Sudan dyes in 6 successive injections of 5 µg/L standard solution were below 8.9%. For samples spiked with 10 µg/kg standards, the proposed method achieved spike recoveries in the range of 77.72%~92.38%. LODs were calculated as 3 times of S/N ratios and the calculated LODs for Sudan I and II were 0.29 µg/L and 0.69 µg/L, respectively.

Sudan dyes are a category of artificially synthesized azo dyes that are not intended for use as food additives. There are 4 major types of Sudan dyes, i.e. Sudan I, Sudan II, Sudan III and Sudan IV. Sudan dyes are potential carcinogens which, when present in food at concentrations higher than 1000 mg, have high probability to induce tumor(s) in animals. Some of their metabolites, in particular, are potentially carcinogenic to humans.
EU had imposed a ban in 1995 on the addition of Sudan dyes into food as colorants. Such practice is also banned in China. In spite of this, food safety scandals concerning Sudan dyes just keep emerging. For example, Sudan I was detected in roasted chicken wings and roasted chicken burgers of a famous chain fast food restaurant in March 2005. Another example, in November 2006, AQSIQ's supervisory inspection results showed two brands of red-yolk duck eggs on the market contained Sudan dyes.

Most of the methods for the determination of Sudan dyes in foods are based on High performance liquid chromatography. In this paper, a method is proposed in reference with the sample pretreatment method specified in the afore-mentioned national standard for detecting Sudan dyes in food by GC-MS/MS in Multiple Reaction Monitoring (MRM) mode. The proposed method effectively eliminated matrix interference and yielded results of high reproducibility. It can serve as a supplement to the standard HPLC method for detecting Sudan dyes in food.

EXPERIMENTAL

Apparatus

GC-MS/MS: GCMS-TQ8030

Conditions of Analysis

Column	: Rxi-5Sil MS, 30 m × 0.25 mm ×0.25 μm
Injection port temperature	: 300 °C
Injection mode	: Splitless injection
Column temperature program	: 90 °C(1 min)→@20 °C/min→290 °C(7 min)
Constant Linear Velocity	: 37 cm/sec
Injection volume	: 1 μL
Ionization mode	: EI
Temperature of ion source	: 230 °C
Temperature of GC-MS interface	: 260 °C
Collision gas	: Argon
Acquisition mode	: MRM as in Table 1

Sample Preparation

Approximately 1 g chili oil was taken, accurately weighed and transferred to a headspace vial, added 5 mL cyclohexane, and subjected to vortex mixer until the chili oil was dissolved. The solution was slowly loaded onto an aluminium oxide chromatographic column, the headspace vial was rinsed with a few cyclohexane for multiple times, and the rinse solvent was combined and loaded on to the chromatographic column. The sample solution was allowed to completely drain, and then 30 mL cyclohexane was used to rinse the column. The rinse solution was discarded, and the column was eluted with 60 mL cyclohexane (containing 5% acetone). The eluent was collected, condensed, and transferred to a volumetric flask with 5 mL acetone in which it was dried with nitrogen flush and brought to the volume of 1.0 mL for analysis with GC-MS/MS.

Table 1 MRM parameters of Sudan dyes

No.	Retention Time	Compound	CAS No.	Quantitative Ion	Qualitative Ion 1	Qualitative Ion 2
1	11.895	Sudan I	842-07-9	248>171(9)	248>143(19)	248>115(28)
2	13.192	Sudan II	3118-97-6	276>247(10)	276>259(8)	276>143(19)

RESULT AND DISCUSSION

Chromatogram of standard samples

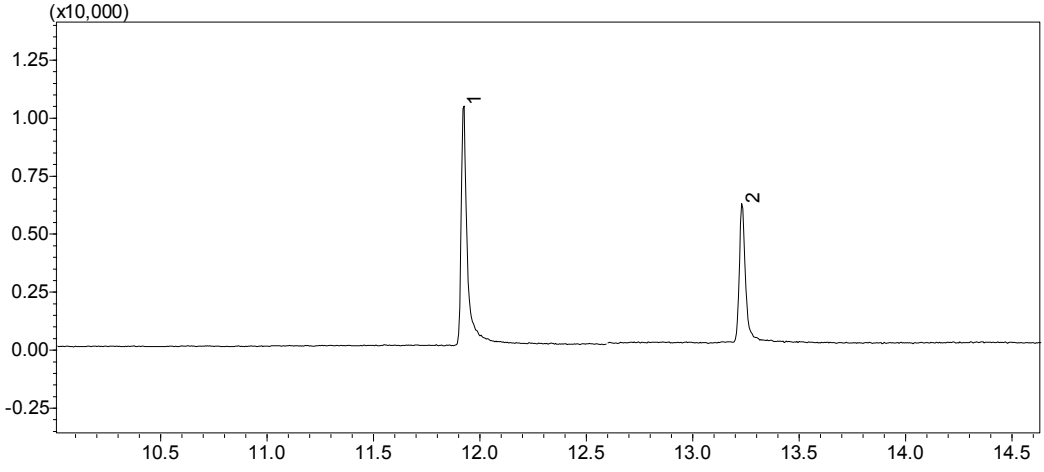
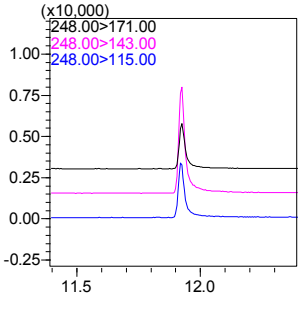


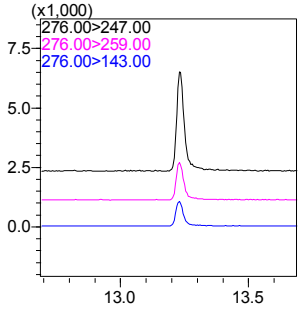
Fig. 1 TIC of a multi-standard solution of Sudan I and Sudan II (10 µg/L)

Calibration curves, repeatability and LODs

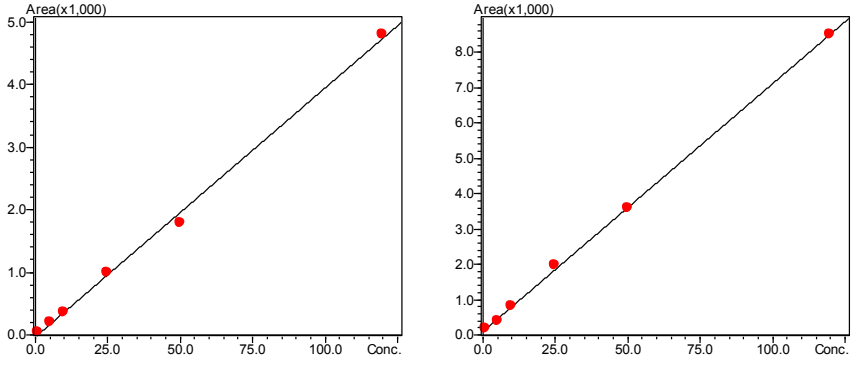
A series of multi-standard solutions of the two Sudan dyes of concentrations 1, 5, 10, 25, 50, 120 µg/L were prepared and subjected to MS analysis in MRM mode. Calibration curves were plotted as shown below, using concentration as abscissa and peak area as ordinate. LODs were calculated as 3 times of the signal-to-noise ratio. To assess the repeatability of peak area, 5 µg/L standard samples were injected 6 times in succession and the RSDs were calculated. The correlation coefficients of standard curves, LODs and RSDs of peak areas are as shown in Table 2.



Sudan I



Sudan II



Sudan I Sudan II

Fig. 2 MRM chromatograms and calibration curves of the 2 Sudan dyes

Table 2 Correlation coefficients of standard curves, LODs and %RSDs of peak areas of the Sudan dyes

No.	Compound	Correlation Coefficient (R^2)	LOD (μg/L)	%RSD (n=6)
1	Sudan I	0.9989	0.29	8.8
2	Sudan II	0.9999	0.69	8.9

Recovery

1 g of sample was spiked with desired amount of multi-standard solution of the Sudan dyes at the spiked level of 10 μg/kg and subjected to 3 replicate assays, the resulted average recoveries were as shown in Table 3.

Table 3 Spike recovery of samples

No.	Compound	Determined value (μg/kg)			Average Recovery %	Recovery RSD%
		1	2	3		
1	Sudan I	8.69	9.42	9.60	92.38	5.2
2	Sudan II	8.01	8.10	7.20	77.72	6.4

CONCLUSION

Sudan I and Sudan II in chili oil were analyzed with the proposed method using Shimadzu GCMS-TQ8030. With the merits of simple operation and good repeatability, the proposed method indicated spike recoveries in the range of 77.72%~92.38% at the spiked level of 10 μg/kg and its LODs for both Sudan I and Sudan II were lower than 1 μg/L. The application of tandem spectrometry in MRM mode for the analysis of Sudan dyes can effectively reduce matrix interference and enhance the sensitivity of analytical method.

C-22 Detection of 11 Preservatives and Antioxidants in Food with GC-MS/MS

INTRODUCTION

A method is proposed in this paper for the detection of 11 preservatives and antioxidants in food with gas chromatograph-triple quadrupole mass spectrometer. The samples were extracted with acetonitrile and analyzed by GC-MS/MS. For the 11 preservatives and antioxidants, the method showed good linearity in the concentration range of 0.5~50 μg/mL, with correlation coefficients all greater than 0.999. The spike recoveries were in the range of 89.7~118.7%. The relative standard deviation of compositions in 5 successive determinations of 5 mg/L standard samples were between 1.8% and 3.3%, showing that the method was of good repeatability.

The food additives are added to preserve the original quality and nutritional value of food. The preservatives can inhibit the growth of microbes and extend the shelf life of food. Most of the preservatives used in food production currently are artificially synthesized and will have some side effects in case of improper use. Some preservatives even contain trace toxins and long-term excessive intake will cause some damage to human health.

Antioxidants are food additives that can prevent or delay the oxidative deterioration of food, improve food stability and extend shelf life. But they have certain hazards to human body. For example, intake of large dose of BHA is carcinogenic and BHT is suspected to inhibit human respiratory enzyme activity.

Therefore, the amount of preservatives and antioxidants in food has increasing cause of food safety attention.

EXPERIMENTAL

Apparatus

GC-MS/MS: Shimadzu GCMS-TQ8030

Conditions of Analysis

Column	: Rtx-WAX 30 m × 0.25 mm × 0.25 μm
Injector temperature	: 250 °C
Column temperature	: 120 °C (1 min)→@20 °C/min→190 °C →@5 °C/min→220 °C(6 min)→@10°C/min →240 °C(6 min)
Carrier gas	: Helium gas

Carrier gas control mode	: CLV
Linear velocity	: 38.1 cm/sec
Injection mode	: Split
Split ratio	: 20:1
Injection volume	: 1 μL
Ionization temperature	: 230 °C
Temperature of GC-MS interface	: 250 °C
Acquisition Mode	: MRM transitions and collision voltages for the compounds are listed in Table 1.

Pretreatment of samples

5 g sample was weighed and transferred to a 50 mL centrifuge tube. Then 15 mL n-hexane saturated acetonitrile was added, homogenized for 1 min, shaken thoroughly for 5 min and subjected to supersonic extraction for 10 min. Centrifuge at 5000 rpm for 10 min. The acetonitrile was collected and extracted twice. The acetonitrile extract was combined (the oil samples should be added with 5 mL acetonitrile saturated n-hexane solution and subjected to vortex for 1 min). The acetonitrile layer was dried with anhydrous sodium sulfate, then dried using N_2 evaporation at 40 °C in water bath and reconstituted with 1 mL acetonitrile to dissolve the residue for detection.

Results and Discussion

3.1 Chromatogram of multi-standard solution

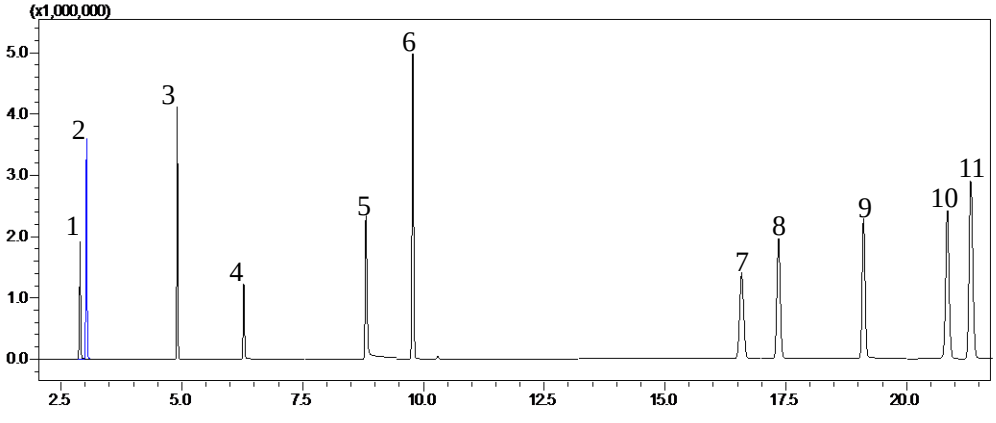


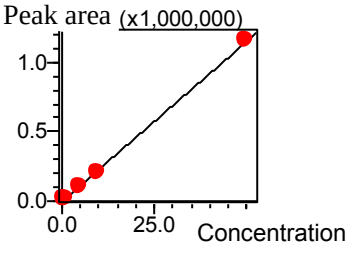
Fig. 1 MRM chromatograms of multi-standard solution of preservatives and antioxidants

Table 1 Compound, retention time, selected transition and collision energy of the preservatives and antioxidants

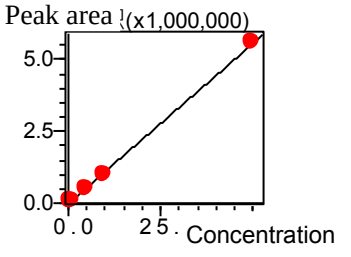
ID	Compound	CAS No.	Retention Time (min)	Target transition (*m/z*)	Collision Energy CE	Reference transition (*m/z*)	Collision Energy CE
1	Propionic acid	79-09-4	2.905	74>56	4	73>55	15
2	Dimethyl Fumarate	624-49-7	3.035	113>85	5	85>53	7
3	2,6-Di-tert-butyl-4-methylphenol	128-37-0	4.900	205>177	7	220>205	10
4	Sorbicacid	110-44-1	6.285	112>97	4	97>69	7
5	Benzoic Acid	65-85-0	8.820	122>105	9	105>77	15
6	Butyl hydroxy anisol	25013-16-5	9.795	165>137	7	137>109	9
7	Methyl p-hydroxybenzoate	99-76-3	16.630	121>93	10	152>121	10
8	Ethyl p-hydroxybenzoate	120-47-8	17.385	121>93	9	138>121	10
9	Propylparaben	94-13-3	19.130	121>93	8	138>121	15
10	TBHQ	1948-33-0	20.890	151>123	9	123>67	10
11	Butyl paraben	94-26-8	21.360	138>121	10	121>93	10

Calibration curve

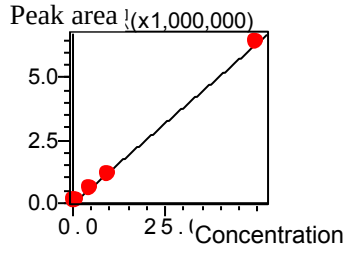
A series of multi-standard solutions of preservatives and antioxidants was prepared at concentrations of 0.5, 1, 5, 10 and 50 µg/mL, respectively; 1 µL was injected for analysis in MRM. The resulted calibration curves and correlation coefficients are listed in Fig. 2.



Propionic acid (r=0.9997)



Dimethyl Fumarate (r=0.9998)



BHT (r=0.9998)

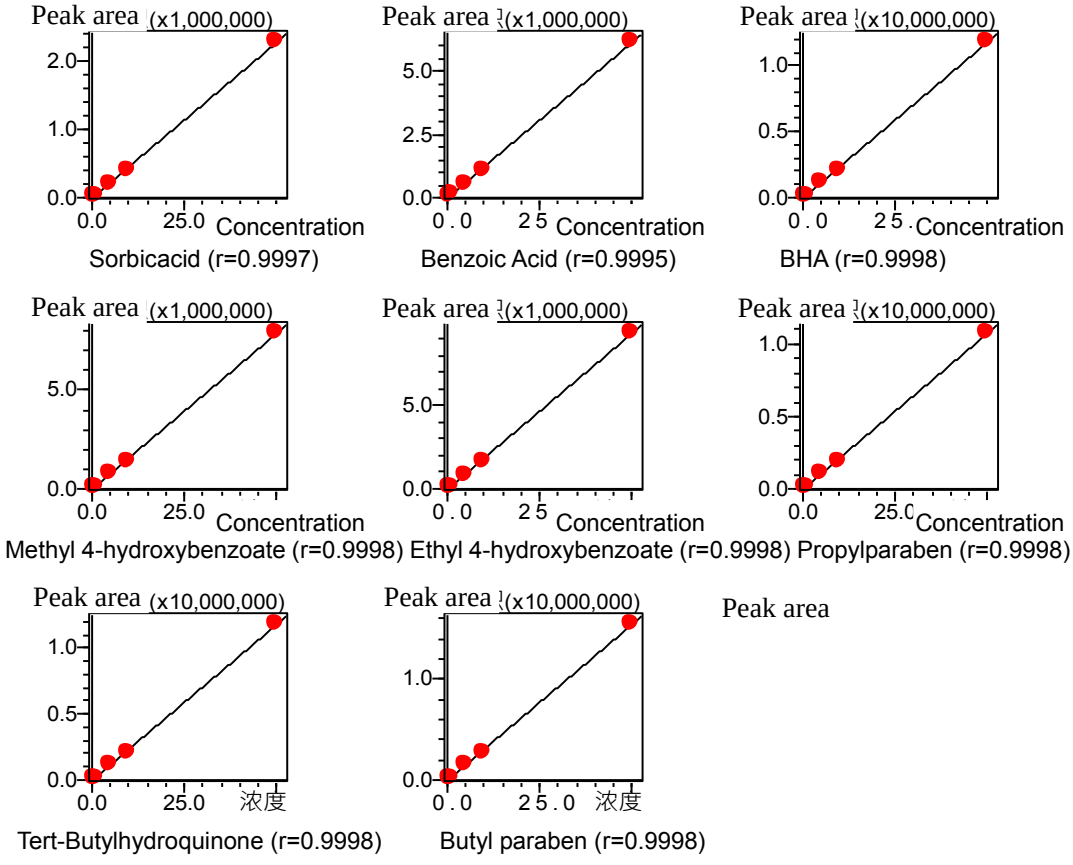


Fig. 2 Calibration curve of preservatives and antioxidants

Repeatability test

5 mg/L multi-standard solution of preservatives and antioxidants was taken and subjected to 5 successive determinations for assessment of peak area repeatability. The results are listed in Table 2.

Table 2 Repeatability of peak area (n=5)

ID	Compound	Peak Area 1	Peak Area 2	Peak Area 3	Peak Area 4	Peak Area 5	RSD(%)
1	Propionic acid	113461	106314	115067	114325	114815	3.3
2	Dimethyl Fumarate	569858	538825	574540	568470	572789	2.6
3	2,6-Di-tert-butyl-4-methylphenol	667074	648930	682345	665418	673549	1.8
4	Sorbicacid	232589	216584	233009	228533	230785	3.0
5	Benzoic Acid	637866	609081	643157	647189	638976	2.4
6	Butyl hydroxy anisol	1232954	1212158	1270942	1234187	1255664	1.8
7	Methyl p-hydroxybenzoate	809206	803479	842494	815948	832464	2.0

8	Ethyl p-hydroxybenzoate	984127	950717	994127	964732	983196	1.8
9	Propyl paraben	108784	1087052	1141569	1105905	1124182	2.1
10	TBHQ	120403	1197834	1257514	1221282	1239227	2.0
11	Butyl paraben	157268	1576406	1649105	1602089	1617199	2.0

Recoveries and LODs

5 g samples with low content of preservatives and antioxidants were accurately weighed and standard solution of preservatives and antioxidants at concentration of 5 mg/L were added. The samples were subjected to extraction according to the above-mentioned steps and 1 μL was injected to access the proposed method's recoveries. The results are listed in Table 3.

Table 3 Recoveries and LODs

ID	Compound	Blank value (mg/L)	Test value (mg/L)	Recovery (%)	LOD (μg/mL)
1	Propionic acid	0.5873	6.0610	109.5	0.016
2	Dimethyl Fumarate	0.0000	5.5733	111.5	0.004
3	2,6-Di-tert-butyl-4-methylphenol	2.3699	8.3045	118.7	0.003
4	Sorbicacid	0.2019	5.5762	107.5	0.006
5	Benzoic Acid	0.8858	6.0051	102.4	0.009
6	Butyl hydroxy anisol	0.0000	4.4826	89.7	0.013
7	Methyl p-hydroxybenzoate	0.0000	5.2296	104.6	0.005
8	Ethyl p-hydroxybenzoate	0.0000	5.2064	104.1	0.054
9	Propyl paraben	0.0000	5.2041	104.1	0.005
10	TBHQ	0.0259	4.9896	99.3	0.008
11	Butyl paraben	0.0000	5.2022	104.0	0.005

LODs

Based on the data of 0.5 μg/mL standard sample, LODs were calculated as 3 times of S/N ratio. The resulted LODs for the preservatives and antioxidants are listed in Table 3.

Sample assay

The commercial soy sauce, fruit juice, caramel treats, preserved fruit, rice noodles and other samples were analyzed. The test chromatograms and results were as follows:

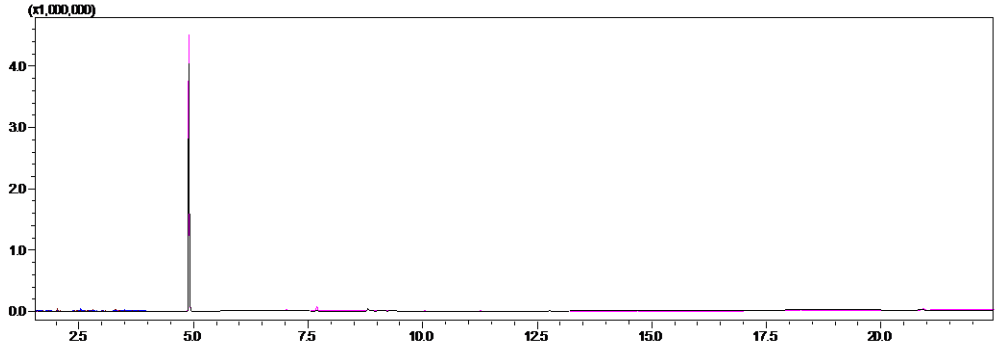


Fig. 3 TIC of soy sauce

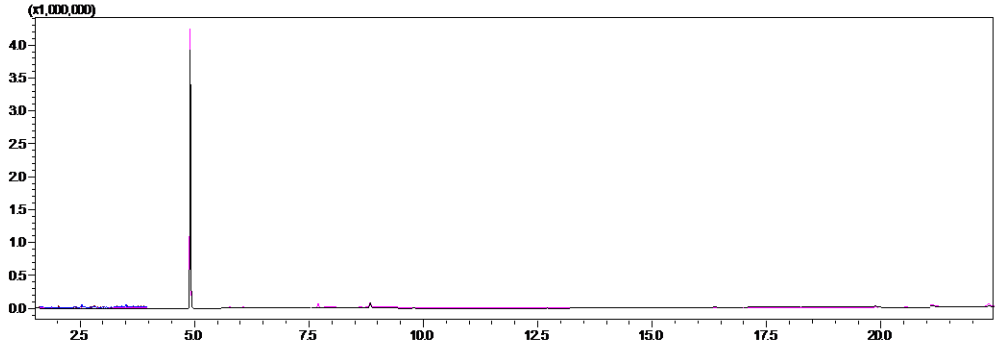


Fig. 4 TIC of fruit juice

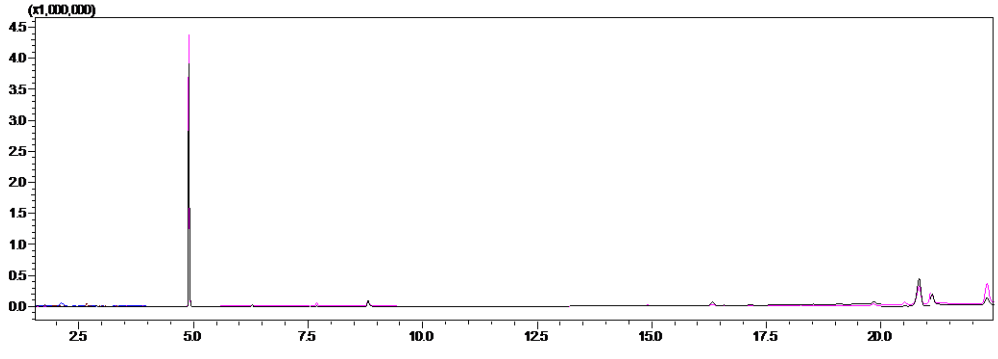


Fig. 5 TIC of caramel treats

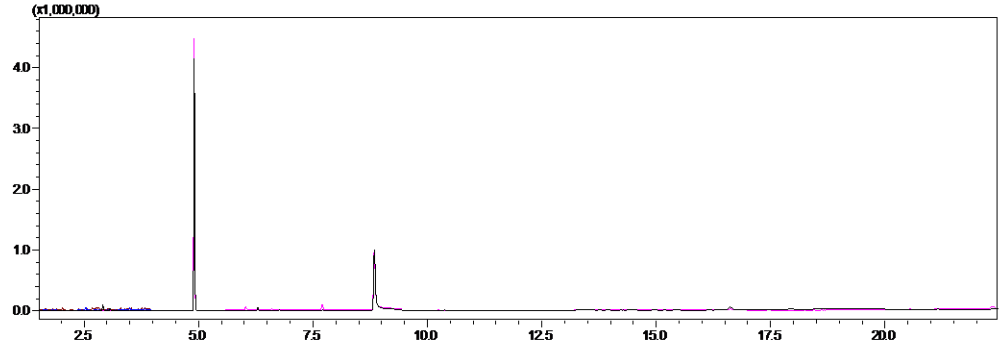


Fig.6 TIC of preserved fruit

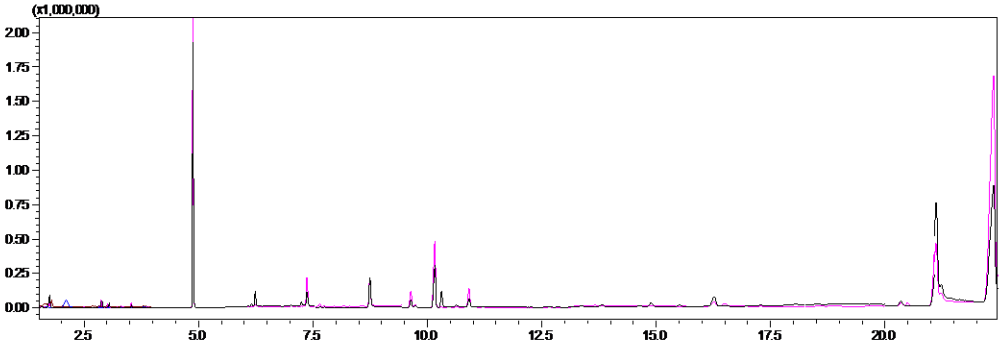


Fig.7 TIC of rice noodles

Table 3 Test results of commercial soy sauce, fruit juice, caramel treats, preserved fruit and rice noodles

ID	Compound	Retention Time (min)	Content (mg/kg)				
			Soy sauce	Fruit juice	Caramel treats	Preserved fruit	Rice noodles
1	Propionic acid	2.905	83.9	N.D	0.3	1.7	0.4
2	Dimethyl Fumarate	3.035	N.D	N.D	N.D	N.D	N.D
3	2,6-Di-tert-butyl-4-methylphenol	4.900	764.8	10.8	11.0	11.6	9.6
4	Sorbicacid	6.285	7.2	N.D	0.3	0.6	1.2
5	Benzoic Acid	8.820	12502.4	0.4	0.5	5.3	8.4
6	Butyl hydroxy anisol	9.795	N.D	N.D	N.D	N.D	N.D
7	Methyl p-hydroxybenzoate	16.630	N.D	N.D	N.D	N.D	N.D
8	Ethyl p-hydroxybenzoate	17.385	N.D	N.D	N.D	N.D	N.D
9	Propyl paraben	19.130	0.8	N.D	N.D	N.D	N.D
10	TBHQ	20.890	0.8	N.D	2.7	N.D	N.D
11	Butyl paraben	21.360	0.6	N.D	N.D	N.D	N.D

CONCLUSION

The method proposed in this paper for determination of preservatives and antioxidants in food with GCMS-TQ8030 was having good linearity for the compounds in concentration range of 0.5~50 µg/mL and provided recoveries in the range of 89.7~118.7%. The RSDs of 5 successive injections of 5 mg/L standard samples were in the range of 1.8~3.3%, indicating that the method was of good precision.

C-23 Determination of 4-Methylimidazole in coke with GC-MS/MS

INTRODUCTION

In this paper, a method was developed for the determination of 4-Methylimidazole in coke with Shimadzu gas chromatograph-triple quadrupole mass spectrometer GCMS-TQ8030. The calibration curves of this method showed good linearity in the concentration range of 5-1000 µg/L, with r=0.9998. The LOD of compounds was 1.11 µg/L and the average spike recovery was 81.75%. The RSD of peak areas for all 5 successive injections of standard samples with the concentration of 5 µg/L was 4.15%.

The 4-Methylimidazole, white or off-white crystalline powder, is an important organic intermediate. The previous researches showed that 4-Methylimidazole could increase the incidence of tumor and leukemia among mice, thus it can be inferred that 4-Methylimidazole may be carcinogenic for human.

4-Methylimidazole in coke is mainly from the caramel color. The caramel color, as a food color, is widely used in food and beverage such as coke, soy sauce, vinegar, yellow rice wine and beer. The caramel color decocted directly from sugar is free of 4-Methylimidazole, however, currently most domestic and international companies produce the caramel color using ammoniac compounds as the catalyst, or using the sugar, ammonia and nitrite under the condition of high temperatures and pressures. During those processes, 4-Methylimidazole could be produced. The maximum residue limits (MRLs) for 4-Methylimidazole in coke from WHO is 200 mg/kg, according to the national standard GB8817-2001 in China, the content of 4-Methylimidazole in caramel color produced by ammonium process shall not exceed 0.02%.

In this paper, a method was developed for the detection of 4-Methylimidazole in coke with Shimadzu triple quadrupole mass spectrometer. The proposed method offered simplicity, accuracy and low LOD. Therefore, it can be used for the determination of 4-Methylimidazole in coke.

EXPERIMENTAL

Apparatus

GC-MS/MS: GCMS-TQ8030

Conditions of Analysis

Column	: InterCap WAX, 30 m×0.25 mm×0.25 µm
Column temperature program	: 80 °C (1 min)→@20 °C/min→150 °C→@5 °C/min →250 °C(2 min)
CID gas	: Argon
Injector temperature	: 250 °C
Injection volume	: 1 µL
Injection mode	: Splitless, 1 min
Control mode	: CLV (Constant Linear Velocity)
Linear velocity of carrier gas	: 36.8 cm/sec
Interface temperature	: 250 °C
Ionization temperature	: 230 °C
Acquisition mode	: MRM

Sample Preparation

The samples were subjected room temperature for 30 min for removal of carbonic acid gas. Transfer 5 mL sample with a graduated cylinder to a 250 mL separating funnel, adjust the pH of samples to pH 11 using the 10 mol/L sodium hydroxide solution, add 40 mL dichloromethane extracts, shake 5 min, allow the system to rest for stratification, and then collect the lower organic phase, repeat the extraction three times using the same method, combine the organic phase and condense it to almost dry, reconstitute to 2 mL with acetone for further test.

RESULTS AND DISCUSSION

Chromatogram

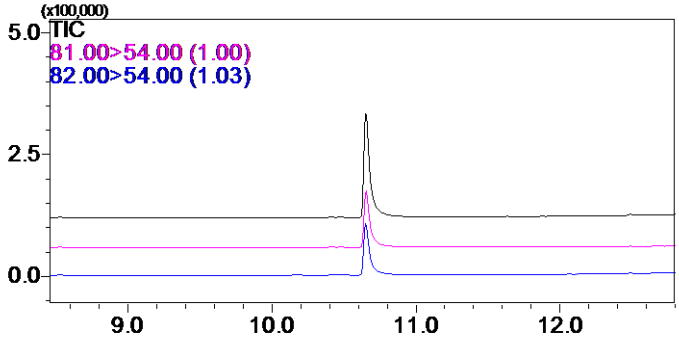


Fig. 1 MRM chromatograms of standard solutions of 4-Methylimidazole (0.5 µg/ml)

Table 1 Retention time and MRM parameters of 4-Methylimidazole

No	Compound	CAS#	Retention Time	Quantitative Ion	CE	Qualitative Ion	CE
1	4-4-Methylimidazole	131-11-3	10.625	82>54	10	81>54	10

Calibration curves

Six levels of standard solutions of 4-Methylimidazole were prepared at concentrations of 5, 50, 100, 200, 500, and 1000 µg/L, respectively. Calibration curves of 4-Methylimidazole were plotted as shown as follows with the concentration as abscissa and peak area of quantitative ion as ordinate.

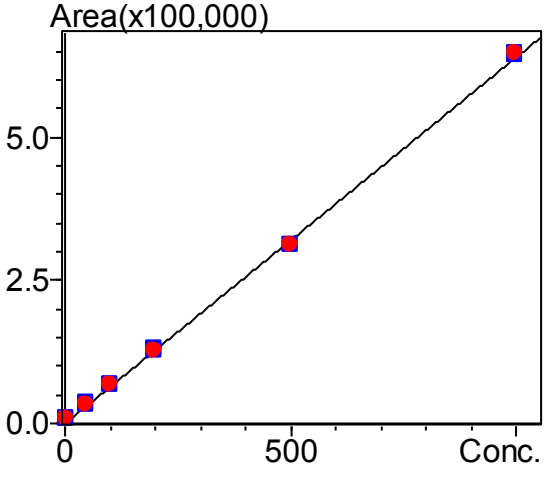


Fig. 2 Calibration curves of 4-Methylimidazole

LODs and repeatability

LODs were calculated with 3 times of S/N ratio using the data of 5 µg/L standard solution and are shown in Table 2. Standard solution at concentration of 5 µg/L was injected 5 consecutive times to calculate the peak area RSD (%) and check the repeatability of the method. The results are shown in Table 2.

Table 2 Correlation coefficient, LOD, peak area repeatability of calibration curve of 4-Methylimidazole (n=5)

Compound	Correlation coefficient	LOD (µg/L)	RSD (%)
4-Methylimidazole	0.9998	1.11	4.15

RECOVERY

A commercial coke product was selected for the determination and recovery test. Three samples were subjected to the pretreatment for analysis at spike concentration of 200 ug/L. The quantitation results and spike recovery of coke samples are shown in Table 3.

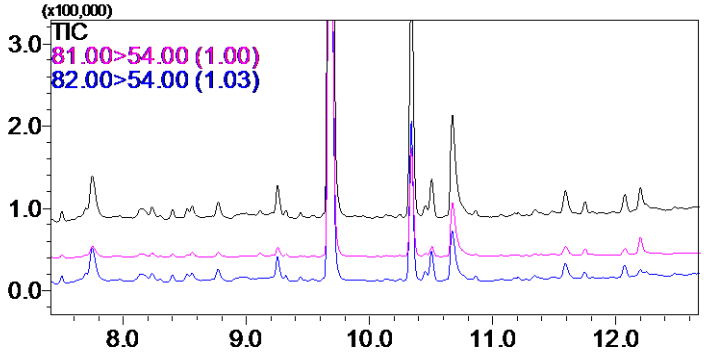


Fig. 3 MRM chromatogram of coke sample

Table 3 Quantitative results of coke samples

Compound	Coke samples		Spiked samples	
	Quantitation results (µg/mL)	Recovery %	Average Recovery %	RSD%(n=3)
4-Methylimidazole	112.40	75.02 85.90 84.34	81.75	7.20

CONCLUSION

A method is proposed for the analysis and determination of 4-Methylimidazole in coke with Shimadzu GCMS-TQ8030. The method is easy to operate, and the linear correlation coefficient was 0.9998 in the concentration range of 5~1000 μg/L, the method's LOD was 1.11 μg/L, the average recovery of spiked samples was 81.75%. The method could meet the requirements for the determination of 4-Methylimidazole in coke.

C-24 Determination of multi-pesticide residues in tea leaves by SPE-GC-MS/MS

INTRODUCTION

An analytical method is proposed for simultaneous determination of 51 pesticides residues in tea leaves with GC-MS/MS and solid phase extraction (SPE) purification. The correlation coefficients of the pesticides were all greater than 0.998 in the concentration range of 5.0~100 µg/L. Assam tea, oolong tea, and black tea samples were spiked with a multi-standard solution of 51 pesticides for spike recovery test. The proposed method achieved spike recoveries in the range of 71~105% for the 51 pesticides at spiked level of 7.5 µg/kg, meeting the requirements for routine analysis of pesticides residues in tea leaves.

In recent years, EU and many developed countries have, out of the consideration of “food safety”, set up more and more rigorous technological barriers which substantially affected tea export.

An analytical method has been proposed for the assay of the residues of 51 pesticides and agrochemicals in tea leaves by GC-MS/MS. However, tea leaves’ composition can be so complicated that their extract might contain a lot of interference that have adverse impact on subsequent analysis. Therefore, it is necessary to subject tea extract to purification before analysis.

A method was proposed in this paper for simultaneous determination of 51 pesticides residues in tea leaves. With the method, tea leaves samples were homogenized and extracted with acetonitrile, and analytes in the resulted extract were purified by solid phase extraction (SPE), separated and analyzed by GC and triple quadrupole mass spectrometry in Multiple Reaction Monitoring (MRM) mode using the matrix matched calibration curves. The proposed method effectively reduced matrix effect and background interference and enhanced analysis sensitivity.

EXPERIMENTAL

Apparatus
GC-MS/MS: GCMS-TQ8030 (Shimadzu)

Conditions of Analysis
Column : Rtx-5 ms, 30 m×0.25 mm×0.25 µm
Injection port temperature : 250 °C
Column temperature program : 50 °C(1 min)→@(25 °C/min)→150 °C
→@(10°C/min)→300 °C(7 min)
CLV mode : 47.2 cm/sec
Injection mode : Splitless (1 min)
Injection volume : 1 µL
High pressure injection : 250 kPa (1 min)
Temperature of ion source : 230 °C
Temperature of GC-MS interface : 250 °C

MRM acquisition parameters were as listed in Table 1

Sample Preparation
Tea leaves were sampled, grinded, sieved through 20 mesh sifter, and then subjected to the following procedures.

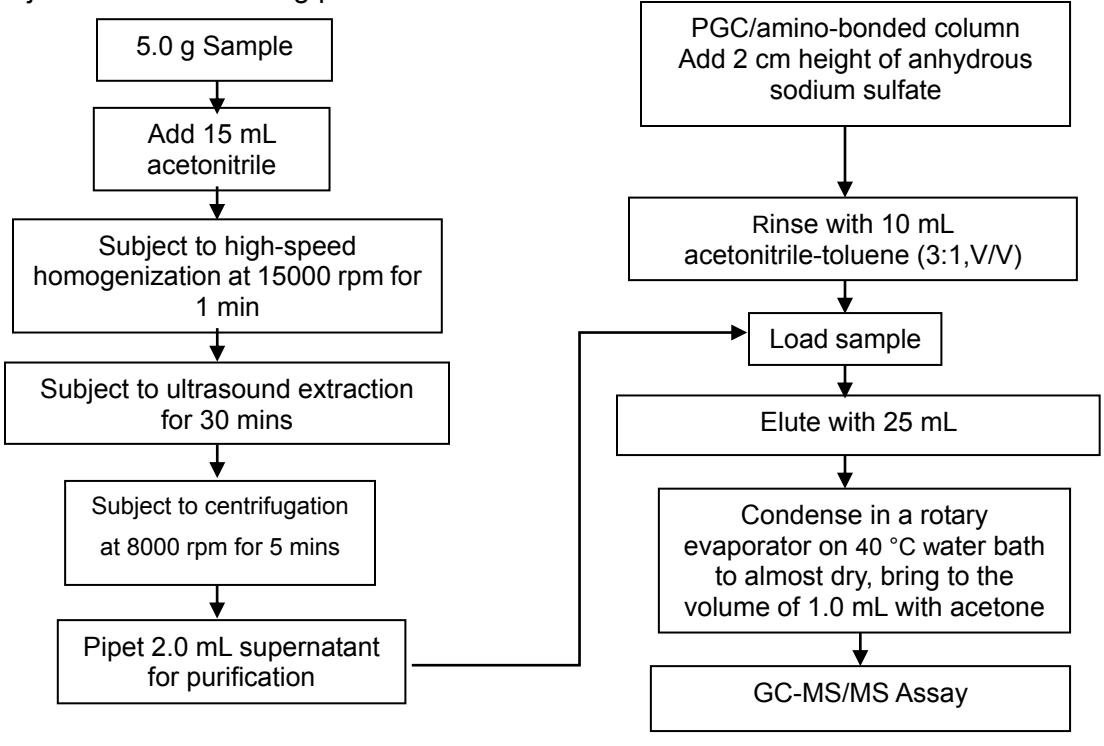


Fig. 1 Sample pre-treatment flow chart

Table 1 Retention time and MRM parameters of pesticides

No.	CAS No.	Compound	Retention Time (min)	Quantitative transition	CE	Qualitative transition	CE
1	62-73 -7	Dichlorvos	6.130	185>93	14	185>109	14
2	13194-48-4	Ethoprophos	10.183	200>158	6	200>114	14
3	3689-24-5	Sulfotep	10.717	322>202	10	322>294	4
4	298-02-2	Phorate	10.838	260>75	8	260>231	4
5	319-84-6	alpha-HCH	10.976	219>183	8	219>145	20
6	319-85-7	beta-HCH	11.541	219>183	8	219>145	20
7	58-89-9	gamma-HCH	11.654	219>183	8	219>145	20
8	13071-79-9	Terbufos	11.700	231>175	14	231>129	26
9	82-68-8	Quintozene	11.750	295>237	16	295>265	12
10	944 - 22 - 9	Fonofos	11.799	246>109	18	246>137	6
11	333-41-5	Diazinon	11.924	304>179	10	304>162	8
12	13171-21-6	Phosphamidon-1	11.991	264>127	14	264>193	8
13	319-86-8	delta-HCH	12.151	219>183	10	219>145	20
14	1897-45-6	chlorothalonil	12.242	266>231	14	266>168	22
15	13171-21-6	Phosphamidon-2	12.726	264>127	14	264>193	8
16	50471-44-8	Vinclozolin	12.898	285>212	12	285>178	14
17	298-00-0	Parathion-methyl	12.924	263>109	14	263>136	8
18	122-14-5	Fenitrothion	13.458	277>260	6	277>109	14
19	121-75-5	Malathion	13.630	173>99	14	173>127	6
20	55-38-9	Fenthion	13.826	278>109	20	278>125	20
21	2921-88-2	Chlorpyrifos	13.855	314>258	14	314>286	8
22	56-38-2	Parathion	13.879	291>109	14	291>137	6
23	43121-43-3	Triadimefon	13.921	208>181	10	208>127	14
24	24353-61-5	Isocarbophos	14.004	289>136	14	289>113	6
25	83733-82-8	Isofenphos-methyl	14.351	199>121	14	241>121	22
26	120068-37-3	Fipronil	14.659	367>213	30	367>255	22
27	13593-03-8	Quinalphos	14.716	157>129	14	157>93	10
28	2597-03-7	Phenthoate	14.717	274>125	20	274>246	6
29	32809-16-8	Procymidone	14.845	283>96	10	283>255	12
30	950-37-8	Methidathion	15.013	145>85	8	145>58	14
31	959-98-8	alpha-Endosulfan	15.249	339>160	18	339>267	8
32	41198-08-7	Profenofos	15.584	337>267	14	337>309	6
33	72-55-9	p,p'-DDE	15.667	246>176	30	246>211	22
34	33213-65-9	beta-Endosulfan	16.340	339>160	18	339>267	8
35	72-54-8	p,p'-DDD	16.459	235>165	24	235>199	14
36	789-02-6	o,p'-DDT	16.525	235>165	24	235>199	14
37	24017-47-8	Triazophos	16.768	257>162	8	257>134	22
38	50-29-3	p,p'-DDT	17.147	235>165	24	235>199	16
39	36734-19-7	Iprodione	17.868	314>245	12	314>56	22
40	82657-04-3	Bifenthrin	18.041	181>166	12	181>153	8
41	732-11-6	Phosmet	18.062	160>133	14	160>77	24

42	39515-41-8	Fenpropathrin	18.182	265>210	12	265>172	14
43	2310-17-0	Phosalone	18.737	182>111	14	182>138	8
44	52645-53-1	Permethrin-1	19.775	183>168	14	183>165	14
45	52645-53-1	Permethrin-2	19.897	183>168	14	183>165	14
46	96489-71-3	Pyridaben	19.927	147>117	22	147>132	14
47	56-72-4	Coumaphos	20.042	362>109	16	362>226	14
48	68359-3 -5	Cyfluthrin-1	20.346	226>206	14	226>199	6
49	68359-3 -5	Cyfluthrin-2	20.437	226>206	14	226>199	6
50	68359-3 -5	Cyfluthrin-3,4	20.518	226>206	14	226>199	6
51	52315-07-8	Cypermethrin-1	20.662	163>127	6	163.>91	14
52	52315-07-8	Cypermethrin-2	20.756	163>127	6	163>91	14
53	52315-07-8	Cypermethrin-3,4	20.864	163>127	6	163>91	14
54	70124-77-5	Flucythrinate-1	20.862	199>157	10	199>107	22
55	70124-77-5	Flucythrinate-2	21.050	199>157	10	199>107	22
56	51630-58-1	Fenvalerate-1	21.580	419>225	6	419>167	12
57	51630-58-1	Fenvalerate-2	21.779	419>225	6	419>167	12
58	69409-94-5	Fluvalinate-1	21.738	250>55	20	250>200	20
59	69409-94-5	Fluvalinate-2	21.796	250>55	20	250>200	20
60	119446-68-3	Difenoconazole-1	22.037	323>265	14	323>202	28
61	119446-68-3	Difenoconazole-2	22.111	323>265	14	323>202	28
62	52918-63-5	Deltamethrin-1	22.123	253>93	20	253>172	8
63	52918-63-5	Deltamethrin-2	22.365	253>93	20	253>172	8

RESULTS AND DISCUSSION

Chromatogram of a multi-standard solution of pesticides

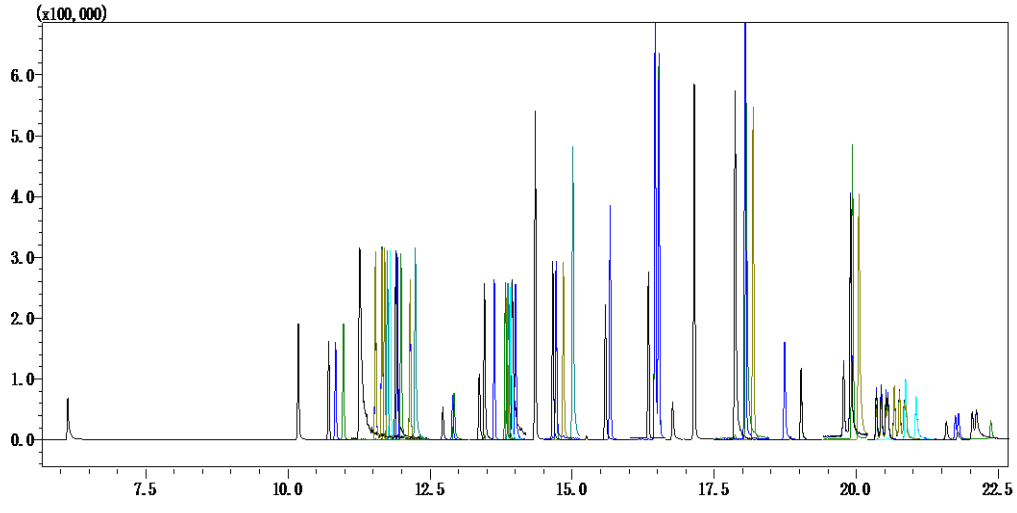


Fig. 2 MRM chromatograms of a multi-standard solution of pesticides (50 µg/L)

Calibration curve

A series of multi-standard blank matrix solutions of concentrations 2.0, 5.0, 10, 20, 50, 100 µg/L were prepared using blank tea matrix solution as solvent. Calibration curves were plotted with concentration as abscissa and peak area as ordinate. The calibration curves and MRM chromatograms of some pesticides are shown in Fig. 3. LODs were calculated as 3 times of S/N (peak to peak). Information on the calibration curves' correlation coefficients and LODs is as given in Table 2.

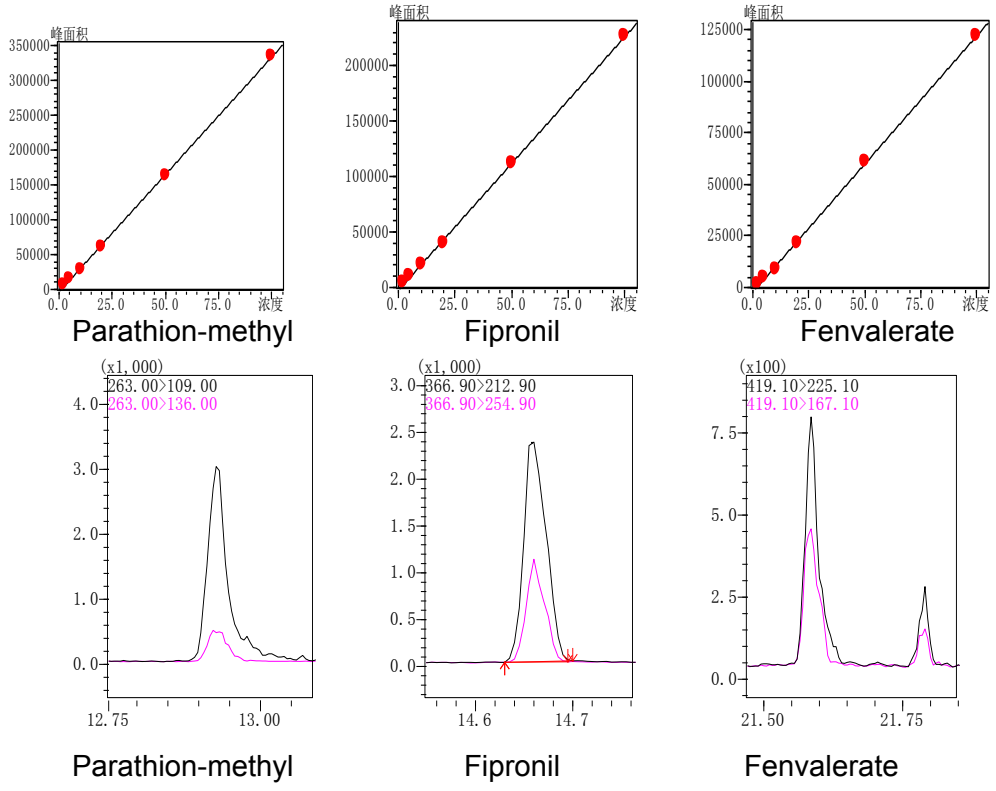


Fig. 3 Calibration curves and MRM chromatograms of representative pesticides (2.0 µg/L)

Table 2. Correlation coefficients of standard curves, LODs and spike recoveries of pesticides

No	Compound	Correlation Coefficient (R^2)	LOD (μg/L)	Assam tea sample Av. recovery(%) RSD(%)	Oolong tea sample Av. recovery(%) RSD(%)	Black tea sample Av. recovery(%) RSD(%)
1	Dichlorvos	0.9998	0.15	71.4 5.7	73.0 1.4	71.2 2.2
2	Ethoprop	0.9998	0.06	73.6 3.7	79.8 3.2	77.0 5.8
3	Sulfotep	0.9998	0.03	79.2 7.2	87.2 3.7	85.4 7.4
4	Phorate	0.9998	0.05	78.4 5.5	85.4 3.3	87.2 6.9
5	alpha-HCH	0.9995	0.02	76.4 4.1	78.8 5.6	79.4 6.9
6	beta-HCH	0.9999	0.10	77.6 4.8	82.4 6.2	83.6 6.4
7	gamma-HCH	0.9997	0.03	82.1 5.4	87.2 8.5	87.4 6.1
8	Terbufos	0.9998	0.10	85.4 6.9	93.4 4.0	91.6 6.1
9	Quintozene	0.9998	0.10	76.4 2.5	76.8 5.6	78.5 8.3
10	Fonofos	0.9999	0.02	83.4 2.0	92.6 1.6	90.6 5.6
11	Diazinon	0.9999	0.04	84.4 1.7	90.8 2.2	90.4 3.4
12	Phosphamidon	0.9998	0.12	84.4 2.1	90.0 3.3	92.6 3.4
13	delta-HCH	0.9998	0.04	82.0 7.9	79.4 4.2	81.0 7.7
14	Chlorothalonil	0.9997	0.06	89.2 8.7	96.4 0.3	92.4 7.5
15	Vinclozoline	0.9995	0.04	79.0 8.1	83.4 5.3	78.6 6.0
16	Parathion-methyl	0.9998	0.20	87.4 8.2	88.0 2.3	90.0 5.3
17	Fenitrothion	0.9995	0.05	89.4 4.7	94.0 3.8	92.4 5.5
18	Malathion	0.9998	0.03	88.4 5.0	95.8 3.5	95.2 2.9
19	Fenthion	0.9998	0.03	84.8 4.7	89.2 2.9	90.0 5.0
20	Chlorpyrifos	0.9999	0.03	85.2 7.2	90.6 3.9	92.0 5.4
21	Parathion	0.9996	0.10	95.4 7.3	98.8 5.8	97.6 3.6
22	Triadimefon	0.9999	0.20	84.4 7.7	89.4 7.4	89.6 8.3
23	Isocarbophos	0.9996	0.10	84.0 7.6	94.8 6.0	88.0 2.1

24	Isofenphos-methyl	0.9999	0.04	85.2 4.6	92.4 3.2	91.8 5.6
25	Fipronil	0.9998	0.05	91.0 3.1	93.6 3.6	93.4 4.1
26	Quinalphos	0.9998	0.55	87.2 6.9	89.0 2.3	90.4 8.7
27	Phenthoate	0.9994	0.04	104.6 7.7	99.0 3.9	93.8 5.3
28	Procymidone	0.9997	0.02	87.4 4.7	90.4 2.6	91.4 4.7
29	Methidathion	0.9994	0.01	95.5 5.7	98.4 2.5	96.4 3.4
30	Endosulfan	0.9998	0.60	76.0 3.3	81.2 6.7	78.5 7.8
31	Profenofos	0.9997	0.04	87.2 7.0	89.0 5.4	87.0 5.5
32	p,p'-DDE	0.9996	0.02	81.8 6.0	84.2 5.2	84.2 5.2
33	p,p'-DDD	0.9999	0.03	83.8 6.9	87.6 4.7	85.2 6.6
34	o,p'-DDT	0.9999	0.03	83.8 2.7	88.2 6.0	88.0 5.9
35	Triazophos	0.9995	0.14	98.8 6.8	105.0 4.0	105.8 3.2
36	p,p'-DDT	0.9999	0.04	84.0 6.9	86.4 5.2	86.4 5.5
37	Iprodione	0.9999	0.20	78.2 7.0	79.8 4.7	76.2 6.4
38	Bifenthrin	0.9999	0.07	79.6 7.7	79.2 5.7	78.0 6.9
39	Phosmet	0.9989	0.20	99.6 4.2	105.0 1.2	101.0 6.7
40	Fenpropathrin	0.9998	0.10	79.2 7.6	79.8 3.9	80.8 6.6
41	Phosalone	0.9997	0.14	91.2 5.6	94.8 5.7	95.8 4.2
42	Permethrin	0.9998	3.10	72.8 7.8	79.3 3.4	75.4 8.4
43	Pyridaben	0.9998	1.00	82.4 4.7	83.4 5.0	83.4 4.0
44	Coumaphos	0.9994	0.10	93.8 8.2	95.0 4.4	89.0 1.0
45	Cyfluthrin	0.9998	3.10	81.6 4.1	88.4 7.3	84.2 7.2
46	Cypermethrin	0.9999	3.60	77.6 4.7	73.2 6.1	76.8 6.2
47	Flucythrinate	0.9998	1.00	84.4 4.1	92.6 8.0	89.2 4.7
48	Fenvalerate	0.9997	2.90	84.0 5.7	93.8 4.0	94.0 7.2
49	Fluvalinate	0.9998	0.90	85.2 2.1	87.0 5.8	91.8 4.6
50	Difenoconazole	0.9996	1.10	76.5 6.3	87.2 4.1	81.2 7.1
51	Deltamethrin	0.9997	2.50	94.0 3.8	93.4 3.8	82.7 7.8

Recovery test

Blank matrix samples of assam tea, oolong tea, and black tea were spiked with a multi-standard solution of pesticides at the spiked level of 7.5 µg/kg and then subjected to the afore-mentioned pretreatment procedures. Three replicate assays were performed on each of the samples and the resulted spike recoveries of analytes from the 3 samples were as shown in Table 2.

CONCLUSION

Tea leaf products were analyzed with the proposed method for determination of 51 pesticides residues using solid phase extraction purification technique and Shimadzu GCMS-TQ8030 gas chromatograph-triple quadrupole mass spectrometer. The method had the merits of good repeatability and high sensitivity and achieved spike recoveries in the range of 71~105% for the 51 pesticides. The experiment showed that tandem mass spectrometry can avoid the interference of impurities, especially in the analysis of complex samples like tea leaves, thereby reducing false positive rate with improved selectivity and detection sensitivity.

C-25 Determination of 13 anti-mould agents in wine by GCMSMS and QuEChERS

INTRODUCTION

An analytical method is proposed for fast detection of 13 anti-mould agents in wine by PTV-GC-MS/MS and QuEChERS. The correlation coefficients of calibration curves of 13 anti-mould agents were all greater than 0.998 in the concentration range of 2.0~100μg/L. A multi-standard solution of the anti-mould agents was spiked into wine samples at the spiked level of 7.0 μg/kg. The proposed method yielded spike recoveries in the range of 82~116% for the 13 anti-mould agents, suggesting that the method is suitable for analyzing anti-mould agents in wine.

Anti-mould agents can inhibit the growth and multiplication of moulds, thereby preventing grapes from spoilage in their growing, storing and transporting process. However, anti-mould agents are toxic to a certain degree and may damage the liver, nervous system, and bone marrow of human beings. Moreover, most anti-mould agents are disinfectants that are apt to retain in wine. Therefore, it is especially critical to quantify the content of anti-mould agents in wine in order to safeguard its quality.

QuEnChERS is a sample pretreatment method commonly used for its speed, convenience and low cost.

In this paper, a detection method is proposed for simultaneous analysis of 13 anti-mould agents in wine by QuEChERS pretreatment method in conjunction with Shimadzu PTV-GC-MS/MS. Based on programmable temperature vaporization (PTV) technology, the proposed method uses low-temperature injection and removes acetonitrile to improve the life of columns. Moreover, triple quadrupole tandem mass spectrometry is an excellent tool reducing matrix interference and enabling high sensitivity and accurate identification and quantitation of target analyte(s) in a complex matrix. The proposed method is simple, fast, sensitive and reliable, meeting the requirements for detecting anti-mould agents in wine.

EXPERIMENTAL

Apparatus

Triple Quadrupole GC-MS: Shimadzu GCMS-TQ8030
(equipped with PTV injector)

Conditions of Analysis

Column : Rxi-5silMS, 30m×0.25mm×0.25μm
Injector temperature : 65°C(1min)→@(200°C/min)→250°C(15 min)
Split valve program :

0~0.9min	Split: 20: 1
0.9~3.5min	Splitless
3.5min	Split: 20: 1

Column temperature program : 40°C(4min)→@(25°C/min)→125°C→
@(10°C/min) →300°C(6 min)
Constant Linear Velocity : 36.2 cm/sec
Injection volume : 2 μL
Ionization : 230°C
Temperature of GC-MS interface : 280°C
Acquisition mode : MRM. Analytical conditions are listed in Table 1.

Sample Preparation

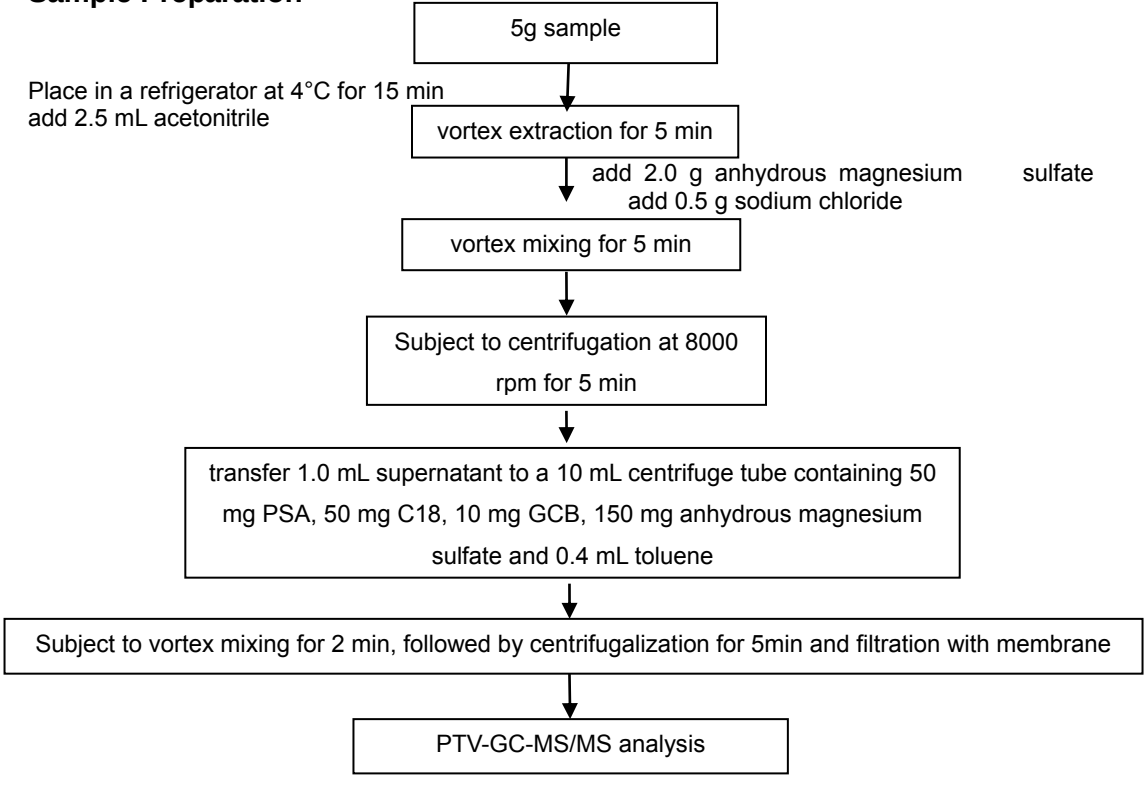


Fig. 1 Sample pretreatment flow chart

Table 1 Retention time and MRM parameters

No.	CAS No.	Compound	Retention Time (min)	Quantitative Transition	CE	Qualitative Transition	CE
1	90-43-7	2-Phenylphenol	13.090	171>141	24	170>115	28
2	122-39-4	Diphenylamine	14.261	169>66	24	169>77	28
3	53112-28-0	Pyrimethanil	16.076	198>158	18	198>158	28
4	1897-45-6	chlorothalonil	16.123	266>231	14	266>168	22
5	57837-19-1	Metalaxyl	17.240	249>190	8	249>146	22
6	43121-43-3	Triadimefon	18.100	208>181	10	208>127	14
7	148-79-8	Thiabendazole	18.900	201>174	16	201>130	26
8	32809-16-8	Procymidone	18.939	283>96	10	283>255	12
9	35554-44-0	Imazalil	19.720	215>173	6	215>159	6
10	60207-90-1	Propiconazole-1	21.215	259>69	14	259>191	8
11	60207-90-1	Propiconazole-2	21.335	259>69	14	259>191	8
12	107534-96-3	Tebuconazole	21.640	250>125	22	250>153	12
13	36734-19-7	Iprodione	22.055	314>245	12	314>56	22
14	67747-09-5	Prochloraz	24.250	180>138	12	308>85	10

RESULTS AND DISCUSSION

MRM chromatogram of a multi-standard solution of the anti-mould agents

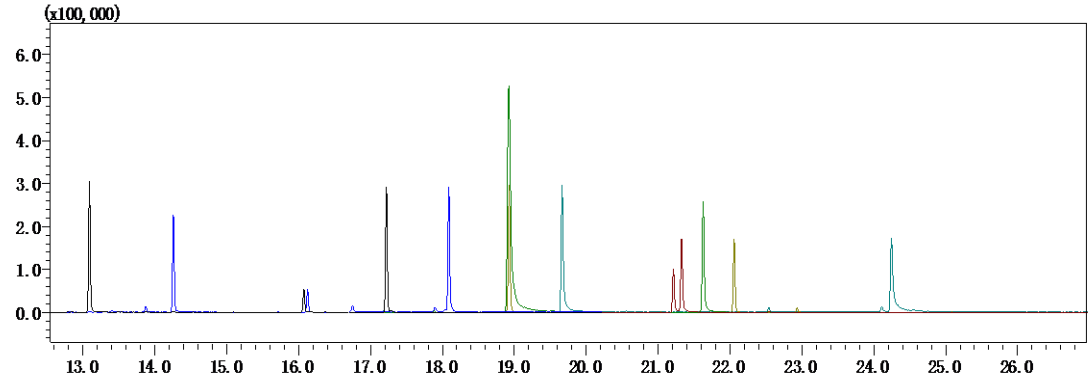


Fig.2 MRM chromatogram of a multi-standard solution (50μg/L) of the anti-mould agents

Calibration curve

A series of multi-standard solutions of the anti-mould agents were prepared at concentrations of 2.0, 5.0, 10, 20, 50, 100 μg/L using blank wine matrix solution as solvent. Calibration curves were plotted with Concentration as abscissa and Peak Area as ordinate. The representative calibration curves and MRM mass chromatogram of some analytes are shown in Fig. 3. LODs were calculated as 3 times of S/N (peak to peak). Information on the calibration curves' correlation coefficients and LODs was given in Table 2.

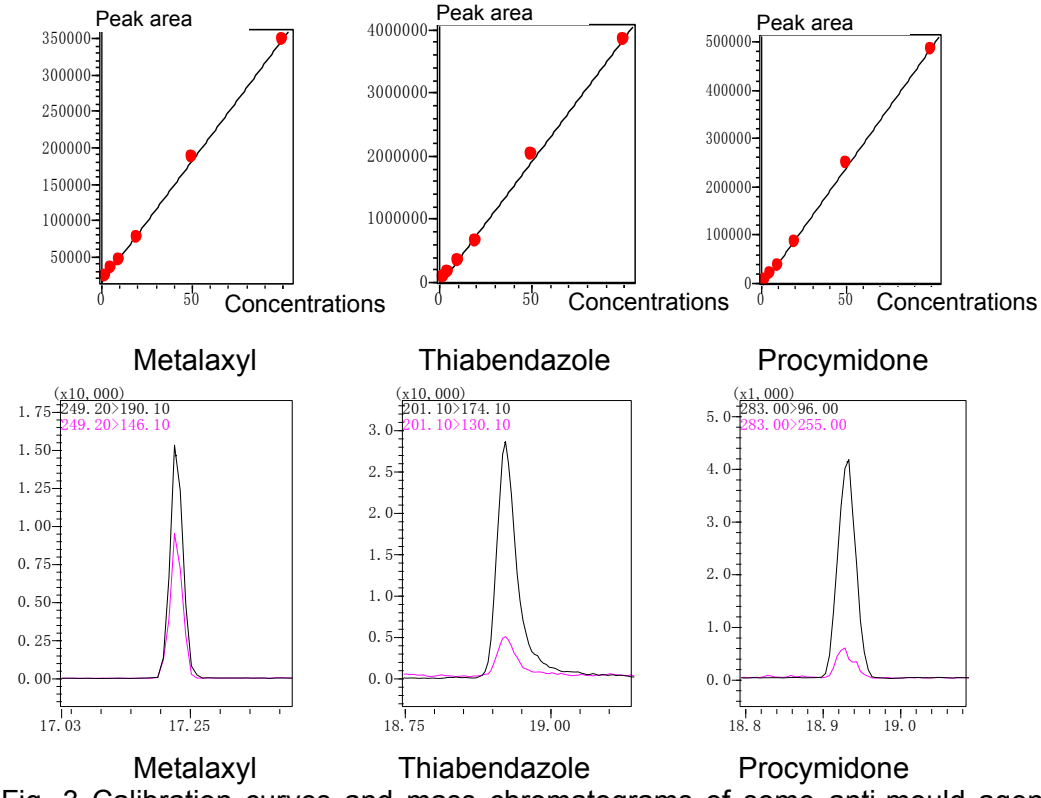


Fig. 3 Calibration curves and mass chromatograms of some anti-mould agents (2.0 μg/L)

Table 2. Correlation coefficients of calibration curves, LODs and spike recoveries of anti-mould agents

No.	Compound	Correlation Coefficient (R^2)	LOD (μg/L)	Spiked Level 7.0μg/kg	
				Average Recovery(%)	RSD(%)
1	2-Phenylphenol	0.9991	0.09	107.5	4.3
2	Diphenylamine	0.9991	0.18	108.6	4.7
3	Pyrimethanil	0.9996	0.04	104.2	2.3
4	Chlorothalonil	0.9982	0.11	82.4	6.8
5	Metalaxyl	0.9996	0.01	115.1	2.7
6	Triadimefon	0.9993	0.05	105.5	4.2
7	Thiabendazole	0.9991	0.02	93.0	3.6
8	Procymidone	0.9994	0.01	104.0	4.7
9	Imazalil	0.9993	0.10	99.8	2.0
10	Propiconazole	0.9994	0.17	96.5	2.1
11	Tebuconazole	0.9992	0.06	98.4	1.5
12	Iprodione	0.9994	0.09	93.6	2.5
13	Prochloraz	0.9986	0.60	91.2	5.6

Recovery test

Blank matrix samples of wine were spiked with a multi-standard solution of anti-mould agents at the spiked level of 7.0μg/kg and then subjected to the afore-mentioned pretreatment procedures. Five replicate assays were performed on each of the samples and the resulted spike recoveries of analytes were shown in Table 2.

Sample assay

A number of off-the-shelf wine samples were assayed with the proposed method and a brand is detected to be with 2-Phenylphenol content at 0.7μg/kg, Diphenylamine at 1.7μg/kg, and Metalaxyl at 2.7μg/kg. Chromatogram of the sample was shown in Fig. 4.

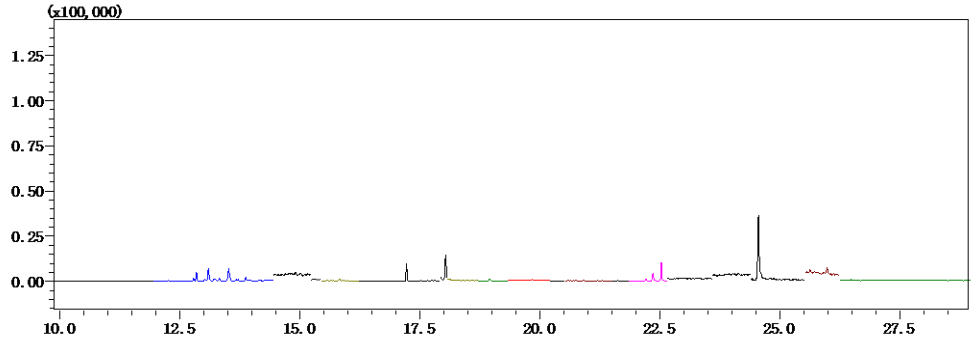


Fig.4 MRM chromatogram of a wine sample

CONCLUSION

13 anti-mould agents in wine were analyzed with the proposed method using QuEChERS pretreatment technology and Shimadzu PTV Triple Quadrupole GC-MS. The method demonstrated good repeatability and high sensitivity for the 13 anti-mould agents, yielding spike recoveries in the range of 82~116% for them.

C-26 Determination of multi-pesticides residue in wine by GCMSMS and QuEChERS

INTRODUCTION

A detection method is proposed for fast analysis of 90 multi-pesticide residues in wine by PTV-GC-MS/MS and QuEChERS. The correlation coefficients of calibration curves of the pesticides were greater than 0.998 for the concentration range of 2.0~100μg/L. A multi-standard solution of the 90 pesticides was added into wine samples for spike recovery test. The proposed method achieved spike recoveries in the range of 71~116% for the 90 pesticides at spiked level of 7.0 μg/kg, meeting the requirements for analysis of pesticide residues in wine.

Wine is an alcoholic drink made from the fermentation of fresh grapes or grape juice. During the field production of grapes, however, a number of pesticides may be used, some of which may still retain in wine and pose a potential threat to consumers' health even though the fermentation process can reduce such pesticide residues. Therefore, it is necessary to carry out study to find out the pesticide residues in wine.

QuEChERS is a sample pretreatment method commonly used for its speed, convenience and low cost.

In this paper, a detection method is proposed for simultaneous analysis of 90 pesticide residues in wine by QuEChERS pretreatment method in conjunction with Shimadzu PTV-GC-MS/MS. Based on programmable temperature vaporization (PTV) technology, the proposed method uses low-temperature injection and removes acetonitrile to improve the life of columns. Moreover, triple quadrupole tandem mass spectrometry has merits such as least matrix interference and high sensitivity, enabling accurate identification of target analyte (s) in a complex matrix. The proposed method is simple, fast, sensitive and reliable, meeting the requirements for pesticide residues analysis in wine.

EXPERIMENTS

Apparatus

Triple Quadrupole GC-MS (equipped with PTV injector) : Shimadzu GCMS-TQ8030

Conditions of Analysis

Column : Rxi-5silMS, 30m × 0.25mm × 0.25µm
Injector temperature : 65°C(1min)→@(200°C/min)→250°C(15 min)
Split valve program :

0~0.9min	Split: 20:1
0.9~3.5min	Splitless
3.5min	Split: 20:1

Column temperature program : 40°C(4min)→@(25°C/min)→125°C →@(10°C/min)→300°C(6 min)
CLV mode : 36.2 cm/sec
Injection volume : 2 µL
Ionization : 230°C
Temperature of GC-MS interface : 280°C
Acquisition mode : MRM as listed in Table 1.

Sample Preparation

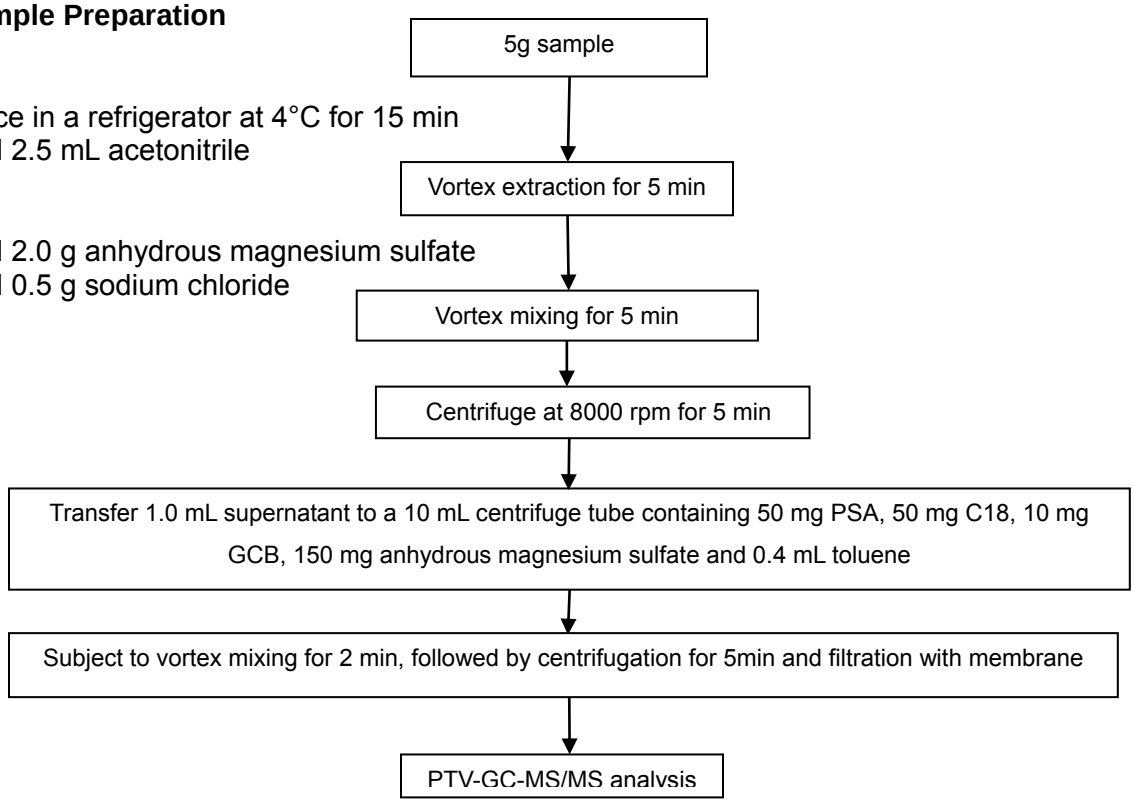


Fig. 1 Sample pretreatment flow chart

Table 1 Retention time and MRM parameters of pesticides

No.	CAS No.	Compound	Retention Time (min)	Quantitative Transition	CE	Qualitative Transition	CE
1	62-73 -7	Dichlorvos	10.016	185>93	14	185>109	14
2	1194-65-6	Dichlobenil	11.120	171>100	24	171>136	14
3	31895-21-3	Thiocyclam	12.935	135>71	8	135>56	24
4	90-43-7	2-Phenylphenol	13.090	171>141	24	170>115	28
5	1113-02-6	Omethoate	13.825	156>80	22	156>141	14
6	122-39-4	Diphenylamine	14.261	169>66	24	169>77	28
7	13194-48-4	Ethoprophos	14.318	200>158	6	200>114	14
8	141-66-2	Dicrotophos	14.585	127>95	18	192>127	10
9	3689-24-5	Sulfotep	14.704	322>202	10	322>294	4
10	6923-22-4	Monocrotophos	14.766	127>95	18	192>127	10
11	95465-99-9	Cadusafos	14.891	159>97	18	127>99	10
12	298-02-2	Phorate	14.967	260>75	8	260>231	4
13	319-84-6	alpha-HCH	15.112	219>183	8	219>145	20
14	60-51-5	Dimethoate	15.333	125>47	14	125>79	8
15	1912-24-9	Atrazine	15.545	215>58	14	215>200	6
16	319-85-7	beta-HCH	15.602	219>183	8	219>145	20
17	82-68-8	Quintozene	15.719	295>237	16	295>265	12
18	58-89-9	gamma-HCH	15.803	219>183	8	219>145	20
19	13071-79-9	Terbufos	15.848	231>175	14	231>129	26
20	23950-58-5	Propyzamide	15.920	173>145	16	173>109	26
21	944 - 22 - 9	Fonofos	15.951	246>109	18	246>137	6
22	333-41-5	Diazinon	15.952	304>179	10	304>162	8
23	13171-21-6	Phosphamidon-1	15.983	264>127	14	264>193	8
24	53112-28-0	Pyrimethanil	16.076	198>158	18	198>158	28
25	1897-45-6	chlorothalonil	16.123	266>231	14	266>168	22
26	319-86-8	delta-HCH	16.338	219>183	10	219>145	20
27	13171-21-6	Phosphamidon-2	16.748	264>127	14	264>193	8
28	709-98-8	Propanil	16.865	161>99	24	161>90	22
29	5598-13-0	Chlorpyrifos-methyl	16.935	286>93	22	286>271	14
30	50471-44-8	Vinclozolin	17.019	285>212	12	285>178	14
31	298-00-0	Parathion-methyl	17.067	263>109	14	263>136	8
32	57837-19-1	Metalaxyl	17.240	249>190	8	249>146	22
33	76-44-8	Heptachlor	17.290	272>237	20	274>239	16
34	122-14-5	Fenitrothion	17.561	277>260	6	277>109	14
35	121-75-5	Malathion	17.716	173>99	14	173>127	6
36	51218-45-2	S-Metolachlor	17.870	238>162	12	238>133	26
37	2921-88-2	Chlorpyrifos	17.884	314>258	14	314>286	8
38	55-38-9	Fenthion	17.957	278>109	20	278>125	20
39	1861-32-1	Chlorthal-dimethyl	17.990	301>223	26	301>273	14

40	309-00-2	Aldrin	18.010	263>193	28	293>220	26
41	56-38-2	Parathion	18.030	291>109	14	291>137	6
42	43121-43-3	Triadimefon	18.100	208>181	10	208>127	14
43	24353-61-5	Isocarbophos	18.112	289>136	14	289>113	6
44	115-32-2	Dicofol	18.204	250>139	14	250>215	8
45	83733-82-8	Isofenphos-methyl	18.417	199>121	14	241>121	22
46	120068-37-3	Fipronil	18.605	367>213	30	367>255	22
47	2597-03-7	Phenthoate	18.858	274>125	20	274>246	6
48	13593-03-8	Quinalphos	18.830	157>129	14	157>93	10
49	148-79-8	Thiabendazole	18.900	201>174	16	201>130	26
50	32809-16-8	Procymidone	18.939	283>96	10	283>255	12
51	950-37-8	Methidathion	19.142	145>85	8	145>58	14
52	50512-35-1	Isoprothiolane	19.705	290>204	6	290>118	14
53	35554-44-0	Imazalil	19.720	215>173	6	215>159	6
54	41198-08-7	Profenofos	19.766	337>267	14	337>309	6
55	41814-78-2	Tricyclazole	19.810	189>162	12	189>135	18
56	19666-30-9	Oxadiazon	19.820	258>175	8	258>112	28
57	72-55-9	p,p'-DDE	19.871	246>176	30	246>211	22
58	143390-89-0	Kresoxim-methyl	19.950	206>116	6	206>131	14
59	60-57-1	Dieldrin	20.025	277>241	8	263>228	24
60	72-20-8	Endrin	20.410	263>191	30	263>193	28
61	563-12-2	Ethion	20.630	231>175	14	231>185	12
62	72-54-8	p,p'-DDD	20.669	235>165	24	235>199	14
63	789-02-6	o,p'-DDT	20.734	235>165	24	235>199	14
64	55814-41-0	Mepronil	20.875	269>119	14	269>227	6
65	24017-47-8	Triazophos	20.892	257>162	8	257>134	22
66	128639-02-1	Carfentrazone-ethyl	21.030	340>312	14	340>151	28
67	60207-90-1	Propiconazole-1	21.215	259>69	14	259>191	8
68	124495-18-7	Quinoxyfen	21.260	237>208	28	237>182	28
69	60207-90-1	Propiconazole-2	21.335	259>69	14	259>191	8
70	50-29-3	p,p'-DDT	21.385	235>165	24	235>199	16
71	107534-96-3	Tebuconazole	21.640	250>125	22	250>153	12
72	36734-19-7	Iprodione	22.055	314>245	12	314>56	22
73	82657-04-3	Bifenthrin	22.204	181>166	12	181>153	8
74	39515-41-8	Fenpropathrin	22.397	265>210	12	265>172	14
75	2310-17-0	Phosalone	22.901	182>102	14	367>154	6
76	68085-85-8	Cyhalothrin-1	22.989	197>141	12	197>161	8
77	86-50-0	Azinphos-methyl	22.995	160>132	6	160>77	20
78	68085-85-8	Cyhalothrin-2	23.174	197>141	12	197>161	8
79	52645-53-1	Permethrin-1	24.007	183>168	14	183>165	14
80	52645-53-1	Permethrin-2	24.132	183>168	14	183>165	14
81	56-72-4	Coumaphos	24.159	362>109	16	362>226	14
82	96489-71-3	Pyridaben	24.198	147>117	22	147>132	14

83	67747-09-5	Prochloraz	24.250	180>138	12	308>85	10
84	134605-64-4	Butafenacil	24.260	331>180	14	331>152	28
85	68359-3 -5	Cyfluthrin-1	24.527	226>206	14	226>199	6
86	68359-3 -5	Cyfluthrin-2	24.621	226>206	14	226>199	6
87	68359-3 -5	Cyfluthrin-3,4	24.688	226>206	14	226>199	6
88	52315-07-8	Cypermethrin-1	24.862	163>127	6	163.>91	14
89	52315-07-8	Cypermethrin-2	24.959	163>127	6	163>91	14
90	70124-77-5	Flucythrinate-1	25.019	199>157	10	199>107	22
91	52315-07-8	Cypermethrin-3,4	25.024	163>127	6	163>91	14
92	76578-14-8	Quizalofop-ethyl	25.055	372>299	14	372>272	10
93	70124-77-5	Flucythrinate-2	25.216	199>157	10	199>107	22
94	51630-58-1	Fenvalerate-1	25.840	419>225	6	419>167	12
95	175013-18-0	Pyraclostrobin	25.950	164>132	14	164>77	28
96	69409-94-5	Fluvalinate-1	25.962	250>55	20	250>200	20
97	69409-94-5	Fluvalinate-2	26.031	250>55	20	250>200	20
98	51630-58-1	Fenvalerate-2	26.082	419>225	6	419>167	12
99	119446-68-3	Difenoconazole-1	26.407	323>265	14	323>202	28
100	119446-68-3	Difenoconazole-2	26.489	323>265	14	323>202	28
101	52918-63-5	Deltamethrin-1	26.520	253>93	20	253>172	8
102	52918-63-5	Deltamethrin-2	26.784	253>93	20	253>172	8
103	13180 -57-3	Famoxadone	27.285	330>224	10	330>196	22

RESULTS AND DISCUSSION

Chromatogram of a multi-pesticide standard solution

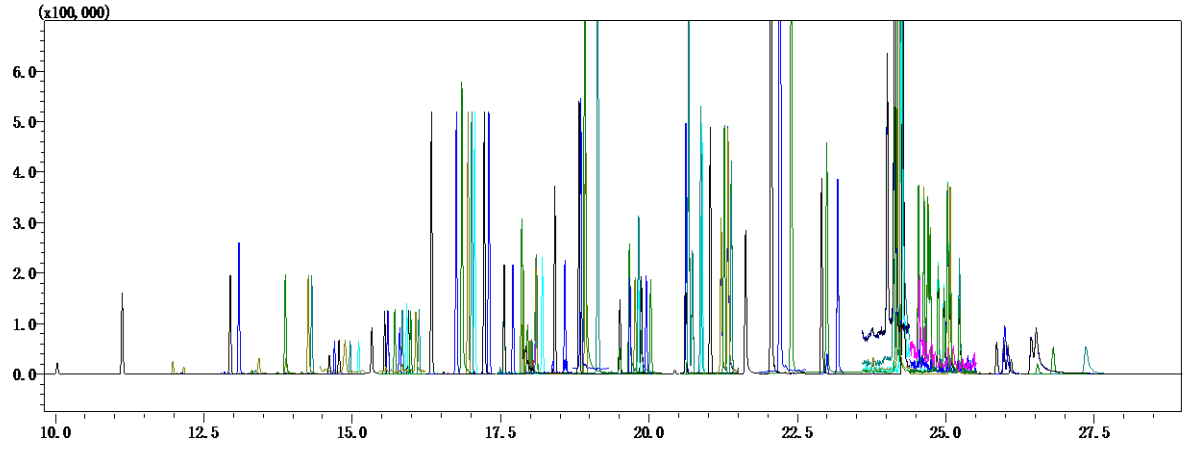


Fig. 2 MRM chromatogram of a multi-pesticide standard solution (50µg/L)

Calibration curves

A series of standard solutions of the pesticides of concentrations of 2.0, 5.0, 10, 20, 50, 100 µg/L were prepared using blank wine matrix solution as solvent. Calibration curves were plotted with concentration as abscissa and peak area as

ordinate. The calibration curves and MRM mass chromatograms of representative pesticides are shown in Fig. 3. LODs were calculated as 3 times of peak to peak ratio (PPR). Information on the calibration curves' correlation coefficients and LODs is given in Table 2.

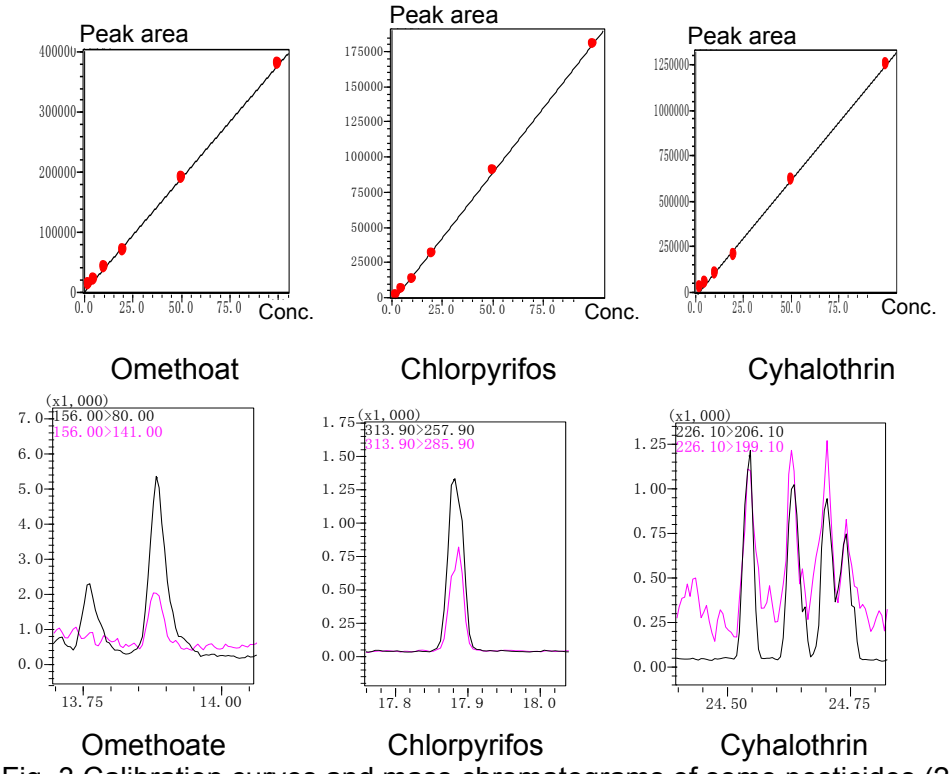


Fig. 3 Calibration curves and mass chromatograms of some pesticides (2.0 μg/L)

Table 2 Correlation coefficients of calibration curves, LODs and spike recoveries of pesticides

No.	Compound	Correlation Coefficient (R^2)	LOD (μg/L)	Spiked Level 7.0μg/kg Average Recovery (%) RSD(%)
1	Dichlorvos	0.9997	0.20	111.1 4.6
2	Dichlobenil	0.9994	0.10	108.4 4.5
3	Thiocyclam	0.9992	0.04	111.1 7.1

4	2-Phenylphenol	0.9991	0.09	107.5 4.3
5	Omethoate	0.9996	0.30	82.4 5.9
6	Diphenylamine	0.9991	0.18	103.3 2.4
7	Ethoprophos	0.9992	0.05	97.3 4.6
8	Dicrotophos	0.9997	0.06	87.6 2.2
9	Sulfotep	0.9991	0.05	104.5 5.5
10	Monocrotophos	0.9995	0.07	80.9 4.7
11	Cadusafos	0.9996	0.08	95.2 4.9
12	Phorate	0.9991	0.06	104.1 4.9
13	alpha-HCH	0.9993	0.09	107.4 4.5
14	Dimethoate	0.9997	0.06	94.6 2.9
15	Atrazine	0.9993	0.09	98.6 2.9
16	beta-HCH	0.9991	0.08	102.1 4.6
17	Quintozene	0.9984	0.10	100.7 4.5
18	gamma-HCH	0.9990	0.10	102.7 4.1
19	Terbufos	0.9988	0.10	102.3 6.0
20	Propyzamide	0.9995	0.30	95.7 5.6
21	Fonofos	0.9991	0.04	102.0 5.0
22	Diazinon	0.9991	0.05	97.8 5.8
23	Phosphamidon	0.9996	0.13	89.5 3.3
24	Pyrimethanil	0.9996	0.04	104.2 2.3
25	Chlorothalonil	0.9982	0.11	81.3 5.5
26	delta-HCH	0.9994	0.03	101.7 4.1
27	Propanil	0.9984	0.02	90.9 3.0
28	Chlorpyrifos-methyl	0.9989	0.05	98.5 4.2
29	Vinclozoline	0.9993	0.05	102.6 4.8
30	Parathion-methyl	0.9993	0.07	101.2 3.8
31	Metalaxyl	0.9996	0.01	115.1 2.7

32	Heptachlor	0.9995	0.09	104.8 5.5
33	Fenitrothion	0.9996	0.05	97.2 4.2
34	Malathion	0.9997	0.05	103.3 5.0
35	S-Metolachlor	0.9997	0.02	107.7 4.7
36	Chlorpyrifos	0.9997	0.06	98.6 3.2
37	Fenthion	0.9994	0.04	100.6 5.8
38	Chlorthal-dimethyl	0.9992	0.05	98.1 5.3
39	Aldrin	0.9989	0.25	105.7 5.8
40	Parathion	0.9995	0.04	103.8 4.1
41	Triadimefon	0.9993	0.05	105.5 4.2
42	Isocarbophos	0.9996	0.05	105.8 4.3
43	Dicofol	0.9992	0.04	110.7 3.5
44	Isofenphos-methyl	0.9996	0.04	111.4 5.3
45	Fipronil	0.9996	0.02	94.8 4.2
46	Phenthoate	0.9995	0.05	97.0 4.9
47	Quinalphos	0.9996	0.40	105.8 5.0
48	Thiabendazole	0.9991	0.02	93.0 3.6
49	Procymidone	0.9994	0.01	104.0 4.7
50	Methidathion	0.9996	0.02	96.4 4.2
51	Isoprothiolane	0.9994	0.04	101.4 3.9
52	Imazalil	0.9993	0.10	99.8 2.0
53	Profenofos	0.9996	0.03	96.1 5.7
54	Tricyclazole	0.9995	0.15	86.3 2.9
55	Oxadiazon	0.9996	0.05	111.6 4.1
56	p, p'-DDE	0.9996	0.04	105.2 4.5
57	Kresoxim-methyl	0.9994	0.06	104.5 4.1
58	Dieldrin	0.9984	0.30	95.4 6.4
59	Endrin	0.9995	0.45	102.9 5.0

60	Ethion	0.9994	0.06	102.6 4.4
61	p, p'-DDD	0.9995	0.03	104.2 5.6
62	o, p'-DDT	0.9996	0.03	108.6 4.8
63	Mepronil	0.9994	0.04	96.9 2.0
64	Triazophos	0.9995	0.10	92.5 4.4
65	Carfentrazone-ethyl	0.9994	0.04	93.3 4.4
66	Propiconazole	0.9993	0.17	96.5 2.1
67	Quinoxyfen	0.9993	0.06	98.6 3.2
68	p, p'-DDT	0.9995	0.05	105.0 3.9
69	Tebuconazole	0.9992	0.06	98.4 1.5
70	Iprodione	0.9994	0.09	93.6 2.5
71	Bifenthrin	0.9996	0.07	95.1 3.9
72	Fenpropathrin	0.9993	0.15	94.5 4.4
73	Phosalone	0.9995	0.03	92.0 3.2
74	Cyhalothrin	0.9994	2.50	92.1 2.7
75	Azinphos-methyl	0.9989	0.12	71.2 6.8
76	Permethrin	0.9991	1.20	92.1 1.8
77	Coumaphos	0.9997	0.20	81.8 2.2
78	Pyridaben	0.9994	0.20	94.7 2.8
79	Prochloraz	0.9986	0.60	91.2 5.6
80	Butafenacil	0.9996	0.03	92.9 3.2
81	Cyfluthrin	0.9994	2.40	93.6 2.2
82	Cypermethrin	0.9992	3.00	92.2 3.0
83	Flucythrinate	0.9989	1.50	90.4 4.0
84	Quizalofop-p-ethyl	0.9991	0.12	92.4 3.6
85	Fenvalerate	0.9994	0.50	87.6 2.0
86	Pyraclostrobin	0.9996	0.60	72.0 5.0
87	Fluvalinate	0.9993	0.40	80.5 4.4

88	Difenoconazole	0.9991	0.60	91.2 4.8
89	Deltamethrin	0.9991	2.00	80.0 2.9
90	Famoxadone	0.9994	0.40	81.9 3.1

Recovery test

Blank matrix samples of wine were spiked with a multi-pesticide standard solution at the spiked level of 7.0μg/kg and then subjected to the afore-mentioned pretreatment procedures. Five replicate assays were performed on each of the samples and the resulted spike recoveries of analytes are shown in Table 2.

Sample assay

A number of off-the-shelf wine samples were assayed and one of the sample was detected for presence of 0.08mg/kg Metalaxyl. Chromatogram of the sample was shown in Fig. 4.

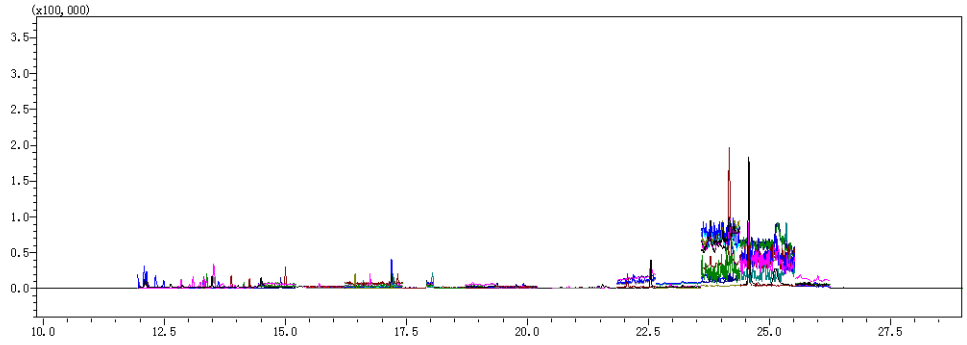


Fig. 4 MRM chromatogram of a wine sample

CONCLUSION

90 pesticide residues in wine were analyzed with the proposed method using QuEChERS extraction method and Shimadzu PTV-GC-MS/MS. The instrument shows good repeatability and high sensitivity and achieved spike recoveries in the range of 71~116% for the 90 pesticides. This method found to be robust and reliable for analysis of wine for multi-pesticide residues at low level.

C-27 Determination of Aryloxyphenoxy propionate herbicides in carrot with GC-MS/MS

INTRODUCTION

A method was proposed in this paper for determination of aryloxyphenoxy propionate (APP) herbicides in carrot with Shimadzu Triple Quadrupole GCMS-TQ8030. The method developed shows calibration curves of good linearity in the concentration range of 1~100μg/L with LODs in the range of 0.07~0.34 μg/L (calculated as 3 times of Signal to Noise Ratio). Six replicate injections of 10μg/L herbicides standard solutions were performed and the %RSDs of the peak areas of the analytes were in the range of 0.81%~2.58%. The method's spike recoveries for the analytes were in the range of 73.94%~110.37%.

Aryloxyphenoxy propionates (APPs) herbicides, a new category of herbicides that have demonstrated good activity in the past two decades, are developed on the basis of phenoxyacetic herbicides. They are a category of highly selective antagonists of phytohormones used for the prevention and removal of annual or perennial weeds of the *Gramineae* family.

Presently in China, MRLs have been set for some APP herbicides, but the MRLs only apply to soybean, beet and edible vegetable oil (e.g. the MRL for quizalofop-p-ethyl in cottonseeds is 0.05mg/kg). By contrast, many countries including EU, USA, and Japan have established MRLs for all APP herbicides in foods (Japan, for example, has set an MRL of 0.01mg/kg for haloxyfop-methyl in poultry eggs).
In this paper, a sensitive and accurate method is proposed for determination of trace residue of APP herbicides in carrot with Shimadzu Triple Quadrupole GCMS-TQ8030.

EXPERIMENTS

Instrument

Triple Quadrupole GC-MS: GCMS-TQ8030

Conditions of Analysis

Column	: Rxi-5Sil MS, 30m×0.25mm×0.25μm
Column temperature program	:50°C(2min)→@30°C/min→180°C→ @5°C/min →280°C(10min)
Injector temperature	: 250°C

Injection mode	: Splitless (1.2min)
Carrier gas control mode	: Constant Linear Velocity (36.3cm/sec)
Carrier gas	: Helium
Collision gas	: Argon
Solvent cut time	: 13.5min
Detector voltage	: Tuning voltage+0.2kv
Interface temperature	: 280°C
Ionization temperature	: 230°C
Acquisition mode	: MRM

Pretreatment of samples

Preparation of test samples

5g test sample was accurately weighed (with a precision of 0.01g) and transferred to a 250mL stopper Erlenmeyer flask, added 15g anhydrous magnesium sulfate, 6g anhydrous sodium acetate and 50mL extraction solvent (n-hexane saturated acetonitrile solution containing 1% glacial acetic acid), subjected to a shaker for 30 min, allowed to rest for 10 min, then filtered into a 150mL concentrator tube. The residue was again added with another 20 mL extraction solvent and subjected to extraction once again. The two filtrates were combined, subjected to rotary evaporation at 40°C to almost dry, the resulted residue was dissolved in 2 mL acetonitrile for cleanup.

Purification

The sample extract was transferred to a small test tube, which was filled with 200mg PSA filler and 250mg graphite carbon filler beforehand, subjected to vortex mixing thoroughly for 1min, filtered through micro-pore film after which the colorant had disappeared.

RESULTS AND DISCUSSION

Standard chromatogram

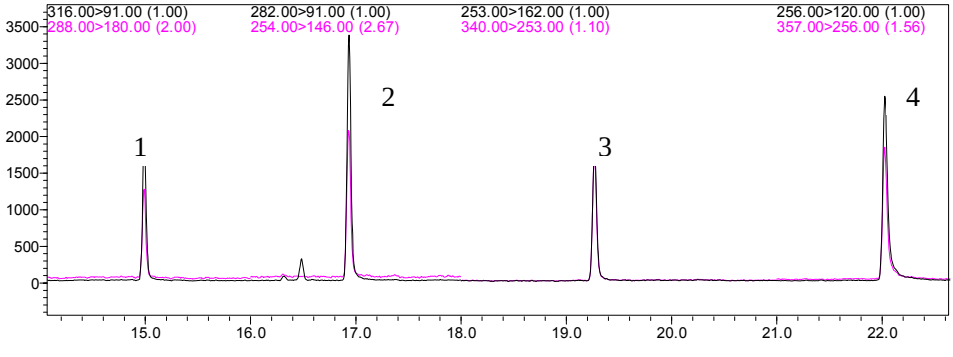


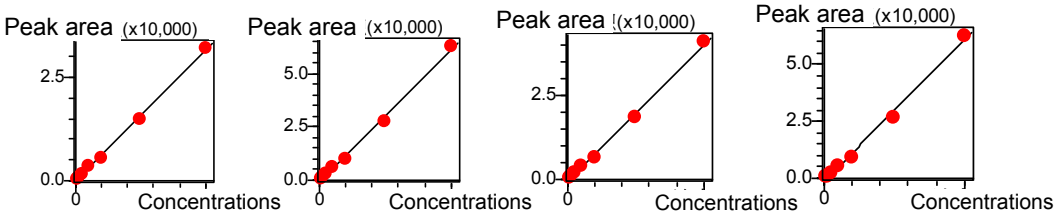
Fig. 1 MRM chromatogram of a multi-residue herbicides (10.00 µg/L)

Table 1 MRM parameters of the herbicides

No.	Analyte	CAS No.	Quantitative Ion	CE	Qualitative Ion	CE
1	Haloxyfop-methyl	69806-34-4	316>91	18	288>180	23
2	Fluazifop-butyl	79241-46-6	282>91	18	254>146	22
3	Diclofop-methyl	51338-27-3	253>162	18	340>253	13
4	Cyhalofop-butyl	122008-85-9	256>120	12	357>256	12

Calibration curve

A series of multi-standard solutions were prepared at concentrations of 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0μg/L, respectively, with acetone as solvent. Linearity was plotted with concentration as abscissa and quantitative ion peak area as ordinate. Instrument LOD was calculated (as 3 times of Signal to Noise) based on the data of 1.0 μg/L standard solution, and the results are as follows.



Haloxyfop-methyl Fluazifop-butyl Diclofop-methyl Cyhalofop-butyl

Table 2. Correlation coefficients and LODs of the analytes (μg/L)

No.	Analyte	Correlation Coefficient	LOD
1	Haloxyfop-methyl	0.9988	0.08
2	Fluazifop-butyl	0.9978	0.22
3	Diclofop-methyl	0.9983	0.07
4	Cyhalofop-butyl	0.9970	0.34

Repeatability

Six replicate injections of 10 μg/L herbicides standard solutions were performed and the %RSDs of the peak area and retention time data of the 6 replicate assays were shown in Table 3.

Table 3 Repeatability test results (n=6)

No.	Analyte	Peak area RSD%	Retention time RSD%
1	Haloxyfop-methyl	0.81	0.02
2	Fluazifop-butyl	1.42	0.02
3	Diclofop-methyl	1.81	0.02
4	Cyhalofop-butyl	2.58	0.01

Recovery test

Off-the-shelf carrots were selected for a recovery test. Samples were added with standards at spiked levels of 5.0, 10.0 and 20.0μg/kg, respectively, and analyzed in accordance with relevant standard. The spike recoveries are shown in Table 4.

Table 4 Recoveries of spiked samples (%)

No.	Analyte	Carrot		
		Spiked level (μg/ kg)		
		5.0	10.0	20.0
1	Haloxyfop-methyl	99.70	88.04	91.60
2	Fluazifop-butyl	108.43	97.58	110.37
3	Diclofop-methyl	79.48	73.94	84.17
4	Cyhalofop-butyl	92.55	89.36	103.67

Sample assay results

Off-the-shelf carrots were purchased from a supermarket and subjected to the afore-mentioned pretreatment procedures and GCMSMS analysis. The observed quantitative results as shown in Table 5.

Table 5. Results of sample quantification

No.	Analyte	Assay results(μg/L)
1	Haloxyfop-methyl	N.D
2	Fluazifop-butyl	N.D
3	Diclofop-methyl	N.D
4	Cyhalofop-butyl	N.D

CONCLUSION

A method is proposed in reference with SN/T 1737.4-2010 *Determination of herbicide residues. Part 4: Determination of aryloxyphenoxypropionate herbicide residues in foodstuff for import and export by GC-MS/MS method*, a standard

issued by China Entry-Exit Inspection and Quarantine Bureau, for determination of APP herbicides residues in carrots using Shimadzu Triple Quadrupole GC-MS (GCMS-TQ8030). The proposed method was easy to operate and of good linearity in the concentration range of 1.0~100.0µg/L on the calibration range. The instrument LOD of 0.07~0.34µg/L (calculated as 3 times of SNR) and a spike recovery in the range of 73.94%~110.37% was obtained. The method is suitable for quantification of APP herbicide residues in carrots.

C-28 Determination of melamine in milk powder by HPLC-tandem mass spectrometry

INTRODUCTION

A method was proposed for determination of melamine in milk powder with Shimadzu LCMS-8030 triple quadrupole mass spectrometer. Samples were extracted, separated with HPLC, and then qualitatively and quantitatively analyzed using LCMS-8030 triple quadrupole mass spectrometer. The method demonstrated satisfactory linearity for melamine in the concentration range of 0.01~0.5 mg/L with a correlation coefficient 0.9998. Precision tests were performed on standard solutions at concentrations of 0.01 mg/L, 0.1 mg/L and 0.5 mg/L, the %RSDs of retention time and peak area for 6 successive injections were below 0.64% and 4.34%, respectively, suggesting that the system was of good precision. The spike recovery test of samples spiked with 0.01 mg/kg of standard solution showed a good recovery of 104.0%. The method's LOQ was 0.005 mg/kg.

Melamine, chemical name of 1,3,5-Triazine-2,4,6-triamine, is an important nitrogen heterocyclic organic industrial chemical commonly used for the production of plastic, glue and flame retardant. It is frequently reported in recent years that law-breakers use melamine as adulterant in raw material for dairy product manufacturing. Triple quadrupole mass spectrometer is provided with multiple reaction monitoring (MRM) mode which can effectively eliminate matrix interference, therefore, it can be used for quantitative analysis of trace amount of melamine. It is stipulated in China's national standard GB/T22388-2008 *Determination of Melamine in Raw Milk and Dairy Products* that LC-MS/MS method shall be used for determination of melamine in milk and dairy products. Furthermore, the method can achieve an LOQ of 0.005 mg/kg for melamine. In this paper， a method was proposed for quantitative assay of melamine in milk powder with Shimadzu LCMS-8030 triple quadrupole mass spectrometer.

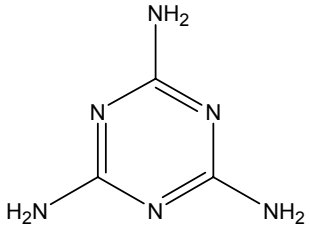


Fig.1 Chemical structure of melamine

EXPERIMENTAL

Instrument

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The detailed configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.41 chromatography workstation.

Conditions of Analysis

LC conditions

Column	: CAPCELL PAK CR (1:4) 2.0 mm I.D.× 150 mm L., 5 µm
Mobile phase A	: The premix of 500 mL 10 mM ammonium acetate aqueous solution and 2 mL acetic acid.
Mobile phase B	: Acetonitrile
Elution mode	: Isocratic, A/B = 40/60 (v/v)
Flow rate	: 0.2 mL/min
Column temperature	: 40 °C
Injection volume	: 10 µL

MS condition

Ionization mode	: ESI (+)
Ionspray voltage	: 4.5 kV
Nebulizing gas	: Nitrogen 3.0 L/min
Drying gas	: Nitrogen 15 L/min
Collision gas	: Argon
DL temperature	: 250 °C
Heater block temperature	: 400 °C

Mode: multiple reaction monitoring (MRM), precursor ion at *m/z* 127.10, quantitative product ion at *m/z* 85.05, qualitative product ion at *m/z* 68.00.

Dwell time : 300 msec
Pause time : 3 msec
MRM parameters : see Table1.

Table 1 MRM parameters of melamine

Name	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
Melamine	127.10	85.05	-12.0	-20.0	-18.0
		68.00*	-12.0	-30.0	-27.0

* refers to qualitative ion.

Preparation of standard solutions and pretreatment of samples

Samples were subjected to pretreatment procedures as stipulated in GB/T22388-2008 *Determination of Melamine in Raw Milk and Dairy Products--Part II LC-MS/MS Method*.

Blank samples were subjected to the pretreatment procedures, and the resulted extract was used to prepare matrix match standards using 10 mg/L melamine stock solution. The working standards at concentrations of 0.01, 0.05, 0.1, 0.2, and 0.5 mg/L were prepared.

RESULTS AND DISCUSSION

Chromatogram of melamine standard sample

The chromatogram of 0.1 mg/L melamine standard sample was as shown in Fig.2, in which the chromatographic peak at retention time of 5.62 min was melamine.

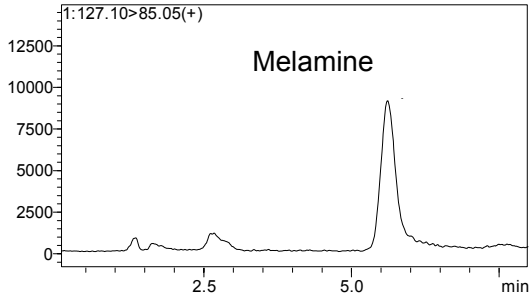


Fig.2 Chromatogram of 0.1 mg/L standard solution

Linearity

A series of standard working solutions at concentrations of 0.01, 0.05, 0.1, 0.2, and 0.5 mg/L were analyzed using the analytical conditions specified above. A calibration curve using equation Y = (1568.71)X + (-1426.24) was plotted (as shown Fig. 3) using concentration as abscissa and peak area as ordinate. The

resulted curve was of good linearity with a correlation coefficient R^2 = 0.9998. The concentration and peak area results of standard solutions are shown in Table 2.

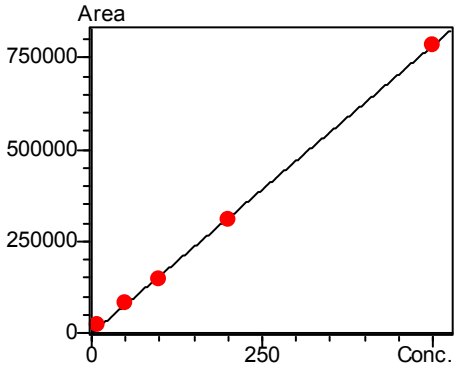


Fig. 3. Calibration curve of melamine

Table 2. Concentrations and peak areas of standard solutions.

Conc. (mg/L)	Area
0.01	19666
0.05	81499
0.1	148105
0.2	786149
0.5	306538

Precision test

The method's precision was assessed by 6 successive injections of standard samples at concentrations of 0.01 mg/L, 0.1 mg/L and 0.5 mg/L, respectively. The resulted %RSDs of retention time and peak areas are shown in Table3.

Table 3 Reproducibility test data of melamine (n=6)

Conc.(mg /L)	%RSD (RT)	%RSD (Area)
0.01	0.64	4.34
0.1	0.18	3.80
0.5	0.34	3.18

Accuracy test

In order to assess the method's accuracy, samples spiked with 0.01 mg/kg standard were analyzed for determination of spike recovery. The MRM chromatogram of blank sample is shown in Fig.4, suggesting that the sample contained trace amount of melamine at concentration of 2.736 μg/kg. The

chromatogram of samples spiked with 0.01 mg/kg standard is shown in Fig.5, showing that the average recovery of two samples spiked with 0.01 mg/kg standard was 104.0%. Samples spiked with 3 mg/kg standard were processed in duplicate and one-fifth of the filtrate obtained at the step of solid-phase extraction was purified. The calculated average recovery was 99.9%, meeting the requirements stipulated in China's national standard that "at the spiked level of 0.01 mg/kg~0.5 mg/kg, the recovery shall be in the range of 80%~110%".

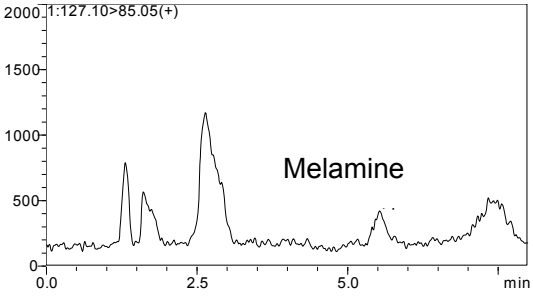


Figure 4. MRM chromatogram of a blank sample

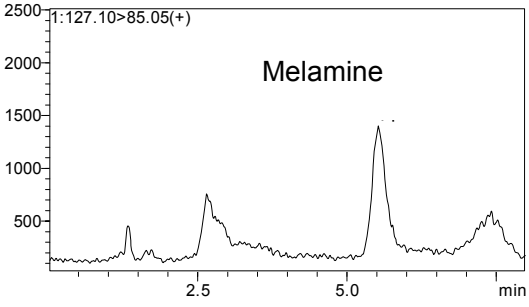


Fig.5 MRM chromatogram of a sample spiked with 0.01 mg/kg of standard

Sensitivity test

Standard working solutions of melamine (0.01, 0.05, 0.1, 0.2 and 0.5 mg/L) were analyzed and workstation software was used to calculate the LOD (S/N=3) of melamine which observed to be 0.0017 mg/L. The calculated LOQ (S/N=10) found to be 0.0052 mg/L. The 0.005 mg/L standard solution of melamine was prepared and injected for analysis. The MRM chromatogram is shown in Fig. 6. The S/N=14.04 was observed.

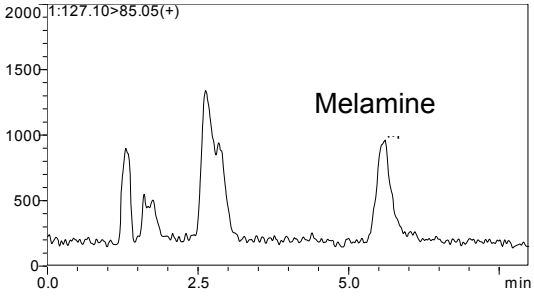


Fig.6 Chromatogram of 0.005 mg/L melamine

CONCLUSION

A method that met the requirements stipulated in China's national standard was proposed for determination of melamine in milk powder with Shimadzu LCMS-8030 triple quadrupole mass spectrometer. The calibration curve had a correlation coefficient higher than 0.999. The %RSDs of retention time and peak areas of standard working solutions at 3 different levels were below 0.64% and 4.34%, respectively. The method's LOQ was 0.005 mg/kg and spike recovery of 0.01 mg/kg sample was 104.0%. Shimadzu LCMS-8030 triple quadrupole mass spectrometer can satisfactorily meet the requirements for determination of melamine in dairy product.

C-29 Determination of Phthalates in Beverage by UFLC- Triple Quadrupole Mass Spectrometry

INTRODUCTION

A method was proposed for the determination of phthalates using Shimadzu ultra fast liquid chromatograph and triple quadrupole mass spectrometer. Samples were, analyzed by LC-30A ultra fast liquid chromatograph, and then quantitatively assayed with LCMS-8030 triple quadrupole mass spectrometer. The calibration curves of 16 phthalates were plotted in the concentration range of 10-500 µg/L using internal standard method. The plotted calibration curves were of satisfactory linearity with correlation coefficients higher than 0.999. Standard solutions at concentrations of 20 µg/L, 50 µg/L, and 100 µg/L were used for precision test. The %RSDs of retention time and peak area of 6 successive injections were below 1.04 % and 4.15 %, respectively, showing that the system had satisfactory precision.

Phthalates are a group of artificially synthesized chemicals which, when added into plastics, can improve the plastics' elasticity. They are a category of common elasticizers extensively present in agricultural film, plastic bags, toys, and rubber tubes. They can be carcinogenic and teratogenic if ingested. On May 24, 2011, Taiwanese media reported that some clouding agent products in food which later found to be elasticizer-tainted. The origin of this problem was that some manufacturer used phthalate elasticizers as clouding agents instead of palm oil in the production of food additives in order to reduce production costs.

A method was developed for the determination of phthalates using Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer.

EXPERIMENTAL

Instruments

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The detailed configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, LabSolutions Ver. 5.41 chromatography workstation.

Conditions of Analysis

LC conditions

Column : Shim-pack XR-ODS III 2.0 mm I.D. × 150 mm L., 2.2 µm
Mobile phase A : 5mM ammonium acetate aqueous solution
Mobile phase B : Methanol
Flow rate : 0.4 mL/min
Column temperature : 45 °C
Injection volume : 10 µL
Elution mode : Gradient elution, see Table 1 for time program.

Table 1 Time program

Time (min)	Module	Command	Value
0.01	Pumps	Pump B Conc.	75
6.50	Pumps	Pump B Conc.	90
7.00	Pumps	Pump B Conc.	100
8.50	Pumps	Pump B Conc.	100
8.60	Pumps	Pump B Conc.	75
10.00	Controller	Stop	

MS condition

Ionization mode : ESI (+)
Ionization voltage : +4.5 kV
Nebulizing gas : Nitrogen 3.0 L/min
Drying gas : Nitrogen 15 L/min
Collision gas : Argon
DL temperature : 250 °C
Heater block temperature : 450 °C
Mode : Multiple Reaction Monitoring (MRM)
Dwell time : 10 ms
Pause time : 3 ms
MRM parameters : see Table 2

Preparation of standard solutions

Standards : A total of 16 standards were used, i.e. dimethylphthalate (DMP), dibutylphthalate (DBP), dimethoxyethyl phthalate (DMEP), dioctyl phthalate (DPP), butyl benzyl phthalate (BBP), bis(2-n-butoxyethyl) phthalate (DBEP), dicyclohexyl phthalate (DCHP), di-2-ethylhexyl phthalate (DEHP), Di-n-octyl phthalate (DNOP), dinonyl phthalate (DNP), dihexylphthalate (DHXP), diethyl phthalate (DIBP), bis(2-ethoxyethyl) phthalate (DEEP), di-iso-decyl phthalate (DIDP), diethyl phthalate (DEP), and diphenyl phthalate (DIPP).

Internal standard substance: Deuterated-di-2-ethylhexyl phthalate (D4-DEHP).

Preparation of standard working solutions: Multi-standard intermediate solution was prepared using methanol as solvent, and then diluted with 50% methanol aqueous solution to get multi-standard working solutions at concentrations of 10, 20, 50, 100, and 500 μg/L.

Preparation of internal standard (IS) working solution: 10 mg/L IS intermediate solution was prepared using methanol as solvent, and then diluted with 50% methanol to get 100 μg/L internal standard working solution.

Sample pretreatment method: 5.0 mL beverage was taken, added with 2.0 mL n-hexane (residue analysis grade), shaken for 2 min, allowed to settle. Then 1.0 mL supernatant was taken and dried under nitrogen flush, added with 50 % methanol aqueous solution and diluted to 1.0 mL and then injected for analysis.

Table 2 MRM Parameters

Compound	Precursor Ion	Product Ion	Q1 Pre Bias	CE (V)	Q3 Pre Bias
DMEP	283.15	59.10	-29	-15.2	-24
		207.1*	-29	-10.0	-26
DMP	195.10	163.1	-22	-11.3	-20
		77.10*	-22	-34.5	-29
DEEP	311.15	73.15	-32	-13.9	-29
		221.15*	-32	-10.0	-28
DEP	223.15	177.15	-40	-10.0	-21
		149.05*	-40	-16.5	-32
DIPP	319.15	225.20	-33	-13.9	-30
		77.10*	-33	-38.4	-29
DIBP	279.20	149.05	-32	-17.8	-32
		205.15*	-32	-10.0	-25
DBP	279.20	149.05	-36	-15.2	-31
		205.15*	-36	-10.0	-25
BBP	313.20	91.15	-33	-31.9	-20
		149.10*	-33	-13.9	-32
DBEP	367.25	101.30	-23	-13.9	-22
		249.25*	-23	-10.0	-20
DPP	307.20	149.10	-35	-13.9	-33
		219.20*	-35	-10.0	-29
DCHP	331.20	149.05	-40	-31.9	-32
		167.05*	-40	-13.9	-20
DHXP	335.25	149.20	-34	-13.9	-33
		233.30*	-34	-10.0	-32
DIDP	447.40	141.25	-21	-12.6	-31
		85.20*	-21	-21.6	-20
D4-DEHP	395.35	153.20	-20	-26.8	-33
		113.30*	-20	-10.0	-25
DEHP	391.35	149.15	-40	-31.9	-32
		113.30*	-40	-10.0	-25
DNOP	391.35	149.15	-40	-19	-33
		261.25*	-40	-10	-20
DNP	419.35	71.20	-20	-22.9	-29
		127.25*	-20	-12.6	-29

Note: *refers to qualitative ion

RESULTS AND DISCUSSION

MRM Chromatograms of Standard Samples

The chromatograms of 10 ng/mL standard solutions were as shown in Fig.1-Fig. 17.

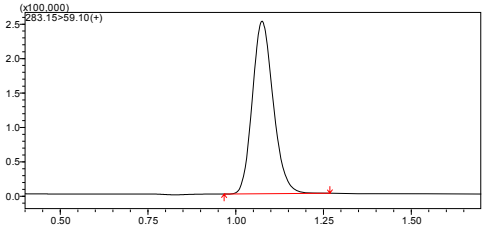


Fig.1 MRM chromatogram of DMEP (283.15>59.10)

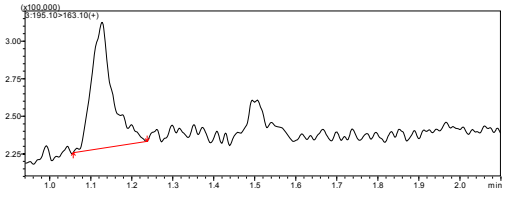


Fig. 2 MRM chromatogram of DMP (195.10>163.10)

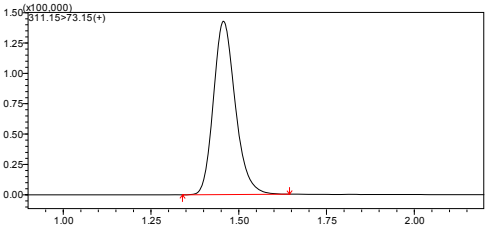


Fig. 3 MRM chromatogram of DEEP (311.15>73.15)

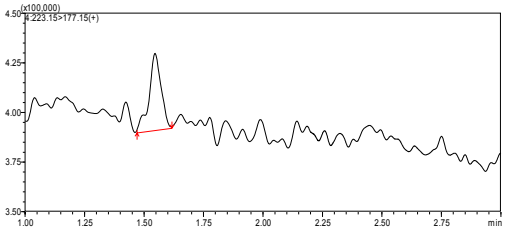


Fig. 4 MRM chromatogram of DEP (223.15>177.15)

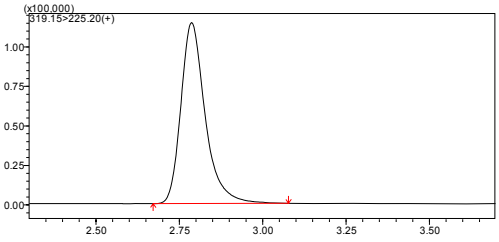


Fig. 5 MRM chromatogram of DIPP (319.15>225.20)

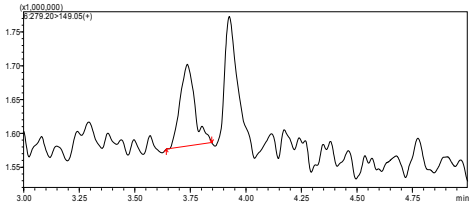


Fig. 6 MRM chromatogram of DIBP (279.20>149.05)

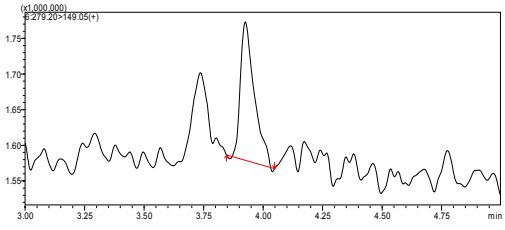


Fig. 7 MRM chromatogram of DBP (279.20>149.05)

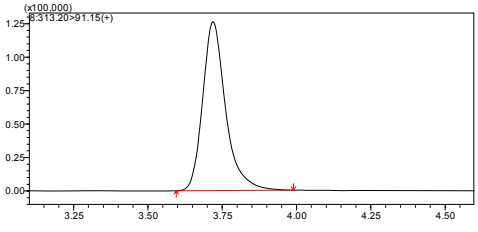


Fig. 8 MRM chromatogram of BBP (313.20>91.15)

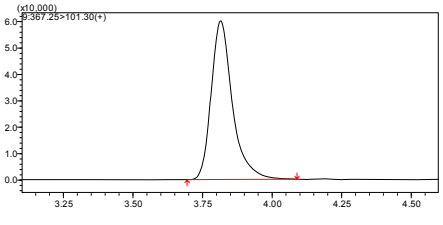


Fig. 9 MRM chromatogram of DBEP (367.25>101.30)

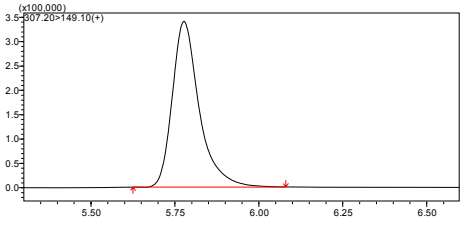


Fig. 10 MRM chromatogram of DPP (307.20>149.10)

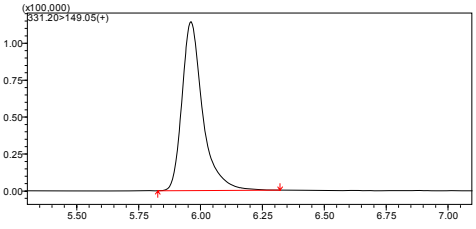


Fig. 11 MRM chromatogram of DCHP (331.20>149.05)

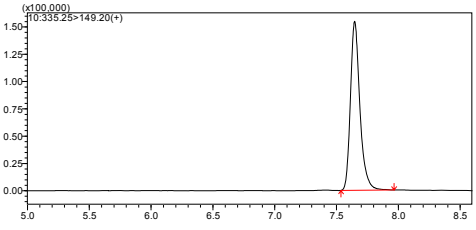


Fig. 12 MRM chromatogram of DHXP (335.25>149.20)

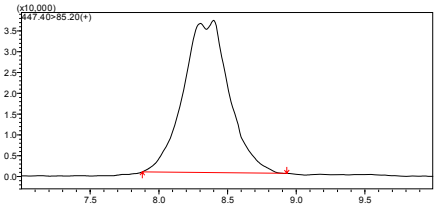


Fig. 13 MRM chromatogram of DIDP (447.40>141.25)

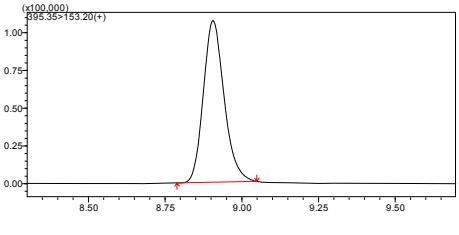


Fig. 14 MRM chromatogram of D4-DEHP (395.35>153.20)

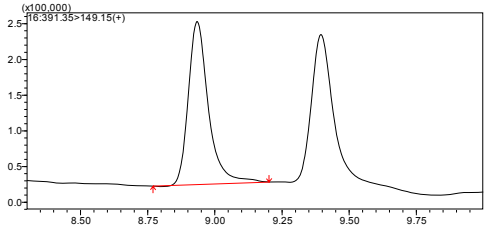


Fig. 15 MRM chromatogram of DEHP (391.35>149.15)

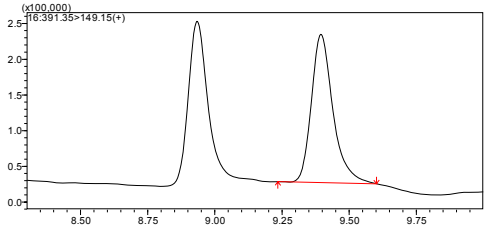


Fig. 16 MRM chromatogram of DNOP (391.35>149.15)

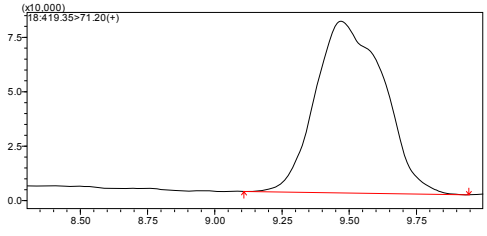


Fig. 17 MRM chromatogram of DNP (419.35>127.25)

Linearity

Multi-standard working solutions at concentrations of 10, 20, 50, 100 and 500 µg/L were analyzed with internal standard method under the analysis conditions as specified before and calibration curves were plotted. The plotted calibration curves were of satisfactory linearity. The details are given in Table 2.

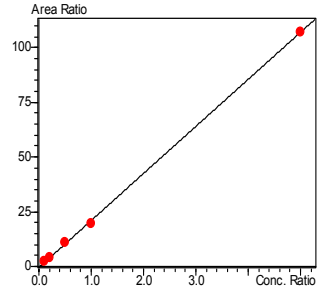


Fig. 18 Calibration curve of DMEP

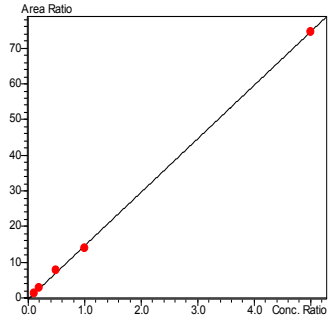


Fig. 19 Calibration curve of DMP

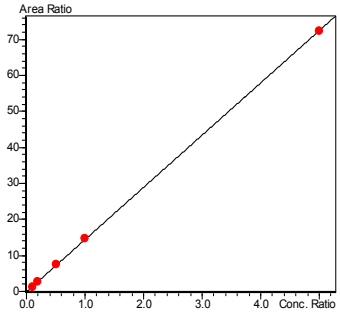


Fig. 20 Calibration curve of DEEP

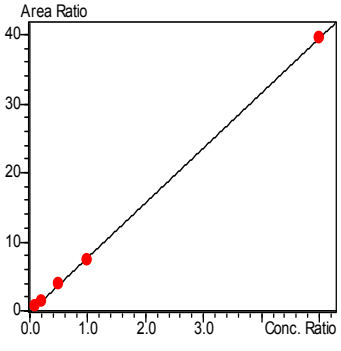


Fig. 21 Calibration curve of DEP

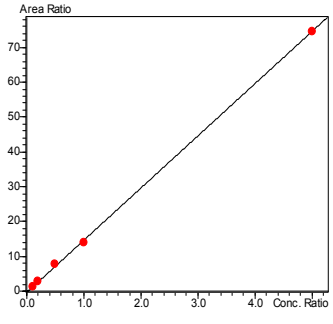


Fig. 22 Calibration curve of DIPP

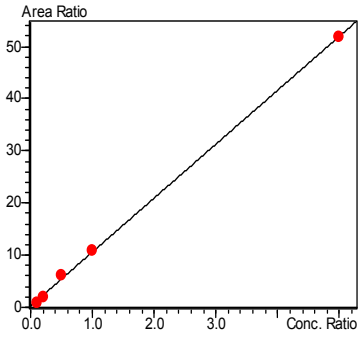


Fig. 23 Calibration curve of DIBP

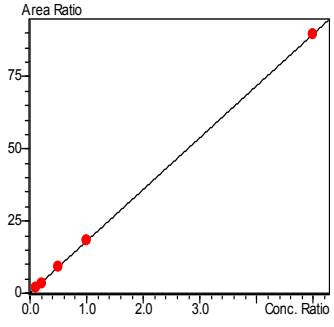


Fig. 24 Calibration curve of DBP

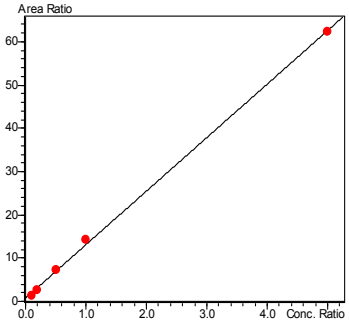


Fig. 25 Calibration curve of BBP

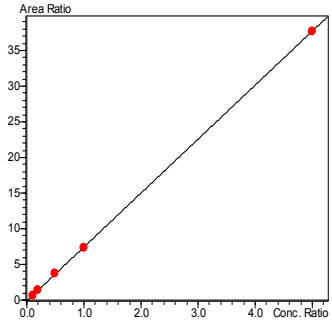


Fig. 26 Calibration curve of DBEP

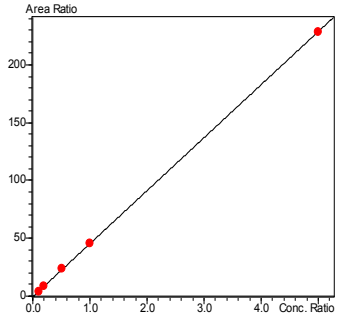


Fig. 27 Calibration curve of DPP

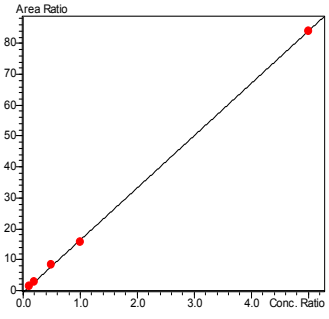


Fig. 28 Calibration curve of DCHP

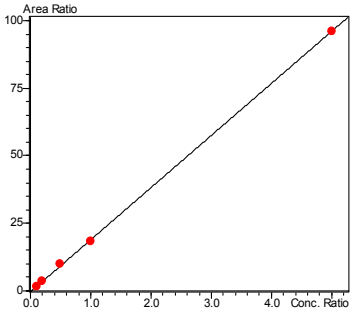


Fig. 29 Calibration curve of DHXP

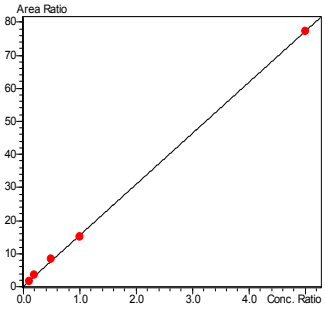


Fig. 30 Calibration curve of DIDP

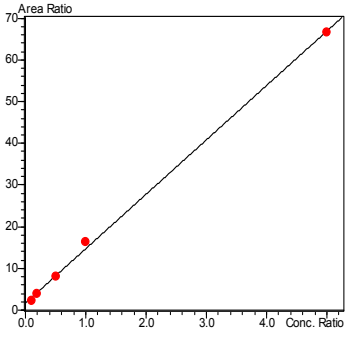


Fig. 31 Calibration curve of DEHP

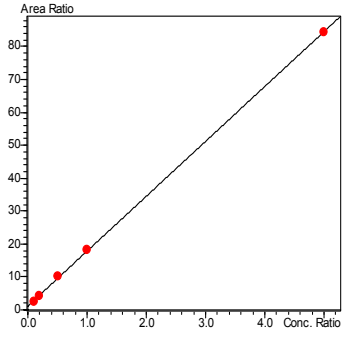


Fig. 32 Calibration curve of DNOP

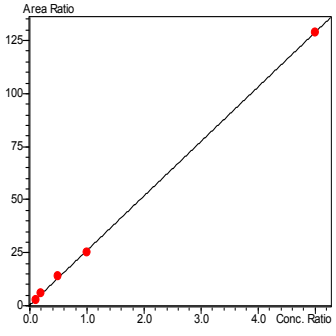


Fig. 33 Calibration curve of DNP

Table 2 Calibration curves and LOQ information of the 16 phthalates

Compound	Calibration Curve	Correlation Coefficient (r)	LOQ (µg/L)	LOD (µg/L)
DMEP	Y = (21.5056)X + (0.507982)	0.9999	0.51	0.17
DMP	Y = (3.84968)X + (0.6499)	0.9993	8.65	2.85
DEEP	Y = (14.5116)X +(-0.0910578)	1.0000	0.13	0.04
DEP	Y = (7.9453)X + (-0.229857)	0.9999	67.80	22.40
DIPP	Y = (14.9911)X + (-0.349504)	0.9999	0.27	0.09
DIBP	Y = (10.3416)X + (0.280682)	0.9997	24.30	8.10
DBP	Y = (17.9339)X + (0.142112)	1.0000	12.90	4.26
BBP	Y = (12.3748)X + (0.731055)	0.9996	0.09	0.03
DBEP	Y = (7.56302)X + (-0.109707)	1.0000	0.30	0.10
DPP	Y = (45.8435)X + (-0.495981)	1.0000	0.12	0.04
DCHP	Y = (16.9033)X + (-0.553426)	0.9999	0.18	0.06
DHXP	Y = (19.2892)X + (-0.36547)	1.0000	0.09	0.03
DIDP	Y = (15.4162)X + (0.149031)	0.9999	0.63	0.21
DEHP	Y = (13.0563)X + (1.66949)	0.9994	0.76	0.25
DNOP	Y = (16.667)X + (1.19159)	1.0000	0.72	0.24
DNP	Y = (25.6942)X + (0.384239)	1.0000	0.69	0.23

Precision test

Multi-standard working solutions at concentrations of 20, 50, and 100 µg/L were injected 6 times in succession to assess the precision of the method. Repeatability of retention time and peak area is shown in Table 3. The results showed that the %RSDs of retention time and peak area data of standard solutions at 3 concentrations (high, medium, low) were 0.03%~1.04 % and 0.19 %~4.15 %, respectively, indicating that the method's precision was satisfactory.

Table 3. Repeatability of 16 phthalates (n=6)

Compound	20 μg/L		50 μg/L		100 μg/L	
	%RSD (RT)	%RSD (Area)	%RSD (RT)	%RSD (Area)	%RSD (RT)	%RSD (Area)
DMEP	0.05	2.68	0.45	0.94	0.12	0.52
DEEP	0.10	2.95	0.68	1.05	0.13	0.86
DMP	0.10	3.90	0.39	4.03	0.14	3.13
DEP	0.53	3.66	0.70	3.02	0.62	3.89
DIPP	0.09	3.28	1.04	0.50	0.10	0.19
DIBP	0.05	2.12	0.93	2.53	0.20	3.65
DBP	0.11	3.09	0.84	3.72	0.13	2.17
BBP	0.09	2.87	0.88	1.04	0.09	0.55
DBEP	0.10	1.91	0.87	1.24	0.08	0.78
DPP	0.07	1.63	0.49	1.24	0.05	1.26
DCHP	0.06	1.23	0.46	1.35	0.04	0.71
DHXP	0.05	1.63	0.31	1.52	0.03	0.76
DIDP	0.44	2.39	0.50	2.34	0.16	1.82
DEHP	0.05	3.54	0.20	3.72	0.05	2.22
DNOP	0.06	2.71	0.26	1.82	0.07	1.82
DIDP	0.06	4.15	0.26	4.11	0.07	2.37

Spike recovery test

An off-the-shelf green tea beverage was taken as matrix for determination of DEHP. DEHP was detected in the off-the-shelf green tea beverage and its content was determined to be 4.0 μg/L; and the MRM chromatogram is shown in Fig. 34. The above-mentioned green tea was spiked with DEHP at spike level of 50 μg/L and then subject to analysis; the concentration of DEHP was assayed to be 49.2 μg/L; after the DEHP content in matrix (4.0 μg/L) was deducted, the recovery of spiked samples was calculated to be 90.4 %. The chromatogram of off-the-shelf green tea samples spiked with standards is shown in Fig. 35.

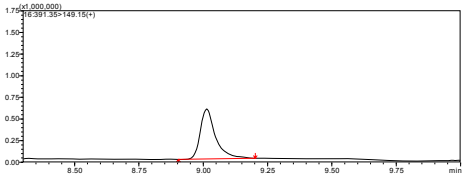


Fig. 34 MRM chromatogram of an off-the-shelf green tea (391.30>149.05)

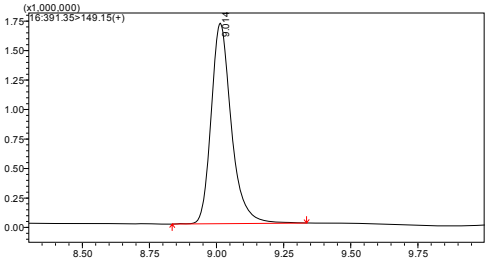


Fig. 35. MRM chromatogram of green tea spiked with standards (391.30>149.05)

REAL SAMPLE ASSAY RESULTS

Four types of off-the-shelf beverage samples were analyzed. Solvent blank was deducted from the quantitative analysis results obtained for the tested samples. Samples whose phthalates content is outside the range of the calibration curve were diluted first before injected for analysis. The quantification results were as shown in Table 4. DEHP was detected at different levels in all 4 types of beverage.

Table 4. Quantification results of the tested samples

Tested sample	Green tea	Sports drink	Guava juice	Milk tea
DEHP concentration (mg/L)	0.004	0.785	0.103	0.081

CONCLUSION

A method was developed for the determination of phthalates in beverage using Shimadzu LC-30A ultra-fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer. The method was fast, reproducible, linear and rugged. The correlation coefficients of all calibration curves were of good linearity and higher than 0.999. DEHP was detected as major contaminant at various levels in all 4 types of the off-the-shelf beverages.

C-30 Determination of Rhodamine B in chili by UFLC-triple quadrupole mass spectrometry

INTRODUCTION

In this paper, a method was proposed for fast and sensitive determination of Rhodamine B in chili with Shimadzu LC-30A ultra-fast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. Extracted samples were analyzed by LC-30A ultra-fast liquid chromatograph, and then quantitatively analyzed using LCMS-8030 triple quadrupole mass spectrometer. The method was of good linearity for Rhodamine B in the concentration range of 0.25～500 μg/L and the correlation coefficients of all calibration curves were greater than 0.999. Precision test was performed on standard solutions at the concentration of 10 μg/L, 100 μg/L, and 1000 μg/L. The %RSDs of retention time and peak area of 6 successive injections were below 0.337% and 1.164%, respectively, suggesting that the method was of good precision. The method demonstrated an LOQ of 0.5 μg/kg and met the requirement of 5 μg/kg in SN/T 2430-2010.

Rose red B also called as Rhodamine B or rose bengal, is a basic fluorescent dye. It is extensively used as a fluorescent reagent in environmental protection, mining, iron and steel industries for fluorimetric analysis. But its application in food is banned in China and EU countries because of its potential carcinogenic and mutagenic actions. Its molecular structure is as shown in Fig.1.

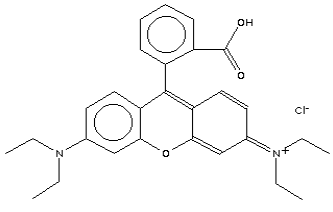


Fig.1 Chemical structure of Rhodamine B

No national standard and industrial standard concerning the assay of Rhodamine B in food had been stipulated in China until 2010 when SN/T 2430-2010 *Determination of Rhodamine B in Food for Import and Export* was promulgated and implemented by the Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China. In this paper, a method is proposed for

fast and accurate determination of Rhodamine B in food with Shimadzu LC-30A ultra-fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer for reference of relevant laboratories.

EXPERIMENTAL

Apparatus

A combined system of Shimadzu ultra-fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The specific configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.41 chromatography workstation.

Conditions of Analysis

LC Conditions

Apparatus : LC-30A
Chromatographic column : Shim-pack XR-ODS III2.0 mm ×50 mm L, 1.6 μm
Mobile phase A : 0.1% aqueous solution of formic acid
Mobile phase B : 0.1% acetonitrile solution of formic acid
Flow rate : 0.3 mL/min
Injection volume : 4 μL
Column temperature : 40 °C
Elution mode : Gradient elution with an initial concentration of 30% phase B., see Table 1 for time program.

Table 1 Time program

Time (min)	Module	Command	Value
2.00	Pumps	B Conc.	90
3.00	Pumps	B Conc.	90
3.10	Pumps	B Conc.	30
4.00	Controller	Stop	

MS condition

Apparatus : LCMS-8030
Ion source : ESI(+)
Ionization voltage : +4.5 kV
Nebulizing gas : Nitrogen 3.0 L/min
Drying gas : Nitrogen 15 L/min
Collision gas : Argon
DL temperature : 250 °C

Heater block temperature	:	400 °C
Mode	:	Multiple reaction monitoring (MRM)
Dwell time	:	20 ms
Pause time	:	3 ms
MRM parameters	:	Listed in Table2

Table 2 MRM parameters of Rhodamine B

Name	Precursor Ion	Product Ion	Q1Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
Rhodamine B	443.25	399.20	-30.0	-45.0	-29.0
		355.10*	-30.0	-50.0	-26.0

* refers to qualitative ion.

Sample Preparation

Preparation of standard solution: 10 mg/L standard intermediate solution was prepared using methanol as solvent and then diluted with water into standard working solutions at concentrations of 0.25, 0.5, 5, 10, 50, 100, and 500 μg/L.

Sample pretreatment method: The same as in SN/T 2430-2010 *Determination of Rhodamine B in Food for Import and Export*, which is a standard promulgated and implemented by the Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

RESULT AND DISCUSSION

MRM Chromatogram of Standard Samples

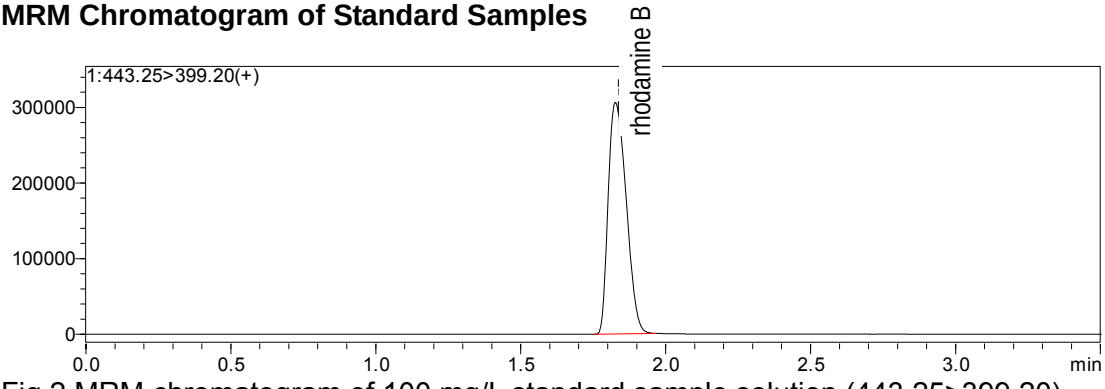


Fig.2 MRM chromatogram of 100 mg/L standard sample solution (443.25>399.20)

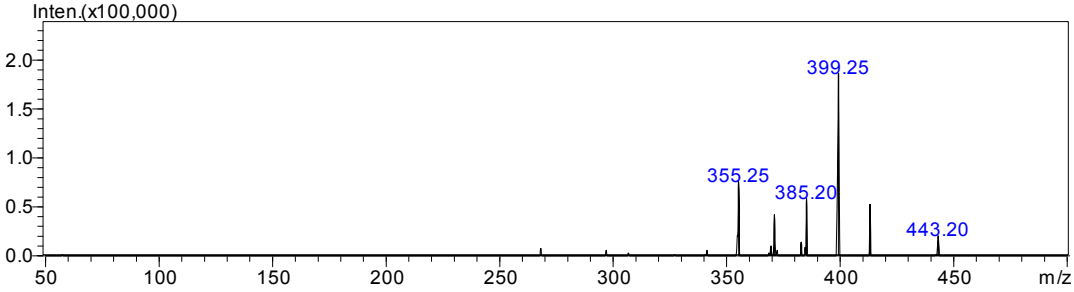
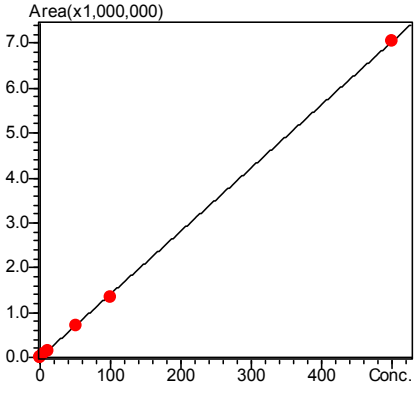


Fig.3 Product ion spectra of 100 µg/L standard sample solution (CE, -50 V)

Linearity

Standard working solutions at concentrations of 0.25, 0.5, 5, 10, 50, 100, and 500 µg/L were analyzed using the analytical conditions specified above. The calibration curve was plotted (as shown in Fig.4 and Fig. 5, which is an enlarged part of lower range of Fig. 4) with concentration as X-axis and peak area as Y-axis. The plotted calibration curve was of good linear relation and its linear equation was Y = (14945)X + (0) and its correlation coefficient was r = 0.9999.



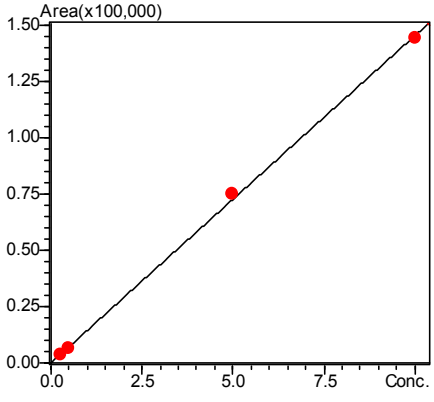


Fig.4 Calibration curve of rhodamine B (full concentration range)
Fig.5 Calibration curve of rhodamine B (low concentration range)

Precision test

Standard working solutions at concentrations of 10, 100, and 1000 µg/L were analyzed for 6 times in succession to assess the method's precision. The repeatability results of retention time and peak area are shown in Table 3. The results showed that the %RSDs of retention time and peak area data of standard solutions at the 3 concentrations fell in the range of 0.556%~1.164% and 0.134%~0.337%, respectively, indicating that the method's precision was satisfactory.

Table 3 Repeatability - retention time and peak area (n=6)

No.	10 µg/L		100 µg/L		1000 µg/L	
	%RSD (Area)	%RSD (RT)	%RSD (Area)	%RSD (RT)	%RSD (Area)	%RSD (RT)
1	4,942	1.836	68,430	1.837	224,177	1.828
2	4,977	1.841	68,741	1.838	224,699	1.831
3	4,828	1.824	69,283	1.826	225,902	1.830
4	4,953	1.833	68,513	1.836	225,709	1.828
5	4,898	1.826	68,694	1.835	220,357	1.829
6	4,865	1.834	68,148	1.835	220,217	1.824
Average	4,911	1.832	68,635	1.834	223,510	1.828
RSD%	1.164	0.337	0.556	0.227	1.153	0.134

Sensitivity test

Rhodamine B was detected in chili blank matrix at concentration of 0.2 µg/kg, yielding a chromatogram as shown in Fig.6. For chili blank sample spiked with Rhodamine B at 0.5 µg/kg, the chromatogram as shown in Fig.7 was obtained. The proposed method had an LOQ of 0.5 µg/kg for Rhodamine B, better than the LOQ of 5.0 µg/kg stipulated in SN/T 2430-2010 *Determination of Rhodamine B in Food for Import and Export*.

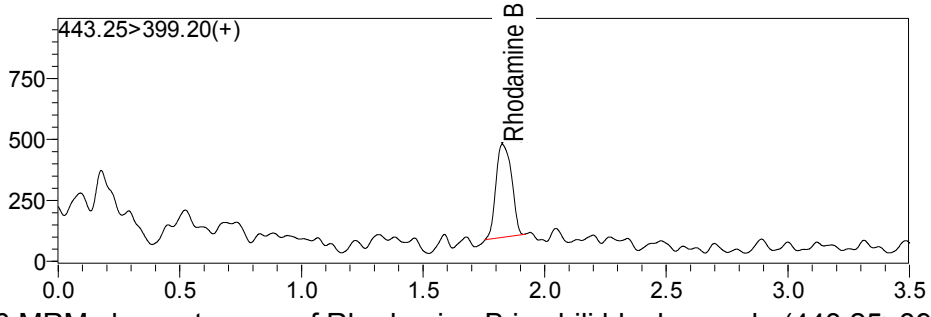


Fig.6 MRM chromatogram of Rhodamine B in chili blank sample (443.25>399.20)

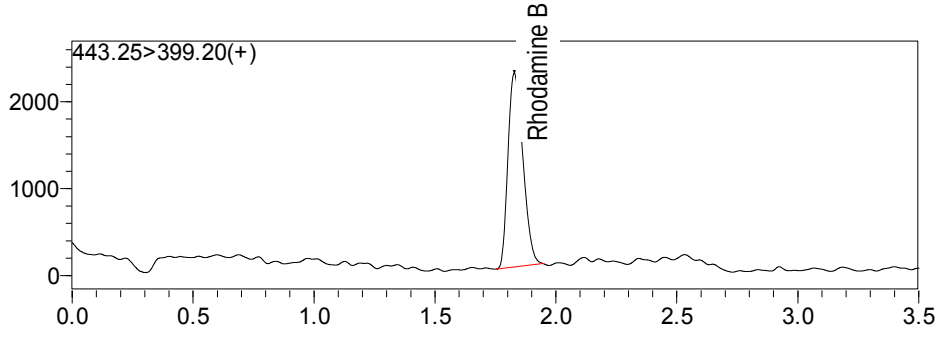


Fig.7 MRM chromatogram of chili sample spiked with Rhodamine B at 0.5 µg/kg (443.25>399.20)

CONCLUSION

A method is proposed for the analysis of Rhodamine B in chili with Shimadzu LC-30A ultra-fast liquid chromatograph-tandem LCMS-8030 triple quadrupole mass spectrometer. The method was fast, precise and having wide linearity range (0.25~500 µg/L). The correlation coefficient of calibration curve was greater than 0.999. The method's LOQ for Rhodamine B in chili was 0.5 µg/kg, sufficient for the 5.0 µg/kg LOQ requirement stipulated in the industrial standard issued by China Entry-Exit Inspection and Quarantine Bureau. It is concluded that the method with Shimadzu ultra-fast liquid chromatograph-tandem mass spectrometer can meet the requirements for the analysis of Rhodamine B in food for import and export.

C-31 Detection of Sodium 4-Chlorophenoxy acetate, 6-Benzylaminopurine and 2, 4-D in Bean Sprouts

INTRODUCTION

In this paper, a method was proposed for fast determination of sodium 4-chlorophenoxyacetate, 6-benzylaminopurine, and 2, 4-D in bean sprouts with ultra-fast liquid chromatograph and triple quadrupole mass spectrometer. Extracted samples were analyzed by ultra-fast liquid chromatograph, and then quantitatively analyzed with triple quadrupole mass spectrometer. The 3 compounds got separated and detected rapidly within 1.5 minutes. The method was of good linearity for 4 samples in the concentration range of 1~100 μg/L and the correlation coefficients of all calibration curves were greater than 0.999. Precision test was performed on 10 μg/L multi-standard solution. The RSDs of retention time and peak area of 6 successive injections were below 0.3% and 4.3%, respectively, suggesting that the system was of good precision. The method can be used satisfactorily for detection of sodium 4-chlorophenoxyacetic, 6-benzylaminopurine, and 2,4-D in bean sprouts.

6-benzylaminopurine (6-BA) is an artificially synthesized cytokine, which is capable of suppressing the decomposition of chlorophyll, nucleic acids, and proteins in plant leaves. Because of its endogenous hormone-like structure and properties, it is widely used as a growth regulator of rootless bean sprouts. Sodium 4-chlorphenoxyacetate (CPA-Na) is a legal food additive that has been extensively used in bean sprouts production. 2, 4-D can be a plant growth regulator when used at low dosage, although it is a poisonous herbicide when used at high concentration. However, it shall not be used as a growth regulator in excess of its statutory limit. The use of excessive amount of the above substances in bean sprouts production may endanger human health. Consumption of excessive 6-BA may cause skin and mucosa irritation, esophageal and gastral mucosa damage, and other symptoms like nausea and vomiting. At present, few reports are available on methods for concurrent detection of multiple growth regulators. In light of this, a method was proposed in this paper for concurrent detection of the above 3 growth regulators by LC/MS/MS to meet the demand for fast detection of these compounds in bean sprouts.

EXPERIMENTAL

Apparatus

A combined system of Shimadzu LC-30A ultra-fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer was used in this experiment. It consists of two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC auto-sampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer and LabSolutions Ver 5.41 chromatography workstation.

Conditions of Analysis

LC Conditions

Column	: Shim-pack XR-ODS (2.0 mm I.D. × 50 mmL., 2.2 μm)
Mobile phase A	: Water
Mobile phase B	: Methanol
Flow rate	: 0.3 mL/min
Injection volume	: 3 μL
Column temperature	: 40 °C
Elution mode	: Binary gradient with initial concentration of 80%B. See Table 1 for time program.

Table 1 Time program

Time(min)	Module	Command	Value
0.50	Pumps	Pump B Conc.	90
0.70	Pumps	Pump B Conc.	100
0.80	Pumps	Pump B Conc.	100
0.81	Pumps	Pump B Conc.	80
1.50	Controller	Stop	

MS conditions

Ionization	: ESI(+) for 6-BA, ESI(-) for CPA-Na and 2, 4-D
Ionization voltage	: +4.5kV, -3.5 kV
Nebulizing gas	: Nitrogen, 3.0 L/min
Drying gas	: Nitrogen, 15.0 L/min
Collision gas	: Argon
DL temperature	: 250 °C
Block heater temperature	: 400 °C
Mode	: MRM
Pause time	: 30 ms
Dwell time	: 1 ms
MRM parameters	: see Table 2

Table 2 MRM parameters

No.	Name	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	6-BA	225.90	91.15	-17.0	-25.0	-18.0
			65.10*	-24	-50	-25
2	CPA-Na	184.90	127.20	19.0	15.0	26.0
			141.20*	18.0	15.0	15.0
3	2,4-D	218.90	161.10	23.0	15.0	17.0
			124.85*	23.0	30.0	24.0

* refers to qualitative ion.

Sample Preparation

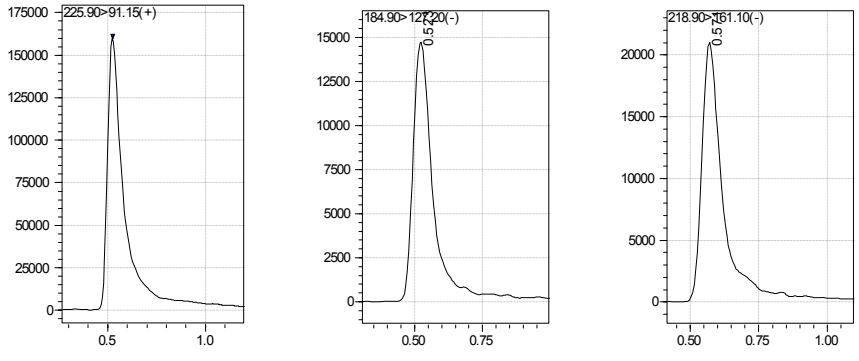
Preparation of standard solution: 1mg/L multi-standard solution was prepared using methanol as solvent and then diluted with methanol into standard working solutions of concentrations of 1, 10, and 100 μg/L.

Sample pretreatment method: Refer to DB11/T 379-2006 *Determination of sodium 4-chlorphenoxyacetate, 6-benzylaminopurine, 2, 4-D, gibberellic acid and thiram residues in soybean sprout and mungbean sprout.* The bean sprout samples used in this experiment were provided by users.

RESULTS AND DISCUSSION

MRM Chromatogram of Standard Samples

MRM chromatograms are shown in Fig. 1.



6-BA CPA-Na 2, 4-D

Fig. 1 MRM chromatograms of compounds

Linearity

Multi-standard working solutions of concentrations of 1, 10, and 100 µg/L were analyzed under the analytical conditions as specified above and calibration curves were plotted as shown in Fig. 2. The calibration curves were of good linearity and their linear equations and correlation coefficients were listed in Table 3.

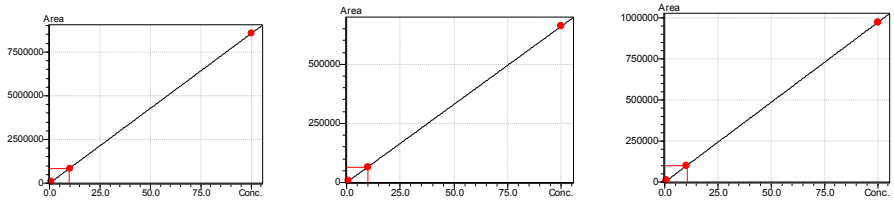


Fig. 2 Calibration curves

Table 3 Parameters of calibration curves of 6-BA, CPA-Na, and 2, 4-D

Name	Calibration Curve	Correlation Coefficient (R^2)
6-BA	Y = (85607.9)X	0.9999
CPA-Na	Y = (6605.55)X	0.9998
2, 4-D	Y = (9741.88)X	0.9999

Precision test

10 µg/L multi-standard working solution was analyzed for 6 times in succession to assess the precision of the method. The repeatability results of retention time and peak area are as shown in Table 4. The results showed that the RSD% of retention time and peak area of 10 µg/L standard solutions were better than 0.3% and 4.3%, respectively, suggesting that the method's precision was satisfactory.

Table 4 Repeatability - retention time and peak area at 10 µg/L (n=6)

Name	R.T. (min)	%RSD (R.T.)	%RSD (Area)
6-BA	0.524	0.116	1.397
CPA-Na	0.526	0.283	4.268
2, 4-D	0.571	0.299	2.157

Sensitivity test

In order to assess the method's sensitivity, blank matrices of bean sprout were spiked with multi-standard solutions to prepare samples at spike level of 10 μg/L. The method's S/N ratios at 10 μg/L and LODs were calculated using LabSolution software (Table 5). The proposed method can meet the requirements specified in DB11/377-2006 *Hygienic standard for soybean sprout and mung bean sprout* (Beijing local standard) for the detection of these 3 compounds at their statutory MRL levels (as shown in Table 5).

Table 5 S/N ratios at 10 μg/L, LODs and MRL requirements

Name	S/N	LOD (μg/L)	MRL (mg/kg)
6-BA	105.55	0.25	≤ 0.2
CPA-Na	119.83	0.36	≤ 1
2, 4-D	92.61	0.28	≤ 0.1

CONCLUSION

A method was proposed for the determination of CPA-Na, 6-BA, and 2,4-D in bean sprouts with ultra-fast liquid chromatography and triple quadrupole mass spectrometry. The proposed method was fast and of good precision. The correlation coefficients of calibration curves of the 3 compounds were all greater than 0.999 in the concentration range of 1~100 μg/L. The method's LODs of the 3 compounds in bean sprout met the requirements specified in DB11/377-2006 *Hygienic standard for soybean sprout and mung bean sprout*.

C-32 Detection of Pesticide Residues in Vegetable with Liquid Chromatography-Triple Quadrupole Mass Spectrometer (LCMS-8030) (1)

INTRODUCTION

In this paper, a method is proposed for fast detection of pesticide residues in vegetable with ultra-fast liquid chromatograph-triple quadrupole mass spectrometer. Mixture of aldicarb-sulfoxide, aldicarb-sulfone, methomyl, aldicarb, carbofuran, carbaryl, diflubenzuron and chlorbenzuron, were quantitatively analyzed in vegetables. The 8 compounds, which were analyzed within 15 minutes, demonstrated good linearity in the concentration range of 1~50 μg/L and correlation coefficients of calibration curves greater than 0.999. Precision test was performed on 5 μg/L multi-standard solution. The RSDs of retention time and peak area of 6 successive injections were better than 0.17% and 5.8%, respectively, suggesting that the method was of good precision. The method met the LOD requirements for detection of the pesticides in chilies satisfactorily.

Food safety is an important public issue which relates to human health and social stability, and detection of pesticide residues in vegetable is an important task in the field of food safety. Considering there are more than 1000 pesticides that have been registered, we are in urgent need of effective methods for detection of pesticide residues. A method for fast detection of the 8 pesticides of aldicarb-sulfoxide, aldicarb-sulfone, methomyl, aldicarb, carbofuran, carbaryl, diflubenzuron and chlorbenzuron by LC/MS/MS was developed for daily routine pesticide residue monitoring.

EXPERIMENTAL

Instruments

A combined system of Shimadzu LC-30A ultra-fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer was used in this experiment. It consists of two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer and LabSolutions Ver 5.41 chromatography workstation.

Conditions of Analysis

LC Conditions

Column : Shim-pack XR-ODSIII (2.0 mm I.D. × 150 mm L., 2.2 µm)
Mobile phase A : Water
Mobile phase B : Methanol
Flow rate : 0.3 mL/min
Injection volume : 5 µL
Column temperature : 40°C
Elution mode : Binary gradient with initial concentration of 10%B. See Table 1 for time program.

Table 1 Time program

Time(min)	Module	Command	Value
0.25	Pumps	B Conc.	10
10.00	Pumps	B Conc.	95
12.00	Pumps	B Conc.	95
12.20	Pumps	B Conc.	10
15.00	Controller	Stop	

MS conditions

Ionization : ESI(+), ESI(-)
Ionization voltage : +4.5kV, -3.5 kV
Nebulizing gas : Nitrogen, 3.0 L/min
Drying gas : Nitrogen, 15 L/min
Collision gas : Argon
DL temperature : 250 °C
Block heater temperature : 400 °C
Mode : MRM
Pause time : 20 ms
Dwell time : 1 ms
MRM parameters : see Table 2

Table 2 MRM parameters

ID#	Name	Ret. Time	Mode	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
1	Aldicarb-sulfoxide	3.16	+	229.10	166.00	-15.0	-10.0	-18.0
					109.05*	-15.0	-15.0	-21.0
2	Aldicarb-sulfone	3.55	+	223.10	86.10	-14.0	-15.0	-16.0
					148.20*	-10.0	-10.0	-15.0
3	Methomyl	4.12	+	163.00	88.05	-16.0	-10.0	-16.0
					106.05*	-16.0	-10.0	-21.0

4	Aldicarb	6.68	+	213.10	89.05	-14.0	-15.0	-18.0
					116.05*	-23.0	-10.0	-23.0
5	Carbofuran	7.52	+	222.00	165.00	-10.0	-10.0	-17.0
					123.05*	-23.0	-20.0	-24.0
6	Carbaryl	7.81	+	202.10	145.05	-13.0	-10.0	-30.0
					127.00*	-13.0	-30.0	-13.0
7	Diflubenzuron	9.90	-	309.10	289.00	14.0	10.0	21.0
					155.90*	14.0	10.0	30.0
8	Chlorbenzuron	10.11	-	307.10	153.90	14.0	10.0	30.0
					125.90*	14.0	25.0	23.0

* refers to qualitative ion.

Sample Preparation

Preparation of standard solution:

1mg/L multi-standard stock solution was prepared using methanol as solvent and then diluted with methanol into multi-standard working solutions of concentrations of 1, 5, and 50 μg/L.

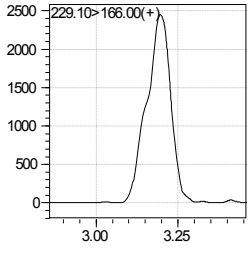
Sample pretreatment method:

Refer to GBT 20769-2006 *Method for determination of 405 pesticides and related chemicals residues in fruits and vegetables—LC-MS-MS method.*

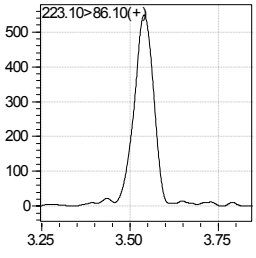
RESULTS AND DISCUSSION

MRM Chromatogram of Standard Pesticides

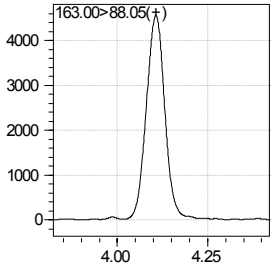
MRM chromatograms of pesticides are shown in Fig. 1.



Aldicarb-sulfoxide



Aldicarb-sulfone



Methomyl

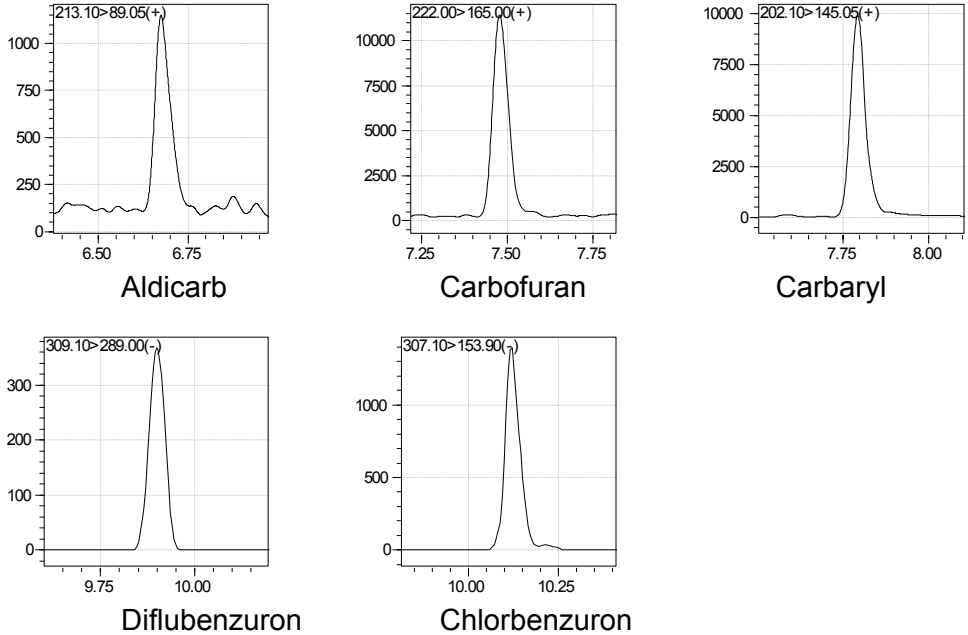
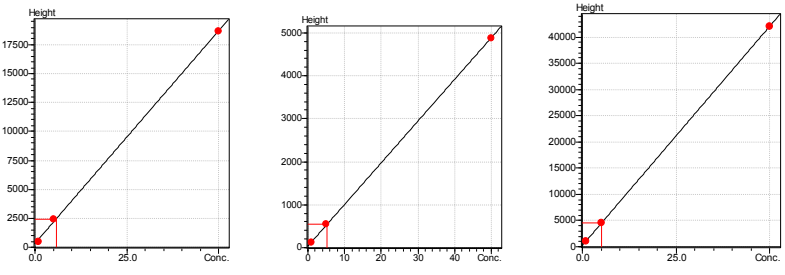


Fig. 1 MRM chromatograms of pesticides

2.1 Linearity

Multi-standard working solutions of concentrations of 1, 5, and 50 µg/L were analyzed under the analytical conditions as specified above and calibration curves were plotted as shown in Fig. 2. The calibration curves were of good linearity and their linear equations and correlation coefficients were listed in Table 3.



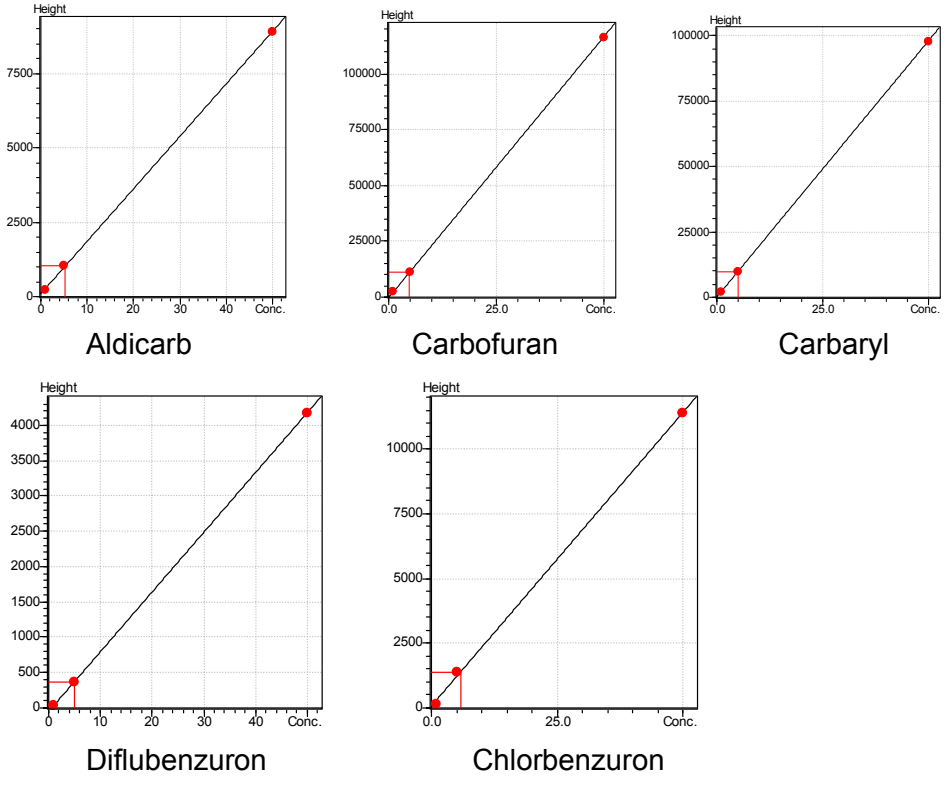


Fig. 2 Calibration curves of pesticides

Table 3 Parameters of the 8 pesticides' calibration curves

No.	Name	Calibration Curve	Correlation Coefficient (R^2)
1	Aldicarb-sulfoxide	Y = (2145.58)X + (423.117)	0.9997
2	Aldicarb-sulfone	Y = (456.441)X + (-134.501)	0.9999
3	Methomyl	Y = (3178.47)X + (157.195)	0.9999
4	Aldicarb	Y = (568.799)X + (159.553)	0.9999
5	Carbofuran	Y = (7309.17)X + (-456.088)	0.9999
6	Carbaryl	Y = (5867.82)X + (384.865)	0.9999
7	Diflubenzuron	Y = (259.812)X + (-164.596)	0.9999
8	Chlorbenzuron	Y = (679.573)X + (89.3587)	0.9999

Precision test

5 µg/L multi-standard working solution was determined for 6 times in succession to assess the precision of the method. The repeatability of retention time and peak area is shown in Table 4. The results showed that the RSDs of retention time and peak area of 5 µg/L standard solutions were better than 0.17% and 5.790%, respectively, suggesting that the method's precision was satisfactory.

Table 4. Repeatability - retention time and peak area at 5 µg/L (n=6)

Sample name	RSD% R.T.	RSD% Area	Sample name	RSD% R.T.	RSD% Area
Aldicarb-sulfoxide	0.165	3.225	Carbofuran	0.036	1.770
Aldicarb-sulfone	0.157	5.790	Carbaryl	0.032	1.925
Methomyl	0.072	1.653	Diflubenzuron	0.034	4.587
Aldicarb	0.073	4.849	Chlorbenzuron	0.031	2.949

Sensitivity test

In order to assess the method's sensitivity, blank matrices of chili were spiked with multi-standard solutions to prepare samples at spike level of 5 µg/L. The S/N ratios at 5 µg/L and LODs were calculated using LabSolution software. The results of aldicarb-sulfoxide, aldicarb-sulfone, methomyl, aldicarb, carbofuran, carbaryl, diflubenzuron and chlorbenzuron at 5 µg/L are summarized in Table 5. All met the LOD requirements specified in GBT 20769-2006 *Method for determination of 405 pesticides and related chemicals residues in fruits and vegetables—LC-MS-MS method*.

Table 5 S/N ratios and LODs at 5 µg/L and MRL requirements

Name	S/N ratio	LOD (µg/L)
Aldicarb-sulfoxide	214.1	0.09
Aldicarb-sulfone	71.1	0.23
Methomyl	285.4	0.06
Aldicarb	68.6	0.28
Carbofuran	384.0	0.05
Carbaryl	412.4	0.04
Diflubenzuron	50.3	0.35
Chlorbenzuron	64.1	0.30

CONCLUSION

A method was proposed for determination of aldicarb-sulfoxide, aldicarb-sulfone, methomyl, aldicarb, carbofuran, carbaryl, diflubenzuron and chlorbenzuron in chilies with ultra-fast liquid chromatograph-triple quadrupole mass spectrometer. The proposed method was of fast analysis speed and good precision. The correlation coefficients of calibration curves were all greater than 0.999 in the concentration range of 1~50 μg/L. The method's LODs of the 8 compounds in chilies met the requirements specified in GBT 20769-2006 *Method for determination of 405 pesticides and related chemicals residues in fruits and vegetables—LC-MS-MS method*.

C-33 Detection of Pesticide Residues in Vegetable with Liquid Chromatography-Triple Quadrupole Mass Spectrometer (LCMS-8030) (2)

INTRODUCTION

In this paper, a method was proposed for fast detection of pesticide residues in vegetable with ultra-fast liquid chromatograph-triple quadrupole mass spectrometer. The mixture of 10 pesticides, i.e. imidacloprid, dimethoate, acetamiprid, dichlorvos, pyrimethanil, triadimefon, fipronil, difenoconazole, chlorpyrifos, and pyridaben, were quantitatively analyzed. The 10 compounds, which were fast analyzed within 15 minutes, demonstrated good linearity in the concentration range of 1~50 μg/L and correlation coefficients of calibration curves greater than 0.999. Precision test was performed on 5 μg/L multi-standard solution. The RSDs of retention time and peak area of 6 consecutive injections were better than 0.07% and 6.0%, respectively, suggesting that the method was of good precision. The method met the LOD requirements for detection of the pesticide residues in cucumbers satisfactorily.

Food safety is an important public issue which relates to human health and social and political stability. In this paper, a method capable of fast detection of the 8 pesticides of imidacloprid, dimethoate, acetamiprid, dichlorvos, pyrimethanil, triadimefon, fipronil, difenoconazole, chlorpyrifos, and pyridaben by LC/MS/MS was developed for daily routine pesticide residue monitoring.

EXPERIMENTAL

Instruments

A combined system of Shimadzu LC-30A ultra-fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer was used in this experiment. It consists of two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer and LabSolutions Ver 5.41 chromatography workstation.

Conditions of Analysis

LC Conditions

Column : Shim-pack XR-ODSIII (2.0 mm I.D. × 150 mmL., 2.2 μm)
Mobile phase A : Water
Mobile phase B : Methanol
Flow rate : 0.3 mL/min
Injection volume : 5 μL
Column temperature : 40 °C
Elution mode : Binary gradient with initial concentration of 10%B. See Table 1 for time program.

Table 1 Time program

Time (min)	Module	Command	Value
0.25	Pumps	B Conc.	10
10.00	Pumps	B Conc.	95
12.00	Pumps	B Conc.	95
12.20	Pumps	B Conc.	10
15.00	Controller	Stop	

MS conditions

Ionization : ESI(+), ESI(-)
Ionization voltage : +4.5kV, -3.5 kV
Nebulizing gas : Nitrogen, 3.0 L/min
Drying gas : Nitrogen, 15.0 L/min
Collision gas : Argon
DL temperature : 250 °C
Heater block temperature : 400 °C
Mode : MRM
Pause time : 20 ms
Dwell time : 1 ms
MRM parameters : see Table 2

Table 2 MRM parameters of 10 pesticides

ID#	Name	R. T.	Mode	Precursor Ion	Product Ion	Q1	CE	Q3
1	Imidacloprid	5.21	+	256.10	208.95	-17.0	-15.0	-22.0
					175.10*	-17.0	-20.0	-19.0
2	Dimethoate	5.68	+	230.10	198.95	-15.0	-10.0	-21.0
					124.90*	-15.0	-20.0	-12.0
3	Acetamiprid	5.71	+	223.10	126.05	-14.0	-20.0	-24.0
					56.05*	-10.0	-15.0	-21.0
4	Dichlorvos	7.40	+	221.00	109.00	-14.0	-15.0	-21.0
					79.00*	-10.0	-30.0	-30.0
5	Pyrimethanil	8.40	+	200.20	82.10	-13.0	-25.0	-15.0
					107.05*	-20.0	-25.0	-20.0
6	Triadimefon	9.39	+	294.10	69.15	-19.0	-25.0	-26.0
					225.10*	-14.0	-15.0	-24.0
7	Fipronil	9.83	-	434.90	329.85	20.0	15.0	23.0
					249.85*	20.0	30.0	25.0
8	Difenoconazole	10.51	+	406.20	251.05	-19.0	-25.0	-28.0
					111.00*	-19.0	-50.0	-21.0
9	Chlorpyrifos	11.28	+	350.00	125.00	-16.0	-20.0	-26.0
					97.00*	-16.0	-35.0	-17.0
10	Pyridaben	11.87	+	365.30	147.10	-17.0	-25.0	-15.0
					309.10*	-17.0	-15.0	-21.0

* refers to qualitative ion.

Sample Preparation

Preparation of standard solutions: 1 mg/L multi-standard stock solution was prepared using methanol as solvent and then diluted with methanol into multi-standard working solutions of concentrations of 1, 5 and 50 µg/L.

Sample pretreatment method: Refer to China standard method, GBT 20769-2006 *Method for determination of 405 pesticides and related chemicals residues in fruits and vegetables—LC-MS-MS method*.

RESULTS AND DISCUSSION

MRM Chromatogram of Standard Samples

MRM chromatograms of pesticides are shown in Fig. 1.

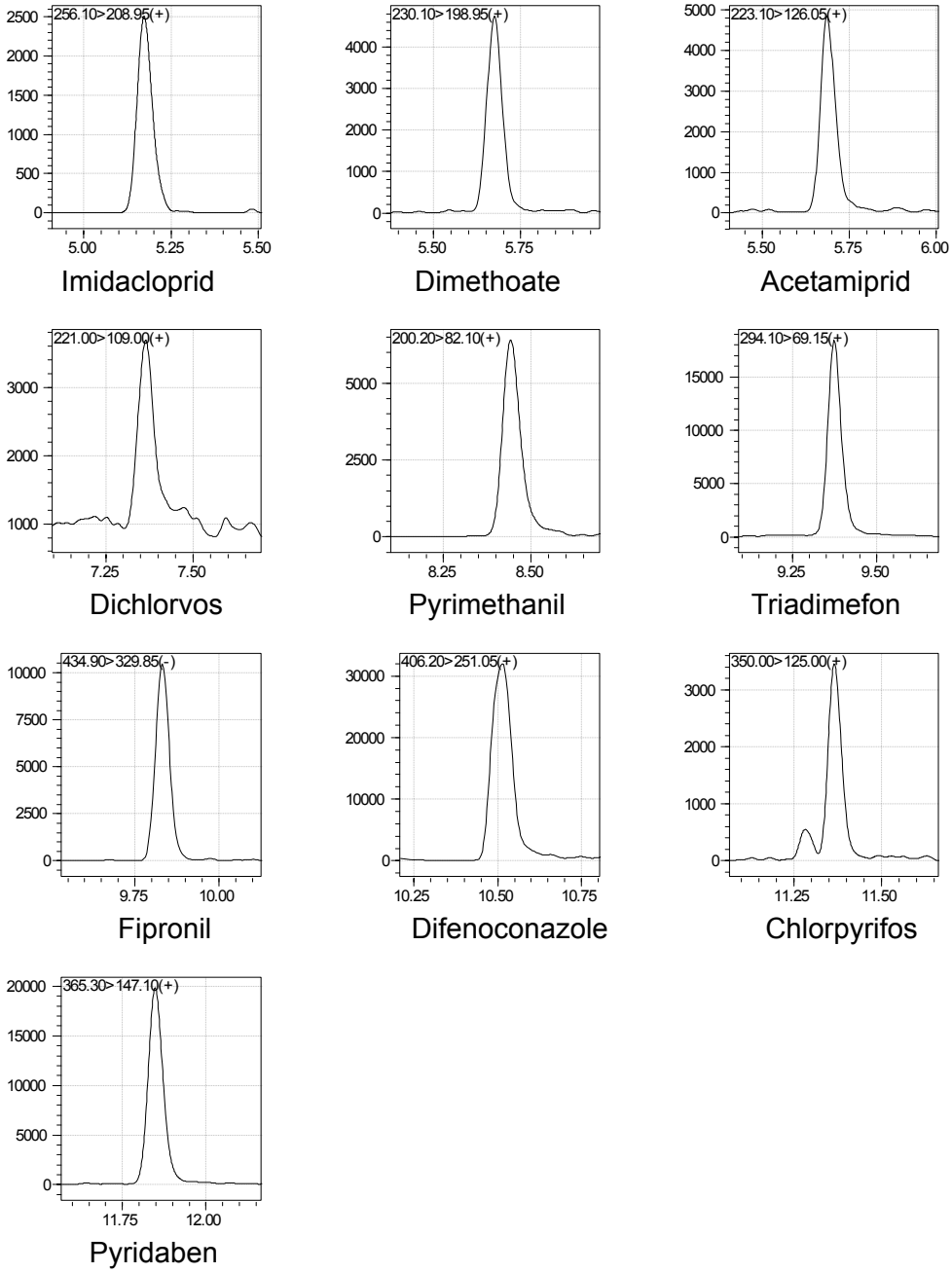
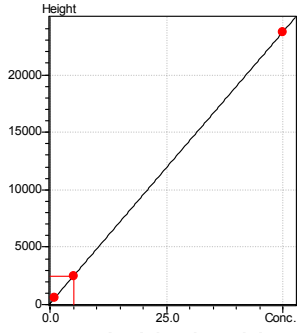


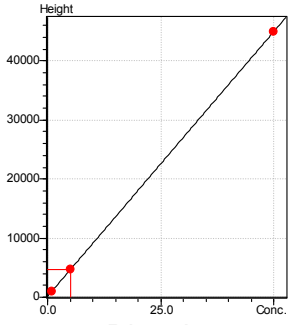
Fig. 1 MRM chromatograms of pesticides

Linearity

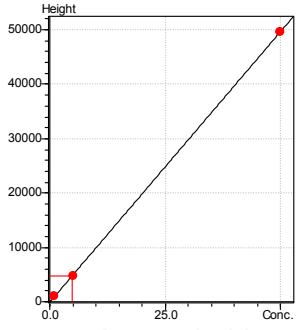
Multi-standard working solutions of concentrations of 1, 5 and 50 µg/L were analyzed under the analytical conditions as specified above and calibration curves are plotted as shown in Fig. 2. The resulted calibration curves were of good linearity and their linear equations and correlation coefficients are listed in Table 3.



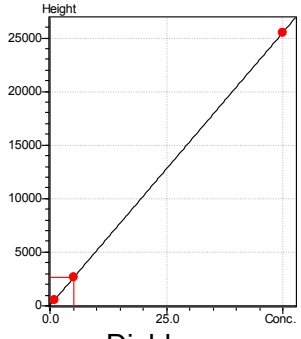
Imidacloprid



Dimethoate



Acetamiprid



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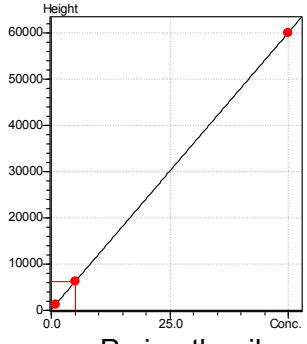
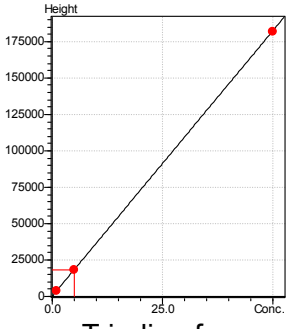
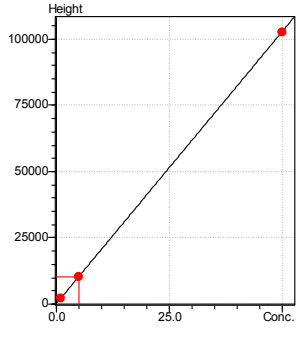


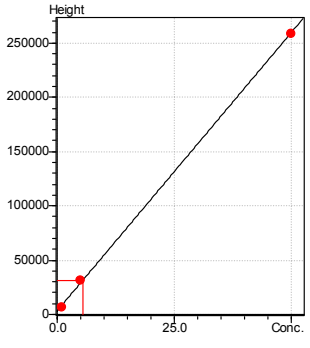
Pyrimethanil



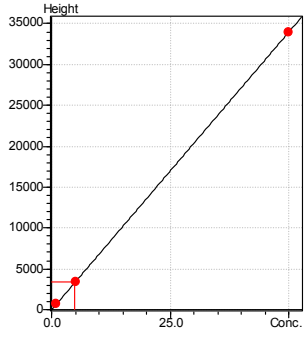
Triadimefon



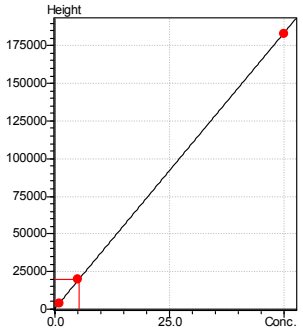
Fipronil



Difenoconazole



Chlorpyrifos



Pyridaben

Fig. 2 Calibration curves

Table 3 Parameters of calibration curves

No.	Name	Calibration Curve	Correlation Coefficient (r)
1	Imidacloprid	Y = (472.778)X + (103.733)	0.9999
2	Dimethoate	Y = (897.469)X + (138.17)	0.9999
3	Acetamiprid	Y = (994.738)X + (-62.8567)	0.9999
4	Dichlorvos	Y = (509.583)X + (45.9658)	0.9999
5	Pyrimethanil	Y = (1196.81)X + (171.778)	0.9999
6	Triadimefon	Y = (3635.22)X + (212.521)	0.9999
7	Fipronil	Y = (2048.14)X + (231.634)	0.9999
8	Difenoconazole	Y = (5109.77)X + (3497.55)	0.9998
9	Chlorpyrifos	Y = (679.885)X + (-14.3331)	0.9999
10	Pyridaben	Y = (3649.43)X + (679.034)	0.9999

Precision test

5 µg/L multi-standard working solution was analyzed for 6 times in succession to assess the precision of the method. Repeatability of retention time and peak area are as shown in Table 4. The results showed that the RSDs of retention time and peak area data of 5 µg/L standard solutions were better than 0.07% and 6.0%, respectively, suggesting that the method's precision was satisfactory.

Table 4 Repeatability - retention time and peak area at 5 µg/L (n=6)

Name	%RSD R. T.	%RSD Area	Name	%RSD R. T.	%RSD Area
Imidacloprid	0.059	2.619	Triadimefon	0.031	2.704
Dimethoate	0.070	3.377	Fipronil	0.028	2.909
Acetamiprid	0.053	4.051	Difenoconazole	0.035	2.132
Dichlorvos	0.028	4.710	Chlorpyrifos	0.039	5.975
Pyrimethanil	0.042	1.712	Pyridaben	0.015	5.257

Sensitivity test

In order to assess the method's sensitivity, blank matrices of cucumber were spiked with multi-standard solutions to prepare samples at spike level of 5 µg/L. The S/N ratios at 5 µg/L and LODs were calculated using LabSolution software. The results of 10 pesticides at 5 µg/L are as shown in Table 5 and all met the LOD requirements in GBT 20769-2006 M*ethod for determination of 405 pesticides and related chemicals residues in fruits and vegetables—LC-MS-MS method*.

Table 5 S/N ratios at 5 µg/L, LODs

Name	S/N	LOD (µg/L)
Imidacloprid	262.76	0.05
Dimethoate	192.79	0.08
Acetamiprid	170.75	0.08
Dichlorvos	25.22	0.47
Pyrimethanil	143.57	0.11
Triadimefon	344.38	0.04
Fipronil	998.15	0.02
Difenoconazole	152.99	0.11
Chlorpyrifos	0.64	2.74
Pyridaben	418.28	0.03

CONCLUSION

A method was proposed for determination of imidacloprid, dimethoate, acetamiprid, dichlorvos, pyrimethanil, triadimefon, fipronil, difenoconazole, chlorpyrifos, and pyridaben in cucumber with ultra-fast liquid chromatograph-triple quadrupole mass spectrometer. The proposed method was of fast and of good precision. The correlation coefficients of calibration curves were all greater than 0.999 in the concentration range of 1~50 μg/L. The method's LODs of the 10 pesticides in cucumbers met the requirements specified in GBT 20769-2006 *Method for determination of 405 pesticides and related chemicals residues in fruits and vegetables—LC-MS-MS method*.

C-34 Detection of Organophosphorus Pesticide Residues in Vegetable with Triple Quadrupole Mass Spectrometer

INTRODUCTION

In this paper, a method was proposed for fast detection of organophosphorus pesticide residues in vegetable with ultra-fast liquid chromatograph-triple quadrupole mass spectrometer. The mixture of 11 pesticides, i.e. methamidophos, acephate, phosmet, malathion, triazophos, isofenphos methyl, parathion, phoxim, phosalone, phorate, and profenofos, were quantitatively analyzed. Those were separated and detected rapidly within 15 minutes. The method was of good linearity for 11 pesticides in the concentration range of 1~50 μg/L and the correlation coefficients of all calibration curves were greater than 0.999. Precision test was performed on 5 μg/L multi-standard solution. The RSDs of retention time and peak area of 6 consecutive injections were better than 0.16% and 6.6%, respectively, suggesting that the method was of good precision. The method met the LOD requirements for detection of organophosphorus pesticide residues in kidney beans.

Detection of pesticide residues in vegetable is an important task in the field of food safety. In this paper, a method capable of fast detection of the 11 pesticides of methamidophos, acephate, phosmet, malathion, triazophos, isofenphos methyl, parathion, phoxim, phosalone, phorate, and profenofos by LC/MS/MS was developed for daily routine pesticide residue monitoring.

EXPERIMENTAL

Instruments

A combined system of Shimadzu LC-30A ultra-fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer was used in this experiment. It consists of two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer and LabSolutions Ver 5.41 chromatography workstation.

Conditions of Analysis

LC Conditions

Column : Shim-pack XR-ODSIII (2.0 mm I.D. × 150 mmL., 2.2 µm)
Mobile phase A : Water
Mobile phase B : Methanol
Flow rate : 0.3 mL/min
Injection volume : 5 µL
Column temperature : 40 °C
Elution mode : Binary gradient with initial concentration of 10%B. See Table 1 for time program.

Table 1 Time program

Time(min)	Module	Command	Value
0.25	Pumps	B Conc.	10
10.00	Pumps	B Conc.	95
12.00	Pumps	B Conc.	95
12.20	Pumps	B Conc.	10
15.00	Controller	Stop	

MS conditions

Ionization : ESI(+), ESI(-)
Ionization voltage : +4.5 kV, -3.5 kV
Nebulizing gas : Nitrogen, 3.0 L/min
Drying gas : Nitrogen, 15 L/min
Collision gas : Argon
DL temperature : 250 °C
Heater block temperature : 400 °C
Mode : MRM
Pause time : 20 ms
Dwell time : 1 ms
MRM parameters : see Table 2

Table 2 MRM parameters of 11 organophosphorus pesticides

ID#	Name	Ret. Time	Mode	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
1	Methamidophos	1.99	+	142.00	94.00	-14.0	-15.0	-18.0
					125.00*	-13.0	-15.0	-24.0
2	Acephate	2.50	+	184.00	142.95	-18.0	-10.0	-15.0
					125.00*	-19.0	-20.0	-13.0
3	Phosmet	8.87	+	318.00	160.00	-15.0	-15.0	-17.0
					77.00*	-15.0	-50.0	-29.0
4	Malathion	9.28	+	331.00	127.05	-15.0	-15.0	-26.0
					99.00*	-15.0	-25.0	-20.0
5	Triazophos	9.49	+	314.10	162.00	-14.0	-20.0	-16.0
					119.00*	-21.0	-35.0	-23.0
6	Isofenphos-methyl	9.993	+	332.25	231.05	-15.0	-15.0	-25.0
					273.10*	-15.0	-10.0	-19.0
7	Parathion	10.05	+	292.10	264.00*	-14.0	-10.0	-30.0
					236.00	-13.0	-15.0	-27.0
8	Phoxim	10.315	+	299.10	77.05*	-14.0	-30.0	-14.0
					128.95	-14.0	-10.0	-28.0
9	Phosalone	10.401	+	367.90	111.00	-17.0	-40.0	-22.0
					181.90*	-17.0	-15.0	-20.0
10	Phorate	10.50	+	261.00	75.00	-17.0	-10.0	-13.0
					199.00*	-12.0	-10.0	-20.0
11	Profenofos	10.86	+	372.90	302.85	-17.0	-20.0	-30.0
					344.80*	-17.0	-15.0	-24.0

* refers to qualitative ion.

Sample Preparation

Preparation of standard solution:

1 mg/L multi-standard stock solution was prepared using methanol as solvent and then diluted with methanol into multi-standard working solutions of concentrations of 1, 5 and 50 μg/L.

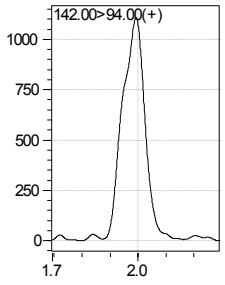
Sample pretreatment method:

Refer to China standard method, GBT 20769-2006 M*ethod for determination of 405 pesticides and related chemicals residues in fruits and vegetables—LC-MS-MS method*.

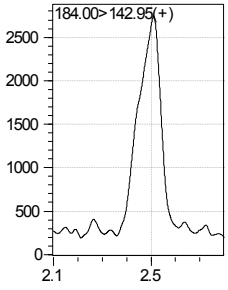
RESULTS AND DISCUSSION

MRM Chromatogram of Standard Samples

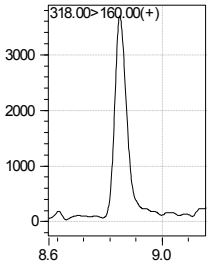
MRM chromatograms of pesticides are shown in Fig. 1.



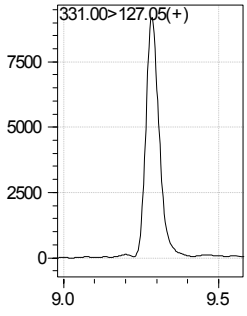
Methamidophos



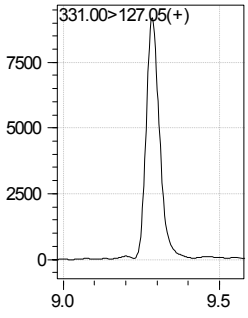
Acephate



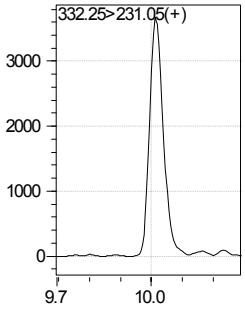
Phosmet



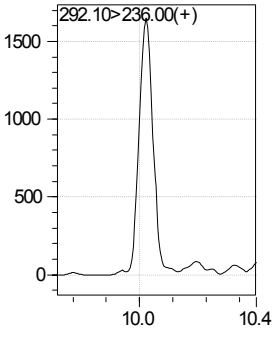
Malathion



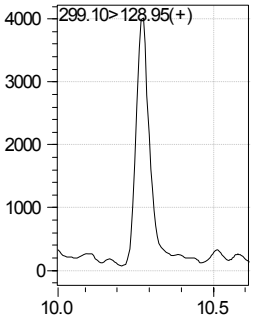
Triazophos



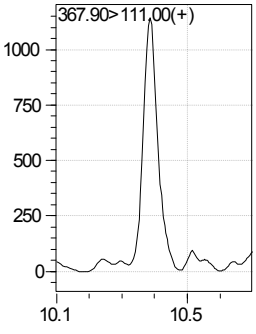
Isofenphos-methyl



Parathion



Phoxim



Phosalone

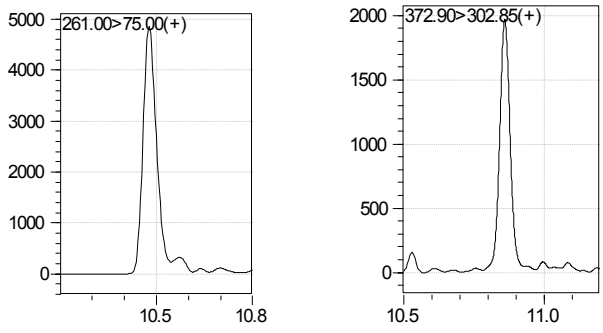
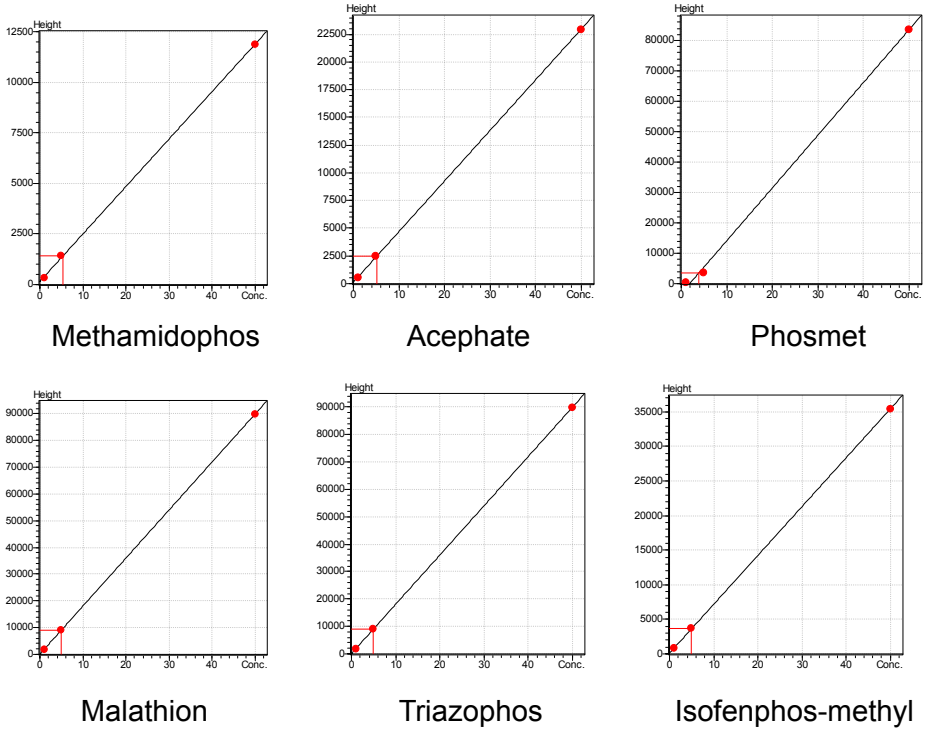


Fig. 1 MRM chromatograms of pesticides

Linearity

Multi-standard working solutions of concentrations of 1.0, 5 and 50 µg/L were analyzed under the analytical conditions as specified above and calibration curves were plotted as shown in Fig. 2. The calibration curves were of good linearity and their linear equations and correlation coefficients are listed in Table 3.



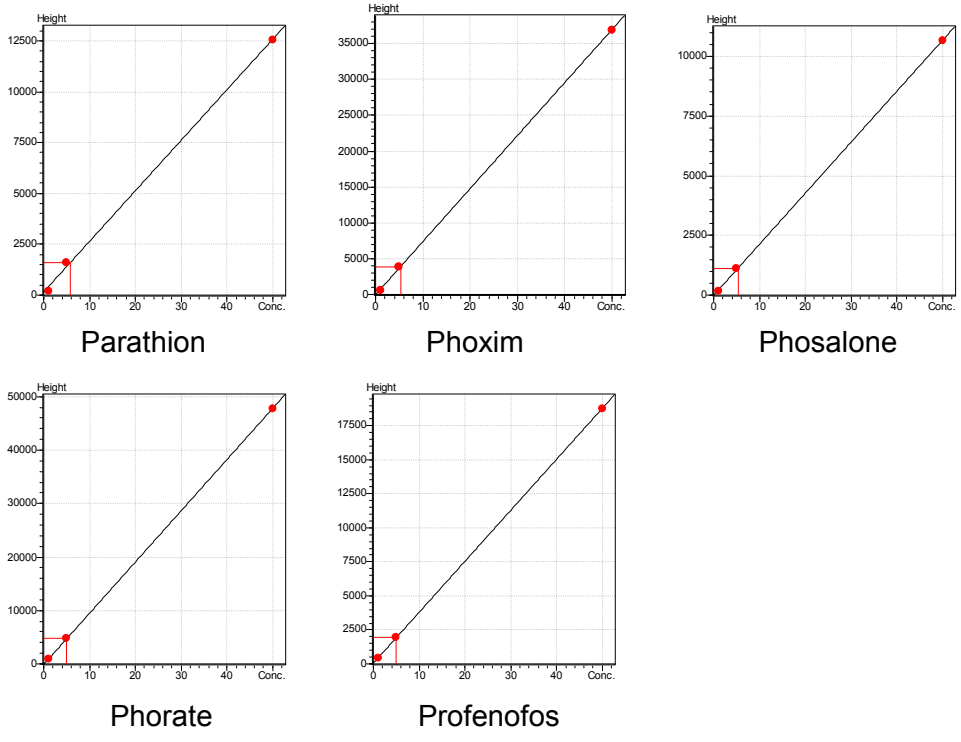


Fig. 2 Calibration curves of pesticides

Table 3 Parameters of calibration curves

No.	Name	Calibration Curve	Correlation Coefficient (R^2)
1	Methamidophos	Y = (238.483)X + (177.461)	0.9998
2	Acephate	Y = (456.367)X + (132.062)	0.9999
3	Phosmet	Y = (1734.39)X + (-3211.27)	0.9992
4	Malathion	Y = (1795.6)X + (104.65)	0.9999
5	Triazophos	Y = (1795.6)X + (104.65)	0.9999
6	Isofenphos-methyl	Y = (706.649)X + (112.75)	0.9999
7	Parathion	Y = (248.284)X + (164.907)	0.9995
8	Phoxim	Y = (739.071)X + (-18.9656)	0.9999
9	Phosalone	Y = (213.732)X + (-6.86764)	0.9999
10	Phorate	Y = (957.961)X + (-86.5895)	0.9999
11	Profenofos	Y = (374.408)X + (52.2537)	0.9999

Precision test

5 µg/L multi-standard working solution was analyzed for 6 times in succession to assess the precision of the method. Repeatability of retention time and peak area were as shown in Table 4. The results showed that the RSD% of retention time and peak area data of 5 µg/L standard solutions fell in the range of 0.022%~0.155% and 1.315%~6.542%, respectively, suggesting that the method's precision was satisfactory.

Table 4 Repeatability - retention time and peak area at 5 µg/L (n=6)

Name	RSD% R.T.	RSD% Area	Name	RSD% R.T.	RSD% Area
Methamidophos	0.155	4.723	Parathion	0.039	6.542
Acephate	0.196	2.638	Phoxim	0.030	2.019
Phosmet	0.025	3.357	Phosalone	0.029	2.590
Malathion	0.023	1.315	Phorate	0.025	4.263
Triazophos	0.022	1.531	Profenofos	0.025	4.585
Isofenphos-methyl	0.036	4.867			

Sensitivity test

In order to assess the method's sensitivity, blank matrices of kidney bean were spiked with multi-standard solutions to prepare samples at spike level of 5 µg/L. S/N ratios at 5 µg/L and LODs were calculated using LabSolution software. The results are shown in Table 5 and all met the LOD requirements in GBT 20769-2006 *Method for determination of 405 pesticides and related chemicals residues in fruits and vegetables—LC-MS-MS method*.

Table 5 S/N ratios at 5 µg/L, LODs

Name	S/N ratio	LOD (µg/L)
Methamidophos	61.16	0.29
Acephate	47.77	0.36
Phosmet	174.73	0.13
Malathion	374.29	0.05
Triazophos	253.84	0.07
Isofenphos-methyl	73.02	0.19
Parathion	66.27	0.22
Phoxim	76.25	0.25
Phosalone	248.84	0.07
Phorate	136.72	0.13
Profenofos	181.48	0.09

CONCLUSION

A method was proposed for determination of methamidophos, acephate, phosmet, malathion, triazophos, isofenphos methyl, parathion, phoxim, phosalone, phorate, and profenofos in kidney beans with ultra-fast liquid chromatograph-triple quadrupole mass spectrometer. The proposed method was fast and reproducible. The correlation coefficients of calibration curves were all greater than 0.999 in the concentration range of 1~50 µg/L. The method's LODs of the 11 compounds in kidney beans met the requirements specified in GBT 20769-2006 *Method for determination of 405 pesticides and related chemicals residues in fruits and vegetables—LC-MS-MS method*.

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C-35 Determination of Diarrheic Shellfish Poisons in Scallops by Triple Quadrupole Mass Spectrometry

INTRODUCTION
In this paper, a method is described for the determination of diarrheic shellfish poisons in scallops with Shimadzu LC-30A ultra-high performance liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer. Extracted samples were analyzed by LC-30A, and then quantified with LCMS-8030. 4 toxins were separated and detected rapidly within 2 minutes. The proposed method was of good linearity for gymnodimine (GYM), 13-desmethyl spirolide C (SPX-1) and pectenotoxin-2 (PTX-2) in the concentration range of 0.001 ~ 0.25 μmol/L and for okadaic acid (OA) in the concentration range of 0.01 ~ 0.25 μmol/L; the correlation coefficients of calibration curves of all toxins were greater than 0.999. Repeatability test was performed with multi-standard solutions at concentrations of 0.005 μmol/L, 0.05 μmol/L and 0.25 μmol/L. The RSDs of retention time and peak area in 6 consecutive injections were below 0.152% and 4.379%, respectively, suggesting that the method was of good precision. PTX-2 and OA were detected in a commercial scallop sample from a downstream city of a river at concentrations of 0.011 and 0.674 μmol/kg, respectively.

Diarrheic shellfish poison (DSP) is a group of liposoluble secondary metabolites having the chemical structure of polyether or macrocyclic lactone that are produced by marine algae or microbes. The first case of DSP poisoning was reported in 1960s in the Netherlands. Since then, DSP poisoning cases have been frequently reported and some 20,000 people have been poisoned. The main symptoms of DSP poisoning include vomiting, diarrhea, and, in serious cases, jaundice and acute atrophic hepatonecrosis. Regulations demanding the detection of DSP by LC/MS have been promulgated one after another since 2002. EU promulgated a regulation 2002/225/EC in 2002 stipulating the detection method and LODs of shellfish poisoning. Japan set up DSP limits of 20 μg/100 g and 200 μg/100 g for intestinal gland of *mytilus edulis* and scallop; China also promulgated a national standard GB/T5009.212-2008 *Determination of diarrhetic shellfish poison in shellfish* in 2008 but the standard used bioanalysis method. Zhejiang Province issued a provincial standard DB33/T 743-2009 *Determination of diarrheic shellfish poisoning residues in fishery products--HPLC-MS/MS method* in 2009. In this paper, a method for fast and accurate determination of DSP in scallops with

Shimadzu LC-30A ultra-high performance liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer was developed.

EXPERIMENTAL

Instruments

A combined system of Shimadzu LC-30A ultra-high performance liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer was used in the experiment. The configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.41 chromatography workstation.

Conditions of Analysis

LC Conditions

Liquid chromatograph : LC-30A system
Column : Shim-pack XR-ODS III (2.0 mmI.D.×50 mmL., 1.6 μm)
Mobile phase A : 10 mM ammonium acetate-0.1% formic acid aqueous solution,
Mobile phase B : Acetonitrile
Flow rate : 0.4 mL/min
Injection volume : 5 μL
Column temperature : 40 °C
Elution mode : Binary gradient with initial concentration of 30%B.
See Table 1 for time program.

Table 1 Time program

Time (min)	Module	Command	Value
0.50	Pumps	B Conc.	30
1.00	Pumps	B Conc.	98
2.00	Pumps	B Conc.	98
2.30	Pumps	B Conc.	30
3.00	Controller	Stop	

MS conditions

Mass spectrometer : LCMS-8030
Ionization : ESI-positive and negative
Interface voltage : +4.5 kV (positive), -3.5 kV (negative)
Nebulizing gas : Nitrogen, 3.0 L/min
Drying gas : Nitrogen, 15 L/min
Collision gas : Argon

DL temperature : 250 °C
Block heater temperature : 400 °C
Mode : MRM
Pause time : 50 ms
Dwell time : 3 ms
MRM parameters : see Table 2

Table 2 MRM Parameters

No.	Name	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
1	Gymnodimine (GYM)	508.55	490.55	-20.0	-25.0	-26.0
			121.35*	-20.0	-50.0	-14.0
2	13-desmethyl spirolide C (SPX-1)	692.65	164.20	-20.0	-50.0	-18.0
			674.45*	-20.0	-35.0	-26.0
3	Pectenotoxin-2 (PTX-2)	876.50	213.20	-22.0	-45.0	-16.0
			823.45*	-22.0	-45.0	-16.0
			349.20*	-22.0	-25.0	-32.0
4	Okadaic acid (OA)	803.30	255.45	30.0	50.0	24.0
			189.20*	30.0	20.0	15.0

* refers to qualitative ion.

Sample Preparation

Preparation of standard solution:

0.25 μmol/L multi-standard solution was prepared using acetonitrile as solvent, and then diluted with water into standard working solutions at concentrations of 0.001, 0.005, 0.010, 0.05, 0.1, 0.2 and 0.25 μmol/L.

Sample pretreatment method:

Refer to Zhejiang provincial standard DB33/T 743-2009 *Determination of diarrheic shellfish poisoning residues in fishery products--HPLC-MS/MS method*.

RESULTS AND DISCUSSION

MRM Chromatogram of Standard Samples

Fig. 1 shows the MRM chromatograms of standard toxins (0.1 µmol/L). The 4 toxins were analyzed within 2 minutes.

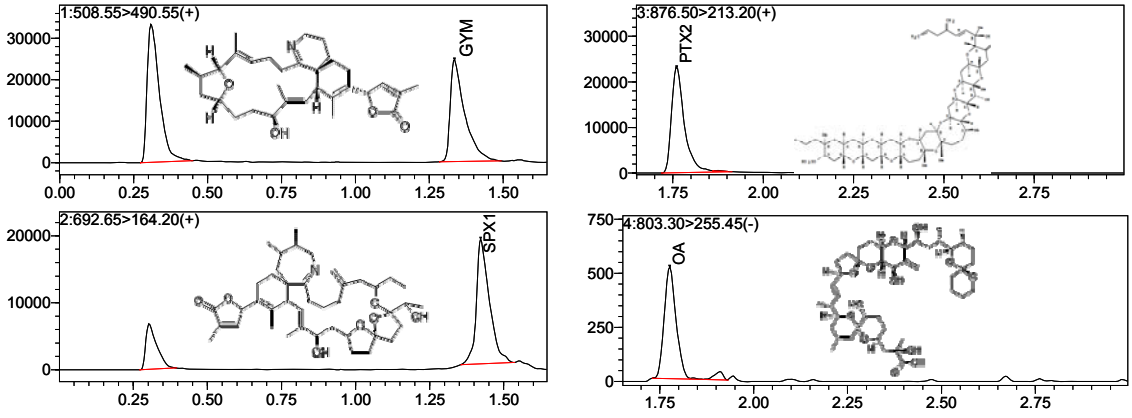


Fig. 1 MRM chromatograms of standard toxins (1µmol/L each)

Linearity

Multi-standard working solutions of concentrations of 0.001, 0.005, 0.010, 0.05, 0.1, 0.2 and 0.25 µmol/L were analyzed using the analytical conditions specified above. Calibration curves were plotted as shown in Figs. 2 - 5 with concentration as abscissa and peak area as ordinate. The calibration curves of GYM, SPX-1 and PTX-2 demonstrated good linearity in the concentration range of 0.001 ~ 0.25 µmol/L. The calibration curve of OA was of good linearity in the concentration range of 0.01 ~ 0.25 µmol/L. Relevant linear equations, correlation coefficients and LODs and LOQs were listed in Table 3.

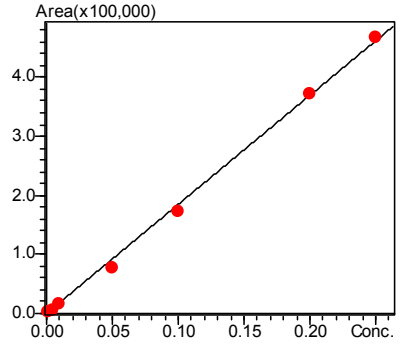


Fig. 2 Calibration curve of GYM

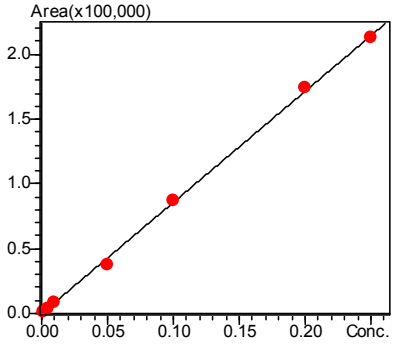


Fig. 3 Calibration curve of SPX-1

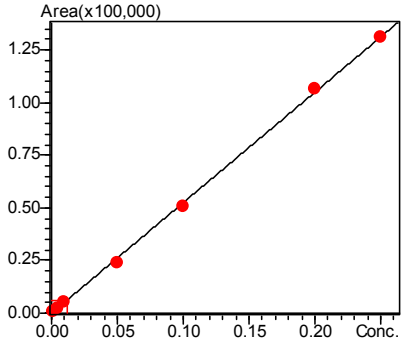


Fig. 4 Calibration curve of PTX-2

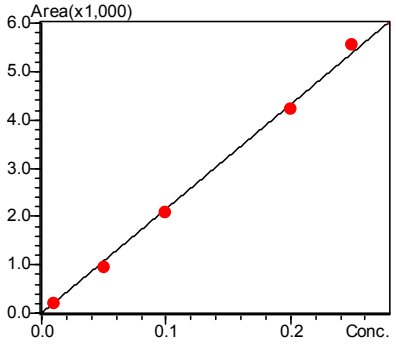


Fig. 5 Calibration curve of OA

Table 3 Parameters of the 4 DSPs' calibration curves

No.	Name	Calibration Curve	Correlation Coefficient (r)	LOD (μmol/L)	LOQ (μmol/L)
1	GYM	Y = (1848000)X	r=0.9994	0.00027	0.00080
2	SPX-1	Y = (857167)X	r=0.9996	0.00020	0.00061
3	PTX-2	Y = (524492)X	r=0.9996	0.00017	0.00051
4	OA	Y = (21601.8)X	r=0.9990	0.00314	0.00952

Repeatability test

Multi-standard solutions at concentrations of 0.005 μmol/L, 0.05 μmol/L and 0.25μmol/L were injected 6 successive times for assessment of the method's precision. The resulted repeatability of retention time and peak area are shown in Table 4. The %RSDs of retention time and peak area data of standard solutions of 3 concentrations were 0.036%~0.152% and 1.405%~4.379%, respectively, showing that the method's precision was satisfactory.

Table 4 Repeatability - retention time and peak area (n=6)

Compound	%RSD (0.005 µmol/L)		%RSD (0.05 µmol/L)		%RSD (0.25 µmol/L)	
	R. T.	Area	R. T.	Area	R. T.	Area
GYM	0.092	2.365	0.073	2.138	0.048	2.277
SPX-1	0.133	3.666	0.036	1.405	0.055	1.814
PTX-2	0.144	2.553	0.052	1.717	0.048	1.920
OA	0.139*	3.996*	0.152	4.379	0.054	2.821

* concentration was 0.01 µmol/L

Real sample

A sample of commercial scallop from a downstream city of a river was analyzed and two DSPs, namely PTX-2 and OA, were detected at concentrations of 0.011 and 0.674 µmol/kg, respectively. The content of OA significantly exceeded 0.02 mg/kg (appr. 0.025 µmol/kg), which is the limit stipulated in Zhejiang's provincial standard.

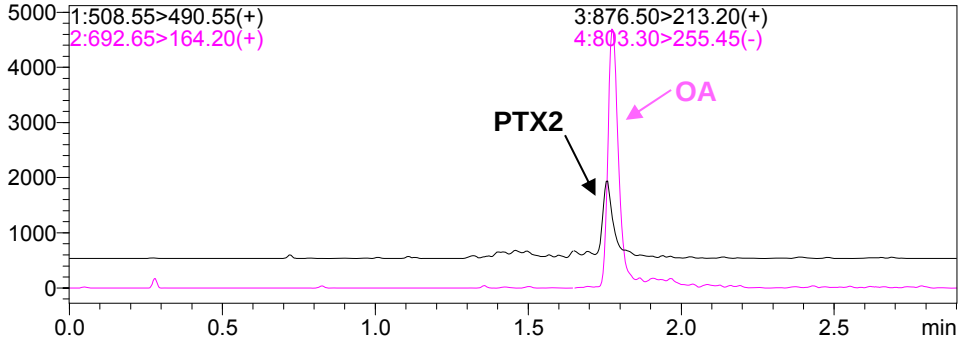


Fig. 6 MRM chromatograms of a scallop sample

CONCLUSION

A method was established for the determination of 4 DSPs in scallops using Shimadzu LC-30A ultra-high performance liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. The method had the merits of fast analysis speed, good repeatability and precision; the calibration curves of GYM, SPX-1 and PTX-2 were having good linearity in the concentration range of 0.001 ~ 0.25 µmol/L, the calibration curve of OA was of good linearity in the concentration range

of 0.01 ~ 0.25 µmol/L; all calibration curves had a correlation coefficient greater than 0.999. PTX-2 and OA were detected in a sample of commercial scallop from a downstream city of a river at concentrations of 0.011 and 0.674 µmol/kg, respectively.

C-36 Determination of substituted urea pesticides residues in food of plant origin with LCMS-8030

INTRODUCTION

A method was developed for determination of substituted urea pesticides residues in food of plant origin using Shimadzu LC-30A ultra-fast liquid chromatograph in conjunction with LCMS-8030 triple quadrupole mass spectrometer. Analytes in samples that had been processed were fast separated by the LC-30A ultra-fast liquid chromatograph within 7 minutes, and then quantitatively analyzed with the LCMS-8030 triple quadrupole mass spectrometer. Linearity, precision, LODs, and LOQs of the method for determination of 4 substituted urea pesticides residues were evaluated. The method demonstrated good linearity for chlortoluron, isoproturon, diuron, and linuron in the concentration range of 5~100 µg/L with correlation coefficients all greater than 0.999. Precision tests were performed on multi-standard solutions of concentrations of 5 µg/L, 20 µg/L and 100 µg/L, respectively. The experiment results showed that the %RSDs of retention time and peak area in 6 successive injections fell in the ranges of 0.07-0.1% and 0.43 ~ 2.41%, respectively, suggesting that the method's precision was satisfactory. The method's LODs were 0.12-0.36 µg/L and LOQs were 0.42~1.2 µg/L.

Developed since the end of World War II, substituted urea pesticides are a category of very important pesticides belonging to systematic soil treatment agents. Presently more than 20 substituted urea pesticides are extensively used abroad in agricultural production. In China, 4 substituted urea pesticides, including chlortoluron, isoproturon, diuron and linuron are commonly used for weeding in fields of corn, wheat, soybean, cotton, peanut, and vegetable and orchards. China has issued regulatory standards on the determination of substituted urea pesticides residues, including *SN/T 2213-2008 Determination of substituted ureas pesticides residues in foodstuffs of plant origin for import and export--LC-MS/MS method*. In this paper, a method is proposed in reference with SN/T 2213-2008 for determination of substituted ureas pesticides residues (chlortoluron, isoproturon, diuron and linuron) in foodstuffs of plant origin with Shimadzu LC-30A ultra-fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer for the

reference of relevant laboratorians.

EXPERIMENTAL

Instruments

A combined system of Shimadzu ultra-fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A system controller, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.42 chromatography workstation.

Analytical conditions

LC Conditions

Apparatus : LC-30A system
Column : Shim-pack XR-ODS III 2.0 mmI.D.×75 mmL., 1.6 μm
Mobile phase A : 0.1% acetic acid aqueous solution
Mobile phase B : Methanol
Flow rate : 0.3 mL/min
Injection volume : 10 μL
Column temperature : 40 °C
Elution mode : Binary gradient with initial concentration of 50% of mobile phase B.

See Table 1 for time program.

Table 1 Time program

Time(min)	Module	Command	Value
7.00	Pumps	B Conc.	100
7.10	Pumps	B Conc.	100
7.11	Pumps	B Conc.	50
10.00	Controller	Stop	

MS conditions

Apparatus : LCMS-8030
Ionization : ESI, positive
Ionization voltage : +4.5 kV
Nebulizing gas : Nitrogen, 3.0 L/min
Drying gas : Nitrogen, 15 L/min
Collision gas : Argon
DL temperature : 250 °C
Heater block temperature : 400 °C
Mode : multiple reaction monitoring (MRM)

Dwell time : 50 ms
Pause time : 3 ms
MRM parameters : see Table 2

Table 2 MRM parameters

No.	Compound	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Chlortoluron	213	72*	-23.0	-22.0	-14.0
			140	-23.0	-24.0	-16.0
2	Isoproturon	207	72*	-24.0	-22.0	-28.0
			165	-24.0	-16.0	-16.0
3	Diuron	233	72*	-16.0	-22.0	-14.0
			160	-17.0	-29.0	-29.0
4	Linuron	249	160*	-13.0	-20.0	-18.0
			182	-13.0	-15.0	-20.0

* refers to quantitative ion.

Sample Preparation

Preparation of standard solution:

Standard substances: chlortoluron, isoproturon, diuron, linuron.

A multi-standard solution of concentration of 10 mg/L was prepared with acetonitrile as solvent and then progressively diluted into standard working solutions of concentrations of 100, 50, 20, 10, and 5 μg/L with methanol/water (1/1).

Sample pretreatment method:

Refer to *SN/T 2213-2008 Determination of substituted ureas pesticides residues in foodstuffs of plant origin for import and export--LC-MS/MS method.*

RESULTS AND DISCUSSION

Mass spectra and MS/MS spectra

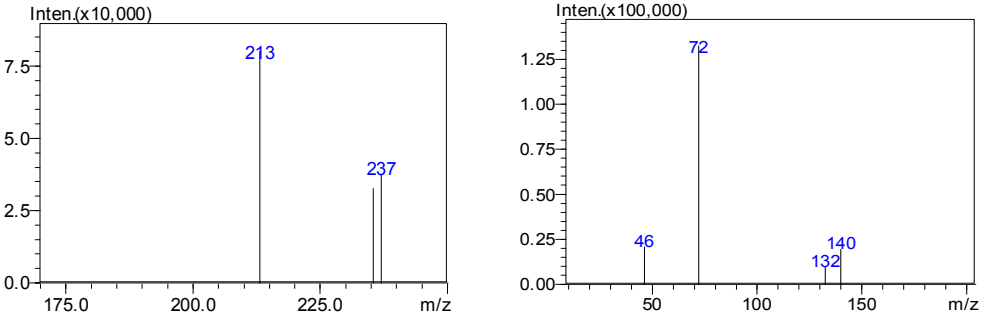


Fig. 1 Mass spectrum (left) and MS/MS spectrum (right) of chlortoluron

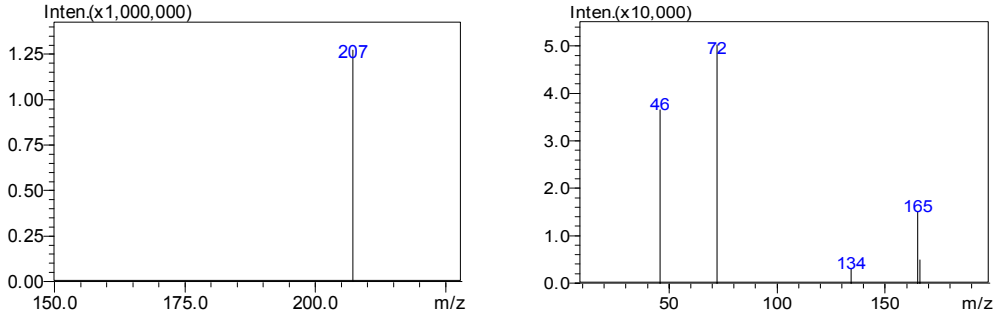


Fig. 2 Mass spectrum (left) and MS/MS spectrum (right) of isoproturon

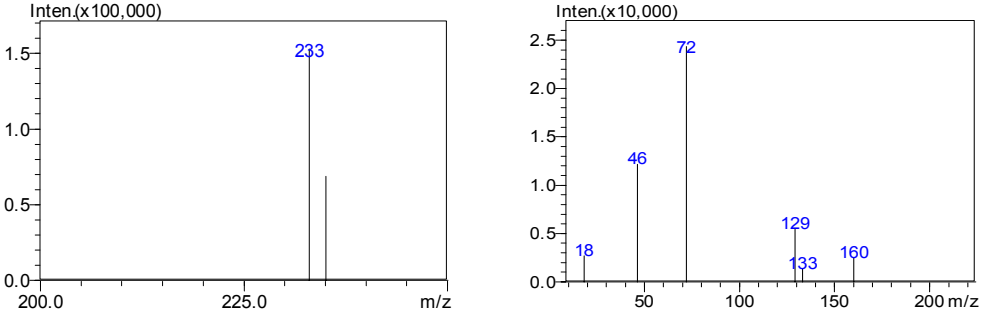


Fig. 3 Mass spectrum (left) and MS/MS spectrum (right) of diuron

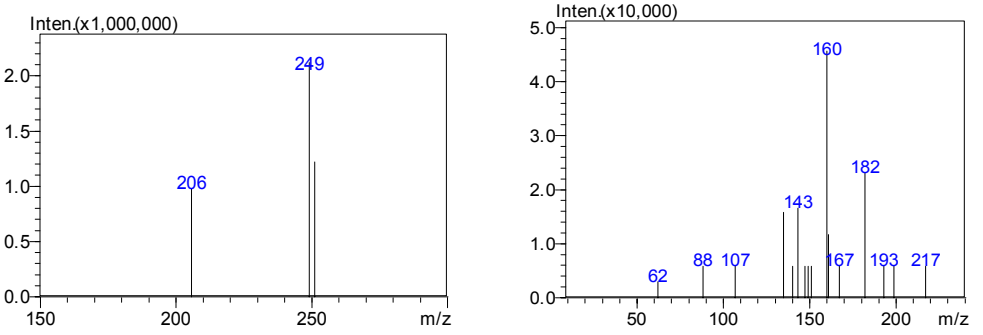


Fig. 4 Mass spectrum (left) and MS/MS spectrum (right) of linuron

MRM chromatogram of standard mixture

MRM chromatogram of 20 µg/L multi-standard mixure is shown in Fig. 5.

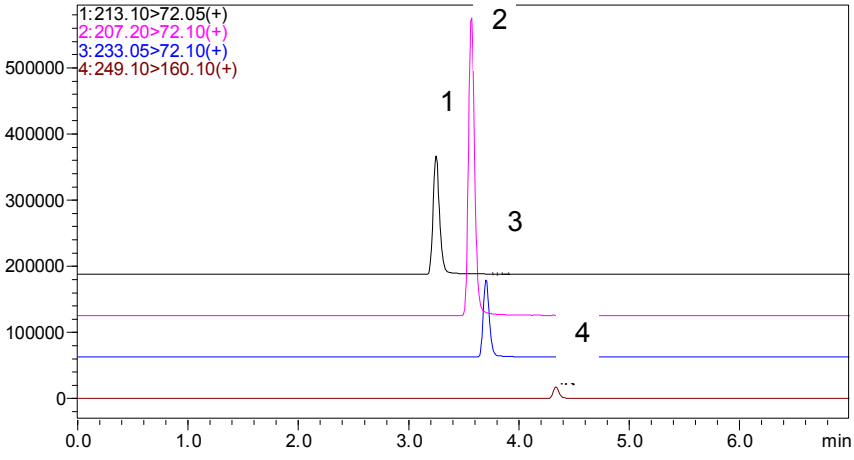


Fig. 5 MRM chromatograms of 20 µg/L multi-standard mixture

Linearity

Multi-standard working solutions of concentrations of 5, 10, 20, 50 and 100 µg/L were analyzed using the analytical conditions specified above. Calibration curves as shown in Figs. 6 – 9 were plotted by the external standard method with concentration as X-axis and peak area as Y-axis. The calibration curves of the 4 substituted urea pesticides were of good linearity in the concentration range of 5~100 µg/L. Their linear equations and correlation coefficients are listed in Table 3.

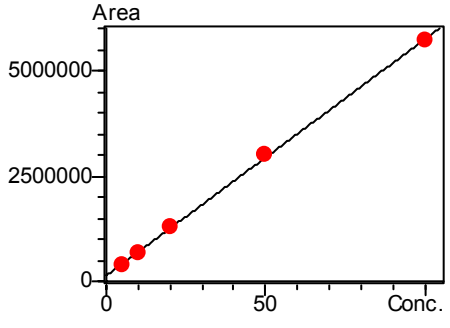


Fig. 6 Calibration curve of chlortoluron

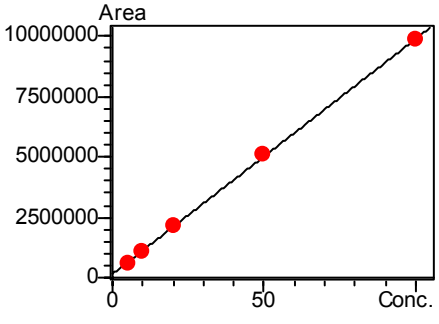


Fig. 7 Calibration curve of isoproturon

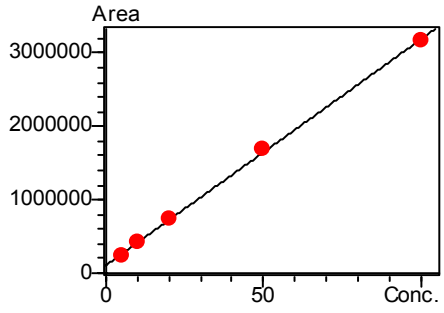


Fig. 8 Calibration curve of diuron

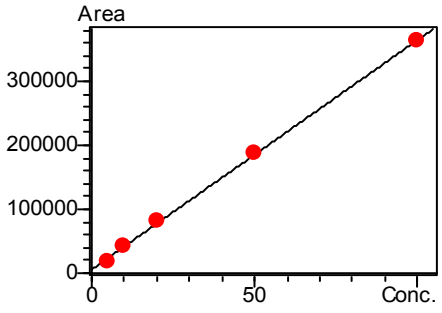


Fig. 9 Calibration curve of linuron

Table 3 Parameters of calibration curves of the 4 substituted urea pesticides

No.:	Compound	Calibration Curve	Correlation Coefficient (r)
1	Chlortoluron	Y = (56446.0)X + (125124)	0.9996
2	Isoproturon	Y = (96846.4)X + (161530)	0.9997
3	Diuron	Y = (30799.5)X + (99640.2)	0.9997
4	Linuron	Y = (3593.43)X + (5576.96)	0.9997

LODs and LOQs

Seven standard samples at concentration of 5.0 µg/L were prepared and then directly injected for analysis. Standard deviation (S) was calculated after excluding outlier(s), and minimum detection limits (MDLs) were determined as 3S and limits of quantification (LOQs) as 10S. The results are shown in Table 4:

Table 4 MDLs and LOQs of the 4 substituted urea pesticides

No.	Compound	Standard deviation (S)	MDL (µg/L)	LOQ (µg/L)
1	Chlortoluron	0.07	0.21	0.70
2	Isoproturon	0.04	0.12	0.40
3	Diuron	0.05	0.15	0.50
4	Linuron	0.12	0.36	1.20

Precision test

Six replicate samples of concentrations of 5 µg/L, 20 µg/L and 100 µg/L were prepared and injected for analysis in succession. The %RSDs of retention time and peak area data of standard solutions of the 4 substituted ureas pesticides fell in the ranges of 0.07-0.1% and 0.43-2.41%, respectively, showing that the method's precision was satisfactory.

Table 5 Repeatability - retention time and peak area (n=6)

No.	Compound	%RSD (5 µg/L)		%RSD (20 µg/L)		%RSD (100 µg/L)	
		R.T	Area	R.T	Area	R.T	Area
1	Chlortoluron	0.08	1.57	0.11	0.43	0.08	0.96
2	Isoproturon	0.09	1.73	0.10	1.17	0.08	1.00
3	Diuron	0.09	1.74	0.11	1.14	0.07	1.36
4	Linuron	0.07	2.41	0.05	1.52	0.09	1.62

Spiked matrix test

In order to assess the method's sensitivity, blank vegetable matrix sample that had been subjected to the sample preparation method as specified in the literature for extraction of pesticides was spiked with multi-standard solution at the spiked level of 10 µg/kg. MRM chromatograms of blank vegetable matrix are shown in Fig. 10 and MRM chromatograms of vegetable matrix spiked with standards are shown in Fig. 11. As can be seen, the system responded well to spiked matrix samples.

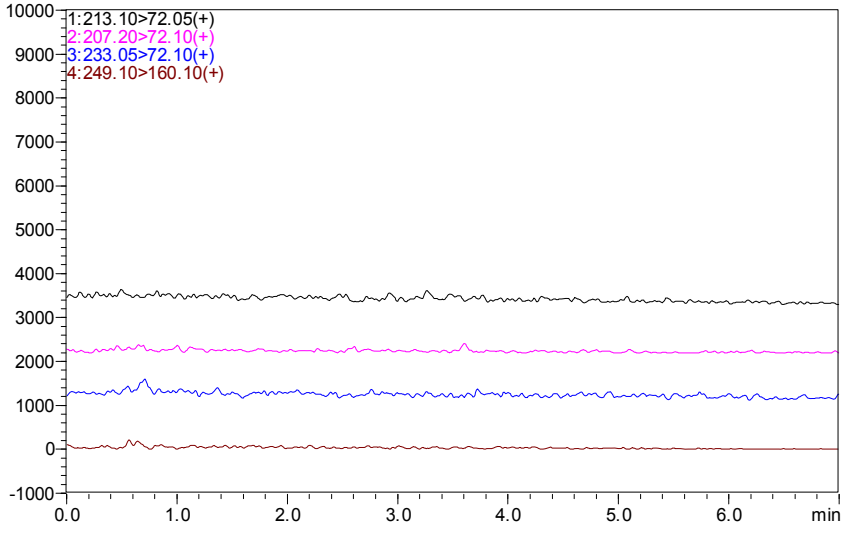


Fig. 10 MRM chromatograms of vegetable blank matrix sample

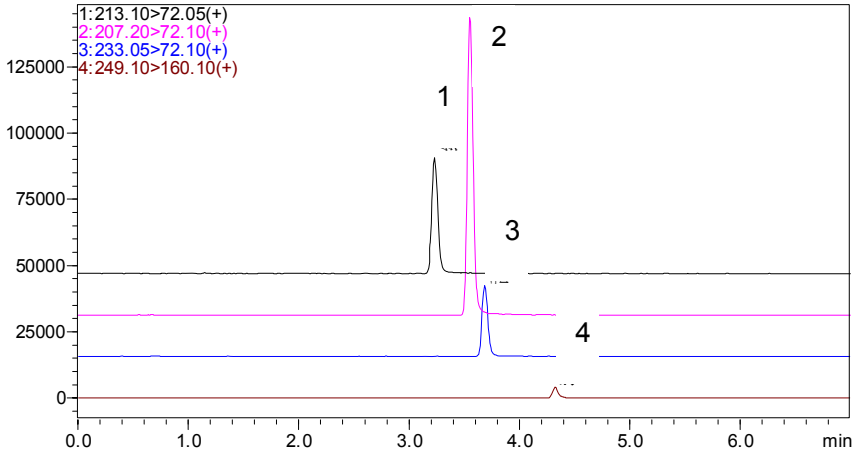


Fig. 11 MRM chromatograms of vegetable matrix sample spiked with standards

CONCLUSION

A method was developed in reference with SN/T 2213-2008 for the determination of substituted urea pesticides residues in food of plant origin using Shimadzu LC-30A ultra-fast liquid chromatograph in conjunction with LCMS-8030 triple quadrupole mass spectrometer. The method demonstrated good linearity for the 4 substituted urea pesticides of chlortoluron, isoproturon, diuron and linuron in the concentration range of 5~100 µg/L with correlation coefficients all greater than 0.999. The method's MDLs were 0.12-0.36 µg/L and LOQs were 0.42~1.2 µg/L. The method worked out well to vegetable matrix samples that had been subjected to pretreatment procedures and spiked with standards and met the MDL requirement of 10 µg/kg stipulated in *SN/T 2213-2008 Determination of substituted ureas pesticides residues in foodstuffs of plant origin for import and export--LC-MS/MS method.*

SHIMADZU
Excellence in Science

C-37 Determination of avermectins residues in foodstuffs of animal origin by ultra-fast liquid chromatography-triple quadrupole mass spectrometry

INTRODUCTION

In this paper, a method is proposed for determination of 4 avermectins residues in foodstuff of animal origin using Shimadzu ultra-fast liquid chromatograph and triple quadrupole mass spectrometer. Analytes in samples that had been subjected to pretreatment were separated by the LC-30A ultra-fast liquid chromatograph, and then quantitatively analyzed with the LCMS-8040 triple quadrupole mass spectrometer. Calibration curves of the 4 avermectins residues were plotted by the external standard method and the plotted calibration curves had a linear range of 1~200 ng/mL and correlation coefficients greater than 0.995. Precision tests were performed on 10 ng/mL, 50 ng/mL and 100 ng/mL multi-standard solutions and the %RSDs of retention time and peak area of 6 successive injections fell in the ranges of 0.10~0.54% and 1.03~5.62%, respectively, suggesting that the method's precision was good.

The avermectins are a series of macrocyclic lactone pesticides separated from the fermentation products of streptomycetes that have potent anthelmintic and insecticidal actions in animals and on plants, including avermectin, eprinomectin, doramectin, ivermectin, etc. All avermectins are highly poisonous and with toxicity similar to organophosphate pesticides. The United States, EU, and Japan have stipulated MRLs for the avermectins. It is hard to vaporize the avermectins due to their relatively high molecular weight. Currently available analytical methods of these drugs mainly include ELISA and HPLC-FLD. In recent years, there are many study reports in China and/or foreign countries on the determination of avermectins residues by HPLC-MS/MS. It is stipulated in *GB/T 21320-2008 Determination of avermectins residues in foodstuffs of animal origin—LC-MS/MS* that the LODs of avermectin, eprinomectin, doramectin, and ivermectin are required to be at least 1.5 μg/kg. In this paper, a method is proposed in reference with *GB/T 21320-2008* for fast determination of 4

avermectins residues in pork with Shimadzu LC-30A ultra-fast liquid chromatograph and LCMS-8040 triple quadrupole mass spectrometer.

EXPERIMENTAL

Instruments

A combined system of Shimadzu ultra-fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8040 was used in the experiment. The configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8040 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.42 chromatography workstation.

Analytical conditions

LC Conditions

Column : Shim-pack XR-ODS III 2.0 mmI.D.×50 mmL., 1.6 μm
Mobile phase A : a mixed aqueous solution of 2mM ammonium acetate and 0.02% formic acid
Mobile phase B : Methanol
Flow rate : 0.4 mL/min
Injection volume : 10 μL
Column temperature : 40 °C
Elution mode : Binary gradient with initial concentration of 80% of phase B.
See Table 1 for time program.

Table 1 Time program

Time (min)	Module	Command	Value
3.00	Pumps	B Conc.	100
4.40	Pumps	B Conc.	100
4.50	Pumps	B Conc.	80
6.00	Controller	Stop	

MS conditions

Ionization : ESI (+)
Ionization voltage : +4.5 kV
Nebulizing gas : Nitrogen, 2.0 L/min
Drying gas : Nitrogen, 15 L/min
Collision gas : Argon
DL temperature : 250 °C

Heater block temperature : 450 °C
Mode : multiple reaction monitoring (MRM)
Dwell time : 100 ms
Pause time : 3 ms
MRM parameters : see Table 2

Table 2 MRM parameters

No.	Compound	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Avermectin	895.60	327.40*	-34.0	-54.0	-11.0
			449.30	-34.0	-51.0	-12.0
2	Eprinomectin	936.60	352.00*	-26.0	-60.0	-13.0
			490.00	-26.0	-55.0	-15.0
3	Doramectin	921.55	353.20*	-34.0	-58.0	-23.0
			449.00	-34.0	-53.0	-30.0
4	Ivermectin	897.50	329.10*	-34.0	-56.0	-15.0
			753.30	-34.0	-45.0	-34.0

* refers to quantitative ion.

Sample Preparation

Preparation of standard solution:

A 1 μg/mL multi-standard stock solution was prepared using acetonitrile as solvent and progressively diluted into a series of standard working solutions of concentrations of 1, 5, 10, 20, 50, 100 and 200 ng/mL with acetonitrile.

Sample pretreatment method:

Refer to *GB/T 21320-2008 Determination of avermectins residues in foodstuffs of animal origin—LC-MS/MS*.

RESULTS AND DISCUSSION

MRM chromatogram of standard mixture

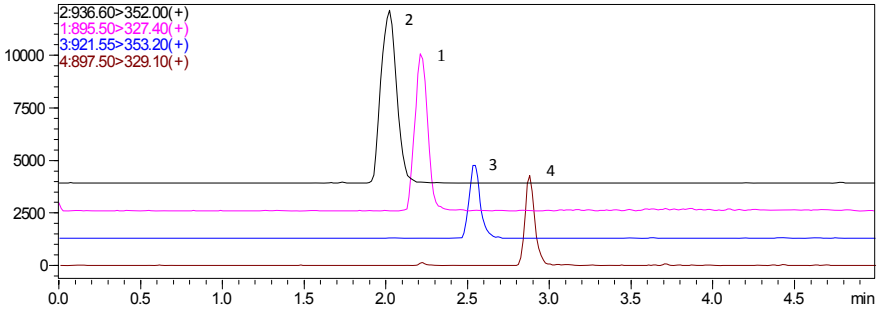


Fig.1 MRM chromatograms of 50 ng/mL multi-standard solution of four avermectins

(1. Avermectin 2. Eprinomectin 3. Doramectin 4. Ivermectin)

Mass spectra and product ions for MRM

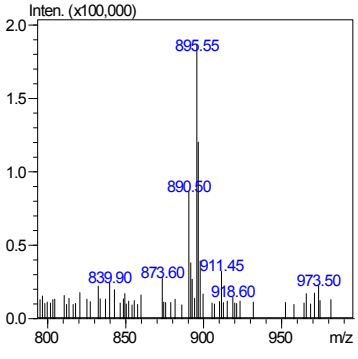


Fig.2 Mass spectrum of Avermectin

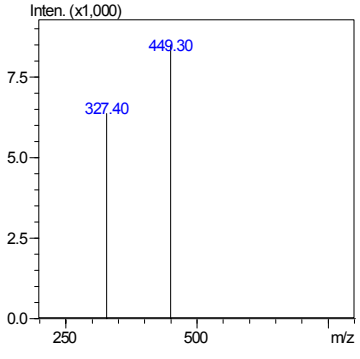


Fig.3 Product ions for MRM (Avermectin)

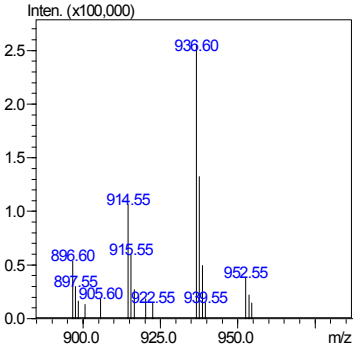


Fig.4 Mass spectrum of Eprinomectin

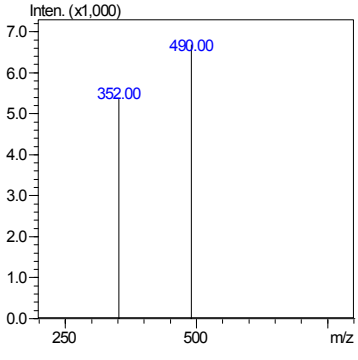


Fig.5 Product ions for MRM (Eprinomectin)

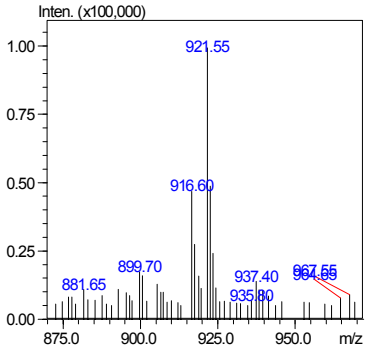


Fig.6 Mass spectrum of Doramectin

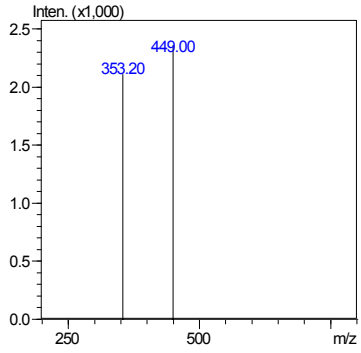


Fig.7 Product ions for MRM (Doramectin)

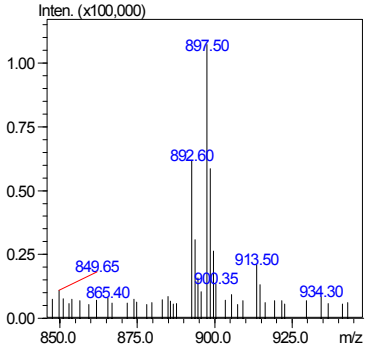


Fig.8 Mass spectrum of Ivermectin

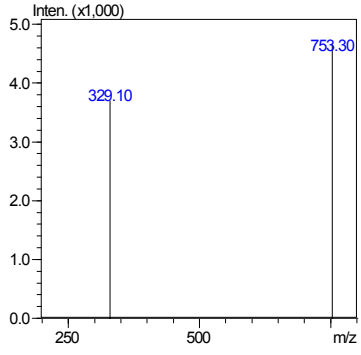


Fig.9 Product ions for MRM (Ivermectin)

Calibration curve

A series of multi-standard working solutions of concentrations of 1, 5, 10, 20, 50, 100 and 200 ng/mL were analyzed using the analytical conditions above. Calibration curves as shown in Figs.10 ~ 13 were plotted by the external standard method with concentration as X-axis and peak area as Y-axis. The calibration curves of the 4 avermectins were of good linearity in the concentration range of 1~200 ng/mL. Their linear equations and correlation coefficients are listed in Table 3.

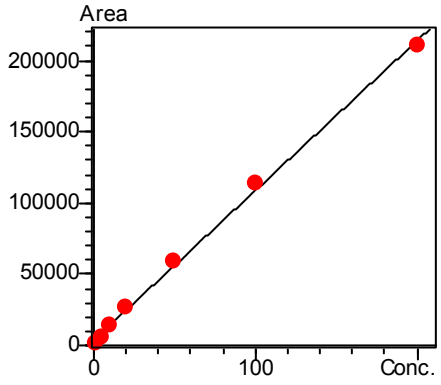


Fig.10 Calibration curve of Avermectin

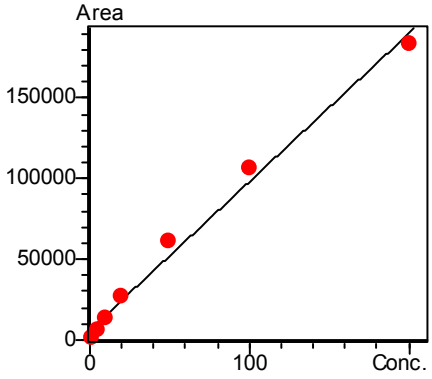


Fig.11 Calibration curve of Eprinomectin

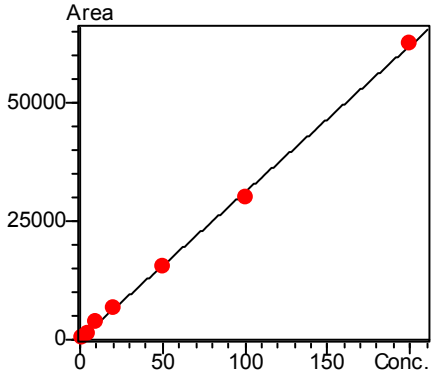


Fig.12 Calibration curve of Doramectin

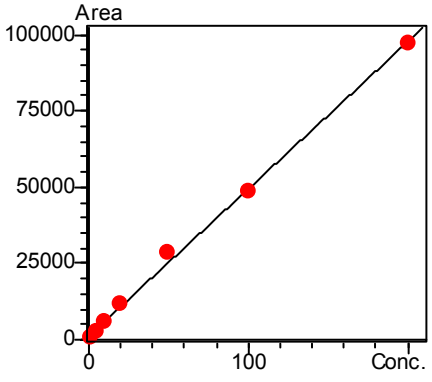


Fig.13 Calibration curve of Ivermectin

Table 3 Parameters of calibration curves of the 4 Avermectins

No.	Compound	Calibration Curve	Correlation Coefficient (R^2)
1	Avermectin	Y = (1050.65)X + (3738.91)	0.9990
2	Eprinomectin	Y = (917.875)X + (6294.14)	0.9954
3	Doramectin	Y = (309.253)X + (126.834)	0.9996
4	Ivermectin	Y = (481.796)X + (1258.76)	0.9992

Precision test

Precision tests were performed by 6 successive injections of multi-standard solutions of concentrations of 10 ng/mL, 50 ng/mL and 100 ng/mL. The %RSDs of peak area and retention time of standards of the 3 concentrations found to be in the range of 1.03~5.62% and 0.10~0.54%, respectively, suggesting that the method's precision was good.

Table 4 Repeatability - retention time and peak area (n=6)

Compound	%RSD (10 ng/mL)		%RSD (50 ng/mL)		%RSD (100 ng/mL)	
	Area	R.T	Area	R.T	Area	R.T
Avermectin	3.48	0.37	3.47	0.17	2.68	0.10
Eprinomectin	2.12	0.54	2.20	0.11	1.03	0.10
Doramectin	5.62	0.40	5.15	0.13	3.97	0.12
Ivermectin	3.03	0.13	3.68	0.11	4.14	0.08

Sensitivity test

In order to assess the method's sensitivity, multi-standard solution of the 4 Avermectins was spiked into blank pork matrix at the spike level of 2 μg/kg. The matrix was then subjected to the sample pretreatment procedures as specified above followed by injection and analysis. The MRM chromatograms of blank pork matrix are shown in Fig. 14 and the MRM chromatograms of spiked pork matrix are as shown in Fig. 15.

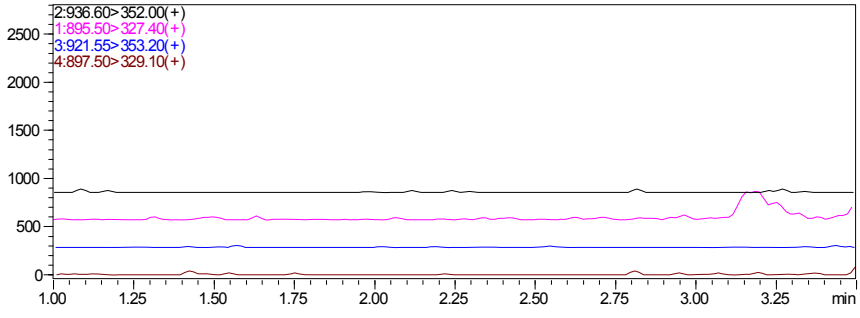


Fig. 14 MRM chromatograms of blank pork matrix

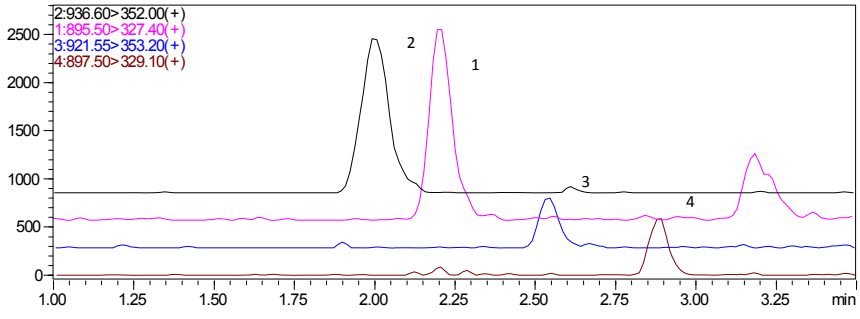


Fig. 15 MRM chromatograms of spiked pork sample
(1. Avermectin 2. Eprinomectin 3. Doramectin 4. Ivermectin)

Spiked pork samples were subjected to 7 replicate assays in accordance with the procedures described and the results were used to calculate standard deviation (S), LOD (=3.14×S), and LOQ (=4×LOD) of the analytes. The results are shown in Table 4.

Table 4 LODs and LOQs of the 4 Avermectins

No.	Compound	Standard Deviation (S)	LOD (μg/kg)	LOQ (μg/kg)
1	Avermectin	0.13	0.40	1.61
2	Eprinomectin	0.11	0.35	1.42
3	Doramectin	0.09	0.29	1.18
4	Ivermectin	0.14	0.44	1.74

CONCLUSION

The proposed method for determination of the 4 Avermectins residues in pork with Shimadzu LC-30A ultra-fast liquid chromatography and LCMS-8040 triple quadrupole mass spectrometer was fast, repeatable and precise. It demonstrated good linearity for Avermectin, Eprinomectin, Doramectin and Ivermectin in the concentration range of 1~200 ng/mL. The method outperformed the LOD requirements in *GB/T 21320-2008 Determination of avermectins residues in foodstuffs of animal origin—LC-MS/MS* and achieved LODs of 0.29~0.44 μg/kg for the Avermectins, making it suitable for the assay of Avermectins residues in foodstuffs of animal origin.

C-38 Determination of Carbendazol and other Pesticide Residues in Wine with UFLC-Triple Quadrupole Mass Spectrometry

INTRODUCTION

A method was developed for the determination of carbendazol, thiophanate methyl and metalaxyl pesticide residues in wine using Shimadzu ultra-fast liquid chromatograph (UFLC) in conjunction with triple quadrupole mass spectrometer. Carbendazol, thiophanate methyl and metalaxyl pesticides in wine were first enriched by solid-phase extraction, then fast separated with LC-30A UFLC, and finally quantitatively analyzed using LCMS-8040 triple quadrupole mass spectrometer. The calibration curves of carbendazol, thiophanate methyl and metalaxyl were plotted by an external standard method and all demonstrated a wide linear range and correlation coefficients greater than 0.999. Precision tests were performed on 5 μg/L, 10 μg/L and 50 μg/L multi-standard solutions and the %RSDs of retention time and peak area of 6 successive injections found to be in the range of 0.030%~0.212% and 0.912%~2.978%, respectively, suggesting that the method's precision was good.

The two common pesticides for grapes, carbendazol and metalaxyl are used by the world's major grape-producing countries. Carbendazol is not allowed in the United States, because of the risk of liver cancer, but thiophanate methyl (with similar molecular structure of carbendazol) is allowed with regulated residue limits. It is understood that the maximum residue limits (MRLs) of metalaxyl and carbendazol have been stipulated by the countries around the world. Taking carbendazol for example, its limit is 3 mg/kg in the wine in Australia, 5 mg/kg in Canada, 0.5 mg/kg in EU, 3 mg/kg in Japan and 10 mg/kg in the United States. At present, there are still no specific standards for pesticide residues in wine in China. High performance liquid chromatography (HPLC)-tandem mass spectrometry, with high selectivity and sensitivity and high accuracy, is suitable for trace analysis of organic residues in complex matrices. A method was developed for the determination of carbendazol, thiophanate methyl and metalaxyl pesticide

residues in wine using Shimadzu LC-30A UFLC in conjunction with LCMS-8040 triple quadrupole mass spectrometer.

EXPERIMENTAL

Instruments

A combined system of Shimadzu UFLC LC-30A and triple quadrupole mass spectrometer LCMS-8040 was used in the experiment. The configuration included two LC-30AD pumps, a DGU-20A_5 online degasser, a SIL-30AC autosampler, a CTO-30A column oven, a CBM-20A communication bus module, a LCMS-8040 triple quadrupole mass spectrometer, and a LabSolutions Ver. 5.50 chromatography workstation.

Conditions of Analysis

LC conditions

Column : Shim-pack XR-ODS III 2.0 mm I.D.× 50 mm L., 1.6 µm
Mobile phase A : 5 mM ammonium acetate aqueous solution
Mobile phase B : Acetonitrile
Flow rate : 0.5 mL/min
Column temperature : 40 °C
Injection volume : 5 µL
Elution mode : Gradient elution with initial concentration of mobile phase B of 15%.,
see Table 1 for the time program.

Table 1 Time program

Time (min)	Module	Command	Value
1.60	Pumps	B Conc.	80
2.00	Pumps	B Conc.	80
2.01	Pumps	B Conc.	15
3.00	Controller	Stop	

MS conditions

Ionization mode : ESI(+)
Ionization voltage : 4.5 kV
Nebulizing gas : Nitrogen, 3.0 L/min
Drying gas : Nitrogen, 20 L/min
Collision gas : Argon
DL temperature : 250 °C
Heater block temperature : 450 °C

Mode : Multiple Reaction Monitoring (MRM)
Dwell time : 50 ms
Pause time : 3 ms
MRM parameters : see Table 2

Preparation of standard solutions

Standard analyte:- carbendazol, thiophanate methyl and metalaxyl.

Preparation of standard working solutions: 1.0 mg/L multi-standard intermediate solution was prepared using methanol as solvent, and then diluted with acetonitrile aqueous solution (15:85, V/V) into multi-standard working solutions of concentrations of 0.5 μg/L, 1 μg/L, 5 μg/L, 10 μg/L, 50 μg/L, 100 μg/L, 200 μg/L and 500 μg/L.

Sample pretreatment method:

Refer to “GBT 23206-2008 Determination of 512 pesticides and related chemical residues in fruit and vegetable juices and fruit wine - LC-tandem MS” for sample extraction and purification methods.

Table 2 Optimized MRM parameters

Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Carbendazol	192.1	160.0*	-20	-18	-30
		132.0	-20	-29	-23
Thiophanate methyl	343.1	151.0*	-17	-20	-28
		310.9	-17	-11	-21
Metalaxyl	280.1	220.1*	-29	-13	-24
		248.1	-29	-11	-28

Note: * refers to quantitative ion

RESULTS AND DISCUSSION

Mass spectrum and MS/MS spectrum

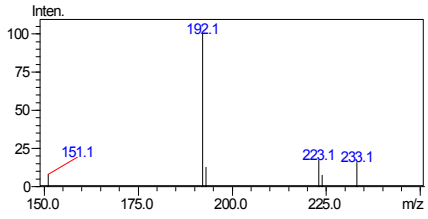


Fig. 1 Mass spectrum of carbendazol

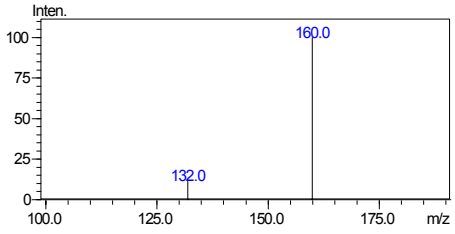


Fig. 2 MS/MS spectrum of carbendazol (CE -2V)

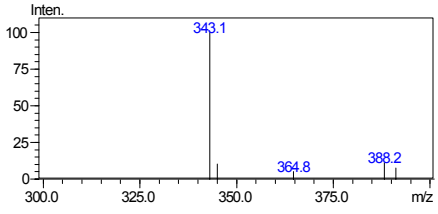


Fig. 3 Mass spectrum of thiophanate methyl

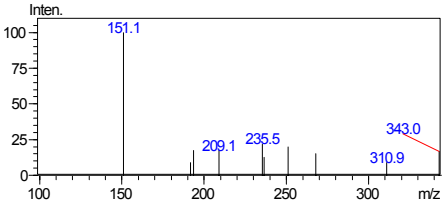


Fig. 4 MS/MS spectrum of thiophanate methyl (CE - 15V)

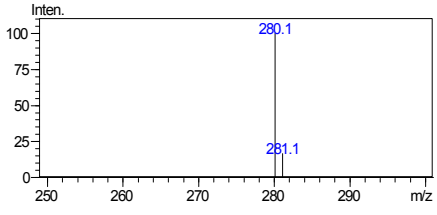


Fig. 5 Mass spectrum of metalaxyl

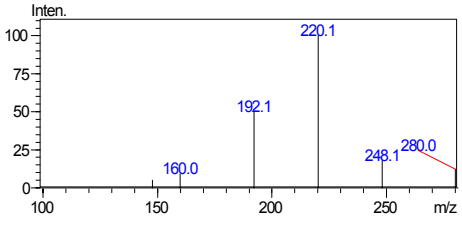


Fig. 6 MS/MS spectrum of metalaxyl (CE - 15V)

MRM chromatogram of standard mixture

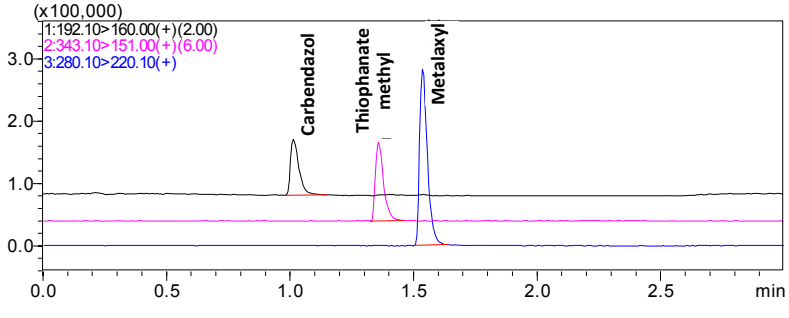


Fig. 7. MRM chromatograms of standard mixture (10 µg/L)

Linear range

Multi-standard solutions at concentrations of 0.5 μg/L, 1 μg/L, 5 μg/L, 10 μg/L, 50 μg/L, 100 μg/L, 200 μg/L and 500 μg/L were subjected to quantitative analysis by external calibration method under the analysis conditions as specified. Calibration curves were plotted as shown in Fig. 8 to Fig. 10 with concentration as abscissa and peak area as ordinate. The calibration curves were of satisfactory linearity and their linear equations and correlation coefficients were shown in Table 3.

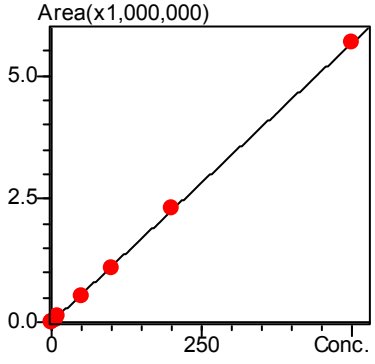


Fig. 8 Calibration curve of carbendazol

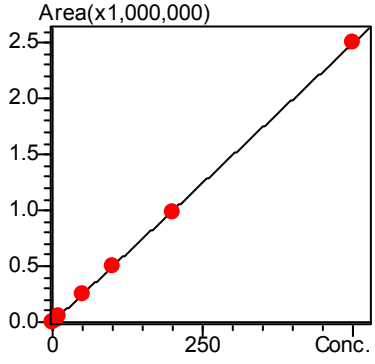


Fig. 9 Calibration curve of thiophanate methyl

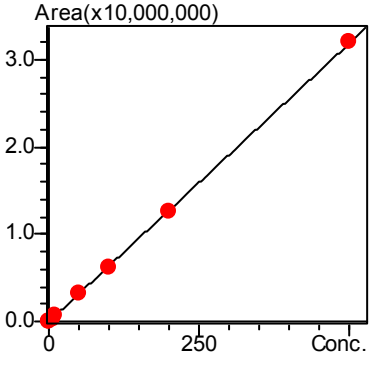


Fig. 10 Calibration curve of metalaxyl

Table 3 Parameters of calibration curves

No.	Compound	Calibration Curve	Linear Range (μg/L)	Correlation Coefficient (r)
1	Carbendazol	Y = (11329.9)X	0.5~500	0.9999
2	Thiophanate methyl	Y = (4990.23)X	0.5~500	1.0000
3	Metalaxyl	Y = (63758.0)X	0.5~500	1.0000

Precision test

Multi-standard working solutions of various concentrations were injected 6 times in succession to assess the method's precision. The resulted repeatability of retention time and peak area was shown in Table 4. The results showed that the %RSDs of retention time and peak area data of standard solutions of various concentrations are in the range of 0.030 %~0.212% and 0.912 %~2.978% respectively, suggesting the method had satisfactory precision.

Table 4 Repeatability - retention time and peak area (n=6)

Compound	%RSD (5 μg/L)		%RSD (10 μg/L)		%RSD (50 μg/L)	
	R.T.	Area	R.T.	Area	R.T.	Area
Carbendazol	0.104	2.978	0.118	1.829	0.212	2.078
Thiophanate methyl	0.174	2.426	0.071	1.539	0.088	0.912
Metalaxyl	0.030	1.995	0.056	1.334	0.055	1.170

Sensitivity test

In order to evaluate the method's sensitivity, 7 standard samples were prepared at concentration of 1.0 μg/L and subjected to 7 replicate injections for analysis, yielding chromatograms as shown in Fig. 11. The MDLs and LLOQs were calculated from the standard deviations(S) of the 7 injections and determinations using the formulae MDL＝3.14×S, LOQ=4×MDL. The assay results were shown in Table 5.

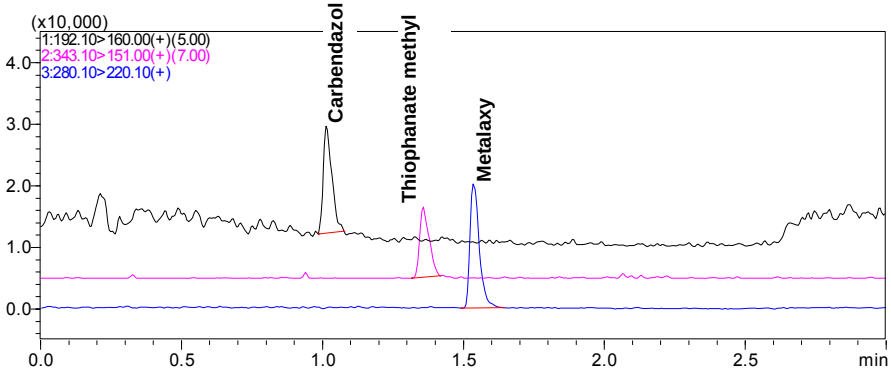


Fig. 11 MRM Chromatograms of Tested Samples (1.0 μg/L)

Table 5 Sensitivity test results

No.	Compound	Standard Deviation (S)	LOD (μg/L)	LOQ (μg/L)
1	Carbendazol	0.05	0.16	0.63
2	Thiophanate methyl	0.06	0.19	0.75
3	Metalaxyl	0.03	0.09	0.38

Recovery test

Wine samples were analyzed for carbendazol, thiophanate methyl and metalaxyl pesticide residues. Above-mentioned pesticide residues were detected in wine samples. The chromatograms are shown in Fig. 12. The detection results were shown in Table 6. The 15 mL wine samples were taken and spiked with standard substances of carbendazol, thiophanate methyl and metalaxyl at spiked concentrations of 40 μg/kg, 8 μg/kg and 40 μg/kg respectively and subjected to quantitative analysis. The resulted chromatogram of the spiked samples was shown in Fig. 13. Carbendazol, thiophanate methyl and metalaxyl pesticide residues in wine samples were spiked in sample and the spiked recoveries were as listed in Table 6. The experiment met the requirements in “GB/T 27404-2008 Criterion on quality control of laboratories - Chemical testing of food” that the recovery was between 60% and 120% when the sample content was less than 0.100 mg/kg.

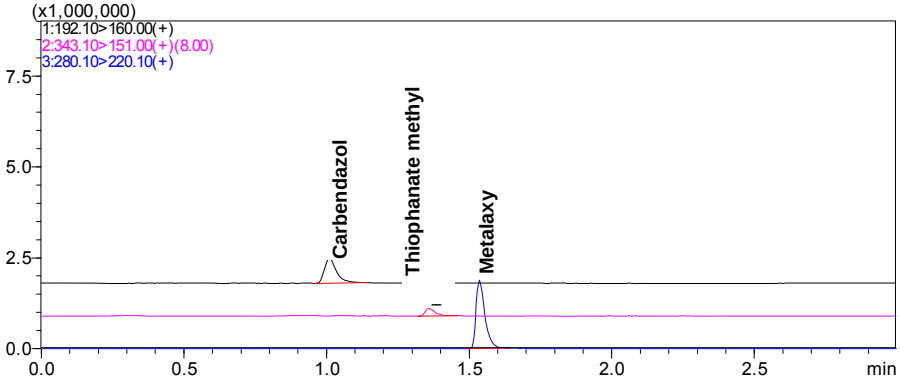


Fig. 12 MRM chromatograms of a wine sample

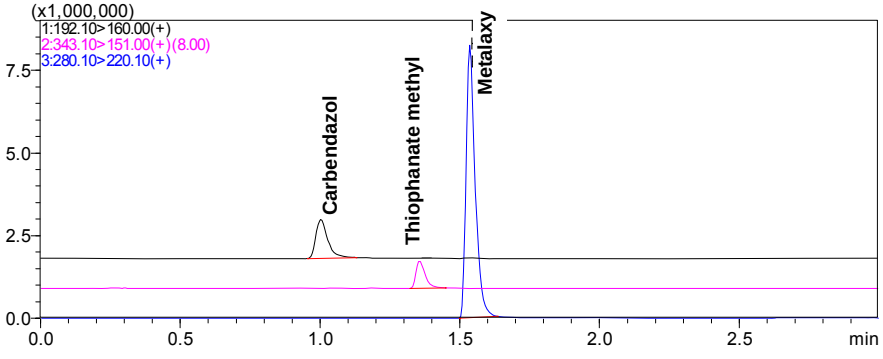


Fig. 12 MRM chromatograms of a spiked wine sample

Table 6 Recovery test results

No.	Compound	Matrix Concentration (μg/kg)	Spiked Concentration (μg/kg)	Tested Concentration (μg/kg)	Recovery (%)
1	Carbendazol	20.43	40	44.53	60.25
2	Thiophanate methyl	1.64	8	6.61	62.13
3	Metalaxyl	8.25	40	40.08	79.58

Actual sample analysis results

Two commercial wine samples were detected for carbendazol, thiophanate methyl and metalaxyl pesticide residues. Above-mentioned pesticide residues were detected. The detection results were shown in Table 7.

Table 7 Detection results of commercial wine samples

No.	Compound	Commercial wine 1 (μg/kg)	Commercial wine 2 (μg/kg)
1	Carbendazol	20.43	23.80
2	Thiophanate methyl	1.64	3.05
3	Metalaxyl	8.25	5.42

CONCLUSION

A method was developed for the determination of carbendazol, thiophanate methyl and metalaxyl pesticide residues in wine using Shimadzu LC-30A UFLC in conjunction with LCMS-8040 triple quadrupole mass spectrometer. With the proposed method, 3 targeted compounds, i.e. carbendazol, thiophanate methyl and metalaxyl were separated and analyzed within 3 min and demonstrated wide linearity range. The correlation coefficients of all calibration curves were greater than 0.9999. Precision tests were performed on 5 μg/L, 10 μg/L and 50 μg/L multi-standard solutions and the %RSDs of retention time and peak area of 6 successive injections were in the range of 0.030%~0.212% and 0.912%~2.978%, respectively, suggesting that the method's precision was good. With the merits of fast analysis speed, high sensitivity and good repeatability, the method is suitable for analysis of carbendazol, thiophanate methyl and metalaxyl pesticide residues in wine.

C-39 Determination of 19 Phthalate Plasticizers in Chinese White Liquor with GC-MS/MS

INTRODUCTION

In this paper, a method was proposed for fast determination of 19 phthalate plasticizers in Chinese white liquor with Shimadzu GCMS-TQ8030. White spirit samples were subjected to water bath heating to remove most ethanol, later subjected to n-hexane extraction, and then injected on GCMSMS for analysis. The results showed that the proposed method was of good linearity in the concentration range of 0.01~2.00 μg/mL with a correlation coefficient greater than 0.997. Its LOD, ranged from 0.02 to 21.45 μg/L. The %RSDs of peak areas of phthalates in 5 successive injections were less than 5 %, and the mean recoveries of spiked samples were between 70% - 125%.

Plasticizers are additives that can improve the flexibility of materials or liquefy the materials. Their application is limited to industrial production. Phthalates, because of their moderate viscosity, high stability, low volatility, good availability, and low cost, are the most extensively used plasticizer.

In November 2012, excessive plasticizers were found in the white spirit of a Chinese brand. Phthalates (DEHP, in particular) belong to a group of substances called environmental hormone which can interfere with the endocrine system. According to available toxicological studies carried out by many countries on DEHP, the substance has a variety of toxicity, mainly reproductive toxicity, developmental toxicity, mutagenicity, carcinogenicity, endocrine toxicity, and immunotoxicity.

Presently, the available methods for determination of phthalate plasticizers in beverage and alcohol mainly involve certain pretreatment (e.g. LLE, SPE, SPME) and qualitative & quantitative analyses by means of GC, GC/MS, LC, and LC/MS. White spirit is a good solvent for plasticizers because of its high solubility in ethanol. This, together with its complicated matrix and the multiplicity of phthalate plasticizers, makes it a bit difficult to analyze qualitatively and quantitatively.

Based on the above consideration, a method was proposed in this paper for fast detection of 19 phthalate plasticizers in white spirit products with Shimadzu triple quadrupole mass spectrometer GCMS-TQ8030. The proposed method is easy to operate and adopts MRM acquisition mode, which can effectively reduce the interference from components in white spirit matrix and help to achieve higher sensitivity.

EXPERIMENTAL

Instruments

GC-MS/MS : GCMS-TQ8030

Conditions of Analysis

Column : Rxi-5 Sil ms, 30 m × 0.25 mm × 0.25 µm
Column temperature program : 90 °C (1 min)-15 °C/min-210 °C (2min)-5 °C/min -250 °C (5 min)-25 °C/min-300 °C(4 min)
Injector temperature : 250 °C
Injection mode : Splitless (1 min)
Carrier gas control mode : CLV (37 cm/sec)
Carrier gas : Helium
Collision gas : Argon
Solvent cut time : 4 min
Detector voltage : Tuning voltage+0.3kV
Interface temperature : 280 °C
Temperature of ion source : 230 °C
Mode : MRM (parameters were as listed in Table 1)

Pretreatment of samples

10 mL white spirit sample was accurately pipetted and transferred to a 25 mL stoppered test tube. Then it was heated on water bath 85 °C for 30 min (shaken evenly several times during the period) and allowed to cool down to room temperature. Accurately 2 mL n-hexane was added and subjected to a vortex mixer for 1min. The whole mixture was allowed settle and then the supernatant was removed for analysis.

RESULTS AND DISCUSSION

Standard chromatogram

The MRM chromatograms of standard solutions spiked with 19 phthalic acid esters (PAEs) were as shown in Fig. 1.

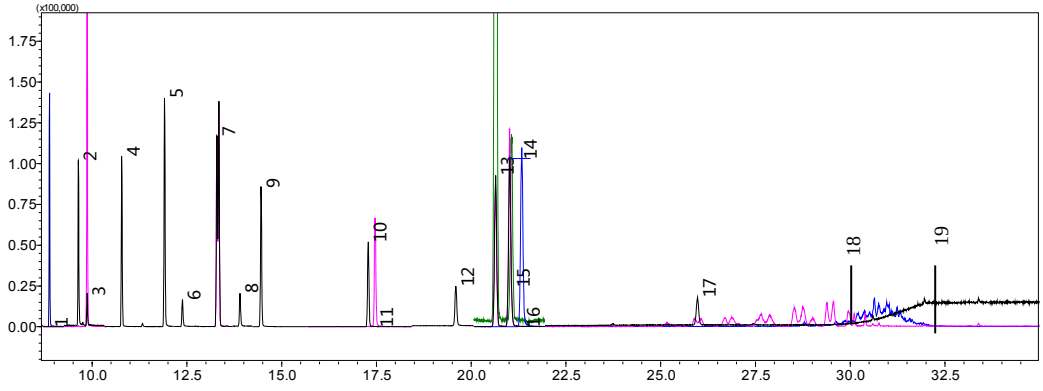


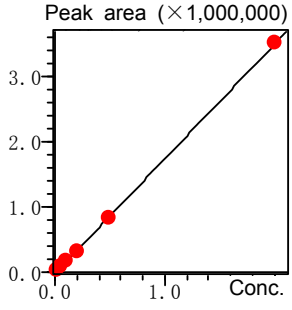
Fig. 1 MRM chromatograms of standard mixture spiked with 19 PAEs (100 μg/L)

Table 1 MRM parameters for the 19 PAEs

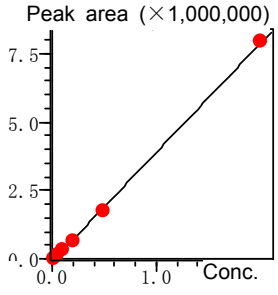
NO.	Composition Name	Quantitative Ion (*m/z*)	CE	Qualitative Ion (*m/z*)	CE
1	DIPP	209>149	10	167>149	10
2	DALP	132>104	7	189>105	17
3	DPRP	209>149	8	191>149	5
4	DIBP	223>149	10	205>149	5
5	DBP	223>149	10	205>149	5
6	DMOEP	207>59	5	176>149	10
7	BMPP	167>149	10	251>149	20
8	DEOEP	176>149	10	176>104	25
9	DAP	237>149	10	219>149	5
10	DHXP	251>149	15	233>149	5
11	BBP	206>149	10	238>104	20
12	DBOEP	193>149	15	176>149	10
13	DCHP	167>149	10	249>149	15
14	DEHP	167>149	10	279>149	15
15	DHP	249>149	10	167>149	15
16	DPP	225>77	25	225>141	20
17	DNOP	279>149	12	279>71	17
18	DINP	293>149	10	293>167	5
19	DIDP	307>149	20	307>167	5

Calibration curve

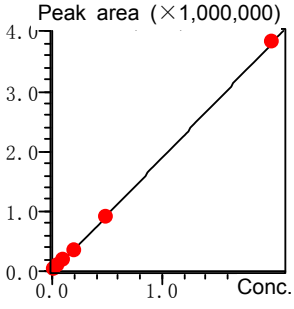
Standard solutions of 19 PAEs at concentrations of 0.01, 0.05, 0.10, 0.20, 0.50, and 2.00 µg/mL were prepared using n-hexane as solvent. Calibration was performed with concentration as abscissa and peak area of quantitative ion as ordinate. Calibration curves for the components as shown below. Based on the data from 0.01 µg/mL standard solution, the LODs of PAEs were calculated. The LODs and the calibration curves' correlation coefficients are shown in the Table 2.



DIPP



DALP



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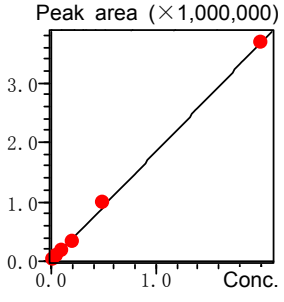

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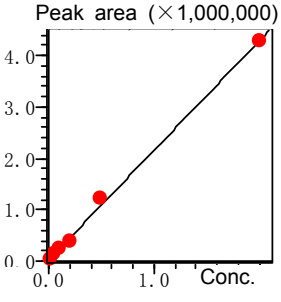

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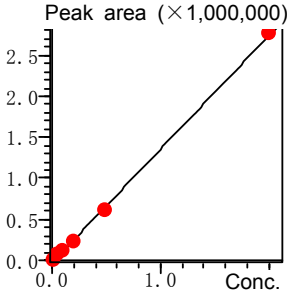

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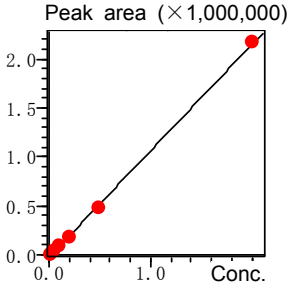
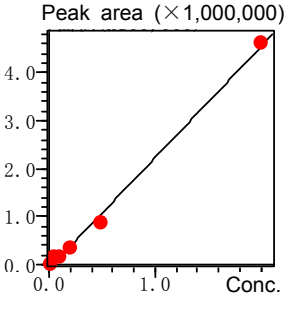


BMPP



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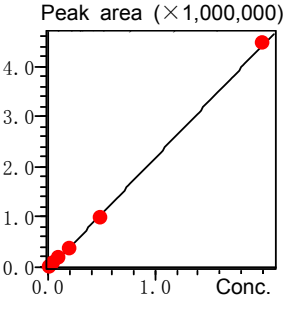

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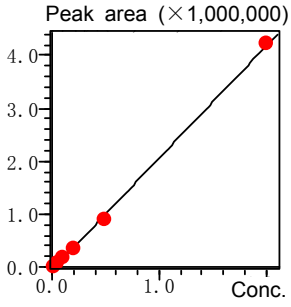
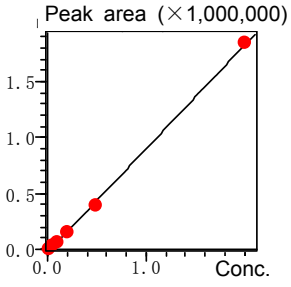


DHXP



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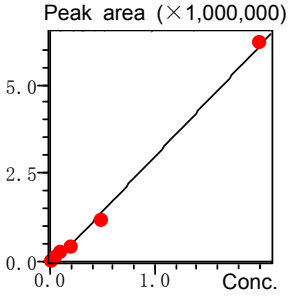
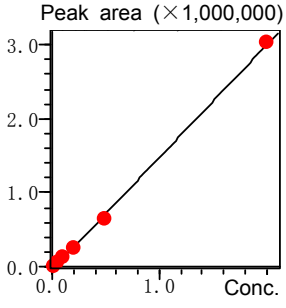


DBOEP



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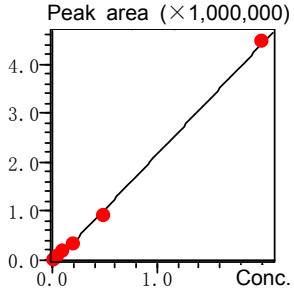

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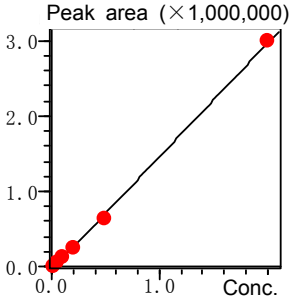

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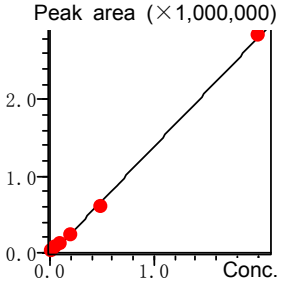

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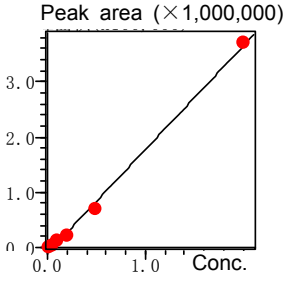

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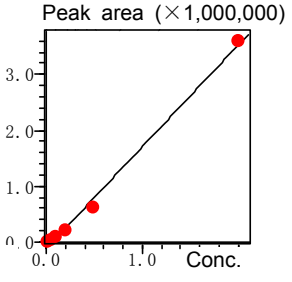
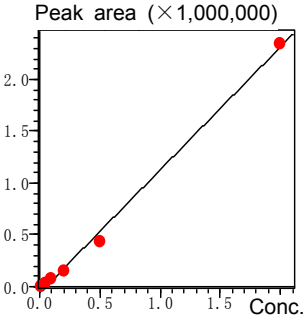


DINP



DIDP

Table 2 Correlation coefficients and LODs of PAEs

No.	Composition Name	Correlation Coefficient	LOD (μg/L)	No.	Compound Name	Correlation Coefficient	LOD (μg/L)
1	DIPP	0.9992	0.05	11	BBP	0.9989	0.11
2	DALP	0.9993	0.82	12	DBOEP	0.9977	0.99
3	DPRP	0.9996	0.09	13	DCHP	0.9989	0.38
4	DIBP	0.9995	0.56	14	DEHP	0.9987	0.13
5	DBP	0.9995	0.18	15	DHP	0.9989	0.02
6	DMOEP	0.9992	1.35	16	DPP	0.9989	0.05
7	BMPP	0.9994	0.04	17	DNOP	0.9984	0.96
8	DEOEP	0.9991	0.25	18	DINP	0.9981	19.78
9	DAP	0.9992	0.19	19	DIDP	0.9973	21.45
10	DHXP	0.9991	0.18				

Repeatability

A sample of white spirit spiked with the PAEs was subject to the pretreatment process, and then analyzed for 5 times in succession. The peak areas and %RSDs of the PAEs were as shown in Table 3.

Table 3 Repeatability test (n=5)

NO.	Composition Name	Peak Area					
		1	2	3	4	5	%RSD
1	DIPP	479584	482465	485211	502038	502742	1.43
2	DALP	1097614	1062900	1073005	1077322	1071242	2.39
3	DPRP	425477	425795	430457	435886	439454	1.40
4	DIBP	291905	294447	299455	305262	309015	1.75
5	DBP	612733	617273	619089	627688	634459	2.00
6	DMOEP	895490	897965	904244	919779	932995	2.00
7	BMPP	905454	907193	915635	930430	949556	3.46
8	DEOEP	7183	7154	7214	7325	7510	2.37
9	DAP	2709581	2922540	2869586	2921568	2963914	2.51
10	DHXP	2951656	2986966	3079669	3110707	2965970	1.97
11	BBP	51628	51294	52072	53626	54325	2.19
12	DBOEP	1326458	1334715	1341204	1364168	1392390	2.15
13	DCHP	1427097	1421760	1436194	1467776	1497104	2.66
14	DEHP	1726367	1734340	1736767	1773467	1816959	2.46
15	DHP	72013	72565	72124	74148	76661	2.67
16	DPP	468750	472541	474631	484062	500594	2.25
17	DNOP	4756421	4788618	4820182	4931999	5060825	2.54

18	DINP	6319083	6370330	6410173	6506059	6721775	1.40
19	DIDP	2489991	2499301	2507544	2565436	2624733	3.56

Recovery test

A white spirit product that is commercially available was selected for the recovery test. Samples spiked with the compositions at concentrations of 0.04, 0.08 and 0.16 μg/mL, respectively, were prepared in parallel in accordance with the sample pretreatment procedures and injected for analysis. The average recoveries of 3 parallel analyses of the samples at all spike concentrations were as shown in Table 4.

Table 4 Recoveries of spiked samples (%)

No.	Composition Name	Spiked Concentration (μg/mL)		
		0.04	0.08	0.16
1	DIPP	83.11	101.06	96.28
2	DALP	75.04	92.10	89.43
3	DPRP	83.41	112.29	92.83
4	DIBP	118.27	99.56	101.68
5	DBP	119.65	98.87	105.51
6	DMOEP	79.88	75.35	72.58
7	BMPP	113.00	125.23	118.91
8	DEOEP	123.12	115.39	109.49
9	DAP	109.08	111.51	104.37
10	DHXP	121.02	115.19	101.99
11	BBP	115.39	123.61	117.77
12	DBOEP	103.97	85.27	90.39
13	DCHP	102.10	83.49	96.10
14	DEHP	113.86	122.98	111.77
15	DHP	125.25	124.33	122.87
16	DPP	108.49	123.55	107.94
17	DNOP	109.86	96.66	96.31
18	DINP	100.74	99.48	109.22
19	DIDP	100.31	107.78	97.97

Sample determination results

A white spirit product that is commercially available was selected and subjected to the pretreatment procedures and analysis. The quantitative analysis results are as shown in Table 5.

Table 5 Quantitative results of the white spirit samples

NO.	PAE Name	Content (μg/mL)
1	DIPP	0.005
2	DALP	N.D
3	DPRP	N.D
4	DIBP	0.009
5	DBP	0.012
6	DMOEP	N.D
7	BMPP	0.008
8	DEOEP	0.009
9	DAP	N.D
10	DHXP	0.009
11	BBP	N.D
12	DBOEP	N.D
13	DCHP	N.D
14	DEHP	N.D
15	DHP	0.012
16	DPP	0.015
17	DNOP	0.011
18	DINP	0.012
19	DIDP	0.013

CONCLUSION

A method was proposed for the analysis of 19 PAEs in Chinese white spirit using Shimadzu GCMS-TQ8030. The proposed method is easy to operate and of good linearity in the range of 0.01~2.00 μg/mL for the calibration curves. The LODs were in the range of 0.02~21.45μg/L and mean recoveries are 70%~125% for spiked samples. It is suitable for fast detection of the 19 PAEs in Chinese white spirit.

C-40 Determination of Melamine in Milk Powder by Pre-column Derivatization GC-MS/MS

INTRODUCTION

A method is proposed in this paper for determination of melamine in dairy products by means of extraction of melamine from milk powder with 50% aqueous solution of methanol and pre-column derivatization in conjunction with triple quadrupole GC-MS. The method is of good linearity in the concentration range of 5~100 μg/L with a correlation coefficient of 0.9999. The method showed an LOD of 0.06 μg/kg and a recovery of spiked samples greater than 97.96%. It is very practical and easy to operate and can be used for fast determination of melamine in milk powder.

Melamine, though frequently referred to as “protein essence” in China, is actually a nitrogen-rich heterocyclic triazine compound which, mainly used for the production of melamine resin, and should never be used in food processing or as a food additive. It is, however, used as a food additive, though illegally, to boost the protein content in foods. It is difficult to analyze using conventional method because the innate defects of the commonly used protein content. Long-term intake of melamine into human body may damage the reproductive system and urinary system and cause vesical and/or renal calculus.

China has paid much attention to the issue of food safety after the “melamine-tainted milk powder” scandal and exerted extra efforts on the detection of melamine in raw milk and dairy products by GB/T22388-2008 *Determination of melamine in raw milk and dairy products*, the national standard in force, in which it is stipulated that the LOQ of GC-MS/MS method for determination of melamine must be less than 5 μg/kg.

Presently many methods, including LC, LC/MS, and GC/MS, are available for determination of melanime. This paper describes a method which effectively reduces background interference and enhances analysis sensitivity by means of the extraction of melamine from milk powder with 50% aqueous solution of methanol and pre-column derivatization and subsequent analysis of melamine by triple quadrupole mass spectrometer in multiple reaction monitoring (MRM) mode. The method is simple, reliable, and of low LOD.

EXPERIMENTAL

Instruments

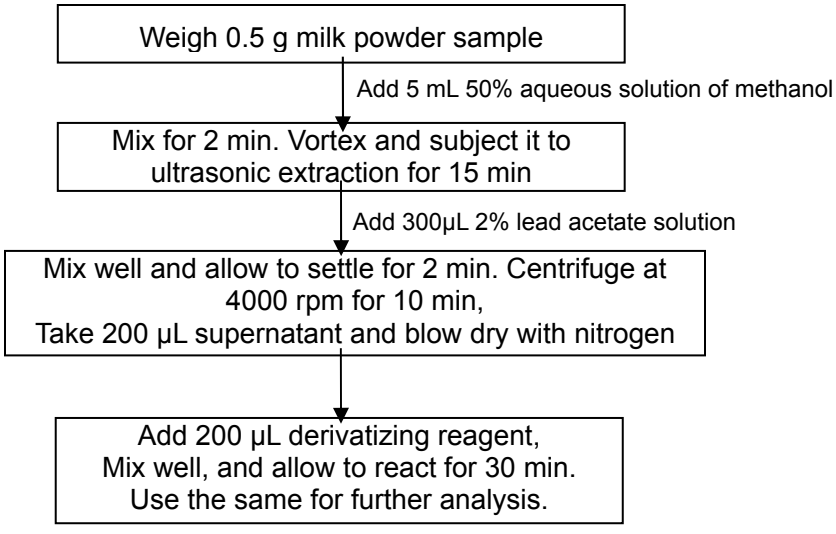
GC-MS/MS : GCMS-TQ 8030

Conditions of Analysis

GC-MS/MS parameters

Column	: Rxi-5Sil MS, 30 m × 0.25 mm × 0.25 µm
Injector temperature	: 250 °C
Injection mode	: Splitless
Carrier gas control mode	: CLV, 36.5 cm/sec
Flow rate	: 1.3 mL/min
Column temperature program	: 75 °C (1 min)→@30 °C/min→220 °C→ @5 °C/min→250 °C(2 min)
Interface temperature	: 250 °C
Temperature of ion source	: 220 °C
Ionization mode	: EI
Mass range	: *m/z* 35~500
Solvent cut time	: 2 min
MRM conditions	: see Table 1

Pretreatment of samples



RESULTS AND DISCUSSION

MRM chromatogram of standards

MRM chromatograms of melamine derivatives are shown in Fig. 1.

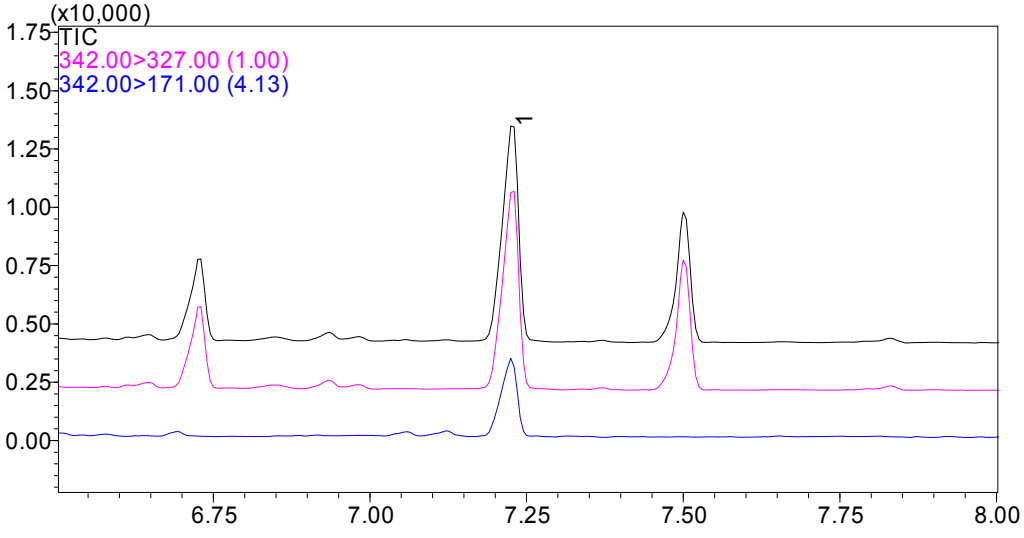


Fig. 1. MRM chromatogram of melamine derivatives (100 μg/L)

Table 1 Retention time and MRM parameters of melamine derivatives

No.	Retention Time (min)	Name	Quantitative Ion (CE)	Qualitative Ion (CE)
1	7.23	Melamine derivatives	342>327（15）	342>171（15）

Calibration curve and LOD

A series of melamine standard solutions were prepared at concentrations of 5, 10, 50, 100 μg/L, respectively, using 50% aqueous solution of methanol as solvent. The calibration curve is shown in Fig. 2. Based on the data of 5 μg/L standard solution, the method's LOD and LOQ were calculated in accordance with the above-mentioned sample pretreatment process. The results were as shown in Table 2.

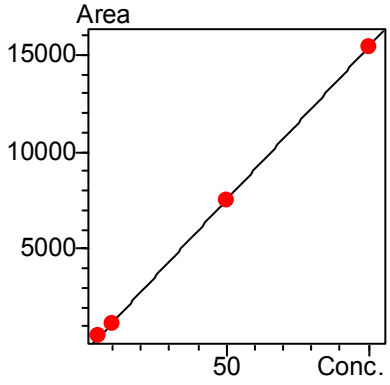


Fig. 2 Calibration curve

Table 2. Correlation coefficients and LODs of the compositions

No.	Name	Correlation Coefficient	LOD (μg/kg)	LOQ (μg/kg)
1	Melamine	0.9999	0.06	0.20

Repeatability test

Repeatability tests were performed on 10 μg/L standard solutions and the results as listed in Table 3, indicates that the method's repeatability is good.

Table 3. Repeatability (n=5)

No.	Peak Area 1	Peak Area 2	Peak Area 3	Peak Area 4	Peak Area 5	%RSD
1	1137	1063	1144	1061	1059	3.99

Sample and recovery test

Melamine standard solution was spiked into two samples (Milk powder 1 and Milk powder 2, both were commercially available branded products) at spike concentration of 5 μg/L and the samples were prepared in accordance with the pretreatment procedures. Three spiked samples were prepared in parallel for each concentration. Results of the recovery test are as shown in Table 4.

Table 4 Spike recovery and reproducibility of samples (n=3)

		Mild powder 1			Mild powder 2		
No.	Name	Mild powder 1 (μg/L)	Spike recovery （%）	Spiked Level RSD%	Mild powder 2 (μg/L)	Spike recovery （%）	Spiked Level RSD%
1	Melamine	N.D.	96.76	4.05	N.D.	101.73	5.06

CONCLUSION

A method was developed for the determination of melamine in milk powder by Shimadzu GCMS-TQ8030. The method is easy to operate and of good linearity in the concentration range of 5~100 μg/L. The LOD found to be 0.06 μg/kg and a recovery of spiked samples is greater than 96%. The proposed method can be used for fast detection of melamine in milk powder.

C-41 Detection of dyes in food by ultra-fast liquid chromatography-triple quadrupole mass spectrometry

INTRODUCTION

A method is proposed for detection of 6 industrial dyes in biscuits with Shimadzu LC-30A ultra-fast liquid chromatograph (UFLC) in conjunction with LCMS-8030 triple quadrupole mass spectrometer. Calibration curves of the 6 industrial dyes were plotted using external standard method. The plotted calibration curves were of satisfactory linearity with correlation coefficients higher than 0.999. Standard solutions of various concentrations were used for precision test. The %RSDs of retention time and peak area data of 6 successive injections were below 0.553% and 4.786%, respectively, showing that the method was of satisfactory precision. The method was of high sensitivity, fast and suitable for detection of residues of basic yellow1, acid orange 20, basic orange 2, acid orange 7, auramine O and acid yellow 36 in food.

Industrial dyes are used for dying fur products, fabric products, and porcelain products in variegated colors. Basic yellow 1, acid orange 20, basic orange 2, acid orange 7, auramine O and acid yellow 36 belong to industrial dyes. All of these dyes are carcinogenic, teratogenic, and mutagenic and cause serious damage to human health. In China, the practice of adding industrial dyes into food is strictly prohibited. However, some profit-driven outlaw merchants still illegally add industrial dyes into food products because these industrial dyes are cheap, stable, and with excellent tenacity. Currently available methods for detection of these dyes include high performance liquid chromatography and liquid chromatography-tandem mass spectrometry. Liquid chromatography is widely used but suffers from poor sensitivity, poor qualitative capacity, and high false positive rate. Liquid chromatography-mass spectrometry is a well-accepted technique for detection of illegally-added dyes in food. In this paper, a method was proposed for detection of 6 industrial dyes, i.e. basic yellow 1, acid orange 20, basic orange 2, acid orange 7, auramine O and acid yellow 36, with a combined system of Shimadzu LC-30A UFLC and LCMS-8030 triple quadrupole mass spectrometer.

EXPERIMENTAL

Instruments

A combined system of Shimadzu ultra-fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, LabSolutionsVer. 5.41 chromatography workstation.

Conditions of Analysis

LC conditions

Column : Shim-pack XR-ODS III 2.0 mm I.D. × 50 mm L., 1.6 µm
Mobile phase A : 10 mM ammonium acetate aqueous solution- formic acid (100:0.1, v/v)
Mobile phase B : Acetonitrile
Flow rate : 0.4 mL/min
Column temperature : 40 °C
Injection volume : 5 µL
Elution mode : Binary gradient with initial concentration of 30% of mobile phase B., see Table 1 for time program

Table 1 Time program

Time(min)	Module	Command	Value
0.8	Pumps	B Conc.	60
1.6	Pumps	B Conc.	60
1.61	Pumps	B Conc.	30
2.5	Controller	Stop	

MS condition

Ionization : ESI (+) for basic yellow 1, basic orange 2 and auramine O
ESI(-) for acid orange 20, acid orange 7 and acid yellow 36
Ionization voltage : +4.5 kV
Nebulizing gas : Nitrogen, 3.0 L/min
Drying gas : Nitrogen, 15 L/min
Collision gas : Argon
DL temperature : 250 °C
Heater block temperature : 400°C
Acquisition mode : Multiple Reaction Monitoring (MRM)

Dwell time : 20 ms
Pause time : 3 ms
MRM parameters : see Table 2

Preparation of standard solutions

A total of 6 standard substances, i.e. basic yellow 1, acid orange 20, basic orange 2, acid orange 7, auramine O and acid yellow 36, were used.

Preparation of standard working solutions: A multi-standard intermediate solution of concentration of 20 mg/L was prepared using methanol as solvent, and then diluted with 30% acetonitrile aqueous solution to get multi-standard working solutions of concentrations of 10, 20, 50, 100, 200 and 500 μg/L.

Sample pretreatment method

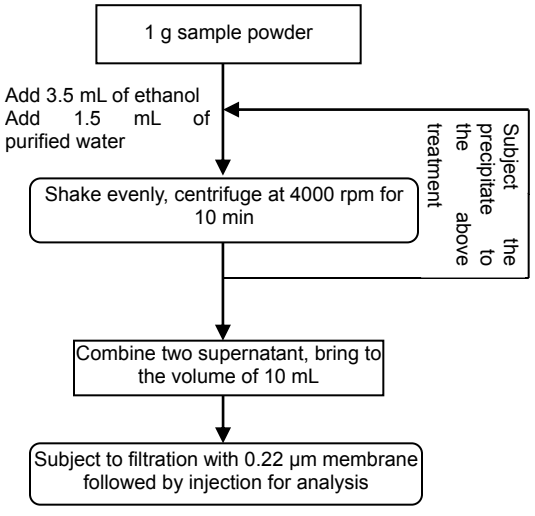


Table 2 MRM parameters Note: * refers to quantitative ion

Compound	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Auramine O	268.4	147.1 *	-30.0	-30.0	-16.0
		131.1	-30.0	-50.0	-26.0
Basic orange 2	213.2	77.2 *	-11.0	-20.0	-17.0
		121.2	-11.0	-20.0	-14.0
Basic yellow 1	283.0	267.1 *	-30.0	-35.0	-30.0
		252.1	-30.0	-50.0	-27.0
Acid yellow 36	352.0	156.1 *	12.0	35.0	29.0
		80.0	12.0	50.0	29.0
Acid orange 7	327.1	171.1 *	12.0	20.0	15.0
		156.0	12.0	30.0	15.0
Acid orange 20	327.1	171.1 *	12.0	20.0	15.0
		107.1	12.0	35.0	15.0

RESULTS AND DISCUSSION

Mass spectrum and MS/MS spectrum

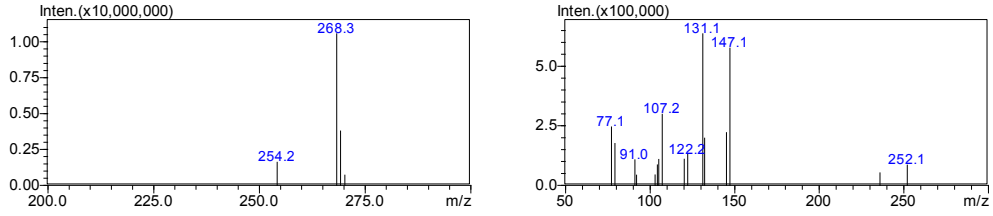


Fig.1 Mass spectrum (left) and MS/MS spectrum (right, CE -45V) of auramine O

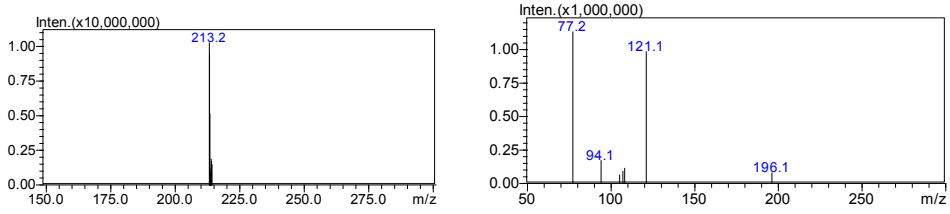


Fig.2 Mass spectrum (left) and MS/MS spectrum (right, CE -20V) of basic orange 2

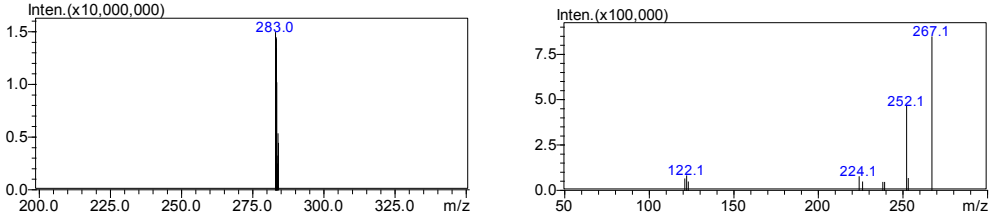


Fig. 3 Mass spectrum (left) and MS/MS spectrum (right, CE -45V) of basic yellow 1

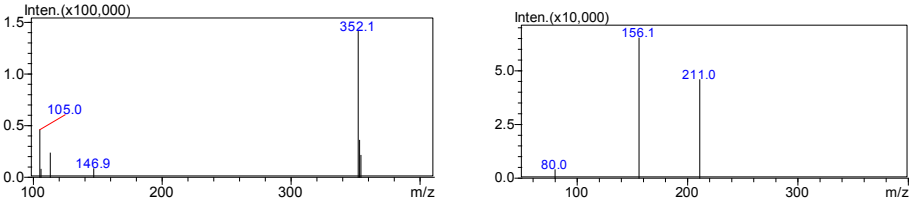


Fig. 4 Mass spectrum (left) and MS/MS spectrum (right, CE 30V) of acid yellow 36

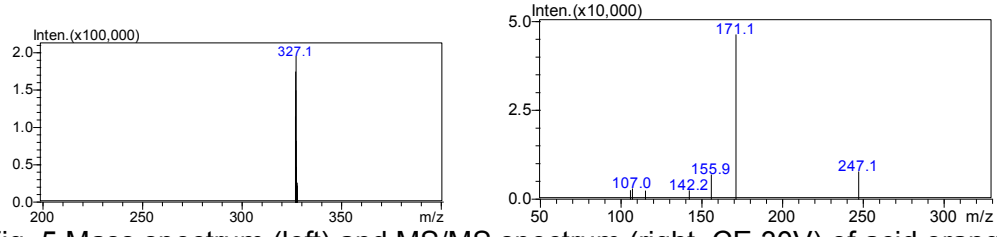


Fig. 5 Mass spectrum (left) and MS/MS spectrum (right, CE 30V) of acid orange 7

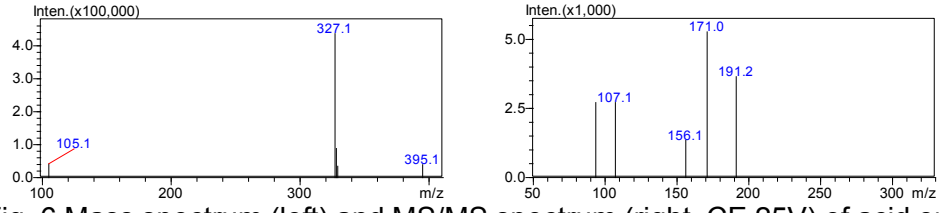


Fig. 6 Mass spectrum (left) and MS/MS spectrum (right, CE 25V) of acid orange 20

MRM chromatogram of standard mixture

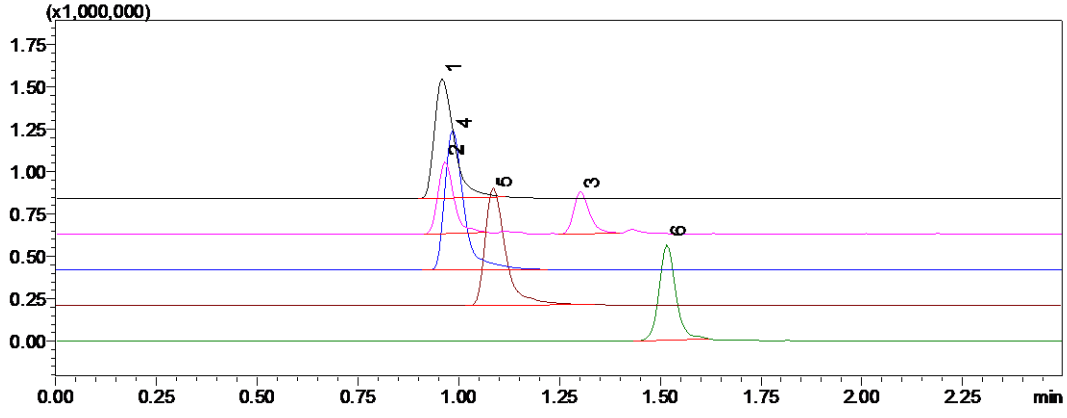


Fig. 7 MRM chromatograms of standard mixture (500 μg/L)

(1. basic yellow 1; 2. acid orange 20; 3. acid orange 7; 4. auramine O; 5. basic orange 2; 6. acid yellow 36)

Linear range

Multi-standard working solutions of various concentrations were quantitatively determined by external standard method under the analytical conditions as specified in 1.2. Calibration curves were plotted as shown in Fig. 8 to Fig. 13 with concentration as abscissa and peak area as ordinate. The calibration curves were of satisfactory linearity and their linear equations and correlation coefficients are shown in Table 3.

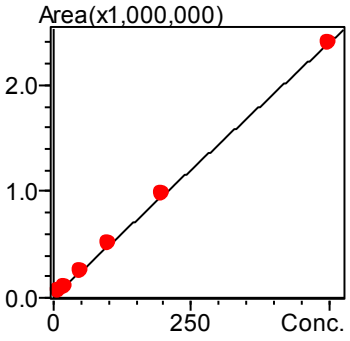


Fig. 8 Calibration curve of basic yellow 1

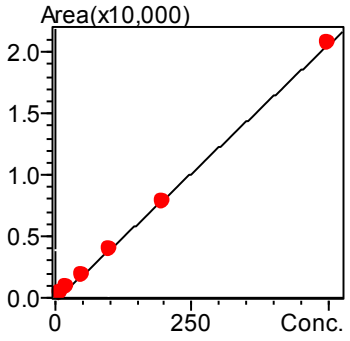


Fig. 9 Calibration curve of acid orange 20

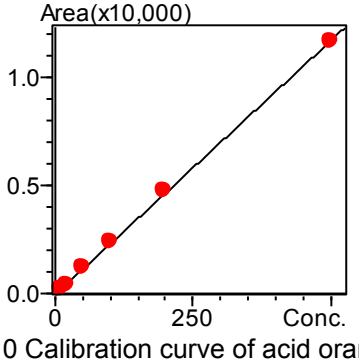


Fig. 10 Calibration curve of acid orange 7

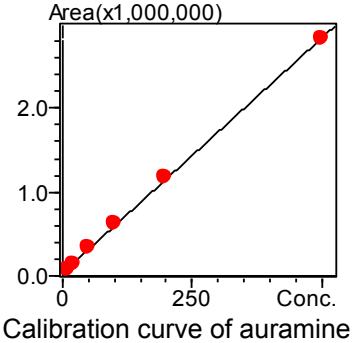


Fig. 11 Calibration curve of auramine O

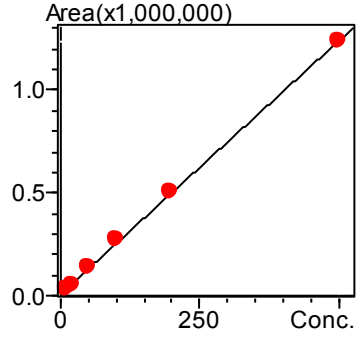


Fig. 12 Calibration curve of basic orange 2

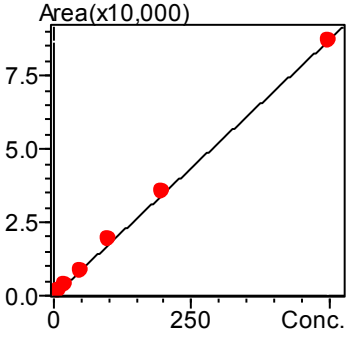


Fig. 13 Calibration curve of acid yellow 36

Table 3 Parameters of calibration curves

No.	Compound	Calibration Curve	Linear Range (µg/L)	Correlation Coefficient (R^2)
1	Basic yellow 1	Y = (4763.02)X + (14280.1)	10~500	0.9999
2	Acid orange 20	Y = (41.6687)X + (-268.801)	10~500	0.9996
3	Acid orange 7	Y = (23.4618)X + (-54.0828)	10~500	0.9999
4	Auramine O	Y = (5609.37)X + (33693.9)	10~500	0.9998
5	Basic orange 2	Y = (2463.23)X + (8577.69)	10~500	0.9999
6	Acid yellow 36	Y = (173.996)X + (101.200)	10~500	0.9997

Precision test

Multi-standard working solutions of various concentrations were determined for 6 times in succession to assess the method's precision. Repeatability of retention time and peak area data is shown in Table 4. The result showed that the %RSDs of retention time and peak area data of standard solutions of various concentrations are in the range of 0.046%~ 0.553% and 1.695%~4.786% respectively, suggesting the system had satisfactory precision.

Table 4 Repeatability - retention time and peak area (n=6)

Compound	%RSD (50 µg/L)		%RSD (100 µg/L)		%RSD (200 µg/L)	
	R.T.	Area	R.T.	Area	R.T.	Area
Basic yellow 1	0.060	3.434	0.111	2.414	0.155	2.277
Acid orange 20	0.553	4.539	0.426	3.900	0.129	1.950
Acid orange 7	0.316	4.786	0.318	3.758	0.106	2.415
Auramine O	0.092	2.114	0.147	3.831	0.087	2.165
Basic orange 2	0.103	2.297	0.165	2.844	0.104	2.410
Acid yellow 36	0.046	4.529	0.070	1.695	0.086	1.832

Sensitivity test

In order to assess the system's sensitivity, a multi-standard working solution of concentration of 10 µg/L was subjected to analysis as per the specified analytical conditions. The S/N ratios and LODs (S/N=3) were calculated with LabSolutions Ver. 5.41. The observed S/N ratios and LODs for basic yellow 1, acid orange 20, acid orange 7, auramine O, basic orange 2 and acid yellow 36 are given in Table 5.

Table 5 S/N ratios and the method's LODs

Compound	S/N	LOD (µg/kg)
Basic yellow 1	108.33	3.05
Acid orange 20	8.63	38.24
Acid orange 7	4.97	66.40
Auramine O	216.69	1.52
Basic orange 2	128.90	2.56
Acid yellow 36	35.93	9.18

Analysis of real samples

2 off-the-shelf biscuit products were taken as samples and subjected to the pretreatment. The analytical findings are shown in Table 6.

Table 6 Results of 2 biscuit samples

Name	Biscuits 1	Biscuits 2
Basic yellow 1	ND	ND
Acid orange 20	ND	ND
Acid orange 7	ND	ND
Auramine O	ND	ND
Basic orange 2	ND	ND
Acid yellow 36	ND	ND

ND: not detected

CONCLUSION

A fast, reproducible and reliable qualitative and quantitative method was established for the determination of 6 industrial dyes in food with Shimadzu LC-30A ultra-fast liquid chromatograph in conjunction with LCMS-8030 triple quadrupole mass spectrometer. The total analysis run time was 2.5 minutes with high precision. It demonstrated linearity over wide range (10~500 µg/L) and the correlation coefficients of all calibration curves were greater than 0.999. The off-the-shelf biscuit products were showed negative detection for basic yellow 1, acid orange 20, basic orange 2, acid orange 7, Auramine O or acid yellow 36.

C-42 Detection of gibberellins in fruits with UFLC-triple quadrupole mass spectrometry

INTRODUCTION

This paper proposes a method for fast detection of 6 gibberellins (GA1, GA3, GA4, GA5, GA7, and GA9) with ultra-high performance liquid chromatograph and Shimadzu triple quadrupole mass spectrometer. Samples were subjected to separation by the ultra high performance liquid chromatograph and quantitatively analyzed with the triple quadrupole mass spectrometer. The 6 compounds were promptly separated and analyzed within 13 minutes. The proposed method demonstrated good linearity for the 6 analytes in the concentration range of 5 - 500 µg/L with correlation coefficients greater than 0.999. Precision test was performed on a 25 µg/L multi-standard solution, the %RSDs of retention time and peak area in 6 successive injections were below 0.170% and 9.10%, respectively. The LLODs by this method were in the range of 0.48 - 1.74 µg/L.

Gibberellins as a phytohormones extensively occurred in plants, can promote the growth, expansion and division of cells and as a result can stimulate the growth of leaves and burgeons. However, people become more and more concerned about the impact of the ever-increasing application of these phytohormones on human health. It has been revealed in recent years that growth regulators residues in crops may pose hazards to human beings if they enter human body via the food chain. In mild cases, they may cause diarrhea; in serious cases, they may impair the immunity of human beings, induce osteoporosis. They may even lead to serious consequences due to their potential teratogenicity, carcinogenicity, and/or mutagenicity. Developed countries have stipulated strict maximum residue limits (MRLs) for these substances. To date, very few methods have been published in China and/or foreign countries on the detection of exogenous plant growth regulators. The published ones mainly involve the analysis of 1 - 2 analytes in such matrices as fruits and vegetables by HPLC and LC-MS. However, HPLC is not suitable for the analysis of trace residues in complicated matrices due to its low resolution and sensitivity. Furthermore, it is impractical to complete structure elucidation with HPLC, which provides no information on the structure of target analytes. Consequently, its application in the analysis of residues is substantially

limited. In contrast, LC-MS/MS not only can achieve the detection and structure elucidation of analytes simultaneously but also can meet the requirements for detection of trace analytes in complicated matrices. Therefore, it has been used for the analysis of residues of exogenous plant growth regulators. In this experiment, a method was established for fast and concurrent detection of 6 exogenous plant growth regulators in fruits with satisfactory sensitivity and repeatability.

EXPERIMENTAL

Instruments

A system of Shimadzu LC-30A ultra-fast liquid chromatograph and LCMS-8040 triple quadrupole mass spectrometer was used in this experiment. It consists of two LC-30AD pumps, a DGU-20A5 online degasser, an SIL-30AC autosampler, a CTO-30A column oven, CBM-20A communications bus module, LCMS-8040 triple quadrupole mass spectrometer and LabSolutions Ver 5.5 chromatography workstation.

Conditions of Analysis

LC Conditions

Apparatus : LC-30A system
Chromatographic column : Shim-pack XR-ODS III 75 mmL×2.0 mm I.D, 1.6 μm
Mobile phase A : 1% formic acid aqueous solution
Mobile phase B : 1% formic acid methanol solution
Flow rate : 0.3 mL/min
Injection volume : 1 μL
Column temperature : 30 °C
Elution mode : Binary gradient with an initial concentration of 30%B, see Table 1 for time program.

Table 1 Time program

Time (min)	Module	Command	Value
0.01	Pumps	Pump B Conc.	30
1.00	Pumps	Pump B Conc.	30
8.00	Pumps	Pump B Conc.	100
10.00	Pumps	Pump B Conc.	100
10.01	Pumps	Pump B Conc.	30
13.00	Controller	Stop	

MS conditions

Instruments	: LCMS-8040
Ionization	: ESI(-)
Ionization voltage	: -3.5 kV
Nebulizing gas and drying gas	: Nitrogen at 3.0 L/min and 15 L/min
Collision gas	: Argon
DL temperature	: 250 °C
Heater block temperature	: 450 °C
Detector voltage	: 1.1 kV
Mode	: Multiple reaction monitoring (MRM)
Dwell time and pause time	: 20 ms, 1 ms

Table 2 MRM parameters of 6 gibberellins

No.	Compound	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
1	GA1	347.00	273.15*	15.0	23.0	28.0
			285.20	15.0	21.0	30.0
2	GA3	345.00	143.20*	15.0	35.0	26.0
			239.20	15.0	15.0	25.0
3	GA4	331.00	225.20	20.0	18.0	24.0
			243.20*	20.0	19.0	25.0
4	GA5	329.00	145.15*	14.0	24.0	26.0
			285.20	14.0	17.0	29.0
5	GA7	329.00	223.25*	14.0	18.0	23.0
			211.25	14.0	29.0	22.0
6	GA9	315.00	271.20*	19.0	20.0	28.0
			253.30	19.0	25.0	16.0

* refers to quantitative ion

Preparation of standard solution

A multi-standard solution of concentration of 1 mg/L was prepared using methanol as solvent and then diluted with methanol into standard working solutions at concentrations of 1, 5, 10, 50, and 100 μg/L.

Sample Preparation

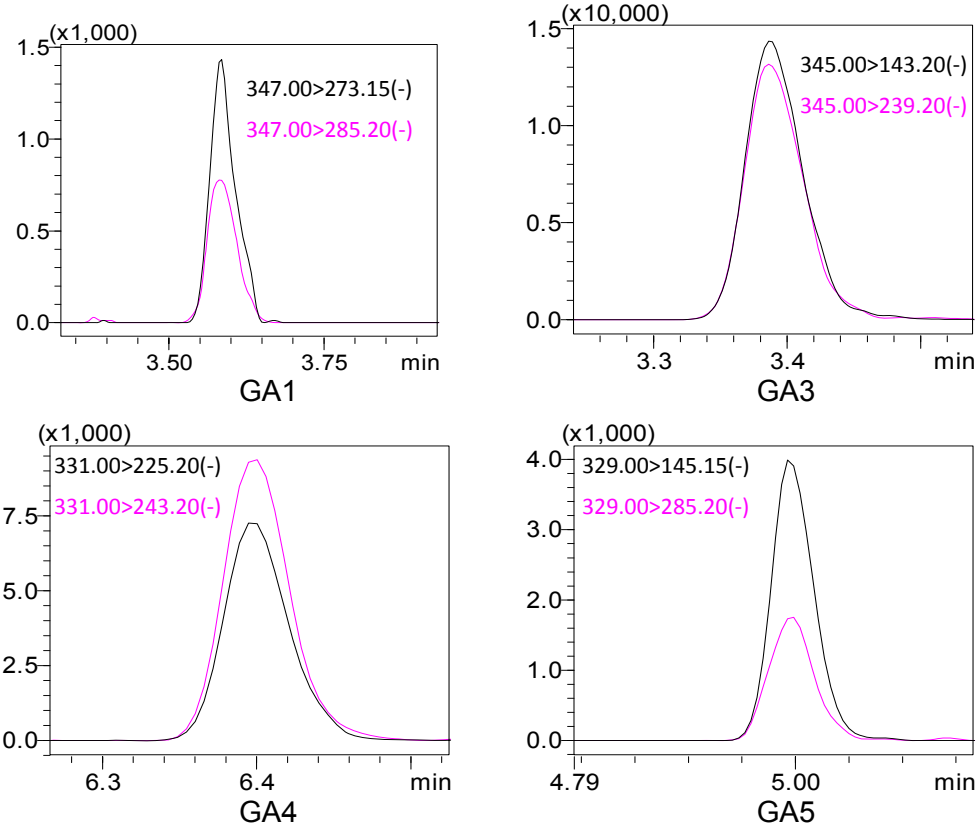
5.0 g sample was weighed (with a precision of 0.001 g), transferred to a 50 mL polypropylene centrifuge tube with screw cap.15 mL of 80% acetonitrile solution was added. The solution was subjected to vortex mixer for 1 min, 1 g NaCl was added. The solution was shaken evenly for 3 min, then centrifuged at 5000 rpm for 5 min; 10 mL of the resulted supernatant acetonitrile solution was taken and

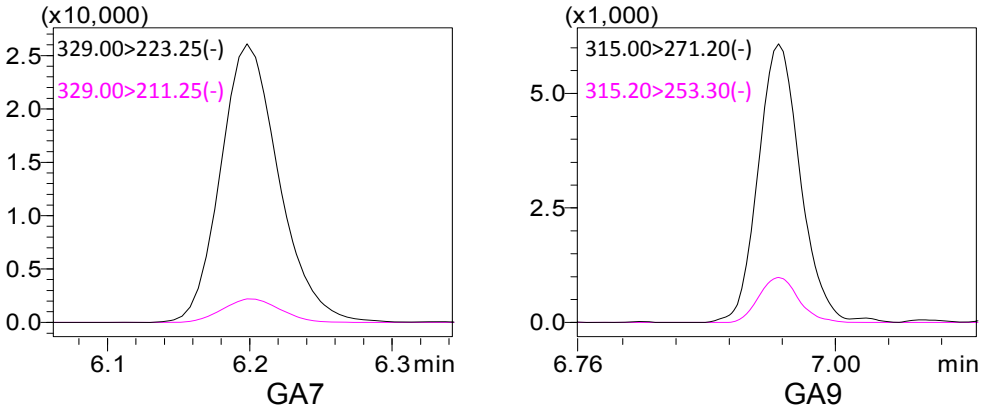
concentrated with 45 °C nitrogen flush to almost dry, dissolved and brought to the volume of 10 mL with methanol and mixed evenly.

A Bond Elut Certify II column bed was rinsed with 3 mL methanol, 3 mL of water and 3 mL of 0.1 mol/L potassium phosphate buffer in succession, prior to the loading of 5 mL extract to the column bed, for cleanup of the analytes by chromatography at a controlled flow rate of 1 drop/s. The column bed was rinsed with 2 mL of 0.1 mol/L phosphate buffer, degassed by vacuum for more than 5 min, then subjected to elution with 2 mL methanol. The eluent was collected in a 15 mL graduated test tube and then concentrated with 45 °C nitrogen flush to almost dry, and then brought to the volume of 1.0 mL with methanol. The resultant solution was filtered with 0.22 μm filter membrane and the filtrate is used for analysis with LC-MS/MS.

RESULT AND DISCUSSION

MRM Chromatogram of Standard Samples





Total ion chromatogram of 50 µg/L standard sample

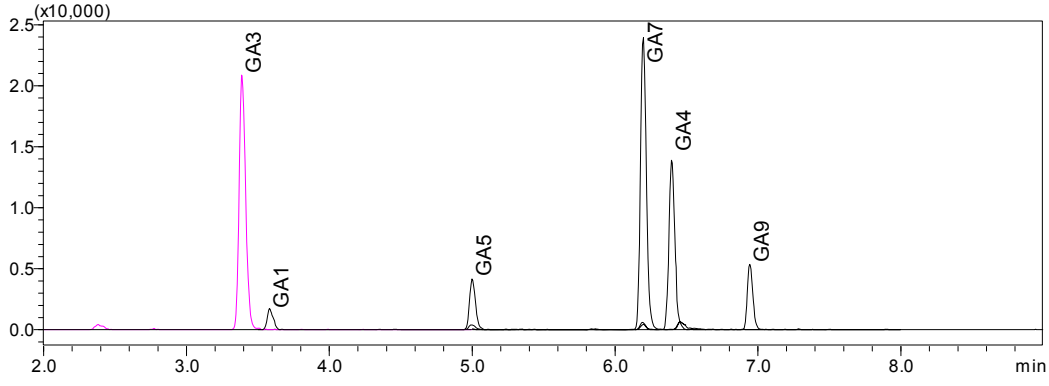


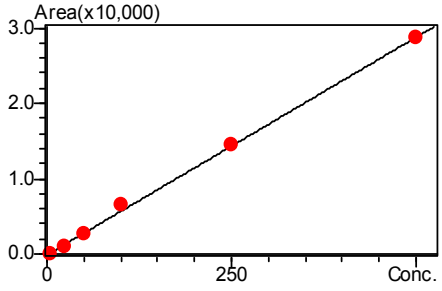
Fig. 1 Total ion chromatogram of 50 µg/L standard sample

Table 3. Retention time of the analytes

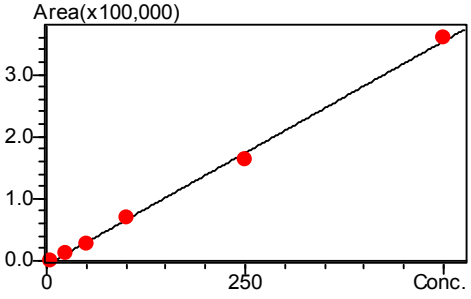
Analyte	GA1	GA3	GA4	GA5	GA7	GA9
Retention Time (min)	3.60	3.39	6.43	5.02	6.22	6.97

Linearity of the calibration curves of standard samples

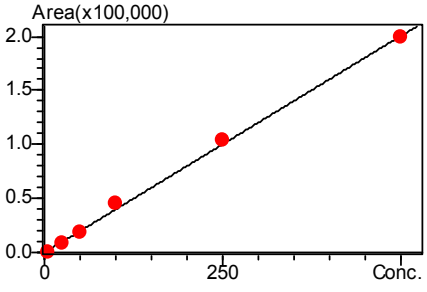
Multi-standard working solutions of concentrations of 5, 25, 50, 100, 250, and 500 µg/L were analyzed under the specified analytical conditions. The calibration curves were plotted as shown in Fig. 2 with concentration as X-axis and peak area as Y-axis. The linear equations and correlation coefficients are listed in Table 4.



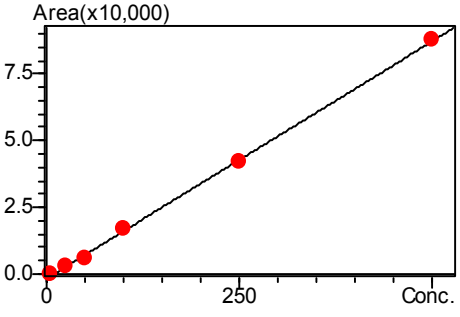
GA1



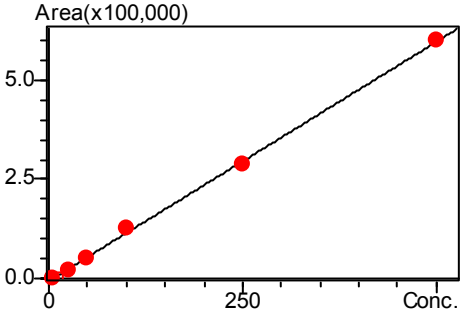
GA3



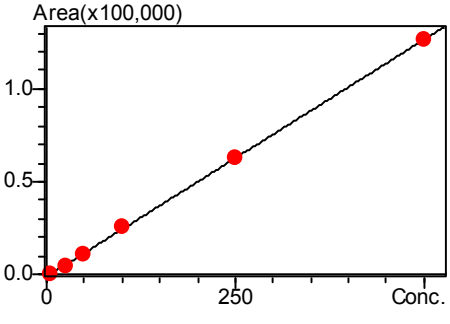
GA4



GA5



GA7



GA9

Fig. 2 Calibration curves

Table 4 Parameters of calibration curves of the 6 analytes

No.:	Name	Calibration Curve	Correlation Coefficient (R^2)
1	GA1	Y = (2890.21)X + (-120.567)	0.9993
2	GA3	Y = (1796.71)X + (-1005.02)	0.9991
3	GA4	Y = (20104.2)X + (-144.793)	0.9993
4	GA5	Y = (8892.73)X + (-1603.98)	0.9996
5	GA7	Y = (60330.4)X + (-4099.67)	0.9994
6	GA9	Y = (12785.0)X + (-1280.44)	0.9997

Repeatability test

50 μg/L multi-standard working solution was analyzed for 6 times in succession to assess the precision of the method. The resulted repeatability data of retention time and peak area are shown in Table 4. The results showed that the %RSDs of retention time and peak area data of 50 μg/L standard solutions are in the range of 0.038% - 0.166% and 1.36% - 9.07%, respectively, suggesting that the method was of satisfactory repeatability.

Table 5. Repeatability - retention time and peak area at 50 μg/L (n=6)

No.	Analyte	R.T. (min)	%RSD R.T.	%RSD Area
1	GA1	3.60	0.166	9.07
2	GA3	3.39	0.105	3.54
3	GA4	6.43	0.042	6.50
4	GA5	5.02	0.070	1.60
5	GA7	6.22	0.057	1.36
6	GA9	6.97	0.038	2.81

Sensitivity test

The 7 blank water samples at spiked level of 5 ng/L were prepared and subjected to the pretreatment procedures for analysis. Standard deviation (S) of the results of the 7 samples were calculated after excluding outlier(s). In the experiment, the t-distribution value is 3.143 and limits of detection (LODs) were calculated using formula S×3.143. The method's LOQs for the analytes were calculated as 4 times of LODs in reference with HJ168-2010 *Environmental monitoring—Technical guideline on drawing and revising analytical method standards*. The results are as shown in Table 6.

Table 6 LODs and LOQs of 6 gibberellins

Analyte	Concentration (μg/L)							Average	Standard	LOD	LOQ
	1	2	3	4	5	6	7	(μg/L)	Deviation(S)	(μg/L)	(μg/L)
GA1	4.96	5.38	5.97	4.87	5.28	6.01	6.29	5.54	0.55	1.74	6.97
GA3	4.99	5.24	5.18	5.57	5.43	5.21	5.47	5.30	0.20	0.63	2.52
GA4	5.32	5.87	5.43	5.56	5.46	5.74	5.97	5.62	0.24	0.76	3.06
GA5	5.78	5.63	5.37	5.96	5.12	5.87	6.32	5.72	0.39	1.24	4.96
GA7	5.21	5.32	5.26	5.49	5.36	5.25	5.64	5.36	0.15	0.48	1.93
GA9	4.85	5.21	5.67	5.75	5.36	5.28	5.75	5.41	0.33	1.05	4.21

Recovery test

Blank apple matrix was spiked with standard working solutions of the 6 gibberellins of various concentrations to get spiked samples of concentrations of 5 μg/L, 50 μg/L, and 250 μg/L, respectively. The resulted recovery data are as shown in Table 7

Real sample analysis results

An apple bought from the market was subjected to the proposed method and the resulted MRM chromatograms as shown in Fig. 3 demonstrated that 3 gibberellins, i.e. GA3, GA4, and GA7, were present in the samples.

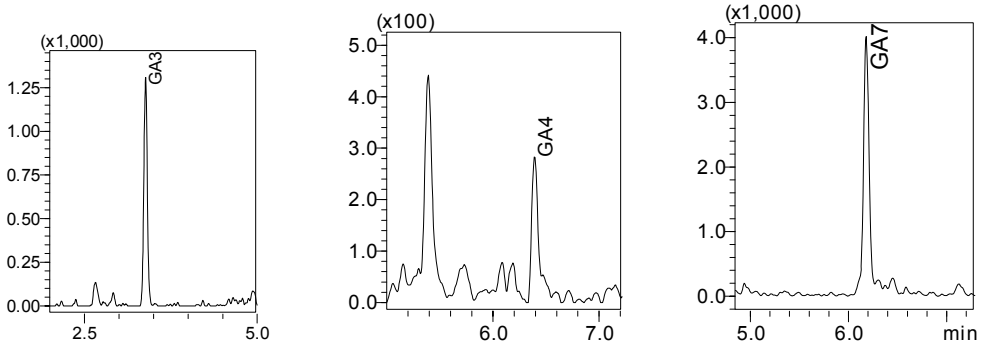


Fig. 3 MRM chromatograms of actual samples

Table 7 Recoveries of samples at various concentrations

Spiked Level (µg/L)	Analyte	Calculated result (µg/L)	Recovery (%)	Average Recovery (%)
5	GA1	3.53	70.6	71.5
		3.62	72.4	
	GA3	3.61	72.2	72.4
		3.63	72.6	
	GA4	3.75	75.0	68.7
		3.12	62.4	
	GA5	3.61	72.2	71.9
		3.58	71.6	
	GA7	3.82	76.4	76.9
		3.87	77.4	
	GA9	3.72	74.4	75.0
		3.78	75.6	
50	GA1	40.3	80.6	82.8
		42.5	85.0	
	GA3	41.9	83.8	84.5
		42.6	85.2	
	GA4	39.2	78.4	79.3
		40.1	80.2	
	GA5	43.6	87.2	86.1
		42.5	85.0	
	GA7	45.1	90.2	87.9
		42.8	85.6	
	GA9	43.7	87.4	86.8
		43.1	86.2	
250	GA1	198	79.2	82.0
		212	84.8	
	GA3	207	82.8	86.4
		225	90.0	
	GA4	218	87.2	90.2
		233	93.2	
	GA5	206	82.4	84.2
		215	86.0	
	GA7	221	88.4	95.6
		257	102.8	
	GA9	225	90.0	88.8
		219	87.6	

CONCLUSION

A method was established for fast detection of 6 gibberellins with ultra-high performance liquid chromatograph and triple quadrupole mass spectrometer. With the advantages of fast analysis and good repeatability, the method yielded calibration curves with correlation coefficients all greater than 0.999 in the range of 50 - 1000 µg/L when used to analyze apple samples. It is suitable for the detection of relevant growth hormones.

C-43 Determination of 7-Aryloxyphenoxy phenoxypropionate Herbicides in Pork and Honey with GC-MS/MS

INTRODUCTION

Aryloxyphenoxypropionate (APP) herbicides were developed on the basis of 2, 4-D and other phenoxypropionate herbicides. They play an important role in global herbicide market because of their high efficacy, low toxicity and broad herbicide spectrum.

Widely used as herbicides, APP get into farm crops and their residues are considered as endocrine disrupting chemicals (EDCs) in the environment. According to study reports, APP herbicides have evident damaging action on testicular spermatogenic cells of rats.

Presently in China, MRLs have been set for some APP herbicides, but the MRLs only apply to soybean, beet and edible vegetable oil (e.g. the MRL for quizalofop-p-ethyl in cottonseeds is 0.05mg/kg). By contrast, many countries including EU, USA, and Japan have established MRLs for all APP herbicides in foods (Japan, for example, has set an MRL of 0.01 mg/kg for haloxyfop-methyl in poultry eggs). China's accession to WTO has far-reaching impact on the country's food import and export. Therefore, it is of special importance to ensure the safety of imported/exported foods, especially farm products.

It is necessary to establish a method that can determine APP herbicide residues in food with high sensitivity and selectivity. In this paper, a sensitive and accurate method was proposed for determination of trace residue of 7 APP herbicides in pork and honey with Shimadzu GCMS-TQ8030.

EXPERIMENTAL

Instruments

GC-MS/MS: GCMS-TQ8030

Conditions of Analysis

Column	: Rtx-5 ms, 30 m × 0.25 mm × 0.25 μm
Column temperature program	: 50 °C (2 min)-30 °C/min-180 °C-5 °C/min-280 °C (10 min)
Injector temperature	: 250 °C
Injection mode	: splitless (1 min)
Carrier gas control mode	: CLV (36.3 cm/sec)
Carrier gas	: Helium
Collision gas	: Argon
Solvent cut time	: 9 min
Detector voltage	: tuning voltage+0.2 kv
Interface temperature	: 280 °C
Temperature of ion source	: 230 °C

Collection mode: MRM (parameters are as listed in Table 1)

Pretreatment of samples

Samples were subjected to pretreatment in reference with a China standard method, SN/T 1737.4-2010 *Determination of aryloxyphenoxypropionate herbicide residues in foodstuff for import and export by GC-MS/MS method*.

Targeted compounds in samples were extracted with n-hexane saturated acetonitrile (containing 1% glacial acetic acid) and the resulted extract was purified by means of matrix solid phase dispersion extraction and then subjected to determination with GC-MS/MS and quantification with external standard method.

RESULTS AND DISCUSSION

MRM chromatograms of standard mixture

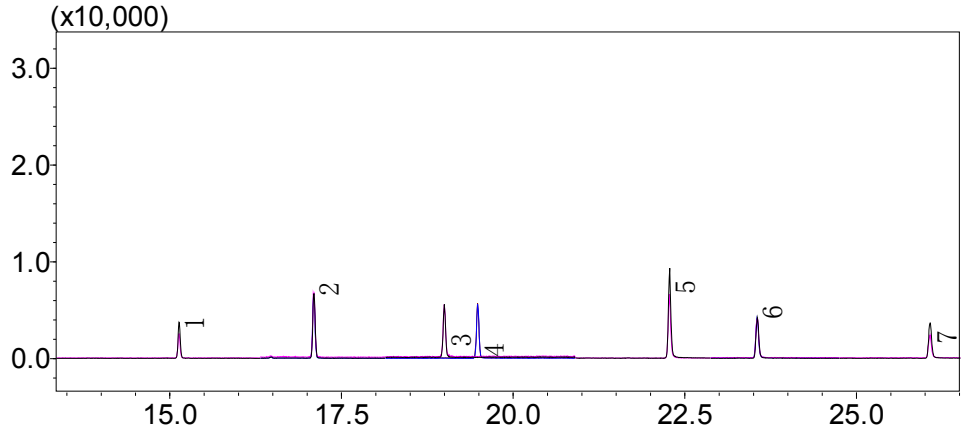


Fig. 1 MRM chromatograms of multi-standard solution of the 7 herbicides (10 μg/L each)

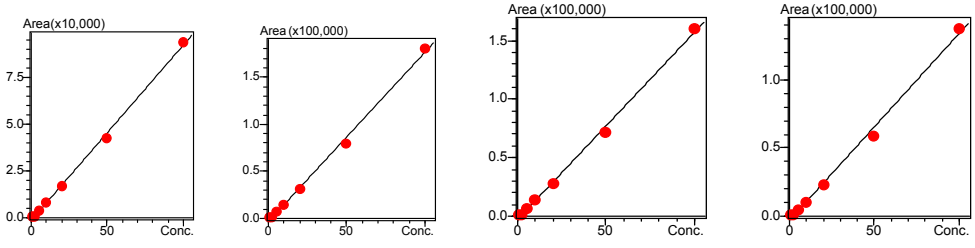
Table 1 MRM parameters of the 7 herbicides

NO.	Compound	Quantitative Ion	CE	Qualitative Ion	CE
1	Haloxyfop-methyl	316>91	18	288>180	23
2	Fluazifop-butyl	282>91	18	254>146	22
3	Clodinafop-propargyl	350>266	12	238>130	22
4	Diclofop-methyl	253>162	18	340>253	13
5	Cyhalofop-butyl	256>120	12	357>256	12
6	Fenoxaprop-p-ethyl	361>288	12	288>91	22
7	Quizalofop-p-ethyl	372>299	13	299>91	20

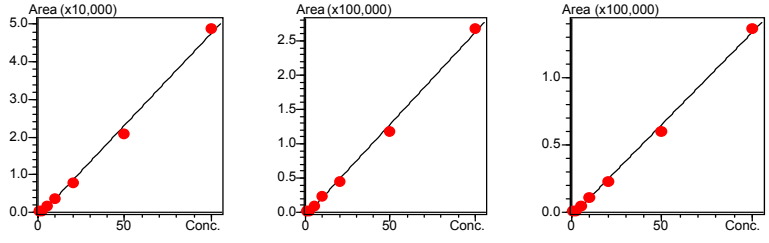
Calibration curve

A series of multi-standard solutions were prepared at concentrations of 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, and 100.0 μg/L, respectively, with acetone as solvent. Linear fitting was performed with concentration as abscissa and quantitative ion peak

area as ordinate. LODs were calculated based on the data of 1.0 μg/L standard solution. The results are shown in Fig. 2 and Table 2.



Haloxyfop-methyl Fluazifop-butyl Clodinafop-propargyl Diclofop-methyl



Cyhalofop-butyl Fenoxaprop-p-ethyl Quizalofop-p-ethyl

Fig. 2 Calibration curves

Table 2. Correlation coefficients and LODs

No.	Compound	Correlation coefficient	LOD (μg/L)	No.	Compound	Correlation coefficient	LOD (μg/L)
1	Haloxyfop-methyl	0.9991	0.31	5	Cyhalofop-butyl	0.9989	0.11
2	Fluazifop-butyl	0.9993	0.07	6	Fenoxaprop-p-ethyl	0.9991	0.27
3	Clodinafop-propargyl	0.9984	0.44	7	Quizalofop-p-ethyl	0.9994	0.13
4	Diclofop-methyl	0.9991	0.16				

Repeatability

A spiked pork sample and a spiked honey sample were prepared and subjected to the pretreatment as specified in 1.3 and injected 5 times in succession for determination of repeatability. As shown in Table 3, %RSDs of peak area were better than 9%.

Table 3 Repeatability test results (n=5)

NO.	Compound	%RSD of peak area	
		Pork	Honey
1	Haloxyfop-methyl	2.55	1.01
2	Fluazifop-butyl	2.05	1.69
3	Clodinafop-propargyl	8.08	1.84
4	Diclofop-methyl	2.42	2.55
5	Cyhalofop-butyl	3.46	3.00
6	Fenoxaprop-p-ethyl	1.05	8.61
7	Quizalofop-p-ethyl	3.07	8.83

Recovery test

The recovery test was performed on a pork product and a honey product purchased from a supermarket. The products were spiked at concentrations of 5.0, 10.0 and 20.0 μg/L, respectively, and prepared in parallel into 3 samples for analysis and the resulted average recoveries of spiked samples are shown in Table 4. Recoveries were ranged between 70% and 130%.

Table 4 Recoveries of spiked samples (%)

NO.	Composition	Pork			Honey		
		Spiked Concentration (μg/L)			Spiked Concentration (μg/L)		
		5.0	10.0	20.0	5.0	10.0	20.0
1	Haloxyfop-methyl	121.15	100.06	104.26	79.03	75.31	87.88
2	Fluazifop-butyl	123.9	105.93	111.59	82.01	77.19	93.02
3	Clodinafop-propargyl	125.15	111.73	125.51	73.25	70.58	76.44
4	Diclofop-methyl	115.79	99.81	107.48	76.96	76.22	77.45
5	Cyhalofop-butyl	126.94	103.76	110.25	70.82	78.15	82.89
6	Fenoxaprop-p-ethyl	77.58	86.46	89.02	109.54	72.44	75.04
7	Quizalofop-p-ethyl	70.77	78.47	77.09	80.24	70.32	71.63

Sample determination results

A pork product and a honey product were purchased from a supermarket and subjected to analysis. Quantitative results are shown in Table 5.

Table 5. Results of sample quantification

NO.	Compound	Determination Results (μg/L)	
		Pork	Honey
1	Haloxyfop-methyl	0.30	0.32
2	Fluazifop-butyl	0.48	0.38
3	Clodinafop-propargyl	N.D	0.81
4	Diclofop-methyl	0.33	0.30
5	Cyhalofop-butyl	0.55	0.51
6	Fenoxaprop-p-ethyl	0.27	N.D
7	Quizalofop-p-ethyl	0.66	N.D

CONCLUSION

A method was proposed in reference with SN/T 1737.4-2010 *Determination of herbicide residues. Part 4:Determination of aryloxyphenoxypropionate herbicide residues in foodstuff for import and export by GC-MS/MS method*, a standard issued by China Entry-Exit Inspection and Quarantine Bureau, for determination of 7 APP herbicide residues in pork and honey with Shimadzu GC-MS/MS (GCMS-TQ8030). The proposed method is easy to operate and of good linearity in the range of 1.0~100.0 µg/L on the calibration curves, with an instrument LOD of 0.07~0.44 µg/L and a mean spike recovery of 70%~130%.The method is suitable for quantification of 7 APP herbicide residues in pork and honey.

C-44 QUANTITATIVE ANALYSIS OF PARA RED IN CHILI MATRIX USING LCMS-8040

INTRODUCTION

Today, there are extensive numbers of synthetic dyes available in markets used not only to enhance colors but also enhance the presentation and acceptability of food products, where no natural colors exist. Unfortunately, many food items manufactuers use these dyes to cover aging effects, to masquerade decay, and/or to disguise poor foodstuffs.
It has been investigated that not only the degradation products of these dyes but also the synthetic precursors and intermediates could be highly dangerous due to their toxic and carcinogenic nature. Dyes of aromatic structures which contain azo linkage, amino or nitro groups cause cancer in experimental animals as well as in humans.
Para red is an azo dye more specifically belonging to the group of sudan dyes. These are used to enhance the color of chili powders which has been strictly prohibited by various government agencies. Keeping this in view, LC/MS/MS technique with the use of APCI probe has been used to develop a sensitive method of quantitation of para red from chili matrix.

EXPERIMENTAL

Instrument parameters

System configuration

HPLC	: Nexera UHPLC system
Pumping unit	: LC-30AD
Column oven	: CTO-20AC
Degassing unit	: DGU-20A$_5$
Autosampler	: SIL-30AC
MS	: LCMS-8040

LC conditions

Guard column : Phenomenex SecurityGuard ULTRA Cartridge

Column : Shim-pack XR-ODS II (100 mm L x 3 mm I.D., 2.2 µm)

Mobile phase : A - 5 mM ammonium formate in water:methanol (80:20 v/v)

B - 5 mM ammonium formate in methanol

Flow rate : 0.8 mL/min

Injection volume : 30 µL

Gradient program :

Time (min)	Pump B conc.
0.01	90
2.00	100
4.00	100
4.01	90
5.00	90

MS conditions

Interface : Atmospheric pressure chemical ionization (APCI); negative

Mode : MRM

Interface temperature : 450 ºC

DL temperature : 250 ºC

Heat block temperature: 300 ºC

Nebulising gas : 4.4 L/min

Drying gas : 3 L/min

Standard preparation

Preparation of matrix matched standards:

Commercially available chili is powdered using mixer grinder. 1.0553 g of this chili powder was mixed with 20 mL acetonitrile using ultra sonicator for 10 mins. Mixture was centrifuged and supernatant was collected. This supernatant was used as a diluent to prepare para red matrix matched

standards at concentration levels of 0.2 ppb, 0.5 ppb, 1 ppb, 2 ppb, 5 ppb and 10 ppb.

RESULTS AND DISCUSSION

Table 1. Results ofquantitative analysis of para red

Compound Name	MRM Transition	Retention Time (min)	Cal Range (ppb)	Correlation coefficient (R^2)	S/N at LOQ Level
Para Red	313.00>241.00	1.330	0.2 - 10	0.9947	30.18

The quantitative results of para red has been reported in Table 1. The chromatograms of different calibration levels and of the blank chili matrix has been shown in Figure 1. Figure 2 represents calibration graph of para red.

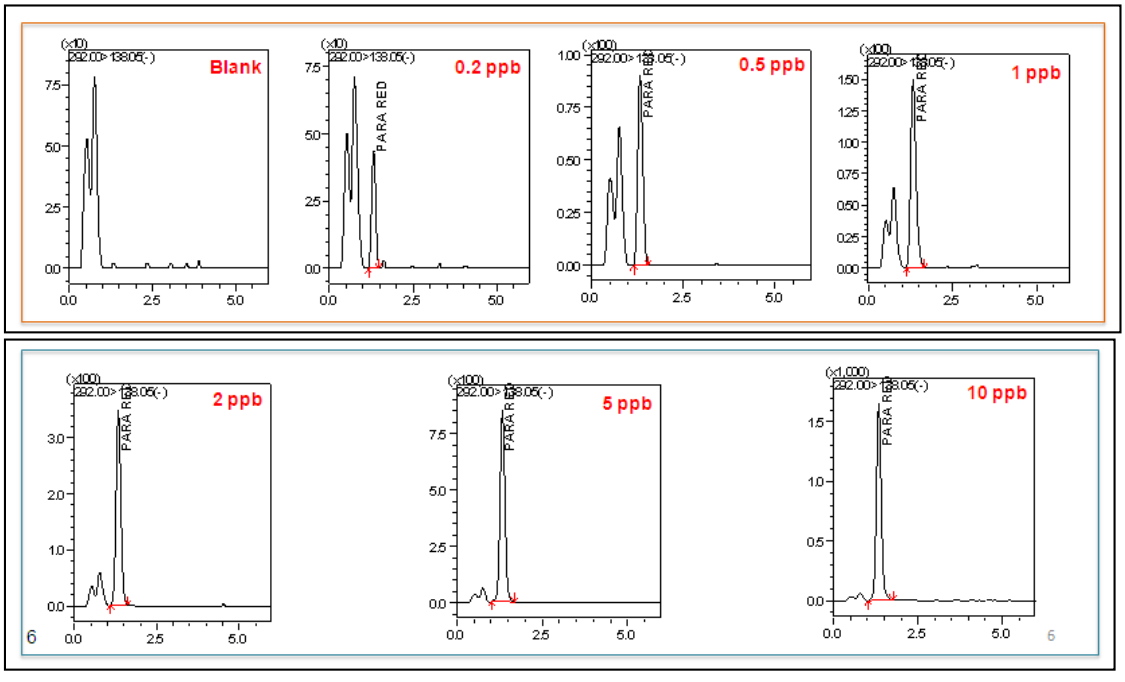
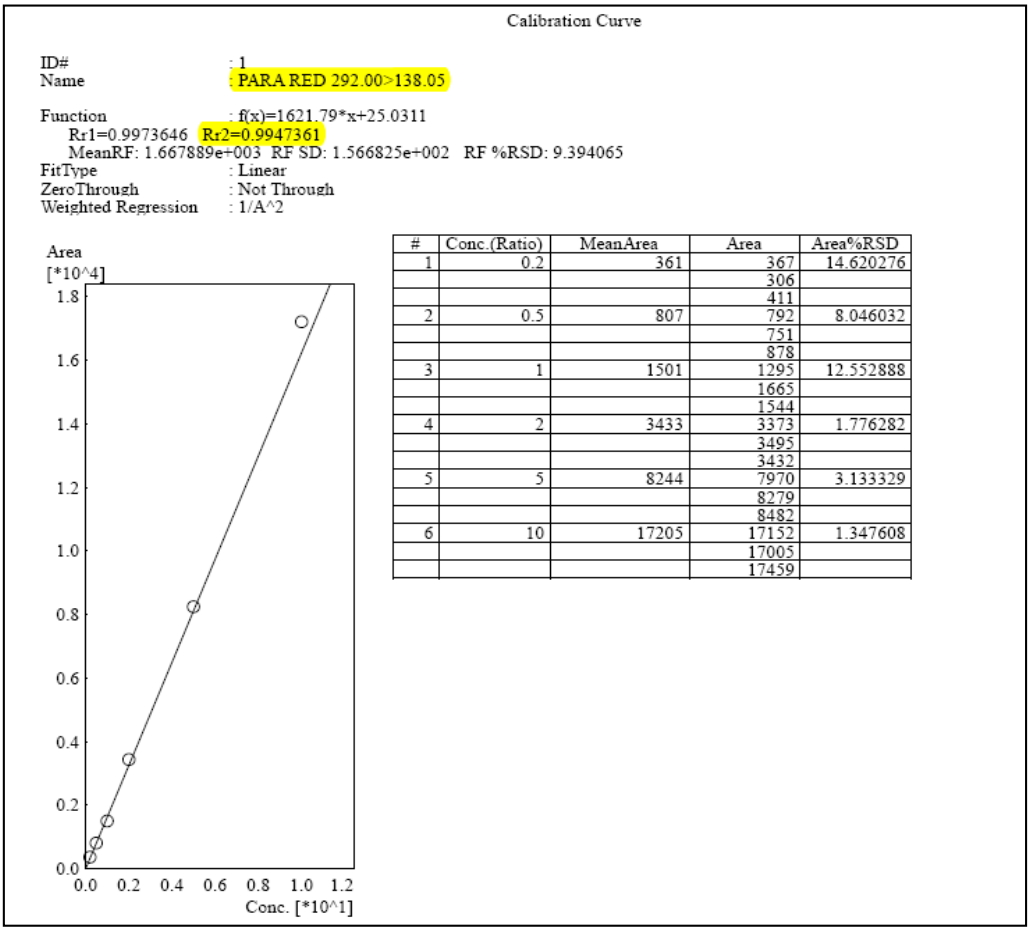


Figure 1. Blank and Standard chromatograms of para red at all calibration levels



#	Conc.(Ratio)	MeanArea	Area	Area%RSD
1	0.2	361	367	14.620276
			306	
			411	
2	0.5	807	792	8.046032
			751	
			878	
3	1	1501	1295	12.552888
			1665	
			1544	
4	2	3433	3373	1.776282
			3495	
			3432	
5	5	8244	7970	3.133329
			8279	
			8482	
6	10	17205	17152	1.347608
			17005	
			17459	

Figure 2. Calibration curve information for para red on LCMS-8040 system

CONCLUSION

Chili powder is a complex matrix and can exhibit matrix effect (either ion suppression or enhancement) during analysis. A calibration curve based on matrix matched standards can, therefore, demonstrate true sensitivity of analyte in presence of matrix. Hence, this method was adopted to generate more reliable and accurate method of quantitation as compared to quantitation against neat standards. Analysis of Para red on LCMS-8040 using mtrix matched standars gave an LOQ of 0.2 ppb in chilli matrix.

GCMS-QP2010 Ultra with HS-20

C-45 Analysis of styrene leached from polystyrene cups using GCMS coupled with Headspace (HS) sampler

INTRODUCTION

Worldwide studies have revealed the negative impacts of household disposable polystyrene cups (Figure 1) on human health and environment.
Molecular structure of styrene is shown in Figure 2. Styrene is considered as a possible human carcinogen by the WHO and International Agency for Research on Cancer (IARC).[1] Migration of styrene from polystyrene cups containing beverages has been observed.[2] Styrene enters into our body through the food we take, mimics estrogens in the body and can therefore disrupt normal hormonal functions. This could also lead to breast and prostate cancer.
The objective of this study is to develop a sensitive, selective, accurate and reliable method for styrene determination using low carryover headspace sampler, HS-20 coupled with Ultra Fast Scan Speed 20,000 u/sec, GCMS-QP2010 Ultra to assess the risk involved in using polystyrene cups.

Figure 1. Polystyrene cup

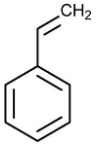


Figure 2. Structure of styrene

EXPERIMENTAL

Extraction of styrene from polystyrene cups

This study was carried out by extracting styrene from commercially available polystyrene cups and recoveries were established by spiking polystyrene cups with standard solution of styrene. Solutions were prepared as follows,

1) Standard Stock Solution
   1000 ppm of styrene standard stock solution in DMF:Water-50:50 (v/v) was prepared. It was further diluted with water to make 100 ppm and 1 ppm of standard styrene solutions.
2) Calibration Curve
   Calibration curve was plotted using standard styrene solutions in the concentration range of 1 to 50 ppb with water as a diluent. 5 mL of each standard styrene solution was transferred in separate 20 mL headspace vials and crimped with automated crimper.
3) Sample Preparation
   150 mL of boiling water (around 100 ^{0}C)[1] was poured into polystyrene cups. The cup was covered with aluminum foil and kept at room temperature for 1 hour. After an hour, 5 mL of sample from the cup was transferred into the 20 mL headspace vial and crimped with automated crimper.

Method was partly validated to support the findings by performing reproducibility, linearity, LOD, LOQ and recovery studies. For validation, solutions of different concentrations were prepared using standard stock solution of styrene (1000 ppm) as mentioned in Table 1.

Table 1 : Method validation parameters

Parameter	Concentration (ppb)
Linearity	1, 2.5, 5, 10, 20, 50
Accuracy/Recovery	2.5, 10, 50
Precision at LOQ level	1
Reproducibility	50

HS-GCMS Analytical Conditions

Samples were analyzed using HS-20 coupled with GCMS-QP2010 Ultra as per the conditions given below

Headspace parameters

Mode	Loop
Oven Temp	80.0 ˚C
Sample Line Temp	130.0 ˚C
Transfer Line Temp	140.0 ˚C
Equilibrating Time	20.0 min
Pressurizing Time	0.5 min
Injection Time	1.0 min
Needle Flush Time	5.0 min
GC Cycle Time	10.0 min

Chromatographic parameters

Colum	Rxi-5Sil MS(30 m x 0.25 mm x 0.25 µm)
Injection Mode	Split
Split Ratio	10.0
Carrier Gas	Helium
Flow Control Mode	Linear Velocity
Linear Velocity	36.3 cm/sec

Column Oven Temp

Rate ˚C /min	Temperature ˚C	Hold time (min
---	50.0	0.00
40.0	200.0	1.00
30.0	280.0	5.00

Mass Spectrometry parameters

Ion Source Temp	200 ˚C
Interface Temp	230 ˚C
Ionization Mode	EI
Mode	SIM
m/z (amu)	103, 104 and 78
Start Time	1.0 min
End Time	5.0 min

RESULTS AND DISCUSSION

Fragmentation of Styrene

Mass spectrum of styrene is shown in Figure 5. From the mass spectrum, base peak of m/z 104 was used for quantitation where as m/z 103 and 78 were used as reference ions.
SIM chromatogram of 50 ppb standard styrene solution with m/z 104, 103 and 78 is shown in Figure 4.
Method validation data is summarized in Table 2. Figures 6 and 7 show overlay of SIM chromatograms for m/z 104 at linearity levels and calibration curve respectively.

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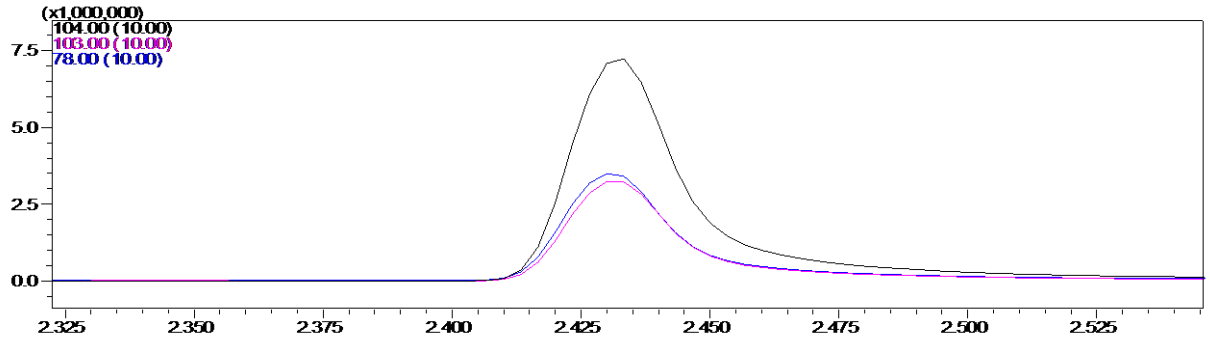


Figure 4: SIM chromatograms of 50 ppb Styrene standard solution

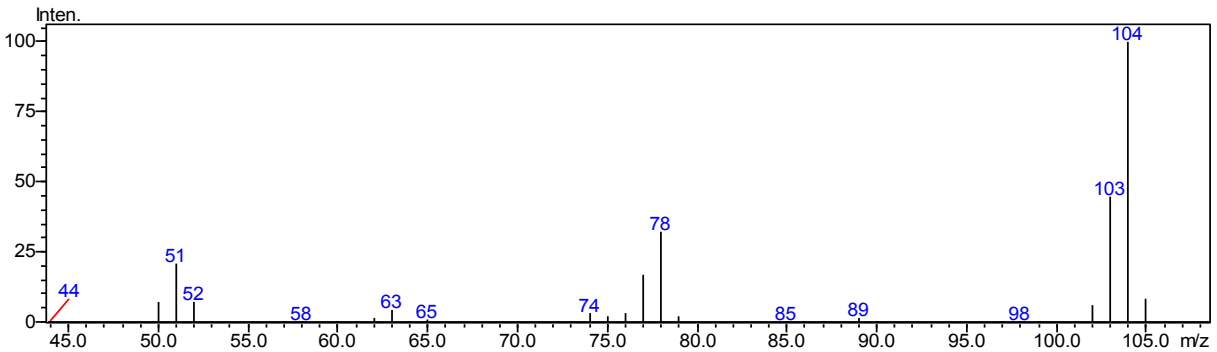


Figure 5: Mass spectrum of Styrene

Summary of the results

Table 2: Result for Method Parameters

No.	Compound Name	Parameter	Concentration	Result
1	Styrene	Reproducibility	50	% RSD : 1.74 (n=6)
2		Linearity	1 – 50	R^2 : 0.9996
3		LOD	1 – 50	0.2 ppb**
4		LOQ		1.0 ppb**
5		Precision at LOQ	1	S/N ratio : 38 (n=6)
				% RSD : 3.2 (n=6)

* Linearity levels – 1, 2.5, 5, 10, 20 and 50 ppb. For linearity, refer Figure 6 and Figure 7

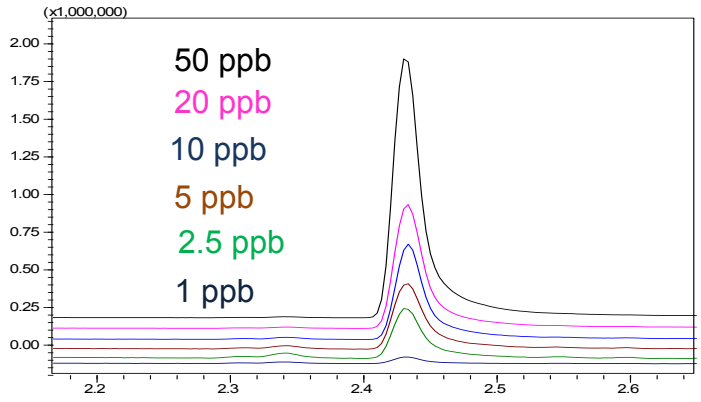


Figure 6. Overlay of SIM chromatograms for m/z 104 at linearity levels

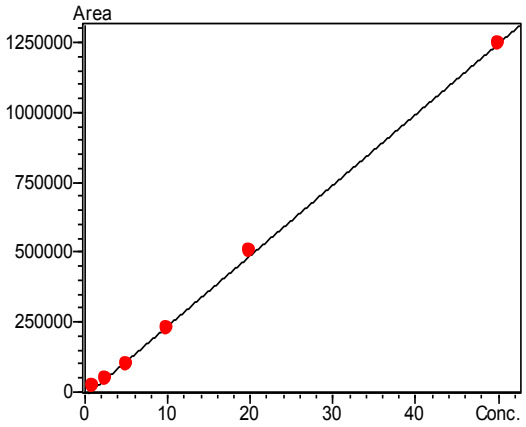


Figure 7. Calibration curve for Styrene

Quantitation of styrene in polystyrene cup sample

Analysis of leachable styrene from polystyrene cups was done as per method described earlier. Recovery studies were carried out by spiking 2.5, 10 and 50 ppb of standard styrene solutions in polystyrene cups. Figure 8 shows overlay SIM chromatogram of spiked and unspiked samples. Table 3 shows the summary of results.

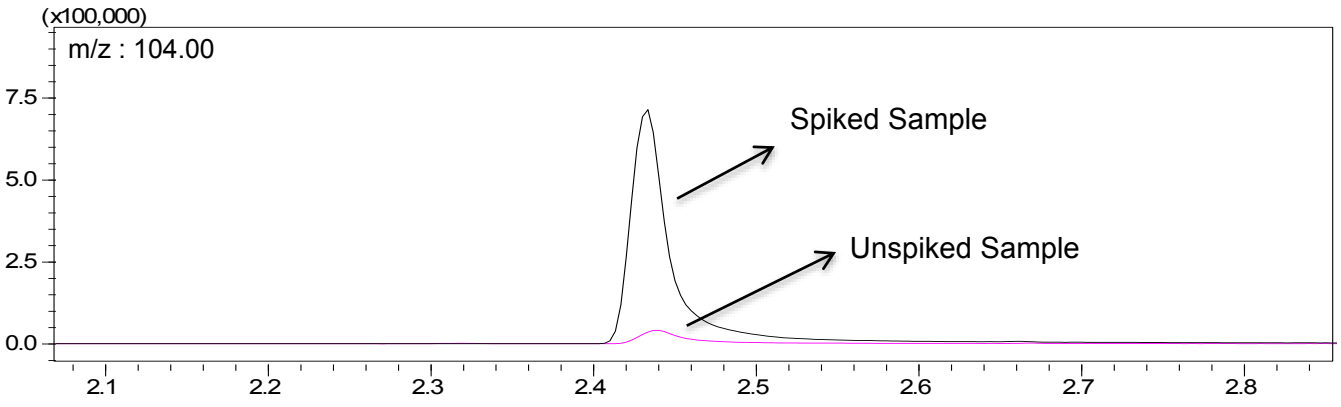


Figure 8. Overlay SIM chromatograms of spiked and unspiked samples

Table 3: Summary of results for sample analysis

Sr. No.	Sample Name	Parameter	Observed Concentration in ppb	Spiked Concentration in ppb	% Recovery
1	Unspiked sample	Precision	9.8	NA	NA
2	Spiked polystyrene cups	Recovery	12.0	2.5	88.0
			18.5	10	87.0
			55.9	50	92.2

CONCLUSION

HS-GCMS method was developed for quantitation of styrene leached from polystyrene cup. Part method validation was performed. Results were obtained for reproducibility, linearity, LOQ and recovery studies.

With low carryover, the characteristic feature of HS-20 headspace, reproducibility even at very low concentration level could be achieved easily.

Ultra Fast Scan Speed 20,000 u/sec is the characteristic feature of GCMS-QP2010 Ultra mass spectrometer, useful for quantitation of styrene at very low level (ppb level) with high sensitivity.

SHIMADZU
Excellence in Science

C-46

LCMS-8040

Quantitation of Methyl parathion from chili matrix using LC/MS/MS

INTRODUCTION

Methyl parathion is an organophosphate (OP) insecticide that has caused many health problems since its introduction in the early 1950s. The World Health Organisation classifies methyl parathion as class 1a 'extremely hazardous' pesticide. Like other organophosphate insecticides, methyl parathion is a cholinesterase inhibitor which is highly toxic when inhaled or ingested, and moderately toxic when adsorbed dermally (it is also readily adsorbed through the skin).

Due to toxicity and rampant use of methyl parathion, it is imperative to analyse this compound at trace level in different matrices. Also, matrix like chili requires analytical methods to be more specific towards the analytes of interest in presence of complex interferences. This has increased the popularity of LC/MS/MS as a suitable analytical tool. High sensitivity of LCMS-8040 system coupled with its ease of maintenance ensures reliable quantitative analysis of methyl parathion from complex matrices like chili. Also, the Ultrafast MRM capabilities of LCMS-8040 (555 MRM transitions/second) along with minimized dwell times (0.8 msec) and pause times (1 msec), makes it well suited for UHPLC analysis where analysis cycle times are reduced so as to achieve high sample throughput.

EXPERIMENTAL

Preparation of matrix matched standards:

Commercially available red chili is powdered using mixer grinder. 1.06 g of this chili powder was mixed with 20 mL acetonitrile using ultra sonicator for 10 mins. Mixture was centrifuged and supernatant was collected. This supernatant was used as a diluent to prepare methyl parathion matrix matched standards at concentration levels of 0.5 ppb, 1 ppb, 2 ppb, 5 ppb and 10 ppb.

Note: chili powder is a complex matrix and can exhibit matrix effect (either ion suppression or enhancement) during analysis. A calibration curve based on matrix matched standards can demonstrate true sensitivity of analyte in presence of matrix. Therefore, this method provides more reliable and accurate method of quantitation as compared to quantitation against neat standards.

Analytical conditions

Nexera parameters

Column	- Shim-pack XR C8 (100 mm L x 3 mm I.D.; 2.2 µ)
Guard column	- Phenomenex SecurityGuard ULTRA Cartridge
Mobile phase	- A: 5 mM ammonium formate in water B: 5 mM ammonium formate in methanol
Flow rate	- 0.6 mL/min
Oven temperature	- 50 ºC
Injection volume	- 30 µL
Gradient program	-

Time in min	A Conc %	B Conc %
0.01	90	10
2.00	0	100
4.00	0	100
4.01	90	10
8.00	Stop	

LCMS-8040 parameters

MS interface	- Atmospheric Pressure Chemical Ionisation
Polarity	- Negative
Nebulizing gas flow	- 1.5 L/min;
Drying gas flow	- 3.0 L/min
Interface temp.	- 450 ºC
Desolvation line temp.	- 250 ºC
Heat block temp.	- 300 ºC
MRM transition	- 247.90>138.20

RESULTS AND DISCUSSION

LC/MS/MS results

LCMS-8040 has a feature of 'Optimisation of method' in which the mass spectrometer selects the best product ion(s) and optimises voltages and collision energies for the precursor to product transition. Utilizing this feature, MRM transitions were optimized and used to determine quantitative sensitivity of methyl parathion. The results obtained are as tabulated in Table 1. Figure 1 shows the calibration curve for methyl parathion. Figure 2 shows a representative chromatogram of methyl parathion at LOQ level.

Table 1: Results for Methyl Parathion matrix match standards with calibration information

Compound name	Retention time (min)	Calibration range* (ppb)	Correlation coefficient (r^2)
Methyl Parathion	2.81	0.5 - 10	0.9993

* n=6 for each level

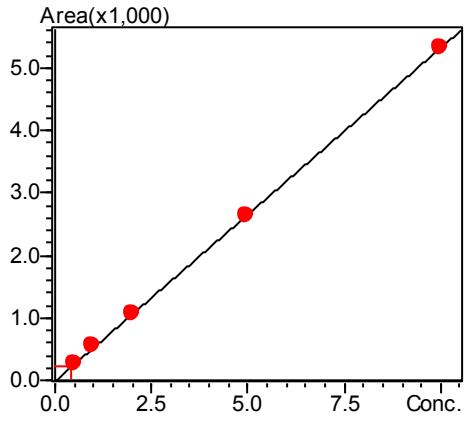


Figure 1: Calibration curve of Methyl parathion

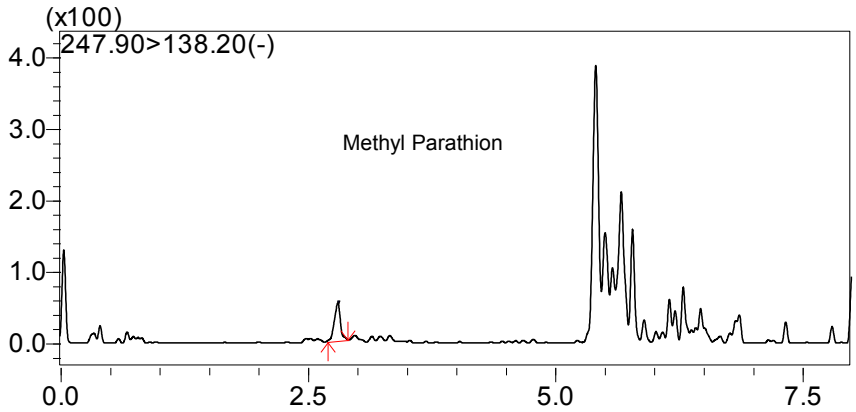


Figure 2: Methyl Paratihon at 0.5ppb

Area repeatability at LOQ level for methyl parathion showed 9.95 % RSD. For the rest of the levels, the area % RSD ranged between 2.3- 6.9% in the increasing order of linearity.

CONCLUSION

With the growing stringency in food safety, guidelines are constantly being revised so as to ensure safety of food products reaching the consumers. Accordingly, LC/MS/MS method for trace level quantitation of Methyl parathion from chili matrix was developed.

SHIMADZU

GC-MS

Gas Chromatograph Mass Spectrometer

C-47 Analysis of Residual Pesticides in Processed Foods Using GC-MS/MS

INTRODUCTION

When analyzing residual pesticides in processed foods, the same analysis method for vegetables cannot be used due to the interference by a large quantity of substances co-extracted from the sample. In such cases, a different sample-preparation method must be considered to remove or minimize the co-extracted substances. However, it is time consuming to develop a optimal sample-preparation method.
This application datasheet presents a study of an analysis of residual pesticides in retort-pouched curry (curry packaged in a sealed pouch) using QuEChERS and GC-MS/MS. QuEChERS method is commonly used for extraction and cleanup of food products prior to GC/MS/MS analysis, due to its speed and simplicity. While GC-MS/MS is able to selectively separate residual pesticides from a large quantity of co-extracted substances.

EXPERIMENTAL

The curry was extracted and prepared for analysis using the QuEChERS method and then spiked with a mixture of 39 pesticides at 0.01 mg/L of all pesticides. The spiked sample was then analyzed by GC-MS and GC-MS/MS using the analytical conditions indicated in Table 1. The SIM (selected ion monitoring) mode was used for the GC-MS and MRM (multiple reaction monitoring) mode was used for the GC-MS/MS.

Table 1 Analysis Conditions

GC-MS :GCMS-TQ8030
Column :Rxi-5Sil MS (30 m length, 0.25 mm I.D., df = 0.25 μm)
Glass Insert :Splitless insert with wool (P/N: 221-48876-03)
[GC]
Injection Temp. : 250 °C
Column Oven Temp. : 50 °C (1 min) → (25 °C/min) → 125 °C → (10 °C/min) → 300 °C (15 min)
Injection Mode : Splitless (high pressure injection at 250 kPa for 1.5 min)
Carrier Gas Control : Linear velocity (47.2 cm/sec)
Injection Volume : 1 μL

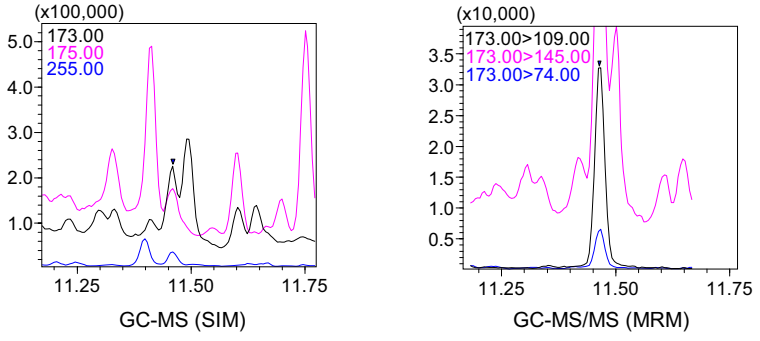
[MS]
Interface Temp. : 250 °C
Ion Source Temp. : 230 °C
Acquisition Mode : MRM (see below)

		Quantitative Transition		Qualitative Transition 1		Qualitative Transition 2	
Compound Name	Retention Time	Precursor>Product	CE	Precursor>Product	CE	Precursor>Product	CE
Mevinphos	7.621	192.0>164.0	5	192.0>127.0	10	192.0>109.0	26
Carbofuran	10.971	164.0>149.0	10	164.0>131.0	16	164.0>103.0	25
Simazine	11.007	201.0>173.0	6	201.0>186.0	6	201.0>138.0	12
gamma-HCH (Lindane)	11.298	219.0>183.0	6	219.0>145.0	20	219.0>181.0	8
Propyzamide	11.464	173.0>145.0	15	173.0>109.0	26	173.0>74.0	30
Diazinon	11.521	304.0>179.0	12	304.0>162.0	6	304.0>195.0	10
delta-HCH	11.838	219.0>183.0	10	219.0>145.0	22	219.0>147.0	22
Ametryn	12.748	227.0>185.0	8	227.0>170.0	12	227.0>212.0	10
Fenitrothion	13.070	277.0>260.0	6	277.0>109.0	18	277.0>125.0	16
Malathion	13.246	173.0>127.0	6	173.0>99.0	15	173.0>145.0	6
Thiobencarb	13.405	257.0>100.0	8	257.0>72.0	24	257.0>224.0	4
Fenthion	13.464	278.0>109.0	20	278.0>125.0	20	278.0>169.0	16
Parathion	13.532	291.0>109.0	15	291.0>137.0	6	291.0>142.0	5
Phthalide	13.726	243.0>215.0	18	243.0>179.0	26	243.0>144.0	40
Fosthiazate-1	13.824	195.0>103.0	8	195.0>139.0	5	195.0>167.0	5
Fosthiazate-2	13.873	195.0>103.0	8	195.0>139.0	5	195.0>167.0	5
Isofenphos	14.200	213.0>121.0	16	213.0>185.0	6	213.0>93.0	28
Procymidone	14.416	283.0>96.0	12	283.0>68.0	24	283.0>255.0	12
Dimepiperate	14.419	145.0>112.0	8	145.0>69.0	16	145.0>84.0	20
Tetrachlorvinphos	14.740	329.0>109.0	20	329.0>314.0	16	329.0>79.0	28
Flutolanil	15.080	173.0>145.0	16	173.0>125.0	26	173.0>95.0	28
Isoprothiolane	15.174	290.0>204.0	6	290.0>118.0	14	290.0>162.0	18
Myclobutanil	15.393	179.0>125.0	15	179.0>152.0	10	179.0>90.0	30
Chlorfenapyr	15.638	247.0>227.0	16	247.0>200.0	26	247.0>177.0	25
Triazophos	16.381	161.0>134.0	8	161.0>106.0	14	161.0>91.0	18
Bifenthrin	17.713	181.0>166.0	12	181.0>165.0	25	181.0>179.0	12
Fenpropathrin	17.892	265.0>210.0	12	265.0>172.0	14	265.0>89.0	26
Pyridaben	19.647	147.0>117.0	22	147.0>119.0	10	147.0>132.0	14
Cypermethrin-1	20.345	163.0>127.0	6	163.0>91.0	15	163.0>109.0	20
Cypermethrin-2	20.448	163.0>127.0	6	163.0>91.0	15	163.0>109.0	20
Cypermethrin-3	20.506	163.0>127.0	6	163.0>91.0	15	163.0>109.0	20
Flucythrinate-1	20.507	451.0>225.0	6	451.0>199.0	15	451.0>157.0	28
Cypermethrin-4	20.546	163.0>127.0	6	163.0>91.0	15	163.0>109.0	20
Flucythrinate-2	20.702	451.0>225.0	6	451.0>199.0	15	451.0>157.0	28
Fenvalerate-1	21.233	419.0>225.0	6	419.0>167.0	14	419.0>125.0	28
Fluvalinate-1	21.351	250.0>200.0	18	250.0>55.0	18	250.0>145.0	26
Fluvalinate-2	21.415	250.0>200.0	18	250.0>55.0	18	250.0>145.0	26
Fenvalerate-2 (Esfenvalerate)	21.435	419.0>225.0	6	419.0>167.0	14	419.0>125.0	28
Imibenconazole	23.008	375.0>260.0	22	375.0>306.0	8	375.0>271.0	18

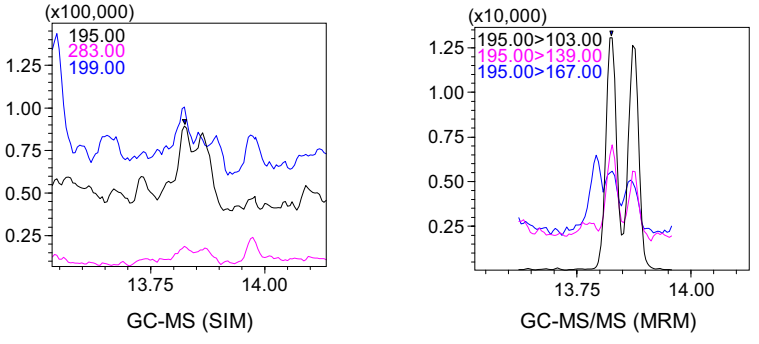
RESULTS AND DISCUSSION

Fig. 1 shows mass chromatograms of the spiked sample extract (0.01 mg/L) using the SIM mode of GC-MS and the MRM mode of GC-MS/MS. Some of the pesticides could not be detected or identified in the SIM of GC-MS because they overlap with co-extracted substances. By contrast, it was possible to selectively detect and identify those same pesticides using the Multiple Reaction Monitoring technique of GC-MS/MS.
Therefore, GC-MS/MS provided an effective means of analyzing residual pesticides in processed foods that contain a large quantity of substances co-extracted from the sample, such as retort-packed curry.

Propyzamide



Fosthiazate-1,2



Isofenphos

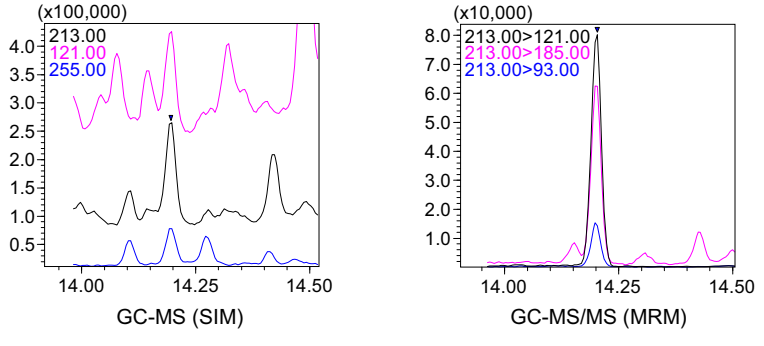


Fig. 1 Mass Chromatograms of Sample Extracts Spiked a mixture of 39 pesticides at 0.01 mg/L

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GC-MS
Gas Chromatograph Mass Spectrometer

C-48 GC-MS/MS Analysis of Pesticides in Drinking Water

INTRODUCTION

According to Japan's list of drinking water quality control substances, pesticides are included as supplemental items subject to analysis. Designed to complement the standards, the Ministry of Health, Labour and Welfare encourages water utilities to monitor pesticide levels and achieve specified targets. Among the 102 listed pesticides, 84 are simultaneously analyzed using solid-phase extraction and GC-MS. In this datasheet, those pesticides were analyzed using GC-MS/MS and Multiple Reaction Monitoring (MRM) mode.

EXPERIMENTAL

Analytical conditions are shown in Table 1.

Table 1: Analytical Conditions

GC-MS	:GCMS-TQ8030
Column	:Rtx-5MS (Length 30 m, 0.25 mm I.D., df=0.25 µm)
Glass liner	:Custom Sky Liner, Splitless Single Taper Gooseneck w/Wool (RESTEK, catalog# 567366)
[GC]	
Injection Temp.	:250°C
Column Oven Temp.	:80°C(2 min)→(20°C /min)→180°C →(5°C /min)→280°C(3 min)
Injection Mode	:Splitless (High Pressure Injection 250 kPa, 2.3 min)
Flow Control Mode	:Linear Velocity (44.5 cm/sec)
Injection Volume	:2 µL
[MS]	
Interface Temp.	:250 °C
Ion Source Temp.	:230 °C
Data Acquisition Mode	:MRM

MRM Monitoring *m/z*

Compound Name	Quantitative Transition Precursor>Product	CE (V)	Qualitative Transition Precursor>Product	CE (V)
Dichlorvos	184.9>109.0	18	184.9>93.0	13
Dichlobenil	170.9>136.0	13	170.9>100.0	23
Etridiazole	210.9>182.9	10	210.9>139.9	20
Chloroneb	205.9>190.9	12	205.9>140.9	19
Isoprocarb	136.1>121.1	9	136.1>103.1	23
Molinate	126.1>55.0	18	126.1>83.1	6
Fenobucarb	150.1>121.1	9	150.1>103.1	23
Trifluralin	306.1>264.0	7	306.1>206.1	17
Benfluralin	292.1>264.0	9	292.1>206.1	14
Pencycuron	180.1>125.0	10	180.1>89.0	29
Dimethoate	125.0>79.0	10	125.0>62.0	8
Simazine	201.1>173.1	6	201.1>186.1	7
Atrazine	215.2>200.1	8	215.2>173.1	6
Diazinon oxon	273.1>137.1	18	273.1>217.0	10
Propyzamide	172.9>144.9	15	172.9>109.0	27
Pyroquilon	173.1>130.1	20	173.1>144.1	23
Diazinon	304.1>179.2	10	304.1>162.1	9
Anthracene-d10 (ISTD)	188.2>160.1	20	188.2>158.1	30
Disulfoton	274.1>88.0	6	274.1>60.0	22
Chlorothalonil	265.9>230.9	19	265.9>169.9	23
Iprobenfos	204.0>91.0	8	204.0>122.0	15
Tolclofos-methyl oxon	249.0>199.0	26	249.0>233.9	15
Fenitorothion oxon	244.0>109.0	16	244.0>90.0	18
Bromobutide	232.2>176.1	10	232.2>114.1	9
Terbucarb	205.2>177.1	8	205.2>145.1	18
Malaoxon	127.1>99.0	7	127.1>109.0	10
Simetryn	213.2>170.1	10	213.2>185.1	7
Tolclofos-methyl	265.0>249.9	15	265.0>219.9	23
Alachlor	188.1>160.1	10	188.1>131.1	22
Metalaxyl	249.2>190.2	6	249.2>146.1	18
Fenthion oxon	262.0>247.0	8	262.0>109.0	26
Dithiopyr	354.1>306.0	7	354.1>286.0	17
Fenitrothion	277.0>260.1	7	277.0>109.0	20
Esprocarb	222.1>91.0	19	222.1>162.2	7
Malathion	173.1>127.1	7	173.1>99.0	18
Thiobencarb	257.1>100.1	7	257.1>72.1	23
Chlorpyrifos oxon	298.0>241.8	14	298.0>269.9	6
Fenthion	278.1>109.0	18	278.1>169.0	18
Chlorpyrifos	314.0>257.9	19	314.0>285.9	7
Isofenphos oxon	229.1>201.0	10	229.1>121.1	24
Phthalide	242.8>214.8	18	242.8>178.9	26
Dimethametryn	212.1>122.1	13	212.1>94.0	22
Pendimethalin	252.1>162.1	11	252.1>191.1	8
Methyldymron	107.1>106.1	13	107.1>77.0	25

Compound Name	Quantitative Transition Precursor>Product	CE (V)	Qualitative Transition Precursor>Product	CE (V)
Isofenphos	213.1>185.1	6	213.1>121.1	18
Captan	149.0>105.1	5	149.0>79.0	19
Dimepiperate	145.1>112.1	9	145.1>69.1	18
Phenthoate	274.0>121.0	11	274.0>125.0	18
Procymidone	283.1>96.0	10	283.1>68.1	24
Butamifos oxon	244.0>216.0	7	244.0>136.1	15
Methidathion	145.0>85.0	8	145.0>58.0	18
9-Bromoanthracene (ISTD)	256.0>177.1	18	256.0>151.1	30
alpha-Endosulfan	240.9>205.9	13	240.9>170.0	26
Butamifos	286.1>202.1	17	286.1>185.0	27
Napropamide	128.1>72.1	7	128.1>100.1	9
Flutolanil	173.0>145.0	18	173.0>95.0	27
Isoxathion oxon	161.1>105.0	11	161.1>77.0	25
Isoprothiolane	290.1>204.1	5	290.1>118.0	14
Pretilachlor	238.1>162.2	11	238.1>146.2	10
Fenthion oxon sulfoxide	262.1>247.1	11	262.1>109.0	22
CNP-amino	287.0>108.1	19	287.0>217.0	13
Fenthion oxon sulfone	294.1>104.1	19	294.1>230.2	8
Buprofezin	172.1>57.1	18	172.1>131.1	6
Isoxathion	312.9>177.0	7	312.9>130.0	17
beta-Endosulfan	240.9>205.9	18	240.9>170.0	23
Fenthion sulfoxide	278.0>109.0	20	278.0>169.1	14
Fenthion sulfone	310.0>109.0	24	310.0>105.1	16
Mepronil	269.1>119.1	18	269.1>227.1	5
Chlornitrofen	318.9>288.9	12	318.9>238.0	10
Edifenphos	310.0>173.0	13	310.0>109.1	25
Propiconazole-1	259.1>69.0	13	259.1>173.0	18
Endosulfate	271.8>236.8	18	271.8>234.8	19
Propiconazole-2	259.0>69.0	11	259.0>172.9	19
EPN oxon	141.0>77.0	18	141.0>51.0	30
Thenylchlor	288.1>141.0	13	288.1>174.1	7
Pyributicarb	165.1>108.1	10	165.1>93.0	25
Iprodione	314.0>244.9	11	314.0>56.0	25
Pyridaphenthion	340.0>199.1	8	340.0>109.0	22
Chrysene-d12 (ISTD)	240.2>236.1	30	240.2>238.2	20
EPN	157.0>77.0	24	157.0>110.0	14
Piperophos	320.2>122.1	10	320.2>81.0	26
Bifenox	341.1>309.9	6	341.1>188.8	19
Anilofos	226.1>184.0	5	226.1>157.0	13
Pyriproxyfen	136.1>78.0	20	136.1>96.0	14
Mefenacet	192.0>136.0	17	192.0>109.0	28
Cafenstrole	188.2>119.1	22	188.2>82.0	20
Etofenprox	163.1>135.1	10	163.1>107.1	19

RESULTS AND DISCUSSION

The standard sample mixture of 84 pesticides at the concentration of 5 μg/L was analyzed 5 times. The overlay mass chromatograms from 5 injections and the repeatability are shown in Fig. 1 and Table 2, respectively.

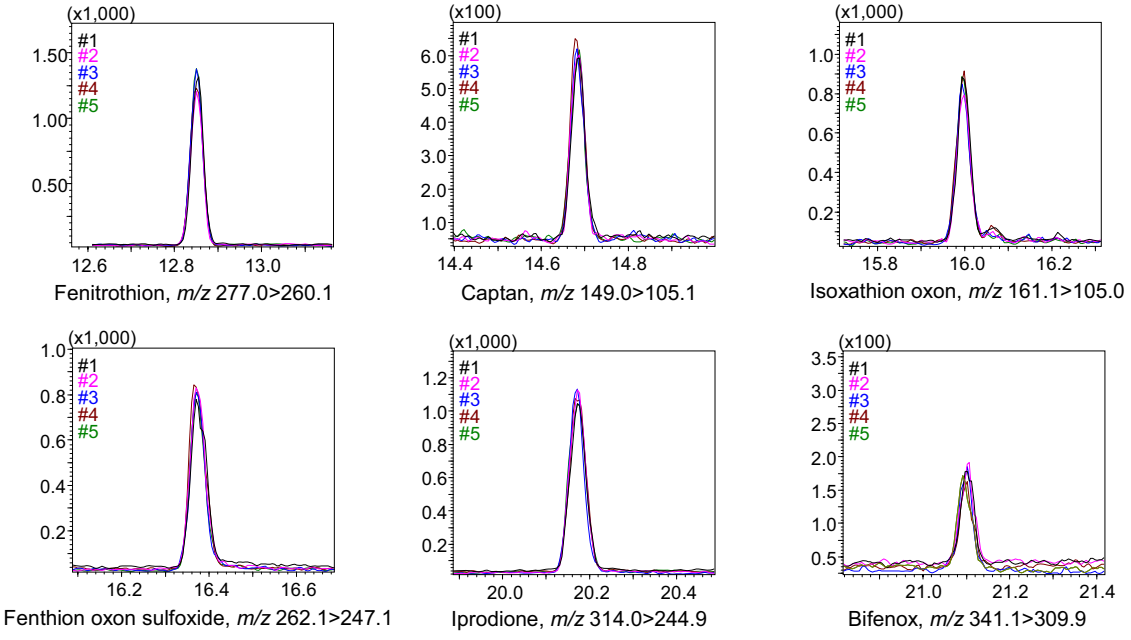

Fig. 1: Overlay mass chromatograms from 5 injections

Table 2: Repeatability (*n*=5, area ratio)

Compound Name	%RSD	Compound Name	%RSD	Compound Name	%RSD
Dichlorvos	1.62	Metalaxyl	3.01	Pretilachlor	7.26
Dichlobenil	0.73	Fenthion oxon	1.88	Fenthion oxon sulfoxide	5.72
Etridiazole	3.38	Dithiopyr	0.66	CNP-amino	1.04
Chloroneb	0.89	Fenitrothion	5.72	Fenthion oxon sulfone	1.19
Isoprocarb	0.47	Esprocarb	1.30	Buprofezin	2.14
Molinate	1.25	Malathion	0.82	Isoxathion	8.25
Fenobucarb	0.65	Thiobencarb	2.83	beta-Endosulfan	5.28
Trifluralin	1.71	Chlorpyrifos oxon	4.14	Fenthion sulfoxide	3.17
Benfluralin	2.09	Fenthion	1.17	Fenthion sulfone	9.61
Pencycuron	0.23	Chlorpyrifos	2.40	Mepronil	3.62
Dimethoate	2.98	Isofenphos oxon	2.03	Chlornitrofen	1.82
Simazine	1.17	Phthalide	1.03	Edifenphos	1.06
Atrazine	3.51	Dimethametryn	1.37	Propiconazole-1	7.70
Diazinon oxon	1.37	Pendimethalin	3.38	Endosulfate	2.98
Propyzamide	1.39	Methyldymron	2.29	Propiconazole-2	5.75
Pyroquilon	1.36	Isofenphos	2.93	EPN oxon	2.31
Diazinon	3.15	Captan	7.46	Thenylchlor	5.43
Disulfoton	3.37	Dimepiperate	3.64	Pyributicarb	0.88
Chlorothalonil	1.57	Phenthoate	2.65	Iprodione	3.03
Iprobenfos	1.29	Procymidone	0.87	Pyridaphenthion	3.78
Tolclofos-methyl oxon	1.56	Butamifos oxon	4.28	EPN	2.85
Fenitorothion oxon	3.75	Methidathion	2.27	Piperophos	5.48
Bromobutide	4.98	alpha-Endosulfan	1.78	Bifenox	7.02
Terbucarb	1.08	Butamifos	5.57	Anilofos	2.48
Malaoxon	2.64	Napropamide	2.38	Pyriproxyfen	2.39
Simetryn	3.14	Flutolanil	1.40	Mefenacet	1.70
Tolclofos-methyl	2.33	Isoxathion oxon	2.71	Cafenstrole	3.14
Alachlor	1.12	Isoprothiolane	4.96	Etofenprox	1.10

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GC-MS
Gas Chromatograph Mass Spectrometer

C-49 Simultaneous Analysis of Residual Pesticides in Foods via the QuEChERS Method Utilizing GC-MS/MS

INTRODUCTION

Analytical standards (0.001 mg/L to 0.1 mg/L), as well as samples (0.01 mg/L) created by pretreating paprika with the QuEChERS method and then adding pesticides to the resulting solution, were measured using the analysis conditions shown in Table 1.

EXPERIMENTAL

The European Union Reference Laboratory (EURL) has reported their results on evaluating the validity of residual pesticide analysis utilizing GC-MS/MS and LC-MS/MS[1]. In their report, the measurement of 66 pesiticides using GC-MS/MS was recommended. This data sheet presents selected results of analysis of these pesticides using the triple quadrupole GCMS-TQ8030.

Table 1 Analytical Conditions

GC-MS :GCMS-TQ8030
Column :Rxi-5Sil MS (30 m length, 0.25 mm I.D., df=0.25 µm)
Glass Liner :Sky Liner, Splitless Single Taper Gooseneck w/Wool (Restek Corporation, catalog # 567366)

[GC]
Injection Temp. :250 °C
Column Oven Temp. :70 °C (2 min) → (25 °C /min) → 150 °C → (3 °C/min) →200 °C → (8 °C/min) → 280 °C (10 min)
Injection Mode :Splitless
Flow Control Mode :Linear velocity (58.1 cm/sec.)
Injection volume :1 µL

[MS]
Interface Temp. :250 °C
Ion Source Temp. :230 °C
Data Acquisition Mode :MRM (See the below.)

MRM Monitoring *m/z*

Compound Name	Quantitative Transition Precursor>Product	CE (V)	Qualitative Transition Precursor>Product	CE (V)	Precursor>Product	CE (V)
Diphenylamine	169.10>77.00	26	169.10>115.10	30		
Ethoprophos	200.00>157.90	6	200.00>114.00	14	200.00>97.00	26
Chlorpropham	213.10>171.10	6	213.10>127.10	18		
Trifluralin	306.10>264.00	8	264.10>206.10	8	264.10>160.10	18
Dicloran	206.00>176.00	12	206.00>124.00	26	176.00>148.00	12
Propyzamide	172.90>144.90	16	172.90>109.00	26		
Chlorothalonil	265.90>230.90	14	265.90>167.90	24	263.90>167.90	24
Diazinon	304.10>179.10	12	179.20>137.20	18		
Pyrimethanil	199.10>184.10	14	199.10>158.10	14		
Tefluthrin	197.10>141.10	26	177.10>127.10	32		
Pirimicarb	238.20>166.10	10	166.10>96.00	14		
Chlorpyrifos-methyl	285.90>270.90	12	285.90>93.00	22		
Vinclozolin	212.10>172.00	14	212.10>144.90	26	212.10>109.00	30
Parathion-methyl	263.10>109.00	18	263.10>81.00	26		
Tolclofos-methyl	265.00>249.90	12	265.00>93.00	24		
Metalaxyl	206.20>162.10	8	206.20>132.10	18		
Fenitrothion	277.10>125.00	18	277.10>109.00	18		
Pirimiphos-methyl	305.10>290.10	12	290.10>125.00	24		
Dichlofluanid	332.00>167.10	6	224.00>123.00	12		
Malathion	173.10>117.00	12	173.10>99.00	18		
Chlorpyrifos	196.90>168.90	14	196.90>107.00	26		
Fenthion	278.10>125.00	22	278.10>109.00	18		
Parathion	291.10>109.00	14	291.10>81.00	26		
Tetraconazole	336.10>218.00	18	336.10>204.00	26		
Pendimethalin	252.20>162.10	12	252.20>161.10	12		
Cyprodinil	225.20>224.10	6	224.20>208.10	18		
(E)-Chlorfenvinphos	323.10>266.90	14	267.00>159.00	18		
Tolylfluanid	137.10>91.00	18	137.10>65.00	26		
Fipronil	367.00>227.90	26	367.00>212.90	26		
Captan	79.00>77.00	8	79.00>51.00	22		
(Z)-Chlorfenvinphos	323.10>266.90	14	267.00>159.00	18		
Phenthoate	274.10>125.00	18	274.10>121.10	12		
Folpet	147.10>103.10	10	147.10>76.00	26		
Procymidone	283.10>96.10	12	283.10>67.10	24		
Methidathion	145.10>85.00	8	145.10>58.00	18		
alpha-Endosulfan	240.90>205.90	14	238.90>203.90	16		
Mepanipyrim	222.20>220.10	8	222.20>193.10	26		
Profenofos	337.10>266.80	16	207.90>63.00	26		
Myclobutanil	179.10>152.00	8	179.10>125.00	16		
Flusilazole	233.10>165.10	18	233.10>152.10	18		

Compound Name	Quantitative Transition Precursor>Product	CE (V)	Qualitative Transition Precursor>Product	CE (V)	Precursor>Product	CE (V)
Buprofezin	172.10>57.10	18	105.10>104.10	4		
Bupirimate	273.10>193.20	8	273.10>108.00	18		
beta-Endosulfan	240.90>205.90	14	238.90>203.90	14		
Oxadixyl	163.10>132.10	10	163.10>117.10	24		
Ethion	231.00>174.90	14	231.00>128.90	26		
Triazophos	161.10>134.10	8	161.10>106.10	14		
Endosulfan sulfate	386.90>252.90	10	386.90>216.90	26		
Propiconazole-1	259.10>190.90	8	259.10>172.90	18	259.10>69.10	12
Propiconazole-2	259.10>190.90	8	259.10>172.90	18	259.10>69.10	12
Tebuconazole	252.10>127.00	24	250.10>125.10	24		
Iprodione	314.10>244.90	12	314.10>56.10	24		
Bromopropylate	340.90>184.90	18	182.90>154.90	16		
Bifenthrin	181.10>166.10	16	181.10>165.10	22	181.10>153.10	10
Fenpropathrin	265.10>210.10	12	181.10>152.10	24	181.10>127.10	26
Fenazaquin	160.20>145.10	8	145.20>115.10	24	145.20>91.10	24
Tebufenpyrad	333.20>276.10	8	333.20>171.00	22		
Tetradifon	355.90>158.90	12	353.90>159.00	12	228.90>200.90	14
Phosalone	182.00>138.00	8	182.00>111.00	18	182.00>102.10	18
Pyriproxyfen	136.10>96.00	12	136.10>78.00	24		
Cyhalothrin	181.10>152.10	24	163.10>127.00	14	163.10>91.00	22
Fenarimol	251.00>139.00	18	139.10>111.00	16		
Acrinathrin	289.10>93.10	12	181.10>152.10	24	208.10>181.10	8
Permethrin-1	183.10>168.10	12	183.10>153.10	18	183.10>115.10	24
Pyridaben	147.20>132.10	14	147.20>117.10	22		
Permethrin-2	183.10>168.10	12	183.10>153.10	18	183.10>115.10	24
Cyfluthrin-1	206.10>151.20	24	163.10>127.10	6	163.10>91.00	14
Cyfluthrin-2	206.10>151.20	24	163.10>127.10	6	163.10>91.00	14
Cyfluthrin-3	206.10>151.20	24	163.10>127.10	6	163.10>91.00	14
Cyfluthrin-4	206.10>151.20	24	163.10>127.10	6	163.10>91.00	14
Cypermethrin-1	181.10>152.10	24	163.10>127.10	6	163.10>91.00	14
Cypermethrin-2	181.10>152.10	24	163.10>127.10	6	163.10>91.00	14
Cypermethrin-3	181.10>152.10	24	163.10>127.10	6	163.10>91.00	14
Cypermethrin-4	181.10>152.10	24	163.10>127.10	6	163.10>91.00	14
Ethofenprox	163.20>135.00	10	163.20>107.10	18		
Fenvalerate-1	125.10>99.00	22	125.10>89.00	22		
tau-Fluvarlinate-1	250.10>200.10	16	250.10>55.00	18		
Fenvalerate-2	125.10>99.00	22	125.10>89.00	22		
tau-Fluvarlinate-2	250.10>200.10	16	250.10>55.00	18		
Deltamethrin-1	252.90>93.10	18	181.10>152.10	24		
Deltamethrin-2	252.90>93.10	18	181.10>152.10	24		

RESULTS AND DISCUSSION

Calibration curves for each pesticide obtained by analyzing six calibration standards (0.001 mg/L to 0.1 mg/L), the mass chromatograms for the 0.01 mg/L samples, and the area repeatability (n=6) for each pesticide obtained from the pesticide-spiked samples (0.01 mg/L) are shown below.

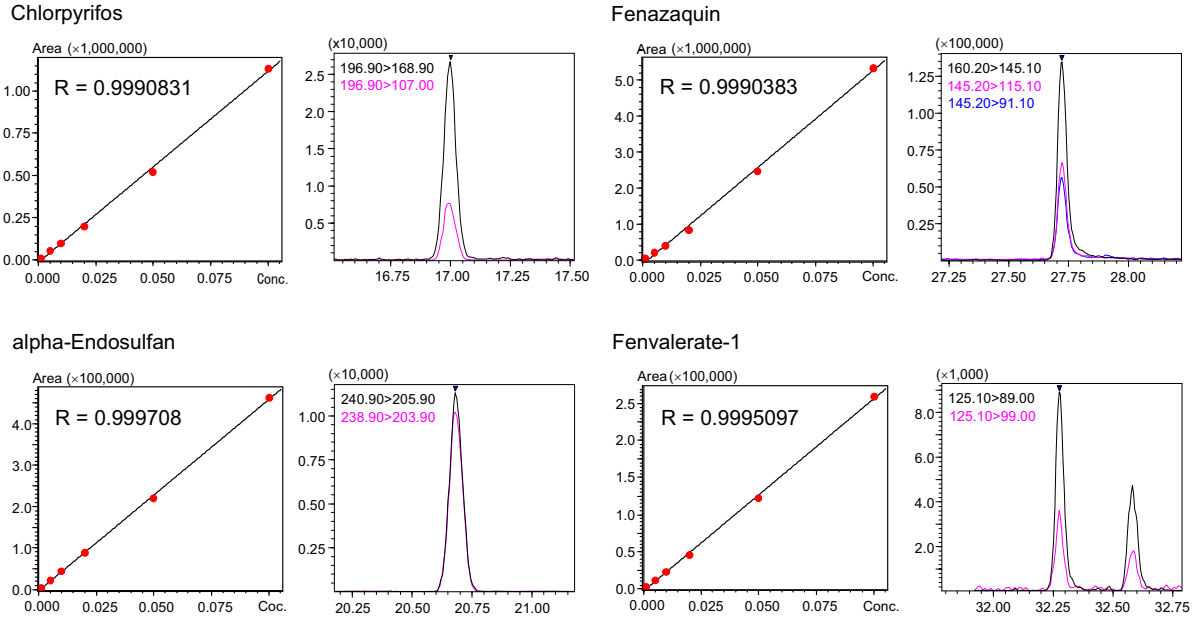

Fig. 1 Calibration Curves for Each Pesticide and the Mass Chromatograms for the 0.01 mg/L Samples

Table 2 Area Reproducibility for Each Pesticide (n=6)

Compound Name	%RSD	Compound Name	%RSD	Compound Name	%RSD	Compound Name	%RSD
Diphenylamine	4.99	Chlorpyrifos	5.23	Buprofezin	4.92	Fenarimol	5.16
Ethoprophos	4.95	Fenthion	5.75	Bupirimate	5.47	Acrinathrin	2.03
Chlorpropham	6.26	Parathion	6.93	beta-Endosulfan	6.29	Permethrin-1	6.34
Trifluralin	5.33	Tetraconazole	6.96	Oxadixyl	5.74	Pyridaben	7.11
Dicloran	6.49	Pendimethalin	6.29	Ethion	6.18	Permethrin-2	6.24
Propyzamide	5.52	Cyprodinil	5.21	Triazophos	3.45	Cyfluthrin-1	4.44
Chlorothalonil	4.46	(E)-Chlorfenvinphos	5.35	Endosulfan sulfate	4.26	Cyfluthrin-2	3.77
Diazinon	5.45	Tolylfluanid	4.81	Propiconazole-1	6.02	Cyfluthrin-3	7.35
Pyrimethanil	3.18	Fipronil	6.76	Propiconazole-2	5.56	Cyfluthrin-4	8.19
Tefluthrin	5.13	Captan	5.74	Tebuconazole	7.59	Cypermethrin-1	8.58
Pirimicarb	5.00	(Z)-Chlorfenvinphos	5.52	Iprodione	1.72	Cypermethrin-2	3.71
Chlorpyrifos-methyl	5.27	Phenthoate	6.40	Bromopropylate	5.71	Cypermethrin-3	8.08
Vinclozolin	6.33	Folpet	6.56	Bifenthrin	5.29	Cypermethrin-4	2.48
Parathion-methyl	5.81	Procymidone	6.40	Fenpropathrin	4.00	Ethofenprox	5.03
Tolclofos-methyl	4.89	Methidathion	6.17	Fenazaquin	4.84	Fenvalerate-1	4.20
Metalaxyl	5.43	alpha-Endosulfan	6.27	Tebufenpyrad	5.62	tau-Fluvarlinate-1	2.16
Fenitrothion	5.10	Mepanipyrim	6.41	Tetradifon	6.09	Fenvalerate-2	5.65
Pirimiphos-methyl	5.35	Profenofos	5.92	Phosalone	5.90	tau-Fluvarlinate-2	2.14
Dichlofluanid	4.04	Myclobutanil	5.46	Pyriproxyfen	5.16	Deltamethrin-1	7.58
Malathion	6.31	Flusilazole	5.63	Cyhalothrin	5.38	Deltamethrin-2	7.32

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GC-MS
Gas Chromatograph Mass Spectrometer

C-50 Easy Screening for Residual Pesticides in Processed Foods Using GC-MS/MS

INTRODUCTION

The analysis of residual pesticides in processed foods using GC-MS/MS, which provides excellent selectivity and sensitivity, has become a focus of attention.
Before starting GC-MS/MS measurements, it is necessary to optimize MRM transitions (precursor ions & product ions) and collision energies (CE) for each pesticide measured, which is extremely labor intensive. Furthermore, in order to calculate quantitative values, it is necessary to prepare standard samples and create calibration curves.
The Quick-DB database contains the optimal MRM conditions (MRM transitions and CE), mass spectra, retention indices, calibration curves and other information. This enables the semi-quantitative analysis of pesticides without using standard samples. Pesticide surrogates are used as the internal standard substances for calibration curves. Favorable quantitative accuracy is achieved by selecting the surrogates suited to each pesticide.
In analyzing residual pesticides in processed foods, which contain a number of contaminants, separating the pesticides from the contaminants can be impossible, even with GC-MS/MS. In this case, an effective approach to separating and detecting the pesticides is to perform the analysis with two columns respectively, which differ in their separation patterns. The information registered in Quick-DB is also compatible with analysis using two different columns for residual pesticides in processed foods. In addition, if the Twin Line MS system is used, the two columns can be attached to the MS unit simultaneously, so data can be sampled from the different columns smoothly, without compromising the MS vacuum.
This data sheet reports on the results of applying Quick-DB and the Twin Line MS system to the analysis of residual pesticides in curry.
Please also refer to Application Data Sheets No. 91 and No. 92. Application Data Sheet No. 91 introduced an example of easy screening for residual pesticides in foods using GCMS, while No. 92 introduced an example of using two columns with different separation patterns for easy screening of residual pesticides in foods.

EXPERIMENTAL

Using the Restek Q-sepTM, commercially-available retort-pouch curry was pretreated via the QuEChERS method. The sample solution obtained was spiked with 230 standard pesticide samples at a concentration of 10 ng/mL. The pesticide-spiked samples were then subjected to Scan/MRM analysis under the analysis conditions registered in Quick-DB. The analysis conditions are shown in Table 1. The two columns indicated in Table 1 were installed to a single GC-MS with the Twin Line MS system. The retention times for the pesticide components were estimated based on the analysis results for the n-alkane standard sample.

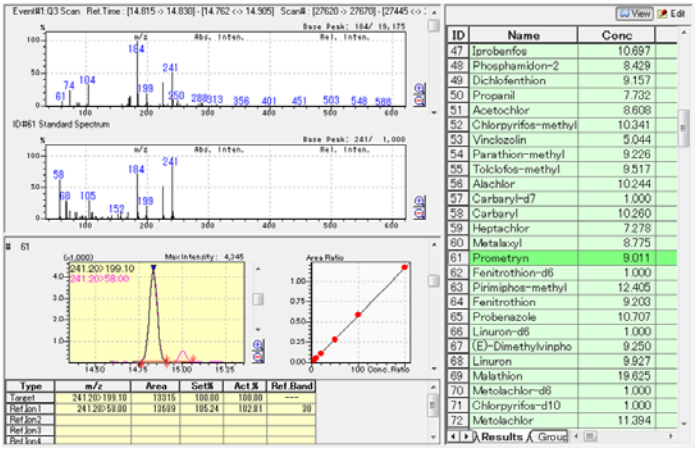
Table 1 Analysis Conditions

GC-MS:	GCMS-TQ8030 (Twin Line MS System)
Column 1:	Rxi-5Sil MS (30 m L., 0.25 mm I.D., df=0.25 µm) (Restek Corporation, P/N: 13623)
Column 2:	Rtx-200MS (30 m L., 0.25 mm I.D., df=0.25 µm) (Restek Corporation, P/N: 15623)
Glass Insert:	Sky Liner, Splitless Single Taper Gooseneck w/Wool (Restek Corporation, P/N: 567366)

[GC]		[MS]	
Vaporization Chamber Temperature:	250 °C	Interface Temp.:	300 °C
Column Oven Temperature:	60 °C (1 min) → (25 °C /min) → 160 °C → (4 °C /min) → 240 °C → (10 °C /min) → 290 °C (11 min)	Ion Source Temp.:	200 °C
		Solvent Elution Time:	1.5 min
Injection Mode:	Splitless	Measurement Mode:	FAAST (Scan/MRM simultaneous measurement)
High Pressure Injection:	250 kPa (1.5 min)		
Carrier Gas Control:	Linear velocity (40.0 cm/sec)	Scan Mass Range:	m/z 50 to 330
Injection Quantity:	2 µL	Scan Event Time:	0.15 sec
		Scan Speed	5,000 u/sec

RESULTS AND DISCUSSION

The liquid food extract spiked with pesticides was analyzed, and data processing was performed with Quick-DB. The analysis results are shown in Fig. 1. When semi-quantitative analysis was performed using the calibration curves registered in Quick-DB, favorable semi-quantitative values were obtained, close to the additive concentration of 10 ng/mL for many of the components.
To evaluate the quantitative accuracy for this analysis method, ratios were calculated for the semi-quantitative values with respect to the additive concentration. Then the pesticides were classified into those with a ratio under 50 %, 50 % to 200 %, and over 200 %, to find the distribution. The results are shown in Fig. 2. A significant 83 % of components had a semi-quantitative value 50 % to 200 % that of the concentration of the standard pesticide samples added. From this, it is evident that semi-quantitative analysis can be performed with high accuracy.



ID	Name	Conc
47	Iprobenfos	10.697
48	Phosphamidon-2	8.429
49	Dichlofenthion	9.157
50	Propanil	7.732
51	Acetochlor	8.608
52	Chlorpyrifos-methyl	10.341
53	Vinclozolin	5.044
54	Parathion-methyl	9.226
55	Tolclofos-methyl	9.517
56	Alachlor	10.244
57	Carbaryl-d7	1.000
58	Carbaryl	10.260
59	Heptachlor	7.278
60	Metalaxyl	8.775
61	Prometryn	9.011
62	Fenitrothion-d6	1.000
63	Pirimiphos-methyl	12.405
64	Fenitrothion	9.203
65	Probenazole	10.707
66	Linuron-d6	1.000
67	(E)-Dimethylvinpho	9.250
68	Linuron	9.927
69	Malathion	19.625
70	Metolachlor-d6	1.000
71	Chlorpyrifos-d10	1.000
72	Metolachlor	11.394

As a result of calculation of the semi-quantitative values using the calibration curves preregistered in Quick-DB, favorable quantitative values were obtained.
(*The concentration of the internal standard is indicated as 1 ng/mL.)

Fig. 1 Analysis Results for the Pesticide-Spiked Samples (10 ng/mL concentration)

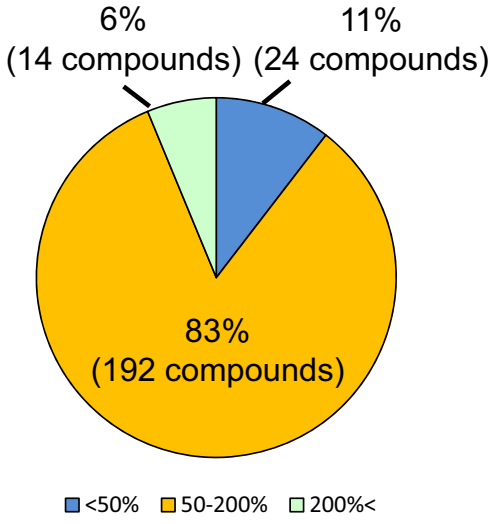


Fig. 2 Percentage Distribution of Semi-Quantitative Values with Respect to the Additive Concentration

In the analysis of residual pesticides in foods, when pesticide peaks are detected, it is necessary to check whether contaminants have been misidentified as pesticides, and whether contaminant overlap has inflated the size of the quantitative values. One confirmation method is to analyze the samples with columns with different separation patterns, and then check that essentially the same quantitative values are obtained for the pesticides detected in the respective columns. As an example, Fig. 3 shows the analysis results for dimethoate. With the Rxi-5Sil MS, there was an impact from contaminants, but with the Rts-200MS, there was not. Semi-quantitative value obtained from the calibration curves registered in Quick-DB was favorable, 9.6 ng/mL, for the use of the Rtx-200MS column. In this way, even for pesticides of which separation from contaminants is difficult, separation is possible if using columns with different separation patterns, enabling highly reliable semi-quantitative analysis.

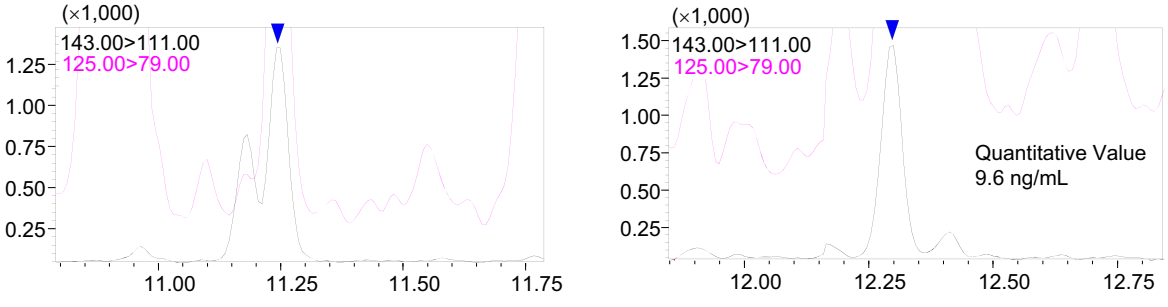


Fig. 3 Chromatograms for Liquid Curry Extract, Spiked with Dimethoate (10 ng/mL Concentration)
(Left: Rxi-5Sil MS; Right: Rtx-200MS)

High-accuracy semi-quantitative analysis was achieved quickly and easily, by attaching two columns to the GCMS-TQ8030 utilizing the Twin Line MS system, and then screening for residual pesticides in processed foods using Quick-DB.

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GC-MS
Gas Chromatograph Mass Spectrometer

C-51 An Investigation of Simultaneous Analysis Methods for 420 Residual Pesticide Compounds in Foods Using GC-MS/MS

INTRODUCTION

Due to its excellent sensitivity and selectivity, GC-MS/MS is utilized for the analysis of residual pesticides in foods. The number of relevant pesticides grows yearly, and has reached 300 to 400 compounds. Due to limitations in MRM-related software functionality and detection sensitivity, analyzing so many pesticides requires the intended pesticides to be divided into multiple method files and then measured method by method. As a result, the number of analyses increases, and places a strain on laboratory productivity. Smart MRM*, the method creation function in GCMSsolution software, automatically creates the optimal methods for the simultaneous analysis of over 400 compounds, while ensuring sensitivity and accuracy are maintained. In addition, MRM analysis can be started without configuring troublesome transition settings by utilizing Smart Pesticides Database, which contains acquisition parameters for 479 compounds. Since this database contains retention indices, retention times can be corrected with a single n-alkane analysis (AART function). Using the corrected retention times allows the user to start creating MRM methods without purchasing or analyzing an analytical standard.

This data sheet reports batch analysis methods for 420 compounds (approximately 1,200 transitions) using Smart Pesticides Database and Smart MRM.

EXPERIMENTAL

Commercially available spinach was pretreated with the QuEChERS method using Restek Q-sep™. Pesticides were added to the sample extract, with the concentration adjusted to 5 ng/mL. The prepared sample was then subjected to MRM analysis for 420 compounds under the analysis conditions registered in Smart Pesticides Database. Table 1 shows the analysis conditions. The retention times for the individual pesticides were corrected using the AART function based on an n-alkane analysis.

Table 1: Analysis Conditions

GC-MS:	GCMS-TQ8040
Column:	SH-Rxi-5Sil MS (30 m long, 0.25 mm I.D., df = 0.25 μm) (Shimadzu, P/N: 221-75954-30)
Glass Insert:	Sky Liner, Splitless Single Taper Gooseneck w/Wool (Restek, P/N: 567366)

[GC]	
Injection Unit Temp.:	250 °C
Column Oven Temp.:	50 °C (1 min) → (25 °C/min) → 125 °C (10 °C/min) → 300 °C (15 min)
Injection Mode:	Splitless
High-Pressure Injection:	250 kPa (1.5 min)
Carrier Gas Control:	Linear velocity (47.2 cm/sec)
Injection Volume:	2 μL

[MS]	
Interface Temp.:	250 °C
Ion Source Temp.:	200 °C
Solvent Elution Time:	1.5 min
Measurement Mode:	MRM
Loop Time:	0.5 sec

RESULTS AND DISCUSSION

Fig. 1 shows the mass chromatograms for malathion, trifloxystrobin, and fenbuconazole. Table 2 shows the area repeatability values for 240 of the 420 compounds (n = 5). By creating suitable MRM analysis methods utilizing Smart MRM, it is possible to analyze even 420 compounds simultaneously with favorable sensitivity and accuracy.

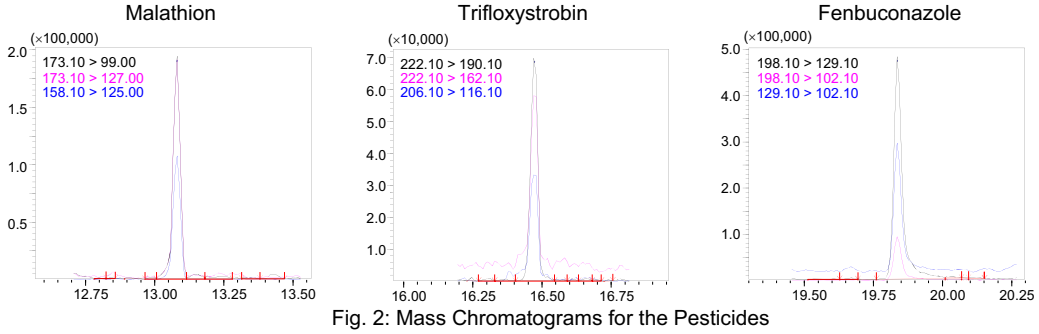

Fig. 2: Mass Chromatograms for the Pesticides

*Supported by GCMSsolution ver. 4.20 and later.

Table 2: Area Repeatability for 240 Compounds (n = 5)

Compound Name	%RSD	Compound Name	%RSD	Compound Name	%RSD	Compound Name	%RSD
Dichlorvos	6.37	Propanil	1.78	Fenothiocarb	2.83	Pyridaphenthion	3.17
Dichlobenil	2.87	Acetochlor	3.83	alpha-Endosulfan	13.10	Iprodione	8.59
EPTC	2.28	Bromobutide	4.15	Butamifos	4.96	Acetamiprid	7.64
Butylate	2.55	Chlorpyrifos-methyl	2.91	Flutriafol	5.72	Phosmet	10.86
Etridiazole	8.94	Vinclozolin	3.91	Fenamiphos	6.49	Bifenthrin	6.12
Methacrifos	3.84	Parathion-methyl	6.53	Napropamide	8.75	EPN	7.46
Clothianidin	9.96	Tolclofos-methyl	2.70	Flutolanil	6.05	Bromopropylate	3.29
Chloroneb	3.36	Simeconazole	4.94	Hexaconazole	8.09	Picolinafen	3.06
Crimidine	3.31	Alachlor	4.17	Prothiofos	3.47	Fenoxycarb	5.28
2-Phenylphenol	2.74	Simetryn	3.24	Fludioxonil	2.39	Bifenazate	3.52
Isoprocarb	4.68	Metalaxyl	2.51	Isoprothiolane	4.10	Etoxazole	7.00
Tecnazene	3.66	Fenchlorphos	2.94	Pretilachlor	3.01	Fenpropathrin	6.37
Omethoate	6.96	Prometryn	4.12	Profenofos	2.96	Fenamidone	2.31
Propoxur	9.13	Pirimiphos-methyl	3.14	Tricyclazole	5.31	Tebufenpyrad	6.29
Propachlor	2.73	Fenitrothion	4.47	Uniconazole	3.63	Bifenox	7.37
Ethoprophos	2.55	Ethofumesate	4.15	Oxadiazon	2.86	Furametpyr	4.71
Ethalfluralin	3.83	(E)-Dimethylvinphos	4.82	Thifluzamide	5.38	Tetradifon	7.80
Chlorpropham	3.21	Bromacil	7.35	Tribufos	2.04	Pentoxazone	5.03
Trifluralin	5.56	Esprocarb	3.06	Myclobutanil	2.62	Phosalone	8.42
Dicrotophos	5.78	Malathion	7.44	Flusilazole	6.76	Leptophos	4.31
Benfluralin	5.09	Quinoclamine	6.49	Oxyfluorfen	12.31	Azinphos-methyl	4.17
Salithion	2.10	Metolachlor	1.79	Bupirimate	3.78	Cyhalothrin-1	9.01
Sulfotep	3.61	Chlorpyrifos	3.63	Buprofezin	5.30	Cyhalothrin-2	8.68
Monocrotophos	5.44	Thiobencarb	7.20	Kresoxim-methyl	2.86	Cyhalofop-butyl	1.48
Cadusafos	3.25	(Z)-Dimethylvinphos	2.82	Carboxin	3.94	Mefenacet	4.52
Phorate	1.99	Diethofencarb	2.10	Diclobutrazol	4.21	Pyrazophos	5.85
alpha-BHC	4.14	Fenthion	6.45	(Z)-Metominostrobin	6.00	Fenarimol	4.05
Thiometon	3.57	Chlorthal-dimethyl	3.06	Azaconazole	2.75	Azinphos-ethyl	3.58
Dicloran	6.15	Fenpropimorph	4.62	Cyflufenamid	9.37	Pyraclofos	7.94
Dimethoate	5.51	Parathion	8.92	Chlorfenapyr	7.41	Fenoxaprop-ethyl	9.11
Furilazole	2.52	Triadimefon	3.61	Isoxathion	9.27	Fluquinconazole	11.11
Carbofuran	8.29	Tetraconazole	6.35	(Z)-Pyriminobac-methyl	2.59	Pyridaben	5.75
Simazine	4.30	Isocarbophos	8.27	Chlorobenzilate	1.33	Butafenacil	4.30
Atrazine	1.34	Nitrothal-isopropyl	6.41	Fensulfothion	6.20	Etobenzanid	2.99
Dimethipin	8.14	Phthalide	5.64	beta-Endosulfan	7.09	Fenbuconazole	2.93
Swep	3.23	Bromophos	3.73	Diniconazole	3.46	Cypermethrin-1	14.35
beta-BHC	1.62	Fosthiazate-1	5.22	Oxadixyl	4.14	Cypermethrin-2	9.04
Chlorbufam	7.21	Fosthiazate-2	8.17	Ethion	3.10	Cypermethrin-3	9.50
Clomazone	3.10	Pendimethalin	6.83	Fluacrypyrim	3.59	Cypermethrin-4	9.03
Quintozene	2.85	(E)-Chlorfenvinphos	3.27	Mepronil	1.35	Halfenprox	4.03
Propazine	6.04	Cyprodinil	4.35	Triazophos	6.34	Flucythrinate-1	7.57
gamma-BHC	4.52	Fipronil	6.31	Chlornitrofen	5.56	Flucythrinate-2	7.78
Terbufos	2.88	Dimethametryn	2.44	Carbophenothion	3.72	Quizalofop-ethyl	5.45
Cyanophos	4.00	Penconazole	4.01	Cyanofenphos	4.13	Etofenprox	4.39
Fonofos	5.74	Chlozolinate	10.85	Trifloxystrobin	2.52	Silafluofen	1.71
Propyzamide	1.97	Tolylfluanid	5.13	Edifenphos	7.31	Fluridone	2.51
Pyroquilon	4.53	Isofenphos	2.82	Norflurazon	4.58	Pyrimidifen	4.83
Diazinon	3.33	Phenthoate	3.43	Propiconazole-1	7.77	Flumioxazin	13.53
Pyrimethanil	3.55	Quinalphos	7.29	Propiconazole-2	7.45	Fenvalerate-1	7.70
Isazofos	3.89	Thiabendazole	3.55	Quinoxyfen	2.34	Fenvalerate-2	5.06
Tefluthrin	2.58	Dimepiperate	2.29	(E)-Pyriminobac-methyl	0.69	Pyraclostrobin	3.40
Terbacil	6.73	Procymidone	1.15	Endosulfan sulfate	12.96	Difenoconazole-1	9.02
Etrimfos	3.20	Bromophos-ethyl	1.78	Lenacil	2.35	Difenoconazole-2	3.94
delta-BHC	7.35	Methidathion	2.84	Chloridazon	7.76	Indoxacarb	13.62
Tri-allate	4.62	Chlorbenside	3.44	Tebuconazole	5.87	Azoxystrobin	8.90
Tebupirimfos	3.79	Propaphos	7.33	Piperonyl butoxide	3.48	Dimethomorph-1	6.48
Iprobenfos	2.12	Tetrachlorvinphos	6.45	Epoxiconazole	0.42	Dimethomorph-2	7.81
Benoxacor	9.77	Trichlamide	13.53	Zoxamide	7.71	Tolfenpyrad	5.71
Dichlofenthion	2.62	Paclobutrazol	3.63	Pyributicarb	5.85	Imibenconazole	7.03
Dimethenamid	2.55	Butachlor	4.03	Chlomethoxyfen	4.34	Cinidon-ethyl	10.40

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GC-MS
Gas Chromatograph Mass Spectrometer

C-52

Simultaneous Analysis of 477 Residual Pesticides in Agricultural Crops Using GC-MS/MS - Part 1

INTRODUCTION

GC-MS/MS systems can measure more than 400 residual pesticides in foods. However, analyzing more than 400 pesticides simultaneously requires a short dwell time (data loading time) during MRM measurements, which results in problems with inadequate sensitivity and the tedious process of creating MRM measurement programs. Consequently, several different methods are used for target pesticides and the same sample is measured multiple times to analyze all components. That can decrease productivity, due to the time required for analyzing all the components involved in the large number of pesticides being inspected. This Application Data Sheet describes a solution to these problems with the creation of a method for simultaneously analyzing 477 components and evaluating the resulting sensitivity and accuracy.

EXPERIMENTAL

Matrix solutions were prepared by processing soy bean, orange, brown rice, and spinach samples according to a pretreatment procedure for residual pesticide analysis, and then purifying them using the GPC Cleanup System (from Shimadzu Corporation).[1)] Measurement sample solutions (1 g/mL sample concentration) were then prepared by spiking the prepared matrix solutions with 477 components (including internal standard substances) to a concentration of 5 ppb (or 200 ppb for the internal standard substances). 19 kinds[*1] of surrogate pesticides were used as the internal standard substances.
The GCMS-TQ8040 combined with the Twin Line MS System was used to measure samples based on the analytical conditions listed in Table 1. Two transitions were specified for each component, one for quantitation and the other for confirmation, and Smart MRM was used to automatically create a measurement program.

Table 1: Analysis Conditions

GC-MS:	GCMS-TQ8040 (Twin Line MS System)
Column 1:	SH-Rxi-5Sil MS (30m L., 0.25 mm I.D., df=0.25 µm) (Shimadzu, P/N: 221-75954-30)
Column 2:	SH-Rtx-200 MS (30m L., 0.25 mm I.D., df=0.25 µm) (Shimadzu, P/N: 221-75811-30)
Glass Insert :	Sky Liner, Splitless Single Taper Gooseneck w/Wool (Restek, P/N: 567366)

[GC]		[MS]	
Injection Temp.:	250 °C	Interface Temp.:	300 °C
Column Oven Temp.:	60 °C (1 min) → (25 °C /min) → 160 °C → (4 °C /min) → 240 °C → (10 °C /min) → 290 °C (11 min)	Ion Source Temp.:	200 °C
		Measurement Mode:	MRM
Injection Mode:	Splitless	Loop Time:	0.4 sec
High Pressure Injection:	250 kPa (1.5 min)	Processing Time Required:	0.3 min
Carrier Gas Control:	Linear Velocity (40.0 cm/sec)		
Injection Volume:	2 µL		

RESULTS AND DISCUSSION

The relationship between the dwell time and retention time in the measurement program created using Smart MRM is shown in Fig. 1. The average dwell time for all components was 12.3 msec, with over 6.5 msec provided even for retention time bands where a high number of pesticides were eluted. Consequently, compared to conventional measurement methods that divide analysis into segments, Smart MRM provides, on average, 2.5 time longer dwell times and makes it easy to create optimal MRM measurement programs.

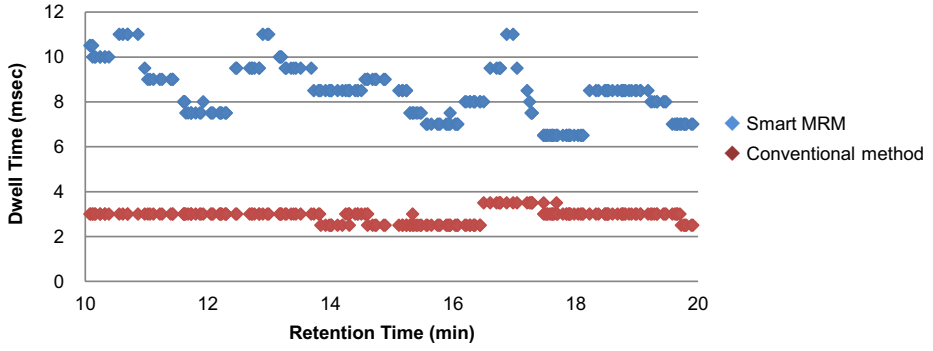

Fig. 1 Relationship Between Retention Time and Dwell Time (for retention times from 10 to 20 minutes)

The repeatability for each matrix was used to evaluate whether the measurement program created using Smart MRM provided adequate sensitivity. The %RSD distribution obtained for each matrix is shown in Fig. 2 and the %RSD values for 100 of the 477 components are tabulated in Table 2. These results show that %RSD (n = 5) was 10 % or less for 88 % of targets (1618 of the 1832 components in four types of matrix), which indicates that high analytical accuracy was achieved when analyzing as many as 477 components simultaneously. By eliminating the need to split the analysis using multiple methods, the number of injections is reduced and productivity increased. This also allows maintenance frequency and costs to be minimized.
Though matrix interference was identified for a few components, high-accuracy detection was possible by using the Twin Line MS system, which uses two columns with different separation characteristics. For information regarding the Twin Line MS System, refer to Application Data Sheet 107.

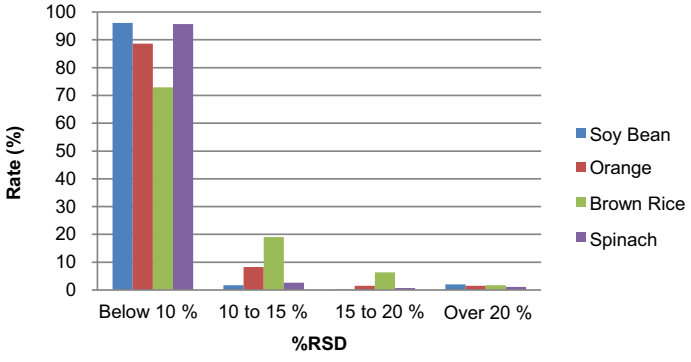

Fig. 2 %RSD Distribution for Each Matrix

Table 2: %RSD (n = 5) of Samples Spiked with Pesticides (5 ppb)

Name of Compound	Soy Bean	Orange	Brown Rice	Spinach	Name of Compound	Soy Beas	Orange	Brown Rice	Spinach	Name of Compound	Soy Bean	Orange	Brown Rice	Spinach
Methamidophos	4.82	7.66	8.84	1.86	Fthalide	6.92	5.01	12.09	4.79	Trifloxystrobin	6.80	8.83	8.84	5.89
Acephate	4.72	4.29	4.85	6.89	Fosthiazate-1	9.53	4.33	14.06	3.16	Tebuconazole	6.63	4.46	9.34	5.18
Propham	4.55	3.84	15.43	3.13	Fosthiazate-2	7.90	3.44	15.15	8.60	Piperonyl butoxide	4.41	3.91	9.63	2.78
Clothianidin	3.66	4.84	7.53	2.92	Pendimethalin	9.80	5.24	9.81	6.47	Acetamiprid	7.56	7.09	8.10	3.44
Chloroneb	4.35	2.74	12.91	5.54	Fipronil	9.68	11.76	9.33	9.72	Iprodione	8.26	3.90	9.09	3.65
Fenobcarb	2.95	3.02	7.72	2.14	Heptachlor-exo-epoxide	5.92	13.72	8.89	7.93	EPN	7.68	9.03	5.85	7.96
Phorate	6.20	4.87	10.62	4.72	Thiabendazole	4.22	3.90	9.34	5.03	Bromopropylate	4.58	3.65	9.49	3.71
Dimethoate	7.03	5.21	8.75	6.91	Captan	14.99	3.41	5.66	10.28	Bifenthrin	4.52	2.87	8.77	2.67
gamma-BHC	9.73	3.38	9.13	7.18	Phenthoate	8.96	1.92	9.77	5.60	Bifenazate	9.05	7.67	9.58	6.96
Cyanophos	5.89	3.82	8.64	4.01	Quinalphos	6.63	5.12	8.38	7.46	Fenpropathrin	8.53	4.96	9.79	9.01
Terbufos	2.89	4.37	7.94	5.04	Procymidone	4.31	5.49	12.58	5.87	Tebufenpyrad	3.66	4.18	9.73	2.88
Diazinon	8.13	4.68	9.35	7.42	Triflumizole	7.71	7.73	7.93	8.74	Tetradifon	8.47	4.02	8.83	7.17
Pyrimethanil	2.80	3.38	8.13	5.52	Chinomethionat	7.98	4.48	11.60	1.82	Azinphos-methyl	7.95	8.06	8.35	5.72
Iprobenfos	3.77	3.83	12.89	3.25	Trichlamide	7.78	3.23	9.93	5.82	Pyriproxyfen	4.25	6.00	5.30	3.39
Benoxacor	7.31	1.86	8.91	4.25	Butachlor	9.05	5.75	8.79	5.25	Fenarimol	1.45	4.13	9.64	2.72
Acetochlor	6.74	6.94	8.74	3.01	Alpha-endosulfan	8.92	3.48	9.39	3.12	Acrinathrin	5.27	9.02	8.37	8.17
Parathion methyl	7.86	4.91	7.77	3.41	Mepanipyrim	4.63	3.89	9.55	3.77	Coumaphos	5.15	6.18	7.79	4.18
Tolclofos-methyl	8.51	7.87	8.79	1.95	Hexaconazole	5.49	8.17	8.81	5.20	Pyridaben	6.42	3.16	7.25	1.59
Carbaryl	4.44	8.21	8.83	6.73	Imazalil	8.84	5.09	8.01	4.24	Cypermethrin-1	8.23	8.70	7.71	1.42
Heptachlor	7.92	3.29	8.59	4.05	Flutolanil	4.88	3.61	9.69	1.93	Boscalid	5.29	14.34	9.02	3.51
Metalaxyl	2.88	6.82	14.92	5.22	Prothiofos	9.31	4.77	10.21	4.80	Cypermethrin-2	8.68	5.80	8.49	7.71
Prometryn	4.48	5.90	8.83	7.87	Isoprothiolane	3.65	4.46	8.04	5.86	Cypermethrin-3	9.28	5.31	8.79	5.44
Pirimiphos-methyl	7.24	9.41	9.11	6.64	Dieldrin	9.55	8.16	9.39	6.59	Cypermethrin-4	4.59	12.36	2.67	7.80
Fenitrothion	9.87	6.55	5.77	7.20	Myclobutanil	4.80	5.72	9.55	2.11	Ethofenprox	4.72	7.17	7.04	3.51
Linuron	7.87	6.27	13.16	4.65	o,p'-DDD	5.51	3.71	11.30	3.02	Silafluofen	3.09	10.17	8.81	2.84
Malathion	9.97	7.47	7.37	2.98	Flusilazole	7.51	7.35	8.85	5.56	Fenvalerate-1	8.28	14.86	9.21	6.41
Metolachlor	3.77	3.78	12.20	4.78	Kresoxim-methyl	6.77	6.34	13.15	3.58	Fenvalerate-2	8.60	16.74	8.23	4.30
Chlorpyrifos	7.22	3.28	9.78	6.08	Chlorfenapyr	10.54	7.30	5.37	7.93	Difenoconazole-1	1.52	9.27	7.86	2.83
Thiobencarb	7.77	2.08	9.59	4.36	Isoxathion	9.10	7.85	12.21	9.12	Difenoconazole-2	5.84	9.25	7.16	7.27
Diethofencarb	5.44	4.17	12.25	6.75	Beta-endosulfan	8.66	8.25	12.65	4.06	Azoxystrobin	5.01	4.22	4.54	5.80
Fenthion	4.11	5.06	9.33	5.36	Ethion	5.81	4.81	9.01	4.22					
Parathion	7.43	8.93	9.05	5.05	Triazophos	6.42	4.64	8.64	2.63	Among Total of 458 Components*2				
Triadimefon	5.66	7.30	9.52	9.15	Edifenphos	7.40	6.61	9.89	7.70	Number of Components with 10 % or Lower	440	406	334	438
Tetraconazole	9.01	8.56	9.70	6.59	Endosulfan sulfate	8.23	4.19	7.14	5.54	Average %RSD (excluding N.D.)	6.62	6.46	9.90	5.55
Dicofol degradation products	4.91	3.99	11.87	7.33	Quinoxyfen	4.23	8.59	12.91	2.30					

Items determined to have 20 % or more overlap (area values) between pesticide-spiked and blank samples are underlined (reference data).
*2 Excludes the 19 internal standard substances.

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GC-MS
Gas Chromatograph Mass Spectrometer

C-53 Simultaneous Analysis of 477 Residual Pesticides in Agricultural Crops Using GC-MS/MS - Part 2

INTRODUCTION

Application Data Sheet No. 106 showed that it is possible to simultaneously analyze 477 components with high sensitivity and high accuracy by using a measurement program created using Smart MRM. However, there were still cases where matrix interference was unavoidable even when using highly selective MRM analysis. Therefore, this Application Data Sheet presents results from analysis using two columns with different separation characteristics: a general-purpose 5 % phenyl / 95 % methylpolysiloxane column and a trifluoropropyl methyl polysiloxane column.
By using the Twin Line MS System, both of these columns can be installed in the same GC-MS/MS system at the same time for continuous analysis without having to release the vacuum or replace columns.

EXPERIMENTAL

Matrix solutions were prepared by processing soy bean, orange, brown rice, and spinach samples according to a pretreatment procedure for residual pesticide analysis, and then purifying them using the GPC Cleanup System (from Shimadzu Corporation).[1)] Measurement sample solutions (1 g/mL sample concentration) were then prepared by spiking the prepared matrix solutions with 477 components (including internal standard substances) to a concentration of 5 ppb (or 200 ppb for the internal standard substances). 19 kinds*[1] of surrogate pesticides were used as the internal standard substances.
The GCMS-TQ8040 combined with the Twin Line MS System was used to measure samples based on the analytical conditions listed in Table 1. Two transitions were specified for each component, one for quantitation and the other for confirmation, and Smart MRM was used to automatically create a measurement program.

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Glass Insert :	Sky Liner, Splitless Single Taper Gooseneck w/Wool (Restek, P/N: 567366)

[GC]		[MS]	
Injection Temp.:	250 °C	Interface Temp.:	300 °C
Column Oven Temp.:	60 °C (1 min) → (25 °C /min) → 160 °C → (4 °C /min) → 240 °C → (10 °C /min) → 290 °C (11 min)	Ion Source Temp.:	200 °C
		Measurement Mode:	MRM
Injection Mode:	Splitless	Loop Time:	0.4 sec
High Pressure Injection:	250 kPa (1.5 min)	Processing Time Required:	0.3 min
Carrier Gas Control:	Linear Velocity (40.0 cm/sec)		
Injection Volume:	2 μL		

Fig. 1: GCMS-TQ8040 with Twin Line MS System

RESULTS AND DISCUSSION

Results from analysis using columns 1 and 2 are shown in Figs. 2 and 3. Due to matrix interference, some pesticide peaks cannot be detected properly with column 1, but using column 2 allows separation of the matrix and results in accurate detection. Furthermore, high-precision analytical results can be obtained even when using column 2.
If a peak is detected in data from column 1, then the data from column 2 can be used to confirm that the peak is from a pesticide.

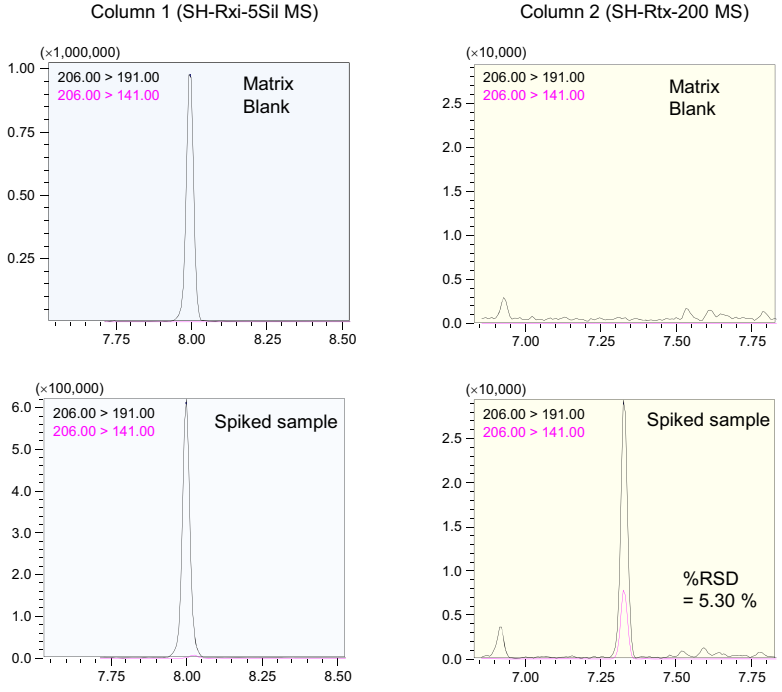

Fig. 2: MRM Chromatograms of Chloroneb in a Soy Bean Sample Using Column 1 (left) and Column 2 (right)

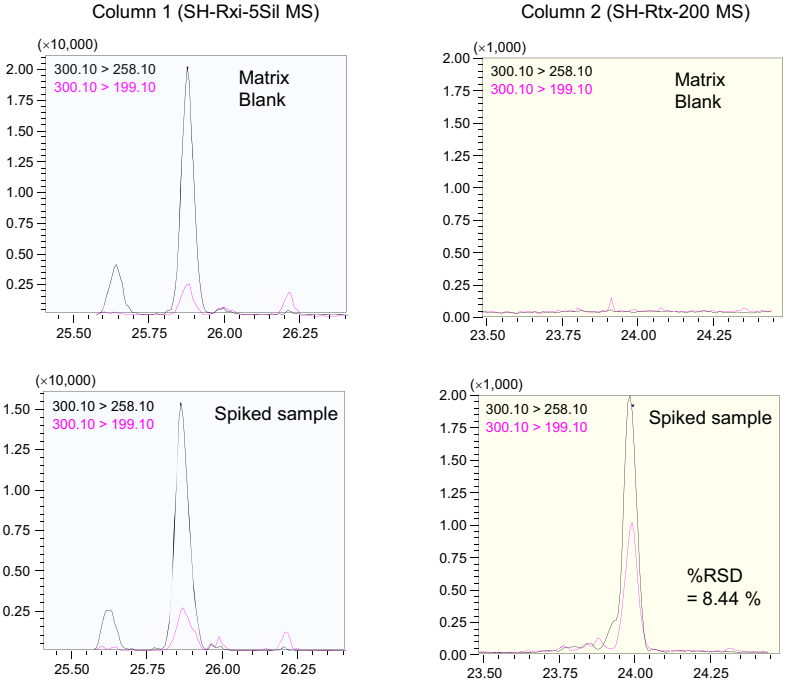

Fig. 3: MRM Chromatograms of Bifenazate in a Brown Rice Sample Using Column 1 (left) and Column 2 (right)

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C-54

Multi-Residue Analysis of Pesticides in Green Tea Using Caffeine Removal Pretreatment

Food

1. Introduction

Green tea is becoming a popular beverage worldwide. Table 1-1 through Table 1-3 show the survey results for worldwide green tea production, and import and export quantities. With about 4 million tons produced worldwide, which is about half that of coffee bean production, China boasts the greatest rate of green tea production, followed by India, Kenya and Sri Lanka.

Sri Lanka is the greatest exporter of green tea, followed by Kenya, India and China. Domestic consumption is very high in India and China, with about 80 % of the production consumed in those countries. Japan also is a high-producing country, but due to even higher consumption, Japan also imports an amount which is equivalent to 50 % of its own production level.

The 27 European Union (EU) countries are the greatest importers, followed by the Russian Federation and the United Kingdom, with very high consumption clearly occurring in Europe.

Due to recent concern regarding food safety among consumers, advances in analytical methods for detecting and quantifying pesticide residues now permit the inspection of many crops for the presence of residual pesticides. With an increasing number of pesticides becoming subject to inspection every year, mass spectrometers are the instrument of choice for conducting simultaneous analyses targeting multiple pesticide residues.

The multi-residue analysis of pesticides in teas has become common worldwide. Caffeine, which is typically present in large quantities, can interfere with detection and quantitation of pesticides and other tea constituents, and is also a source of contamination in analytical instrumentation.

The development of an analytical method for multi-residue analysis of pesticides in green tea by gas chromatography with mass spectrometry (GCMS) is reported in this Application Note. A novel technique was employed to easily and efficiently eliminate caffeine to avoid any adverse effect on pesticide recoveries.

Table 1-1 Tea Production (2009)

Rank	Area	Production (tonnes)
1	China	1375780
2	India	972700
3	Kenya	314100
4	Sri Lanka	290000
5	Turkey	198601
6	Viet Nam	185700
7	Indonesia	146440
8	Japan	86000
9	Argentina	71715
10	Thailand	63707
11	Bangladesh	59500
12	Malawi	52559
13	Uganda	48663
14	Iran (Islamic Republic of)	165717
15	United Republic of Tanzania	32000
16	Myanmar	30500
17	Zimbabwe	20862
18	Rwanda	20000

Table 1-2 Tea Export (2009)

Rank	Area	Production (tonnes)
1	Sri Lanka	288528
2	Kenya	331594
3	China	305352
4	India	203863
5	EU(27)ex.int	29882
6	United Kingdom	27741
7	Germany	25301
8	Indonesia	92304
9	United Arab Emirates	23681
10	Viet Nam	82416
11	Malawi	47356
12	Belgium	7859
13	Argentina	69816
14	United Republic of Tanzania	30438
15	Russian Federation	9713
16	Netherlands	18158
17	Poland	8609
18	Uganda	44446

Table 1-3 Tea Import (2009)

Rank	Area	Production (tonnes)
1	EU(27)ex.int	249930
2	Russian Federation	182149
3	United Kingdom	145960
4	United States of America	110861
5	United Arab Emirates	75255
6	Egypt	80304
7	Pakistan	96932
8	Iran (Islamic Republic of)	51733
9	Japan	43301
10	Saudi Arabia	20331
11	Syrian Arab Republic	30651
12	Germany	44267
13	Canada	17353
14	France	17695
15	Poland	41784
16	Morocco	54400
17	Ukraine	26915
18	Netherlands	29982

Reference : Food and Agriculture Organization of The United Nations , FAOSTAT
http://faostat.fao.org/default.aspx

2. Maximum Pesticide Residue Levels in Tea and Analytical Method

The levels of pesticide residues in food are established in various countries around the world using Maximum Residue Levels (MRL) and Tolerance values. Methods of regulation vary depending on the country, but the applicable pesticides are recorded, their appropriate usage conditions are specified, and their MRL values are set for foods. Although these have been determined based on impact assessments on the human body, reference values are set in consideration of the various types of food products, as intake varies depending on the type of food.
Reference values are set by the EU and Japan for many pesticides used in tea production. In the EU, MRL are specified for each agricultural product under Regulation (EC) No. 396/2005 Annexes. In Japan, the Ministry of Health, Labour and Welfare establishes MRL values for each product, and these can be viewed on the Ministry's home page.
The analytical method follows the Official Method of the AOAC INTERNATIONAL (AOAC). The United States Food and Drug Administration (FDA) publishes the Pesticide Analytical Manual (PAM), which specifies multi-residue methods as well as methods for individual pesticide compounds. The PAM multi-residue simultaneous analysis methods include methods for Non-fatty Foods and for Fatty Foods. Japan's Ministry of Health, Labour and Welfare categorizes the multi-residue analytical method based on whether the product is a cereal or a fruit, and a test method for tea is also indicated. Fig. 1 illustrates the analysis flow specified in the test method indicated by Japan's Ministry of Health, Labour and Welfare. In Japan, the system by which pesticides are regulated in foods is referred to as a "positive list system," which is referred to below as Japan's Positive List Test Method.
In this investigation, 250 target pesticide compounds were analyzed following Japan's Positive List Test Method, and using the Shimadzu GCMS QP-2010 Plus shown below. Fig. 2 shows GCMS chromatogram of a 1 mg/L (ppm) standard mixture of the 250 pesticides. Analytical conditions are shown in Table 2.

Regulation (EC) No 396/2005
http://ec.europa.eu/food/plant/protection/pesticides/community_legislation_en.htm

Pesticide Analytical Manual (PAM)
http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/PesticideAnalysisManualPAM/default.htm

Japan's Ministry of Health, Labour and Welfare
http://www.mhlw.go.jp/english/topics/foodsafety/positivelist060228/index.html

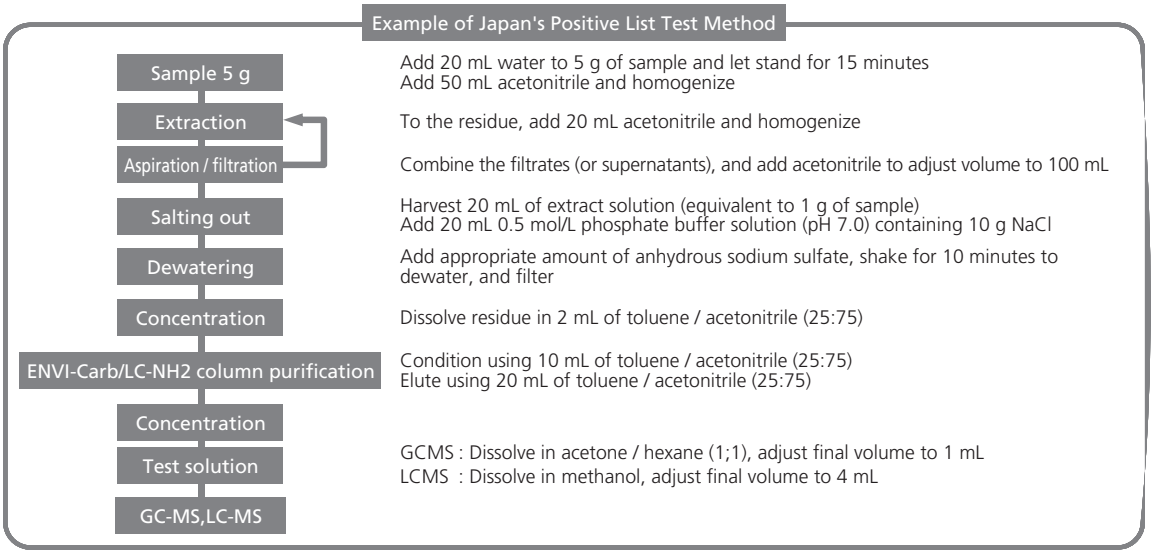

Fig. 1 Flow Diagram of Japan's Positive List Test Method

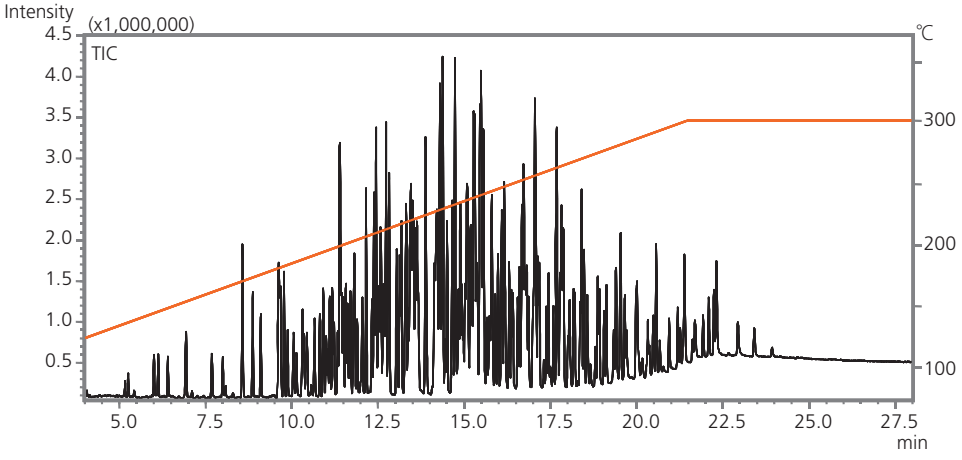

Fig. 2 Total Ion Chromatogram (TIC) of 250 Pesticides Analyzed by GCMS

Table 2 Analytical Conditions

Instrument	: Shimadzu GCMS-QP2010 Plus	Oven program	: 50 °C (1 minute) 25 °C/minute to 125 °C (0 minute) 10 °C/minute to 300 °C (10 minutes)
Inlet	: 1-µL injection volume High-pressure splitless mode (250 kPa, 1.5 minute) 250 °C	Interface	: 250 °C
Column	: Rtx-5MS, 30 mL. × 0.25 mmI.D, df 0.25 µm Helium carrier gas, constant linear velocity (47.0 cm/second)	MS operation	: Electron Impact (EI) ionization Full scan mode, *m/z* 45-550

3. Analysis of Tea

Analysis of pesticide residues in commercially available green tea was conducted using the method conditions described above. After pretreatment according to Japan's Positive List Test Method, analysis by GCMS indicated that none of the 250 target pesticides were detected in the real-world sample. The Total Ion Chromatogram (TIC) obtained from analysis of the green tea extract is shown in Fig. 3. Ideally, method verification should be performed using a sample known to contain one or more of the target pesticides within the calibration range of the method. Since an actual tea sample contaminated with the target compounds was not available, a spike and recovery test was used to verify detection of pesticides and validate the method. A standard solution of pesticides was added to the green tea at a known concentration during the homogenization step. The extract was analyzed by GCMS and individual pesticide peaks were quantified against a calibration curve to verify recovery of the spiked pesticides.

The standard mixture of pesticides was added to the sample at a concentration of 100 µg/L (ppb).The recovery rate (%) obtained in this test was used to assess the test method. Table 3 shows the pesticides for which good recovery was obtained, at 70 % - 120 %.

Analytical interferences such as pigments, proteins, waxes, and other high molecular weight materials are co-extracted from the analytical sample along with the pesticides. Despite the absence of pesticide peaks in the chromatogram of Fig. 3, many peaks were detected. Because caffeine is present in large quantities in tea, its peak, which is seen to elute in the retention time range of 8 – 10 minutes, interferes with the detection of several pesticides in this portion of the chromatogram. Caffeine, which is present at high concentrations in coffee and various teas, including green tea and black tea, behaves much like the targeted pesticide compounds during extraction and cleanup, and is therefore difficult to eliminate using the pretreatment process that was used here.

Depending on the type of solid phase cartridge used as an extract clean-up step, caffeine can be retained, thereby allowing its elimination from the sample solution. Fig. 4 shows an example of caffeine reduction through the use of a Florisil® column. However, due to the similar characteristics of caffeine and pesticides, they are both likely to remain in the cartridge with this processing. Thus, a separate step would be required to elute the pesticides from the cartridge, which would increase the pretreatment time. It would also require two separate GCMS analyses for each sample of green tea. Thus, finding a simplified procedure for caffeine removal was a major priority with respect to the analysis of pesticide residues in tea.

Shimadzu GCMS-QP2010 Plus Used in This Study

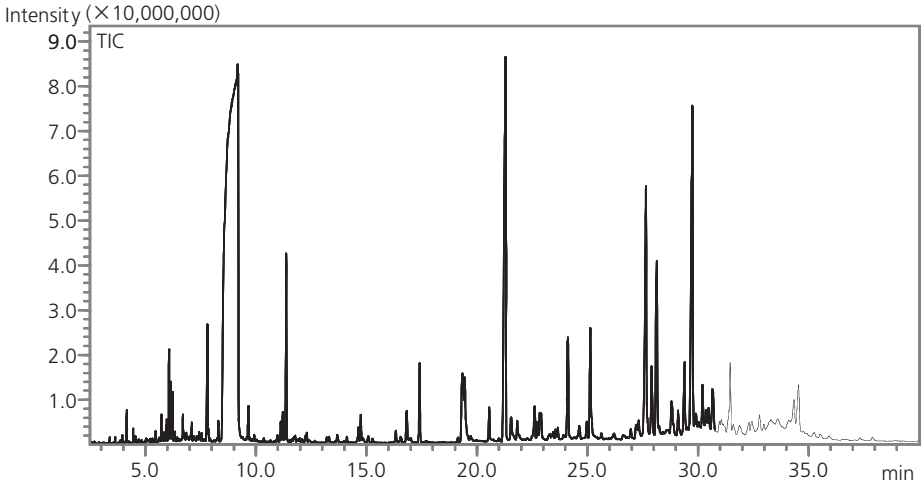

Fig. 3 GCMS Chromatogram of a Green Tea Extract

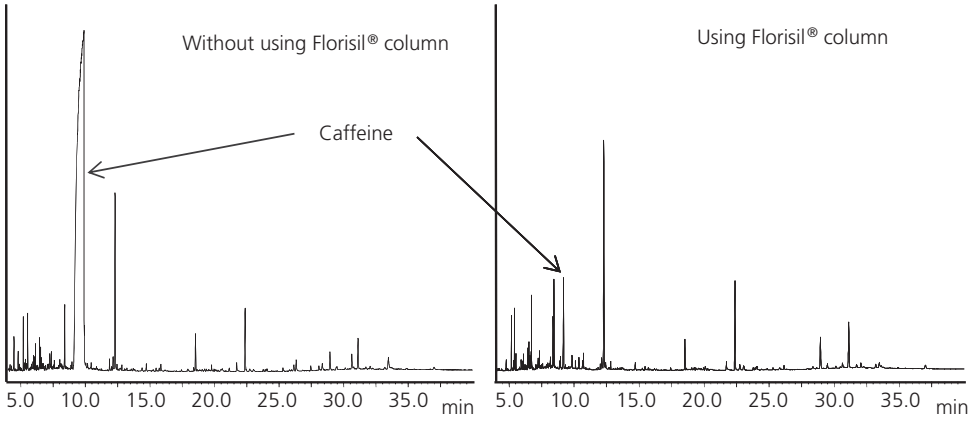

Fig. 4 Caffeine Reduction Due to Florisil® Column

Table 3 Green Tea Sample Spike and Recovery Test Results

Pesticide Name	Recovery (%)
EPTC	104
Mevinphos	86
Etridiazole	102
Chloroneb	97
XMC	106
Fenobucarb	88
Tecnazene	92
Propoxur	93
Propachlor	100
Diphenylamine	103
Ethoprophos	103
Chloropropham	103
Ethalfluralin	103
Trifluralin	113
Bendiocarb	92
Benfluralin	111
Cadusafos	100
alpha-BHC	99
Hexachlorobenzene	96
Dicloran	103
Dimethoate	90
Carbofuran	78
Atrazine	72
Propazine	79
beta-BHC	96
gamma-BHC	96
Propetamphos	101
Terbufos	101
Cyanophos	111
Quintozene	110
Pyroquilon	84
Pyrimethanil	93
Diazinone	100
Phosphamidon-1	73
Prohydrojasmon-1	103
Tefluthrin	102
delta-BHC	103
Triallate	109
Iprobenfos	111
Pirimicarb	90
Benoxacor	107
Benfuresate	111
Dichlofenthion	96
Propanil	89
Bromobutide	108
Spiroxamin-1	82
Acetochlor	102

Pesticide Name	Recovery (%)
Vinclozolin	93
Parathion-methyl	101
Chlorpyrifos-methyl	98
Tolclofos-methyl	107
Carbaryl	106
Alachlor	116
Heptachlor	75
Prometryn	100
Metalaxyl	86
Spiroxamin-2	89
Terbutryn	114
Malathion	108
Thiobencarb	111
Chlorpyrifos	109
Diethofencarb	110
Aldrin	92
Metolachlor	95
Fenpropimorph	98
Fenthion	104
(Z)-Dimethylvinphos	100
Parathion	120
Triadimefon	110
Isofenphos oxon	111
Chlorthal-dimethyl	109
Nitrothal-isopropyl	106
Bromophos	98
Fthalide	105
Diphenamid	91
Fosthiazate-2	99
E-Chlorfenvinphos	106
Dimethametryn	103
Penconazole	100
Heptachlor epoxide (A)	93
Oxy-Chlordane	96
(Z)-Pyrifenox	102
Heptachlor epoxide (B)	84
alpha-Chlorfenvinphos	117
Diclocymet-1	110
Quinalphos	100
Phenthoate	102
Zoxamide deg.	106
Procymidone	100
trans-Chlordane	105
Methidathion	70
Diclocymet-2	105
(E)-Pyrifenox	92
Tetrachlorvinphos	105

Pesticide Name	Recovery (%)
Fenamiphos	97
Flutolanil	114
Hexaconazole	113
Imazalil	92
Isoprothiolane	105
Profenofos	110
Tribufos	114
Pretilachlor	109
Uniconazole P	102
p,p'-DDE	104
Oxadiazon	113
Dieldrin	86
Oxyfluorfen	112
Flamprop-methyl	82
Myclobutanil	98
Buprofezin	84
Imibenconazole-debenzyl	70
Flusilazole	95
Thifluzamide	106
Bupirimate	88
Kresoxim-methyl	101
Isoxathion	117
Cyproconazole	92
Chlorfenapyr	115
Fenoxanil	115
Chlorobenzilate	106
beta-Endosulfan	91
Fensulfothion	94
(Z)-Pyriminobac-methyl	120
p,p'-DDD	107
o,p'-DDT	104
Mepronil	106
Fluacrypyrim	115
Triazophos	91
Benalaxyl	99
Edifenphos	87
Quinoxyfen	74
Propiconazole-1	104
Trifloxystrobin	115
Norflurazon	88
Lenacil	90
Endsulfan sulfate	91
p,p'-DDT	109
Propiconazole-2	95
(E)-Pyriminobac-methyl	103
Tebuconazole	97
Diclofop-methyl	115

Pesticide Name	Recovery (%)
Thenylchlor	111
Diflufenican	99
Propargite	101
Piperonyl butoxide	102
Zoxamide	70
Mefenpyl-diethyl	101
Iprodione	114
Pyridaphenthion	110
Bifenthrin	105
Bromopropylate	119
Phosmet	93
EPN	92
Tebufenpyrad	112
Bifenox	112
Anilofos	91
Phenothrin-2	102
Tetradifon	102
Phosalone	93
Pyriproxyfen	96
Cyhalothrin-1	104
Cyhalofop-butyl	100
Mefenacet	90
Cyhalothrin-2	120
Fenarimol	95
Pyrazophos	105
Pyraclofos	90
Fenoxaprop-ethyl	77
Bitertanol-1	89
trans-Permethrin	109
Bitertanol-2	101
cis-Permethrin	106
Pyridaben	97
Cyfluthrin-1	93
Cafenstrole	83
Fenbuconazole	88
Halfenprox	112
Flucythrinate-1	104
Flucythrinate-2	99
Fenvalerate-1	91
Fluvalinate-1	114
Fenvalerate-2	108
Fluvalinate-2	98
Difenoconazole-1	87
Difenoconazole-2	88
Flumiclorac-pentyl	96
Tolfenpyrad	93
Imibenconazole	91

4. Investigation of Caffeine Removal Prior to Detection of Pesticide Residues in Tea by GCMS

The presence of a large amount of caffeine in tea not only interferes with the detection of pesticides by GCMS, it is a source of contamination of the injection port liner and the GC column. Furthermore, it can sometimes affect the analysis results, shifting the retention times of pesticides in the same chromatographic region as caffeine. The method which employs a solid phase cartridge, as shown in Fig. 4, is time-consuming, and increases the number of analyses. For this study caffeine was removed using a simple procedure that exploits the physical properties of caffeine.

4.1 Caffeine Removal Study

Fig. 5 shows the structural formula of caffeine. The high solubility of caffeine in polar solvents permits large amounts of caffeine to be dissolved in the extraction solvent, e.g. acetone. Moreover, as the temperature of the solution rises, even greater amounts of caffeine are dissolved.

Japan's Positive List Test Method for GCMS analysis of pesticides specifies an extraction solvent having a 1:1 ratio of acetone and hexane. Here we considered the physical property of polarity, with acetone as a polar solvent, and hexane a non-polar solvent. Because the polar solvent accounts for half of the ratio of the solution, this solvent mixture is thought to permit caffeine to dissolve easily. Therefore, it was decided to use hexane alone as the solvent, eliminating the use of acetone.

In addition, a lower solution temperature was considered to provide the benefit of impeding the dissolution of caffeine, and therefore the sample extracts were stored in a freezer. This freezing of the solution is referred to below as "freeze processing."

When the sample solution was freeze processed, deposits became suspended in solution (Fig. 6). By applying centrifugation, these deposits were precipitated, and analysis of the supernatant was equivalent to analysis without most of the caffeine. This process was applied to a green tea sample for confirmation of the effect.

To confirm the effect on pesticide recovery using hexane as the extraction solvent along with the application of freeze processing, pesticides were added to a green tea sample solution that was previously subjected to the described pretreatment, and then we applied the change in solvent (using only hexane) in addition to freeze processing. No adverse effect on recovery was observed.

C8H10N4O2
Mol. Wt.:194.19

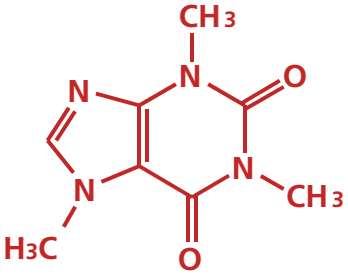

Fig. 5 Structure of Caffeine

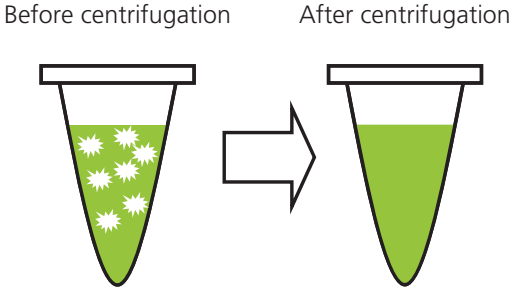

Fig. 6 Status at Vial Tip Before and After Centrifugation

4.2 Caffeine Removal Study Results

After preparing a caffeine-saturated hexane solution, the effect of freeze processing on caffeine removal was evaluated. The chromatograms of caffeine generated before and after processing are shown in Fig. 7, and the caffeine peak area ratio comparison is shown in Table 4. The caffeine content was reduced by 63.5 % as a result of freeze processing.

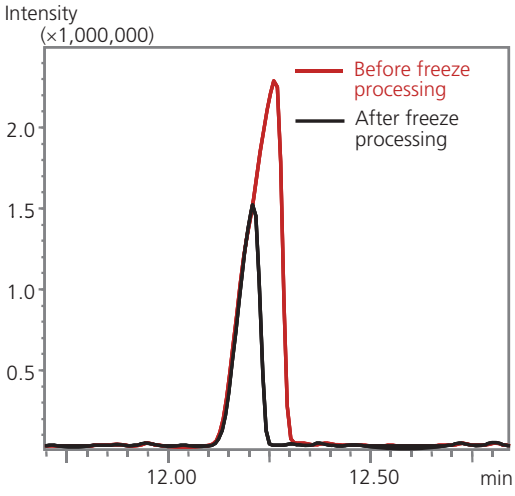

Fig. 7 Effect of Freeze Processing

Table 4 Comparison of Caffeine Area Ratios Before and After Freeze Processing

Before Freeze Processing	After Freeze Processing	Reduction Rate (%)
5209488	1906330	63.5

When freeze processing is conducted, deposits become suspended in the solution. Since centrifugal separation is known to effectively remove these suspended particles, centrifugation time was investigated to determine its effect on the separation. However, any rise in temperature that would occur during longer centrifugation would lessen the effect of freeze processing. Fig. 8 shows the results of the study of centrifugal separation time, allowing for precipitation of deposits following freeze processing. It took at least 1 minute to attain centrifugal separation, but as the centrifugal separation time increased beyond 1 minute, the caffeine area values increase accordingly, until they become constant after 3 minutes. Thus, at 3 minutes, it is thought that the effect of freeze processing would become counterproductive. From this result, we determined that the optimum time required for centrifugal separation is 1 minute.

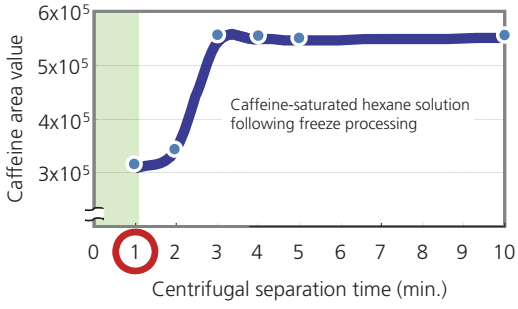

Fig. 8 Result of Investigation of Centrifugal Separation Time

4.3 Applying Caffeine Removal Operation to an Actual Sample

The caffeine removal operations described in the above study were employed to remove caffeine from a real-world tea sample. After processing commercially available tea according to Japan's Positive List Test Method, the solvent that had been specified for use in the obtained final solution was replaced with hexane, followed by freeze processing (-20 °C) and centrifugal separation (1 minute). The obtained supernatant was then analyzed by GCMS. Fig. 9 shows a flow chart of Japan's Positive List Test Method + caffeine removal operations.

Fig. 10 shows the TIC chromatograms obtained before and after caffeine removal by freeze processing. Prior to the removal processing, the caffeine peak is detected as a broad peak that exceeds the column load capacity, but after caffeine removal, a sharp caffeine peak within the column load range is seen, indicating that most of the caffeine was removed. As for the stability of the caffeine removal operation, the repeatability of caffeine area values following caffeine removal operations is shown in Table 5. Caffeine removal by freeze processing can thus be considered to be a stable process that provides good repeatability.

Caffeine removal by solvent replacement and freeze processing was thus confirmed, however actual target pesticides might be removed along with the caffeine. Therefore, spike and recovery testing was performed with respect to the above caffeine removal operation. A test solution prepared using Japan's Positive List Test Method was spiked with a standard mixture of pesticides, and after subjecting this solution to the above described caffeine removal processing, the pesticide recovery rates were obtained.

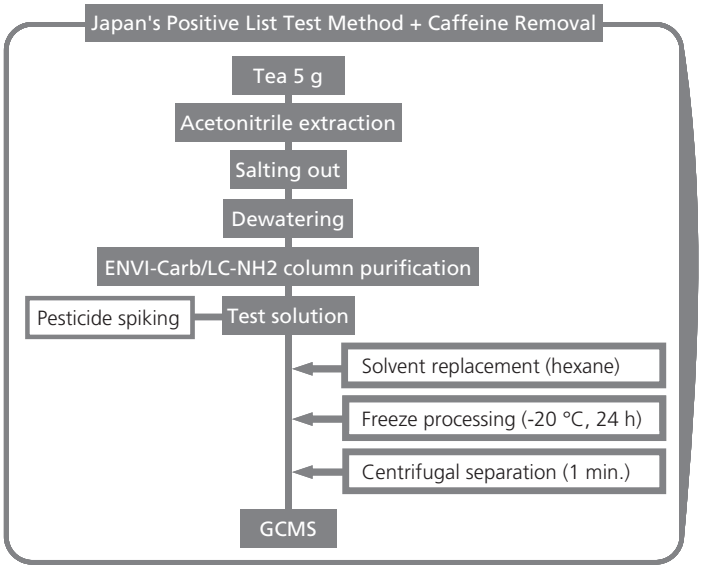

Fig. 9 Flow Diagram of Japan's Positive List Test Method + Caffeine Removal

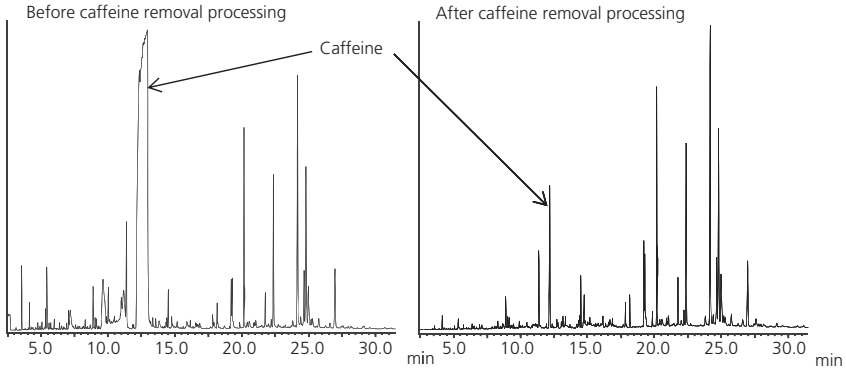

Fig. 10 Effect of Caffeine Removal by Freeze Processing

Table 5 Repeatability of Caffeine Removal Effect

	1	2	3	Average Value	CV%
Caffeine Area Values	4963206	5010227	4996268	4989900	0.48

Fig. 11 shows the pesticide spike and recovery test results obtained after caffeine removal processing. Recovery for most compounds fell between 80 and 120 %, and it was confirmed that switching to a hexane solvent and use of freeze processing did not result in significant loss of pesticides.

Not only was the caffeine peak drastically reduced as a result of the caffeine removal processing, other contaminant substances were eliminated. Removal of these contaminants lessened the adverse effects on the pesticide peak shapes. Fig. 12 shows examples of improved peak detection.

In the case of Mevinphos and Fosthiazate, the interfering peaks before these compounds were removed to allow clear detection of these pesticides. As for Propoxur, the *m/z* 152 peak shape was very different from the *m/z* 110 peak, but this improved as a result of freeze processing. Carbofuran, which co-eluted with another peak, became a single Carbofuran peak.

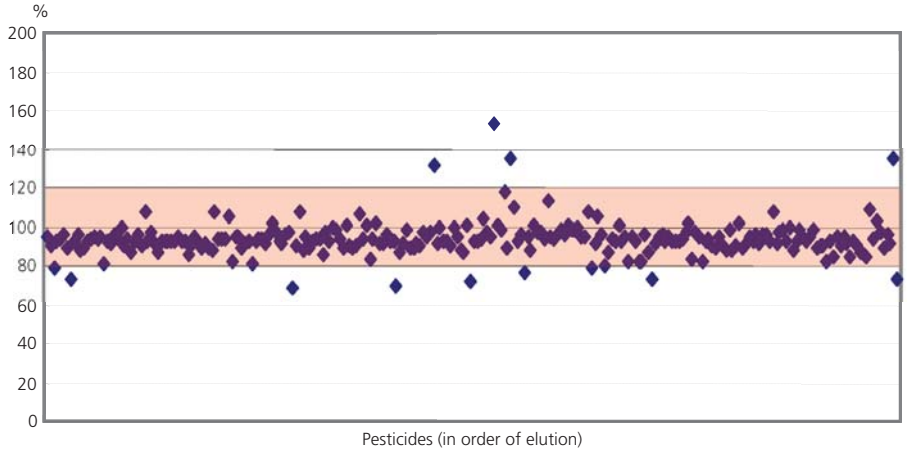

Fig. 11 Pesticide Spike and Recovery Test Results Due to Caffeine Removal Processing

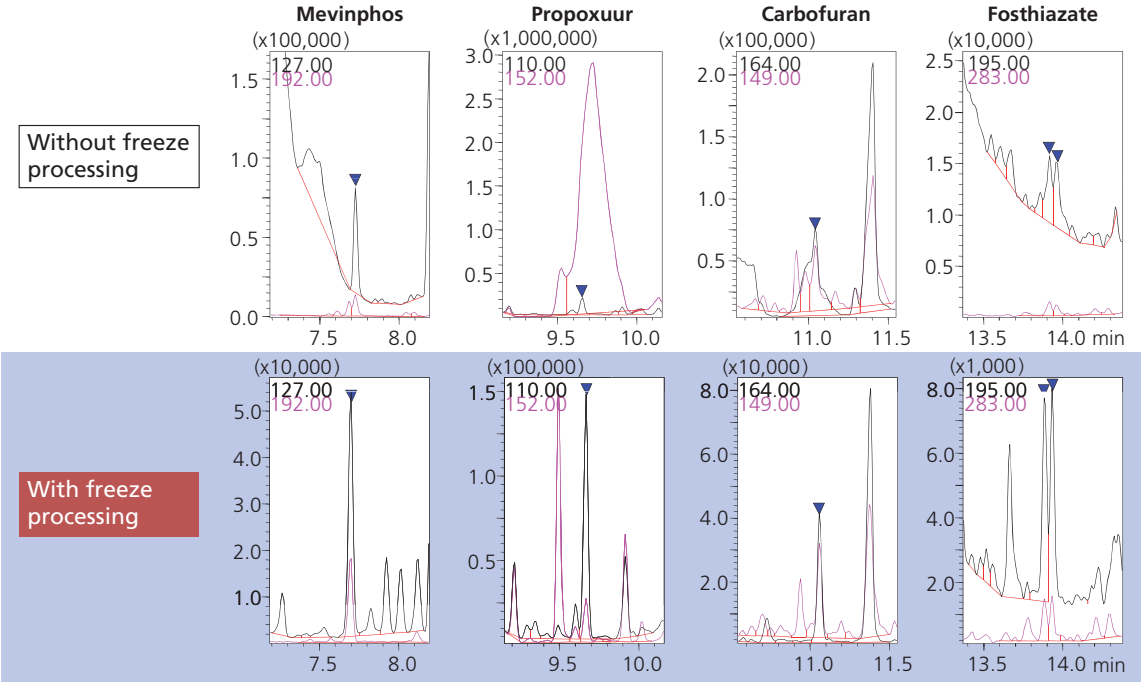

Fig. 12 Examples of Effect of Caffeine Removal Processing (green tea 0.1 ppm)

5. Conclusion

Pesticide residue analysis in tea using Japan's Positive List Test Method for GCMS simultaneous analysis yields good results, but during pesticide analysis by GCMS, the large amount of caffeine that remains in these tea samples can contaminate the GC injection port and column. Similarly, the presence of caffeine can co-elute with and mask the presence of pesticide residues in the same region of the chromatogram. To eliminate the interfering effects of caffeine, the solvent was changed from acetone : hexane (1:1) to 100 % hexane followed by freeze processing, exploiting the physical properties of caffeine, thereby efficiently decreasing the presence of caffeine in the sample. Further, utilizing this removal process, a method was developed for removing caffeine while retaining good recovery and minimal loss of the target pesticides.

SHIMADZU
Excellence in Science

C-55 Determination of 55 pesticide residues in animal derived foods by GPC-GCMS

INTRODUCTION

Removing fats from samples like animal derived foods of high fat content are critical for analysis. At present commonly pretreatment methods are liquid-liquid separation and solid phase extraction. These pretreatment methods are cumbersome and often selective extraction for pesticides. Determination of all kinds of pesticide residues needs different methods of pretreatment. Gel permeation chromatography (GPC) according to volume exclusion principle is used for separation of different molecular weight, so can effectively remove fats and pigments in samples. This method has been applied for sample pretreatment. Off-line GPC method is generally used. Using a lot of organic solvents and operation is cumbersome. The method application has some restrictions.

In this paper, the samples were extracted from homogenized tissue with acetonitrile-water and purified by QuEChERS method, then the determination of 55 pesticide residues in supernatant by on-line GPC-GC/MS was conducted. The calibration curves of 55 pesticide residues were linear in the range of 0.01~0.20 mg/L with good correlation coefficients more than 0.996. The recoveries of the method ranged from 61% to 124%. The method was simple, rapid and reliable, and could meet the requirement the simultaneous determination of 55 pesticide residues in animal derived foods.

EXPERIMENTAL

Instrument: Shimadzu GPC-GCMS

Experimental conditions:

GPC conditions:

Chromatographic column：Shodex CLNpak EV-200 (2.1 mm x 150 mm)

Mobile phase：acetone/cyclohexane (3/7，V/V)

Flow rate：0.1 mL/min

Column temperature：40 ℃

Injection volume：10 μL

GCMS conditions:

Chromatographic column：inert quartz tube：5m x 0.53 mm

Precolumn：Rxi-5 sil MS，5 m x 0.25 mm, 0.25 μm

Analytical column：Rxi-5 sil MS, 25m x 0.25 mm, 0.25 μm

Column temperature: 82 ℃ (5 min)_8 ℃/min_300 ℃ (7.75 min)

Injection temperature:120 ℃ (5 min)_100 ℃/min_250 ℃ (33.7 min)

Pressure: 120 kPa (0 min)_100 kPa/min_180 kPa (4.4 min) _(-49.8 kPa/min)_120 kPa (33.8 min)

Flow control mode：pressure

Column flow：1.75 mL/min

Ion source temperature: 230 ℃

Interface temperature: 300 ℃

Acquisition mode: SIM, conditions are shown in Table 1

Sample preparation

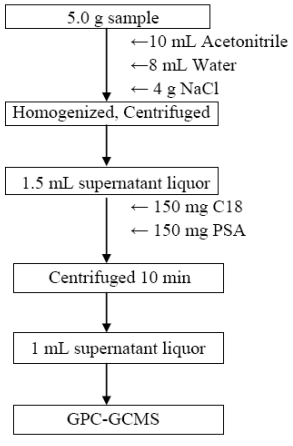

Fig. 1 Schematic flow diagram of the sample preparation

RESULTS AND DISCUSSION

Chromatogram of standard sample

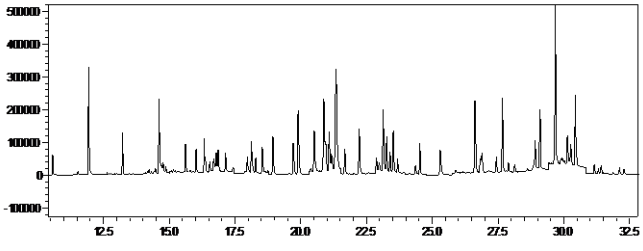

Fig. 2 Chromatograms of mix standards (0.1 mg/L)

Calibration curve , LOD and Recovery

The standard mixture of 55 pesticides was spiked into blank pork solution. The calibration curves of 55 pesticide residues were linear in the range of 0.01~ 0.2 mg/L with good correlation coefficients more than 0.996. The limit of detection were 0.001~0.01 mg/kg (calculated by the S/N=3). The recoveries of the method ranged from 61% to 124%. These results are shown in Table 1.

CONCLUSION

In this paper, the samples were extracted from homogenized tissue with acetonitrile-water and purified by QuEChERS method. 55 pesticide residues in supernatant were determined by on-line GPC-GC/MS. The method was simple,rapid and reliable, and could meet the requirement the simultaneous determination and analysis of 55 pesticide residues in animal derived foods.

Table 1. Retention times, selected ion,correlation coefficients(r), limits of detection(LODs) , recoveries and RSDs of the 55 pesticides（n=3）

No.	Compound	CAS	t_R/min	Monitor ion (*m/z*)	Correlation coefficients(r)	LOD (mg/kg)	Pork Recovery(%)	Pork RSD(%)	Fish Recovery(%)	Fish RSD(%)
1	Dichlorvos	62-73-7	10.624	185*，187，220	0.998	0.003	94.6	0.6	76.2	2.2
2	Dichlobenil	1194-65-6	11.994	171*，173，136	0.997	0.001	87.4	4.1	84.2	6.3
3	Butylate	2008-41-5	13.253	146*，174，217	0.993	0.003	78.7	5.8	71.6	6.6
4	2-Phenylphenol	90-43-7	14.638	170*，169，141	0.993	0.001	90.6	4.0	81.6	5.5
5	Tecnazene	117-18-0	15.633	203*，215，261	0.998	0.003	73.3	7.4	62.6	9.4
6	Chlorethoxyfos	54593-83-8	16.028	153*，263，299	0.997	0.001	87.4	3.2	80.3	5.8
7	Ethalfluralin	55283-68-6	16.546	276*，316，333	0.996	0.002	90.0	3.1	75.4	5.6
8	Chlorpropham	101-21-3	16.693	154*，129，171	0.993	0.01	91.6	4.6	77.2	2.6
9	Trifluralin	1582-09-8	16.788	306*，264，248	0.997	0.003	97.8	3.9	80.3	8.5
10	Benfluralin	1861-40-1	16.861	292*，294，293	0.992	0.001	95.1	5.5	80.3	7.0
11	Phorate	298-02-2	17.145	260*，121，231	0.993	0.004	84.0	8.2	66.9	5.5
12	Quintozene	82-68-8	17.974	237*，295，249	0.997	0.003	75.7	7.2	65.2	6.9
13	Lindane	58-89-9	18.125	181*，183，217	0.995	0.004	82.0	2.5	78.5	8.4
14	Terbufos	13071-79-9	18.289	231*，186，288	0.998	0.003	85.7	1.5	79.8	0.3
15	Diazinon	333-41-5	18.538	304*，179，152	0.9998	0.003	96.4	3.1	91.6	4.4
16	Tefluthrin	79538-32-2	18.941	177*，197，178	0.998	0.001	87.2	7.4	89.2	6.6
17	Chlorpyrifos-methyl	5598-13-0	19.716	286*，290，288	0.997	0.001	94.2	2.1	89.7	6.5
18	Chlorothalonil	1897-45-6,	19.898	266*，264，268	0.998	0.003	86.5	7.4	82.0	8.4
19	Tolclofos-methyl	57018-04-9	19.898	265*，267，250	0.997	0.001	94.1	6.0	90.2	4.5
20	Pirimiphos-methyl	29232-93-7	20.506	290*，276，305	0.9990	0.001	97.6	2.9	94.7	2.2
21	Chlorpyrifos	2921-88-2	20.947	314*，316，286	0.998	0.001	83.1	6.4	120.8	2.0
22	Metolachlor	51218-45-2	20.873	238*，162，240	0.997	0.001	112.3	1.8	89.5	4.2
23	Fenthion	55-38-9	21.073	278*，169，279	0.995	0.003	95.4	5.2	95.7	0.5
24	Parathion	56-38-2	21.157	291*，218，235	0.996	0.003	83.6	5.1	86.9	3.2
25	Isocarbophos	24353-61-5	21.279	230*，136，289	0.997	0.001	79.4	7.0	85.7	7.7
26	Dicofol	115-32-2	21.339	139*，141，250	0.9997	0.001	79.5	1.9	67.0	1.4
27	Isofenphos-methyl	83733-82-8	21.666	199*，241，231	0.995	0.001	97.1	2.1	94.7	4.2
28	Quinalphos	13593-03-8	22.216	146*，156，157	0.998	0.003	93.8	7.5	88.3	4.9
29	alpha-Endosulfan beta-Endosulfan	959-98-8 33213-65-9	22.882 24.352	243*，241，339 241*，339，195	0.998	0.01	68.8	6.0	95.7	2.7
30	Napropamide	15299-99-7	23.124	271*，128，100	0.998	0.007	97.0	7.6	83.0	9.0
31	Prothiofos	34643-46-4	23.260	309*，267，269	0.997	0.01	109.3	9.3	120.8	3.8
32	Pprofenofos	41198-08-7	23.393	339*，337，374	0.9993	0.001	90.8	9.1	90.6	4.6
33	Oxadiazon	19666-30-9	23.512	175*，258，302	0.995	0.001	91.2	6.7	88.4	7.4
34	Oxyfluorfen	42874-03-3	23.680	252*，300，361	0.998	0.003	101.1	6.3	95.2	6.6
35	Ethion	563-12-2	24.520	231*，153，384	0.996	0.01	97.8	4.1	92.8	7.9
36	Quinoxyfen	124495-18-7	25.326	237*，272，307	0.998	0.003	77.9	9.0	70.2	9.0
37	Bbifenthrin	82657-04-3	26.614	181*，166，165	0.997	0.003	76.9	5.4	62.7	9.1
38	Bromopropylate	18181-80-1	26.642	341*，185，183	0.997	0.003	90.6	2.8	79.9	6.1
39	Etoxazole	153233-91-1	26.817	204*，300，359	0.997	0.001	94.9	7.5	90.4	7.7
40	Fenpropathrin	64257-84-7	26.849	181*，265，349	0.994	0.01	99.7	5.1	81.8	4.5
41	Fenamidone	161326-34-7	26.891	268*，238，237	0.992	0.003	118.9	6.6	114.2	7.4
42	Phosalone	2310-17-0	27.437	182*，184，367	0.994	0.003	123.9	9.6	117.3	1.6
43	Pyriproxyfen	95737-68-1	27.665	136*，226，186	0.998	0.003	95.4	8.4	80.1	6.5
44	Cyhalothrin-1 Cyhalothrin-2	91465-08-6 91465-08-6	27.600 27.892	181*，197，208 181*，197，208	0.993	0.007	78.9	3.2	107.2	9.5
45	Fenarimol	60168-88-9	28.134	219*，251，330	0.997	0.007	90.6	4.2	100.4	8.5
46	Permethrin-1 Permethrin-2	54774-45-7 54774-45-7	28.908 29.080	183*，163，184 183*，163，184	0.995	0.003	85.8	7.7	66.1	6.8
47	Pyridaben	96489-71-3	29.094	147*，148，309	0.993	0.001	92.8	9.6	70.2	5.7
48	Boscalid	188425-85-6	30.163	140*，342，344	0.994	0.003	83.8	3.5	88.2	3.8
49	Quizalofop-p-ethyl	94051-08-8	30.271	299*，372，243	0.996	0.003	83.9	9.9	98.0	6.6
50	Etofenprox	80844-07-1	30.432	163*，183，376	0.998	0.001	85.4	5.3	70.0	5.9
51	Pyridalyl	179101-81-6	30.481	204*，146，176	0.994	0.007	70.8	3.0	61.3	3.7
52	Fenvalerate-1 Fenvalerate-2	51630-58-1 51630-58-1	31.169 31.430	167*，125，419 167*，125，419	0.996	0.01	95.1	6.3	93.0	8.9
53	Fluvalinate-1 Fluvalinate-2	102851-06-9 102851-06-9	31.316 31.410	250*，252，502 250*，252，502	0.993	0.003	100.9	2.0	101.5	6.7
54	Deltamethrin-1 Deltamethrin-2	52918-63-5 52918-63-5	31.875 32.124	181*，253，172 181*，253，172	0.993	0.01	81.3	5.9	78.2	2.8
55	Flumiclorac-pentyl	87546-18-7	32.295	308*，423，318	0.992	0.001	77.2	2.7	89.5	3.1

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C-56 Determination of Phthalates in vegetables by GPC–GCMS

INTRODUCTION

Phthalates (PAEs) are a class of compounds which can be added to plastics to increase its flexibility, transparency, durability and longevity. They can be used in electronics industry, agriculture adjuvant, building materials, toys, food packaging materials and textiles etc. Because of its medium viscosity, high stability, low volatility, easily accessible, low cost and other features, they are currently the most widely used plasticizer.

In 2011 PAEs events broke out in Taiwan drinks , and in 2012 the same thing happened to a certain brand of liquor. And recently it was reported that “vegetables wrapped in tape” in the supermarkets may contain PAEs. And this caused more and more consumers pay great attention to the PAEs.

PAEs were classified as one kind of suspected environmental hormone. Their toxicity is mainly estrogen and anti-androgen activity which can cause endocrine disorder and reproductive function hinder in the organism. Therefore, PAEs had been restricted used in the relevant national standards such as drinking water, toys, packaging materials and food etc.

In this report a method was developed using Shimadzu’s GPC-GCMS to determine 22 kinds of PAEs in vegetables. This method is sensitive, easy to operate and can be applied to quickly detect PAEs in vegetables.

EXPERIMENTAL

Instrument: Shimadzu GPC-GCMS.

Experimental conditions:

GPC conditions:
Chromatographic column: Shodex CLNpak EV-200（2.1 mm × 150 mm）
Mobile phase: acetone/cyclohexane（3/7，V/V）
Flow rate: 0.1 mL/min
Column temperature: 40 ℃
Sample size: 20 μL

GCMS conditions:
Chromatographic column: inert quartz tube: 5 m × 0.53 mm
Precolumn: WondaCap WAX, 5m × 0.25 mm × 0.25 μm
Analytical column: WondaCap WAX, 25m × 0.25 mm × 0.25 μm
Column oven temperature: 82 ℃ (5 min)_8 ℃/min_150 ℃ (0 min)_25 ℃/min_240 ℃ (5 min)
Injection temperature:120 ℃ (5 min)_100 ℃/min_280 ℃ (15.8 min)
Pressure: 120 kPa (0 min)_100 kPa/min_180 kPa (4.4 min)_(-49.8 kPa/min)_120 kPa (15.9 min)
Purge flow: 5.0 mL/min_(-10 mL/min)_0 mL/min (6 min)_10 mL/min_5 mL/min (15.1 min)
Sampling time: 7 min；
Solvent cut time: 9.7 min
Interface temperature: 250 ℃
Ion source temperature: 200 ℃
Acquisition mode: SIM, acquisition conditions are shown in Table 1.

Sample preparation

Weigh accurately 1.0 g grinded vegetables into 25 mL centrifuge tube, add 5 mL water, mix and exact for 30 min. Then add 2 mL hexane, mix and vortex for 3 min, then take the supernatant fluid for sample analysis.

Table 1. Characteristic fragment ions of PAEs(m/z).

No.	Compound name	CAS	R.T.	Target Ion	Ref. Ion 1	Ref. Ion 2
1	DMP	131-11-3	14.400	163	133	194
2	DEP	84-66-2	16.642	149	177	176
3	DIPRP	605-45-8	17.633	149	209	150
4	DAP	131-17-9	18.925	149	104	189
5	DPRP	131-16-8	19.308	149	209	191
6	DIBP	84-69-5	20.583	149	167	205
7	DBP	84-74-2	21.833	149	205	223
8	DMEP	117-82-8	22.333	149	104	176
9	DIPP	605-50-5	23.192	149	219	237
10	BMPP	146-50-9	23.342	167	149	251
11	DEEP	605-54-9	23.750	149	104	176
12	DPP	131-18-0	24.175	149	219	237
13	DHXP	84-75-3	26.367	149	233	251
14	BBP	85-68-7	26.475	149	91	206
15	DBEP	117-83-9	27.750	149	101	193
16	DCHP	84-61-7	28.292	149	167	249
17	DHP	3648-21-3	28.392	265	149	247
18	DEHP	117-81-7	28.483	279	149	167
19	DPHP	84-62-8	28.592	225	104	153
20	DNOP	117-84-0	30.292	149	261	279
21	DINP	68515-48-0	30.908	293	149	127
22	DIDP	26761-40-0	31.217	307	149	141

Chromatogram of standard sample :

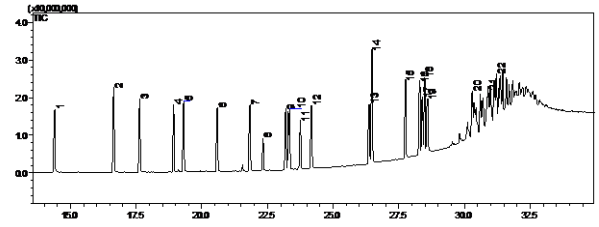

Figure 1. Total Ion Chromatogram of standard sample (1.0 mg/L)

RESULT AND DISCUSSION

Standard chromatography.
Dilute the standard solution to 1.0 μg/ml with Hexane. The Total Ion Chromatogram of standard sample was shown in Figure 1. The CAS number, retention time, and target ion of each compounds are listed in Table 2.

Calibration curve & Repeatability.
Dilute the standard stock solution into 0.005, 0.05, 0.1, 0.5, 1.0 μg/mL. Some of the calibration curves obtained are shown in Figure 2. The correlation coefficients, the RSD% of 7 consecutive tests of 0.05 μg/mL standard samples and the detection limit calculated according to the data of 0.005 μg/mL standard sample(3 S/N) are listed in Table 2.

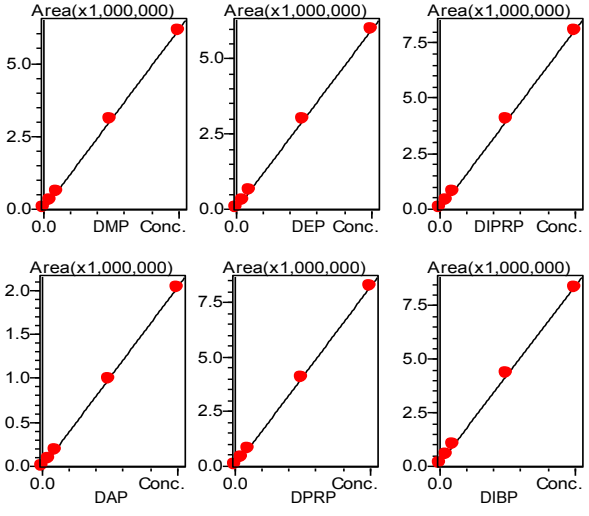

Figure. 2. Calibration curve of some compounds

Sample & recovery results
Add PAEs standard (0.1, 0.2, and 0.4 mg/kg) into sample (Lettuce, canola and celery) before sample pretreatment in accordance with the processing steps and calculate recovery rate. The results were between 60% ~ 130%.

Table 2. Correlation coefficients, RSD, LOD of PAEs

No.	Compound name	Correlation Coefficients	RSD%	LOD(μg/L)
1	DMP	0.9999	4.86	0.10
2	DEP	0.9999	4.37	0.10
3	DIPRP	0.9999	4.23	0.10
4	DAP	0.9999	3.70	0.18
5	DPRP	0.9999	4.26	0.10
6	DIBP	0.9999	4.02	0.10
7	DBP	0.9998	4.14	0.10
8	DMEP	0.9993	3.91	2.23
9	DIPP	0.9998	3.23	0.10
10	BMPP	0.9999	2.35	0.20
11	DEEP	0.9997	3.65	0.56
12	DPP	0.9996	2.42	0.10
13	DHXP	0.9995	4.52	0.11
14	BBP	0.9997	4.91	0.33
15	DBEP	0.9998	3.71	1.03
16	DCHP	0.9997	4.65	0.27
17	DHP	0.9998	4.08	3.09
18	DEHP	0.9995	4.73	0.10
19	DPHP	0.9999	4.53	0.14
20	DNOP	0.9996	4.56	0.45
21	DINP	0.9996	4.59	1.73
22	DIDP	0.9994	4.60	2.03

CONCLUSION

A quick, easy and reliable method for determination PAEs in vegetables by Shimadzu's GPC-GCMS is developed. This method is sensitive, easy to operate and can be applied to quickly detect PAEs in vegetables.

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C-57 Determination of Chlorine Propanol in Soy Sauce by GPC–GCMS

INTRODUCTION

In recent years, the production technology of soy sauce has great changes with the increasing demand for spices. The production process includes brewing, acidolysis and compound categories. Because they can greatly reduce the production cost and time, acidolysis and blended technology have gradually replaced brewing. However, if the condition control improperly in the process of acidolysis, chlorine propanol will be increased. Chlorine propanol was mainly in the produced in the hydrolysis process of vegetable protein. So that foods made from acid hydrolyzed vegetable protein always contain differing levels of chlorine propanol. Some research results showed that 2, 3-dichloro-1-propanol can cause kidney damage, more likely to cause the reproductive system disease. Above all, it is particularly important to study detection method of chlorine propanol.

Ingredients of soy sauce are relatively complex, the pre-treatment methods mainly conclude liquid-liquid extraction, matrix solid-phase dispersion, solid phase extraction, solid phase micro-extraction, etc., The operation of traditional sample purification is time-consumed with high labor intensity. According to the China regulation, SN/T 0548.1 2002, inspection method of 1, 3-dichloro-2-propyl alcohol and 2, 3-dichloro-1-propanol in export soy sauce by GC/ECD is performed without derivatization steps using external standard method for quantitative. The determination limit of 1, 3-DCP and 2, 3-DCP is 0.05 mg/kg. In this paper, liquid-liquid extraction combined with online gel chromatography is used for sample detection. Sample preparation of this method is simple, efficiency, automation more advanced and suitable for 2, 3-dichloro-1-propanol detection in soy sauce.

EXPERIMENTAL

Instrument: Shimadzu GPC-GCMS.

Experimental conditions:

GPC conditions:
Chromatographic column：Shodex CLNpak EV-200 (2.1 mm x 150 mm)
Mobile phase：acetone/cyclohexane (3/7, V/V)
Flow rate：0.1 mL/min
Column temperature：40 ºC
Sample size：20 µL
GCMS conditions:
Chromatographic column：inert quartz tube：5 m x 0.53 mm
Precolumn：WondaCap WAX, 5 m x 0.25 mm x 0.25 µm
Analytical column：WondaCap WAX, 25m x 0.25 mm x 0.25 µm
Column oven temperature: 82 ºC(5min)_8 ºC/min_150 ºC(0min)_25 ºC/min_240 ºC(5min)
Injection temperature:120 ºC(5min)_100 ºC/min_280 ºC(15.8min)
Pressure: 120 kPa(0min)_100 kPa/min_180 kPa(4.4min)_(-49.8 kPa/min)_120 kPa (15.9min)
Purge flow：5.0mL/min_(-10mL/min)_0 mL/min(6min)_10mL/min_5mL/min (15.1min)
Sampling time: 7min；
Solvent cut time：9.7min
Interface temperature: 250 ºC
Ion source temperature: 200 ºC
Acquisition mode：SIM, acquisition conditions are shown in Table 1.

Sample preparation: Weigh accurately 0.5 g soy sauce in 10 mL centrifuge tube, add 0.5 mL anhydrous ethanol, mix and add 1.0 g anhydrous sodium sulfate and 0.5 g sodium chloride, vortex and mix for 3 min, then add 0.5 mL ethyl acetate, vortex and mix for 1 min, let stand for 10 min, take the supernatant fluid for sample analysis.

Chromatogram of standard sample :

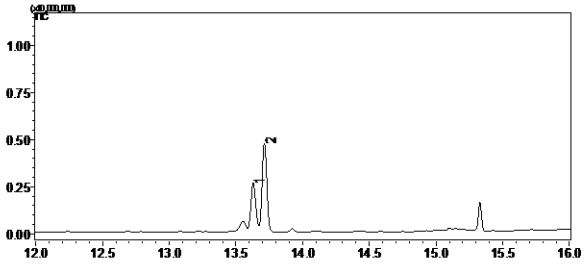

Figure 1. Total ion chromatogram of standard sample (0.5 mg/L)

Table 1. Characteristic fragment ions of 2, 3-dichloro-1-propanol.

No.	Retention time	Compound name	CAS	Quantitation ion	Qualification ions
1	13.638	d5-2,3-DCP	-	46	65、97
2	13.724	2,3-DCP	616-23-9	62	64、92

RESULTS AND DISCUSSION

Calibration curve and reproducibility

Dilute standard solution with ethyl acetate. The concentration of the solution is 0.01, 0.05, 0.1, 0.5 and 1 mg/L. The correlation coefficients (R=0.9999) indicated good correlations between the concentrations of the investigated compounds and their peak areas within the test ranges (Figure 3). Precision of the method was measured by analyzing the same sample (0.01 mg/L) six times. The overall RSD of analysis was 0.94%. The LOD (S/N=3) of 2, 3-dichloro-1-propanol was 0.12 µg/kg.

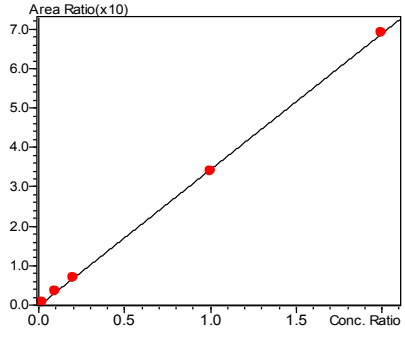

Figure 2. Calibration curve of 2, 3-dichloro-1-propanol

Table 2. Correlation coefficients, RSD, LOD of 2, 3-dichloro-1-propanol

No.	Compound name	correlation coefficients	RSD %	LOD
1	2, 3-dichloro-1-propanol	0.9999	0.94	0.12

Sample detection and recovery results
Add 2,3-dichloro-1-propanol isotope and its internal standard into sample (0.01, 0.1, and 0.2 mg/kg) before sample pretreatment in accordance with the processing steps and calculate recovery rate. The results shown in table 3:

No.	Compound name	Originals (mg/kg)	Recovery (%)			Average (%)	RSD% (n=3)
			0.01 mg/kg	0.1 mg/kg	0.2 mg/kg		
1	2, 3-dichloro-1-propanol	N.D	109.2	105.6	106.1	106.9	1.85

Note：N.D means 2, 3-dichloro-1-propanol was undetected

CONCLUSIONS

In this paper, Shimadzu GPC-GCMS was used to determine 2, 3-dichloro-1-propanol in soy sauce during the analysis. Calibration curve showed good linearity (r=0.9999) within the test ranges. The detection limit was 0.12μg/kg(S/N=3). The average recovery was between 106.9%. The established method in this study was simple, feasible and practical, and could be applied to rapid detection of 2,3-dichloro-1-propanol in soy sauce.

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LCMS-8040 UFMS

C-58 Analysis of Dicyandiamide and Melamine in Milk Powders by LC/MS/MS Method

INTRODUCTION

Melamine was found to be used as a protein-rich adulterant first in pet-food in 2007, and then in infant formula in 2008 in China [1]. The outbreak of the melamine scandal that killed many dogs and cats as well as led to death of six infants and illness of many had caused panic in publics and great concerns in food safety worldwide. Melamine was added into raw milk because of its high nitrogen content (66%) and the limitation of the Kjeldahl method for determination of protein level indirectly by measuring the nitrogen content. In fact, in addition to melamine and its analogues (cyanuric acid etc), a number of other nitrogen-rich compounds was reported also to be potentially used as protein-rich adulterants, including amidinourea, biuret, cyromazine, dicyandiamide, triuret and urea [2]. Recently, low levels of dicyandiamide (DCD) residues were found in milk products from New Zealand [3-4]. Instead of addition directly, the DCD present in the milk products was explained to be due to that cows eating the grass "contaminated by DCD" may produce milk containing traces of DCD residues. Dicyandiamide is a toxic agrichemical compound and could be used to promote the growth of pastures where cows graze. We report here a LC/MS/MS method for sensitive detection and quantification of both dicyandiamide (DCD) and melamine in infant milk powder samples.

EXPERIMENTAL

Preparation of standards and Samples

Dicyandiamide (DCD) and melamine were obtained from Sigma Aldrich. Amicon Ultra-4 (MWCO 5K) centrifuge filtration tubes (15 mL) were obtained from Millipore. Stock solutions of DCD and melamine were prepared in pure water. A set of calibrants (0.5, 1.0, 2.5, 5 and 10 ppb) was prepared from the stock solutions using of ACN/water (90/10) as diluent. The milk powder sample was pre-treated according to a FDA method [1] with some modification as illustrated in Figure 1. The final clear sample solution was injected into LC/MS/MS for analysis.

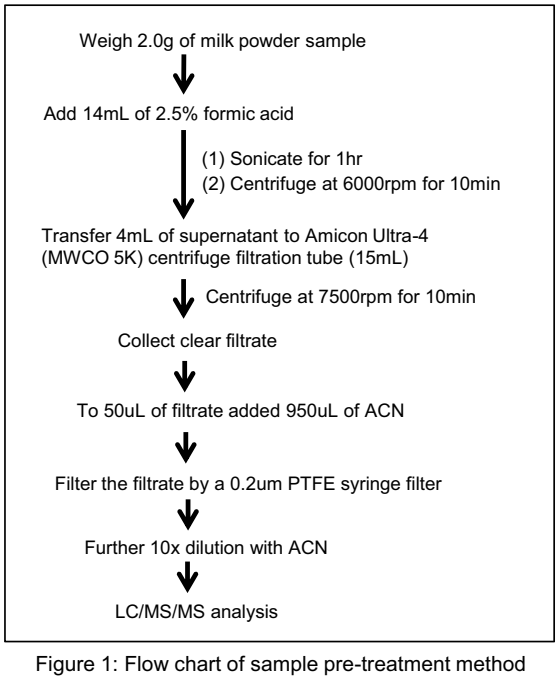


Figure 1: Flow chart of sample pre-treatment method

A LCMS-8040 triple quadrupole LC/MS/MS (Shimadzu Corporation, Japan) was used in this work. The system is consisted of a high pressure binary gradient UHPLC coupled with a LCMS-8040 system. An Alltima HP HILIC column was used for separation of DCD and melamine with a gradient program developed in house. The details of the LC and MS conditions are shown in Table 1.

Table 1: Analytical conditions of DCD and melamine in milk powders on LCMS-8040

LC conditions

Column	2.1mm x 150 mm, Alltech
Flow Rate	0.2 mL/min
Mobile Phase	A :0.1 % formic acid in H2O/ACN (5:95 v/v) B :20mM Ammonium Formate in H2O/ACN (50:50 v/v)
Elution Mode	Gradient elution: 5% (0.01 to 3.0 min) ➔ 95% (3.5 to 5.0 min) ➔ 5% (5.5 to 9.0min)
Oven Temperature	40°C
Injection Volume	5 uL

MS conditions

Interface	ESI
MS mode	Positive
Block Temperature	400°C
DL Temperature	300°C
CID Gas	Ar (230kPa)
Nebulizing Gas Flow	N_2, 2.0L/min
Drying Gas Flow	N_2, 15.0L/min
MRM	DCD: 85.1 ➔ 68.05, 43.00 Melamine: 127.1 ➔ 85.10, 68.05

RESULTS AND DISCUSSION

MRM optimization

MRM optimization of DCD and melamine were performed using an automated MRM optimization program of the LabSolutions. The precursors were the protonated ions of DCD and melamine. Two optimized MRM transitions of each compound were selected and used for quantitation and confirmation. The MRM transitions and parameters are shown in Table 2.

Table 2: MRM transitions and optimized parameters

Name	RT (min)	Transition (m/z)	Voltage (V)		
			Q1 Pre Bias	CE	Q3 Pre Bias
DCD	2.55	85.1 > 68.1	-15	-21	-26
		85.1 > 43.0	-15	-17	-17
MEL	6.29	127.1 > 85.1	-26	-20	-17
		127.1 > 68.1	-26	-27	-26

Method & Performance Evaluation

A LC/MS/MS method was developed for quantitation of DCD and melamine based on the MRM transitions in Table 2. Under the HILIC separation conditions (Table 1), DCD and melamine eluted at 2.55 min and 6.29 min as sharp peaks (see Figures 4 & 5). Figures 2 and 3 show the calibration curves of DCD and melamine standard in neat solutions and in milk matrix solutions (spiked). The linearity with correlation coefficient (R^2) greater than 0.997 across the calibration range of 0.5~10.0 ng/mL was obtained for both compounds in both neat solution and matrix (spiked).

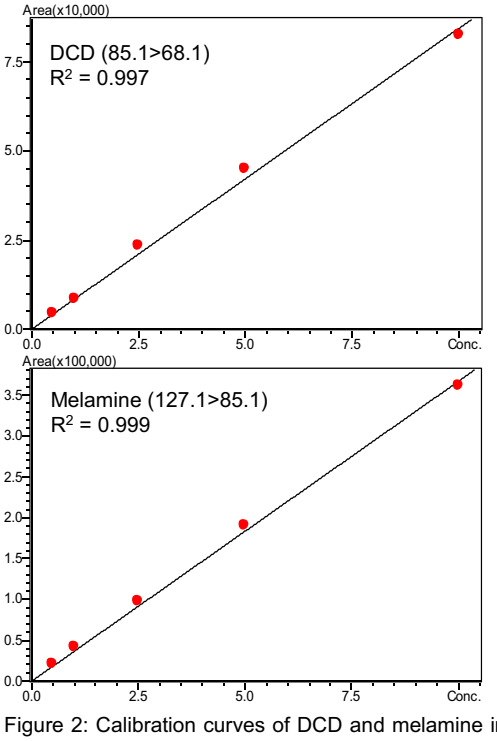


Figure 2: Calibration curves of DCD and melamine in neat solution

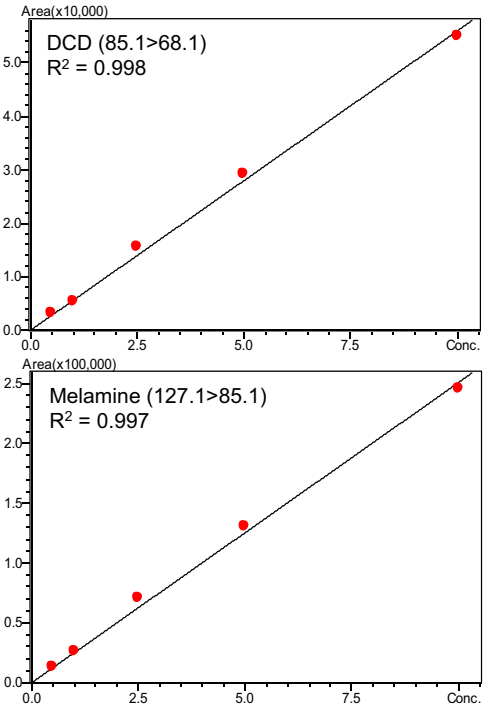


Figure 3: Calibration curves of DCD and melamine spiked in milk powder matrix

The repeatability of the method was evaluated at the levels of 0.5 ng/mL and 1.0 ng/mL. Figures 4 & 5 show the MRM chromatograms of DCD and melamine of six consecutive injections of 0.5 ng/mL level with and without matrix. The peak area %RSD for the two analytes were lower than 9.2% (see Table 3).

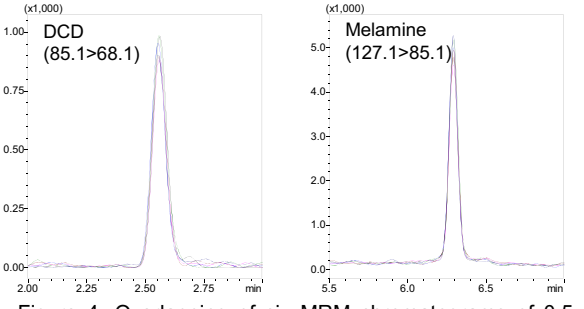


Figure 4: Overlapping of six MRM chromatograms of 0.5 ng/mL DCD and melamine in neat solution

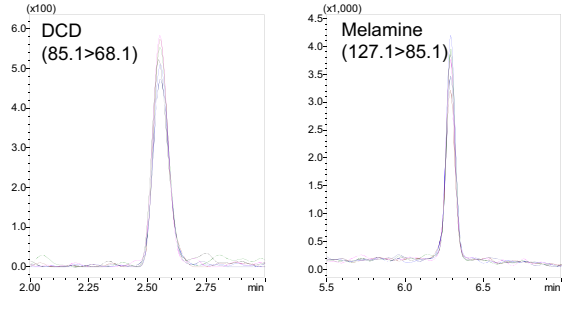


Figure 5: Overlapping of six MRM chromatograms of 0.5 ng/mL DCD and melamine in milk powder matrix

Table 3: Results of repeatability and sensitivity evaluation of DCD and melamine (n=6)

Sample	Compd.	Conc. (ng/mL)	%RSD	LOD (ng/mL)	LOQ (ng/mL)
In solvent	DCD	0.5	5.9	0.03	0.10
		1.0	5.3		
	MEL	0.5	5.5	0.03	0.09
		1.0	2.6		
In matrix	DCD	0.5	5.9	0.05	0.16
		1.0	8.2		
	MEL	0.5	9.2	0.05	0.15
		1.0	2.4		

The LOD and LOQ were estimated from the results of 0.5 ng/mL in both neat and matrix solution. The LOD and LOQ results were summarized in Table 3. The method achieved LOQs (in matrix) of 0.16 and 0.15 ng/mL (ppb) for DCD and melamine, respectively.

Tables 4 & 5 show the results of matrix effect and recovery of the method. The matrix effects for DCD and melamine in the whole concentration ranges were at 64%~70% and 62%~73%, respectively. The recovery was determined by comparing the results of pre-spiked and post-spiked mixed samples of DCD and melamine in the milk powder matrix (2.5 ng/mL each compound). The chromatograms of these samples are shown in Figure 6. The recovery of DCD and melamine were determined to be 102% and 105% respectively.

Table 4: Matrix effect (%) of DCD and melamine in milk powder matrix

Conc. (ng/mL)	0.5	1	2.5	5	10
DCD	70.4	65.4	66.9	64.8	66.6
MEL	62.2	62.5	73.1	68.9	68.0

Table 5: Recovery of DCD and melamine determined with spiked sample of 2.5 ng/mL

Compound	Pre-spiked Area	Post-spiked Area	Recovery (%)
DCD	14,393	13,987	102.9
MEL	65,555	62,659	104.6

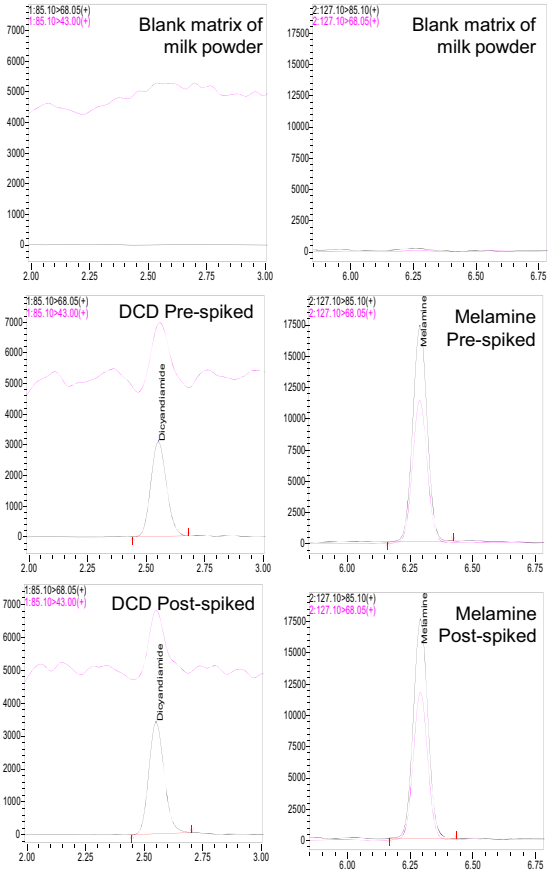


Figure 6: MRM peaks of DCD and melamine in pre- and post-spiked samples of 2.5 ng/mL (each). Noted that, DCD and melamine were not detected in the blank matrix of milk powder sample (top).

CONCLUSION

A high sensitivity LC/MS/MS method was developed on LCMS-8040 for detection and quantitation of dicyandiamide (DCD) and melamine in milk powders. The method performance was evaluated using infant milk powders as the matrix. The method achieved LOQ of ~0.16 ng/mL for both compounds in the matrix, allowing its application in simultaneous analysis of melamine, a protein adulterant in relatively high concentration, and dicyandiamide residues in trace concentration in milk powders samples.

C-59 SIMULTANEOUS ANALYSIS OF 200 PESTICIDES USING LC/MS/MS IN FOOD

INTRODUCTION

In this data set, 200 pesticide residues in fruits and vegetables were analyzed based on the LC/MS/MS mass spectral library of pesticide established by Shimadzu and the related LC analysis conditions, the calibration curve, the LOD, the precision and the recovery were examined. Also the product ion scanning experiments by the MRM trigger (synchronized survey scan) were made and the scanning results of product ions were obtained at the same time of the MRM analysis. Based on the ultra fast switching speed (15 msec) between the positive and negative ions of LCMS-8040, the data set can simultaneously determine the positive and negative ions. The qualitative and the quantitative results can be obtained at the same time after one single injection. Attributed to the ultra fast scanning speed of LCMS-8040 (15000 u/sec), the trigger of product ion scanning can be effectively realized while such a high throughput (200 compounds and 400 MRM channels) detection was performed, which ensured the achievement of more reliable qualitative data together with the quantitative analysis. The fast qualitative detection can be made by searching and comparing the second mass spectrum obtained with the pesticide mass spectrum library. The structural formula, molecular weight and other information of the target compound can be given at the same time

EXPERIMENTAL

Sample information and pre-treatment

Standard solution:

Stock solutions of 200 compounds each with a concentration of 100 mg/L were prepared using methanol or n-hexane. Then a mixture of stock solutions of all these standard solutions with a concentration of 0.5 mg/L was prepared using the mobile phase.

Actual samples:

Three samples including cucumbers, apples and tomatoes were used in the experiment. Refer to GB/T 20769-2008 for the pre-treatment method, as shown below:

Extraction:

Weigh accurately 20 g sample and transfer it to an 80 mL centrifuge tube. Add 40 mL acetonitrile and homogenize and extract the sample with a homogenize machine for 1 minute. Add 5 g sodium chloride, homogenize and extract it again for 1 minute, and centrifuge at the speed of 3800 rpm for 5 minutes. Take 20 mL supernatant (which equals to 10 g sample), and evaporate to about 1mL by rotary evaporator in water bath at 40 °C for further purification for 1 minute, and centrifuge at the speed of 3800 rpm for 5 minutes.

Purification:

Load about 2 cm high of anhydrate sodium sulphate onto a Sep-Pak Vac column. Place the column on a fixed mount with a pear-shaped bottle under it. Pre-wash the column with 4 mL of acetonitrile/toluene (3:1) before the sample is added. When the liquid level reaches the top of sodium sulphate, transfer the concentrated sample to the purification column rapidly and collect it with several pear-shaped bottles. Wash the sample bottle with 2 mL of acetonitrile/toluene (3:1) 3 times and combine the solutions into the column. Add a 50 mL liquid reservoir on the column and elute the pesticides and related chemicals with 25 mL of acetonitrile/toluene (3:1). Collect all the eluate in a pear-shaped bottle, and evaporate to about 0.5 mL by rotary evaporator in water bath at 40 °C. Evaporate the concentrated solution using a nitrogen evaporator to dryness and add 1 mL of acetonitrile/water (3:2) rapidly. Vortex and filter it using a 0.2 μm membrane filter and inject into the LC/MS/MS system.

Instrument parameters:

The data was collected using the LCMS-8040 triple quadrupole LC/MS/MS system. The configuration included:

Pump	: LC-30AD×2
Online degassing unit	: DGU-20A$_5$
Auto-sampler	: SIL-30AC
System controller	: CBM-20A
Column oven	: CTO-30A

LC conditions

Column	: Shim-pack XR-ODSIII (150 mmL × 2.0 mmI.D., 2.2 μm)
Flow rate	: 0.4 mL/min
Injection volume	: 5 μL
Column temperature	: 40 °C
Mobile phase	: A - 2 mmol/L ammonium acetate + 0.02 % formic acid aqueous solution; B - acetonitrile
Gradient	: Binary gradient with initial concentration of 10% of mobile phase B; the time sequence is shown below:

Time (min)	B.Conc
1	10
4	50
20	75
22	95
25	95
26	10
30	10

MS Conditions

Ion source : ESI

Interface voltage of ion source : Positive – (+ 4.5 kV); Negative – (- 3.5 kV)

Nebulizing gas : Nitrogen, 3.0 L/min

Drying gas : Nitrogen, 20 L/min

Collision gas : Argon

DL temperature : 250 °C

Block heater temperature : 450 °C

Pesticides studied were as per Table 1, and monitored as per Table 2.

Table 1: Pesticide information and monitoring ion

No.	Name	CAS#	Molecular formula	Molecular weight	Detected ion
1	Acephate	30560-19-1	$C_4H_{10}NO_3PS$	183.2	$[M+H]^+$
2	Acetamiprid	135410-20-7	$C_{10}H_{11}ClN_4$	222.7	$[M+H]^+$
3	Acetochlor	34256-82-1	$C_{14}H_{20}ClNO_2$	269.8	$[M+H]^+$
4	Alachlor	15972-60-8	$C_{14}H_{20}ClNO_2$	269.8	$[M+H]^+$
5	Aldicarb	116-06-3	$C_7H_{14}N_2O_2S$	190.3	$[M+Na]^+$
6	Aldicarb-sulfone	1646-88-4	$C_7H_{14}N_2O_4S$	222.3	$[M+Na]^+$
7	Aldicarb-sulfoxide	1646-87-3	$C_7H_{14}N_2O_3S$	206.3	$[M+Na]^+$
8	Ametryn	834-12-8	$C_9H_{17}N_5S$	227.3	$[M+H]^+$
9	Anilofos	64249-01-0	$C_{13}H_{19}ClNO_3PS_2$	367.9	$[M+H]^+$
10	Atrazine	1912-24-9	$C_8H_{14}ClN_5$	215.7	$[M+H]^+$
11	Azamethiphos	35575-96-3	$C_9H_{10}ClN_2O_5PS$	324.7	$[M+H]^+$
12	Azimsulfuron	120162-55-2	$C_{13}H_{16}N_{10}O_5S$	424.4	$[M+H]^+$
13	Azinphos-methyl	86-50-0	$C_{10}H_{12}N_3O_3PS_2$	317.3	$[M+H]^+$
14	Azoxystrobin	131860-33-8	$C_{22}H_{17}N_3O_5$	403.4	$[M+H]^+$
15	Benalaxyl	71626-11-4	$C_{20}H_{23}NO_3$	325.4	$[M+H]^+$
16	Bendiocarb	22781-23-3	$C_{11}H_{13}NO_4$	223.2	$[M+H]^+$
17	Bensulfuron methyl	83055-99-6	$C_{16}H_{18}N_4O_7S$	410.4	$[M+H]^+$
18	Bitertanol	55179-31-2	$C_{20}H_{23}N_3O_2$	337.4	$[M+H]^+$
19	Brodifacoum	56073-10-0	C31H23BrO3	523.4	[M+H]+

20	Bupirimate	41483-43-6	C13H24N4O3S	316.4	[M+H]+
21	Buprofezin	69327-76-0	C16H23N3OS	305.4	[M+H]+
22	Butachlor	23184-66-9	C17H26ClNO2	311.9	[M+H]+
23	Butafenacil	134605-64-4	C20H18ClF3N2O6	474.8	[M+H]+
24	Butralin	33629-47-9	C14H21N3O4	295.3	[M+H]+
25	Cadusafos	95465-99-9	C10H23O2PS2	270.4	[M+H]+
26	Carbaryl	63-25-2	C12H11NO2	201.2	[M+H]+
27	Carbendazim	10605-21-7	C9H9N3O2	191.2	[M+H]+
28	Carbofuran	1563-66-2	C12H15NO3	221.3	[M+H]+
29	Carbofuran-3-hydroxy	16655-82-6	C12H15NO4	237.3	[M+H]+
30	Carboxin	5234-68-4	C12H13NO2S	235.3	[M+H]+
31	Cartap Hydrochloride	15263-52-2	C7H16ClN3O2S2	273.8	[M+H]+
32	Chlorfluazuron	71422-67-8	C20H9Cl3F5N3O3	540.7	[M+H]+
33	Chlorimuron-ethyl	90982-32-4	C15H15ClN4O6S	414.8	[M+H]+
34	Chlormequat	999-81-5	C5H13Cl2N	158.1	[M+H]+
35	Chlorotoluron	15545-48-9	C10H13ClN2O	212.7	[M+H]+
36	Chlorpyrifos	2921-88-2	C9H11Cl3NO3PS	350.5	[M+H]+
37	Chlorsulfuron	64902-72-3	C12H12ClN5O4S	357.8	[M+H]+
38	Chromafenozide	143807-66-3	C24H30N2O3	394.5	[M+H]+
39	Cinosulfuron	94593-91-6	C15H19N5O7S	413.4	[M+H]+
40	Clethodim	99129-21-2	C17H26ClNO3S	359.9	[M+H]+
41	Clomazone Dimethazone	81777-89-1	C12H14ClNO2	239.7	[M+H]+
42	Clomeprop	84496-56-0	C16H15Cl2NO2	324.2	[M+H]+
43	Cloquintocet-1- methylh-	99607-70-2	C18H22ClNO3	335.8	[M+H]+
	exyl ester				
44	Clothianidin	210880-92-5	C6H8ClN5O2S	249.7	[M+H]+
45	Coumatetralyl	5836-29-3	C19H16O3	292.3	[M+H]+
46	Cyflufenamid	180409-60-3	C20H17F5N2O2	412.4	[M+H]+
47	Cyproconazole	94361-06-5	C15H18ClN3O	291.8	[M+H]+
48	Cyprodinil	121552-61-2	C14H15N3	225.3	[M+H]+

49	Dazomet	533-74-4	C5H10N2S2	162.3	[M+H]+
50	Diazinon	333-41-5	C12H21N2O3PS	304.4	[M+H]+
51	Dichlorvos	62-73-7	C4H7Cl2O4P	221	[M+H]+
52	Diethofencarb	87130-20-9	C14H21NO4	267.3	[M+H]+
53	Diethyltoluamide	134-62-3	C12H17NO	191.3	[M+H]+
54	Dimepiperate	61432-55-1	C15H21NOS	263.4	[M+H]+
55	Dimethirimol	5221-53-4	C11H19N3O	209.3	[M+H]+
56	Dimethoate	60-51-5	C5H12NO3PS2	229.3	[M+H]+
57	Dimethomorph	110488-70-5	C21H22ClNO4	387.9	[M+H]+
58	Diniconazole	76714-88-0	C15H17Cl2N3O	326.2	[M+H]+
59	Dinotefuran	165252-70-0	C7H14N4O3	202.2	[M+H]+
60	Diuron	330-54-1	C9H10Cl2N2O	233.1	[M+H]+
61	Dymron	42609-52-9	C17H20N2O	268.4	[M+H]+
62	Epoxiconazole	135319-73-2	C17H13C1FN3O	329.8	[M+H]+
63	Esprocarb	85785-20-2	C15H23NOS	265.4	[M+H]+
64	Ethametsulfuron-methyl	97780-06-8	C15H18N6O6S	410.4	[M+H]+
65	Ethiofencarb	29973-13-5	C11H15NO2S	225.3	[M+H]+
66	Ethion	563-12-2	C9H22O4P2S4	384.5	[M+H]+
67	Ethoprophos	13194-48-4	C8H19O2PS2	242.3	[M+H]+
68	Fenamiphos	22224-92-6	C13H22NO3PS	303.4	[M+H]+
69	Fenarimol	60168-88-9	C17H12Cl2N2O	331.2	[M+H]+
70	Fenobucarb	3766-81-2	C12H17NO2	207.3	[M+H]+
71	Fenoxycarb	72490-01-8	C17H19NO4	301.3	[M+H]+
72	Fenpropimorph	67564-91-4	C20H33NO	303.5	[M+H]+
73	Fenpyroximate	111812-58-9	C24H27N3O4	421.5	[M+H]+
74	Flocoumafen	90035-08-8	C33H25F3O4	542.5	[M+H]+
75	Fluazifop-p-butyl	79241-46-6	C19H20F3NO4	383.4	[M+H]+
76	Flusilazole	85509-19-9	C16H15F2N3Si	315.4	[M+H]+
77	Flutriafol	76674-21-0	C16H13F2N3O	301.3	[M+H]+
78	Fonofos	994-22-9	C10H15OPS2	246.3	[M+H]+
79	Forchlorfenuron	68157-60-8	C12H10ClN3O	247.7	[M+H]+

80	Fosthiazate	98886-44-3	C9H18NO3PS2	283.4	[M+H]+
81	Furathiocarb	65907-30-4	C18H26N2O5S	382.5	[M+H]+
82	Hexaconazole	79983-71-4	C14H17Cl2N3O	314.2	[M+H]+
83	Hexazinone	51235-04-2	C12H20N4O2	252.3	[M+H]+
84	Hexythiazox	78587-05-0	C17H21ClN2O2S	352.9	[M+H]+
85	Imazapyr	81334-34-1	C13H15N3O3	261.3	[M+H]+
86	Imibenconazole	86598-92-7	C17H13Cl3N4S	411.7	[M+H]+
87	Imidacloprid	105827-78-9	C9H10ClN5O2	255.7	[M+H]+
88	Indoxacarb	144171-61-9	C22H17ClF3N3O7	527.8	[M+H]+
89	Iprobenfos	26087-47-8	C13H21O3PS	288.3	[M+H]+
90	Iprovalicarb	140923-17-7	C18H28N2O3	320.4	[M+H]+
91	Isazofos	42509-80-8	C9H17ClN3O3PS	313.7	[M+H]+
92	Isoprocarb	2631-40-5	C11H15NO2	193.2	[M+H]+
93	Isoprothiolane	50512-35-1	C12H18O4S2	290.4	[M+H]+
94	Isoproturon	34123-59-6	C12H18N2O	206.3	[M+H]+
95	Kresoxim-methyl	143390-89-0	C18H19NO4	313.4	[M+H]+
96	Linuron	330-55-2	C9H10Cl2N2O2	249.1	[M+H]+
97	Lodosulfuron-methyl	144550-36-7	C14H13IN5NaO6S	529.2	[M+H]+
	-sodium				
98	Malachite green oxalate	2437-29-8	2(C23H25N2).2(C2HO4).	927	[M+H]+
	salt		C2H2O4		
99	Malathion	121-75-5	C10H19O6PS2	330.4	[M+H]+
100	Mefenacet	73250-68-7	C16H14N2O2S	298.4	[M+H]+
101	Mefenoxam	70630-17-0	C15H21NO4	279.3	[M+H]+
102	Metalaxyl	57837-19-1	C15H21NO4	279.3	[M+H]+
103	Metazachlor	67129-08-2	C14H16ClN3O	277.8	[M+H]+
104	Methamidophos	10265-92-6	C2H8O2NPS	141.1	[M+H]+
105	Methiocarb	2032-65-7	C11H15NO2S	225.3	[M+H]+
106	Methomyl	16752-77-5	C5H10N2O2S	162.2	[M+H]+
107	Methoxyfenozide	161050-58-4	C22H28N2O3	368.5	[M+H]+
108	Metolachlor	51218-45-2	C15H22ClNO2	283.8	[M+H]+

109	Metolcarb	1129-41-5	C9H11NO2	165.2	[M+H]+
110	Metsulfuron-methyl	74223-64-6	C14H15N5O6S	381.4	[M+H]+
111	Mevinphos	26718-65-0	C7H13O6P	224.2	[M+H]+
112	Molinate	2212-67-1	C9H17NOS	187.3	[M+H]+
113	Monocrotophos	6923-22-4	C7H14NO5P	223.2	[M+H]+
114	Myclobutanil	88671-89-0	C15H17ClN4	288.8	[M+H]+
115	Naproanilide	52570-16-8	C19H17NO2	291.3	[M+H]+
116	Napropamide	15299-99-7	C17H21NO2	271.4	[M+H]+
117	Omethoate	1113-02-6	C5H12NO4PS	213.2	[M+H]+
118	Oxycarboxin	5259-88-1	C12H13NO4S	267.3	[M+H]+
119	Paclobutrazol	76738-62-0	C15H20ClN3O	293.8	[M+H]+
120	Penconazole	66246-88-6	C13H15Cl2N3	284.2	[M+H]+
121	Pencycuron	66063-05-6	C19H21ClN2O	328.8	[M+H]+
122	Pendimethalin	40487-42-1	C13H19N3O4	281.3	[M+H]+
123	Phorate	298-02-2	C7H17O2PS3	260.4	[M+H]+
124	Phosalone	2310-17-0	C12H15ClNO4PS2	367.8	[M+H]+
125	Phosemet	732-11-6	C11H12NO4PS2	317.3	[M+H]+
126	Phoxim	14816-18-3	C12H15N2O3PS	298.3	[M+H]+
127	Piperonyl Butoxide	3/6/1951	C19H30O5	338.4	[M+NH4]+
128	Pirimicarb	23103-98-2	C11H18N4O2	238.3	[M+H]+
129	Pirimiphos methyl	29232-93-7	C11H20N3O3PS	305.3	[M+H]+
130	Pretilachlor	51218-49-6	C17H26ClNO2	311.9	[M+H]+
131	Prochloraz	67747-09-5	C15H16Cl3N3O2	376.7	[M+H]+
132	Profenofos	41198-08-7	C11H15BrClO3PS	373.6	[M+H]+
133	Promecarb	2631-37-0	C12H17NO2	207.3	[M+H]+
134	Prometryne	7287-19-6	C10H19N5S	241.4	[M+H]+
135	Propamocarb	24579-73-5	C9H20N2O2	188.3	[M+H]+
136	Propanil	709-98-8	C9H9Cl2NO	218.1	[M+H]+
137	Propargite	2312-35-8	C19H26O4S	350.5	[M+NH4]+
138	Propiconazole	60207-90-1	C15H17Cl2N3O2	342.2	[M+H]+
139	Propoxur	114-26-1	C11H15NO3	209.2	[M+H]+
140	Propyzamide	23950-58-5	C12H11Cl2NO	256.1	[M+H]+

145	Pyridaphenthion	119-12-0	C14H17N2O4PS	340.3	[M+H]+
146	Pyrimethanil	53112-28-0	C12H13N3	199.3	[M+H]+
147	Pyriproxyfen	95737-68-1	C20H19NO3	321.4	[M+H]+
148	Quinalphos	13593-03-8	C12H15N2O3PS	298.3	[M+H]+
149	Quizalofop-p-ethyl	100646-51-3	C19H17ClN2O4	372.8	[M+H]+
150	Rimsulfuron	122931-48-0	C14H17N5O7S2	431.4	[M+Na]+
151	Simeconazole	149508-90-7	C14H20FN3OSi	293.4	[M+H]+
152	Spinosad	131929-60-7	C41H65NO10	732	[M+H]+
153	Spirodiclofen	148477-71-8	C21H24Cl2O4	411.3	[M+H]+
154	Sulfotep	3689-24-5	C8H20O5P2S2	322.3	[M+H]+
155	Tebufenozide	112410-23-8	C22H28N2O2	352.5	[M+H]+
156	Tebufenpyrad	119168-77-3	C18H24ClN3O	333.9	[M+H]+
157	Temephos	3383-96-8	C16H20O6P2S3	466.5	[M+H]+
158	Terbufos	13071-79-9	C9H21O2PS3	288.4	[M+H]+
159	Terbuthylazine	5915-41-3	C9H16ClN5	229.7	[M+H]+
160	Terbutryn	886-50-0	C10H19N5S	241.4	[M+H]+
161	Tetrachlorvinphos	961-11-5	C10H9Cl4O4P	366	[M+H]+
162	Tetramethrin	7696-12-0	C19H25NO4	331.4	[M+H]+
163	Thiabendazole	148-79-8	C10H7N3S	201.3	[M+H]+
164	Thiacloprid	111988-49-9	C10H9ClN4S	252.7	[M+H]+
165	Thiamethoxam	153719-23-4	C8H10ClN5O3S	291.7	[M+H]+
166	Thiodicarb	59669-26-0	C10H18N4O4S3	354.5	[M+H]+
167	Thiophanate-methyl	23564-05-8	C12H14N4O4S2	342.4	[M+H]+
168	Tolclofos methyl	57018-04-9	C9H11Cl2O3PS	301.1	[M+H]+
169	Tralkoxydim	87820-88-0	C20H27NO3	329.4	[M+H]+
170	Triadimenol	55219-65-3	C14H18ClN3O2	295.8	[M+H]+
171	Triallate	2303-17-5	C10H16Cl3NOS	304.7	[M+H]+
172	Triazophos	24017-47-8	C12H16N3O3PS	313.3	[M+H]+
173	Trichlorphon	52-68-6	C4H8Cl3O4P	257.4	[M+H]+
174	Tricyclazole	41814-78-2	C9H7N3S	189.2	[M+H]+
175	Tridemorph	24602-86-6	C19H39NO	297.5	[M+H]+
176	Trifloxystrobin	141517-21-7	C20H19F3N2O4	408.4	[M+H]+

177	Triflumizole	99387-89-0	C15H15ClF3N3O	345.8	[M+H]+
178	Triflumuron	64628-44-0	C15H10ClF3N2O3	358.7	[M+H]+
179	Triticonazole	131983-72-7	C17H20ClN3O	317.8	[M+H]+
180	Warfarin	81-81-2	C19H16O4	308.3	[M+H]+
181	2,4-D D3	202480-67-9	C8H3Cl2D3O3	224.1	[M-H]-
182	2,4-DB	94-82-6	C10H10Cl2O3	249.1	[M-H]-
183	2,4-Dichlorophenoxy-acetic Acid	94-75-7	C8H6Cl2O3	221	[M-H]-
184	Acifluorofen	50594-66-6	C14H7ClF3NO5	361.7	[M-H]-
185	Bromadiolone	28772-56-7	C30H23BrO4	527.4	[M-H]-
186	Bromoxynil	1689-84-5	C7H3Br2NO	276.9	[M-H]-
187	Chiptox	94-74-6	C9H9ClO3	200.6	[M-H]-
188	Chloramphenicol	56-75-7	C11H12Cl2N2O5	323.1	[M-H]-
189	Chlorobenzuron	196791-54-5	C14H10Cl2N2O2	309.2	[M-H]-
190	Dicamba	1918-00-9	C8H6Cl2O3	221	[M-H]-
191	Dichlorprop	120-36-5	C9H8Cl2O3	235.1	[M-H]-
192	Diflufenican	83164-33-4	C19H11F5N2O2	394.3	[M-H]-
193	Fludioxonil	131341-86-1	C12H6F2N2O2	248.2	[M-H]-
194	Flufenoxuron	101463-69-8	C21H11ClF6N2O3	488.8	[M-H]-
195	Fomesafen	72178-02-0	C15H10ClF3N2O6S	438.8	[M-H]-
196	Gibberellic Acid	77-06-5	C19H22O6	346.4	[M-H]-
197	Hexaflumuron	86479-06-3	C16H8Cl2F6N2O3	461.1	[M-H]-
198	Lufenuron	103055-07-8	C17H8Cl2F8N2O3	511.2	[M-H]-
199	MCPP Acid	7085-19-0	C10H11ClO3	214.7	[M-H]-
200	Teflubenzuron	83121-18-0	C14H6Cl2F4N2O2	381.1	[M-H]-

Table 2: MRM analysis parameters for the detection of 200 pesticides

No.	Name	Mode	RT (min.)	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE (V)	Q3 Pre Bias(V)
1	Acephate	ESI+	2.015	184.2	143.0*	-20	-8	-15
					95.0	-20	-23	-16
2	Acetamiprid	ESI+	4.542	223.1	126.1*	-30	-22	-30
					56.1	-30	-15	-23
3	Acetochlor	ESI+	10.557	270.1	148.2*	-30	-19	-16
					133.1	-30	-33	-23
4	Alachlor	ESI+	10.480	270.1	238.1*	-30	-10	-26
					162.2	-30	-19	-30
5	Aldicarb	ESI+	5.079	213.1	89.1*	-24	-17	-16
					151.6	-24	-9	-16
6	Aldicarb-sulfone	ESI+	3.424	245.1	166.1*	-12	-15	-17
					109.1	-12	-20	-19
7	Aldicarb-sulfoxide	ESI+	2.928	229.0	166.1*	-25	-11	-17
					109.1	-25	-16	-20
8	Ametryn	ESI+	7.151	228.1	186.1*	-30	-18	-19
					68.1	-30	-39	-27
9	Anilofos	ESI+	12.662	368.0	199.0*	-18	-15	-21
					125.0	-18	-31	-22
10	Atrazine	ESI+	6.303	216.1	174.1*	-30	-17	-18
					96.1	-30	-25	-17
11	Azamethiphos	ESI+	5.391	325.0	183.1*	-16	-16	-19
					112.1	-16	-38	-20
12	Azimsulfuron	ESI+	6.031	425.1	182.1*	-21	-17	-19
					139.0	-21	-46	-24
13	Azinphos-methyl	ESI+	8.019	318.1	132.1*	-15	-14	-23
					261.0	-15	-7	-28
14	Azoxystrobin	ESI+	8.614	404.1	372.1*	-30	-14	-26
					329.0	-30	-31	-23
15	Benalaxyl	ESI+	11.910	326.2	148.2*	-16	-21	-15
					294.1	-16	-11	-20
16	Bendiocarb	ESI+	5.748	224.1	167.1*	-25	-9	-18
					109.1	-25	-19	-20
17	Bensulfuron methyl	ESI+	6.815	411.1	149.2*	-20	-19	-28
					182.2	-20	-20	-19
18	Bitertanol	ESI+	10.130	338.2	269.2*	-17	-9	-29
					99.1	-17	-15	-18
19	Brodifacoum	ESI+	22.047	523.1	335.0*	-26	-22	-23
					256.2	-26	-38	-27
20	Bupirimate	ESI+	11.245	317.1	108.0*	-30	-26	-19
					210.2	-30	-23	-22
21	Buprofezin	ESI+	18.799	306.1	201.1*	-30	-11	-22
					116.1	-30	-16	-12
22	Butachlor	ESI+	17.850	312.2	238.1*	-15	-12	-26
					147.2	-15	-36	-28
23	Butafenacil	ESI+	11.479	475.1	331.0*	-23	-19	-23
					180.0	-23	-40	-18
24	Butralin	ESI+	19.840	296.2	240.1*	-14	-12	-25
					222.1	-14	-21	-24
25	Cadusafos	ESI+	12.953	271.1	159.0*	-30	-14	-29
					97.0	-30	-37	-18

26	Carbaryl	ESI+	6.071	202.1	145.1*	-22	-9	-26
					127.1	-22	-27	-22
27	Carbendazim	ESI+	3.650	192.1	160.1*	-30	-17	-30
					132.1	-30	-30	-24
28	Carbofuran	ESI+	5.809	222.1	165.1*	-25	-11	-17
					123.1	-25	-21	-22
29	Carbofuran-3-hydroxy	ESI+	4.295	238.1	163.1*	-27	-14	-17
					181.2	-27	-10	-19
30	Carboxin	ESI+	6.339	236.1	143.0*	-27	-14	-15
					124.0	-27	-20	-22
31	Cartap Hydrochloride	ESI+	0.647	238.1	73.0*	-27	-27	-29
					150.0	-27	-14	-27
32	Chlorfluazuron	ESI+	20.152	540.0	382.9*	-26	-21	-27
					158.0	-26	-20	-30
33	Chlorimuron-ethyl	ESI+	8.350	415.1	186.0*	-20	-20	-19
					83.1	-20	-43	-15
34	Chlormequat	ESI+	0.649	122.1	58.1*	-30	-29	-23
					63.0	-30	-22	-24
35	Chlorotoluron	ESI+	5.975	213.1	72.0*	-23	-21	-28
					140.1	-24	-23	-24
36	Chlorpyrifos	ESI+	18.590	351.9	199.9*	-27	-18	-21
					97.0	-27	-33	-18
37	Chlorsulfuron	ESI+	5.700	358.1	141.1*	-18	-17	-15
					167.0	-17	-18	-30
38	Chromafenozide	ESI+	10.322	395.3	175.1*	-19	-16	-18
					339.2	-19	-7	-17
39	Cinosulfuron	ESI+	5.478	414.1	183.1*	-20	-18	-19
					157.1	-20	-20	-16
40	Clethodim	ESI+	16.310	360.2	164.1*	-18	-19	-17
					268.1	-18	-11	-30
41	Clomazone Dimethazone	ESI+	7.359	240.1	125.0*	-27	-19	-22
					89.1	-27	-50	-16
42	Clomeprop	ESI+	15.824	324.1	120.2*	-16	-21	-21
					203.0	-16	-16	-21
43	Cloquintocet-1-methyl-hexyl ester	ESI+	16.034	336.1	238.0*	-30	-15	-26
					192.0	-30	-29	-20
44	Clothianidin	ESI+	4.248	250.0	169.1*	-29	-12	-17
					132.0	-29	-14	-24
45	Coumatetralyl	ESI+	8.980	293.1	175.1*	-30	-22	-18
					91.1	-30	-32	-17
46	Cyflufenamid	ESI+	15.196	413.2	295.1*	-20	-16	-30
					203.0	-20	-40	-20
47	Cyproconazole	ESI+	7.971	292.1	70.1*	-30	-20	-27
					125.1	-30	-30	-22
48	Cyprodinil	ESI+	10.923	226.1	93.1*	-30	-34	-16
					108.1	-30	-27	-19
49	Dazomet	ESI+	3.867	163.0	120.0*	-18	-13	-21
					90.1	-18	-10	-16
50	Diazinon	ESI+	13.332	305.0	169.1*	-30	-19	-18
					153.1	-30	-20	-16
51	Dichlorvos	ESI+	5.400	221.0	109.1*	-23	-16	-11
					79.1	-23	-27	-30

52	Diethofencarb	ESI+	8.199	268.1	226.1*	-30	-8	-24
					180.1	-30	-17	-19
53	Diethyltoluamide	ESI+	6.395	192.1	119.1*	-30	-16	-22
					91.1	-30	-30	-16
54	Dimepiperate	ESI+	14.975	264.1	146.1*	-29	-7	-15
					91.1	-29	-36	-16
55	Dimethirimol	ESI+	4.365	210.2	71.1*	-30	-31	-28
					140.1	-30	-21	-26
56	Dimethoate	ESI+	4.495	230.0	199.0*	-26	-9	-21
					125.0	-26	-22	-22
57	Dimethomorph	ESI+	7.529	388.1	301.0*	-19	-20	-21
					165.1	-19	-34	-30
58	Diniconazole	ESI+	10.678	326.1	70.0*	-16	-25	-28
					159.0	-16	-30	-30
59	Dinotefuran	ESI+	3.032	203.1	129.1*	-22	-12	-22
					113.1	-22	-10	-12
60	Diuron	ESI+	6.391	233.0	72.0*	-26	-21	-27
					160.1	-26	-26	-29
61	Dymron	ESI+	8.912	269.2	151.1*	-30	-12	-16
					91.1	-30	-40	-16
62	Epoxiconazole	ESI+	8.810	330.1	121.2*	-17	-21	-22
					141.1	-17	-18	-25
63	Esprocarb	ESI+	17.232	266.1	91.1*	-30	-24	-16
					71.1	-30	-14	-28
64	Ethametsulfuron-methyl	ESI+	5.877	411.1	196.1*	-20	-16	-21
					168.1	-20	-28	-17
65	Ethiofencarb	ESI+	6.339	226.1	107.1*	-26	-15	-19
					164.1	-26	-8	-30
66	Ethion	ESI+	19.150	385.0	199.0*	-19	-10	-22
					143.0	-19	-26	-25
67	Ethoprophos	ESI+	9.194	243.1	131.0*	-26	-20	-23
					97.0	-27	-32	-17
68	Fenamiphos	ESI+	8.513	304.1	217.1*	-15	-22	-23
					202.0	-15	-36	-21
69	Fenarimol	ESI+	8.373	331.0	268.1*	-16	-22	-28
					259.1	-17	-26	-26
70	Fenobucarb	ESI+	7.871	208.1	95.1*	-23	-13	-17
					126.0	-23	-9	-23
71	Fenoxycarb	ESI+	10.370	302.1	88.1*	-15	-21	-16
					116.1	-15	-10	-12
72	Fenpropimorph	ESI+	5.710	304.2	147.2*	-30	-30	-27
					119.1	-30	-39	-22
73	Fenpyroximate	ESI+	19.458	422.2	366.1*	-30	-15	-26
					138.1	-30	-33	-26
74	Flocoumafen	ESI+	20.331	543.2	159.1*	-26	-43	-29
					355.2	-26	-21	-25
75	Fluazifop-p-butyl	ESI+	18.129	384.2	282.2*	-19	-21	-30
					328.1	-19	-17	-23
76	Flusilazole	ESI+	9.593	316.1	247.1*	-30	-18	-27
					165.1	-30	-29	-30
77	Flutriafol	ESI+	5.941	302.1	123.0*	-15	-28	-22
					109.0	-15	-31	-19
78	Fonofos	ESI+	13.684	247.1	109.0*	-27	-19	-19
					137.1	-26	-10	-14

79	Forchlorfenuron	ESI+	6.017	248.1	129.1*	-30	-17	-23
					93.1	-30	-34	-17
80	Fosthiazate	ESI+	6.141	284.1	228.0*	-30	-10	-24
					104.1	-30	-21	-19
81	Furathiocarb	ESI+	17.142	383.2	195.1*	-27	-19	-21
					252.1	-27	-13	-27
82	Hexaconazole	ESI+	10.030	314.1	70.2*	-15	-21	-28
					159.2	-15	-29	-30
83	Hexazinone	ESI+	5.039	253.2	171.1*	-30	-15	-18
					85.1	-30	-31	-15
84	Hexythiazox	ESI+	18.652	353.1	228.0*	-18	-15	-24
					168.1	-18	-25	-30
85	Imazapyr	ESI+	3.787	262.1	217.1*	-29	-19	-23
					69.1	-29	-28	-27
86	Imibenconazole	ESI+	15.337	411.0	125.1*	-20	-31	-22
					171.0	-20	-20	-18
87	Imidacloprid	ESI+	4.396	256.1	175.1*	-29	-17	-18
					209.1	-29	-14	-22
88	Indoxacarb	ESI+	15.371	528.1	249.1*	-26	-17	-27
					293.0	-26	-15	-21
89	Iprobenfos	ESI+	10.018	289.1	205.0*	-30	-10	-22
					91.1	-30	-21	-16
90	Iprovalicarb	ESI+	8.427	321.2	119.1*	-30	-19	-22
					203.1	-30	-8	-22
91	Isazofos	ESI+	11.311	314.1	162.1*	-15	-16	-17
					120.1	-15	-27	-21
92	Isoprocarb	ESI+	6.707	194.1	95.0*	-21	-14	-17
					137.1	-22	-10	-14
93	Isoprothiolane	ESI+	10.122	291.1	231.1*	-14	-11	-25
					189.1	-14	-21	-20
94	Isoproturon	ESI+	6.304	207.1	72.0*	-23	-21	-28
					165.1	-23	-13	-17
95	Kresoxim-methyl	ESI+	11.958	314.1	235.1*	-16	-15	-25
					222.2	-16	-13	-24
96	Linuron	ESI+	8.137	249.0	160.1*	-27	-17	-17
					182.1	-28	-14	-19
97	Lodosulfuron-methyl-sodium	ESI+	6.836	530.0	163.1*	-26	-17	-17
					389.9	-26	-18	-27
98	Malachite Green Oxalate salt	ESI+	5.003	329.1	313.1*	-30	-35	-22
					208.1	-30	-36	-22
99	Malathion	ESI+	10.060	331.0	127.1*	-17	-12	-13
					99.0	-17	-23	-18
100	Mefenacet	ESI+	8.899	299.1	120.1*	-15	-27	-21
					148.1	-15	-14	-15
101	Mefenoxam	ESI+	6.351	280.1	220.2*	-30	-13	-24
					248.1	-30	-10	-27
102	Metalaxyl	ESI+	6.350	280.1	220.2*	-30	-13	-24
					192.2	-30	-18	-20
103	Metazachlor	ESI+	6.959	278.1	210.1*	-30	-10	-22
					134.1	-30	-22	-24
104	Methamidophos	ESI+	1.550	142.1	94.0*	-16	-15	-17
					125.1	-16	-16	-23
105	Methiocarb	ESI+	7.869	226.1	169.1*	-25	-10	-18
					121.1	-25	-18	-23

106	Methomyl	ESI+	3.665	163.1	88.0*	-18	-8	-16
					106.1	-18	-10	-19
107	Methoxyfenozide	ESI+	9.738	369.2	149.1*	-18	-16	-16
					313.1	-18	-8	-22
108	Metolachlor	ESI+	10.397	284.1	252.1*	-30	-14	-27
					176.2	-30	-25	-19
109	Metolcarb	ESI+	5.382	166.1	109.1*	-18	-12	-20
					107.1	-18	-25	-19
110	Metsulfuron-methyl	ESI+	5.431	382.1	167.1*	-19	-16	-18
					141.1	-19	-15	-26
111	Mevinphos	ESI+	4.271	225.0	127.1*	-25	-17	-23
					193.1	-25	-8	-20
112	Molinate	ESI+	9.151	188.1	126.1*	-21	-13	-13
					98.1	-20	-20	-18
113	Monocrotophos	ESI+	3.506	224.1	127.1*	-25	-15	-13
					193.0	-25	-8	-20
114	Myclobutanil	ESI+	8.560	289.1	70.1*	-30	-21	-28
					125.1	-30	-30	-22
115	Naproanilide	ESI+	11.075	292.1	171.1*	-30	-14	-18
					120.1	-30	-24	-22
116	Napropamide	ESI+	9.498	272.2	129.2*	-30	-16	-23
					171.1	-30	-17	-18
117	Omethoate	ESI+	2.571	214.1	183.0*	-23	-10	-19
					155.0	-23	-14	-28
118	Oxycarboxin	ESI+	4.959	268.1	175.0*	-29	-14	-18
					147.0	-29	-25	-27
119	Paclobutrazol	ESI+	7.533	294.1	70.1*	-15	-21	-28
					125.1	-15	-40	-22
120	Penconazole	ESI+	10.094	284.1	159.0*	-14	-27	-30
					70.0	-14	-17	-27
121	Pencycuron	ESI+	14.050	329.1	125.1*	-17	-26	-22
					218.1	-17	-15	-23
122	Pendimethalin	ESI+	18.557	282.2	212.1*	-30	-10	-23
					194.0	-30	-18	-20
123	Phorate	ESI+	14.235	261.0	75.0*	-29	-10	-30
					143.0	-29	-18	-15
124	Phosalone	ESI+	14.364	368.0	182.1*	-30	-14	-19
					111.0	-30	-39	-20
125	Phosemet	ESI+	8.396	318.0	160.0*	-16	-13	-17
					77.1	-16	-54	-30
126	Phoxim	ESI+	14.25	299.0	77.1*	-30	-26	-30
					129.1	-30	-10	-13
127	Piperonyl Butoxide	ESI+	16.830	356.3	177.1*	-24	-13	-19
					119.0	-24	-37	-22
128	Pirimicarb	ESI+	5.764	239.2	72.1*	-30	-25	-30
					182.2	-30	-19	-30
129	Pirimiphos methyl	ESI+	14.855	306.1	108.1*	-30	-31	-19
					95.0	-30	-29	-17
130	Pretilachlor	ESI+	15.040	312.2	252.2*	-15	-16	-28
					176.2	-15	-28	-18
131	Prochloraz	ESI+	10.826	376.0	308.0*	-19	-11	-21
					266.0	-19	-17	-29
132	Profenofos	ESI+	15.112	372.9	302.8*	-18	-19	-30
					345.0	-18	-12	-24

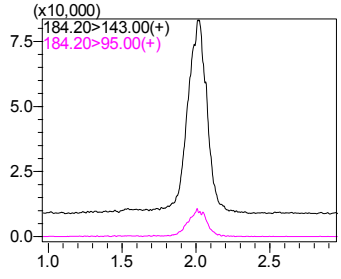
133	Promecarb	ESI+	8.371	208.2	109.1*	-22	-15	-19
					151.1	-22	-8	-16
134	Prometryne	ESI+	8.838	242.2	158.1*	-30	-23	-29
					200.2	-30	-17	-22
135	Propamocarb	ESI+	0.800	189.2	102.1*	-30	-20	-23
					144.1	-30	-12	-15
136	Propanil	ESI+	7.466	218.0	162.0*	-24	-15	-17
					127.0	-24	-26	-23
137	Propargite	ESI+	20.428	368.2	231.2*	-26	-11	-25
					175.2	-26	-17	-19
138	Propiconazole	ESI+	10.763	342.1	159.1*	-17	-30	-29
					205.1	-17	-18	-21
139	Propoxur	ESI+	5.745	210.1	168.1*	-23	-7	-18
					111.1	-23	-13	-20
140	Propyzamide	ESI+	9.190	256.1	190.0*	-28	-13	-20
					173.0	-28	-20	-18
141	Pyraclostrobin	ESI+	13.467	388.1	194.1*	-19	-13	-21
					163.1	-19	-24	-30
142	Pyrazolynate	ESI+	14.186	439.0	91.1*	-21	-40	-16
					173.0	-21	-20	-18
143	Pyrazosulfuron-ethyl	ESI+	7.889	415.1	182.1*	-21	-18	-19
					139.1	-21	-42	-24
144	Pyridaben	ESI+	21.820	365.1	309.1*	-18	-12	-22
					147.1	-18	-25	-27
145	Pyridaphenthion	ESI+	9.088	341.1	189.1*	-17	-22	-20
					205.1	-23	-22	-22
146	Pyrimethanil	ESI+	7.810	200.1	107.0*	-30	-25	-19
					168.1	-30	-29	-30
147	Pyriproxyfen	ESI+	18.032	322.1	96.1*	-30	-14	-10
					185.1	-30	-22	-20
148	Quinalphos	ESI+	11.902	299.0	163.1*	-15	-20	-30
					147.1	-15	-21	-27
149	Quizalofop-p-ethyl	ESI+	15.753	373.1	299.1*	-19	-19	-21
					91.1	-19	-32	-16
150	Rimsulfuron	ESI+	5.706	454.1	299.0*	-22	-19	-21
					178.1	-22	-19	-19
151	Simeconazole	ESI+	8.410	294.1	70.1*	-15	-21	-28
					135.1	-15	-21	-24
152	Spinosad	ESI+	6.034	732.5	142.2*	-40	-31	-25
					98.2	-40	-55	-17
153	Spirodiclofen	ESI+	22.107	411.1	71.2*	-21	-16	-28
					313.1	-21	-11	-22
154	Sulfotep	ESI+	13.952	323.0	115.0*	-16	-31	-20
					171.1	-16	-15	-18
155	Tebufenozide	ESI+	11.364	353.2	133.1*	-18	-20	-24
					297.1	-18	-8	-15
156	Tebufenpyrad	ESI+	15.810	334.2	117.1*	-16	-38	-21
					145.0	-16	-27	-26
157	Temephos	ESI+	18.448	467.0	419.0*	-23	-19	-30
					341.0	-23	-32	-24
158	Terbufos	ESI+	17.788	289.0	103.2*	-14	-9	-18
					57.1	-14	-24	-24
159	Terbuthylazine	ESI+	7.977	230.1	174.1*	-30	-16	-18
					110.0	-30	-29	-19

160	Terbutryn	ESI+	9.135	242.1	186.1*	-30	-23	-30
					91.0	-30	-26	-16
161	Tetrachlorvinphos	ESI+	10.362	364.9	127.1*	-27	-14	-13
					203.9	-27	-38	-21
162	Tetramethrin	ESI+	16.592	332.2	164.2*	-16	-22	-30
					135.2	-16	-19	-24
163	Thiabendazole	ESI+	3.942	202.0	175.1*	-30	-24	-30
					131.1	-30	-33	-24
164	Thiacloprid	ESI+	4.924	253.0	126.1*	-28	-20	-22
					99.0	-28	-43	-17
165	Thiamethoxam	ESI+	3.916	292.0	211.1*	-30	-11	-22
					181.1	-30	-23	-19
166	Thiodicarb	ESI+	5.512	355.1	88.1*	-17	-20	-16
					108.1	-17	-16	-11
167	Thiophanate-methyl	ESI+	5.446	343.1	151.2*	-17	-20	-16
					311.1	-17	-10	-22
168	Tolclofos methyl	ESI+	14.288	301.1	175.1*	-15	-29	-30
					125.2	-20	-17	-13
169	Tralkoxydim	ESI+	18.386	330.2	284.2*	-16	-13	-30
					138.1	-16	-20	-25
170	Triadimenol	ESI+	7.481	296.1	70.1*	-15	-11	-29
					99.2	-15	-15	-17
171	Triallate	ESI+	19.889	304.0	143.0*	-15	-27	-25
					86.0	-15	-17	-16
172	Triazophos	ESI+	10.221	314.1	162.2*	-23	-19	-17
					119.2	-23	-35	-21
173	Trichlorphon	ESI+	4.098	256.9	109.0*	-29	-17	-19
					220.9	-29	-10	-23
174	Tricyclazole	ESI+	4.583	190.0	163.0*	-21	-21	-30
					136.0	-21	-26	-24
175	Tridemorph	ESI+	6.569	298.3	130.1*	-30	-27	-24
					98.1	-30	-30	-18
176	Trifloxystrobin	ESI+	15.575	409.1	186.1*	-20	-18	-20
					145.0	-20	-44	-26
177	Triflumizole	ESI+	12.951	346.1	278.1*	-17	-13	-30
					73.2	-17	-17	-30
178	Triflumuron	ESI+	12.197	359.1	156.0*	-17	-16	-30
					139.0	-17	-33	-26
179	Triticonazole	ESI+	7.971	318.1	70.1*	-16	-21	-28
					125.1	-16	-39	-23
180	Warfarin	ESI+	7.620	309.1	163.1*	-15	-14	-17
					251.1	-15	-19	-27
181	2,4-D D3	ESI-	6.102	222.0	164.1*	15	12	30
					127.1	15	28	22
182	2,4-DB	ESI-	7.932	247.0	161.1*	17	11	29
					125.2	17	28	21
183	2,4-Dichlorophenoxy-acetic Acid	ESI-	6.117	219.0	161.1*	15	11	30
					125.0	15	26	22
184	Acifluorofen	ESI-	9.122	360.0	316.1*	26	9	21
					195.0	26	26	19
185	Bromadiolone	ESI-		527.1	250.1*	38	38	23

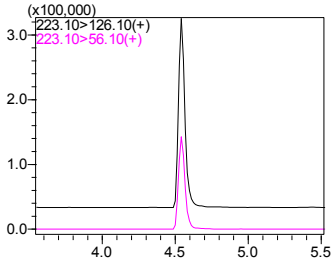
			15.093		93.1	38	42	17
186	Bromoxynil	ESI-	6.252	275.9	81.0*	19	33	27
					79.1	19	45	27
187	Chiptox	ESI-	6.169	199.1	141.1*	14	12	26
					105.1	14	28	21
188	Chloramphenicol	ESI-	4.714	321.0	152.2*	22	17	27
					257.2	22	11	16
189	Chlorobenzuron	ESI-	10.597	307.0	154.2*	21	11	29
					126.2	21	22	25
190	Dicamba	ESI-	4.800	219.0	175.1*	25	7	30
					144.9	25	10	25
191	Dichlorprop	ESI-	6.863	233.0	161.0*	16	11	29
					125.1	16	28	20
192	Diflufenican	ESI-	14.913	393.1	272.1*	28	22	28
					329.1	28	16	21
193	Fludioxonil	ESI-	7.985	247.1	180.2*	17	29	30
					126.2	17	32	22
194	Flufenoxuron	ESI-	18.862	487.1	156.1*	17	15	30
					467.1	17	10	21
195	Fomesafen	ESI-	10.285	437.0	195.2*	30	40	18
					286.1	30	24	17
196	Gibberellic Acid	ESI-	4.021	345.1	143.1*	24	37	27
					239.3	24	16	15
197	Hexaflumuron	ESI-	14.209	459.0	438.9*	16	12	29
					175.1	16	36	29
198	Lufenuron	ESI-	17.249	509.0	326.0*	36	17	21
					339.0	36	11	22
199	Mcpp Acid	ESI-	6.926	213.1	141.1*	14	14	26
					105.0	14	30	15
200	Teflubenzuron	ESI-	14.441	379.0	339.0*	13	11	22
					359.0	13	6	24

RESULTS AND DISCUSSION

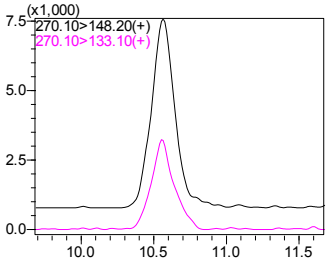
The mixed standard solutions were tested under the analysis conditions as specified above and the chromatograms of the pesticide standard solutions of 50 µg/L are shown below in Figure 1.



1.Acephate

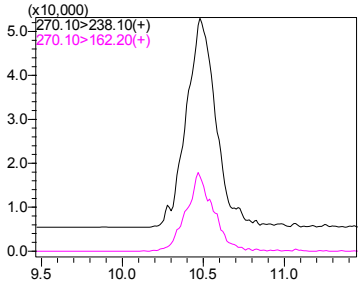


2.Acetamiprid

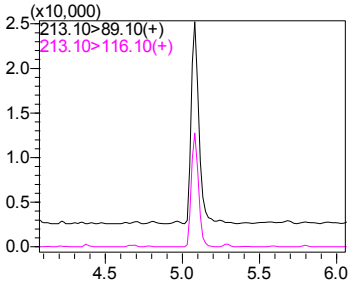


Acetochlor

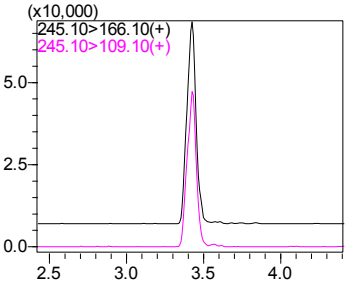
3.



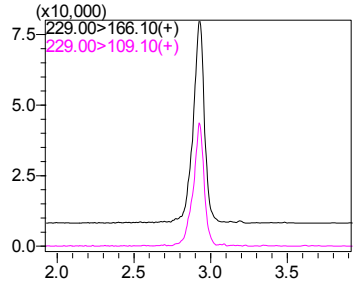
4. Alachlor



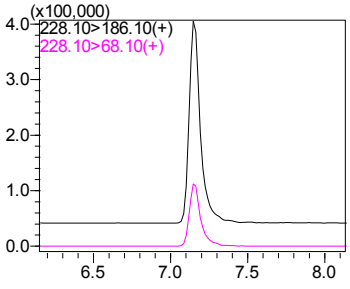
5.Aldicarb



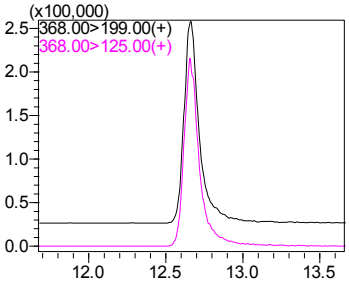
6. Aldicarb-sulfone



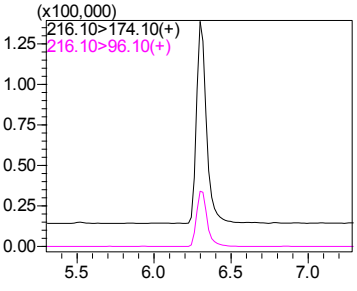
7. Aldicarb-sulfoxide



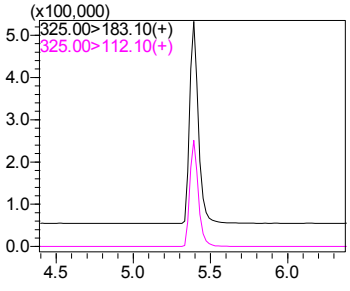
8. Ametryn



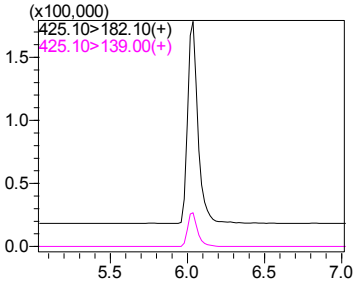
9.Anilofos



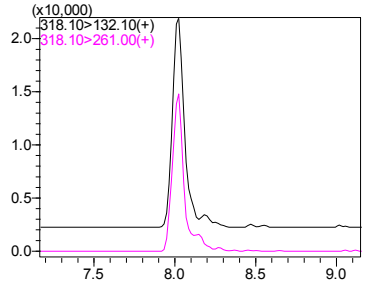
10.Atrazine



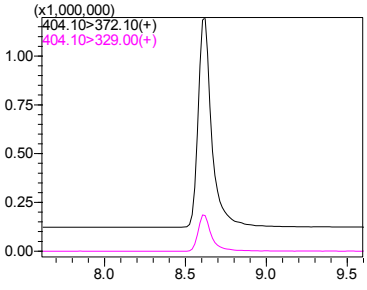
11. Azamethiphos



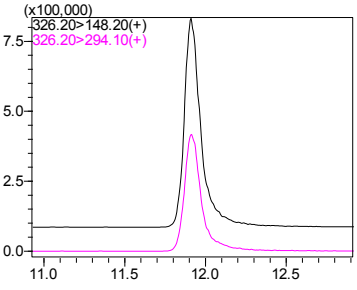
12. Azimsulfuron



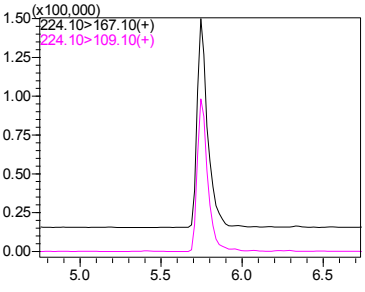
13.Azinphos-methyl



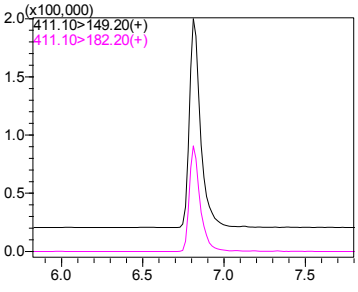
14.Azoxystrobin



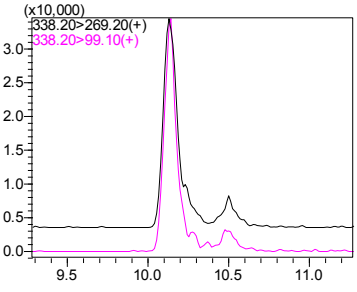
15.Benalaxyl



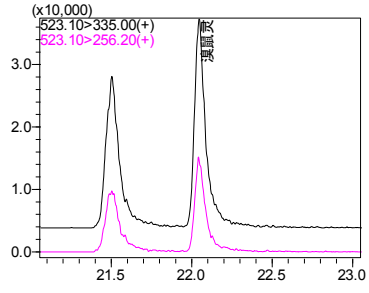
16.Bendiocarb



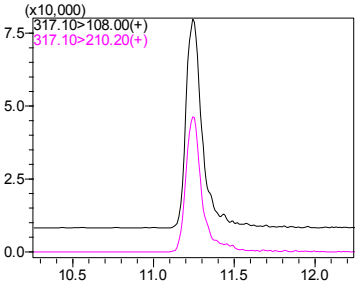
17. Bensulfuron methyl



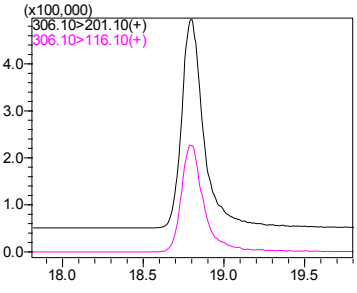
18. Bitertanol



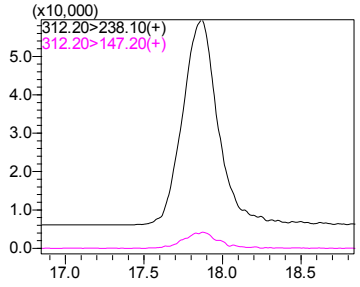
19. Brodifacoum



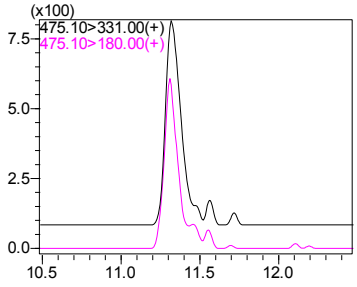
20. Bupirimate



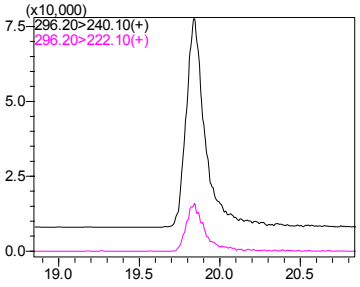
21.Buprofezin



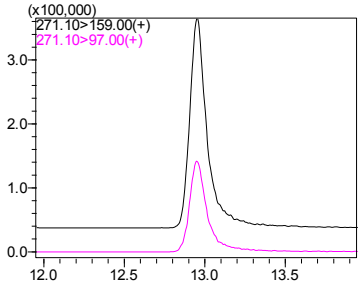
22. Butachlor



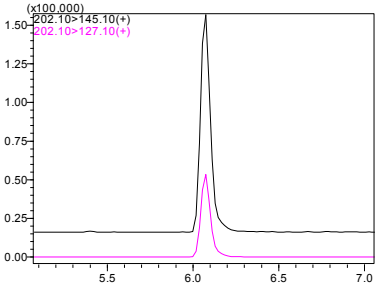
23. Butafenacil



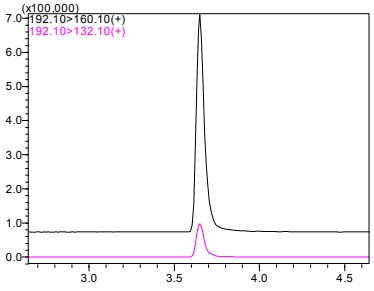
24. Butralin



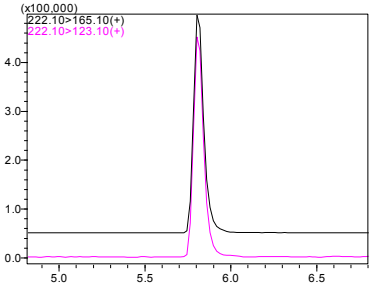
25.Cadusafos



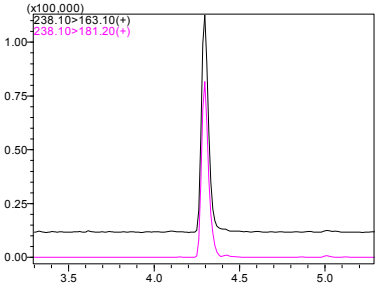
26. Carbaryl



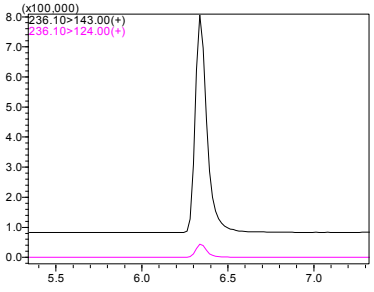
27.Carbendazim



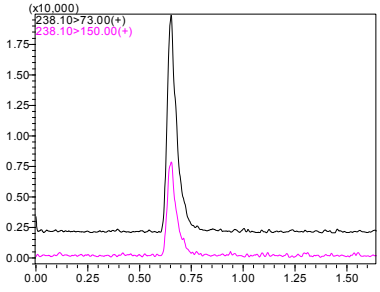
28. Carbofuran



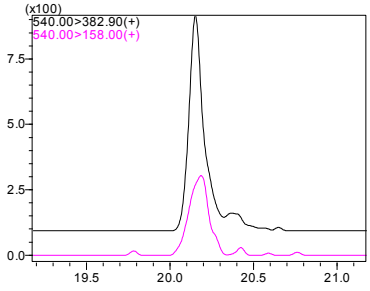
29. Carbofuran-3-hydroxy



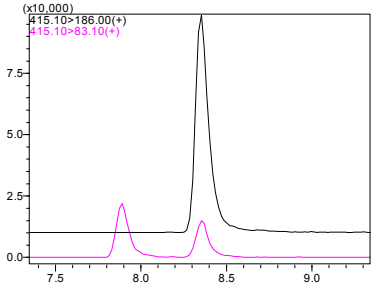
30.Carboxin



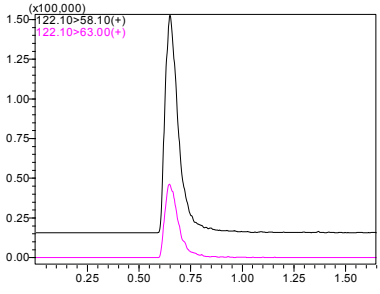
31. Cartap Hydrochloride



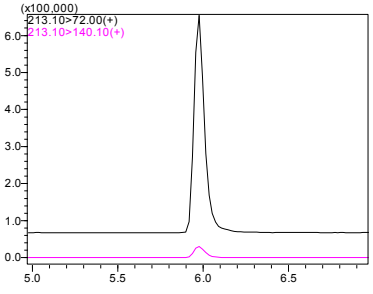
32. Chlorfluazuron



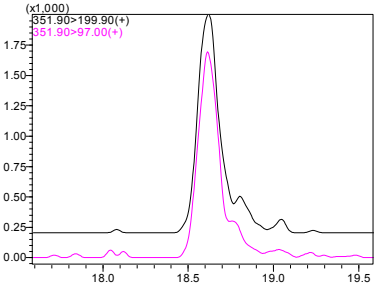
33. Chlorimuron-ethyl



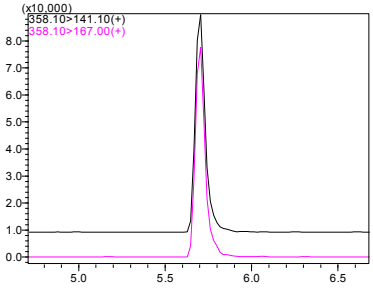
34. Chlormequat



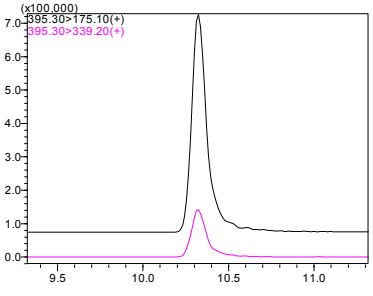
35. Chlorotoluron



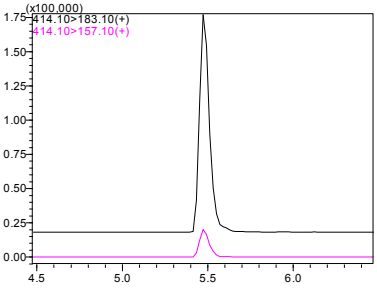
36. Chlorpyrifos



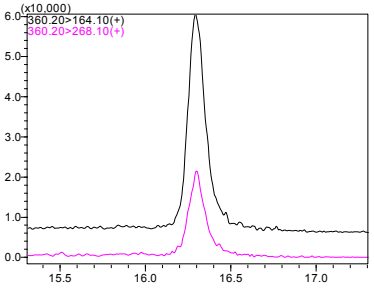
37.Chlorsulfuron



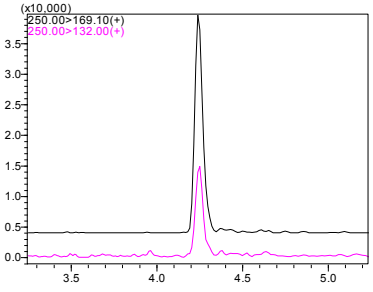
38.Chromafenozide



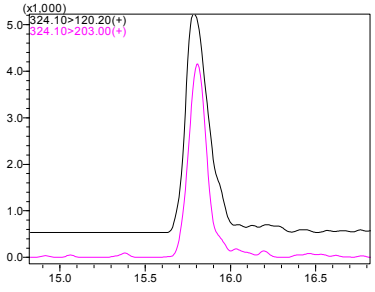
39.Cinosulfuron



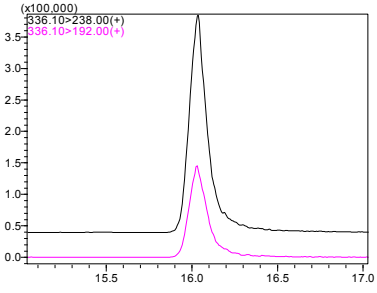
40. Clethodim



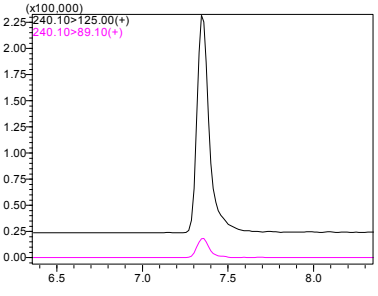
41. Clomazone dimethazone



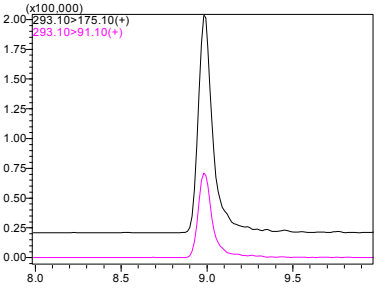
42. Clomeprop



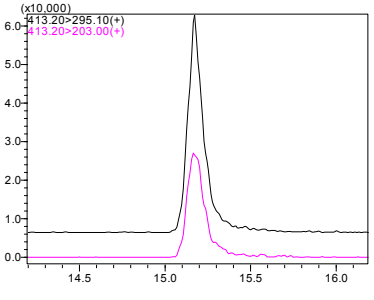
43. Cloquintocet-1-methylhexyl ester



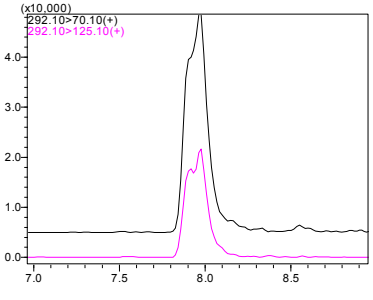
44.Clothianidin



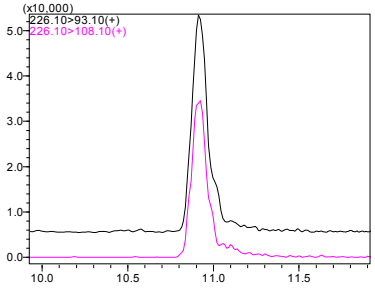
45. Coumatetralyl



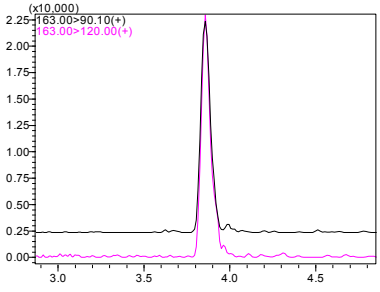
46. Cyflufenamid



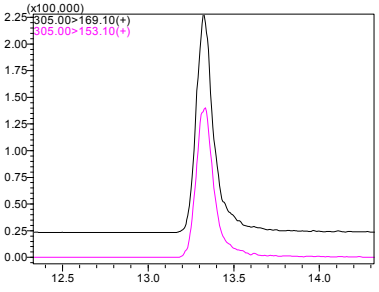
47. Cyproconazole



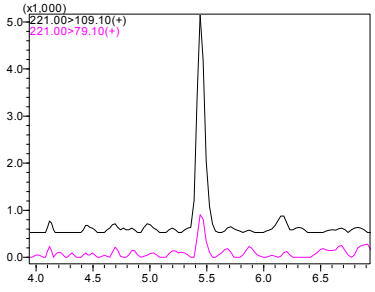
48. Cyprodinil



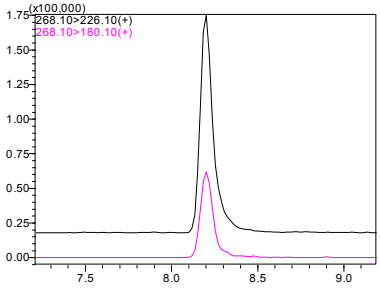
49. Dazomet



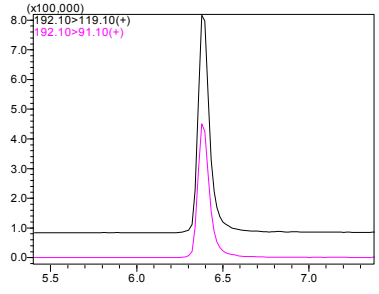
50. Diazinon



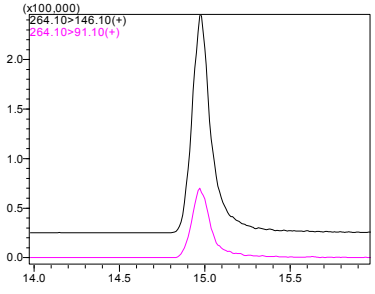
51. Dichlorvos



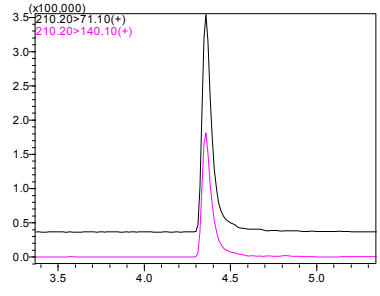
52. Diethofencarb



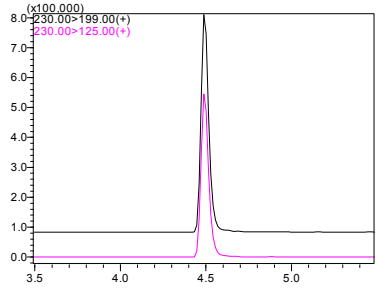
53. Diethyltoluamide



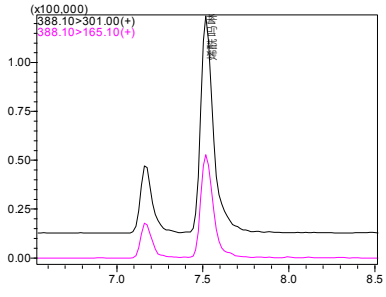
54. Dimepiperate



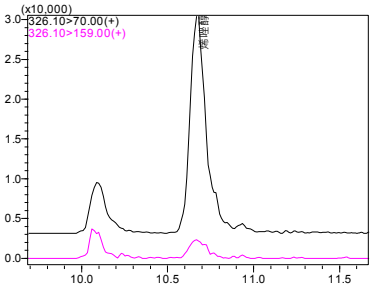
55. Dimethirimol



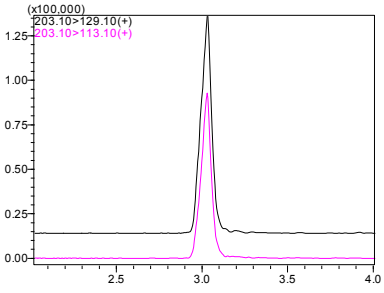
56. Dimethoate



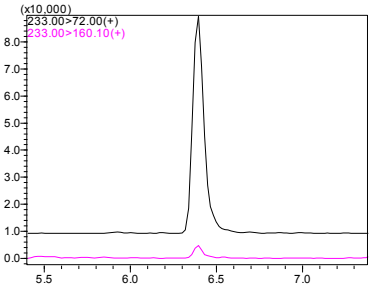
57. Dimethomorph



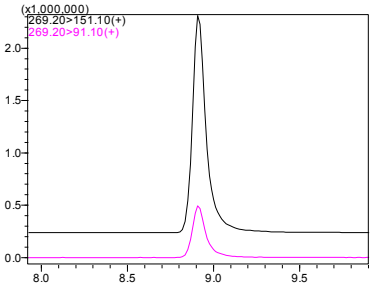
58. Diniconazole



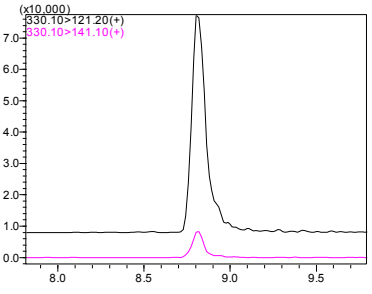
59. Dinotefuran



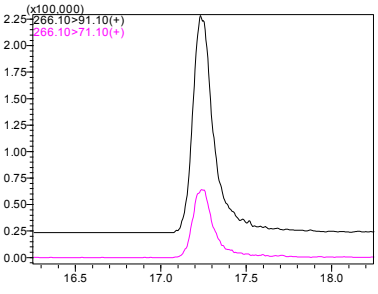
60. Diuron



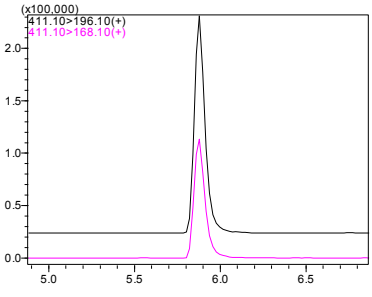
61. Dymron



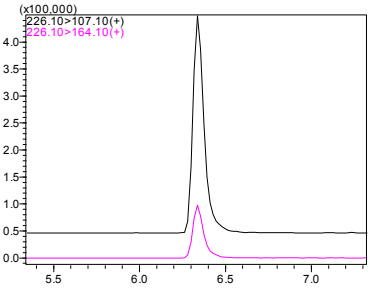
62. Epoxiconazole



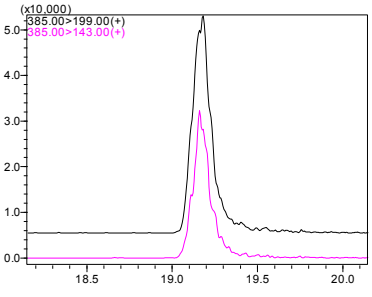
63. Esprocarb



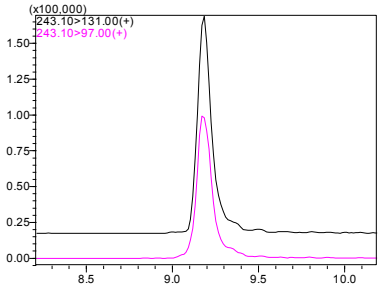
64. Ethametsulfuron-methyl



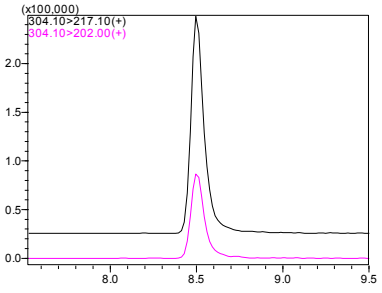
65. Ethiofencarb



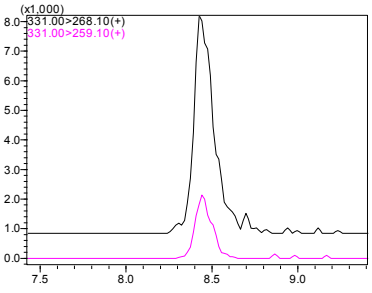
66. Ethion



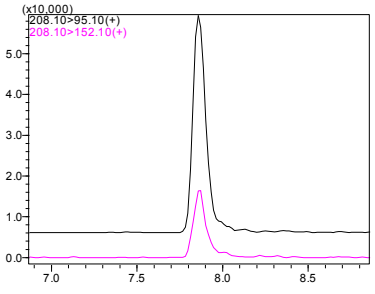
67. Ethoprophos



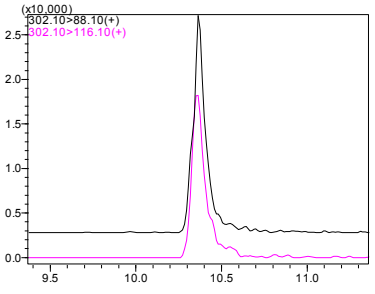
68. Fenamiphos



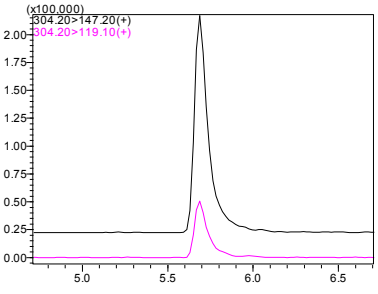
69. Fenarimol



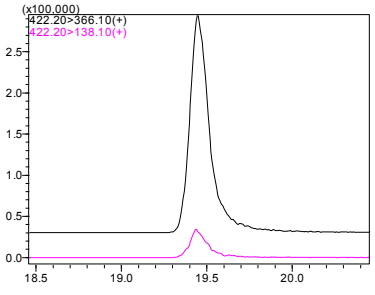
70. Fenobucarb



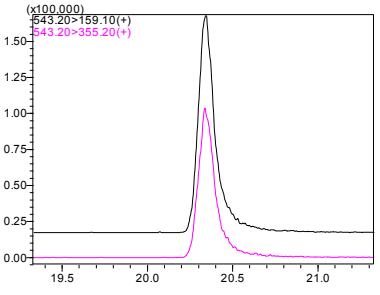
71. Fenoxycarb



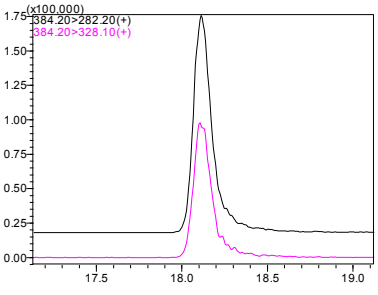
72. Fenpropimorph



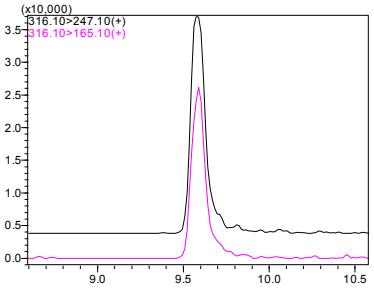
73. Fenpyroximate



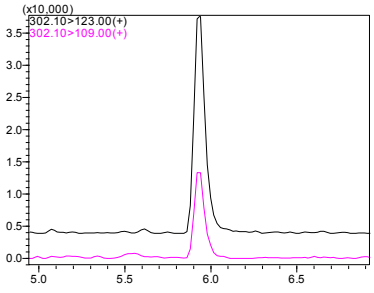
74. Flocoumafen



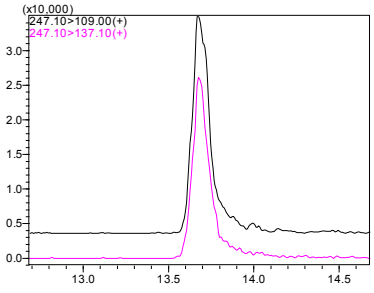
75. Fluazifop-p-butyl



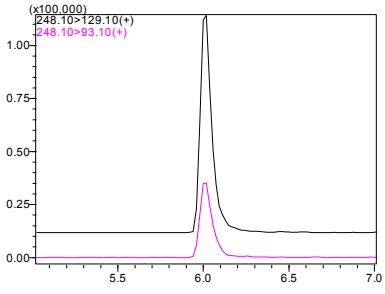
76. Flusilazole



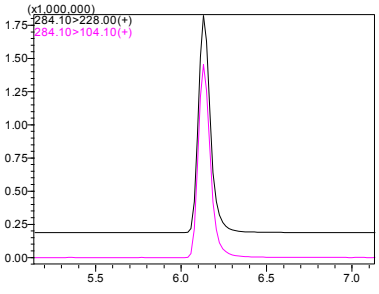
77. Flutriafol



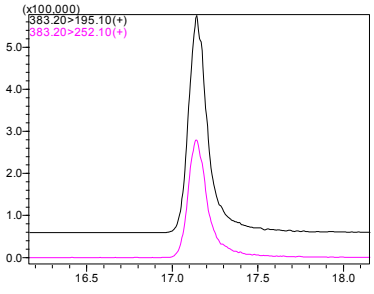
78. Fonofos



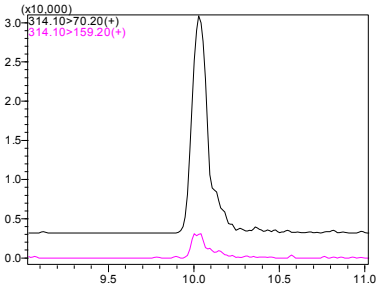
79. Forchlorfenuron



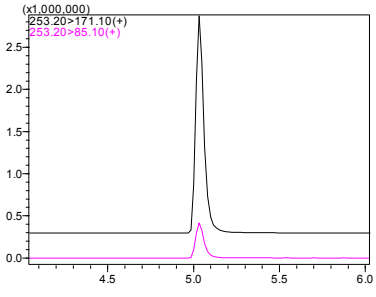
80. Fosthiazate



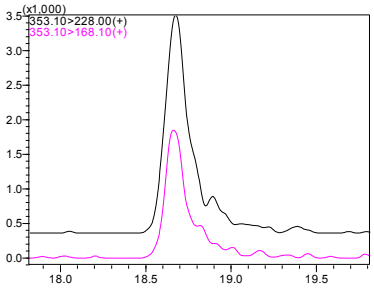
81. Furathiocarb



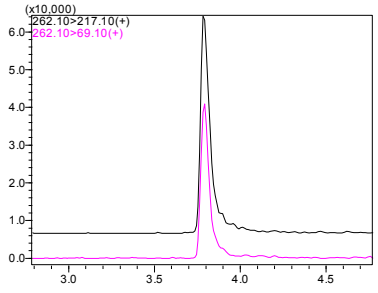
82. Hexaconazole



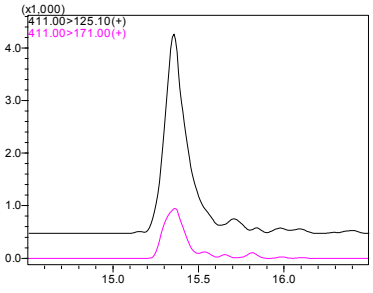
83. Hexazinone



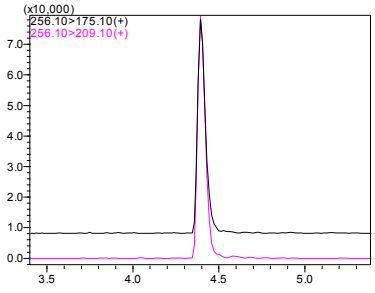
84. Hexythiazox



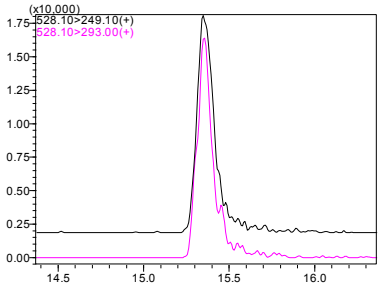
85. Imazapyr



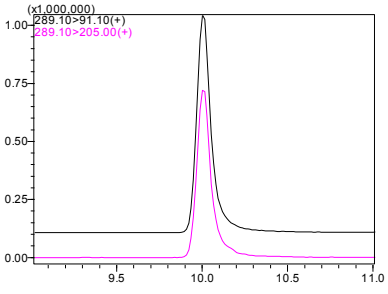
86. Imibenconazole



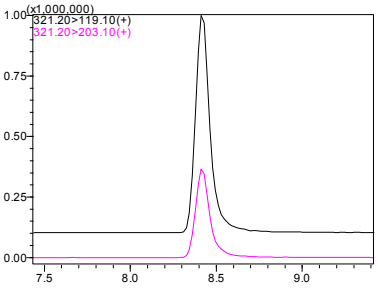
87. Imidacloprid



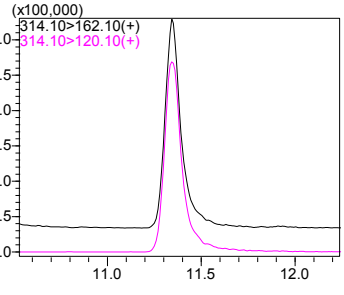
88. Indoxacarb



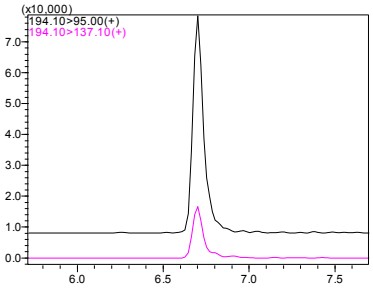
89. Iprobenfos



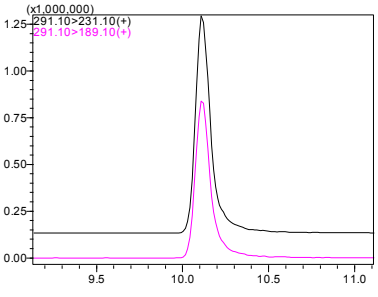
90. Iprovalicarb



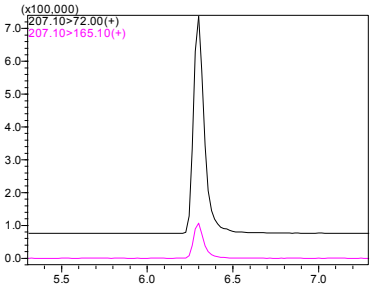
91. Isazofos



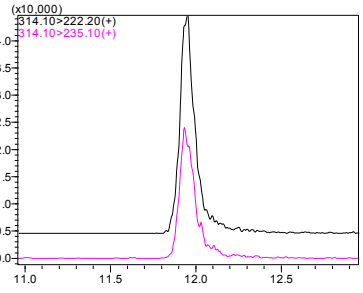
92. Isoprocarb



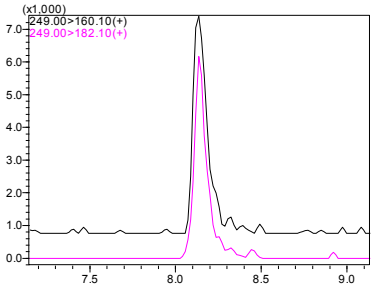
93. Isoprothiolane



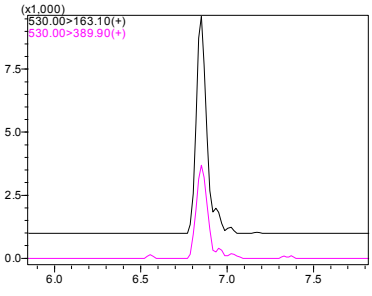
94. Isoproturon



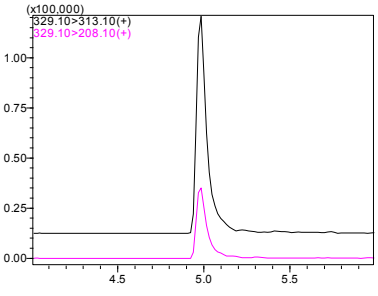
95. Kresoxim-methyl



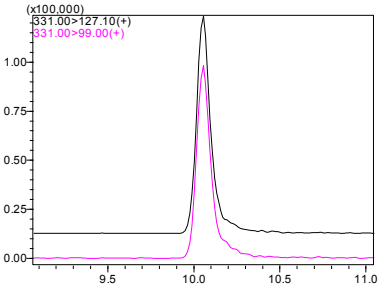
96. Linuron



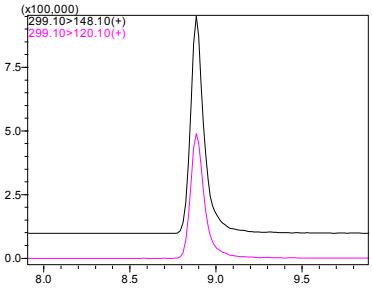
97. Lodosulfuron-methyl-sodium



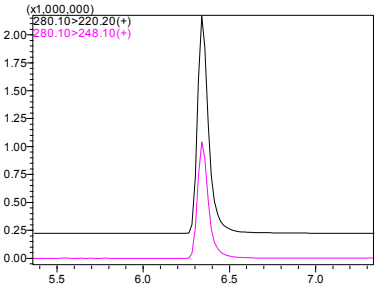
98.Malachite Green Oxalate salt



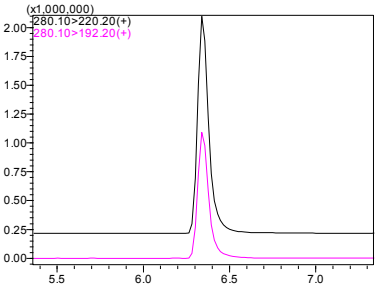
99. Malathion



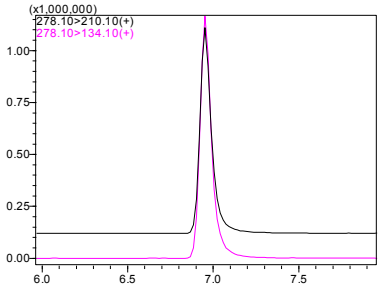
100.Mefenacet



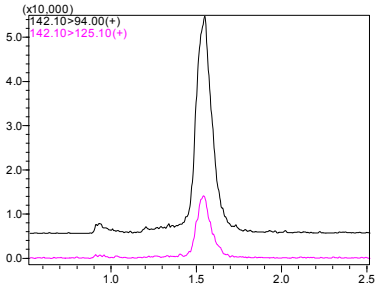
101.Mefenoxam



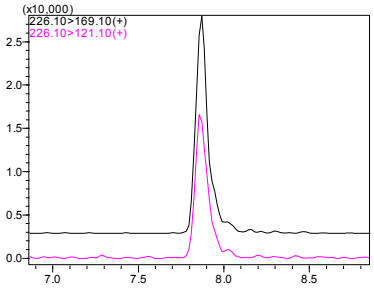
102.Metalaxyl



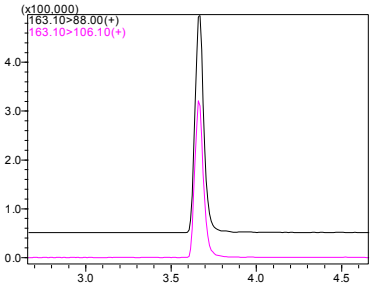
103. Metazachlor



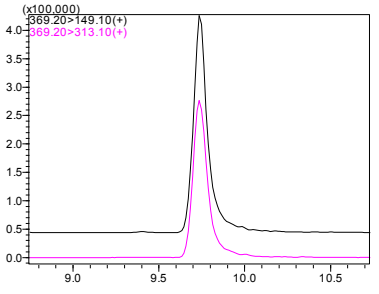
104. Methamidophos



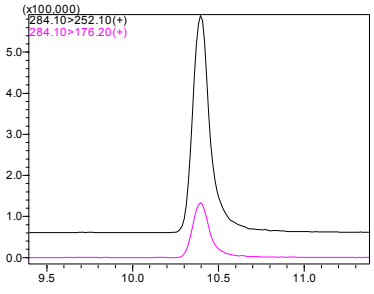
105. Methiocarb



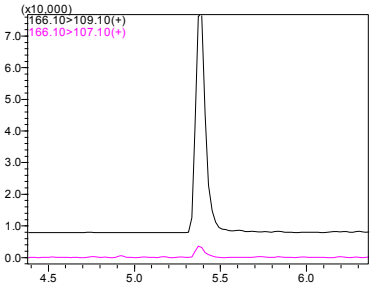
106. Methomyl



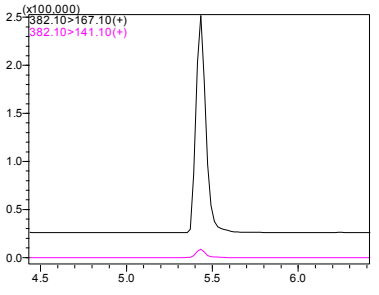
107. Methoxyfenozide



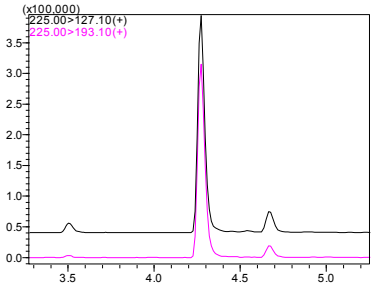
108. Metolachlor



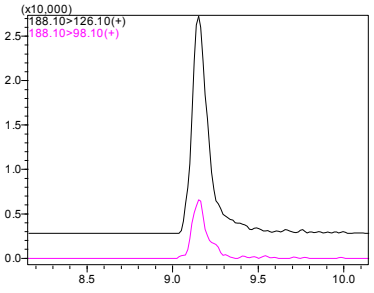
109. Metolcarb



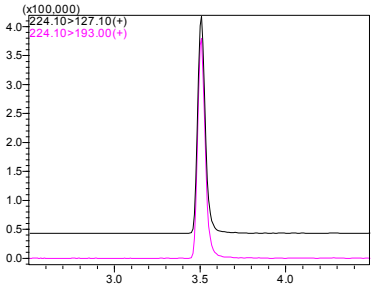
110. Metsulfuron-methyl



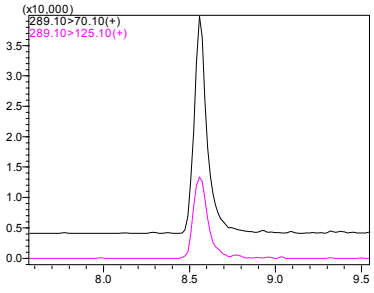
111. Mevinphos



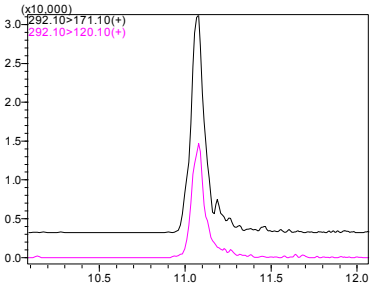
112.Molinate



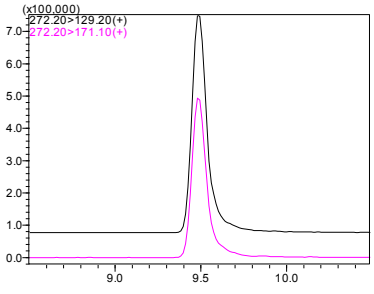
113. Monocrotophos



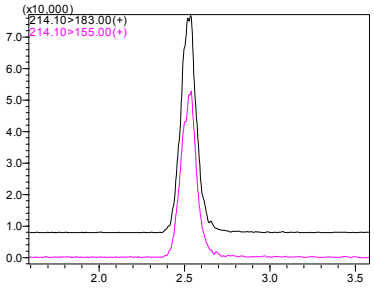
114. Myclobutanil



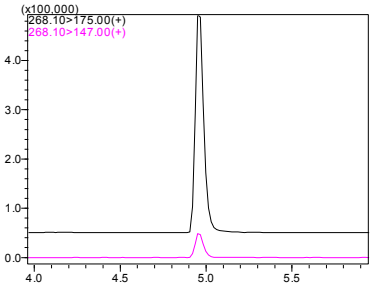
115. Naproanilide



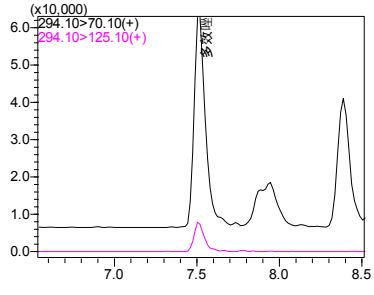
116. Napropamide



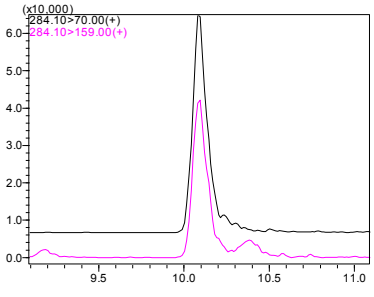
117. Omethoate



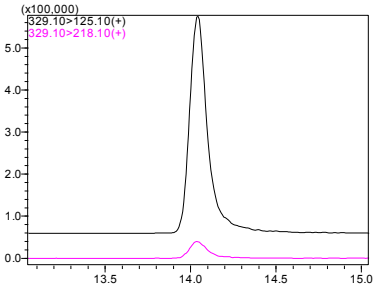
118. Oxycarboxin



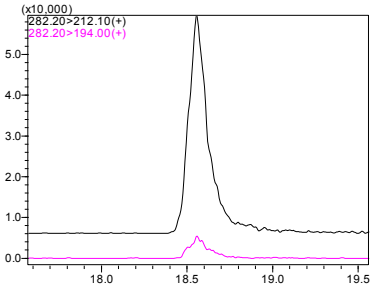
119. Paclobutrazol



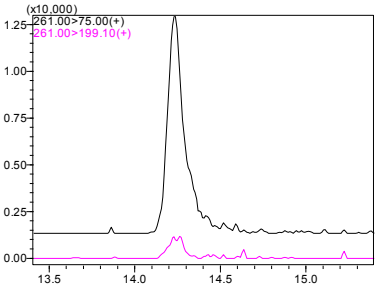
120. Penconazole



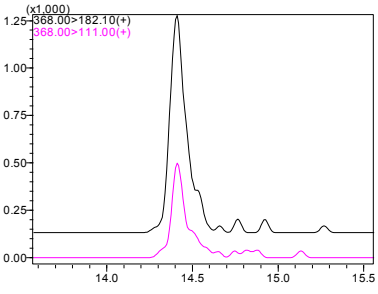
121. Pencycuron



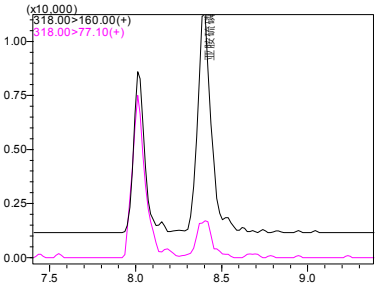
122. Pendimethalin



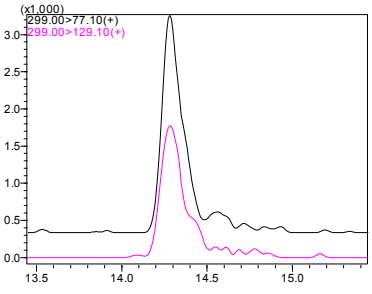
123. Phorate



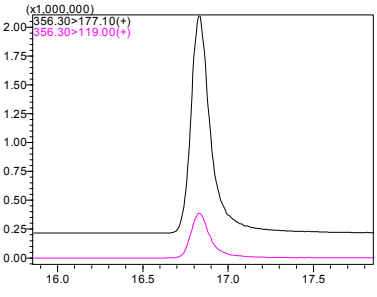
124. Phosalone



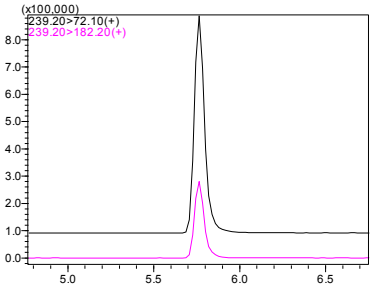
125. Phosemet



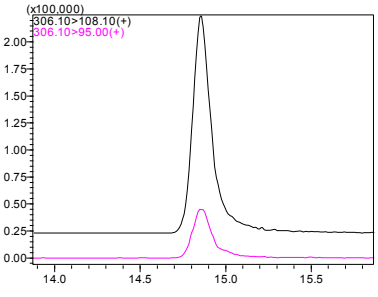
126. Phoxim



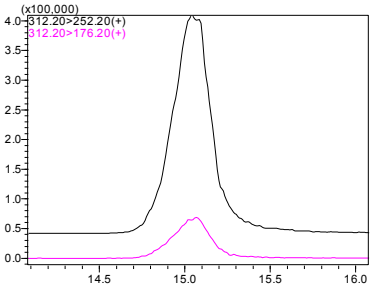
127. Piperonyl butoxide



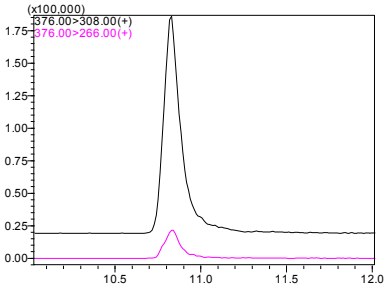
128. Pirimicarb



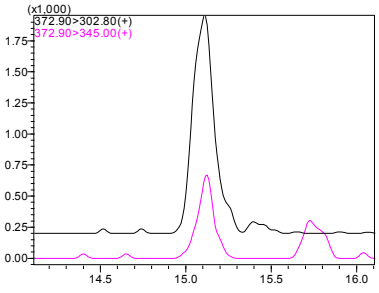
129. Pirimiphos methyl



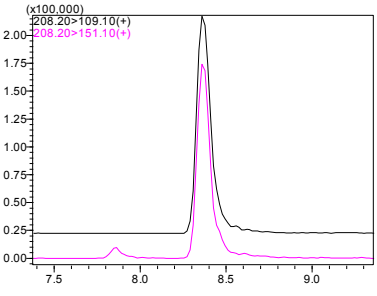
130. Pretilachlor



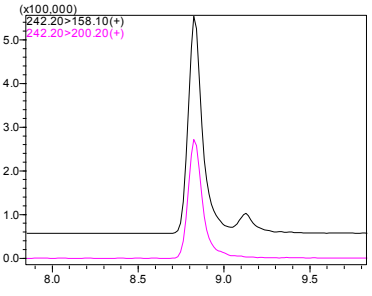
131. Prochloraz



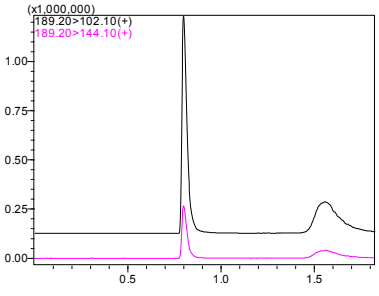
132. Profenofos



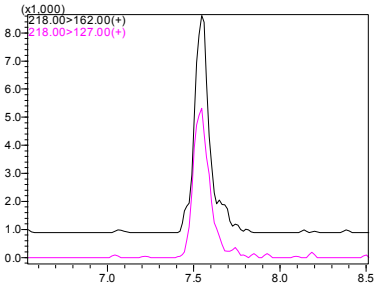
133. Promecarb



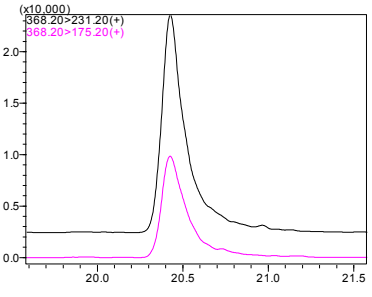
134. Prometryne



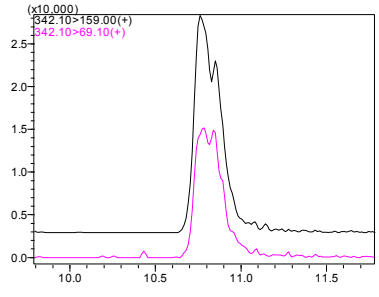
135. Propamocarb



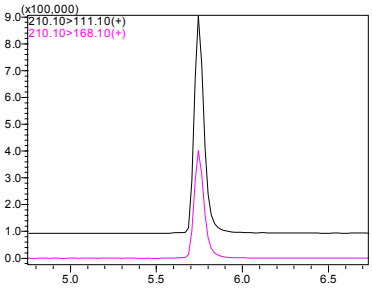
136. Propanil



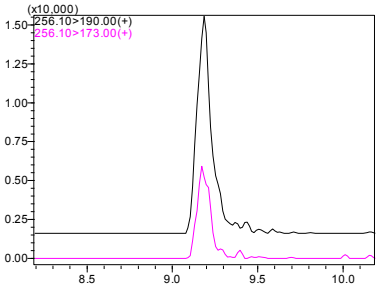
137. Propargite



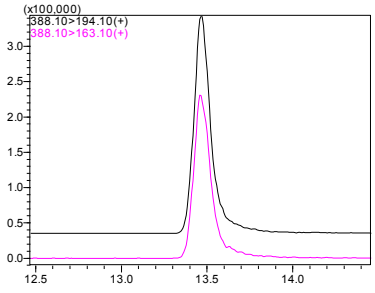
138. Propiconazole



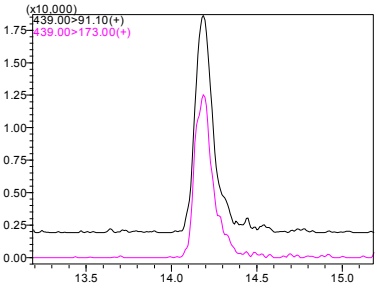
139. Propoxur



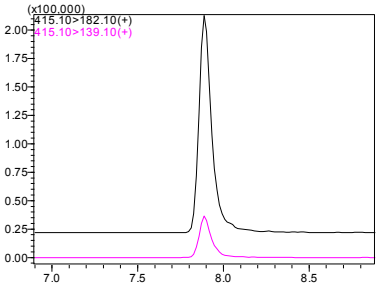
140. Propyzamide



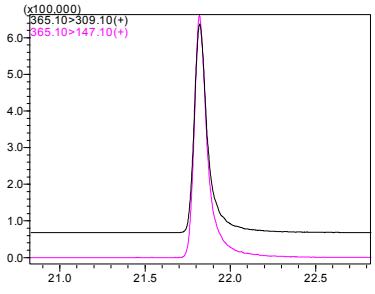
141. Pyraclostrobin



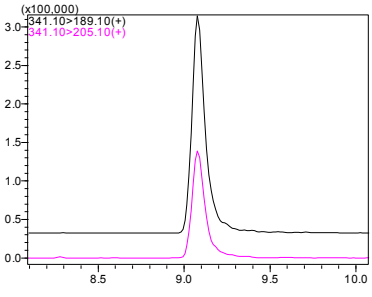
142. Pyrazolynate



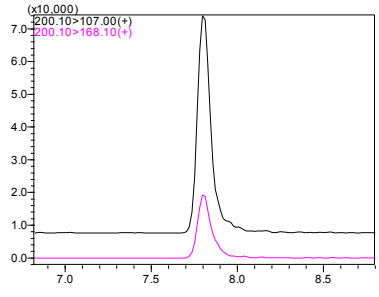
143. Pyrazosulfuron-ethyl



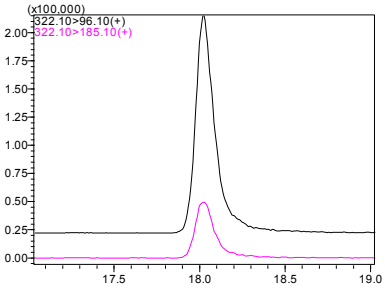
144. Pyridaben



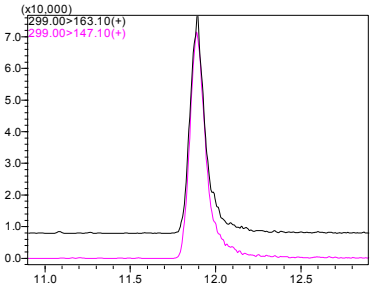
145. Pyridaphenthion



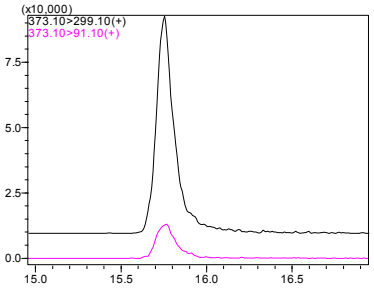
146.Pyrimethanil



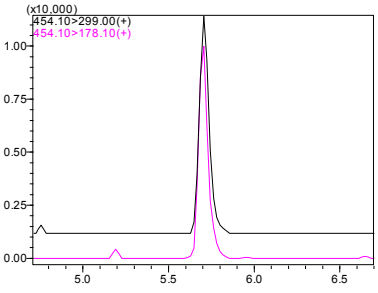
147. Pyriproxyfen



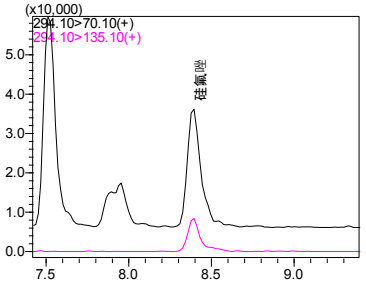
148. Quinalphos



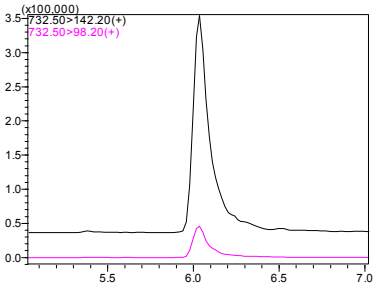
149. Quizalofop-p-ethyl



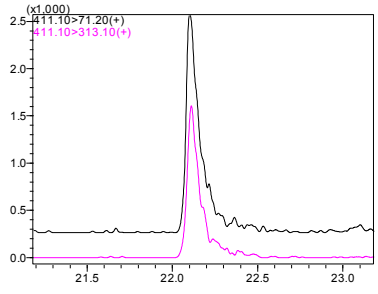
150. Rimsulfuron



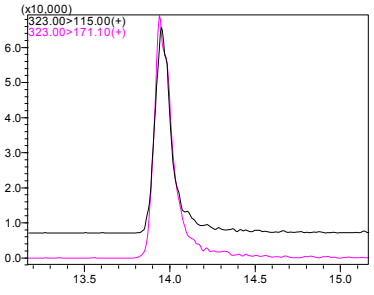
151. Simeconazole



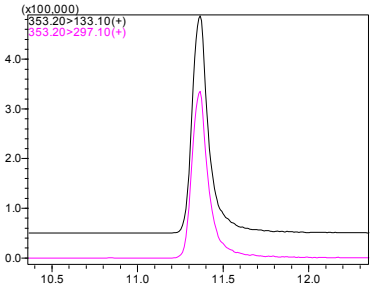
152. Spinosad



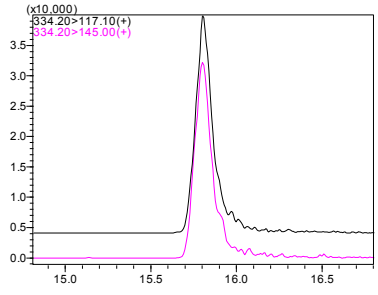
153. Spirodiclofen



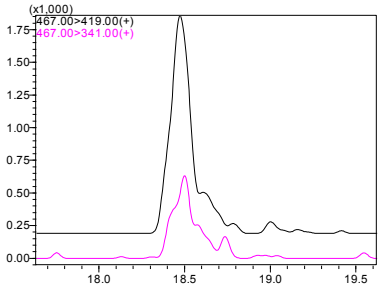
154. Sulfotep



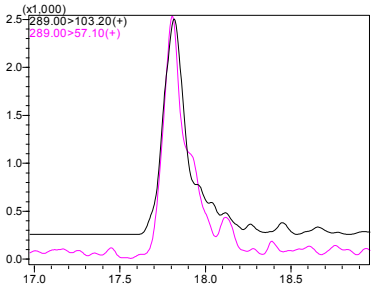
155. Tebufenozide



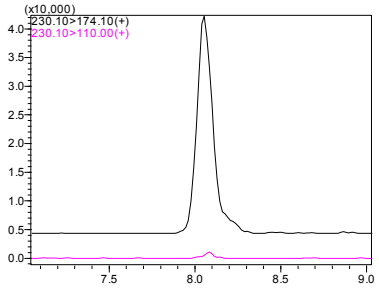
156. Tebufenpyrad



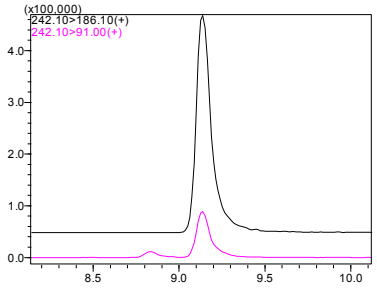
157. Temephos



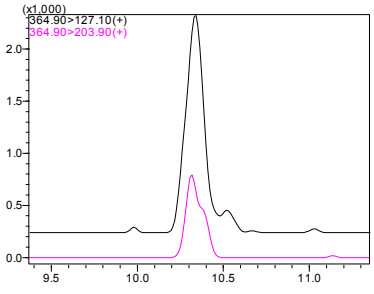
158. Terbufos



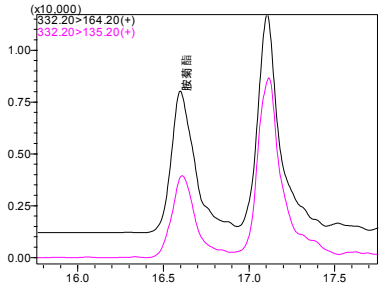
159. Terbuthylazine



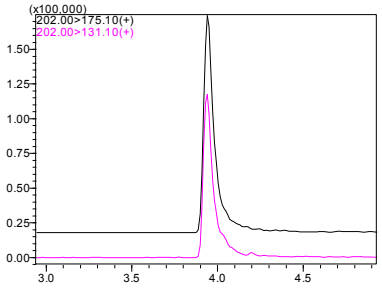
160. Terbutryn



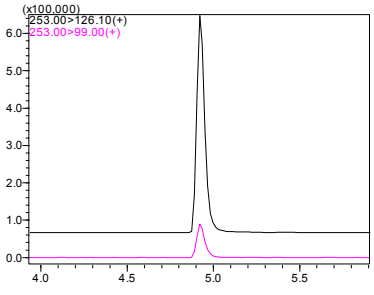
161. Tetrachlorvinphos



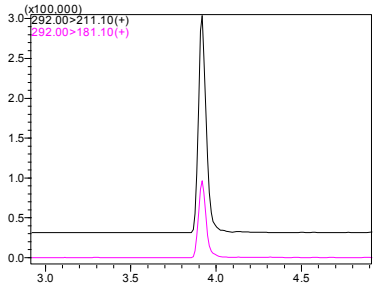
162. Tetramethrin



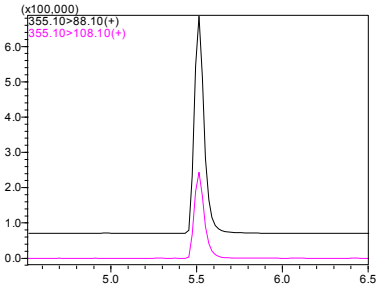
163. Thiabendazole



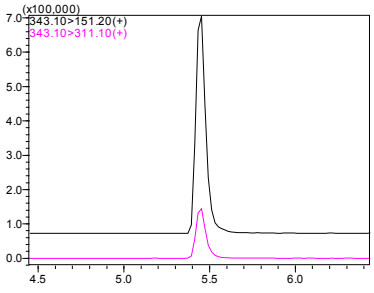
164. Thiacloprid



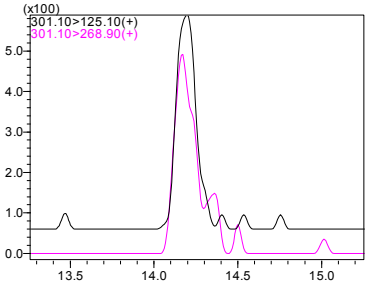
165. Thiamethoxam



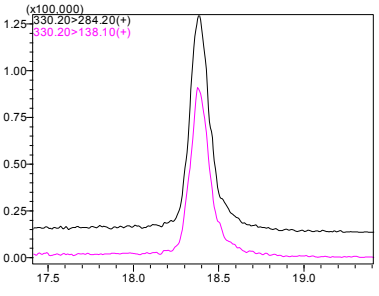
166. Thiodicarb



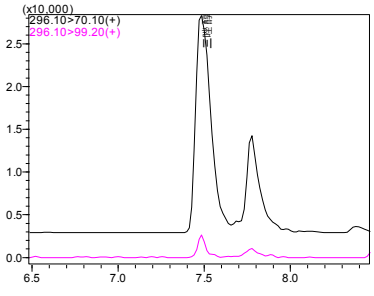
167. Thiophanate-methyl



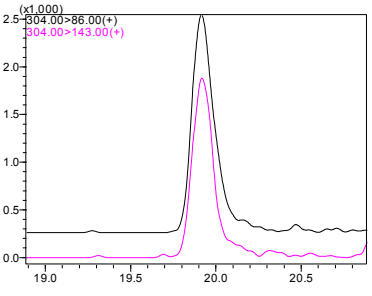
168. Tolclofos methyl



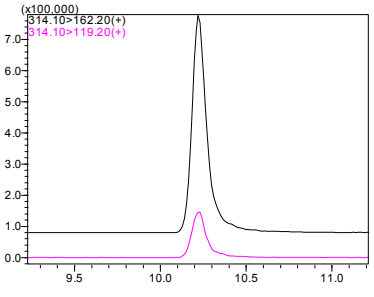
169. Tralkoxydim



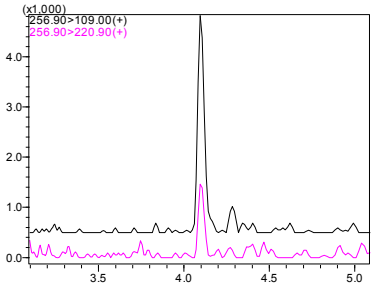
170. Triadimenol



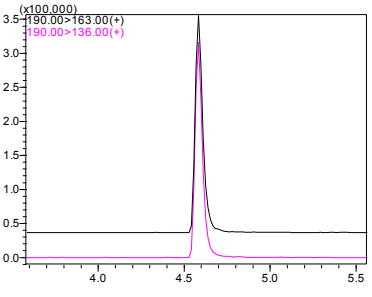
171. Triallate



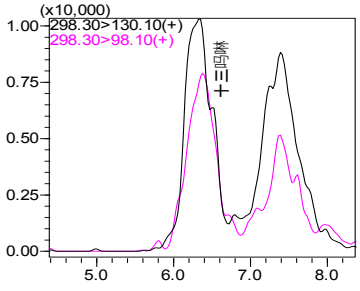
172. Triazophos



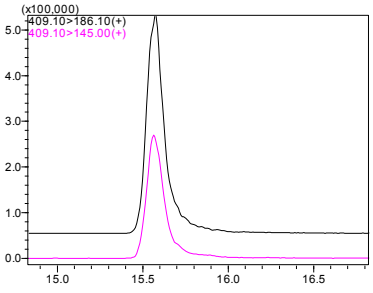
173. Trichlorphon



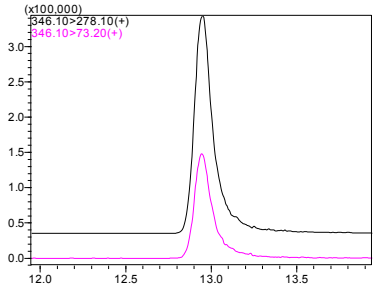
174. Tricyclazole



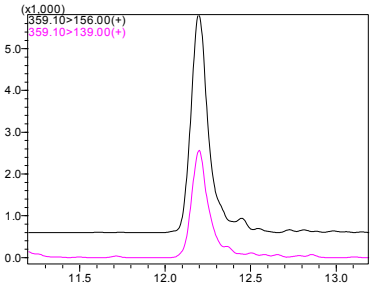
175. Tridemorph



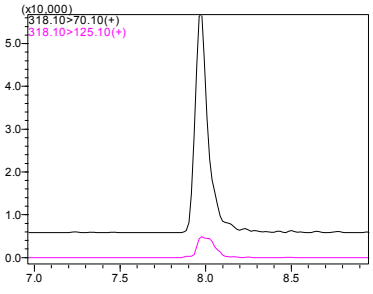
176. Trifloxystrobin



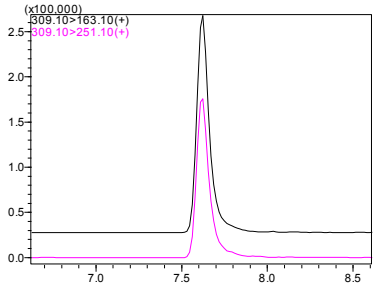
177. Triflumizole



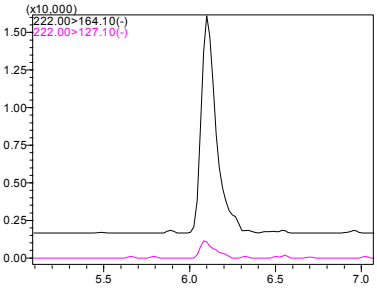
178. Triflumuron



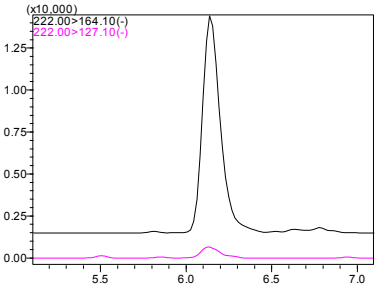
179.Triticonazole



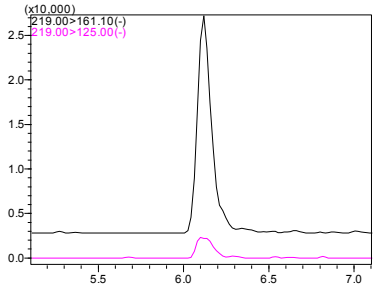
180.Warfarin



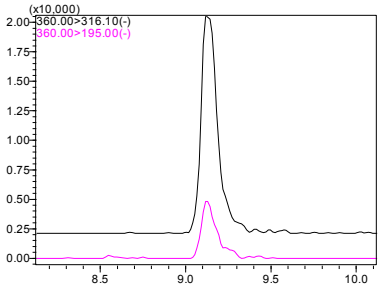
181. 2,4-D D3



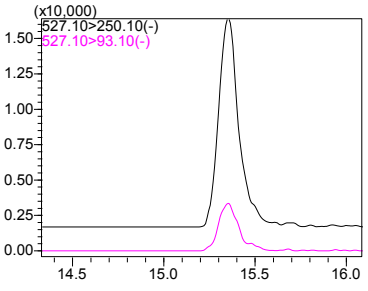
182. 2, 4-DB



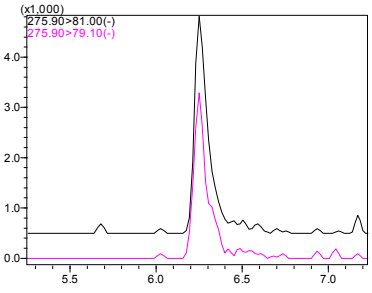
183. 2,4-Dichlorophenoxyacetic Acid



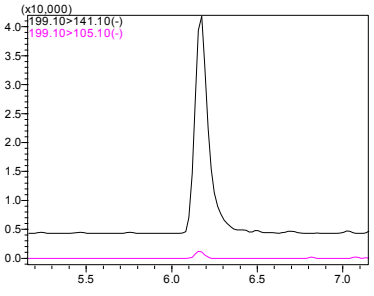
184. Acifluorofen



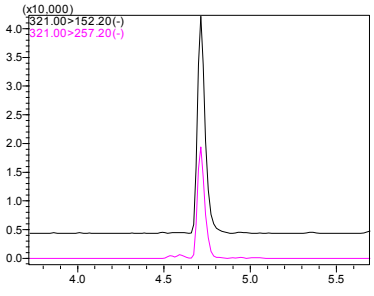
185. Bromadiolone



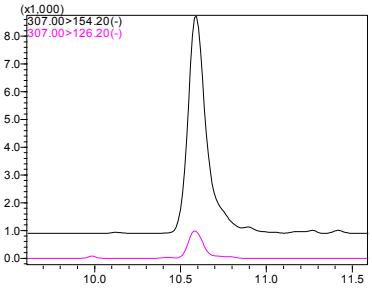
186. Bromoxynil



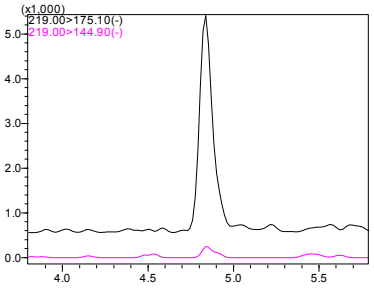
187. Chiptox



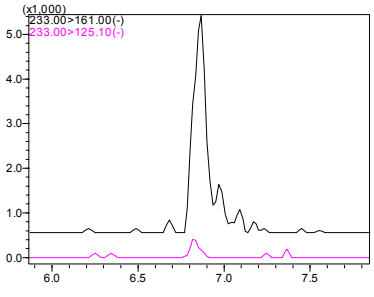
188. Chloramphenicol



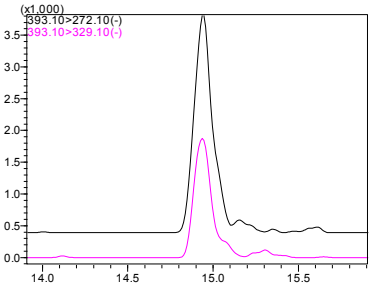
189. Chlorobenzuron



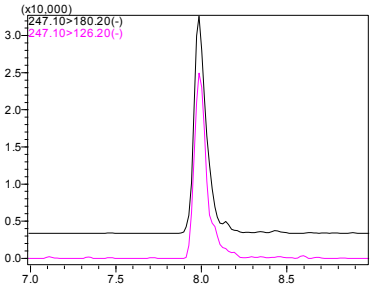
190. Dicamba



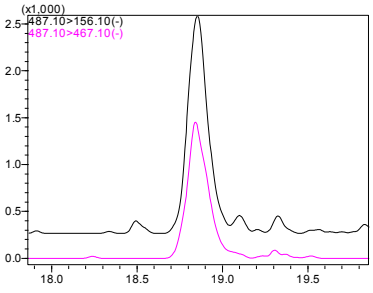
191. Dichlorprop



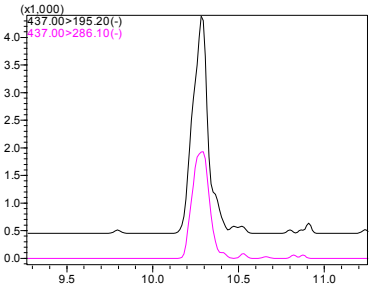
192. Diflufenican



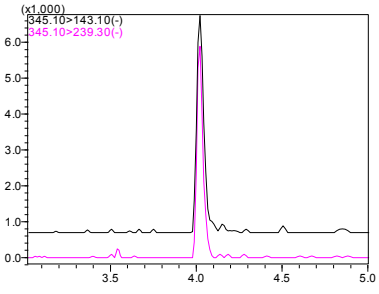
193.Fludioxonil



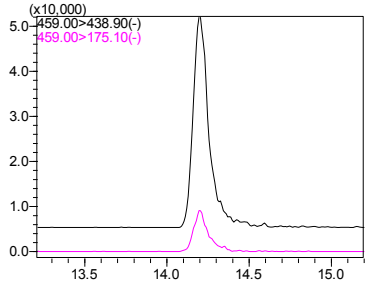
194. Flufenoxuron



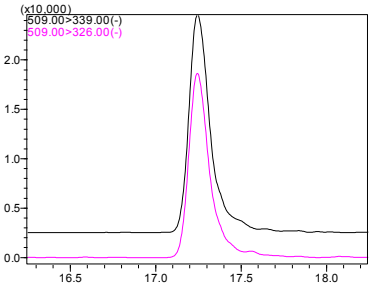
195. Fomesafen



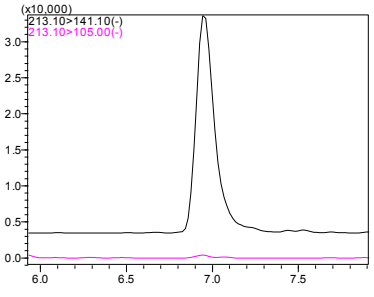
196. Gibberellic Acid



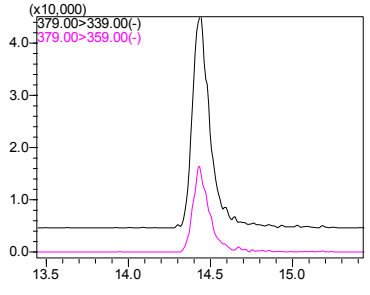
197. Hexaflumuron



198. Lufenuron



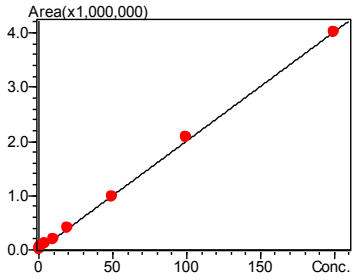
199. MCPP Acid



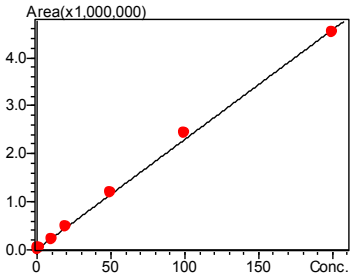
200. Teflubenzuron

Figure 1: Chromatograms for 200 pesticides mixed standard 50 ug/L

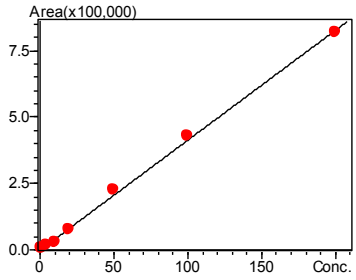
A series of standard working solutions with a concentration ranging between 0.5-200 µg/L was prepared with the mixed standard. Calibration curves were plotted using the external standard as shown in the following Figure 2 with the concentration as abscissa and the peak area as ordinate.



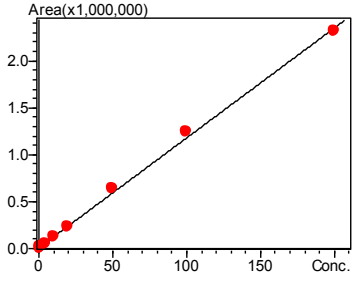
1.Acephate



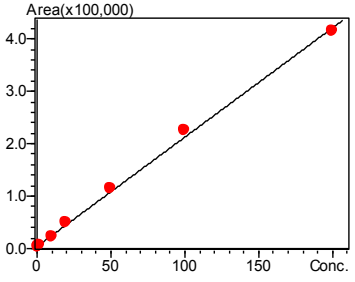
2.Acetamiprid



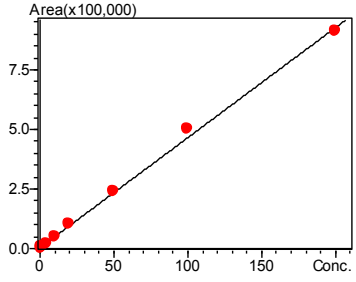
3. Acetochlor



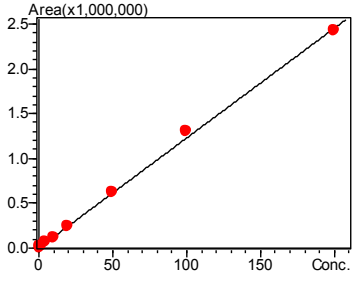
4. Alachlor



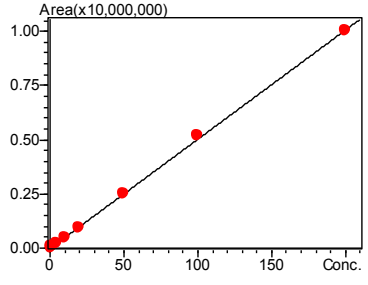
5. Aldicarb



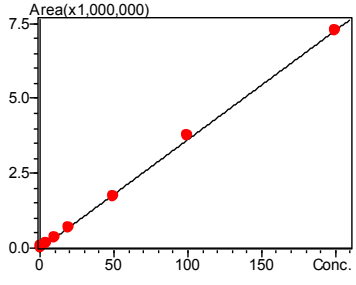
6. Aldicarb-sulfone



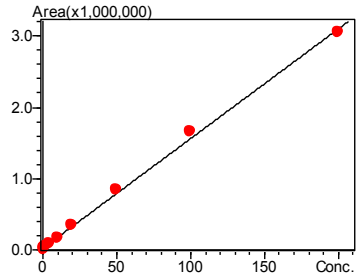
7. Aldicarb-sulfoxide



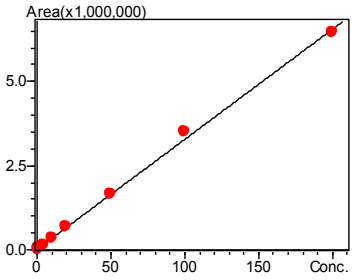
8. Ametryn



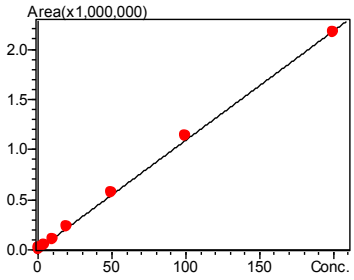
9. Anilofos



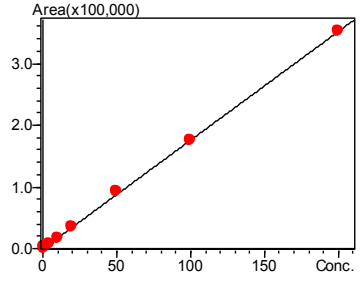
10. Atrazine



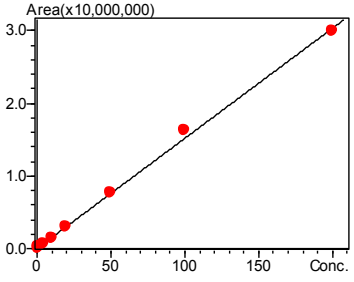
11. Azamethiphos



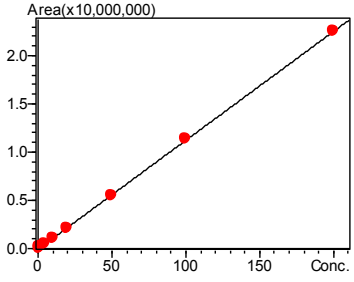
12. Azimsulfuron



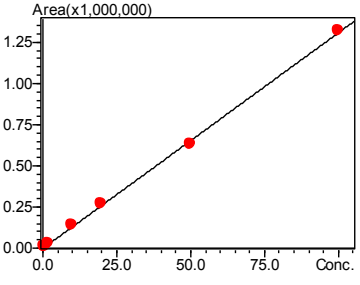
13. Azinphos-methyl



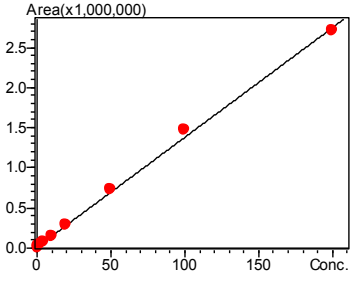
14.Azoxystrobin



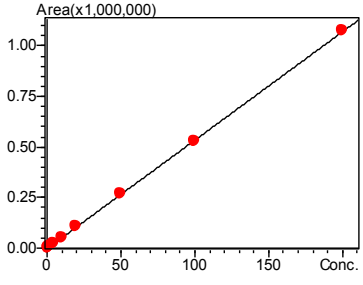
15. Benalaxyl



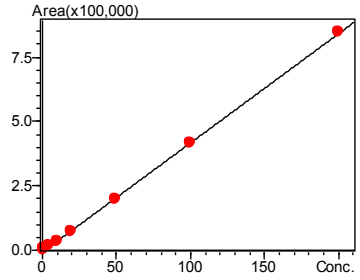
16. Bendiocarb



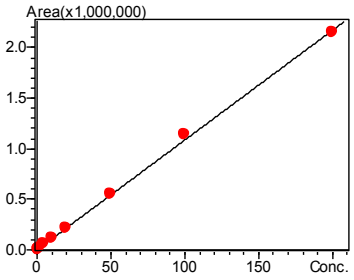
17. Bensulfuron methyl



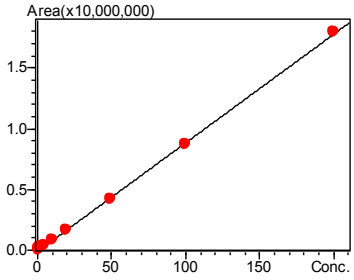
18. Bitertanol



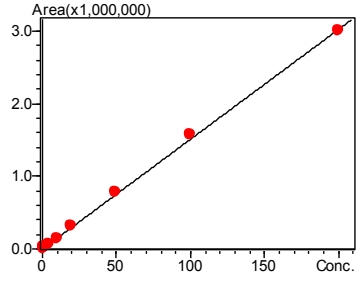
19. Brodifacoum



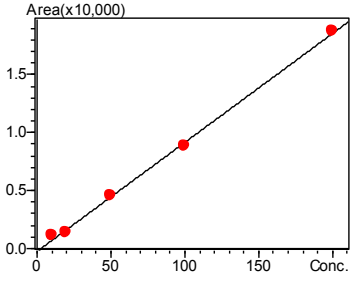
20. Bupirimate



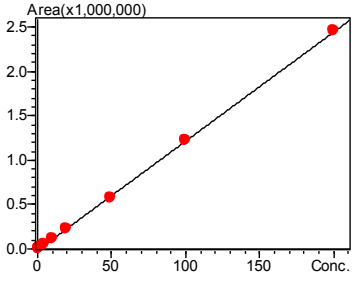
21. Buprofezin



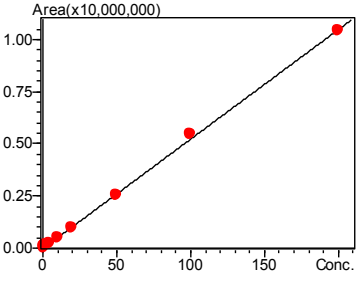
22. Butachlor



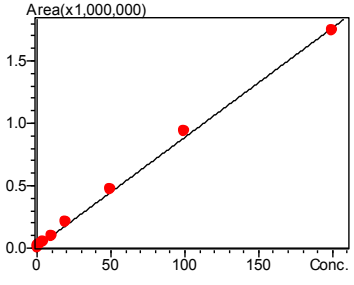
23. Butafenacil



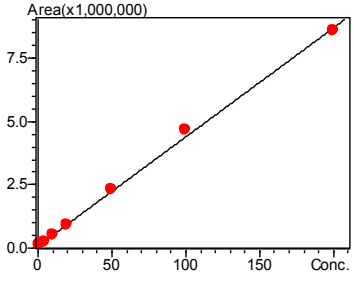
24. Butralin



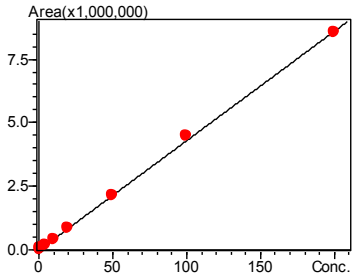
25. Cadusafos



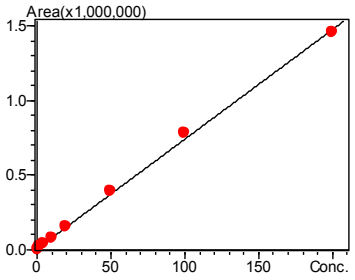
26. Carbaryl



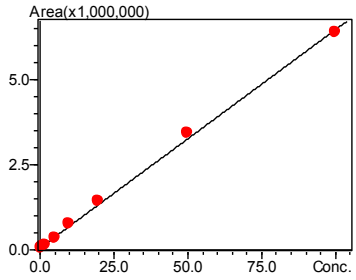
27. Carbendazim



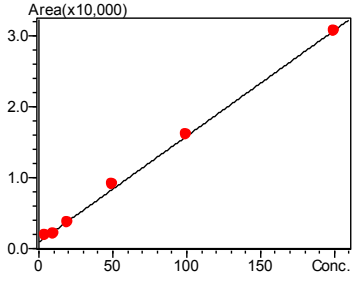
28. Carbofuran



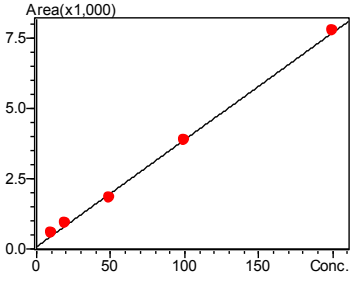
29. Carbofuran-3-hydroxy



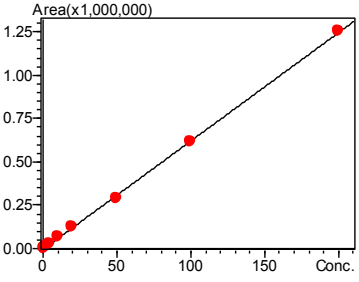
30. Carboxin



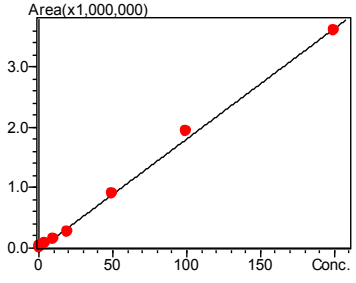
31. Cartap Hydrochloride



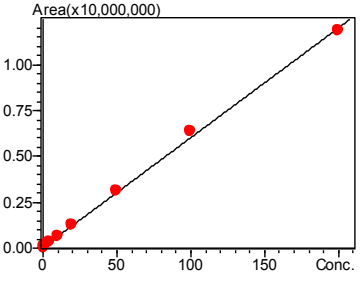
32. Chlorfluazuron



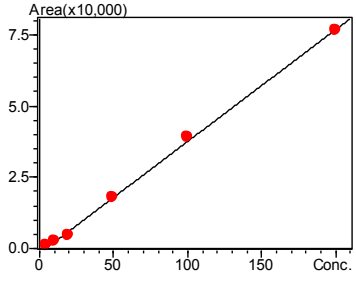
33. Chlorimuron-ethyl



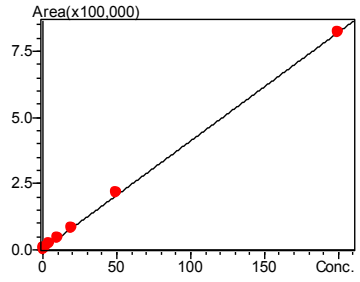
34. Chlormequat



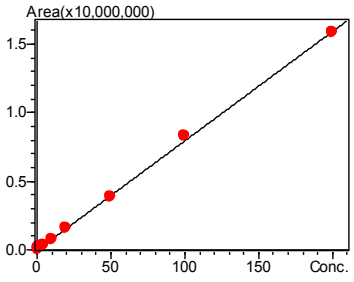
35. Chlorotoluron



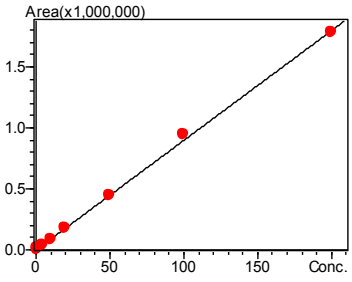
36. Chlorpyrifos



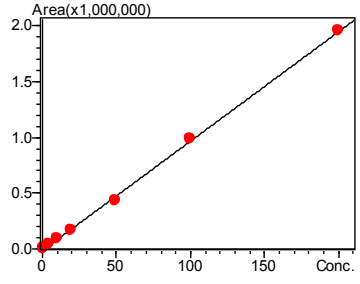
37.Chlorsulfuron



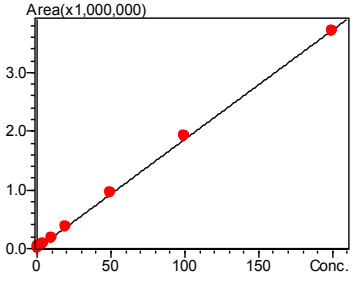
38.Chromafenozide



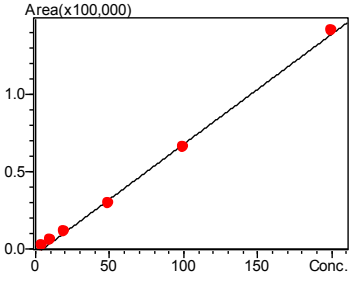
39.Cinosulfuron



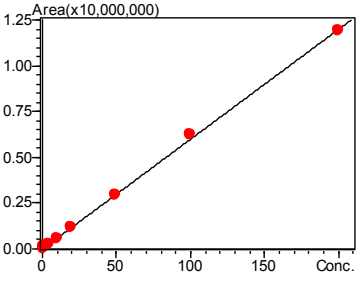
40. Clethodim



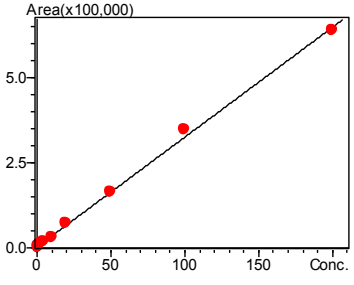
41. Clomazone Dimethazone



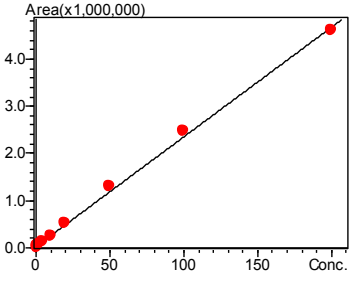
42. Clomeprop



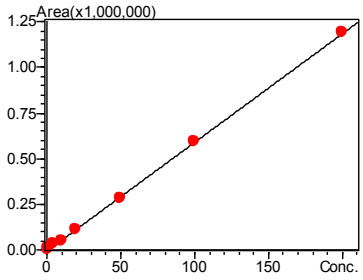
43. Cloquintocet-1-methylhexyl ester



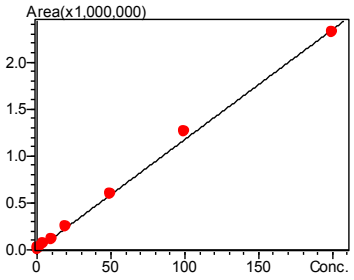
44. Clothianidin



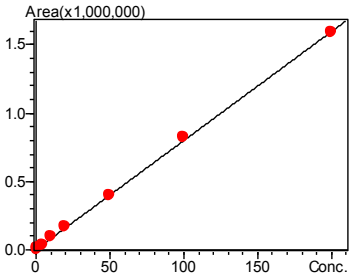
45. Coumatetralyl



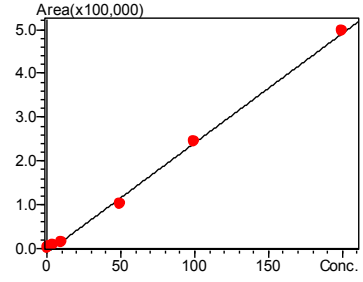
46. Cyflufenamid



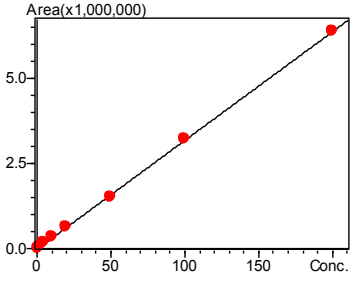
47. Cyproconazole



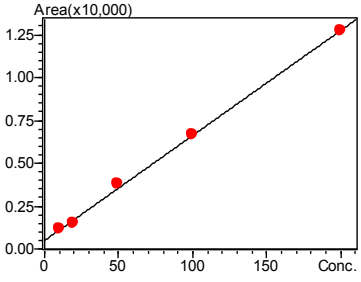
48. Cyprodinil



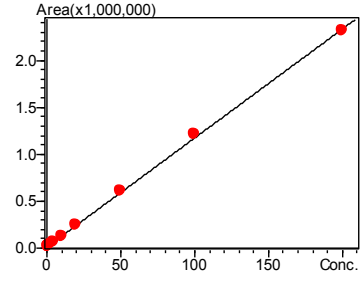
49. Dazomet



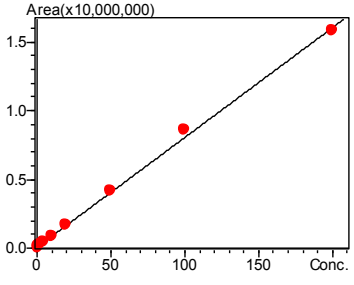
50.Diazinon



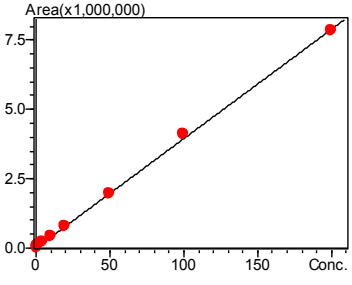
51. Dichlorvos



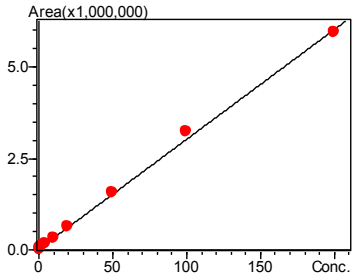
52. Diethofencarb



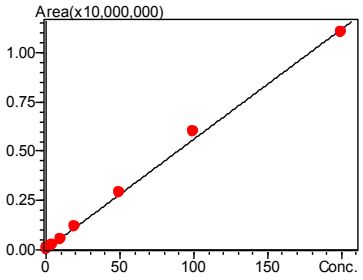
53. Diethyltoluamide



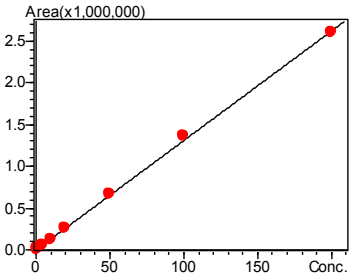
54. Dimepiperate



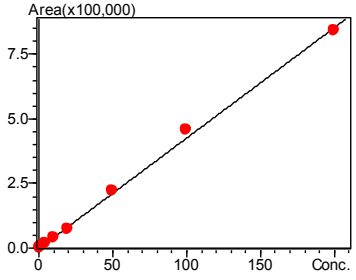
55. Dimethirimol



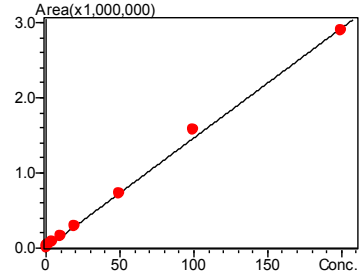
56. Dimethoate



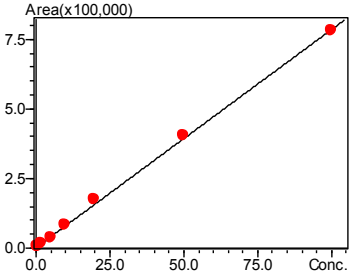
57. Dimethomorph



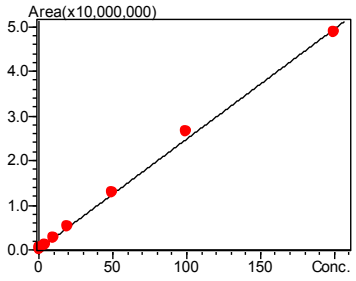
58. Diniconazole



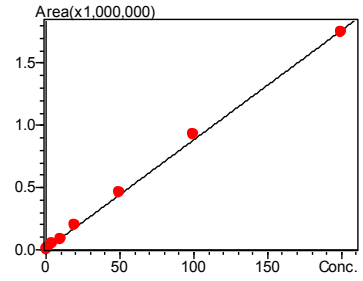
59. Dinotefuran



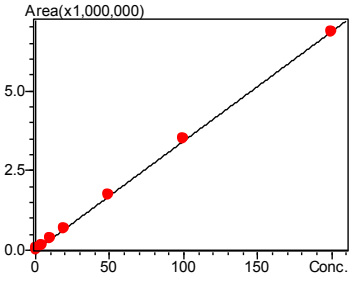
60. Diuron



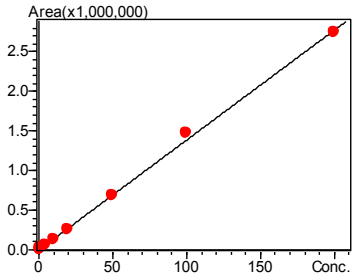
61. Dymron



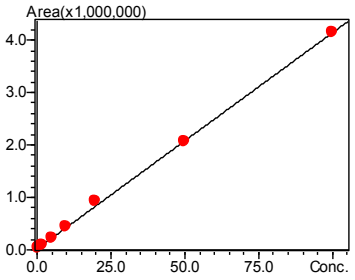
62. Epoxiconazole



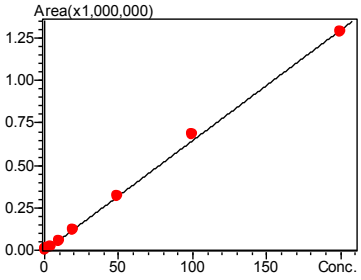
63. Esprocarb



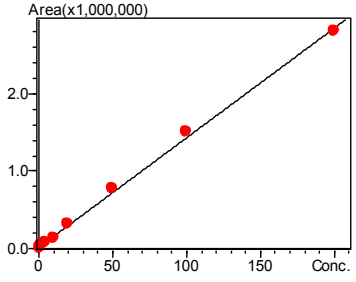
64. Ethametsulfuron-methyl



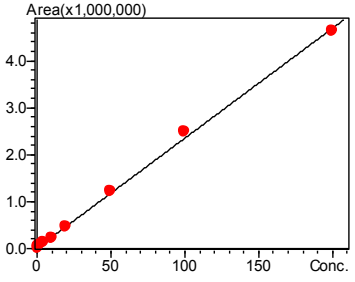
65. Ethiofencarb



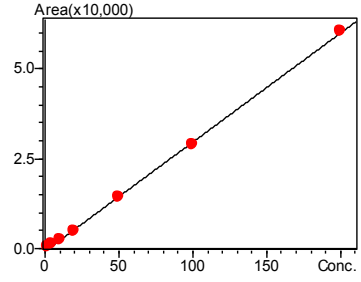
66. Ethion



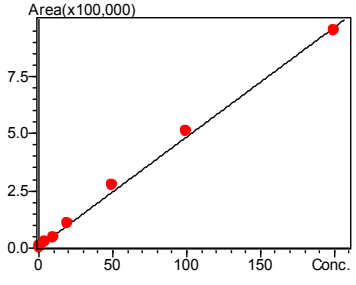
67. Ethoprophos



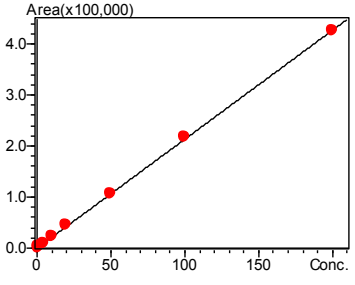
68. Fenamiphos



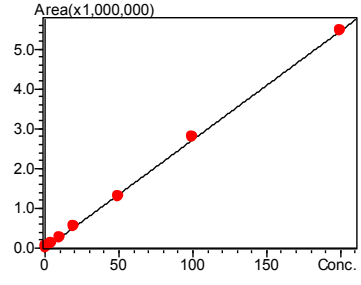
69. Fenarimol



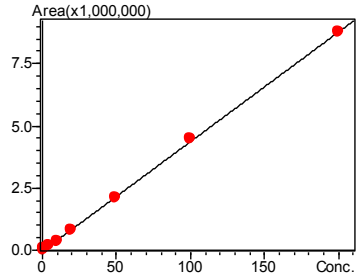
70. Fenobucarb



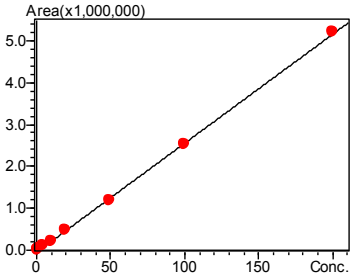
71. Fenoxycarb



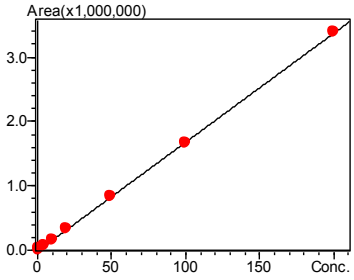
72. Fenpropimorph



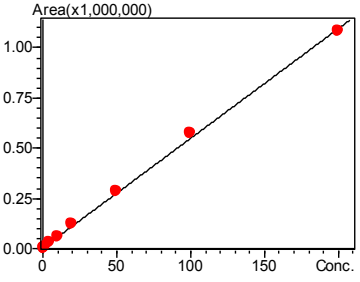
73. Fenpyroximate



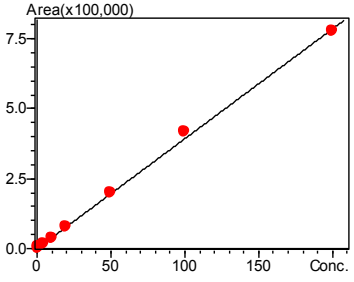
74. Flocoumafen



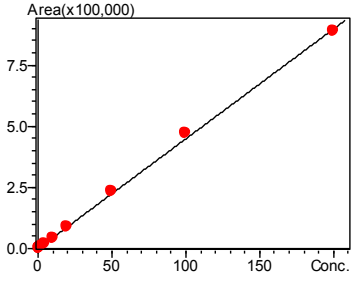
75. Fluazifop-p-butyl



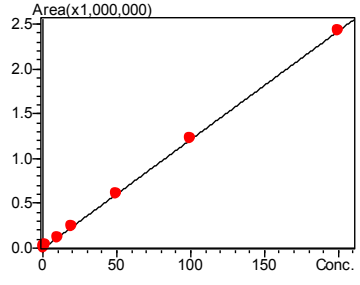
76. Flusilazole



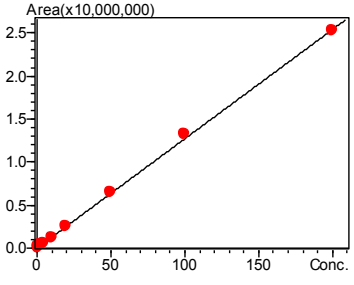
77. Flutriafol



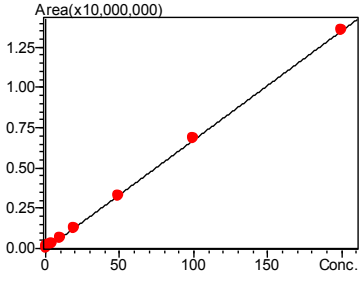
78. Fonofos



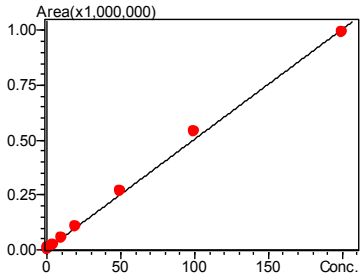
79. Forchlorfenuron



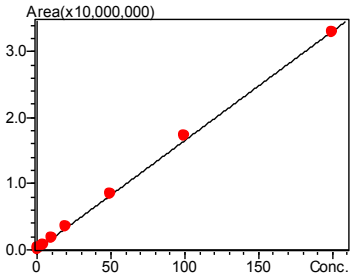
80. Fosthiazate



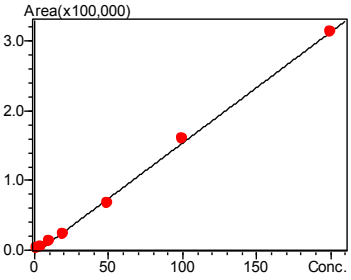
81. Furathiocarb



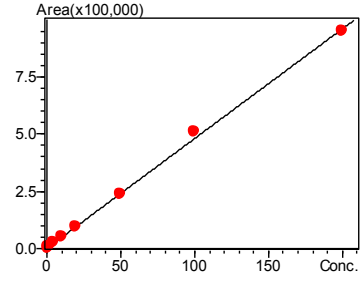
82. Hexaconazole



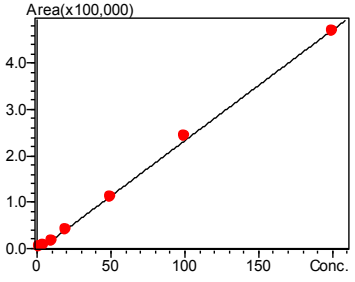
83. Hexazinone



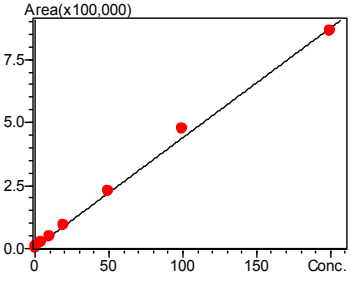
84. Hexythiazox



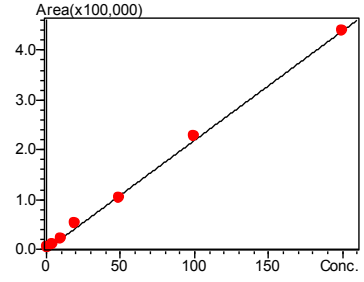
85. Imazapyr



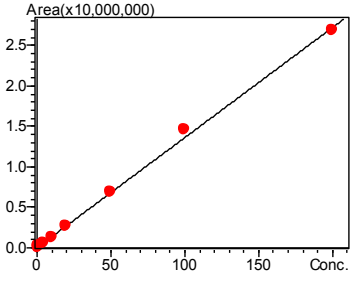
86.Imibenconazole



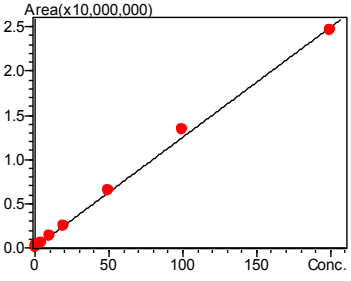
87. Imidacloprid



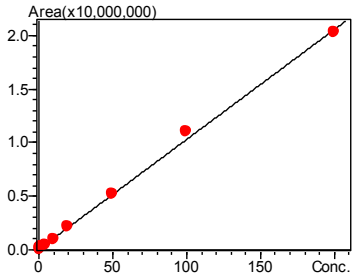
88. Indoxacarb



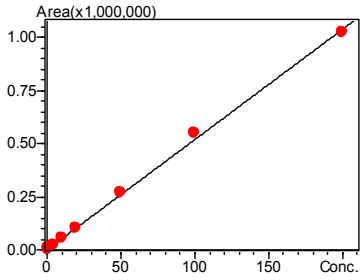
89. Iprobenfos



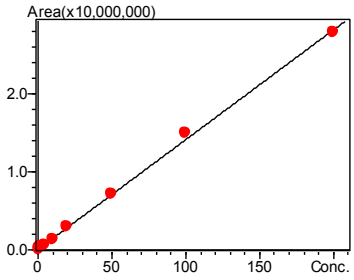
90. Iprovalicarb



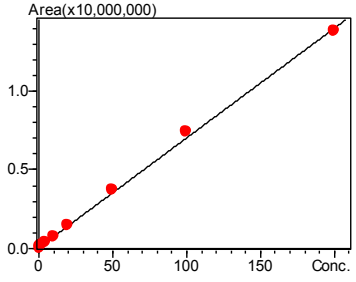
91. Isazofos



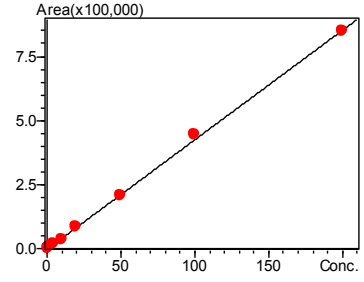
92. Isoprocarb



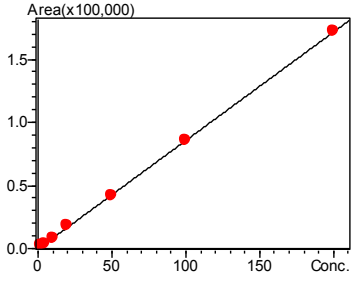
93. Isoprothiolane



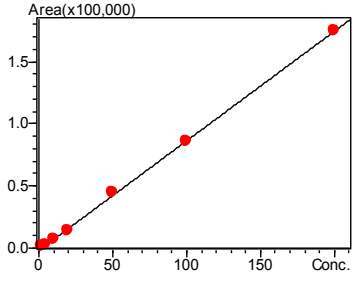
94. Isoproturon



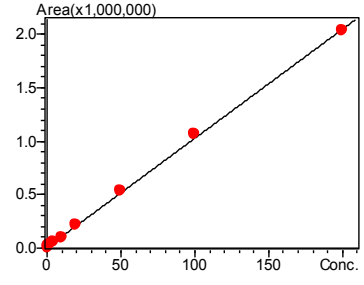
95. Kresoxim-methyl



96. Linuron

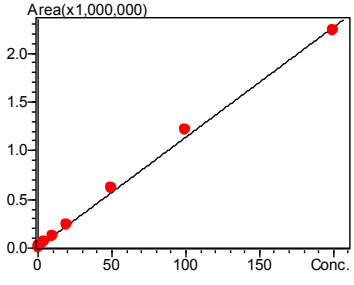


97. Lodosulfuron-methyl-sodium

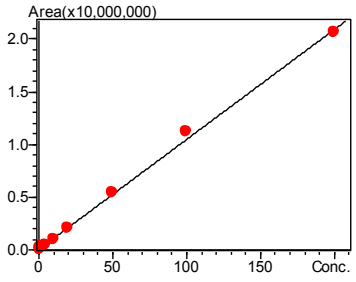


98.

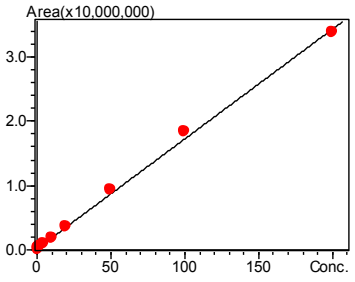
Malachite Green Oxalate salt



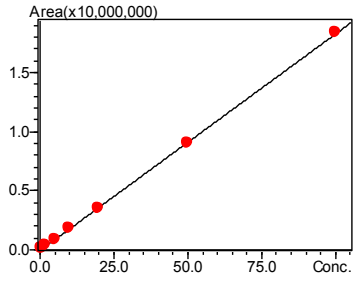
99. Malathion



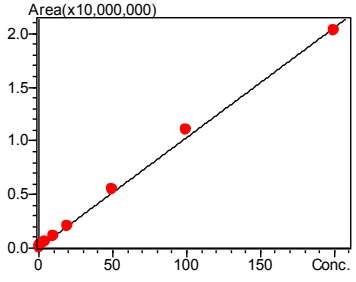
100.Mefenacet



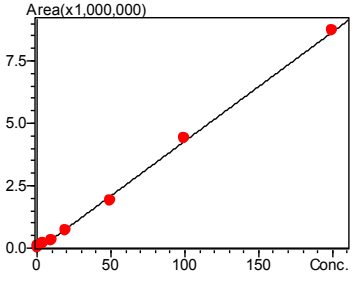
101.Mefenoxam



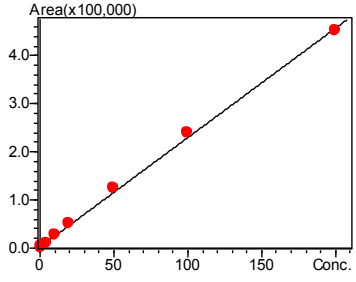
102.Metalaxyl



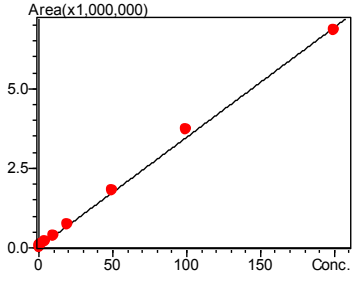
103. Metazachlor



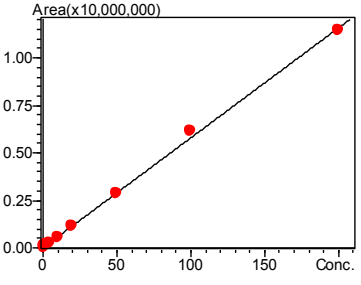
104. Methamidophos



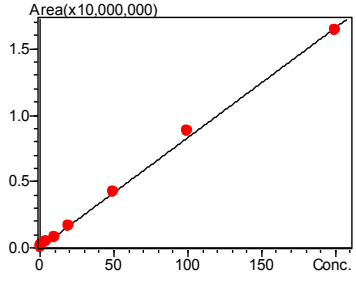
105. Methiocarb



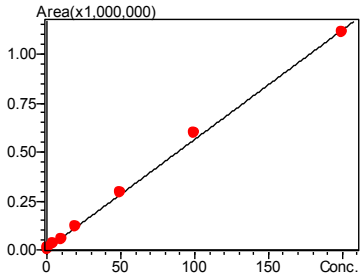
106. Methomyl



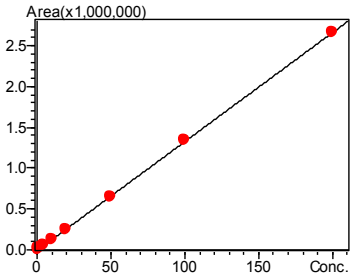
107. Methoxyfenozide



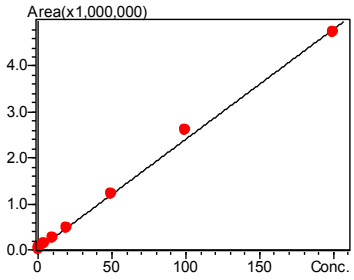
108. Metolachlor



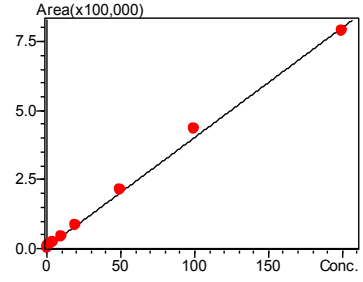
109. Metolcarb



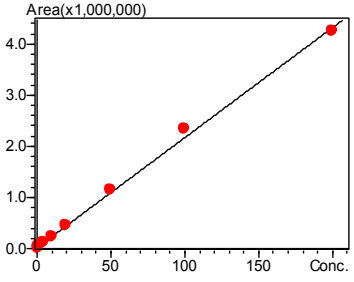
110. Metsulfuron-methyl



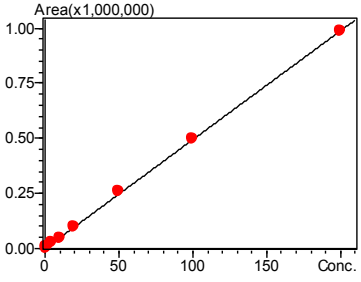
111. Mevinphos



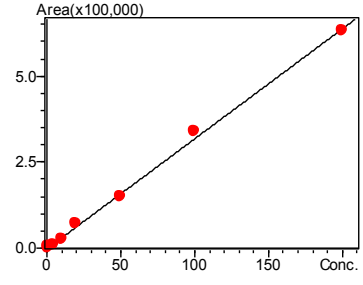
112.Molinate



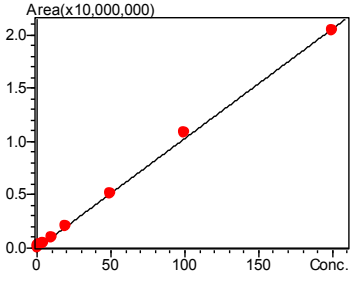
113. Monocrotophos



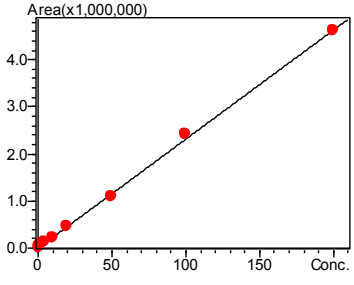
114. Myclobutanil



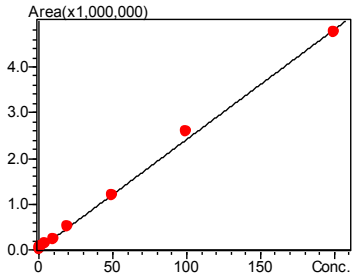
115. Naproanilide



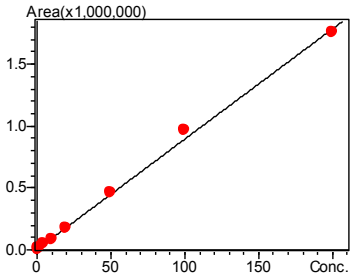
116. Napropamide



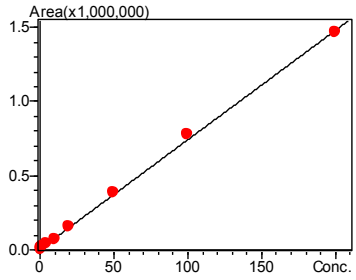
117. Omethoate



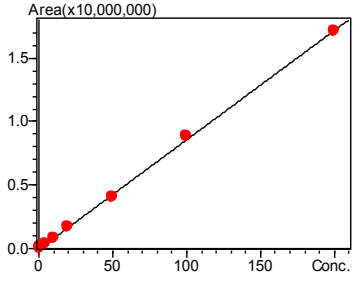
118. Oxycarboxin



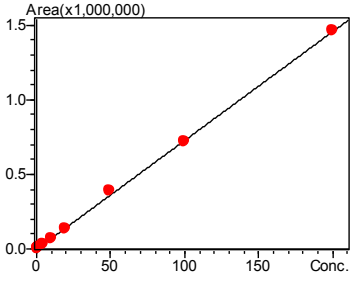
119. Paclobutrazol



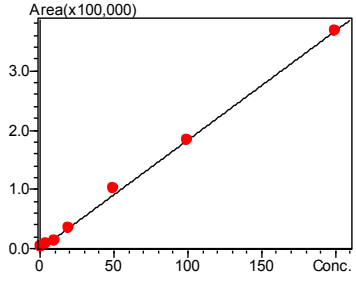
120. Penconazole



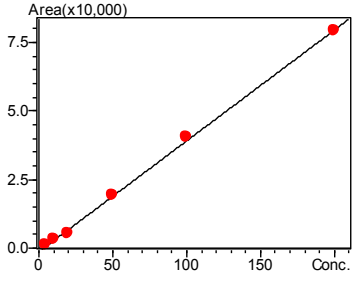
121. Pencycuron



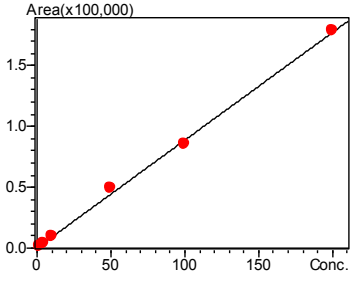
122. Pendimethalin



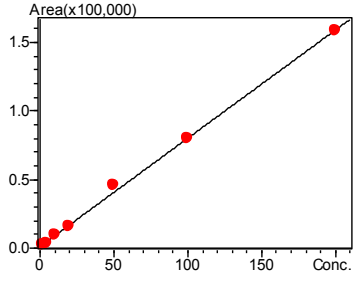
123. Phorate



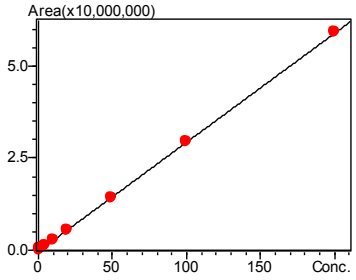
124. Phosalone



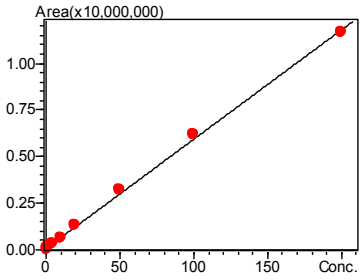
125. Phosemet



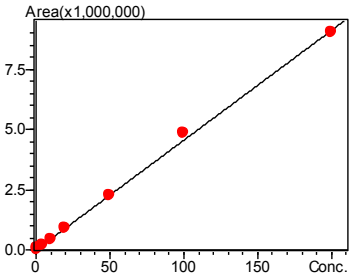
126. Phoxim



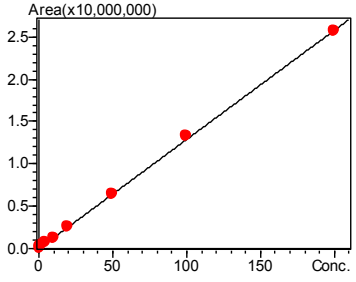
127. Piperonyl butoxide



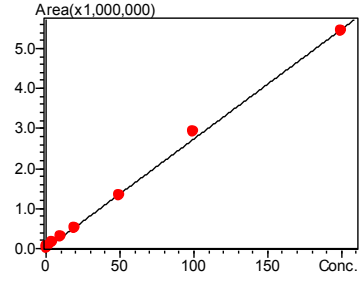
128. Pirimicarb



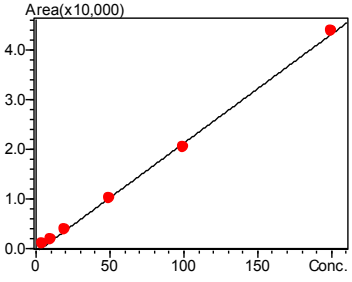
129. Pirimiphos methyl



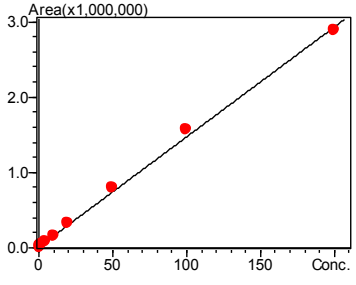
130. Pretilachlor



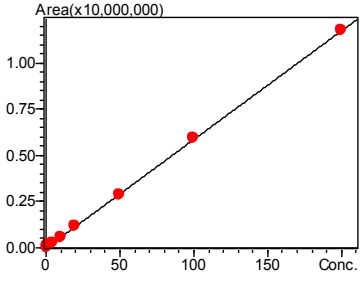
131. Prochloraz



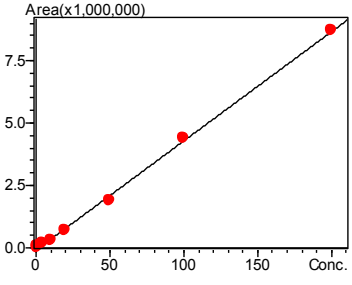
132. Profenofos



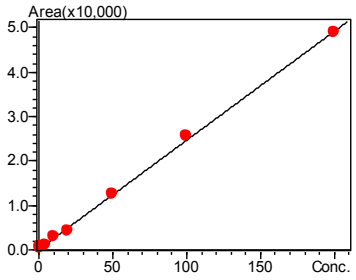
133. Promecarb



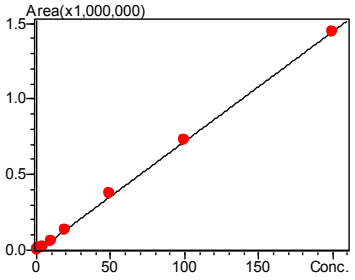
134. Prometryne



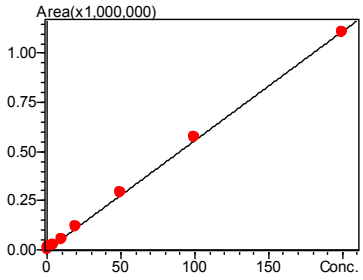
135. Propamocarb



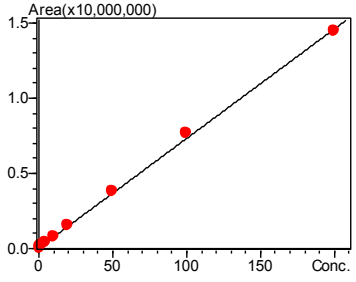
136. Propanil



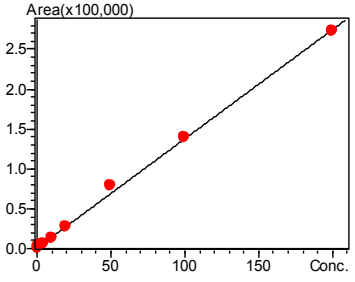
137. Propargite



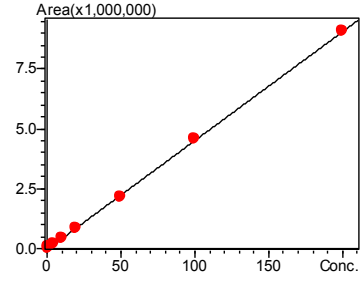
138. Propiconazole



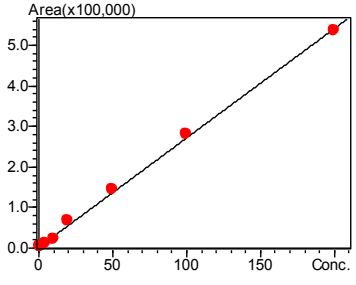
139. Propoxur



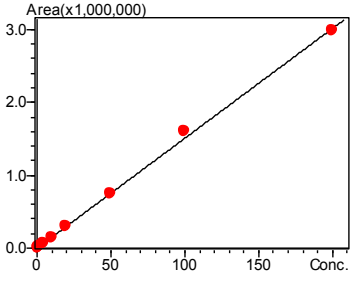
140. Propyzamide



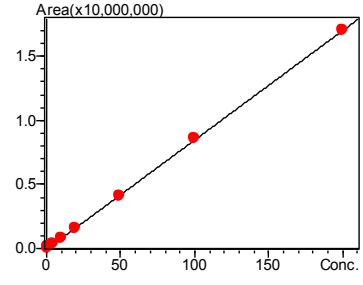
141. Pyraclostrobin



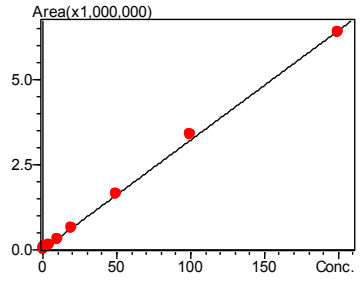
142. Pyrazolynate



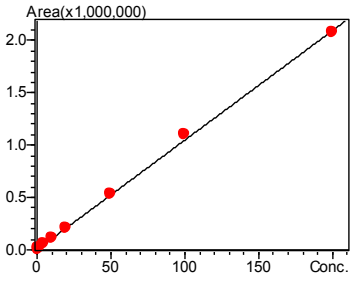
143. Pyrazosulfuron-ethyl



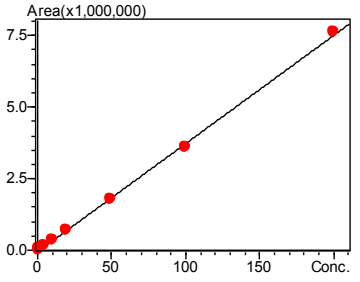
144. Pyridaben



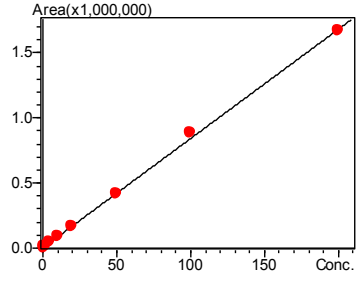
145. Pyridaphenthion



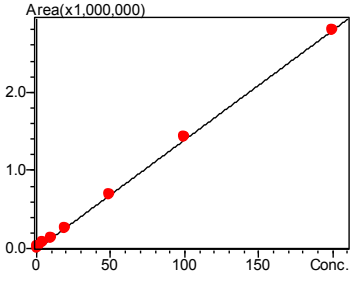
146.Pyrimethanil



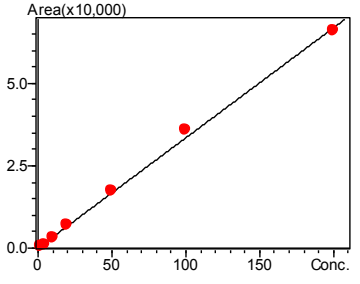
147. Pyriproxyfen



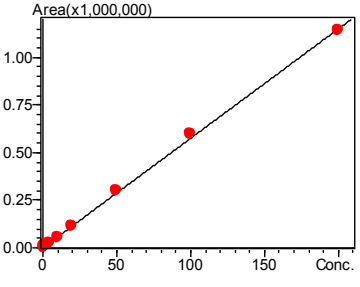
148. Quinalphos



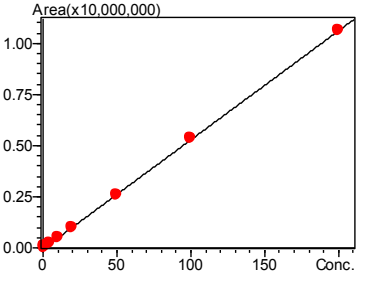
149. Quizalofop-p-ethyl



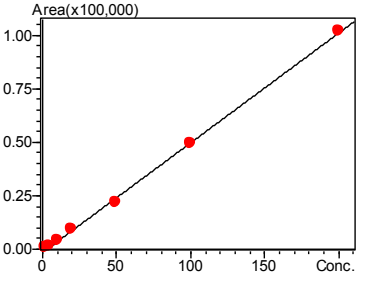
150. Rimsulfuron



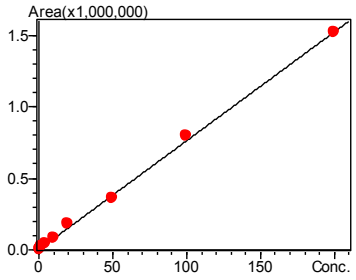
151. Simeconazole



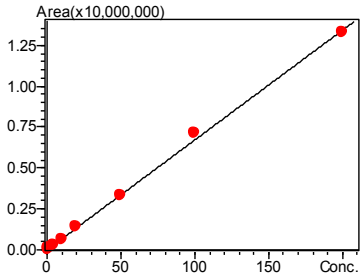
152. Spinosad



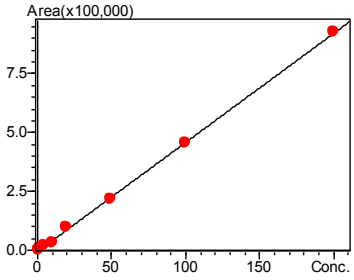
153. Spirodiclofen



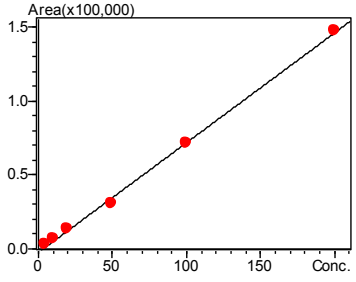
154. Sulfotep



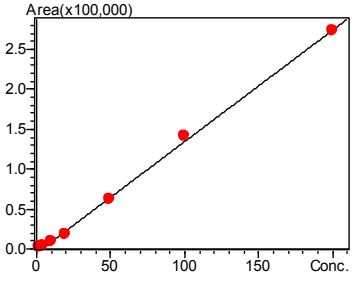
155. Tebufenozide



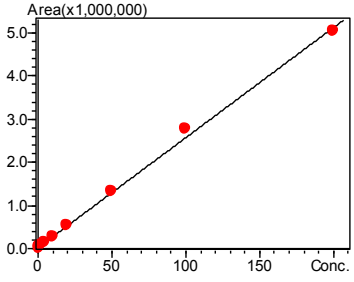
156. Tebufenpyrad



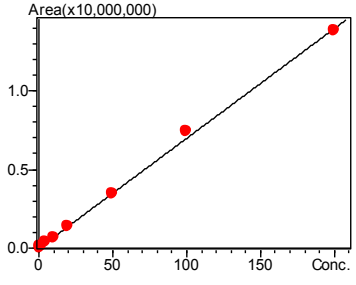
157. Temephos



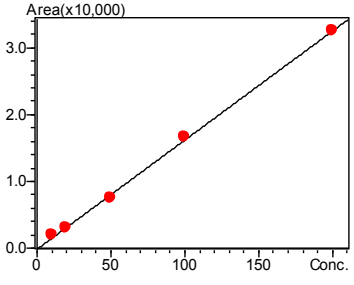
158. Terbufos



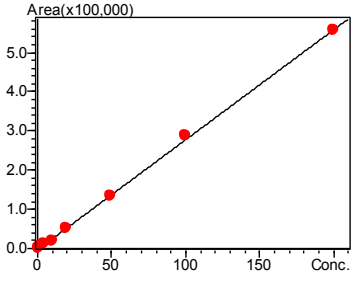
159. Terbuthylazine



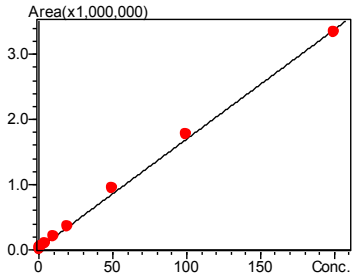
160. Terbutryn



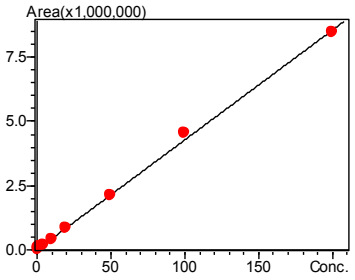
161. Tetrachlorvinphos



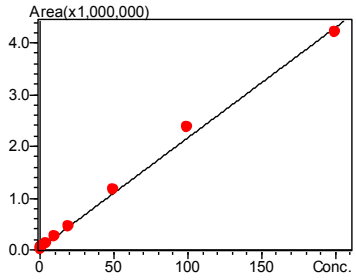
162. Tetramethrin



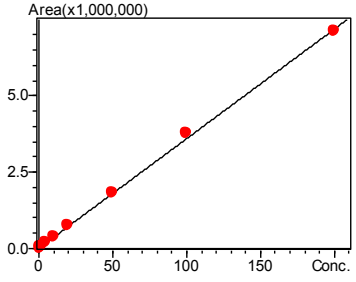
163. Thiabendazole



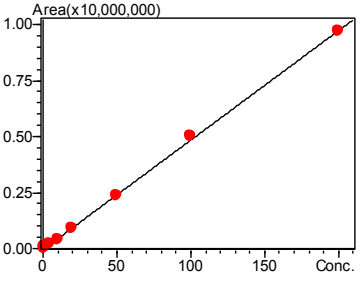
164. Thiacloprid



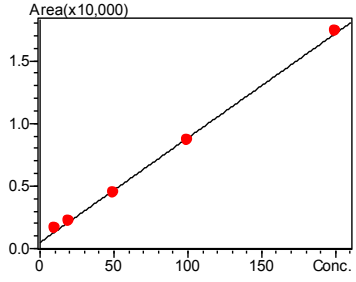
165. Thiamethoxam



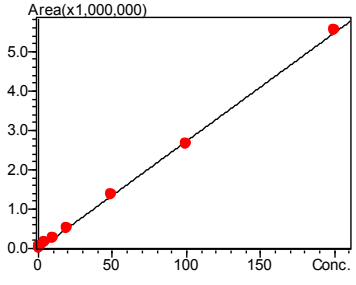
166. Thiodicarb



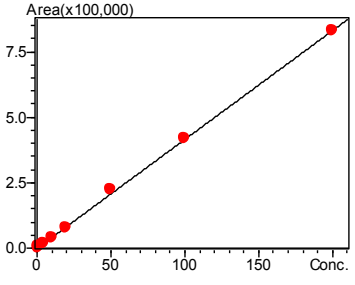
167. Thiophanate-methyl



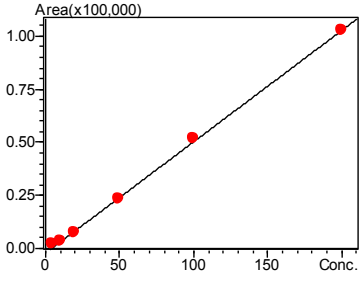
168. Tolclofos methyl



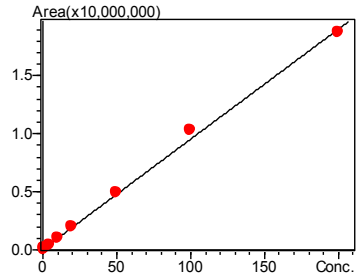
169. Tralkoxydim



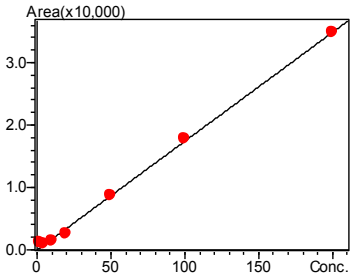
170. Triadimenol



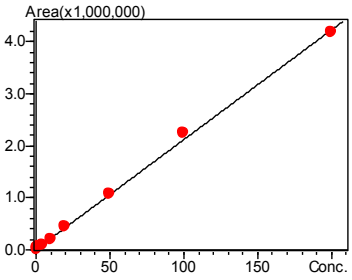
171. Triallate



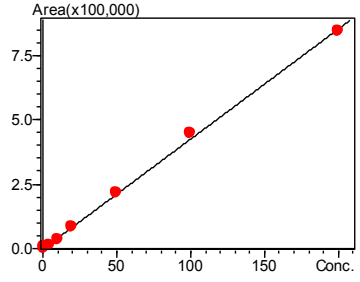
172. Triazophos



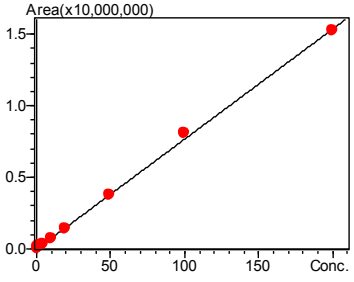
173. Trichlorphon



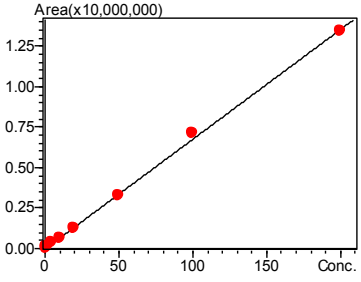
174. Tricyclazole



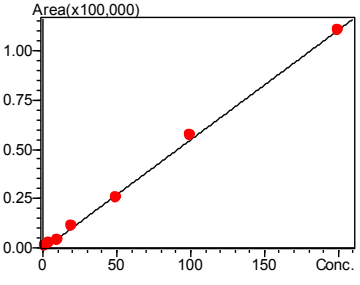
175. Tridemorph



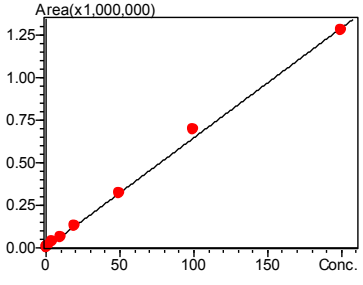
176. Trifloxystrobin



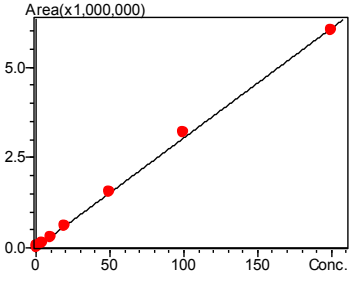
177. Triflumizole



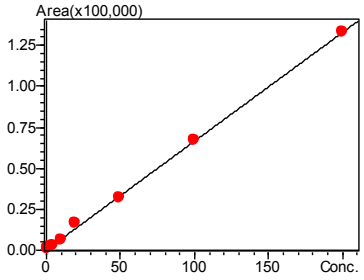
178. Triflumuron



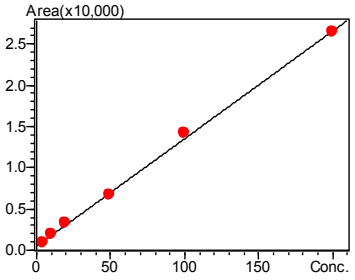
179.Triticonazole



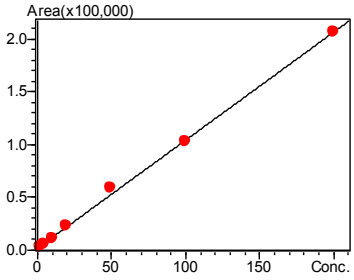
180.Warfarin



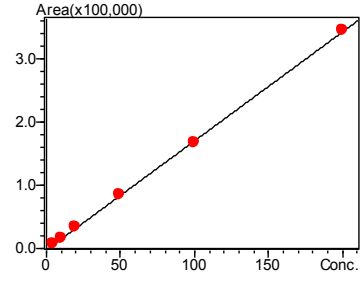
181. 2,4-D D3



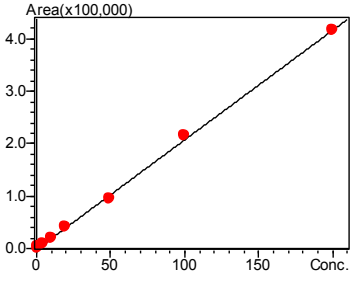
182. 2,4-DB



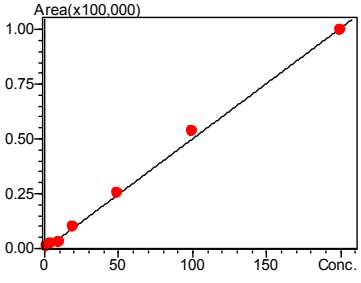
183. 2,4-Dichlorophenoxyacetic Acid



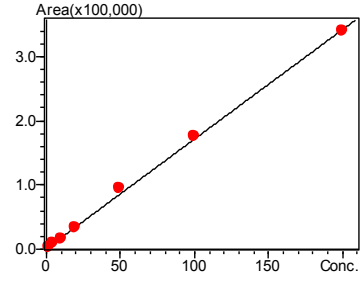
184. Acifluorofen



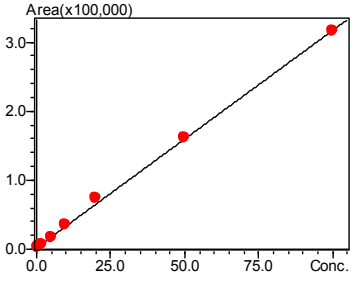
185. Bromadiolone



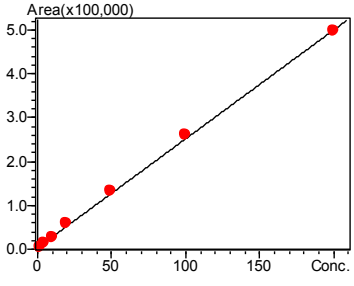
186. Bromoxynil



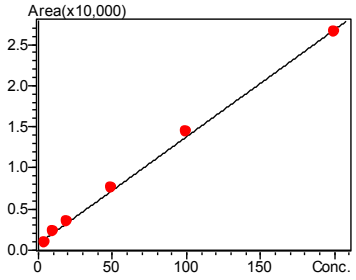
187. Chiptox



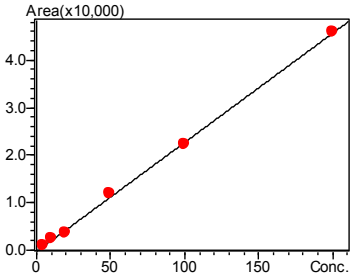
188. Chloramphenicol



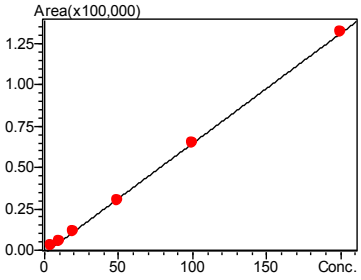
189. Chlorobenzuron



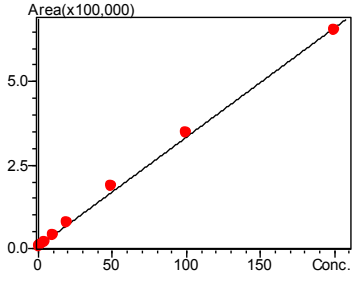
190. Dicamba



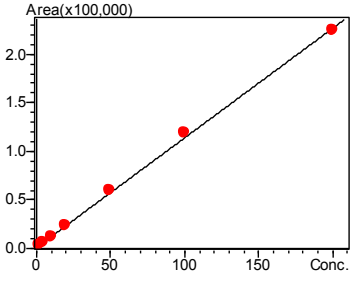
191. Dichlorprop



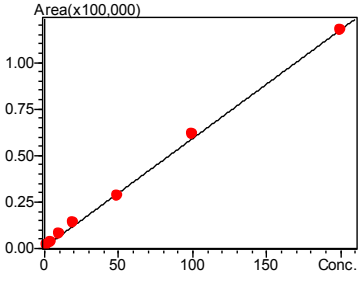
192. Diflufenican



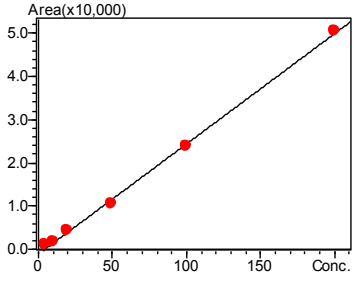
193. Fludioxonil



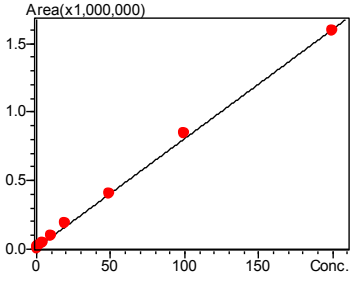
194. Flufenoxuron



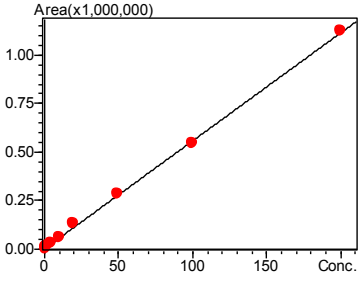
195. Fomesafen



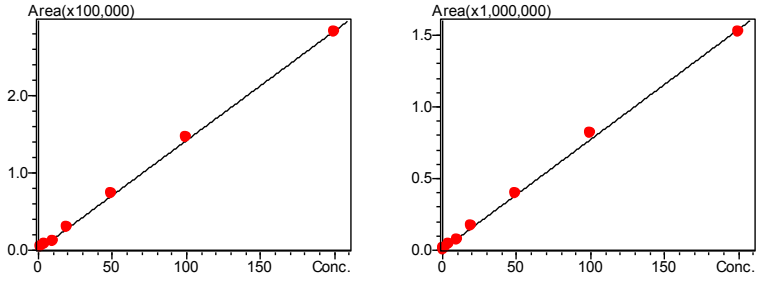
196. Gibberellic Acid



197. Hexaflumuron



198. Lufenuron



199. MCPP acid　　200. Teflubenzuron

Figure 2: Calibration curve for 200 pesticides mixed standard

The LOD calculation: Analyzed the lowest concentration of standard curve of compounds and calculated the SNR. The apparatus's LOD was calculated with 3 times of SNR. Prepared the blank matrix solution with the tomato sample, and analyzed the spiked matrix solution of 0.1-5 μg/kg. The method's LOQ was calculated with 10 times of SNR.

The plotted curves were of satisfactory linearity, linear equation, correlation coefficient and LOD are as shown in the Table 3 below.

Table 3: Calibration curve and LOD's of 200 pesticides

No.	Name	Correlation coefficient	Linear equation	Linear Range (μg/L)	Apparatu LOD (μg/L)	Metho LOQ (μg/kg)
1	Acephate	0.9999	Y = (20139.7)X + (-5356.19)	0.5~200	0.26	0.10
2	Acetamiprid	0.9993	Y = (22851.2)X + (13751.1)	0.5~200	0.04	0.02
3	Acetochlor	0.9993	Y = (4147.05)X + (-1351.08)	1~200	0.40	0.10
4	Alachlor	0.9992	Y = (11711.7)X + (8222.57)	0.5~200	0.07	0.02
5	Aldicarb	0.9991	Y = (2085.25)X + (4108.96)	0.5~200	1.14	0.24
6	Aldicarb-sulfone	0.9990	Y = (4618.79)X + (3619.22)	0.5~200	0.09	0.03

7	Aldicarb-sulfoxide	0.9994	Y = (12250.0)X + (3410.06)	0.5~200	0.05	0.03
8	Ametryn	0.9999	Y = (50403.8)X + (-15375.9)	0.5~200	0.07	0.01
9	Anilofos	0.9998	Y = (36490.2)X + (-27423.8)	0.5~200	0.05	0.06
10	Atrazine	0.9991	Y = (15395.5)X + (22273.2)	0.5~200	0.17	0.03
11	Azamethiphos	0.9991	Y = (32709.3)X + (14146.2)	0.5~200	0.03	0.02
12	Azimsulfuron	0.9998	Y = (10897.3)X + (3218.17)	0.5~200	0.06	0.01
13	Azinphos-methyl	0.9999	Y = (1762.28)X + (-475.964)	0.5~200	0.37	0.39
14	Azoxystrobin	0.9993	Y = (151561)X + (20127.3)	0.5~200	0.01	0.01
15	Benalaxyl	0.9999	Y = (112924)X + (-66706.1)	0.5~200	0.06	0.01
16	Bendiocarb	0.9997	Y = (13097.3)X + (-1948.47)	0.5~100	0.13	0.04
17	Bensulfuron methyl	0.9993	Y = (13665.2)X + (14335.8)	0.5~200	0.72	0.23
18	Bitertanol	1.0000	Y = (5365.00)X + (-2589.72)	0.5~200	0.68	0.32
19	Brodifacoum	0.9998	Y = (4241.04)X + (-7406.21)	0.5~200	0.14	0.04
20	Bupirimate	0.9995	Y = (10854.2)X + (697.365)	0.5~200	0.07	0.02
21	Buprofezin	0.9998	Y = (89359.9)X + (-98748.6)	0.5~200	0.05	0.01
22	Butachlor	0.9998	Y = (15122.9)X + (-2326.88)	0.5~200	0.09	0.03

23	Butafenacil	0.9993	Y = (94.0313)X + (-243.009)	10~200	3.13	3.05
24	Butralin	0.9999	Y = (12291.0)X + (-14326.7)	0.5~200	0.06	0.06
25	Cadusafos	0.9996	Y = (52509.7)X + (-27075.8)	0.5~200	0.07	0.01
26	Carbaryl	0.9993	Y = (8780.66)X + (9919.16)	0.5~200	0.26	0.06
27	Carbendazim	0.9993	Y = (43051.0)X + (89016.7)	1~200	0.16	0.05
28	Carbofuran	0.9997	Y = (43058.8)X + (-11874.4)	0.5~200	0.06	0.03
29	Carbofuran-3-hydroxy	0.9994	Y = (7363.23)X + (6704.75)	0.5~200	0.31	0.10
30	Carboxin	0.9992	Y = (64113.1)X + (57449.9)	0.5~100	0.01	0.03
31	Cartap Hydrochloride	0.9994	Y = (149.580)X + (920.401)	5~200	2.56	2.52
32	Chlorfluazuron	0.9992	Y = (38.1054)X + (79.2276)	10~200	5.96	2.75
33	Chlorimuron-ethyl	0.9998	Y = (6254.21)X + (-4669.72)	0.5~200	0.08	0.02
34	Chlormequat	0.9990	Y = (18313.3)X + (-23730.9)	0.5~200	0.36	0.08
35	Chlorotoluron	0.9994	Y = (59875.8)X + (42009.6)	0.5~200	0.09	0.02
36	Chlorpyrifos	0.9994	Y = (392.470)X + (-1692.30)	5~200	1.90	0.38
37	Chlorsulfuron	0.9999	Y = (4103.32)X + (1442.62)	0.5~200	0.37	0.10
38	Chromafenozide	0.9997	Y = (79803.6)X + (-28763.4)	0.5~200	0.02	0.01
39	Cinosulfuron	0.9996	Y = (8997.41)X +	0.5~200	0.08	0.03

			(-1710.09)			
40	Clethodim	0.9995	Y = (9813.44)X + (-14167.6)	0.5~200	0.05	0.05
41	Clomazone Dimethazone	0.9998	Y = (18630.4)X + (-1647.69)	0.5~200	0.02	0.04
42	Clomeprop	0.9992	Y = (710.200)X + (-3436.52)	5~200	1.76	0.79
43	Cloquintocet-1-methylexyl ester	0.9997	Y = (60173.6)X + (-14006.9)	0.5~200	0.02	0.04
44	Clothianidin	0.9991	Y = (3233.39)X + (2509.82)	0.5~200	0.47	0.49
45	Coumatetralyl	0.9991	Y = (23242.5)X + (27896.9)	0.5~200	0.03	0.03
46	Cyflufenamid	0.9998	Y = (5958.67)X + (-6544.81)	0.5~200	0.18	0.09
47	Cyproconazole	0.9992	Y = (11720.1)X + (3418.55)	0.5~200	0.22	0.07
48	Cyprodinil	0.9998	Y = (7973.99)X + (1978.79)	0.5~200	0.40	0.14
49	Dazomet	0.9990	Y = (2501.59)X + (-9078.76)	1~200	0.66	0.07
50	Diazinon	0.9999	Y = (31930.3)X + (-16106.8)	0.5~200	0.01	0.02
51	Dichlorvos	0.9994	Y = (61.3014)X + (492.279)	10~200	6.70	3.79
52	Diethofencarb	0.9997	Y = (11607.9)X + (11387.8)	0.5~200	0.32	0.22
53	Diethyltoluamide	0.9993	Y = (79943.4)X + (63810.1)	0.5~200	0.04	0.01
54	Dimepiperate	0.9997	Y = (39447.4)X + (3718.42)	0.5~200	0.06	0.02
55	Dimethirimol	0.9993	Y = (30050.7)X + (21635.8)	0.5~200	0.07	0.02

56	Dimethoate	0.9992	Y = (55848.9)X + (26206.1)	0.5~200	0.07	0.02
57	Dimethomorph	0.9998	Y = (13116.6)X + (-2309.89)	0.5~200	0.04	0.07
58	Diniconazole	0.9991	Y = (4273.37)X + (-1549.44)	0.5~200	0.33	0.23
59	Dinotefuran	0.9993	Y = (14619.3)X + (3801.66)	0.5~200	0.06	0.02
60	Diuron	0.9997	Y = (7835.16)X + (1847.00)	0.5~100	0.15	0.07
61	Dymron	0.9991	Y = (246705)X + (158769)	0.5~200	0.01	0.00
62	Epoxiconazole	0.9996	Y = (8801.47)X + (4813.78)	0.5~200	0.06	0.05
63	Esprocarb	0.9999	Y = (34385.0)X + (-9941.46)	0.5~200	0.05	0.07
64	Ethametsulfuron-methyl	0.9994	Y = (13881.3)X + (-1589.33)	0.5~200	0.02	0.02
65	Ethiofencarb	0.9998	Y = (41267.8)X + (15848.2)	0.5~100	0.06	0.04
66	Ethion	0.9995	Y = (6519.60)X + (-5265.53)	0.5~200	0.08	0.06
67	Ethoprophos	0.9993	Y = (14174.9)X + (15071.9)	0.5~200	0.07	0.05
68	Fenamiphos	0.9993	Y = (23409.3)X + (10202.8)	0.5~200	0.05	0.02
69	Fenarimol	0.9997	Y = (303.283)X + (-728.986)	2~200	2.33	2.64
70	Fenobucarb	0.9990	Y = (4791.08)X + (6489.96)	0.5~200	0.05	0.09
71	Fenoxycarb	0.9999	Y = (2134.24)X + (-225.561)	0.5~200	0.23	0.83
72	Fenpropimorph	0.9999	Y = (27336.5)X +	0.5~200	0.12	0.01

			(-15737.8)			
73	Fenpyroximate	0.9998	Y = (44094.1)X + (-39958.7)	0.5~200	0.06	0.01
74	Flocoumafen	0.9997	Y = (25999.0)X + (-46622.8)	0.5~200	0.03	0.02
75	Fluazifop-p-butyl	1.0000	Y = (16916.9)X + (-13961.0)	0.5~200	0.04	0.06
76	Flusilazole	0.9995	Y = (5462.62)X + (3003.80)	0.5~200	0.20	0.05
77	Flutriafol	0.9995	Y = (3932.09)X + (-213.483)	0.5~200	0.30	0.15
78	Fonofos	0.9996	Y = (4488.10)X + (-296.035)	0.5~200	0.39	0.16
79	Forchlorfenuron	1.0000	Y = (12127.3)X + (-3157.53)	0.5~200	0.13	0.05
80	Fosthiazate	0.9998	Y = (126978)X + (7917.73)	0.5~200	0.03	0.01
81	Furathiocarb	0.9999	Y = (67852.6)X + (-51374.7)	0.5~200	0.04	0.03
82	Hexaconazole	0.9992	Y = (5019.50)X + (2919.82)	0.5~200	0.33	0.05
83	Hexazinone	0.9998	Y = (165248)X + (32066.9)	0.5~200	0.01	0.01
84	Hexythiazox	0.9992	Y = (1592.02)X + (-5122.54)	0.5~200	0.19	0.05
85	Imazapyr	0.9993	Y = (4797.39)X + (1403.27)	0.5~200	0.15	0.03
86	Imibenconazole	0.9995	Y = (2387.31)X + (-5881.42)	2~200	0.72	0.32
87	Imidacloprid	0.9990	Y = (4361.95)X + (3715.16)	0.5~200	0.40	0.05
88	Indoxacarb	0.9995	Y = (2199.98)X + (-1101.68)	1~200	0.23	0.06

89	Iprobenfos	0.9992	Y = (136038)X + (25808.2)	0.5~200	0.03	0.01
90	Iprovalicarb	0.9992	Y = (124516)X + (67310.1)	0.5~200	0.02	0.01
91	Isazofos	0.9993	Y = (102530)X + (50439.1)	0.5~200	0.03	0.01
92	Isoprocarb	0.9993	Y = (5190.76)X + (519.422)	0.5~200	0.01	0.02
93	Isoprothiolane	0.9994	Y = (140986)X + (57499.5)	0.5~200	0.03	0.01
94	Isoproturon	0.9993	Y = (69662.0)X + (59947.3)	0.5~200	0.06	0.02
95	Kresoxim-methyl	0.9997	Y = (4277.69)X + (-3139.00)	0.5~200	0.17	0.06
96	Linuron	0.9999	Y = (860.121)X + (-356.746)	2~200	1.28	0.24
97	Lodosulfuron-methyl-sodium	0.9997	Y = (882.395)X + (-1800.17)	2~200	1.47	0.30
98	Malachite Green Oxalate salt	0.9997	Y = (10217.7)X + (5595.89)	0.5~200	0.02	0.02
99	Malathion	0.9991	Y = (11300.2)X + (8280.00)	0.5~200	0.14	0.02
100	Mefenacet	0.9993	Y = (104353)X + (45004.7)	0.5~200	0.04	0.01
101	Mefenoxam	0.9992	Y = (170839)X + (172060)	0.5~200	0.03	0.01
102	Metalaxyl	0.9999	Y = (183439)X + (-65716.4)	0.5~100	0.04	0.01
103	Metazachlor	0.9993	Y = (102553)X + (59610.0)	0.5~200	0.06	0.01
104	Methamidophos	0.9997	Y = (8990.49)X + (-4822.46)	0.5~200	0.14	0.04
105	Methiocarb	0.9994	Y = (2274.59)X +	0.5~200	0.36	0.03

			(2331.75)			
106	Methomyl	0.9992	Y = (34535.0)X + (20190.5)	0.5~200	0.05	0.01
107	Methoxyfenozide	0.9993	Y = (57907.8)X + (4560.01)	0.5~200	0.06	0.01
108	Metolachlor	0.9994	Y = (82876.2)X + (26609.7)	0.5~200	0.03	0.01
109	Metolcarb	0.9994	Y = (5599.78)X + (4612.83)	0.5~200	0.13	0.07
110	Metsulfuron-methyl	0.9999	Y = (13353.8)X + (-10256.7)	0.5~200	0.04	0.01
111	Mevinphos	0.9990	Y = (23994.8)X + (9670.52)	0.5~200	0.13	0.02
112	Molinate	0.9990	Y = (3985.61)X + (4090.55)	0.5~200	0.21	0.07
113	Monocrotophos	0.9991	Y = (21519.3)X + (18784.8)	0.5~200	0.06	0.02
114	Myclobutanil	0.9999	Y = (4940.93)X + (86.5981)	0.5~200	0.25	0.06
115	Naproanilide	0.9991	Y = (3197.96)X + (-2052.45)	0.5~200	0.30	0.17
116	Napropamide	0.9995	Y = (102974)X + (-9866.26)	0.5~200	0.10	0.02
117	Omethoate	0.9996	Y = (23182.2)X + (-3287.15)	0.5~200	0.09	0.04
118	Oxycarboxin	0.9992	Y = (24047.2)X + (9462.62)	0.5~200	0.15	0.06
119	Paclobutrazol	0.9990	Y = (8907.81)X + (4385.73)	0.5~200	0.04	0.08
120	Penconazole	0.9996	Y = (7383.56)X + (4632.82)	0.5~200	0.15	0.06
121	Pencycuron	0.9997	Y = (86153.7)X + (-51940.5)	0.5~200	0.02	0.00

122	Pendimethalin	0.9998	Y = (7293.91)X + (-2595.18)	0.5~200	0.07	0.07
123	Phorate	0.9994	Y = (1846.74)X + (-1396.75)	1~200	0.78	0.77
124	Phosalone	0.9996	Y = (404.600)X + (-1313.82)	5~200	1.11	0.70
125	Phosemet	0.9993	Y = (885.758)X + (169.132)	2~200	0.58	0.13
126	Phoxim	0.9993	Y = (789.841)X + (1237.22)	2~200	0.78	0.34
127	Piperonyl Butoxide	0.9999	Y = (295634)X + (-301122)	0.5~200	0.03	0.01
128	Pirimicarb	0.9994	Y = (58717.3)X + (65531.2)	0.5~200	0.04	0.02
129	Pirimiphos methyl	0.9995	Y = (45685.8)X + (-8375.59)	0.5~200	0.05	0.02
130	Pretilachlor	0.9999	Y = (128991)X + (-12292.8)	0.5~200	0.06	0.01
131	Prochloraz	0.9994	Y = (27411.3)X + (-3253.01)	0.5~200	0.05	0.03
132	Profenofos	0.9995	Y = (219.765)X + (-739.919)	5~200	0.93	0.32
133	Promecarb	0.9991	Y = (14584.7)X + (14099.9)	0.5~200	0.05	0.02
134	Prometryne	1.0000	Y = (58967.2)X + (-24208.2)	0.5~200	0.04	0.03
135	Propamocarb	0.9993	Y = (43730.0)X + (-90291.3)	0.5~200	0.03	0.02
136	Propanil	0.9996	Y = (245.591)X + (124.967)	0.5~200	1.14	0.42
137	Propargite	0.9998	Y = (7261.67)X + (-7369.98)	0.5~200	0.19	0.09

138	Propiconazole	0.9999	Y = (5555.40)X + (546.403)	0.5~200	0.15	0.06
139	Propoxur	0.9996	Y = (72778.2)X + (45915.6)	0.5~200	0.04	0.01
140	Propyzamide	0.9992	Y = (1370.77)X + (582.254)	0.5~200	0.29	0.01
141	Pyraclostrobin	0.9999	Y = (45491.3)X + (-39058.3)	0.5~200	0.04	0.04
142	Pyrazolynate	0.9994	Y = (2695.65)X + (2194.22)	0.5~200	1.10	0.49
143	Pyrazosulfuron-ethyl	0.9994	Y = (15083.0)X + (-176.493)	0.5~200	0.02	0.01
144	Pyridaben	0.9999	Y = (85384.1)X + (-75867.7)	0.5~200	0.02	0.01
145	Pyridaphenthion	0.9996	Y = (32118.8)X + (6829.50)	0.5~200	0.03	0.01
146	Pyrimethanil	0.9997	Y = (10471.8)X + (1792.60)	0.5~200	0.08	0.04
147	Pyriproxyfen	0.9996	Y = (37809.4)X + (-55810.8)	0.5~200	0.06	0.02
148	Quinalphos	0.9997	Y = (8403.39)X + (2837.82)	0.5~200	0.32	0.06
149	Quizalofop-p-ethyl	0.9999	Y = (14022.6)X + (-11980.1)	0.5~200	0.12	0.02
150	Rimsulfuron	0.9991	Y = (334.051)X + (49.0635)	2~200	0.76	0.48
151	Simeconazole	0.9997	Y = (5749.53)X + (663.959)	0.5~200	0.28	0.06
152	Spinosad	0.9999	Y = (53322.9)X + (-52767.3)	0.5~200	0.02	0.02
153	Spirodiclofen	0.9996	Y = (512.912)X + (-1546.76)	2~200	1.30	0.64
154	Sulfotep	0.9995	Y = (7600.48)X +	0.5~200	0.11	0.08

			(2063.93)			
155	Tebufenozide	0.9994	Y = (67114.4)X + (5074.21)	0.5~200	0.03	0.01
156	Tebufenpyrad	0.9998	Y = (4635.50)X + (-5273.57)	0.5~200	0.36	0.04
157	Temephos	0.9992	Y = (742.437)X + (-2404.83)	5~200	0.21	0.13
158	Terbufos	0.9992	Y = (1396.80)X + (-5083.74)	2~200	0.32	0.15
159	Terbuthylazine	0.9990	Y = (25513.2)X + (19139.6)	0.5~200	0.04	0.02
160	Terbutryn	0.9994	Y = (69766.5)X + (-441.687)	0.5~200	0.05	0.01
161	Tetrachlorvinphos	0.9995	Y = (163.464)X + (-162.508)	10~200	4.87	1.53
162	Tetramethrin	0.9997	Y = (2808.55)X + (-3142.39)	0.5~200	0.14	0.05
163	Thiabendazole	0.9993	Y = (16790.1)X + (22086.4)	0.5~200	0.10	0.01
164	Thiacloprid	0.9994	Y = (42772.6)X + (6335.87)	0.5~200	0.02	0.01
165	Thiamethoxam	0.9991	Y = (21557.6)X + (25802.7)	0.5~200	0.05	0.02
166	Thiodicarb	0.9996	Y = (35876.2)X + (13550.9)	0.5~200	0.15	0.03
167	Thiophanate-methyl	0.9998	Y = (48876.6)X + (-46276.6)	0.5~200	0.02	0.01
168	Tolclofos methyl	0.9994	Y = (83.4547)X + (495.131)	10~200	4.12	4.09
169	Tralkoxydim	0.9998	Y = (27511.1)X + (-23889.4)	0.5~200	0.11	0.07
170	Triadimenol	0.9998	Y = (4158.85)X + (-933.707)	0.5~200	0.20	0.08

171	Triallate	0.9996	Y = (523.938)X + (-2145.63)	5~200	2.42	1.66
172	Triazophos	0.9991	Y = (94856.8)X + (52933.8)	0.5~200	0.03	0.01
173	Trichlorphon	0.9992	Y = (174.617)X + (-50.1544)	2~200	3.01	1.32
174	Tricyclazole	0.9994	Y = (21113.4)X + (9499.72)	0.5~200	0.10	0.03
175	Tridemorph	0.9995	Y = (4271.61)X + (-2465.53)	0.5~200	0.81	0.44
176	Trifloxystrobin	0.9995	Y = (76911.5)X + (-17015.0)	0.5~200	0.05	0.02
177	Triflumizole	0.9995	Y = (67795.0)X + (-30218.6)	0.5~200	0.06	0.03
178	Triflumuron	0.9995	Y = (556.993)X + (-888.556)	2~200	0.88	0.29
179	Triticonazole	0.9993	Y = (6465.98)X + (461.280)	0.5~200	0.11	0.04
180	Warfarin	0.9995	Y = (30364.9)X + (4729.49)	0.5~200	0.02	0.01
181	2,4-D D3	0.9995	Y = (663.410)X + (180.247)	0.5~200	1.90	1.01
182	2,4-DB	0.9993	Y = (130.489)X + (476.071)	5~200	4.84	4.89
183	2,4-Dichlorophenoxy-acetic Acid	0.9993	Y = (1023.47)X + (1941.71)	2~200	1.81	0.60
184	Acifluorofen	0.9999	Y = (1719.05)X + (-2265.60)	5~200	1.09	0.41
185	Bromadiolone	0.9995	Y = (2092.79)X + (-1857.26)	0.5~200	0.48	0.44
186	Bromoxynil	0.9992	Y = (505.761)X + (-449.983)	2~200	2.26	0.34

187	Chiptox	0.9995	Y = (1704.10)X + (1150.94)	2~200	0.55	0.22
188	Chloramphenicol	0.9994	Y = (3146.94)X + (2250.30)	0.5~100	0.04	0.03
189	Chlorobenzuron	0.9997	Y = (2481.70)X + (3547.83)	2~200	0.84	0.20
190	Dicamba	0.9992	Y = (129.907)X + (808.724)	5~200	3.83	2.46
191	Dichlorprop	0.9996	Y = (229.913)X + (-305.127)	5~200	8.09	4.73
192	Diflufenican	0.9999	Y = (665.723)X + (-1901.24)	5~200	1.74	2.12
193	Fludioxonil	0.9992	Y = (3276.79)X + (6419.89)	1~200	0.51	0.29
194	Flufenoxuron	0.9995	Y = (1128.24)X + (1106.96)	2~200	1.69	0.38
195	Fomesafen	0.9994	Y = (584.662)X + (691.904)	2~200	2.61	0.60
196	Gibberellic Acid	0.9994	Y = (255.153)X + (-1133.97)	5~200	3.32	0.26
197	Hexaflumuron	0.9995	Y = (7975.32)X + (9337.25)	0.5~200	0.17	0.08
198	Lufenuron	0.9997	Y = (5560.83)X + (2266.43)	0.5~200	0.24	0.23
199	MCPP Acid	0.9997	Y = (1417.79)X + (255.322)	2~200	1.65	0.43
200	Teflubenzuron	0.9993	Y = (7682.99)X + (4669.33)	0.5~200	0.10	0.09

CONCLUSION

A rapid method for simultaneous quantitative analysis of 200 pesticides in cucumber, tomato and apples was developed as per regulatory requirements with Quantifier and Qualifier ions. LCMS-8040 owing to its speed and precision gave linearity >0.999 for all the analytes.

C-60 ANALYSIS OF MULTIPLE PESTICIDE RESIDUES IN SALAD USING TRIPLE QUADRUPOLE GC/MS/MS SYSTEM

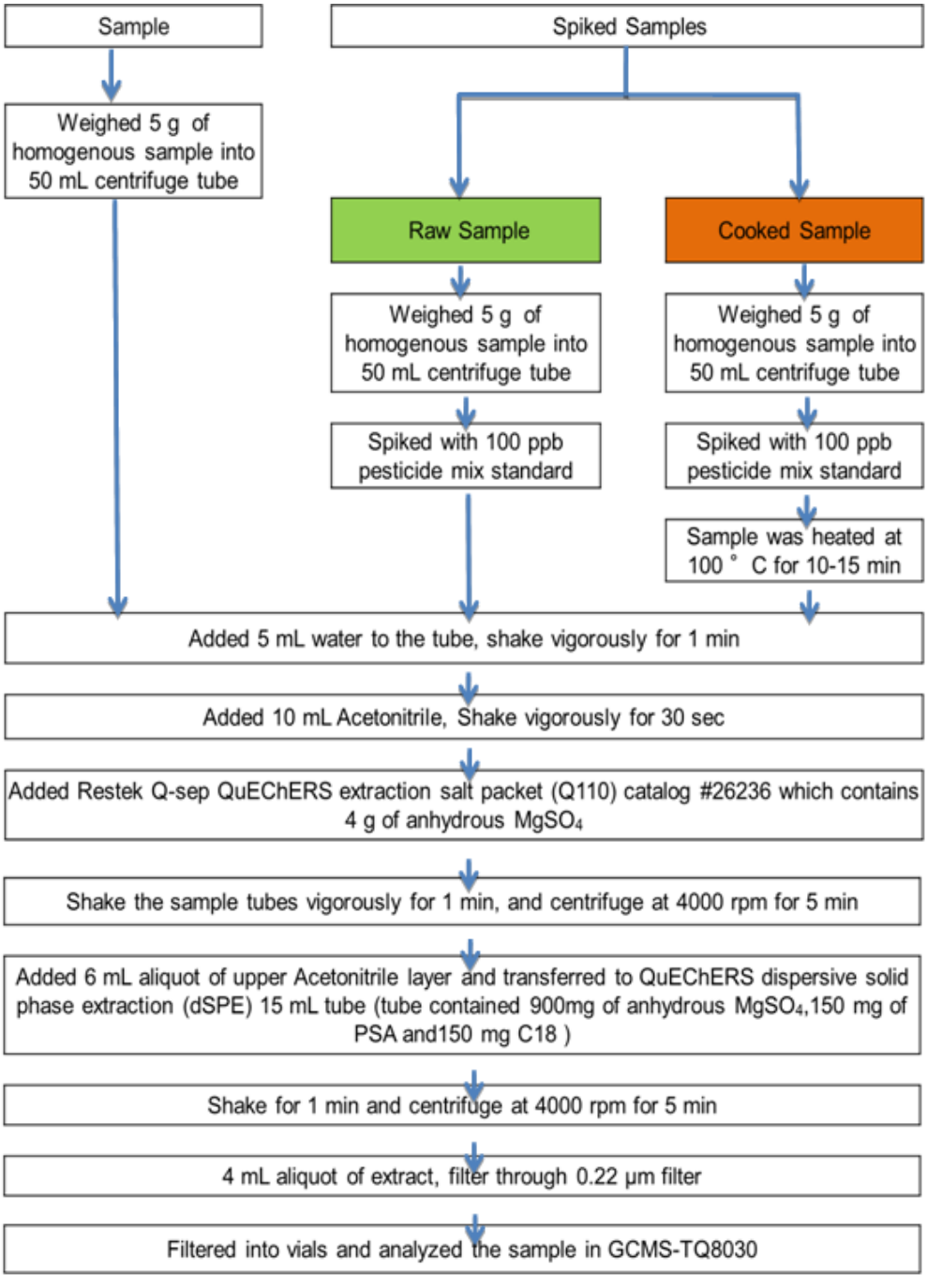
INTRODUCTION

Pesticides are used in agriculture to protect crops from insects, fungi and weeds. Exposures to pesticides in different countries have different regulations to control usage and its content in consumer products. So it has become essential to analyze multiple pesticides in a single run to ensure fast and reliable testing method. Generally most of the cooked products have low risk of pesticide contamination as they get degraded at high temperature. Salad is usually consumed directly without being cooked and this increases the risk of exposure to multi pesticide residues.

The objective of the current study is to develop a fast, sensitive, selective, accurate and reliable method of analysis for multi pesticide residues in pre-cooked and post-cooked salad separately by using Shimadzu GCMS-TQ8030, employing QuEChERS method for extraction, so as to determine the risk of pesticides in salad.

Methodology

Sample information and pre-treatment



Instrument parameters

GC conditions

Column	: Rxi-5Sil MS (30 m x 0.25 mm x 0.25 µm)
Injection Mode	: Split
Split ratio	: 5.0
Carrier gas	: Helium
Flow Control Mode	: Linear Velocity
Linear Velocity	: 40.2 cm/sec
Column Flow	: 1.2 mL/min
Injection Volume	: 2.0 µL

PTV Temp. Program

: Rate °C / min	Temp. °C	Hold time (min)
	150.0	0.0
300.0	290.0	41.0

Column Temp. Program

: Rate °C / min	Temp. °C	Hold time (min)
	70.0	2.0
25.0	150.0	0.0
3.0	200.0	0.0
8.0	280.0	10.0

MS conditions:

Ion Source Temp	: 230.0 ˚C
Interface Temp	: 280.0 ˚C
Ionization Mode	: EI
Mode	: MRM

List of Pesticides analysed (Table 1.)

Table 1: List of Pesticides analysed

Sr. No.	Pesticides	Sr. No.	Pesticides	Sr. No.	Pesticides
1	3-Chloroaniline	11	Propoxur	21	Beta hch
2	Novaluron	12	Diphenylamine	22	Atrazine
3	Diflubenzuron	13	Trifluralin	23	Monolinuron
4	Dichlobenil	14	Benfluralin	24	Clomazone
5	3,4-Dichloraniline	15	Monocrotophos	25	Lindane
6	Trichlorfon	16	Alpha hch	26	Terbufos

7	cis 1,2,3,6-Tetrahydrophthalimide	17	Pencycuron DEG.	27	Diazinon
8	Molinate	18	Dimethoate	28	Chlorothalonil
9	Omethoate	19	Carbofuran	29	Paraoxon methyl
10	Fenobucarb	20	Simazine	30	Delta hch
31	Etrimfos	63	Procymidone	95	Trifloxystrobin
32	Tri-allate	64	Triflumizole	96	Chloridazon
33	Fenchlorphos oxon	65	Methidathion	97	Fluopicolide
34	Fenchlorphos	66	Chlordane trans	98	Triphenyl phosphate
35	Metribuzin	67	Bromophos-ethyl	99	Diclofop
36	Vinclozolin	68	Alpha endosulfan	100	Captafol
37	Parathion methyl	69	Fenamiphos	101	Diflufenican
38	Alachlor	70	Hexaconazole	102	Oxycarboxin
39	Carbaryl	71	Isoprothiolane	103	Spiromesifen
40	Heptachlor	72	Profenofos	104	Iprodione
41	Metalaxyl/ Metalaxyl M	73	p,p-DDE	105	Carbosulfan
42	Chlorpyriphos methyl	74	Fipronil sulphone	106	Phosmet
43	Methiocarb	75	Oxadiazon	107	Bromopropylate
44	Dichlofluanid	76	Myclobutanyl	108	Bifenthrin
45	Chlorpyriphos oxon	77	Iprovalicarb	109	Methoxychlor
46	Malathion	78	Flusilazole	110	Dicofol
47	Metholachlor-s	79	Buprofezin	111	Fenazaquin
48	Aldrin	80	Oxyfluorfen	112	Phenothrin
49	Thiobencarb	81	Kresoxim-methyl	113	Tetradifon
50	Chlorpyriphos ethyl	82	Iprovalicarb-1 & 2	114	Phenothrin
51	Fenthion	83	Chlorfenapyr	115	Lambda-cyhalothrin
52	Triadimefon	84	Cyproconazole-1 & 2	116	Acrinathrin
53	Flufenacet	85	Endrin	117	Permethrin-1
54	4,4- Dichlorobenzophenone	86	Beta endosulfan	118	Permethrin-2
55	Tetraconazole	87	Fenthion	119	Cyfluthrin-1
56	Pendimethalin	88	Oxadiargyl	120	Cyfluthrin-2

57	Penconazole	89	Fenthion sulphone	121	Cyfluthrin-3
58	Fipronil	90	o,p-DDT	122	Boscalid
59	Chlorfenvinphos	91	Benalaxyl/benalaxyl M	123	Etofenprox
60	Captan	92	Carfentrazone	124	Fenvalerate
61	Quinalphos	93	Edifenfos	125	Dimethomorph
62	Folpet	94	Endosulfan sulphate	126	Dimethomorph

RESULTS AND DISCUSSION

For MRM scanning, well resolved pesticides were grouped together. Standard solution mixture of about 1 ppm concentration was prepared and injected using programmable temperature vaporization (PTV) technique to determine precursor ions for individual pesticide. Further product ion scan was taken for individual pesticide from the standard mixture followed by appropriate optimization of collision energy to obtain their characteristic MRM transitions. Based on MRM transitions, mixture of 126 pesticides was analyzed in a single run as in Figure 1.

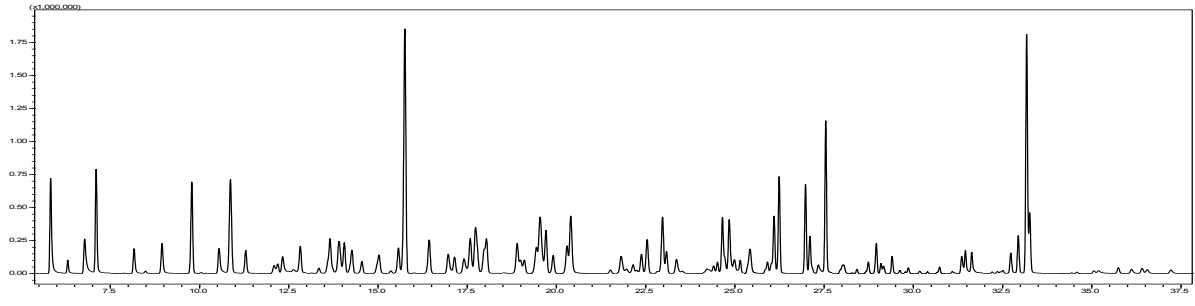


Figure 1. TIC for Pesticides Standard mixture (200 ppb)

Reproducibility of all the listed pesticides was studied and results found are as follows (Table 2)

Table 2: Reproducibility (100 ppb)

Sr. No.	%RSD Range	Number of pesticides
1	1 - 2	15
2	2 - 5	75
3	5 - 10	25
4	10 - 20	11

Linearity was plotted from LOQ concentration of 10 ppb to 100 ppb and recovery was calculated by spiking known pesticides concentration of 100 ppb as depicted in Table 3 to 6.

Table 3: Linearity (10 ppb to 200 ppb)

Sr. No.	R^2	Number of pesticides
1	Above 0.99	114
2	Less than 0.99	12

Table 4: Recovery data

Sr. No.	%Recovery Range	Number of pesticides
1	90 – 110%	60
2	80 – 120%	84
3	70 – 130%	113
4	Less than 70%	13

Table 5: LOD data

Sr. No.	LOD Range	Number of pesticides	S/N Ratio range
1	0.4 - 5 ppb	99	8 - 91
2	5 - 10 ppb	15	94 - 185
3	10- 20 ppb	10	197- 327
4	20- 30 ppb	02	380 & 484

Table 6: LOQ data

Sr. No.	LOQ Range	Number of pesticides	S/N Ratio range
1	1 - 10 ppb	68	24 - 182
2	10 - 20 ppb	40	186 - 361
3	20 - 30 ppb	06	377 - 441
4	30 - 80 ppb	12	560 - 1466

CONCLUSION

- A method is developed for quantification of more than 100 pesticides at very low concentration level in Salad matrix sample by using GC/MS/MS technique with QuEChERS method.
- Ultra fast scanning, Ufsweeper and advance scanning speed protocol (ASSP) technique enabled sensitive, selective, fast, reproducible, linear and accurate pesticides analysis.
- It is safe to consume post-cooked salad rather than the pre-cooked, as the pesticide concentration levels were found to be greatly reduced in cooked salad sample.

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C-61 MULTI-RESIDUE PESTICIDE ANALYSIS FROM DRIED CHILI POWDER USING LC/MS/MS

INTRODUCTION

Pesticide residues existing in foodstuffs can cause serious health problems when consumed. LC/MS/MS methods have been increasingly employed in sensitive quantification of pesticide residues in foods and agriculture products. However, matrix effect is a phenomenon seen in Electro Spray Ionization (ESI) LC/MS/MS analysis that impacts the data quality of the pesticide analysis, especially for complex spice/herb samples.
Chili powder is one such complex matrix that can exhibit matrix effect (either ion suppression or enhancement). A calibration curve based on matrix matched standards can demonstrate true sensitivity of analyte in presence of matrix. Therefore, this approach was used to obtain more reliable and accurate data as compared to quantitation against neat (solvent) standards. Multiresidue, trace level analysis in complex matrices is challenging and tedious. Feature of automatic MRM optimization in LCMS-8040 makes method development process less tedious. UFsweeper^{TM}II technology in the system ensures least crosstalk, which is very crucial for multiresidue pesticide analysis. In addition, the lowest dwell time and pause time along with Ultra fast polarity switching (UFswitching) enables accurate, reliable and high sensitive quantitation.

EXPERIMENTAL

Sample information and pre-treatment

Commercially available red chili was powdered using mixer grinder. To 1 g of this chili powder, 20 mL water:methanol (1:1 v/v) was added and the mixture was sonicated for 10 mins. The mixture was centrifuged and supernatant was collected. This supernatant was used as diluent to prepare

pesticide matrix matched standards at concentration levels of 0.01 ppb, 0.02 ppb, 0.05 ppb, 0.1 ppb, 0.2 ppb, 0.5 ppb, 1 ppb, 2 ppb, 5 ppb, 10 ppb and 20 ppb. Each calibration level was then filtered through 0.2 µ nylon filter and used for the analysis.

Instrument parameters

Column : Shim-pack XR-ODS (75 mm L x 3 mm I.D.; 2.2 µm)
Guard column : Phemomenex SecurityGuard ULTRA Cartridge
Mobile phase : A: 5 mM ammonium formate in water:methanol (80:20 v/v)
B: 5 mM ammonium formate in water:methanol (10:90 v/v)
Flow rate : 0.2 mL/min
Oven temperature : 40 °C
Gradient program (B%)

Time (min)	B.Conc
0.01	45
1.00	45
13	100
18	100
19	45
23	45

Injection volume : 15 µL
MS interface : ESI
Polarity : Positive and negative
MS gas flow : Nebulizing gas 2 L/min; Drying gas 15 L/min
MS temperature : Desolvation line 250 °C; Heat block 400 °C
MS analysis mode : Staggered MRM

RESULTS AND DISCUSSION

LC/MS/MS method was developed for analysis of 80 pesticides belonging to different classes like carbamate, organophosphate, urea, triazines etc. in a single run. LOQ was determined for each pesticide based on following criteria –

A. % RSD for area < 16% (n=3),

B. % accuracy between 80-120 % and

C. Signal to noise ratio (s/n) > 10.

LOQ achieved for 80 pesticides have been summarized in Table 2 and results for LOQ and linearity for each pesticide have been given in Table 3. Representative MRM chromatogram of pesticide mixture at 1 ppb level is shown in Figure 1. Representative MRM chromatograms at LOQ level for different classes of pesticides are shown in Figure 2.

Table 1. Summary of LOQ achieved

LOQ (in ppb)	**0.01**	**0.02**	**0.05**	**0.1**	**0.2**	**0.5**	**1**
No.of pesticides	1	1	3	8	17	24	26

Table 2. Results of LOQ and linearity for pesticide analysis

Sr. No.	**Name of compound**	**MRM Transition**	**Polarity**	**LOQ (ppb)**	**Linearity (R^2)**
1	Spinosyn D	746.20>142.10	Positive	0.01	0.9987
2	Fenpyroximate	421.90>366.10	Positive	0.02	0.9915
3	Bifenazate	301.00>198.00	Positive	0.05	0.9947
4	Spinosyn A	732.20>142.10	Positive	0.05	0.9974
5	Spiromesifen	371.00>273.10	Positive	0.05	0.9957
6	Acetamiprid	222.90>126.00	Positive	0.1	0.9910
7	Carbofuran	221.70>123.00	Positive	0.1	0.9971

8	Dimethoate	229.80>198.90	Positive	0.1	0.9970
9	Dimethomorph I	387.90>301.00	Positive	0.1	0.9991
10	Dimethomorph II	387.90>301.00	Positive	0.1	0.9992
11	Isoproturon	207.00>72.10	Positive	0.1	0.9984
12	Pirimiphos methyl	305.70>108.00	Positive	0.1	0.9997
13	Trifloxystrobin	408.90>186.00	Positive	0.1	0.9989
14	Anilophos	367.70>198.85	Positive	0.2	0.9974
15	Atrazine	215.90>174.00	Positive	0.2	0.9985
16	Carboxin	235.90>143.00	Positive	0.2	0.9952
17	Cyazofamid	324.85>108.10	Positive	0.2	0.9971
18	Edifenphos	310.60>111.00	Positive	0.2	0.9997
19	Ethion	384.70>198.80	Positive	0.2	0.9957
20	Fipronil	434.70>330.00	Negative	0.2	0.9973
21	Linuron	248.80>159.90	Positive	0.2	0.9945
22	Metolachlor	283.90>252.00	Positive	0.2	0.9966
23	Oxycarboxin	267.90>174.90	Positive	0.2	0.9995
24	Phosalone	367.80>181.90	Positive	0.2	0.9987
25	Phosphamidon	299.90>173.90	Positive	0.2	0.9997
26	Thiacloprid	252.90>126.00	Positive	0.2	0.9976
27	Thiobencarb	257.90>125.10	Positive	0.2	0.9977
28	Thiodicarb	354.90>88.00	Positive	0.2	0.9906
29	Triadimefon	293.90>196.90	Positive	0.2	0.9994
30	Tricyclazole	189.90>162.90	Positive	0.2	0.9977

31	Aldicarb	208.10>116.05	Positive	0.5	0.9962
32	Benfuracarb	411.10>190.10	Positive	0.5	0.9981
33	Bitertanol	338.00>99.10	Positive	0.5	0.9935
34	Buprofezin	305.70>201.00	Positive	0.5	0.9933
35	Clodinafop propargyl	349.90>266.00	Positive	0.5	0.9978
36	Chlorantraniliprole	483.75>452.90	Positive	0.5	0.9994
37	Diclofop methyl	357.90>280.80	Positive	0.5	0.9976
38	Flufenacet	363.70>193.90	Positive	0.5	0.9997
39	Flusilazole	315.90>247.00	Positive	0.5	0.9983
40	Hexaconazole	313.90>70.10	Positive	0.5	0.9996
41	Hexythiazox	352.90>227.90	Positive	0.5	0.9909
42	Iodosulfuron methyl	507.70>167.00	Positive	0.5	0.9971
43	Iprobenfos	288.70>205.00	Positive	0.5	0.9981
44	Malaoxon	314.90>99.00	Positive	0.5	0.9996
45	Malathion	330.90>284.90	Positive	0.5	0.9997
46	Mandipropamid	411.90>356.20	Positive	0.5	0.9952
47	Metalaxyl	280.00>220.10	Positive	0.5	0.9996
48	Methabenzthiazuron	221.70>150.00	Positive	0.5	0.9957
49	Methomyl	162.90>88.00	Positive	0.5	0.9988
50	Oxadiazon	362.15>303.00	Positive	0.5	0.9963
51	Penconazole	283.90>70.10	Positive	0.5	0.9992
52	Phorate	260.80>75.00	Positive	0.5	0.9987
53	Phorate sulfoxide	276.80>96.90	Positive	0.5	0.9991

54	Thiophanate methyl	342.90>151.00	Positive	0.5	0.9996
55	Avermectin B1a	890.30>305.10	Positive	1	0.9990
56	Carpropamid	333.70>139.00	Positive	1	0.9985
57	Clomazone	241.90>127.00	Positive	1	0.9967
58	Clorimuron ethyl	415.30>186.00	Positive	1	0.9965
59	Cymoxanil	198.90>128.10	Positive	1	0.9949
60	Diafenthiuron	385.00>329.10	Positive	1	0.9961
61	Diflubenzuron	310.80>158.00	Positive	1	0.9982
62	Dodine	228.10>60.00	Positive	1	0.9980
63	Emamectin benzoate	886.30>158.10	Positive	1	0.9983
64	Fenamidone	311.90>236.10	Positive	1	0.9997
65	Fenarimol	330.70>268.00	Positive	1	0.9900
66	Fenazaquin	306.95>57.10	Positive	1	0.9992
67	Flonicamid	229.90>202.70	Positive	1	0.9971
68	Flubendiamide	680.90>254.05	Negative	1	0.9993
69	Forchlorfenuron	247.90>129.00	Positive	1	0.9956
70	Kresoxim methyl	331.00>116.00	Positive	1	0.9996
71	Paclobutrazol	293.90>70.10	Positive	1	0.9974
72	Pencycuron	328.90>125.00	Positive	1	0.9943
73	Pendimethalin	281.90>212.10	Positive	1	0.9932
74	Profenofos	372.70>302.70	Positive	1	0.9966
75	Propargite	368.00>231.10	Positive	1	0.9950
76	Propoxur	209.90>110.90	Positive	1	0.9987

77	Pyrazosulfuron ethyl	414.90>182.00	Positive	1	0.9992
78	Pyriproxyfen	321.90>96.10	Positive	1	0.9975
79	Simazine	201.90>103.90	Positive	1	0.9992
80	Thiomethon	246.80>89.10	Positive	1	0.9989

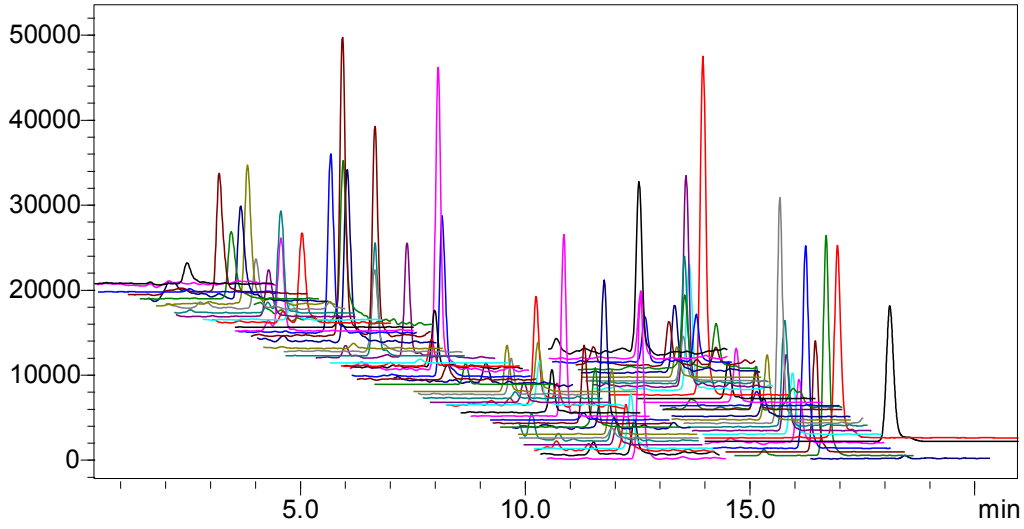


Figure 1. MRM chromatogram of pesticide mixture at 1 ppb level

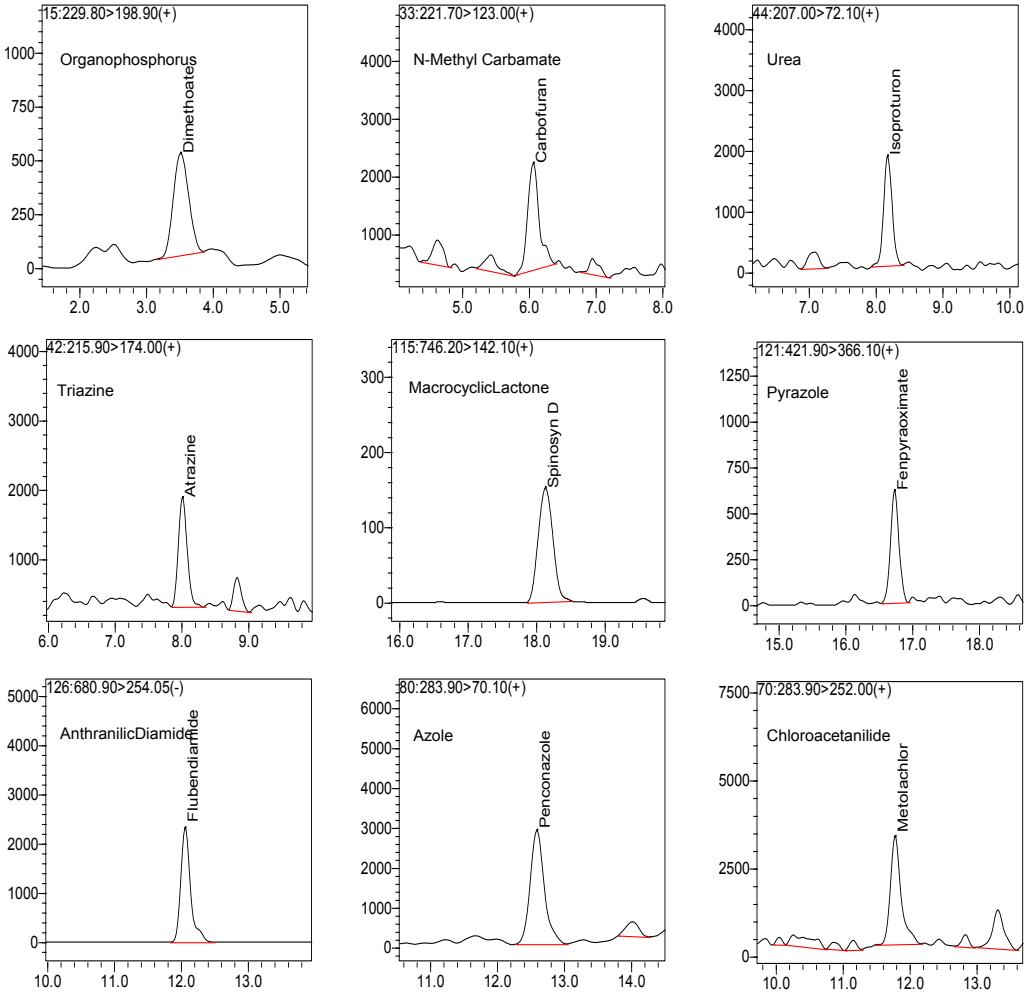


Figure 2. Representative MRM chromatograms at LOQ level from different classes of pesticides

CONCLUSION

- A highly sensitive method was developed for analysis of 80 pesticides belonging to different classes, from dried chili powder in a single run.
- Ultra high sensitivity, ultra fast polarity switching (UFswitching), low pause time and dwell time along with UFsweeper^{TM} II technology enabled sensitive, selective, accurate and reproducible multiresidue pesticide analysis from complex matrix like dried chili powder.

C-62 MULTI PESTICIDE RESIDUE ANALYSIS IN TOBACCO BY GC/MS/MS USING QUECHERS AS AN EXTRACTION METHOD

INTRODUCTION

India is the world's second largest producer (after China) and consumer (after Brazil) of tobacco with nearly $ 1001.54 million revenue generated annually from its export. In countries like India, with tropical-humid climate, the incidences of insect attacks and disease infestations are frequent and application of pesticides for their management is almost obligatory. Like any other crop, tobacco (*Nicotiana tabacum Linn.*), one of the world's leading high-value crop, is also prone to pest attacks, and the farmers do apply various pesticides as a control measure.

The residues of pesticides applied on tobacco during its cultivation may remain in the leaves at harvest that may even sustain post harvest processing treatments and could appear in the final product. Thus, monitoring of pesticide residues in tobacco is an important issue of critical concern from public health and safety point of view demanding implementation of stringent regulatory policies.

To protect the consumers by controlling pesticide residue levels in tobacco, the Guidance Residue Levels (GRL) of 118 pesticides have been issued by the Agro-Chemical Advisory Committee (ACAC) of the Cooperation Center for Scientific Research Relative to Tobacco (CORESTA).

Tobacco is a complex matrix and hence requires selective extraction and extensive cleanup such as QuEChERS (Quick Easy Cheap Effective Rugged Safe) to ensure trace level detection with adequate precision and accuracy. The objective of the present study was to develop an effective, sensitive and economical multi-pesticide residue analysis method for 203 pesticides in tobacco as listed in Table 1.

EXPERIMENTAL

Sample Extraction

Take 2 g of dry powdered tobacco leaves (Figure 1).
Add 18 mL of water containing 0.5 % acetic acid.
Homogenize the sample and Keep it for 30 min.

Add 10 mL ethyl acetate. Immediately, put 10 g sodium sulfate.

Homogenize it thoroughly at 15000 rpm for 2 min.

Centrifuge at 5000 rpm for 5 min for phase separation.

Draw 3 mL of ethyl acetate upper layer from the extract for further cleanup.

Add 1 mL toluene to it and vortex for 0.5 min.

Add cleanup mixture [PSA (150 mg), C18 (150 mg), GCB (75 mg) and anhydrous $MgSO_4$ (300 mg)] and vortex for 2 min.

Centrifuge the mixture at 7000 rpm for 7 min.

Collect the supernatant and filter through a 0.2 µm PTFE membrane filter.

Instrument parameters

GC conditions

Column	: Rxi-5Sil MS (30 m L x 0.25 mm I.D.; 0.25 µm)
Injection Mode	: Splitless
Sampling Time	: 2.0 min
Split Ratio	: 5.0
Carrier Gas	: Helium
Flow Control Mode	: Linear Velocity
Linear Velocity	: 40.2 cm/sec

Column Flow : 1.2 mL/min
Injection Volume : 2.0 µL
Injection Type : High Pressure Injection
Total Program Time : 41.87 min

Column Temp. Program :

Rate (°C / min)	Temp. (°C)	Hold time (min)
	70.0	2.00
25.00	150.0	0.00
3.00	200.0	0.00
8.00	280.0	10.00

MS conditions
Ion Source Temp. : 230.0 °C
Interface Temp. : 280.0 °C
Ionization Mode : EI
Acquisition Mode : MRM (for pesticides as mentioned in Table 1)

Table 1. List of 198 pesticides analysed in Tobacco

Sr. No.	Pesticide	Sr. No.	Pesticide	Sr. No.	Pesticide	Sr. No.	Pesticide
1	2,6-Dichlorobenzamide	46	Clodinafop propargyl	91	Famoxadone	136	Methiocarb
2	2-Phenylphenol	47	Clomazone	92	Fenamidone	137	Metholachlor-s
3	3,4-Dichloraniline	48	Crimidine	93	Fenarimol	138	Methoxychlor
4	3-Chloroaniline	49	Cyanophos	94	Fenbuconazole	139	Metribuzin
5	4-Bromo 2-Chloro phenol	50	Cyfluthrin-1	95	Fenchlorphos	140	Mevinphos
6	4,4- Dichlorobenzophenone	51	Cyfluthrin-2	96	Fenchlorphos oxon	141	Monolinuron
7	Acetochlor	52	Cyfluthrin-3	97	Fenhexamid	142	Myclobutanyl
8	Acrinathrin	53	Cyfluthrin-4	98	Fenobucarb	143	Napropamide
9	Alachlor	54	Cyhalofop-butyl	99	Fenoxycarb	144	Nitrapyrin
10	Aldrin	55	Cypermethrin-2	100	enthionsulphoxide	145	Oxadiargyl
11	Azinphos-ethyl	56	Cypermethrin-3	101	Fenvalerate	146	Oxadiazon
12	Azinphos-methyl	57	Cypermethrin-4	102	Fipronil	147	Oxycarboxin
13	Azoxystrobin	58	Cyprodinil	103	Fipronil sulphone	148	p,p-DDE
14	Barban	59	Delta-HCH	104	Flucythrinate-1	149	Parathion-ethyl
15	Beflubutamid	60	Demeton-s-methyl	105	Flucythrinate-2	150	Parathion-methyl

16	Benfluralin	61	Demeton-S-methyl sulphone	106	Flufenacet	151	Penconazole
17	Benoxacor	62	Dialifos	107	Flumoixazine	152	Pencycuron (Deg.)
18	Beta-endosulfan	63	Diazinon	108	Fluquinconazole	153	endimethalin
19	Bifenox	64	Dichlobenil	109	Flurochloridone-1	154	Permethrin-1
20	Bifenthrin	65	Dichlofluanid	110	Flurochloridone-2	155	Permethrin-2
21	Bitertanol	66	Diclofop	111	Flutolanil	156	Pethoxamid
22	Boscalid	67	Dicloran	112	Flutriafol	157	Phosalone
23	Bromacil	68	Dieldrin	113	Fluxapyoxad	158	Phosmet
24	Bromophos-ethyl	69	Diethofencarb	114	Folpet	159	Pirimicarb
25	Bromopropylate	70	Difenoconazole-1	115	Fuberidazole	160	Pretilachlor
26	Bromuconazole-1	71	Difenoconazole-2	116	Heptachlor	161	Procymidone
27	Bromuconazole-2	72	Diflubenzuron	117	Hexaconazole	162	Profenofos
28	Butralin	73	Diflufenican	118	Iprobenfos	163	Propanil
29	Butylate	74	Dimethipin	119	Isoprocarb	164	Propaquizafop
30	Carbaryl	75	Dimethomorph-1	120	Isoprothiolane	165	Propazine
31	Carbofuran	76	Dimethomorph-2	121	Isopyrazam	166	Propham
32	Carfentrazone	77	Dimoxystrobin	122	Isoxaben	167	Propiconazol
33	Chlordane-trans	78	Diniconazole	123	Lactofen	168	Propisoclor
34	Chlordecone	79	Dinoseb	124	Lambda-cyhalothrin	169	Propyzamide
35	Chlorfenvinphos	80	Dinoterb	125	Malaoxon	170	Proquinazid
36	Chlormephos	81	Dioxathion	126	Malathion	171	Pyraflufenethyl
37	Chlorobenzilate	82	Edifenfos	127	Mepanipyrim	172	Pyrazophos
38	Chloroneb	83	Endosulfan sulphate	128	Mepronil	173	Pyrimethanil
39	Chlorothalonil	84	Endrin	129	Metalaxyl	174	Pyriprooxyfen
40	Chlorpyriphos-ethyl	85	Epoxiconazole	130	Metalaxyl M	175	Pyroquilon
41	Chlorpyriphos-methyl	86	Ethalfluralin	131	Metazachlor	176	Quinoxyfen
42	Chlorpyriphos-oxon	87	Ethoprophos	132	Metconazole	177	Simazine
43	Chlorthal-dimethyl	88	Etoxazole	133	Methabenzthiazuron	178	Spirodiclofen
44	Cinidon-ethyl	89	Etridiazole	134	Methacrifos	179	Sulfotep
45	Cis 1,2,3,6tetrahydrophthalimide	90	Etrimfos	135	Methidathion	180	Swep

181	Tebufenpyrad	187	Tetradifon	193	Triazophos	199	Triflusulfuron
182	Tebupirimfos	188	Thiobencarb	194	Tricyclazole	200	Triticonazole
183	Tebuthiuron	189	Tolylfluanid	195	Trifloxystrobin	201	Valifenalate
184	Tefluthrin	190	Tralkoxydim	196	Triflumizole	202	Vinclozolin
185	Terbacil	191	Triadimefon	197	Triflumuron	203	Zoxamide (Deg.)
186	Tetraconazole	192	Tri-allate	198	Trifluralin		

RESULTS AND DISCUSSION

For MRM optimisation, well resolved pesticides were grouped together. Standard solution mixture of approximately 1 ppm concentration was prepared and analyzed in Q3 scan mode to determine the precursor ion for individual pesticides. Selected precursor ions were allowed to pass through Q1 & enter Q2, also called as Collision cell. In Collision cell, each precursor ion was bombarded with collision gas (Argon) at different energies (called as Collision Energy (CE)) to produce fragments (product ions). These product ions were further scanned in Q3 to obtain their mass to charge ratio. For each precursor ion, product ion with highest intensity and its corresponding CE value was selected, thereby assigning a characteristic MRM transition to every pesticide. Based on MRM transitions, the mixture of 203 pesticides was analyzed in a single run (Figure 1).

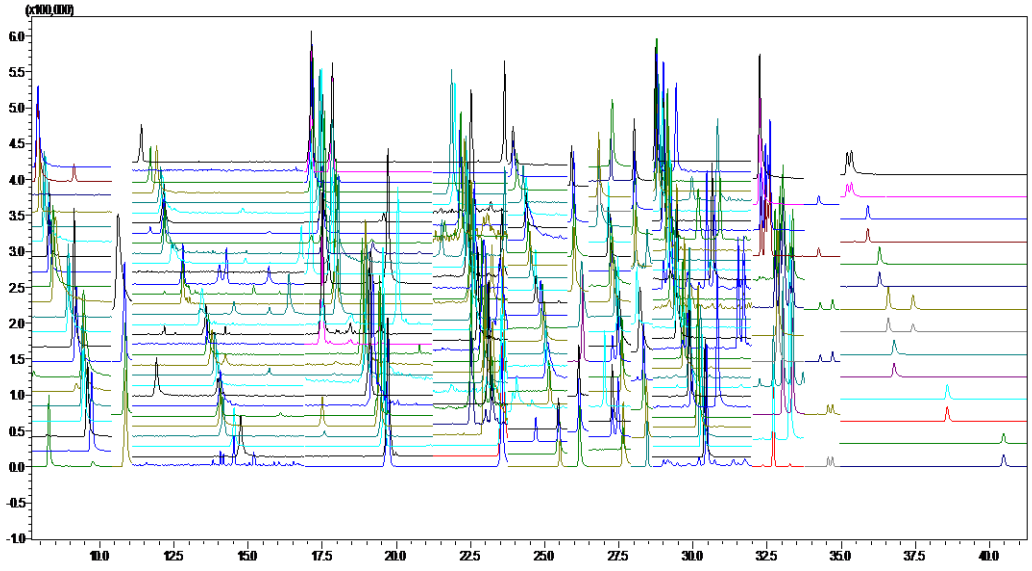
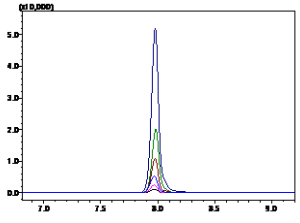
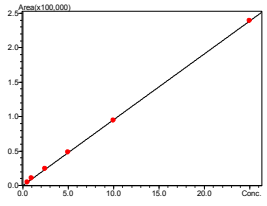
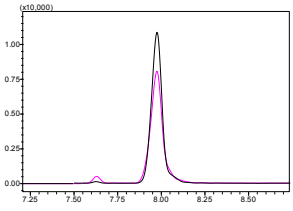


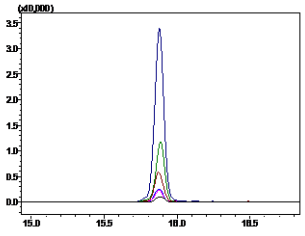
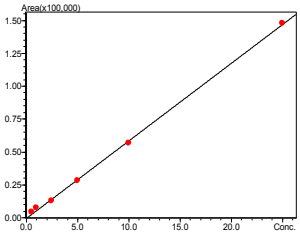
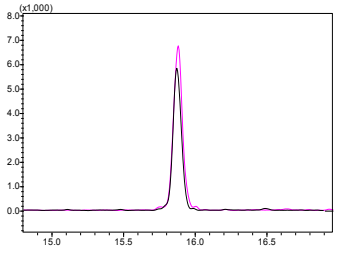
Figure 1. MRM Chromatogram for 203 pesticides mixture

Method was partly validated for each pesticide with respect to linearity (0.5 to 25 ppb), reproducibility, LOQ and recovery. The validation summary for two pesticides namely Mevinphos and Parathion-ethyl (Sr. Nos.140 and 149 in Table 1) is shown in Figures 4 and 5. The summary data of linearity and LOQ for 203 pesticides is given in Table 2 and 3 respectively.

Calibration overlay	Linearity curve	Recovery overlay
		

Linearity (R^2)	LOD (ppb)	LOQ (ppb)	S/N at LOQ	% RSD at LOQ (n=6)	% Recovery at LOQ
0.9999	0.3	1	173	6.93	89.28

Figure 2.Summary data for Mevinphos

Calibration overlay	Linearity curve	Recovery overlay
		

Linearity (R^2)	LOD (ppb)	LOQ (ppb)	S/N at LOQ	% RSD at LOQ (n=6)	% Recovery at LOQ
0.9993	1.5	5	93	4.05	109.10

Figure 3.Summary data for Parathion- ethyl

Table 2. Linearity summary

Sr. No.	Linearity (R^2)	Number of pesticides
1	0.9950 - 1.0000	193
2	0.9880 - 0.9950	10

Table 3. LOQ summary

Sr. No.	LOQ (ppb)	Number of pesticides	% RSD range (n=6)	S/N Ratio range	% Recovery range
1	1	15	6 – 15	16 – 181	70 – 130
2	5	18	3 – 15	19 – 502	
3	10	158	0.95 – 15	10 – 14255	
4	25	12	1 – 10	19 – 660	

CONCLUSION

- A highly sensitive method was developed for quantitation of 203 pesticides in complex tobacco matrix by using Shimadzu GCMS-TQ8030.
- The MRM method developed for 203 pesticides can be used for screening of pesticides in various food commodities. For 90 % of the pesticides, the LOQ of 10 ppb or below was achieved.
- Ultra Fast scanning, UFsweeper® and ASSP™ features enabled sensitive, selective, fast, reproducible, linear and accurate method of analysis.

SHIMADZU
Excellence in Science

C-63 MULTI-RESIDUE ANALYSIS OF 210 PESTICIDES IN FOOD SAMPLES BY TRIPLE QUADRUPOLE UHPLC-MS/MS

INTRODUCTION

Pesticides and their metabolites are of great concern to society as they are harmful to human health, pollute natural resources and disturb the equilibrium of the ecosystem. Consequently, stricter food safety regulations are being enforced around the world, placing pesticide analysis laboratories under increasing pressure to expand the list of targeted pesticides, detect analytes at lower levels and with greater precision, reduce analysis turnaround times, and all the while maintaining or reducing costs. In this study a method was successfully developed for the quantitation of 210 commonly analysed pesticides in food samples using the Nexera UHPLC and LCMS-8040. Initial validation was performed to demonstrate instrument capabilities. Limits of detection (LOD) for 90 % of compounds were less than 0.001 mg kg-1 (1 ppb) and all compounds were less than 0.01 mg kg-1 (10 ppb) for both the quantifying and qualifying transitions using only a 2 µL injection. Repeatability at the 0.01 mg kg-1 reporting level was typically less than 5 % RSD for compounds and correlation coefficients were typically greater than 0.997 in a variety of studied food extracts. Consequently, the LCMS-8040 is ideally suited for routine monitoring of pesticides below the 0.01 mg kg-1 default level set by EU and Japanese legislation.

EXPERIMENTAL

A stock of pesticides was obtained from the Food and Environment Agency, UK, at a concentration of 0.01 mg kg-1 (for each pesticide) in acetone:acetonitrile 1:1. Linearity was investigated over a nine-point calibration with samples ranging from 0.5 µg kg-1 - 0.2 mg kg-1 (0.5 – 200

ppb) analysed in duplicate; calibration samples were injected once in increasing order and once in decreasing order. Linearity was assessed with four calibration curves prepared by serial dilution of: (1) acetonitrile, (2) dried fruit extract, (3) lettuce extract and, (4) pear extract. Instrumental area repeatability was determined by replicate (n=6) injection of pear matrix at 0.01 mg kg-1. LC-MS mobile phase solvents and additives were all of LC–MS quality and purchased from Sigma–Aldrich.

Food extracts were supplied by the Food and Environment Agency, UK, following established QuEChERS protocols. QuEChERS is acronym for Quick Easy Cheap Effective Rugged Safe and is a widely used sample preparation technique for the extraction of pesticides from food. Food samples included dried fruit, lettuce and pear, with the final extracts prepared in 100% acetonitrile.

Pesticide limits of detection were calculated based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136,10 using a standard deviation of 7 replicates in pear matrix at a concentration value that corresponds to an instrument signal to noise ratio in the range of 2.5 to 5 and a Student's t 99% confidence interval:

MDL= St9N-1, 1-α= 0.99)%s.d.

Where, t(n-1,1-α=0.99) = Student's t value for the 99% confidence level with n-1 degrees of freedom (t = 3.14 for 7 replicates), n = number of replicates, and s.d. = standard deviation of the replicate analyses.

Instrument parameters

LC conditions

UHPLC	: Nexera UHPLC system
Column	: Shim-pack XR-ODS III (150 mm L x 2 mm I.D.; 2.2 µm)
Column temp.	: 40 °C
Mobile phase	: A - Water with 5 mM ammonium formate and 0.01 % formic acid B - Methanol with 5 mM ammonium formate and 0.01 % formic acid

Gradient :

Time (min)	B.Conc
0.01	95
16.00	100
18.00	100
18.10	95
20.0	95

Flow rate : 0.4 mL/min
Injection volume : 32 µL (stacked injection: 2 µL sample+ 30 µL water)
Needle wash : 1000 µL Methanol

MS conditions
Ionisation :ESI-Positive and negative(15 msec. polarity switch)
SRM : Dwell time 5 msec. Pause time 1 msec.
Desolvation line : 250 ºC
Heating block : 400 ºC
Drying gas : 15 L/ min
Nebulising gas : 2 L/ min

SRM optimization : 1:1 water:methanol with 10 mM ammonium acetate
Flow rate : 0.5 mL min-1
Flow injection analysis : (No column fitted)
Injection volume : 0.2 µL (0.01 mg kg-1 pesticide std sol)
Mobile phase screening: Carrier 1:1 water:methanol
Flow rate: 0.3 mL min-1
Flow injection analysis (No column fitted) 5µL injection (0.01 mg kg-1 pesticide standard solution) 1µL air gap.
(see text for mobile phase compositions)

RESULTS AND DISCUSSION

Target precursor and product ions were selected based on recommendations from the Food and Environment Agency, UK, and data from the EURL DataPool. Typically the protonated or deprotonated molecule was used for the precursor ion. In order to try to prevent interference of SRM transitions from matrix, product ions greater than m/z 100 were selected wherever possible as they are typically more diagnostic. Analyte specific MS parameters (Q1 pre-bias (V), Q3 pre-bias (V) and collision energy) were optimised using automated flow injection analysis. Briefly, this involves placing pesticide standards into the auto-sampler, from where they are then rapidly injected into the MS with a different parameter optimised on each injection. Each compound was optimised in only a few minutes using the automated software provided in LabSolutions. This allowed large numbers of compounds to be optimised overnight; this is in stark contrast to traditional time-consuming infusion in order to optimise parameters. The compounds studied and their associated transitions are shown in Table 1.

Table 1. MRM transitions optimised for 210 pesticides

COMPOUND	CAS	Transitions		PEAR EXTRACT				
				RT (MIN)	LOD for Transitions (ppb)		%RSD (10PPB)	R^2
		1	2		1	2		
Avermectin B1a	71751-41-2	891 > 305	891 > 567	16.4	0.35	0.56	5.0	0.9975
Acephate	30560-19-1	184 > 143	184 > 49	3.0	0.17	0.31	1.0	0.9999
Acetamiprid	135410-20-7	223 > 126	223 > 99	7.2	0.50	1.00	1.1	0.9979
Acrinathrin	101007-06-1	559 > 208	559 > 181	16.1	1.32	2.36	4.4	0.9990
Alachlor	15972-60-8	270 > 238	270 > 162	13.4	0.09	0.26	1.5	0.9995
Aldicarb	116-06-3	208 > 116	208 > 89	8.5	0.05	0.10	1.7	0.9998
Aldicarb sulfone	1646-88-4	240 > 223	240 > 86	4.3	0.17	0.13	1.8	0.99
Aldicarb sulfoxide	1646-87-3	207 > 89	207 > 132	3.9	0.22	0.36	2.3	1.0000
Amidosulfuron	120923-37-7	370 > 261	370 > 139	9.3	0.14	0.22	2.8	0.9984

Asulam	3337-71-1	231 > 156	231 > 92	3.4	0.72	2.03	3.8	0.9979
Atrazine	1912-24-9	216 > 174	216 > 104	11.1	0.10	0.22	2.4	0.9989
Azinphos-methyl	86-50-0	318 > 132	318 > 77	11.8	0.50	0.50	2.7	0.9903
Azoxystrobin	131860-33-8	404 > 372	404 > 344	12.1	0.03	0.30	2.1	0.9989
Bendiocarb	22781-23-3	224 > 109	224 > 167	9.8	0.10	0.09	1.5	0.9996
Benthiavalicarb-isopropyl	177406-68-7	382 > 180	382 > 116	12.7	0.12	0.41	0.9	0.9997
Bispyribac sodium	125401-92-5	453 > 297	453 > 179	12.1	1.41	5.43	7.4	0.9954
Boscalid	188425-85-6	343 > 307	343 > 140	12.5	0.81	1.19	4.6	0.9968
Bromoxynil*	1689-84-5	274 > 79	276 > 81	9.9	2.24	2.61	4.5	0.9968
Bromuconazole	116255-48-2	376 > 159	376 > 70	13.0	0.72	1.79	2.9	0.9994
Butachlor	23184-66-9	312 > 238	312 > 57	15.3	0.29	0.39	1.6	0.9998
Butocarboxim	34681-10-2	208 > 75	208 > 191	8.4	0.13	0.87	3.1	0.9999
Butocarboxim sulfone	34681-23-7	223 > 106	223 > 166	4.1	2.63	3.23	9.7	0.9949
Butocarboxim sulfoxide	34681-24-8	207 > 88	207 > 75	3.7	0.22	0.21	1.9	0.9999
Carbaryl	63-25-2	202 > 145	202 > 127	10.3	0.13	0.22	2.4	0.9988
Carbendazim	10605-21-7	192 > 160	192 > 132	7.1	0.50	1.00	1.1	0.9996
Carbofuran	1563-66-2	222 > 165	222 > 123	11.1	0.12	0.18	0.7	0.9993
Carboxin	5234-68-4	236 > 143	236 > 87	10.2	0.09	0.25	0.9	0.9991
Chlorantraniliprole*	500008-45-7	482 > 284	482 > 177	11.8	0.50	1.00	2.3	0.9979
Chlorfenvinfos	470-90-6	361 > 155	361 > 99	14.0	0.28	0.49	2.3	0.9966
Chloridazon	1698-60-8	222 > 92	222 > 104	7.2	0.20	0.18	3.2	0.9990
Chlorotoluron	15545-48-9	213 > 72	213 > 46	10.8	0.05	0.13	1.3	0.9967
Chromafenozide	143807-66-3	395 > 175	395 > 91	13.0	0.05	0.60	1.0	0.9977
Clethodim	99129-21-2	360 > 164	360 > 268	14.7	0.08	0.45	0.7	0.9970
Clofentezine	74115-24-5	303 > 138	303 > 102	14.4	4.03	5.76	9.5	0.9967
Clothianidin	210880-92-5	250 > 132	250 > 169	6.5	0.25	0.12	1.6	0.9978
Cyazofamid	120116-88-3	325 > 108	325 > 261	13.3	0.39	3.74	2.4	0.9964
Cycloxydim	101205-02-1	326 > 280	326 > 180	14.8	0.33	0.73	1.0	0.9989
Cyflufenamid	180409-60-3	413 > 295	413 > 241	14.2	0.27	0.29	2.9	0.9982

Cymoxanil	57966-95-7	199 > 128	199 > 111	7.7	2.99	3.52	5.5	0.9960
Cyproconazole	113096-99-4	292 > 70	292 > 125	12.8	0.41	0.60	3.5	0.9988
Cyprodinil	121552-61-2	226 > 93	226 > 108	13.9	0.89	0.91	1.3	0.9990
Cyromazine	66215-27-8	167 > 85	167 > 125	2.2	2.57	4.79	7.4	0.9994
Demeton-S-methyl sulfoxide	301-12-2	247 > 169	247 > 109	5.0	0.01	0.03	1.2	0.9999
Demeton-S-methyl sulfone	17040-19-6	263 > 169	263 > 109	5.3	0.03	0.10	3.1	0.9999
Desmedipham	13684-56-5	318 > 182	318 > 136	11.6	0.08	0.33	0.5	0.9971
Diclobutrazol	75736-33-3	328 > 70	330 > 70	13.8	0.17	0.20	2.7	0.9988
Diethofencarb	87130-20-9	268 > 226	268 > 124	12.2	0.06	0.12	2.2	0.9996
Difenoconazole	119446-68-3	406 > 251	406 > 188	14.5	0.18	0.53	2.6	0.9994
Diflubenzuron	35367-38-5	311 > 158	311 > 141	13.5	2.21	7.48	9.2	0.9936
Dimethoate	60-51-5	230 > 125	230 > 199	7.0	0.05	0.07	1.6	0.9997
Dimethomorph	110488-70-5	388 > 301	388 > 165	12.7	0.29	0.41	2.5	0.9991
Dimoxystrobin	149961-52-4	327 > 205	327 > 116	13.7	0.12	0.14	0.5	0.9997
Dinotefuran	165252-70-0	203 > 129	203 > 157	3.9	0.10	0.22	2.9	0.9994
Disulfoton sulfoxide	2497-07-6	291 > 213	291 > 97	10.8	0.05	0.15	2.6	0.9980
Diuron	330-54-1	233 > 72	235 > 72	11.4	0.09	0.26	0.6	0.9971
DMPF	33089-74-6	163 > 107	163 > 122	4.8	1.00	2.00	2.5	0.9910
Dodine	2439-10-3	228 > 71	228 > 60	13.5	0.30	0.54	1.7	0.9946
Epoxiconazole	135319-73-2	330 > 121	330 > 101	13.3	0.12	0.37	2.5	0.9998
Ethiofencarb	29973-13-5	226 > 107	226 > 169	10.6	0.18	0.59	0.7	0.9994
Ethiofencarb sulfone	53380-23-7	275 > 107	275 > 201	6.2	0.02	0.16	0.9	0.9999
Ethiofencarb sulfoxide	53380-22-6	242 > 107	242 > 185	6.5	0.02	0.02	0.9	0.9999
Ethirimol	23947-60-6	210 > 140	210 > 98	10.8	0.14	0.24	1.8	0.9977
Etofenprox	80844-07-1	394 > 177	394 > 359	16.9	0.03	0.06	3.1	0.9983
Fenamidone	161326-34-7	312 > 92	312 > 236	12.4	0.06	0.18	1.9	0.9988
Fenamiphos	22224-92-6	304 > 217	304 > 202	13.5	0.05	0.28	1.9	0.9970
Fenamiphos sulfone	31972-44-8	336 > 266	336 > 188	10.2	0.31	0.25	4.3	0.9961
Fenamiphos sulfoxide	31972-43-7	320 > 108	320 > 171	10.0	0.18	0.52	3.3	0.9976

Fenbuconazole	114369-43-6	337 > 125	337 > 70	13.4	0.23	0.40	5.0	0.9964
Fenhexamid	126833-17-8	302 > 97	302 > 55	13.1	0.75	0.95	0.9	0.9944
Fenoxycarb	79127-80-3	302 > 88	302 > 116	13.6	0.10	0.20	2.4	0.9989
Fenpropimorph	67564-91-4	304 > 147	304 > 117	14.1	0.05	0.13	1.6	0.9995
Fenpyroximate	111812-58-9	422 > 366	422 > 215	15.9	0.02	0.17	1.2	0.9997
Fenthion sulfoxide	3761-41-9	295 > 109	295 > 280	10.1	0.18	0.27	1.5	0.9985
Fenthion sulfone	3761-42-0	311 > 109	311 > 125	10.4	3.75	3.61	9.8	0.9974
Fipronil*	120068-37-3	435 > 330	435 > 250	13.5	0.11	0.35	4.1	0.9998
Fluazifop acid*	69335-91-7	328 > 282	328 > 91	11.8	0.55	3.61	7.1	0.9983
Fluazinam*	79622-59-6	463 > 416	463 > 398	15.2	0.20	0.27	2.7	0.9994
Fludioxonil*	131341-86-1	247 > 126	247 > 180	12.4	1.00	1.00	4.2	0.9974
Flufenacet	142459-58-3	364 > 152	364 > 194	13.2	0.04	0.06	1.6	0.9986
Flufenoxuron	101463-69-8	489 > 158	489 > 141	15.7	0.24	0.63	8.2	0.9989
Fluometuron	2164-17-2	233 > 72	233 > 46	10.6	0.12	0.14	1.3	0.9996
Fluopicolide	239110-15-7	383 > 173	383 > 145	12.7	0.05	0.17	2.1	0.9967
Fluoxastrobin	361377-29-9	459 > 427	459 > 188	13.1	0.19	0.22	1.7	0.9987
Fluroxypyr*	69377-81-7	253 > 195	255 > 197	7.8	1.13	1.75	5.7	0.9993
Flutriafol	76674-21-0	302 > 70	302 > 123	11.1	0.29	0.43	3.2	0.9984
Fosthiazate	98886-44-3	284 > 104	284 > 228	10.7	0.05	0.12	2.7	0.9985
Furathiocarb	65907-30-4	383 > 195	383 > 252	15.1	0.07	0.13	1.8	1.0000
Halofenozide	112226-61-6	331 > 105	331 > 275	12.3	0.05	0.05	1.7	0.9947
Halosulfuron-methyl*	100784-20-1	435 > 182	437 > 182	11.5	0.30	0.96	3.1	0.9968
Haloxyfop acid*	69806-34-4	360 > 288	362 > 290	13.3	6.20	6.86	13.4	0.9999
Heptenophos	23560-59-0	251 > 127	251 > 89	11.4	0.15	1.36	4.7	0.9982
Hexythiazox	78587-05-0	353 > 228	353 > 168	15.6	2.25	1.02	4.5	0.9956
Imazalil	35554-44-0	297 > 159	297 > 69	11.8	0.30	0.48	3.5	0.9988
Imidacloprid	138261-41-3	256 > 209	256 > 175	6.4	0.50	0.50	1.9	0.9966
Indoxacarb	144171-61-9	528 > 203	528 > 150	14.5	0.40	0.37	3.9	0.9964
Ioxynil*	1689-83-4	370 > 127	370 > 215	11.0	0.12	1.00	3.6	0.9961
Iprovalicarb	140923-17-7	321 > 119	321 > 203	13.1	0.06	0.23	2.5	0.9981
Isazofos	42509-80-8	314 > 120	314 > 162	12.9	0.04	0.13	2.2	0.9994

Isocarbofos	24353-61-5	307 > 231	307 > 121	11.4	0.07	0.12	2.7	0.9991
Isofenphos	25311-71-1	346 > 245	346 > 217	14.3	0.17	0.13	1.7	0.9991
Isofenphos-methyl	99675-03-3	332 > 231	332 > 273	13.8	0.03	0.13	1.2	0.9996
Isoprocarb	2631-40-5	194 > 95	194 > 137	11.1	0.20	0.49	1.9	0.9990
Isoprothiolane	50512-35-1	291 > 189	291 > 231	12.6	0.10	0.09	0.9	0.9994
Isoproturon	34123-59-6	207 > 72	207 > 46	11.3	0.10	0.11	1.7	0.9996
Isoxaben	82558-50-7	333 > 165	333 > 150	12.6	0.02	0.06	0.9	0.9989
Kresoxim-methyl	143390-89-0	314 > 116	314 > 206	13.8	0.15	0.18	3.3	0.9991
Lenacil	2164-08-1	235 > 153	235 > 136	11.2	0.18	0.64	2.2	0.9987
Linuron	330-55-2	249 > 160	249 > 182	12.2	3.15	3.20	3.7	0.9979
Lufenuron*	103055-07-8	509 > 339	509 > 175	15.2	0.35	2.39	3.8	0.9918
Malathion	121-75-5	348 > 127	348 > 331.2	12.6	0.04	0.31	1.0	0.9989
Mandipropamid	374726-62-2	412 > 328	412 > 356	12.5	0.11	0.45	4.2	0.9991
Mecarbam	2595-54-2	330 > 227	330 > 97	13.2	0.15	0.30	2.0	0.9992
Mepanipyrim	110235-47-7	224 > 106	224 > 77	13.1	0.19	0.39	3.6	0.9993
Mepronil	55814-41-0	270 > 119	270 > 91	12.7	0.05	0.07	1.1	0.9972
Mesosulfuron-methyl	208465-21-8	504 > 182	504 > 83	10.9	0.27	0.96	3.4	0.9996
Metaflumizone	139968-49-3	507 > 178	507 > 287	15.1	2.63	3.42	6.6	0.9986
Metalaxyl	57837-19-1	280 > 220	280 > 192	11.3	0.04	0.06	1.9	0.9998
Metamitron	41394-05-2	203 > 175	203 > 104	7.0	0.21	0.44	2.3	0.9990
Metconazole	125116-23-6	320 > 70	322 > 125	14.2	0.10	0.30	3.6	0.9976
Methabenzthiazuron	18691-97-9	222 > 165	222 > 150	11.1	0.11	0.19	0.9	0.9989
Methamidophos	10265-92-6	142 > 94	142 > 125	2.3	0.06	0.69	1.3	0.9991
Methiocarb	2032-65-7	226 > 121	226 > 169	12.3	0.10	0.28	2.9	0.9948
Methiocarb sulfoxide	2635-10-1	242 > 122	242 > 170	6.9	0.04	0.15	1.5	0.9996
Methomyl	16752-77-5	163 > 88	163 > 106	5.0	0.10	0.10	0.8	0.9996
Methoxyfenozide	161050-58-4	369 > 149	369 > 313	12.7	0.50	1.00	1.7	0.9980
Metobromuron	3060-89-7	259 > 148	259 > 91	10.9	0.35	0.63	3.2	0.9987
Metolachlor	51218-45-2	284 > 252	284 > 176	13.4	0.06	0.31	1.5	0.9962
Metolcarb	1129-41-5	166 > 109	166 > 94	9.1	0.12	0.29	2.4	0.9996
Metosulam	139528-85-1	418 > 175	418 > 140	10.1	0.24	0.23	2.2	0.9968

Metoxuron	19937-59-8	229 > 72	229 > 156	8.7	0.04	0.30	1.4	0.9997
Metrafenone	220899-03-6	409 > 209	409 > 227	14.4	0.09	0.10	1.3	0.9993
Metsulfuron-methyl	74223-64-6	382 > 167	382 > 77	9.2	0.19	0.97	1.2	0.9982
Mevinphos	7786-34-7	225 > 127	225 > 193	7.1	0.05	0.16	2.5	0.9998
Molinate	2212-67-1	188 > 126	188 > 55	12.9	2.08	1.25	3.1	0.9956
Monocrotophos	6923-22-4	224 > 193	224 > 127	5.6	0.72	1.35	4.8	0.9991
Monuron	150-68-5	199 > 72	199 > 46	9.4	0.13	0.21	1.6	0.9995
Myclobutanil	88671-89-0	289 > 70	289 > 125	12.8	0.23	0.44	2.6	0.9990
Neoquassin	76-77-7	391 > 373	391 > 207	10.2	0.29	1.63	2.3	0.9970
Nitenpyram	120738-89-8	271 > 126	271 > 225	4.7	0.15	0.29	2.6	1.0000
Nuarimol	63284-71-9	315 > 252	315 > 81	12.2	0.75	2.66	2.8	0.9990
Omethoate	1113-02-6	214 > 125	214 > 183	3.6	0.16	0.18	1.6	0.9998
Oxadixyl	77732-09-3	296 > 279	296 > 219	9.0	0.25	0.26	1.7	0.9999
Oxamyl	23135-22-0	237 > 72	237 > 90	4.6	0.03	0.10	1.5	0.9999
Paclobutrazol	76738-62-0	294 > 70	294 > 125	12.6	0.18	2.74	2.4	0.9982
Penconazole	66246-88-6	284 > 70	284 > 159	13.9	0.17	0.20	2.6	0.9992
Pencycuron	66063-05-6	329 > 125	329 > 218	14.4	0.03	0.39	1.5	0.9992
Phenmedipham	13684-63-4	318 > 168	318 > 136	11.8	0.36	0.32	1.0	0.9949
Phenthoate	2597-03-7	321 > 79	321 > 247	13.7	0.32	0.55	2.3	0.9993
Phorate sulfone	2588-04-7	293 > 171	293 > 97	11.0	0.51	0.26	3.4	0.9964
Phorate sulfoxide	2588-05-8	277 > 97	277 > 199	10.8	0.26	0.13	0.9	0.9979
Phosphamidon	297-99-4	300 > 174	300 > 127	9.3	0.10	0.19	1.0	0.9998
Phoxim	14816-18-3	299 > 77	299 > 129	14.1	0.25	0.30	2.0	0.9992
Picolinafen	137641-05-5	377 > 238	377 > 145	15.2	0.26	1.38	5.4	0.9999
Picoxystrobin	117428-22-5	368 > 145	368 > 205	13.5	0.12	0.17	1.3	0.9994
Pirimicarb	23103-98-2	239 > 72	239 > 182	10.8	0.05	0.10	2.1	0.9996
Pirimicarb-desmethyl	152-16-9	225 > 72	225 > 168	8.5	0.04	0.04	1.7	0.9996
Prochloraz	67747-09-5	376 > 308	376 > 70	14.3	0.10	0.19	2.8	0.9987
Profenofos	41198-08-7	375 > 305	375 > 347	15.0	0.30	0.38	2.6	0.9997
Promecarb	2631-37-0	208 > 109	208 > 151	12.5	0.44	0.42	3.1	0.9993
Prometryn	7287-19-6	242 > 158	242 > 200	13.1	0.07	0.08	1.6	0.9998

Propamocarb free base	24579-73-5	189 > 102	189 > 74	3.1	0.23	0.22	1.4	0.9984
Propaquizafop	111479-05-1	444 > 100	44 > 371	15.2	0.15	0.85	1.2	0.9990
Propiconazole	60207-90-1	342 > 159	342 > 69	14.0	0.23	0.60	3.6	0.9998
Propoxur	114-26-1	210 > 111	210 > 168	9.7	0.07	0.08	2.6	0.9998
Propyzamide	23950-58-5	256 > 190	258 > 192	12.7	1.83	1.94	6.0	0.9915
Prosulfuron	94125-34-5	420 > 141	420 > 167	11.7	0.43	0.82	2.0	0.9940
Prothioconazole	178928-70-6	312 > 70	314 > 70	13.4	0.16	0.50	2.3	0.9952
Pymetrozine	123312-89-0	218 > 105	218 > 79	5.0	0.05	0.39	2.9	0.9994
Pyraclostrobin	175013-18-0	388 > 194	388 > 163	14.2	0.50	1.00	1.9	0.9996
Pyrethrin I	121-21-1	329 > 161	329 > 105	15.9	0.25	1.20	2.3	0.9998
Pyrethrin II	121-29-9	373 > 161	373 > 133	14.6	0.70	2.27	4.2	0.9992
Pyrimethanil	53112-28-0	200 > 107	200 > 82	12.3	0.10	0.50	0.9	0.9999
Pyriproxyfen	95737-68-1	322 > 96	322 > 185	15.5	0.07	0.10	0.6	0.9999
Quassia	76-78-8	389 > 223	389 > 163	9.1	0.57	0.80	2.7	0.9968
Quinmerac	90717-03-6	222 > 204	222 > 141	6.8	0.09	0.45	1.8	0.9966
Quinoxyfen	124495-18-7	308 > 197	308 > 162	15.6	0.18	0.23	3.2	0.9998
Rimsulfuron	122931-48-0	432 > 182	432 > 325	10.0	0.31	0.64	2.8	0.9989
Rotenone	83-79-4	395 > 213	395 > 192	13.5	0.44	0.52	3.5	0.9976
Spinosyn A	131929-60-7	733 > 142	733 > 98	14.1	0.03	0.19	1.6	0.9997
Spinosyn D	131929-63-0	747 > 142	747 > 98	14.6	0.20	0.97	3.3	1.0000
Spiromesifen	283594-90-1	388 > 273	388 > 371	15.6	0.05	0.34	2.3	0.9998
Spiroxamine	118134-30-8	298 > 144	298 > 100	11.7	0.08	0.18	2.1	0.9999
Sulcotrione	99105-77-8	329 > 139	329 > 69	7.5	0.70	5.00	4.3	0.9969
Tebuconazole	107534-96-3	308 > 70	310 > 70	13.9	0.10	0.34	2.1	0.9993
Tebufenozide	112410-23-8	353 > 133	353 > 297	13.5	0.04	0.10	1.5	0.9980
Tebufenpyrad	119168-77-3	334 > 117	334 > 147	15.2	0.30	0.28	0.9	0.9998
Teflubenzuron*	83121-18-0	379 > 339	379 > 359	15.3	0.29	0.40	3.6	0.9973
Terbufos sulfone	56070-16-7	321 > 97	321 > 171	12.1	0.55	0.52	3.8	0.9956
Terbufos sulfoxide	10548-10-4	305 > 187	305 > 97	12.1	0.09	0.09	1.3	0.9989
Tetraconazole	112281-77-3	372 > 159	372 > 70	13.2	0.29	0.55	2.6	0.9950

Thiabendazole	148-79-8	202 > 175	202 > 131	8.2	2.50	2.50	1.5	0.9987
Thiacloprid	111988-49-9	253 > 126	253 > 90	7.9	0.10	0.50	1.0	0.9991
Thiamethoxam	153719-23-4	292 > 211	292 > 181	5.3	0.04	0.08	2.4	0.9995
Thiodicarb	59669-26-0	355 > 88	355 > 108	10.6	0.08	0.18	1.1	0.9991
Thiophanate-methyl	23564-05-8	343 > 151	343 > 311	9.7	0.25	0.62	1.1	0.9967
Tolfenpyrad	129558-76-5	384 > 197	384 > 91	15.3	0.28	0.73	3.0	0.9983
Triadimefon	43121-43-3	294 > 69	294 > 197	12.8	0.24	0.31	2.6	0.9985
Triadimenol	55219-65-3	296 > 70	298 > 70	13.1	0.24	0.54	3.7	0.9982
Triasulfuron	82097-50-5	402 > 141	402 > 167	9.6	0.42	0.36	1.5	0.9993
Triazamate acid*	112143-82-5	287 > 198	287 > 170	10.1	0.09	0.26	4.4	0.9996
Triazophos	24017-47-8	314 > 162	314 > 119	12.9	0.02	0.12	1.5	0.9992
Triclopyr*	55336-06-3	256 > 198	254 > 196	11.1	1.95	1.81	8.9	0.9969
Tricyclazole	41814-78-2	190 > 136	190 > 163	8.3	0.10	0.20	2.3	0.9993
Trifloxystrobin	141517-21-7	409 > 186	409 > 145	14.6	0.02	0.05	1.2	0.9994
Triflumizole	68694-11-1	346 > 278	346 > 43	14.8	0.09	0.09	1.3	0.9996
Triflumuron*	64628-44-0	357 > 154	357 > 176	14.2	1.76	3.12	4.6	0.9991
Triforine	26644-46-2	435 > 390	437 > 392	11.7	0.92	3.53	4.8	0.9963
Triticonazole	131983-72-7	318 > 70	320 > 70	13.2	0.40	0.41	1.9	0.9993
Zoxamide	156052-68-5	336 > 187	336 > 159	14.0	0.09	0.29	1.3	0.9951
2,4-D*	94-75-7	219 > 161	219 > 125	10.3	1.09	5.00	9.7	0.9980

*Negative electrospray ionization

In order to assess the performance of the LCMS-8040 for real samples, limits of detection, linearity and repeatability were determined in food extracts. Linearity was assessed from 0.5 – 200 ppb in four types of sample: (1) acetonitrile, (2) dried fruit extract, (3) lettuce extract and, (4) pear extract. All 210 pesticides achieved excellent correlation coefficients greater than 0.99 in all four types of matrix with typical values greater than 0.997. Correlation coefficients are listed in Table 1 for all pesticides in pear extract.

Pesticide limits of detection were calculated based on the method described by the US-EPA (see experimental section). Limits of detection were assessed for both the quantifying transition and the qualifying transition and

are listed in Table 1. All of the studied pesticides presented LODs less than the 0.01 mg kg-1 reporting level for both transition 1 and 2.

A limit of detection less than 0.001 mg kg-1 (1ppb) was achieved for the quantifying transition and less than 0.002 mg kg-1 (2 ppb) for the qualifying transition for 90 % of compounds: thereby highlighting the excellent sensitivity of the LCMS-8040 for pesticide analysis. Furthermore, these limits of detection were achieved with an injection volume of only 2 µL. Therefore, detection limits could be reduced even further with larger injection volumes. An injection volume of 2 µL was used in the study to allow the injection of 100 % acetonitrile extracts without detriment to early eluting peak shapes.

Repeatability was assessed at the 0.01 mg kg-1 reporting level as peak area % RSD for six replicate injections in pear extracts. Repeatability less than 5 % RSD was achieved for 92 % of the 210 pesticides studied. All of the studied compounds presented repeatability less than 10 % RSD, with exception of haloxyfop acid (13.4 %).

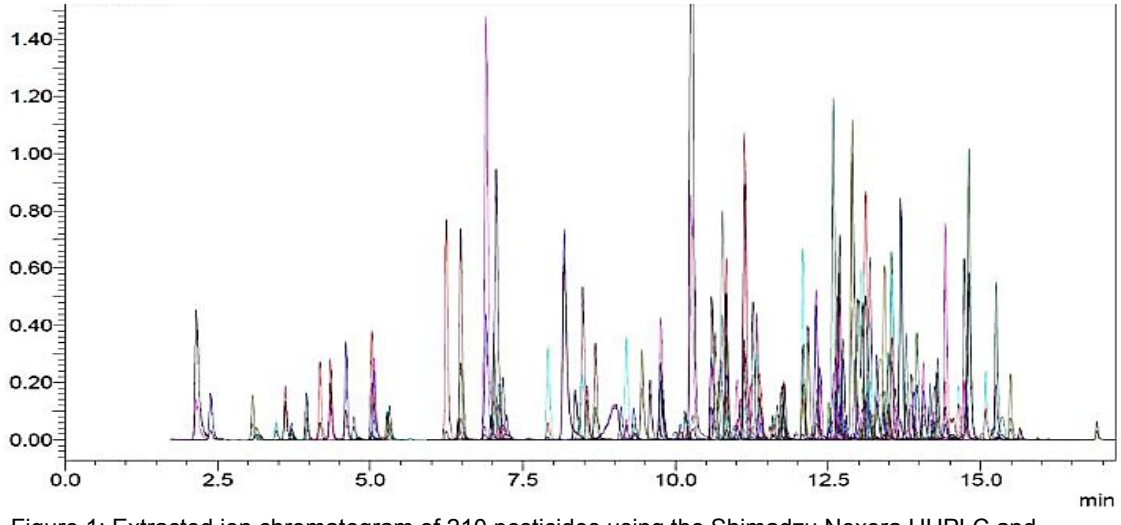


Figure 1: Extracted ion chromatogram of 210 pesticides using the Shimadzu Nexera UHPLC and the Shimadzu LCMS-8040; 2 µL injection of a 0.05 mg/kgstandard solution.

CONCLUSION

The results of the developed methodology show that the Shimadzu LCMS-8040 triple quadrupole can achieve excellent sensitivity, linearity and repeatability in food extracts for over 200 commonly analysed pesticides. Limits of detection were less than 0.01 mg kg-1 (10 ppb) for both the quantifying and qualifying transitions for all compounds studied, while for 90% of compounds was less than 0.001 mg kg-1 (1ppb) (quantifying transition) and 0.002 mg kg-1 (2 ppb) (qualifying transition); thereby providing excellent response, especially given that the injection volume was only 2 µL. The sensitivity of the LCMS-8040 was able to meet the 0.01 mg kg-1 (10 ppb) requirements of regulatory guidelines such as those established by the EU and Japan. Repeatability at the 0.01 mg kg-1 reporting level was less than 5 % for nearly all compounds and correlation coefficients greater than 0.99 for all compounds in a variety of food samples. Consequently, the LCMS-8040 is ideally suited for routine monitoring of pesticides in regulatory laboratories.

C-64 PESTICIDE RESIDUE ANALYSIS FROM PET FOOD SAMPLE USING GC/MS/MS

INTRODUCTION

The Codex Committee on Pesticide Residues (CCPR) gives recommendations to the Codex Alimentarius Commission regarding maximum limits for pesticide residues for specific food items.
A Codex Maximum Limit for Pesticide Residues (MRL) is the maximum concentration of pesticide residue (expressed as mg/kg), recommended by the Codex Alimentarius Commission that is legally permissible in food commodities and animal feeds. Foods derived from commodities that comply with MRLs are expected to be toxicologically acceptable and safe for consumption by humans. Codex MRLs which are applied in international trade are derived from evaluations conducted by the Joint Meeting on Pesticide Residues (JMPR).
A GC/MS/MS method has been developed here for analysis of 15 pesticide residues from pet food sample.

EXPERIMENTAL

Instrument parameters

GCMSMS : Shimadzu GCMS-TQ8030 with AOC-20i+s
GC conditions

Column Details	: Rxi-5 Sil MS (30m X 0.25mm ID X 0.25um)
Injector Port Temperature	: 280 °C
Injection Mode	: Splitless
Split Ratio	: 5.0
Injection Volume	: 2.0 µl
Flow Control Mode	: Linear Velocity
Column Flow	: 1.20 mL/min
Linear Velocity	: 40.2 cm/sec

Detector : Mass spectrometer
Carrier Gas : Helium

MS conditions
Interface Temperature : 280 °C
Ion Source Temperature : 230 °C
Detector gain : 1.50 kV
Ionization mode : EI
Mode : MRM

MRM parameters : MRM transitions & Collision energies for 14 were taken from 'Shimadzu Pesticide Database Ver.-1.03'. For 'Ethoxyquin', the MRM and CE was optimized. The same have been tabulated below.

No.	Name	RT	Target MRM	CE	Ref MRM	CE
1	Methamidophos	5.956	141.0>95.0	8	141.0>126.0	4
2	alpha-HCH	12.530	218.9>182.9	8	218.9>144.9	20
3	Ethoxyquin	13.284	202.15>174.10	16	202.15>145.10	21
4	beta-HCH	13.691	218.9>182.10	9	218.9>144.10	21
5	gamma-HCH (Lindane)	13.962	218.9>182.9	8	218.9>144.9	20
6	delta-HCH	15.342	218.9>182.9	10	218.9>144.9	20
7	Chlorpyrifos-methyl	16.828	285.9>93.0	22	285.9>270.9	14
8	Heptachlor	17.416	271.8>236.9	20	271.8>117.0	32
9	Pirimiphos-methyl	18.467	305.1>180.1	8	305.1>290.1	12
10	Malathion	19.092	173.1>99.0	14	173.1>127.0	6
11	Aldrin	19.235	262.9>193.0	28	262.9>203.0	26
12	Heptachlor-epoxide	21.383	352.8>262.9	14	352.8>281.9	12
13	Dieldrin	24.416	276.9>241.0	8	276.9>170.0	38
14	Endrin	25.203	262.9>191.0	30	262.9>193.0	28
15	o,p'-DDT	27.226	235.0>165.0	24	235.0>199.0	16

Sample preparation

2 gm of powdered sample (pet food) was given QuEChERS treatment to obtain clean extract in acetonitrile. The extract was evaporated & reconstituted in ethyl acetate. This sample extract was used to prepare matrix match calibration standards.

Standard preparation

Sample extract obtained was used as a diluent to prepare matrix match calibration standards for linearity. The linearity was plotted for 0.00, 0.05, 0.25, 0.50, 1.00, 2.50, 5.00 & 10.00 ppb levels. Sample extract was used as a 0.00 ppb calibration level to plot standard addition calibration curve.

RESULTS AND DISCUSSION

Representative chromatogram at 5 ppb level and the quantitative summary of matrix match calibration has been shown in Figure 1 and Table 1 respectively. The pet food sample was analysed in triplicates and plotted on the matrix matched calibration curve. The result of pet food sample analysis has been shown in table 2.

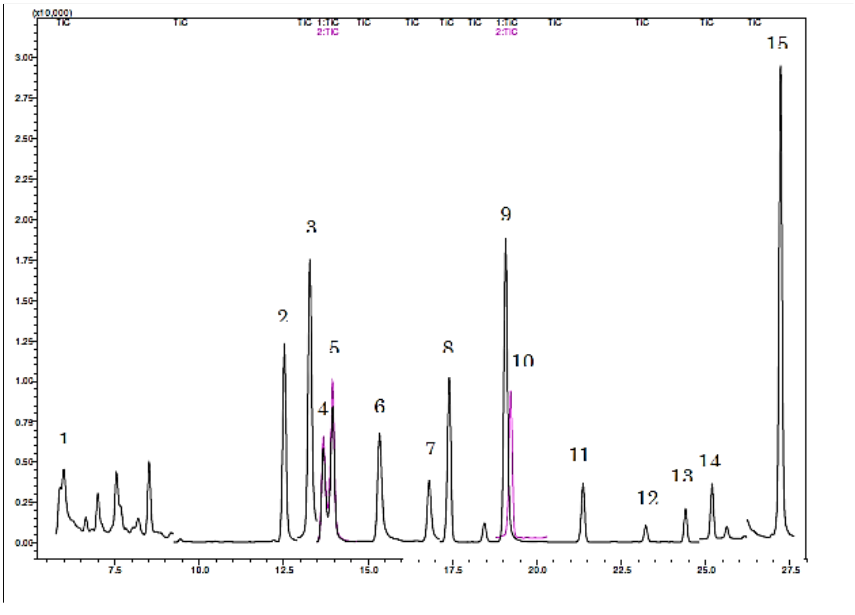


Figure 1. The Total Ion Chromatogram (TIC) for 5 ppb calibration level

Table 1. The Quantitative summary data using matrix match calibration

No.	Name	LOD (ppb)	LOQ (ppb)	*RSD at LOQ (n=6)	Avg SN at LOQ	Avg Area at LOQ	**R^2
1	Methamidophos	0.076	0.25	5.47	15.67	1209	0.9875
2	alpha-HCH	0.015	0.05	15.29	25.30	580	0.99985
3	Ethoxyquin	0.076	0.25	6.53	1069.40	8251	0.985
4	beta-HCH	0.076	0.25	10.27	81.91	638	0.99931
5	gamma-HCH (Lindane)	0.015	0.05	16.48	33.63	321	0.99948
6	delta-HCH	0.076	0.25	12.57	32.07	1181	0.99977
7	Chlorpyrifos-methyl	0.076	0.25	8.17	27.32	515	0.99986
8	Heptachlor	0.015	0.05	15.01	38.40	701	0.99990
9	Pirimiphos-methyl	0.152	0.5	8.49	19.63	328	0.99859
10	Malathion	0.015	0.05	7.97	20.17	697	0.99979
11	Aldrin	0.076	0.25	13.66	16.34	609	0.99957
12	Heptachlor-epoxide	0.076	0.25	13.54	28.85	559	0.99977
13	Dieldrin	0.076	0.25	16.72	11.68	272	0.99975
14	Endrin	0.152	0.5	8.81	27.19	2122	0.99902
15	o,p'-DDT	0.076	0.25	8.61	223.16	7629	0.99968

Table 2. The results of pet food analysis

No.	Name	LOD (ppb)	LOQ (ppb)	*Conc. in Sample-1 (in ppb)				Remarks
				Sample-1_01	Sample-1_02	Sample-1_03	Avg	
1	Methamidophos	0.015	0.05	ND	ND	ND	ND	ND
2	alpha-HCH	0.015	0.05	ND	ND	ND	ND	ND
3	Ethoxyquin	0.076	0.25	ND	ND	ND	ND	ND
4	beta-HCH	0.076	0.25	ND	ND	ND	ND	ND
5	gamma-HCH (Lindane)	0.015	0.05	ND	ND	ND	ND	ND
6	delta-HCH	0.076	0.25	ND	ND	ND	ND	ND
7	Chlorpyrifos-methyl	0.076	0.25	ND	ND	ND	ND	ND
8	Heptachlor	0.015	0.05	ND	ND	ND	ND	ND
9	Pirimiphos-methyl	0.152	0.5	ND	ND	ND	ND	ND
10	Malathion	0.015	0.05	ND	ND	ND	ND	ND
11	Aldrin	0.076	0.25	ND	ND	ND	ND	ND
12	Heptachlor-epoxide	0.076	0.25	ND	ND	ND	ND	ND
13	Dieldrin	0.076	0.25	ND	ND	ND	ND	ND
14	**Endrin**	**0.152**	**0.5**	**0.330**	**0.330**	**0.330**	**0.330**	**Below quantitation limit**
15	**o,p'-DDT**	**0.076**	**0.25**	**0.045**	**0.045**	**0.045**	**0.045**	**ND**

RESULTS AND DISCUSSION

- Peak shape of 'Methamidophos' and 'Ethoxyquin' shows high matrix interference at such low concentration level due to which the R^2 values are not as good as other pesticides.
- In 'Sample-1', out of 15 pesticides analyzed, 14 were found below detection limit, hence, 'Not Detected (ND)'. Only 'Endrin' was found to be present in between detection and quantitation limit.

CONCLUSION

Shimadzu's GCMS-TQ8030 equipped with Ultra fast scanning speed and UFsweeper™ technology enables high sensitivity and high specificity analysis. These features of GCMS-TQ8030 enabled it to detect and quantify pesticide residues at very low concentration levels in presence of complex matrix suchas pet food.

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C-65 PESTICIDE RESIDUE ANALYSIS FOR 25 PESTICIDES FROM CUCUMBER USING GCMS-TQ8040

INTRODUCTION

Peasticides are widely used for agricultural purposes and generally prove low threat to human since they get destroyed during the process of cooking. Cucumbers, however, are generally consumed raw and hence, are at high risk of introducing the pesticides into human body. Hence, considering the severe health hazards they cause, it is important to monitor the pesticide levels in raw foodstuffs. Here, QueChers method has been used for extraction of pesticides which have then been analysed using GCMS-TQ8040, triple quadrupole GCMS system by Shimadzu corporation.

EXPERIMENTAL

Instrument parameters

System	: Shimadzu GCMS-TQ8040 with AOC-20i+s
GC conditions	
Column Details	: Rxi-5 Sil MS (30m X 0.25mm ID X 0.25um)
Injector Port Temperature	: 280 °C
Injection Mode	: Splitless
Split Ratio	: 5.0
Injection Volume	: 2.0 µL
Flow Control Mode	: Linear velocity
Column Flow	: 1.69 mL/min
Linear Velocity	: 47.2 cm/sec
Detector	: Mass spectrometer
Carrier Gas	: Helium

MS conditions

Interface Temperature	: 280 °C
Ion Source Temperature	: 200 °C
Detector gain	: 1.50 kV
Ionization mode	: EI
Mode	: MRM

Standard preparation

500 ppb solution of 25 standard pesticide mixture was used to prepare a calibration range of 1, 5, 10 and 25 ppb by using ethyl acetate as a diluent.

Sample preparation

The cucumber samples blank and spiked were extracted using Quechers extraction method. Extracted samples were quantified against the calibration curve plotted by using solvent standards.

Known spiked (25 ppb) sample preparation:

5 g of unspiked cucumber sample was taken and spiked with 250 µL of 500 ppb standard pesticide mixture to obtain 25 ppb spiked sample. This was further extracted using Quechers extraction method and analyzed against calibration standards.

RESULTS

For most of the pesticides, 6 MRM transitions & collision energies were taken from 'Shimadzu Pesticide Database Ver.-1.03'. Based on the interference of the sample matrix, out of these 6 MRM tranitions, one was used as a Target MRM (refer Table 1)

All the calibration standards and samples viz. (i) Unspiked cucumber, (ii) Spiked cucumber analyzed in duplicate and (iii) Known spiked cucumber were analyzed by using MRM method created using SmartMRM feature of GCMS solutions 4.20.

Table 1. Results of pesticide residue content in cucumber with MRM transitions of the analytes

				Conc. (in ppb)			
				A	B		C
ID	Pesticide Name	Ret. Time (min)	Target MRM	Unspiked	Unknown spiked-01	Unknown spiked-02	25 ppb Spiked
1	Monocrotophos	10.468	127.10>95.00	ND	2.3	2.4	30
2	alpha-HCH	10.765	218.90>182.90	ND	2.4	2.0	19
3	Atrazine	11.24	215.10>58.00	ND	2.0	1.8	28
4	beta-HCH	11.256	218.90>182.90	ND	2.7	2.6	18
5	gamma-HCH (Lindane)	11.445	218.90>182.90	ND	2.3	2.3	17
6	Paraoxon methyl	11.862	230.05>200.05	0.65	0.9	1.0	32
7	delta-HCH	11.983	218.90>182.90	ND	2.0	2.0	18
8	Malaoxon	12.651	194.95>124.90	6.14	8.4	7.9	43
9	Malathion	12.653	173.10>99.00	ND	2.6	3.1	49
10	Parathion-methyl	12.715	263.00>109.00	ND	1.5	1.2	25
11	Alachlor	12.756	188.10>160.10	ND	2.4	2.5	30
12	Butachlor	12.822	188.10>160.10	ND	2.8	2.9	33
14	o,p'-DDE	12.873	246.00>176.00	ND	2.3	2.5	22
13	Aldrin	13.258	262.90>193.00	ND	2.5	2.4	23
15	Chlorpyrifos	13.238	313.90>257.90	1.40	1.9	1.7	30
16	alpha-Endosulfan	15.089	194.90>160.00	ND	3.4	3.7	23
17	p,p'-DDE	15.488	246.00>176.00	ND	2.7	2.5	24
18	Dieldrin	15.606	276.90>241.00	ND	3.6	3.7	25
19	o,p'-DDD	15.614	235.00>165.00	ND	2.4	2.3	26
20	beta-Endosulfan	16.194	194.90>160.00	ND	2.5	2.6	27
21	Ethion	16.257	230.90>174.90	ND	1.2	1.7	47
22	o,p'-DDT	16.275	235.00>165.00	ND	2.2	2.1	33
23	p,p'-DDD	16.33	235.00>165.00	ND	3.2	2.8	29
24	Endosulfan sulfate	16.91	386.80>288.80	ND	2.2	2.1	31
25	p,p'-DDT	16.984	235.00>165.00	ND	2.9	2.5	35

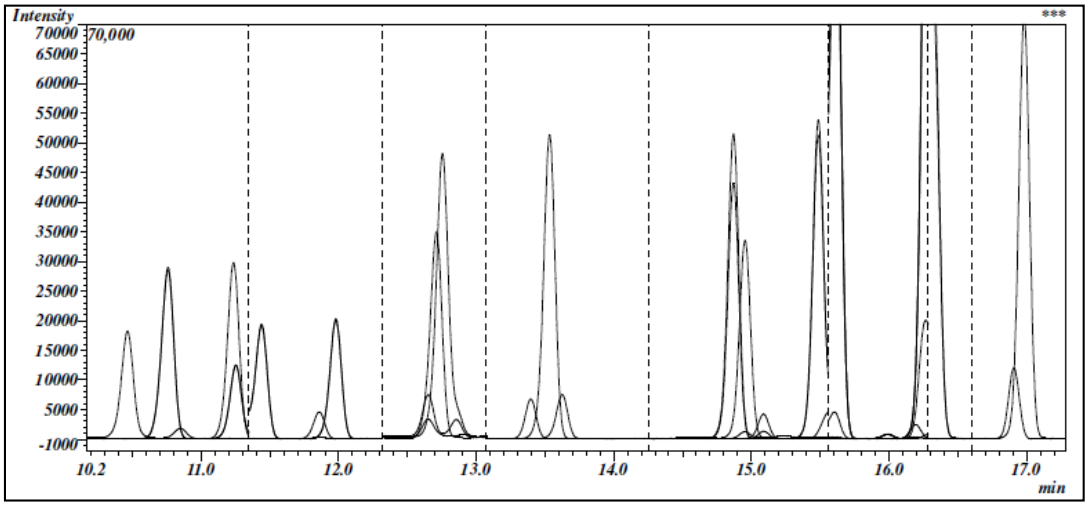

Figure 1. Chromatogram of cucumber extract spiked to obtain 25 ppb resultant pesticide concentration

RESULTS AND DISCUSSION

Recovery studies were carried out and were found to fall within the range of 70 – 130 %. This proves that the spiking procedure and extraction procedure followed at our end was correct.

CONCLUSION

Shimadzu's GCMS-TQ8040 along with SmartMRM Pesticide database enabled automated
setting up of MRM method for analysis. The extraction and determination of the pesticide content in the given cucumber sample was performed and efficiency of extraction was confirmed with known concentration spike-recovery studies.

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C-66 QUANTITATIVE ANALYSIS OF METHYL PARATHION AND PROFENOPHOS IN CARDAMOM MATRIX USING GCMS-TQ8030

INTRODUCTION

Cardamom is amongst the most highly contaminated produce in the State of Kerala which is known as the Spice capital of India. Every farmer uses at least four pesticides for the crop. The levels of pesticide residues in cardamom had been going up in recent years and in some samples tested by KAU, a cocktail of residues of 12 pesticides had been found. Quinalphos, belonging to the organophosphates group, was the biggest contaminant in cardamom, followed by profenophos, methyl parathion and triazophos.
In this note, methyl parathion and profenophos have been analysed by GC/MS/MS from cardamom matrix using standard addition method.

EXPERIMENTAL

Instrument parameters

System	: GCMS-TQ8030
GC conditions	
Column	: Rxi-5 Sil MS (30 m x 0.25 mm I.D. x 0.25 µm
Carrier gas	: Helium
Injector Temp	: 250 °C
Injection mode	: Splitless
Sampling time	: 2.00 min
Split ratio	: 5
Column flow	: 1.20 mL/min
Flow control mode	: Linear velocity

Column Oven temp. program :

Rate (°C/min)	Final Temp (°C)	Hold time (min)
0.00	70.00	2.00
25.00	150.00	0.00
3.00	200.00	0.00
8.00	290.00	10.44

Injection volume : 5 µL
Detector : MS

MS conditions
Interface temp. : 280 °C
Ion soure temp. : 230 °C
Detector gain : 1.70 kV
Ionisation mode : EI
Acqiuisition mode : MRM

MRM teansition	: Methyl parathion	263.00 > 109.00; CE = 14
	Profenophos	336.90 > 266.90; CE = 14

Standard preparation

The Cardamom extract was used as a diluent to prepare Methyl parathion and Profenophos matrix matched standards at concentration levels of 0.00 ppb (SPL Extract Blank), 0.25 ppb, 0.5 ppb, 1.0 ppb, 2.5 ppb and 5.0 ppb. Sample matrix showed the presence of target compounds, hence 'Standard Addition method' of calibration was used for analysis.

RESULTS AND DISCUSSION

The results of quantitative analysis for methyl parathion and profenophos has been tabulated in Table 1. Chromatograms and calibration graphs of methyl parathion and profenophos have been shown in Figures 1 & 2.

Table 1. Quantitative results of methyl parathion and profenophos

No.	Compound Name	MRM Transition	Retention Time (min)	Calibration Range	Correlation coefficient (r2)	S/N at LOQ Level	Conc. at LOQ Level (ppb)
1.	Methyl Parathion	263.00>109.00	17.322	0 ppb – 5ppb	0.9994	23.75	1.01
2.	Profenophos	336.90>266.90	24.439	0 ppb – 5ppb	0.9999	135.90	0.52

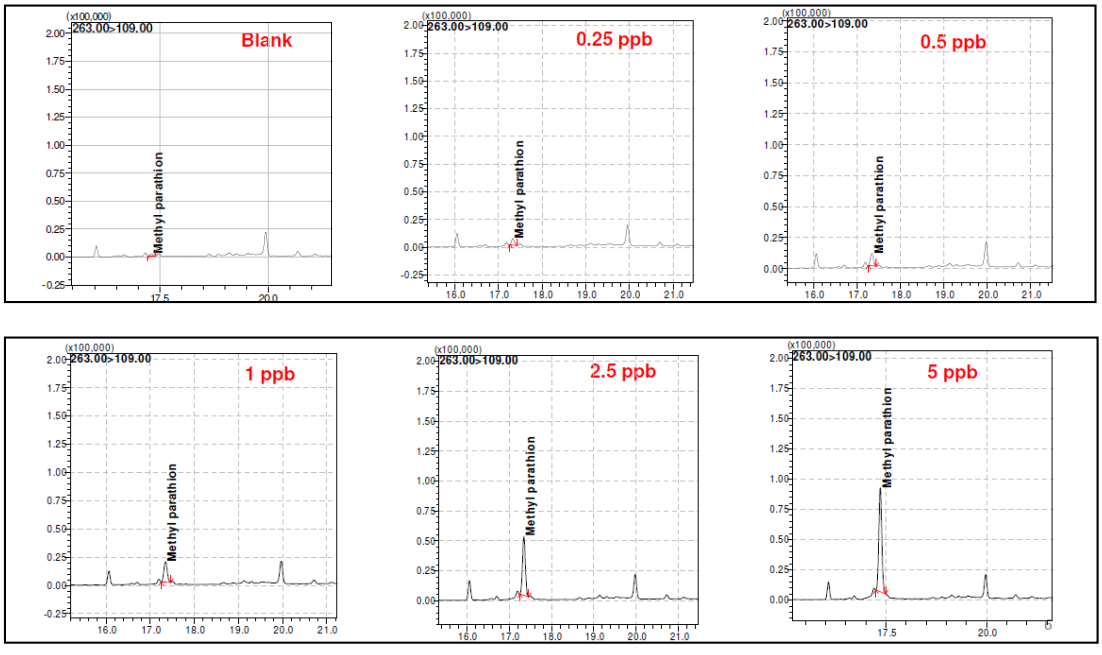
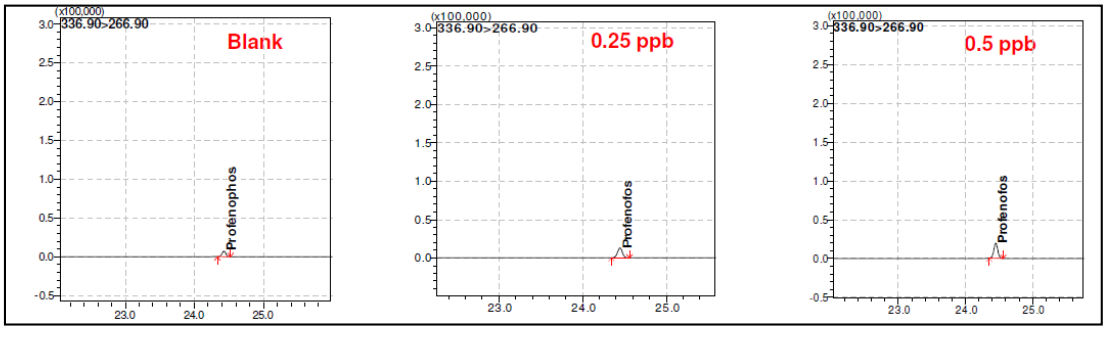


Figure 1a. Matrix match standard chromatograms of methyl parathion



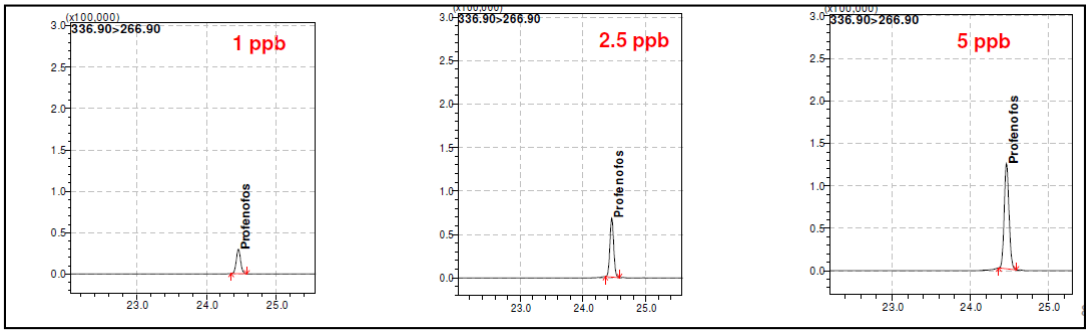
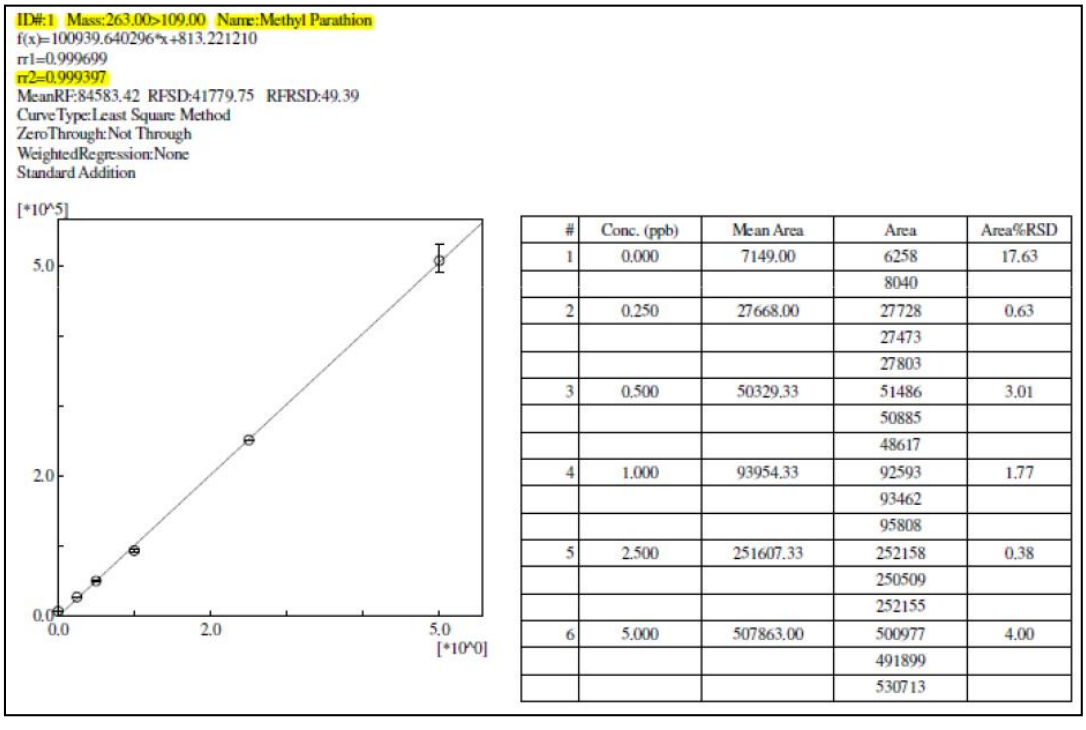
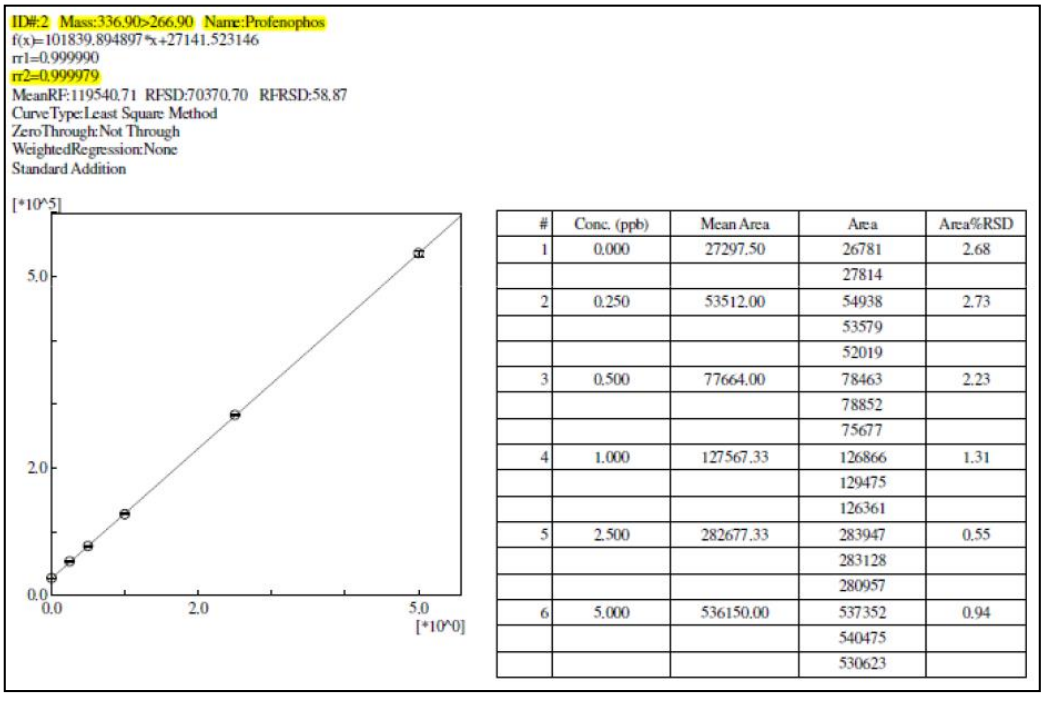


Figure 1b. Matrix match standard chromatograms of profenophos



#	Conc. (ppb)	Mean Area	Area	Area%RSD
1	0.000	7149.00	6258	17.63
			8040	
2	0.250	27668.00	27728	0.63
			27473	
			27803	
3	0.500	50329.33	51486	3.01
			50885	
			48617	
4	1.000	93954.33	92593	1.77
			93462	
			95808	
5	2.500	251607.33	252158	0.38
			250509	
			252155	
6	5.000	507863.00	500977	4.00
			491899	
			530713	

Figure 2a. Calibration curve of methyl parathion



#	Conc. (ppb)	Mean Area	Area	Area%RSD
1	0.000	27297.50	26781	2.68
			27814	
2	0.250	53512.00	54938	2.73
			53579	
			52019	
3	0.500	77664.00	78463	2.23
			78852	
			75677	
4	1.000	127567.33	126866	1.31
			129475	
			126361	
5	2.500	282677.33	283947	0.55
			283128	
			280957	
6	5.000	536150.00	537352	0.94
			540475	
			530623	

Figure 2b. Calibration curve of profenophos

CONCLUSION

A method for analysis of methyl parathion and profenophos from cardamom matrix was successfully established with good sensitivity using GCMS-TQ8030.

Cardamom is a complex matrix and can exhibit matrix effect (either ion suppression or enhancement) during analysis. A calibration curve based on matrix matched standards can demonstrate true sensitivity of analyte in presence of matrix. Therefore, this method provides more reliable and accurate method of quantitation as compared to quantitation against neat standards.

C-67 QUANTITATIVE ANALYSIS OF METHYL PARATHION, QUINALPHOS AND PROFENOPHOS IN CHILI MATRIX USING GC/MS/MS

INTRODUCTION

Chili powder is always considered as a very complex matrix especially due to its pigments which co-elutes during extraction. In the presence of such complex matrix, use of MRM mode in triple quadrupole mass spectrometer is a method of choice to reduce matrix interference and carry out more targeted and specific quantiation of target analytes. This note explains the quantitative analysis of methyl parathion, quinalphos and profenophos from chili matrix using GC/MS/MS system.

EXPERIMENTAL

Instrument parameters

System	: GCMS-TQ8040
GC conditions	
Column	: Rxi-5 Sil MS (30 m x 0.25 mm I.D. x 0.25 µm)
Carrier gas	: Helium
Injector Temp	: 280 °C
Injection mode	: Splitless
Sampling time	: 2.00 min
Split ratio	: 5
Column flow	: 1.20 mL/min
Flow control mode	: Linear velocity

Column Oven temp. program :

Rate (°C/min)	Final Temp (°C)	Hold time (min)
0.00	70.00	2.00
25.00	150.00	0.00
3.00	200.00	0.00
8.00	280.00	10.00

Injection volume : 2 µL
Detector : MS

MS conditions
Interface temp. : 280 °C
Ion soure temp. : 230 °C
Detector gain : 1.80 kV
Ionisation mode : EI
Acqiuisition mode : MRM

MRM teansition	: Methyl parathion	263.00 > 109.00; CE = 14
	Profenophos	336.90 > 266.90; CE = 14
	Quinalphos	157.10 > 102.00; CE = 24

Preparation of Matrix matched standards

2 g sample + 8 mL d/w, soak for 30 min. Add 10 mL acetonitrile, 4 g $MgSO_4$ and 1 g NaCl and vortex for 2 min. Centrifuge at 5000 rpm for 5 min. Take 2 mL supernatant in tube containing 150 mg of $MgSO_4$, 50 mg PSA, 50 mg C18 and 7.5 mg GCB. Then vortex for 2 min and centrifuge at 5000 rpm for 5 min. Take 1 mL supernatant and dry under nitrogen flow. Reconstitute it in 1 mL ethyl acetate. Vortex and centrifuge. This supernatant was used as diluent to prepare methyl parathion, quinalphos and profenophos matrix matched standards at concentration levels of 0.5 ppb, 1.0 ppb, 2.0 ppb, 5.0 ppb, 10.0 ppb.

RESULTS AND DISCUSSION

Table 1. Results of quantitative analysis of pesticides in chili matrix

No.	Compound Name	MRM Transition	Retention Time (min)	Calibration Range	Correlation coefficient (r2)	LOQ	S/N at LOQ Level
1.	Methyl Parathion	263.00>109.00	17.598	0.5 ppb – 10ppb	0.9996	**0.5 ppb**	**153**
2.	Quinalphos	157.10>102.00	22.572	0.5 ppb – 10ppb	0.9996	**0.5 ppb**	**150**
3.	Profenophos	336.90>266.90	24.765	0.5 ppb – 5.0ppb	0.9962	**0.5 ppb**	**323**

Table 1 shows the quantitative results of methyl parathion, quinalophos and profenophos in chili matrix. The chromatograms and calibration graphs are shown below in Figures 1 & 2 respectively.

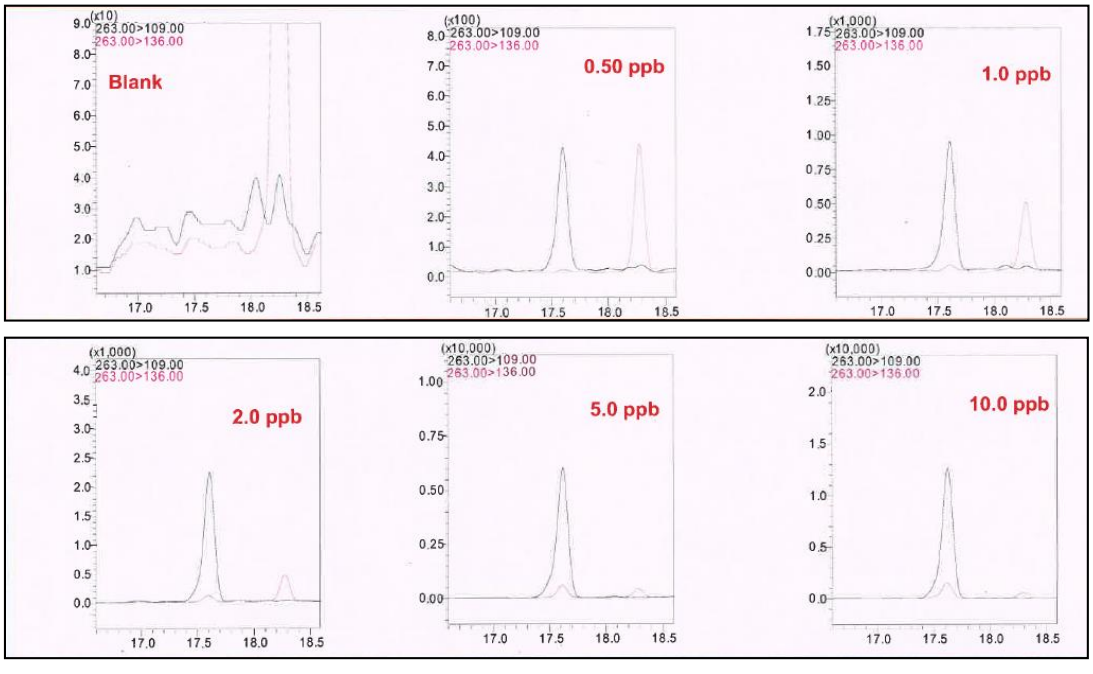


Figure 1a. Chromatograms of matrix matched standard of methyl parathion at different conc. levels

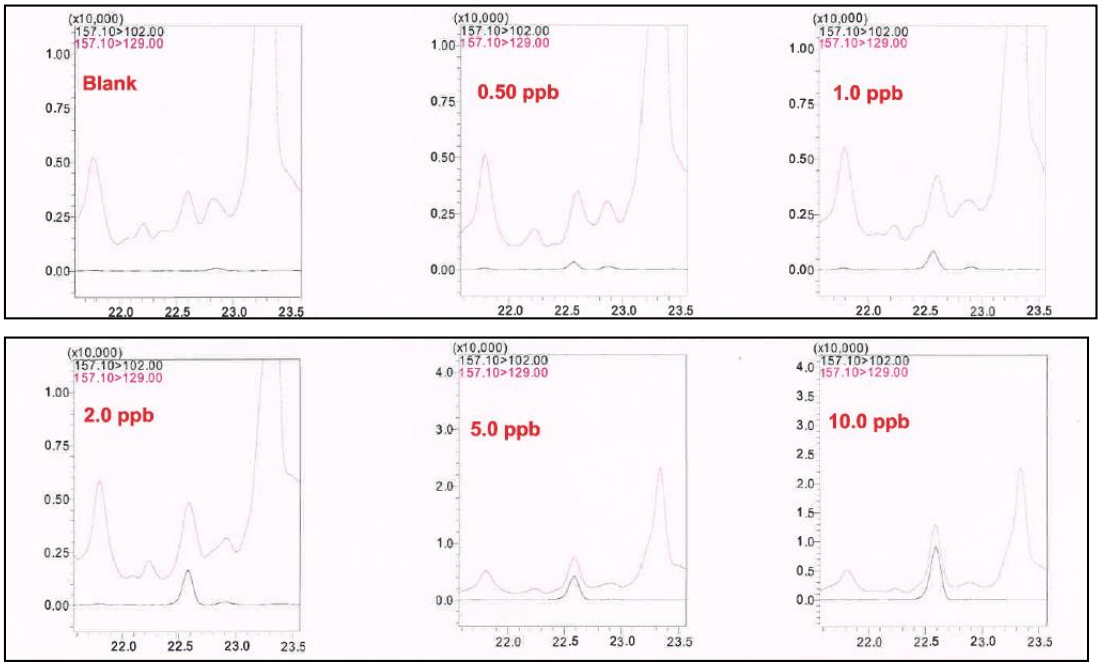


Figure 1b. Chromatograms of matrix matched standard of quinalphos at different conc. levels

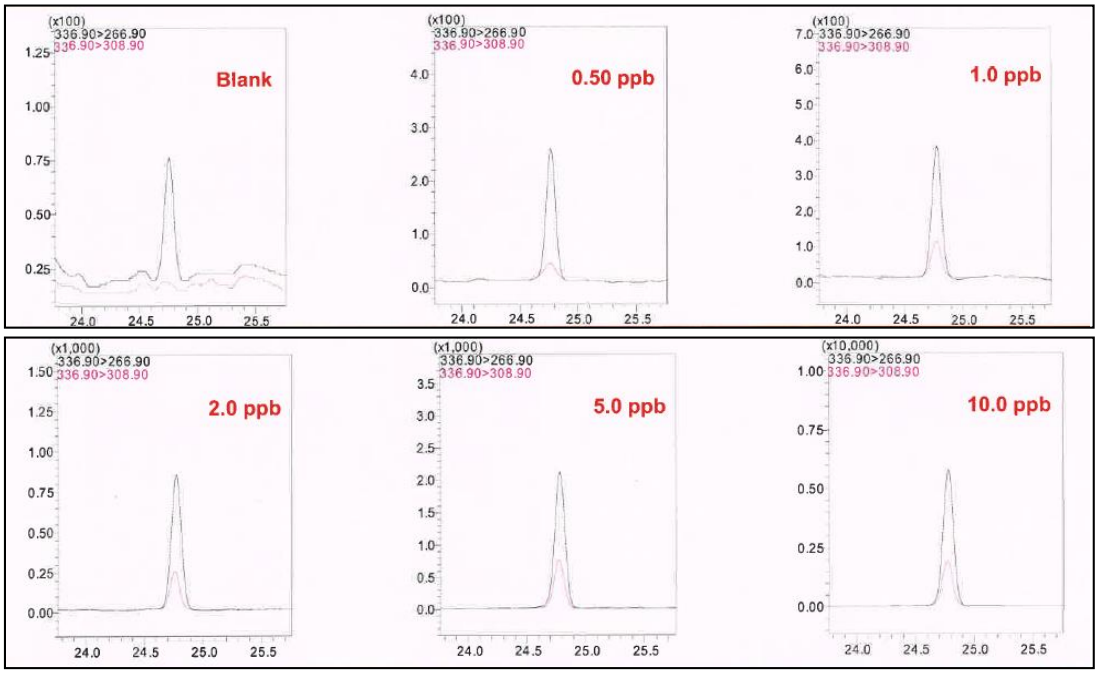
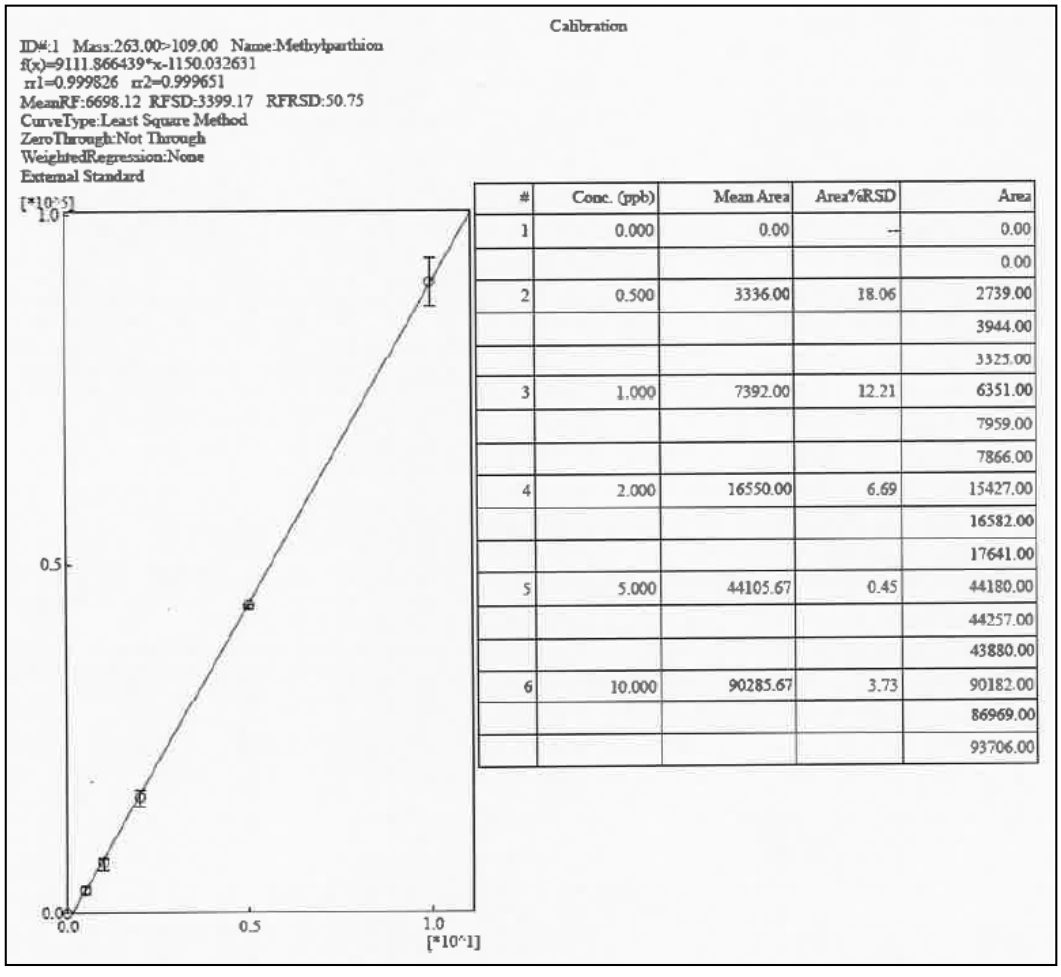
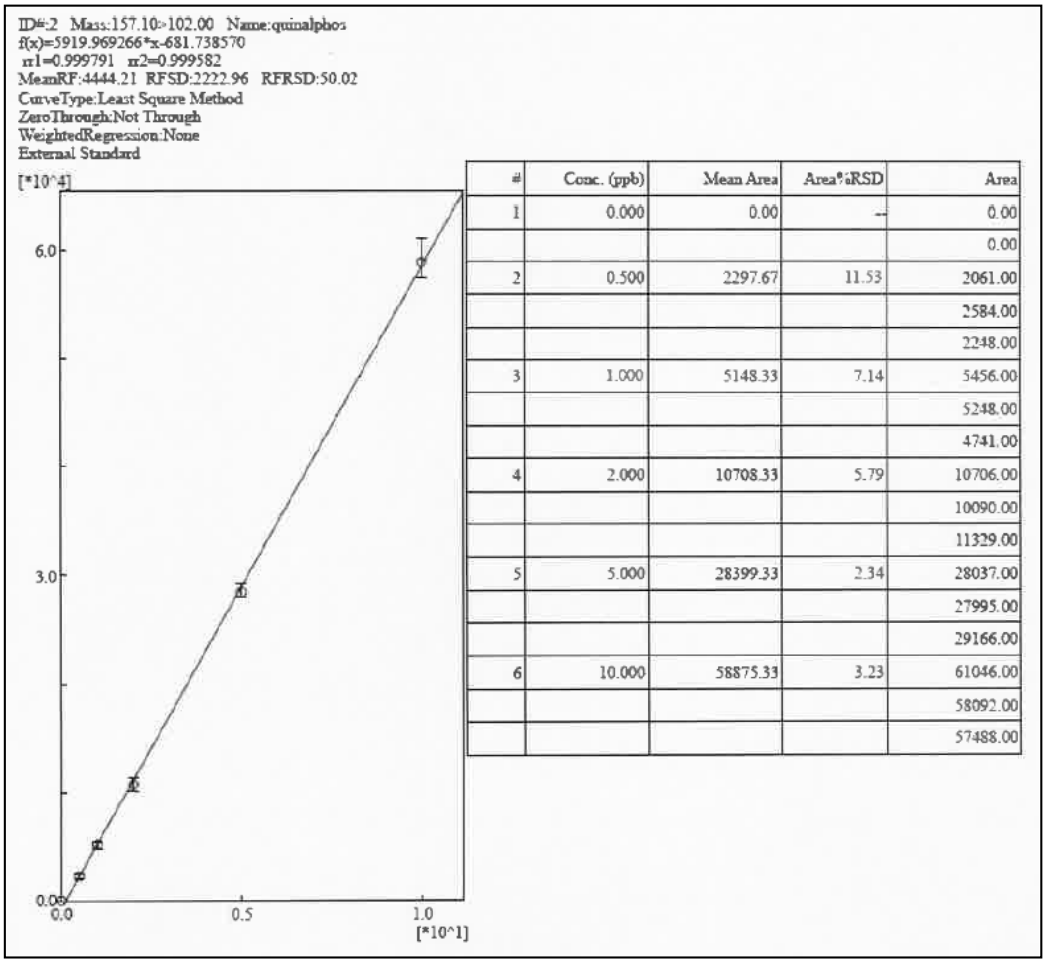


Figure 1c. Chromatograms of matrix matched standard of profenophos at different conc. levels



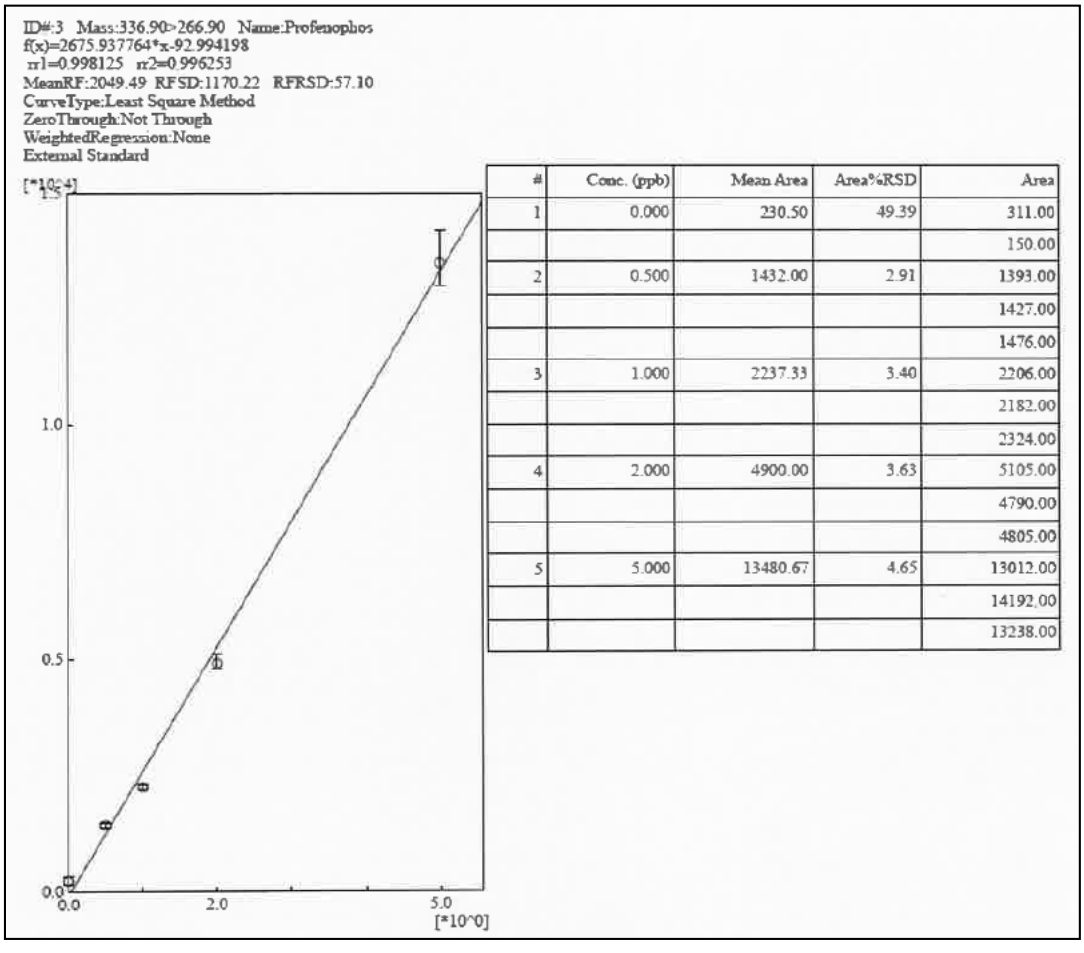
#	Conc. (ppb)	Mean Area	Area%RSD	Area
1	0.000	0.00	--	0.00
				0.00
2	0.500	3336.00	18.06	2739.00
				3944.00
				3325.00
3	1.000	7392.00	12.21	6351.00
				7959.00
				7866.00
4	2.000	16550.00	6.69	15427.00
				16582.00
				17641.00
5	5.000	44105.67	0.45	44180.00
				44257.00
				43880.00
6	10.000	90285.67	3.73	90182.00
				86969.00
				93706.00

Figure 2a. Matrix matched calibration curve of methyl parathion



#	Conc. (ppb)	Mean Area	Area%RSD	Area
1	0.000	0.00	--	0.00
				0.00
2	0.500	2297.67	11.53	2061.00
				2584.00
				2248.00
3	1.000	5148.33	7.14	5456.00
				5248.00
				4741.00
4	2.000	10708.33	5.79	10706.00
				10090.00
				11329.00
5	5.000	28399.33	2.34	28037.00
				27995.00
				29166.00
6	10.000	58875.33	3.23	61046.00
				58092.00
				57488.00

Figure 2b. Matrix matched calibration curve of quinalphos



#	Conc. (ppb)	Mean Area	Area%RSD	Area
1	0.000	230.50	49.39	311.00
				150.00
2	0.500	1432.00	2.91	1393.00
				1427.00
				1476.00
3	1.000	2237.33	3.40	2206.00
				2182.00
				2324.00
4	2.000	4900.00	3.63	5105.00
				4790.00
				4805.00
5	5.000	13480.67	4.65	13012.00
				14192.00
				13238.00

Figure 2c. Matrix matched calibration curve of profenophos

CONCLUSION

A method of analysis for methyl parathion, quinalphos and profenophos from chili matrix was successfully established with good sensitivity using GCMS-TQ8040.

Spices are a complex matrix and can exhibit matrix effect (either ion suppression or enhancement) during analysis. A calibration curve based on matrix matched standards can demonstrate true sensitivity of analyte in presence of matrix. Therefore, this method provides more reliable and accurate method of quantitation as compared to quantitation against neat standards.

SHIMADZU
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C-68 Targeted Screening and Quantification of Pesticide Residuals in Tobaccos by Ultra Fast LC/MS/MS

INTRODUCTION

Pesticides are used widely during cultivation of plants including tobaccos. Although they are not foodstuffs, there has been a global concern in the tobacco industry and public about pesticide residues being taken into the body. LC/MS/MS methods have been employed increasingly in detection and quantification of pesticide residuals in foods and agriculture products. One of the challenges in food safety analysis is the large numbers of pesticides on the watching lists imposed by authorities, which leads to screening analysis using conventional MRM method on triple quadrupole LC/MS/MS to be difficult. The new generation ultra-fast LC/MS/MS technique introduced recently features with ultra fast MRM speed (>500 MRM transitions per second), which enhances greatly the capacity of MRM method when it is used for screening analysis [1]. Both screening analysis and quantification could be carried out using a single method on the same system. Here we report an example of screening analysis and quantification of pesticides in tobaccos using a ready-to-use Method Package on LCMS-8040 system

EXPERIMENTAL

Three samples of dried tobacco leaves labelled as A, B and C were obtained from a manufacture for this study. The QuEChERS (Restek) method was employed for extraction of pesticides from the samples for screening analysis using a ready-to-use MRM based Method Package developed by Shimadzu [2]. The details of the pre-treatment method are described in Figure 1. The Method Package includes the complete analytical conditions from retentions and MRM transitions of 167 pesticides which are on the Positive List (Japanese Regulation). By applying this method directly, screening analysis for the pesticides listed could be easily carried out without any method development efforts. The results of this direct screening analysis were treated as the preliminary results, which were required to be further confirmed. The LC/MS/MS system used was LCMS-8040 (Shimadzu Corporation, Japan) coupled with Nexera UHPLC. The details of the LC and MS conditions are shown in Table 1.

The purposes of this study are in two aspects. Since the pesticides included in the Method Package (167 in current version) may be not sufficient for particular projects running in a testing laboratory, expanding of the compound list of the Method Package may become a task to be carried out for users. Therefore, the first aspect of the study was to practise the procedure of adding new registration of some concerned pesticides which are not in the pesticide list. The second aspect was to validate and quantify the screening results obtained from the direct screening analysis. Nine concerned pesticides standards were obtained from Sigma-Aldrich. A mixed stock solution of the nine pesticides standards (Tables 2 & 3) was prepared and spiked into the extraction solution of Samples A. The pesticide standard in the mixed stock solution was 2500ng/mL and was further diluted into calibration series.

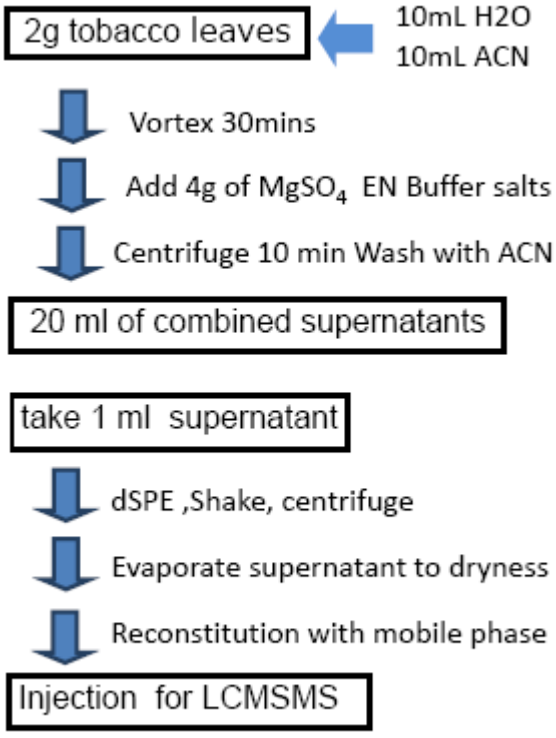

Figure 1: QuEChERS Extraction

Table 1: MRM Based Screening Method

LC Conditions	
Instrument	Shimadzu LCMS-8040
Column	Shim-pack FC-ODS 150 x 2mm, 3um
Mobile Phase	A: 5 mmol/L Ammonium Acetate – Water B: 5 mmol/L Ammonium Acetate – Methanol
Gradient (B Conc.)	15%, 0min → 40% 1~3.5 min → 50%, 6 min → 55%, 8 min → 95%, 17.5min ~ 30 min → 15%, 30.01 → 40min (stop)
Flow rate	0.2 mL/min
Oven temperature	40°C
Injection volume	5 µL

MS Conditions	
MS interface	ESI
MS mode	Two MRM for each compound
Heat Block temp.	400°C
DL temp.	250°C
Nebulizing Gas	1.5 L/min
Drying Gas	10 L/min

Table 2: New registration of Pesticides onto the Shimadzu Method Package

Additional Pesticide	RT (min)	MRM	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Clomazone	14.050	240.1>125.1	-28	-19	-26
		240.1 > 89.1	-28	-45	-18
Profenofos	18.442	373.0>303.0	-27	-17	-23
		373.0>345.1	-27	-12	-19
Chlorpyrifos	19.299	350.0>198.1	-26	-18	-23
		350.0>125.1	-26	-20	-26
Flumetralin	19.416	422.1>143.1	-30	-25	-30
		422.1>107.0	-30	-60	-22
Pendimethalin	19.439	282.2>212.1	-20	-10	-25
		282.2>194.1	-20	-18	-22
Butralin	19.656	296.2>240.2	-22	-13	-28
		296.2>222.2	-22	-21	-27

RESULTS AND DISCUSSION

Targeted Screening Analysis using ready-to-use method package

The quantitative MRM method established was then applied to the samples by processing the raw data in the Postrun program of the LabSolutions. The quantification results in extract solutions and converted to tobacco leaves in ng per gram are shown in Table 7.

The ready-to-use Method Package for pesticides consists of 167 pesticides for screening analysis. In addition to these pesticides, six other concerned pesticides as shown in Table 2 were registered onto the Method Package using their standards and tested under the same conditions. As a result, the modified method package was expanded to include 173 compounds with completed parameters of retentions and optimized MRMs.

The expanded method package was applied to the samples A, B and C for screening analysis of the 173 pesticides. The results were summarized in Table 3. It can be seen that a total of six pesticides were found in the three samples at significant levels as shown in Figure 2. The screening results are considerably reliable because of matching of two MRM transitions as well as retention of each pesticide found. However, further confirmation by spiked samples of the found pesticides is required to reach solid conclusions.

Validation and Quantification of Screening Results

It is necessary to use spiked samples to validate the screening results above. At the same time, quantification of the found pesticides could be obtained if calibration curves were established using spiked samples.

A series of spiked samples were prepared by adding mixed standards (six pesticides) into the extact solutions of samples A, B and C. The chromatograms of sample A and its 100 ppb spiked samples are shown in Figure 3. It can be seen that the MRM peak pairs of every found pesticide (except Methomyl) in sample A were in accordance with the spiked samples in terms of MRM peak ratio and peak intensity which increased proportionally with the spiked amount. This results firmly confirm the screening results above.

MRM calibration curves of the six found pesticides were established using the spiked samples into Sample A as matrix. The calibrations curves with excellent linearity for ranges from 1 ppb to 500 (or 2500) ppb are shown in Figure 4. The performance of the MRM quantification method was evaluated and the results are summarized in Tables 4-6. The repeatability of the method was evaluated and the RSD (%, n=6) of peak area obtained for 50 ng/mL and 10 ng/mL concentration were found to be below 5% except chlorpyrifos (11.5%).

Table 6 shows the results of matrix effect (ME), recovery (RE) and process efficiency (PE) of the six pesticides in Samples A, B and C by the QuEChERS pre-treatment and MRM quantification method. Both post-spiked and pre-spiked samples were prepared and analyzed using the MRM quantification method established. The results indicates that the current method from sample pre-treatment by the modified QuEChERS method and MRM quantification method generated considerably excellent results in low matrix effect, high recovery and high process efficiency.

Table 3: Screening results using the expanded method package of 173 pesticides

No	Found Pesticides	Result of Screening Analysis		
		Sample A	Sample B	Sample C
1	Methomyl	Detected	-	-
2	Thiodicarb	Detected	-	Detected
3	Azoxystrobin	Detected	-	-
4	Profenofos	Detected	Detected	Detected
5	Chlorpyrifos	-	-	Detected
6	Butralin	-	Detected	-

Note: pesticide No. 4-6 were new registrations into the Method Package in this study

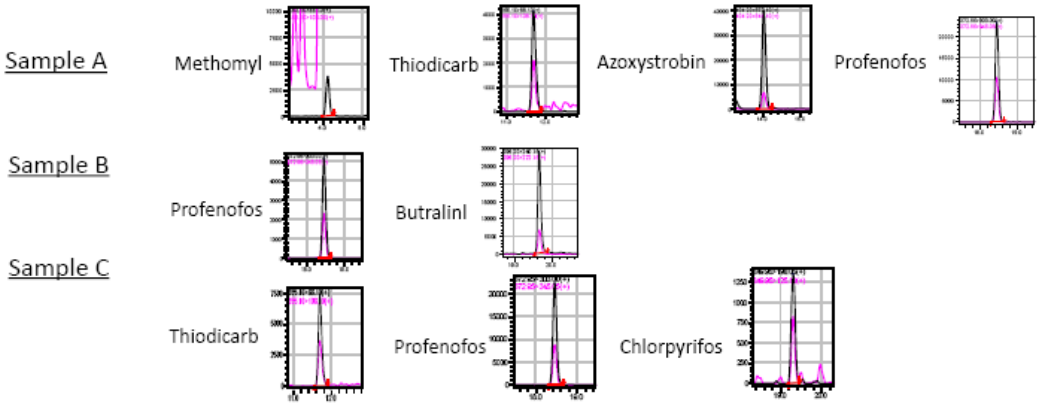

Figure 2: MRM peak pairs of found pesticides in Samples A, B and C using the expanded method package of 173 pesticides on LCMS-8040

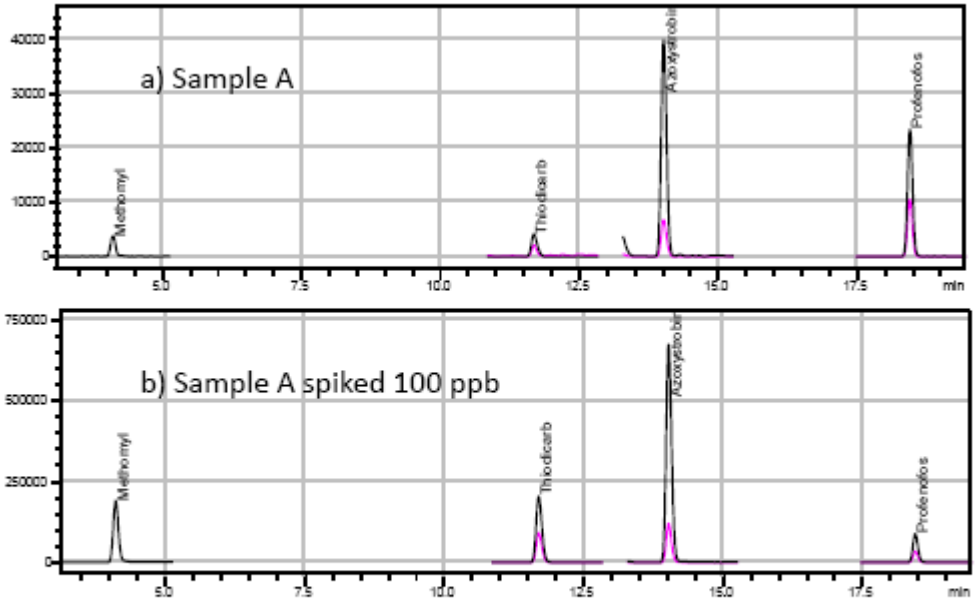

Figure 3: MRM Chromatograms of Sample A (a) and Sample A spiked with 100 ppb pesticide standards (b)

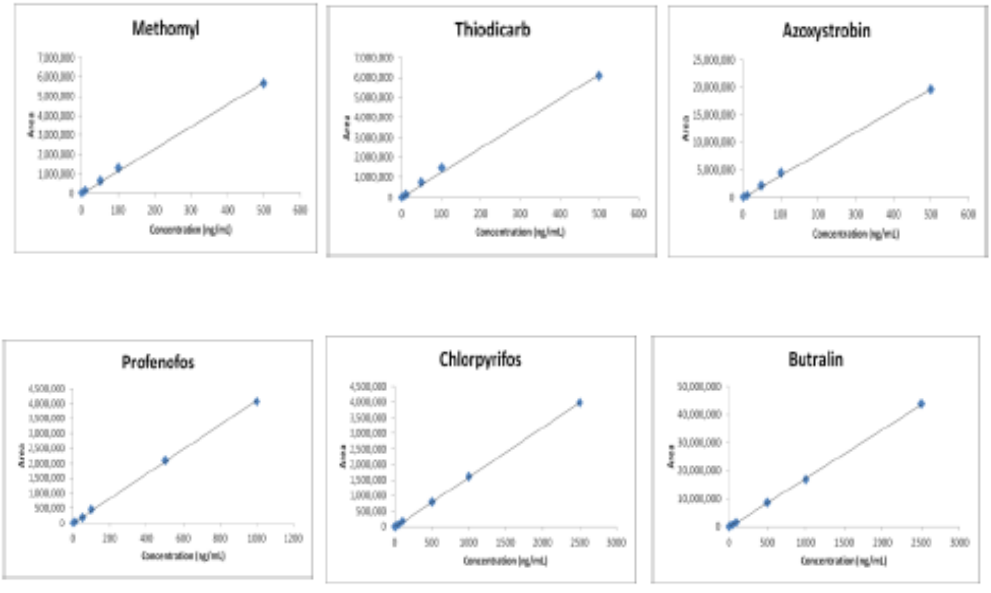

Figure 4: Calibration Curves of six pesticides in tobacco leave extract by MRM method.

Table 4: Range and linearity of calibration curves

No.	Compounds	Ret. Time	Calibration	
			Range (ppb)	R^2
1	Methomyl	4.110	1 – 500	0.9984
2	Thiodicarb	11.693	1 – 500	0.9965
3	Azoxystrobin	14.019	1 – 500	0.9987
4	Profenofos	18.442	1 – 1000	0.9996
5	Chlorpyrifos	19.299	1 – 2500	0.9998
6	Butralin	19.656	1 – 2500	0.9998

Table 5: Repeatability of MRM method

No.	Compounds	% RSD (10ppb, n=6)		% RSD (50ppb, n=6)	
		Ret Time	Area	Ret Time	Area
1	Methomyl	0.127	3.22	0.025	2.39
2	Thiodicarb	0.055	1.18	0.030	1.74
3	Azoxystrobin	0.031	0.97	0.009	2.13
4	Profenofos	0.032	2.92	0.024	2.47
5	Chlorpyrifos	0.042	11.46	0.019	2.08
6	Butralin	0.017	3.22	0.014	4.33

Table 6: Matrix effect (ME), recovery (RE) and process efficiency (PE)

No.	Compounds	Average (%), n=3		
		ME	RE	PE
1	Methomyl	98.0	84.7	82.9
2	Thiodicarb	113.4	89.2	101.0
3	Azoxystrobin	115.4	81.0	91.2
4	Profenofos	104.5	85.7	89.4
5	Chlorpyrifos	95.1	83.2	79.1
6	Butralin	88.3	80.0	70.7

The calculation of ME, RE and PE

Matrix Effect (%): $$ME = \frac{\text{Conc.of Post Spiked} - \text{Conc.of Sample blank}}{\text{Conc. of Standard}} \times 100\%$$

Recovery (%): $$RE = \frac{\text{Conc.of Pre Spiked} - \text{Conc.of Sample blank}}{\text{Conc.of Post Spiked} - \text{Conc.of Sample blank}} \times 100\%$$

Process Efficiency (%): $$PE = \frac{\text{Conc.of Pre Spiked} - \text{Conc.of Sample blank}}{\text{Conc. of Standard}} \times 100\%$$

The quantitative MRM method established was then applied to the samples by processing the raw data in the Postrun program of the LabSolutions. The quantification results in extract solutions and converted to tobacco leaves in ng per gram are shown in Table 7.

Table 7: Quantification results of pesticides in tobacco leaves samples by MRM method

No	Found pesticide	Quantification result in extract solution (ppb)			Quantification result in tobacco leaves (ng/g)		
		Sample A	Sample B	Sample C	Sample A	Sample B	Sample C
1	Methomyl	2.20	-	-	22.00	-	-
2	Thiodicarb	2.48	-	4.06	24.80	-	40.60
3	Azoxystrobin	6.92	-	-	69.20	-	-
4	Profenofos	36.41	8.18	36.10	364.10	81.80	361.00
5	Chlorpyrifos	-	-	6.20	-	-	62.00
6	Butralin	-	10.10	-	-	101.00	-

CONCLUSION

This study shows that the ready-to-use MRM based Method Package provides an easy and reliable workflow for both screening analysis and quantification of residual pesticides in tobacoo leaves on the LCMS-8040. Because of the expandability of the Method Package, users can add easily any desired new registration of pesticides or other concerned compounds. This flexibility makes it even more valuable in research, manufacture and testing laboratories targeting for different groups of pesticides in tobacco leaves etc. The procedure as well as the strategy of using the Method Package for both targeted screening analysis followed by quantification of found pesticides can be applied to other food safety analysis.

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C-69 High Sensitivity Analysis of Acrylamide in Potato Chips by LC/MS/MS with Modified QuEChERS Sample Pre-treatment Procedure

INTRODUCTION

Acrylamide was found to form in fried foods like potato-chips via the so-called Maillard reaction of asparagine and glucose (reducing sugar) at higher temperature (120°C) in 2002 [1,2]. The health risk of acrylamide present in many processing foods became a concern immediately, because it is known that the compound is a neurotoxin and a potential carcinogen to humans [3]. Various analytical methods, mainly LC/MS/MS and GC/MS based methods, were established and used in analysis of acrylamide in foods in recent years [4]. We present a novel LC/MS/MS method for quantitative determination of acrylamide in potato chips with using a modified QuEChERS procedure for sample extraction and clean-up, achieving high sensitivity and high recovery. A small sample injection volume (1uL) was adopted purposely to reduce the potential contamination of samples to the interface of MS system, so as to enhance the operation stability in a laboratory handling food samples with high matrix contents.

EXPERIMENTAL

Acrylamide and isotope labelled acrylamide-d3 (as internal standard) were obtained from Sigma-Aldrich. The QuEChERS kits were obtained from RESTEK. A modified procedure of the QuEChERS was optimized and used in the sample extraction of acrylamide (Q-sep Q100 packet, original unbuffered) in potato chips and clean-up of matrix with d-SPE tube (Q-sep Q250, AOAC 2007.01). Acrylamide and acrylamide-d3 (IS) stock solutions and diluted calibrants were prepared using water as the solvent. Method development and performance evaluation were carried out using spiked acrylamide samples in the extracted potato chip matrix. A LCMS-8040 triple quadrupole LC/MS/MS (Shimadzu Corporation, Japan) was used in this work. A polar-C18 column of 2.5µm particle size was used for fast UHPLC separation with a gradient elution method. Table 1 shows the details of analytical conditions on LCMS-8040 system,.

Table 1: LC/MS/MS analytical conditions of LCMS-8040 for acrylamide

LC condition

Column	Phenomenex Synergi 2.5u Polar-Rp 100A (100 x 2.00mm)
Flow Rate	0.2 mL/min
Mobile Phase	A: water B: 0.1% formic acid in Methanol
Elution Mode	Gradient elution, B%: 1% (0 to 1 min) ➔ 80% (3 to 4.5 min) ➔ 1% (5.5 to 10min)
Oven Temp.	40°C
Injection Vol.	1.0 µL

MS Interface condition

Interface	ESI
MS mode	Positive, MRM, 2 transitions each compound
Block Temp.	400°C
DL Temp.	200°C
CID Gas	Ar (230kPa)
Nebulizing Gas Flow	N2, 1.5L/min
Drying Gas Flow	N2, 10.0L/min

QuEChERS Sample Pre-treatment

The details of a modified QuEChERS procedure for potato chips are shown in Figure 1. Hexane was used to defat potato chips, removing oils and non-polar components. In the extraction step with Q-sep Q100Packet extraction salt (contain 4g MgSO4 & 0.5g NaCl), additional 4g of MgSO4 was added to absorb the water completely (aqueous phase disappeared). Acrylamide is soluble in both aqueous and organic phases. With this modification, high recovery of acrylamide was obtained. It is believed that this is because complete removal of water in the mixed extract solution could promote acrylamide transferring into the organic phase. Dispersive SPE tube was used as PSA to remove organic acids which may decompose acrylamide in the process.

Method Development

As acrylamide is a more polar compound, a Polar-RP type column was selected. Isotope labeled internal standard (acrylamide-d_3) was used to compensate the variation of acrylamide peak area caused by system fluctuation and inconsistency in sample preparation of different batches.

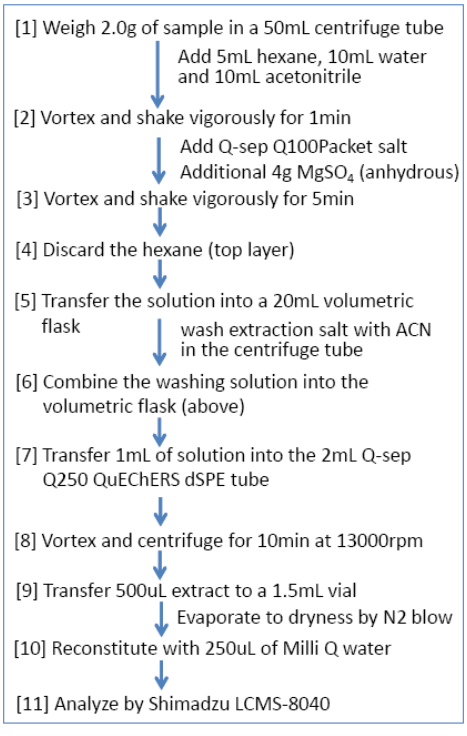


Figure 1: Flow chart of sample pre-treatment with modified QuEChERS.

The obtained extract solution of potato chips was used as "blank" and also matrix for preparation of post-spiked calibrants for establishment of calibration curve with IS (acrylamide-d_3). To obtain reliable results, the blank and each post-spiked calibrant as shown in Table 3 were injected three times and the average peak area ratios were calculated and used.

Table 3: Acrylamide spiked samples and peak area ratios of measured by IS method

Acrylamide post-spiked	IS post-spiked	Conc. Ratio Calculated	Area Ratio measured*
L0, Blank	50ppb	0	0.6033
L1, 1ppb		0.02	0.6120
L2, 5ppb		0.10	0.6786
L3, 10ppb		0.20	0.8239
L4, 50ppb		1.00	1.7686
L5 100ppb		2.00	2.8196
L6, 500ppb		10.00	11.8330

= Area (acrylamide) / Area (IS)

Table 2: MRM transitions and CID voltages

Name	MRM (m/z)	CID Voltage (V)		
		Q1	CE	Q3
Acrylamide-d_3	75.1 > 58.0*	-29	-15	-22
	75.1 > 30.1	-29	-24	-30
Acrylamide	72.1 > 55.0*	-17	-16	-24
	72.1 > 27.1	-17	-22	-30

MRM transition as quantifier

RESULTS AND DISCUSSION

It was found that the potato chips used as "blank" in this study was not free of acrylamide. Instead, it contained 27.1 ng/mL of acrylamide in the extract solution. A linear calibration curve was established with an intercept of 0.594 at zero spiked concentration (L0) as shown in Figure 2. Good linearity with correlation coefficient (R2) greater than 0.9999 across the range of 1.0 ng/mL– 500.0 ng/mL was obtained.

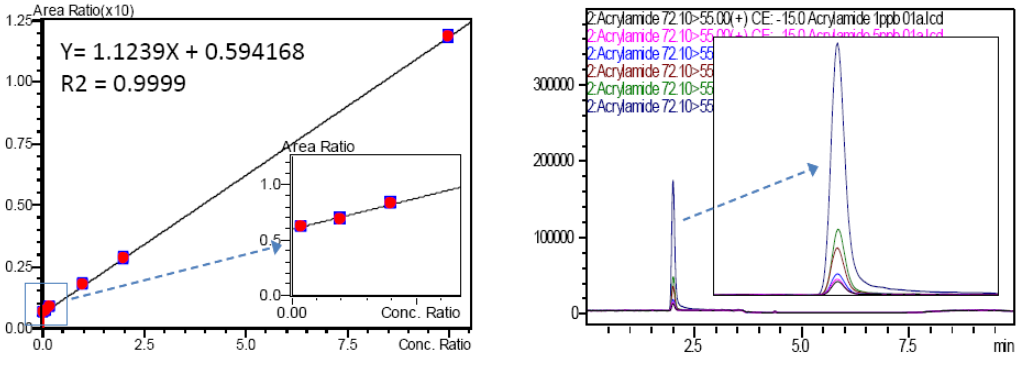


Figure 2: Calibration curve (left) and MRM peaks (right) of acrylamide spiked into potato chips matrix, 1-500 ppb with 50 ppb IS added.

Method Performance Evaluation

It was hard to estimate the LOD and LOQ of the analytical method due to the presence of acrylamide (27.1 ng/mL) in the "blank" (extract of potato chips). However, as reported also by other researchers, it is difficult to obtain potato chips free of acrylamide actually. To obtain actual concentration, it is normally subtracting the background content of acrylamide of a "blank" sample used as reference from a measurement of testing sample. The same way was used to estimate actual S/N value in this work. As a result, the LOD and LOD of acrylamide of this method with 1ul injection volume were estimated to be lower than 1ng/mL and 3ng/mL, respectively. This is consistence with the results estimated with the IS.

Table 4: Repeatability Test Results (n=6)

spiked Sample	Compound	Conc. (ng/mL)	%RSD
L2	Acrylamide	5	3.5
	Acrylamide-d_3	50	3.8
L4	Acrylamide	50	3.9
	Acrylamide-d_3	50	3.6

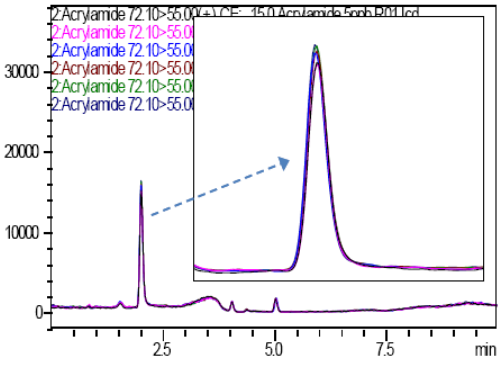


Figure 3: Overlay MRM chromatograms of 5 ng/mL acrylamide spiked in potato chips extract (total: 27.1+5 = 32.1 ng/mL)

The repeatability of the method was evaluated with L2 and L4 spiked samples. The results are shown in Table 4 and Figure 3. The peak area %RSD of acrylamide and IS were below 4%.

The matrix effect (M.E.), recovery efficiency (R.E.) and process efficiency (P.E.) of the method were determined with a duplicate set of spiked samples of 50 ng/mL level

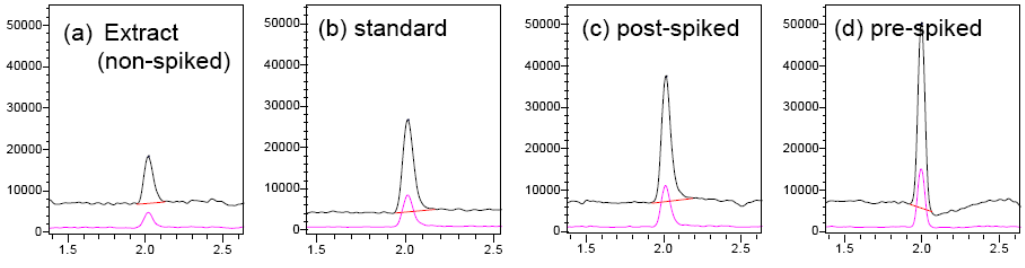


Figure 4: The MRM peaks of acrylamide detected in "blank" extract of potato chips (a), neat standard of 50ppb (b) post-spiked sample of 50ppb (c) and pre-spiked sample of 50ppb.

except for the non-spiked sample. The chromatograms of "set 2", i.e., non-spiked extract, pre-spiked, post-spiked and the standard in neat solution are shown in Figure 4. Noted that, the existing acrylamide in the extract of the potato chips used as reference was accounted for 27.1 ng/mL, corresponding to 135.5 ng per gram of potato chips. The average R.E, M.E and P.E of the method for extraction and analysis of acrylamide obtained are shown in Table 6.

Table 6: Method evaluation of at 50.0ng/mL concentration in potato chips matrix

Parameter	Set 1	Set 2	Average
R.E.	104.7%	112.0%	108.4%
M.E.	96.5%	84.6%	90.5%
P.E.	100.8%	94.5%	97.6%

Conclusions

Acrylamide is formed unavoidably in starch-rich food in cooking and processing at high temperature like potato chips, French fries, cereals and roasted coffee etc. The analysis method established in this work can be used to monitor the levels of acrylamide in processing food accurately and reliably. The QuEChERS method is proven to be fast and effective in extraction of acrylamide from potato chips. The excellent performance of the method in terms of sensitivity, linearity, repeatability and recovery are related to the outstanding performance of the LC/MS/MS used which features ultra fast mass spectrometry (UFMS) technology. The high sensitivity of the method allows the analysis to be performed with a very small injection volume (1µL or below), which would be a great advantage in running heavily food samples with high matrix contents and strong matrix effects. Maintenance of the interface of a mass spectrometer could also be reduced significantly.

C-70 An analytical method for environmental pollutants using GC×GC-MS/MS with ultra-fast MRM switching mode

INTRODUCTION

Generally, gas chromatograph mass spectrometer (GC-MS) is used for the analysis of environmental pollutants. The number of environmental pollutants dramatically increases these days, the number of analysis methods together with sample pretreatment methods largely increases. These methods require specialized experience and advanced technique for sample pretreatment and data analysis for environmental analysis.

To resolve such complexity and difficulty, the analysis system for multitarget analysis with high separation ability and highly sensitive/selective detection has been developed. Comprehensive two dimensional gas chromatograph (GC×GC) and triple quadrupole mass spectrometer (MS/MS) were coupled, and the analysis of PCBs in environmental samples by the system has been investigated.

EXPERIMENTAL

Materials
STD : 19 types of PCBs (0.1 – 100 pg), 14 types of 13C-PCBs (ISTD, 100 pg)

2-Chlorobiphenyl (PCB1)
4-Chlorobiphenyl (PCB3)
2,6-Dichlorobiphenyl (PCB10)
4,4'-Dichlorobiphenyl (PCB15)
2,2',6-Trichlorobiphenyl (PCB19)
3,4,4'-Trichlorobiphenyl (PCB37)
2,2',6,6'-Tetrachlorobiphenyl (PCB54)
3,3',4,4'-Tetrachlorobiphenyl (PCB77)
2,2',4,6,6'-Pentachlorobiphenyl (PCB104)
3,3',4,4',5-Pentachlorobiphenyl (PCB126)
2,2',4,4',6,6'-Hexachlorobiphenyl (PCB155)
3,3',4,4',5,5'-Hexachlorobiphenyl (PCB169)
2,2',3,4',5,6,6'-Heptachlorobiphenyl (PCB188)
2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB189)
2,2',3,3',5,5',6,6'-Octachlorobiphenyl (PCB202)
2,3,3',4,4',5,5',6-Octachlorobiphenyl (PCB205)
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (PCB206)
2,2',3,3',4,5,5',6,6'-Nonachlorobiphenyl (PCB208)
Decachlorobiphenyl (PCB209)

^{13}C-3,4,4',5-Tetrachlorobiphenyl (PCB81-L)
^{13}C-3,3',4,4'-Tetrachlorobiphenyl (PCB77-L)
^{13}C-2,3,3',4,4'-Pentachlorobiphenyl (PCB105-L)
^{13}C-2,3,4,4',5-Pentachlorobiphenyl (PCB114-L)
^{13}C-2,3',4,4',5-Pentachlorobiphenyl (PCB118-L)
^{13}C-2',3,4,4',5-Pentachlorobiphenyl (PCB123-L)
^{13}C-3,3',4,4',5'-Pentachlorobiphenyl (PCB126-L)
^{13}C-2,3,3',4,4',5-Hexachlorobiphenyl (PCB156-L)
^{13}C-2,3,3',4,4',5'-Hexachlorobiphenyl (PCB157-L)
^{13}C-2,3',4,4',5,5'-Hexachlorobiphenyl (PCB167-L)
^{13}C-3,3',4,4',5,5'-Hexachlorobiphenyl (PCB169-L)
^{13}C-2,2',3,3',4,4',5-Heptachlorobiphenyl (PCB170-L)
^{13}C-2,2',3,4,4',5,5'-Heptachlorobiphenyl (PCB180-L)
^{13}C-2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB189-L)

Sample : Fly ash (NIES CRM No.17) - Rough extracted solution

Analytical Condition

GC-MS : GCMS-TQ8030

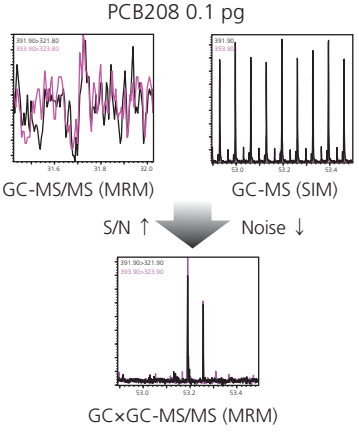
Column : 1st Rxi-5MS (30 m length, 0.25 mm I.D., df = 0.25 µm)
[GC] 2nd BPX-50 (2.5m length, 0.1 mm I.D., df = 0.1 µm)
Injection Temp. : 250°C
Column Oven Temp. : 80°C - (1 min) - (4°C/min) - 310°C (10 min)
Injection Mode : Splitless
Flow Control Mode : Pressure (270 kPa (1 min) - (3.4 kPa/min) - 465.5 kPa (10 min))
Injection Volume : 1 µL
Modulation Period : 4 sec (320°C, 0.3 sec)
[MS]
Interface Temp. : 250°C
Ion Source Temp. : 200°C
Acquition Mode : MRM
Sampling Rate : 70 Hz
MRM monitoring m/z

Compound Name	Quantitative Transition		Qualitativve Transition	
	Precursor > Product	CE (V)	Precursor > Product	CE (V)
Chlorobiphenyl	188.0>152.0	24	190.0>152.0	24
Dichlorobiphenyl	222.0>152.0	24	224.0>152.0	24
Trichlorobiphenyl	256.0>186.0	24	258.0>186.0	24
Tetrachlorobiphenyl	289.9>219.9	24	291.9>221.9	24
Pentachlorobiphenyl	323.9>253.9	24	325.9>255.9	24
Hexachlorobiphenyl	357.9>287.9	27	359.9>289.9	27
Heptachlorobiphenyl	391.9>321.9	30	393.9>323.9	30
Octachlorobiphenyl	427.8>355.8	30	429.8>357.8	30
Nonachlorobiphenyl	461.8>391.8	30	463.8>393.8	30
Decachlorobiphenyl	495.7>425.7	30	497.7>427.7	30
^{13}C-Tetrachlorobiphenyl	301.9>231.9	24	303.9>233.9	24
^{13}C-Pentachlorobiphenyl	335.9>265.9	24	337.9>267.9	24
^{13}C-Hexachlorobiphenyl	369.9>299.9	27	371.9>301.9	27
^{13}C-Heptachlorobiphenyl	403.9>333.9	30	405.9>335.9	30

RESULTS AND DISCUSSION

2D chromatograph of STD 0.1 pg

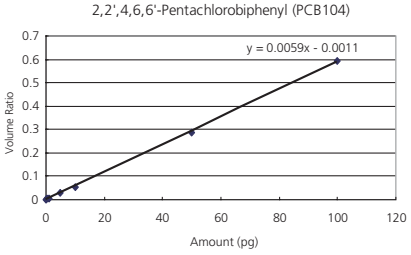
PCB208 0.1 pg

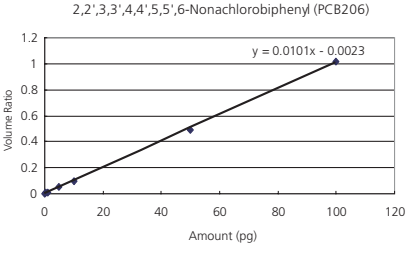


Instrument Detection Limit (IDL) & Calibration Curve

Compound Name	IDL (pg)	R
2-Chlorobiphenyl (PCB1)	0.014	0.9990
4-Chlorobiphenyl (PCB3)	0.012	0.9999
2,6-Dichlorobiphenyl (PCB10)	0.015	1.0000
4,4'-Dichlorobiphenyl (PCB15)	0.020	0.9999
2,2',6-Trichlorobiphenyl (PCB19)	0.012	0.9999
3,4,4'-Trichlorobiphenyl (PCB37)	0.040	0.9997
2,2',6,6'-Tetrachlorobiphenyl (PCB54)	0.035	1.0000
3,3',4,4'-Tetrachlorobiphenyl (PCB77)	0.036	0.9990
2,2',4,6,6'-Pentachlorobiphenyl (PCB104)	0.032	0.9999
3,3',4,4',5-Pentachlorobiphenyl (PCB126)	0.028	0.9996
2,2',4,4',6,6'-Hexachlorobiphenyl (PCB155)	0.034	0.9993
3,3',4,4',5,5'-Hexachlorobiphenyl (PCB169)	0.091	0.9992
2,2',3,4',5,6,6'-Heptachlorobiphenyl (PCB188)	0.041	0.9998
2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB189)	0.056	0.9999
2,2',3,3',5,5',6,6'-Octachlorobiphenyl (PCB202)	0.071	0.9996
2,3,3',4,4',5,5',6-Octachlorobiphenyl (PCB205)	0.039	0.9996
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (PCB206)	0.073	0.9999
2,2',3,3',4,5,5',6,6'-Nonachlorobiphenyl (PCB208)	0.073	0.9990
Decachlorobiphenyl (PCB209)	0.032	0.9992

IDLs were calculated by CV% (0.1 pg, n=5).

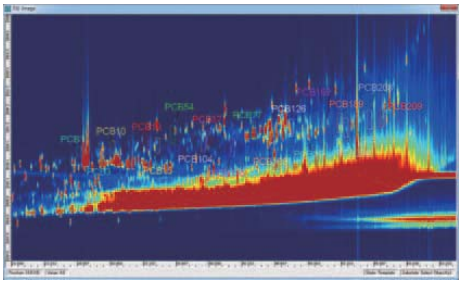




High sensitivity and good accuracy were achieved by ultra fast MRM switching (min. Dwell time < 1 msec).

Result of Fly ash

GC×GC-MS (Scan)



GC×GC-MS/MS (SIM)

Matrices were remaining and would cause miss detecting blobs.

GC×GC-MS/MS (MRM)

GC×GC-MS/MS system enabled to separate and remove almost matrices.

Quantification Result

Compound Name	Amount (pg)	Compound Name	Amount (pg)
2-Chlorobiphenyl (PCB1)	2.139	2,2',4,4',6,6'-Hexachlorobiphenyl (PCB155)	0.509
4-Chlorobiphenyl (PCB3)	25.386	3,3',4,4',5,5'-Hexachlorobiphenyl (PCB169)	6.671
2,6-Dichlorobiphenyl (PCB10)	0.482	2,2',3,4',5,6,6'-Heptachlorobiphenyl (PCB188)	0.653
4,4'-Dichlorobiphenyl (PCB15)	5.223	2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB189)	6.593
2,2',6-Trichlorobiphenyl (PCB19)	0.273	2,2',3,3',5,5',6,6'-Octachlorobiphenyl (PCB202)	1.088
3,4,4'-Trichlorobiphenyl (PCB37)	16.231	2,3,3',4,4',5,5',6-Octachlorobiphenyl (PCB205)	5.654
2,2',6,6'-Tetrachlorobiphenyl (PCB54)	N.D.	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (PCB206)	4.978
3,3',4,4'-Tetrachlorobiphenyl (PCB77)	20.530	2,2',3,3',4,5,5',6,6'-Nonachlorobiphenyl (PCB208)	14.820
2,2',4,6,6'-Pentachlorobiphenyl (PCB104)	0.325	Decachlorobiphenyl (PCB209)	14.154
3,3',4,4',5-Pentachlorobiphenyl (PCB126)	18.254		

※Reference Value

CONCLUSION

- GC×GC-Ultra fast switching MRM analysis achieved high sensitivity and good quantitative performance.
- Sample pretreatment is expected to be easier because almost matrices could be separated and removed by high separation capacity and high selectivity.
- Although target compounds were only PCBs in this study, another pollutants (ex. Dioxins, Cl-PAHs) will be added and validated in the future plan.
- This GC×GC-MS/MS system can accept sharp peaks of GC×GC without lacking of sampling rate in case of increasing target compounds because GCMS-TQ8030 has ultrafast switching ability (Max. 600 trans/sec).

⇒Quick and high sensitive multi-target analysis will be enabled using this GC×GC-MS/MS system.

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GCMS

C-71 Quantitative Analysis of Dibenzo-p-dioxins (PCDD) and Polychlorinated-p-Dibenzofurans (PCDF) in Foodstuff and animal Feed using the GCMS-TQ8030 tandem mass spectrometer

Hans-Ulrich Baier (Shimadzu Europa GmbH)

INTRODUCTION

Contaminations of food and feed with persistent organic pollutants (POP) are determined routinely by various analytical technologies. Dioxins and dioxin like substances belong to this category. They are regarded to have high degree of toxicity to humans. The majority of dioxin contamination of humans is done via the food chain. Dioxins are introduced via several ways into the food products. As an example eggs can be contaminated via the feed of hens. The current methods to determine the amount of dioxins and dioxin like substances is described in American and European legislations [1,2]. In the past mainly gas chromatography coupled to high resolution mass spectrometry with isotopic dilution has been used as analytical method for analyzing and quantifying dioxins. Since June 2014 the EU regulation also allows gas chromatography coupled to tandem quadrupole mass spectrometry (GCMSMS) as a confirmatory method. Dioxins as referred to in this regulation cover a group of 75 polychlorinated dibenzo-para-dioxin (PCDD) congeners and 135 polychlorinated dibenzofuran (PCDF) congeners, of which 17 are of toxicological concern. Polychlorinated biphenyls (PCBs) are a group of 209 different congeners which can be divided into two groups according to their toxicological properties: 12 congeners exhibit toxicological properties similar to dioxins and are therefore often termed dioxin-like PCBs (DL-PCBs). The other PCBs do not exhibit dioxin-like toxicity and have a different toxicological profile.

There have been several publications where the suitability of GCMS [3] or GCMSMS [4] has been tested in the past. Based on those data the new EU regulation included GCMSMS as an alternative for quantitative confirmation of dioxins and PCBs. In this application more than 50 samples of different matrices were split and analysed by the Shimadzu GCMS-TQ8030 triple quadrupole mass spectrometer and the Waters Autospec GCHRMS.

EXPERIMENTAL

Calibration standards of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-p-furans with appropriate ^{13}C isotope labelled internal standards were supplied by Greyhound chromatography (Wellington). ^{13}C labelled internal standards were spiked before sample preparation and used for quantification. Additionally, ^{13}C labelled recovery standards were added before instrumental analysis.

The schematic diagram of sample preparation is shown in figure 1.

For this application samples prepared and measured with HRMS were also analysed with the Shimadzu GCMS-TQ8030 tandem mass spectrometer1). The chromatographic column was a 5% phenyl with 60 m, 0.25 mm, 0.1 µm film MS column. Injection volume was 2 µl into the SPL-2010 in splitless mode. Each compound was measured with 2 MSMS transitions.

The mass spectrometer time settings are shown in table 1. Table 2 shows the target compounds and their internal standards which were measured together with one quantifier and one qualifier MSMS transition.

	start T	end T		Event Time (s)	MRM (Quant)	CE	MRM(Qual)	CE
13C TCDF ISTD	20	28	MRM	0,01	315.95>251.95	33	317.95>253.95	33
TCDD-2378	20	28	MRM	0,3	319.90>256.90	24	321.90>258.90	24
13C TCDD-2378 ISTD	20	28	MRM	0,01	331.90>268.00	24	333.90>270.00	24
13C TCDD-1234 ISTD	20	28	MRM	0,01	331.90>268.00	24	333.90>270.00	24
TCDF	20	28	MRM	0,3	303.90>240.95	33	305.90>242.95	33
PeCDF-23478	28	35	MRM	0,3	339.90>276.90	35	337.90>274.90	35
13C PeCDF-23478 ISTD	28	35	MRM	0,01	351.90>287.90	35	349.90>285.90	35
PeCDD	28	35	MRM	0,3	355.90>292.90	25	353.90>290.90	25
13C PeCDD ISTD	28	35	MRM	0,01	365.90>301.90	25	367.90>303.90	25
HxCDF	35	38	MRM	0,2	373.80>310.90	35	375.80>312.90	35
13C HxCDF ISTD	35	38	MRM	0,01	385.80>321.90	35	387.80>323.90	35
HxCDD	35	38	MRM	0,2	389.80>326.90	25	391.80>328.80	25
13C HxCDD ISTD	35	38	MRM	0,01	399.90>335.90	25	401.80>337.90	25
HpCDD	38	40	MRM	0,2	423.80>360.80	25	425.80>362.80	25
13C HpCDD ISTD	38	40	MRM	0,01	435.80>371.80	25	437.80>373.80	25
HpCDF	38	40	MRM	0,2	407.80>344.80	36	409.80>346.80	36
13C HpCDF ISTD	38	40	MRM	0,01	419.80>355.90	36	421.80>357.90	36
OCDF	40	43	MRM	0,2	441.80>378.80	35	443.80>380.80	35
13C OCDF ISTD	40	43	MRM	0,01	453.80>389.80	35	455.80>391.80	35
OCDD	40	43	MRM	0,2	457.70>394.70	26	459.70>396.70	26
13C OCDD ISTD	40	43	MRM	0,01	469.80>405.80	26	471.80>407.80	26

Table 1: MRM table with measuring window (start T, end T), event time in seconds and transitions with collision energy CE. Total number of time segments is 5. The MS resolution was set to Q1: high/unit (depending on peak) and Q3: low

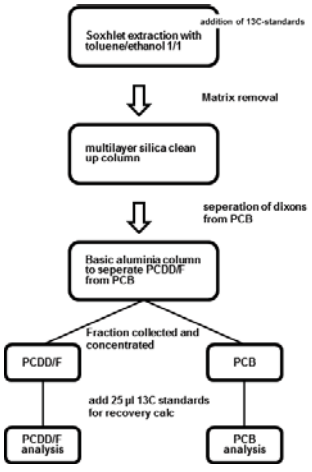


Figure 1: flow diagram of real world sample preparation

The other instrument parameters were: Splitless injection into a splitless liner (Shimadzu) at an injector temperature of 280 °C. GC oven temperature started at 130 °C for 1 min, 20 °C/min to 190 °C, 8 min, 2 °C/min to 220 °C, 3 min, 6 °C/min to 244 °C. The pneumatics were operated in the constant linear velocity mode at 34.7 cm/sec. Interface and ion source temperature were at 280 °C and 230 °C, respectively.

RESULTS AND DISCUSSION

In figure 2 the results recorded with a standard are shown. The target compounds are shown together with the calibration curves. The concentrations are: Tetra: 0.1 pg/µl, Penta, Hexa, Hepta: 0.2 pg/µl, Octa: 0.5 pg/µl.

The compounds with retention times and quantifier/qualifier transitions are shown in table 2.

The calibration ranges used were 0.1 pg/µl – 10 pg/µl for Tetra and Penta, 0.2 pg/µl – 20 pg/µl for Hexa and Hepta, 0.5 pg/µl – 50 pg/µl for OCDD and OCDF with R^2 > 0.999.

Eight replicates were done on the lowest standard. The RSD% was below 3%. The instrument detection limit is calculated from the following formula:

$IDL = t_{\alpha n}\, n \cdot RSDx\ (amount\ standard)/100\%$

$t_{\alpha n} = 2.998$ (student t table, $\alpha = 0.01$ (99% confident level)

The IDL calculated by that formula is 16.78 fg (Tetra, Penta).

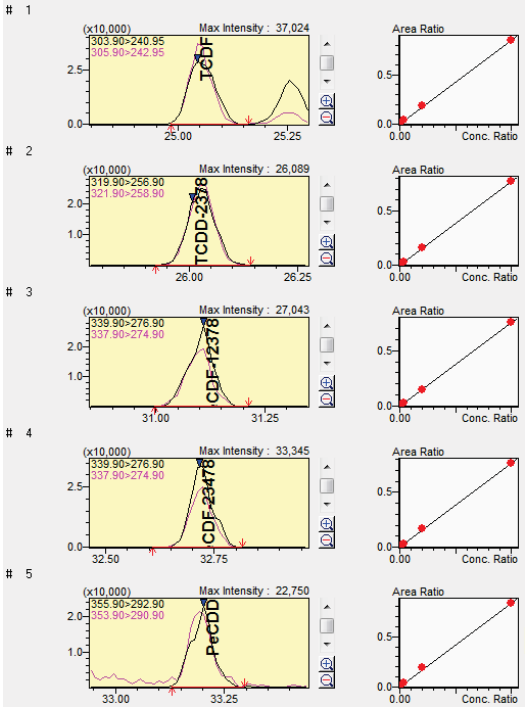
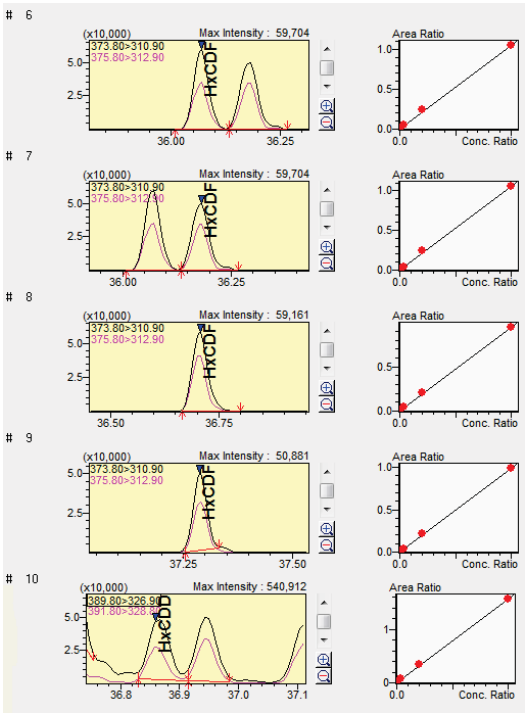
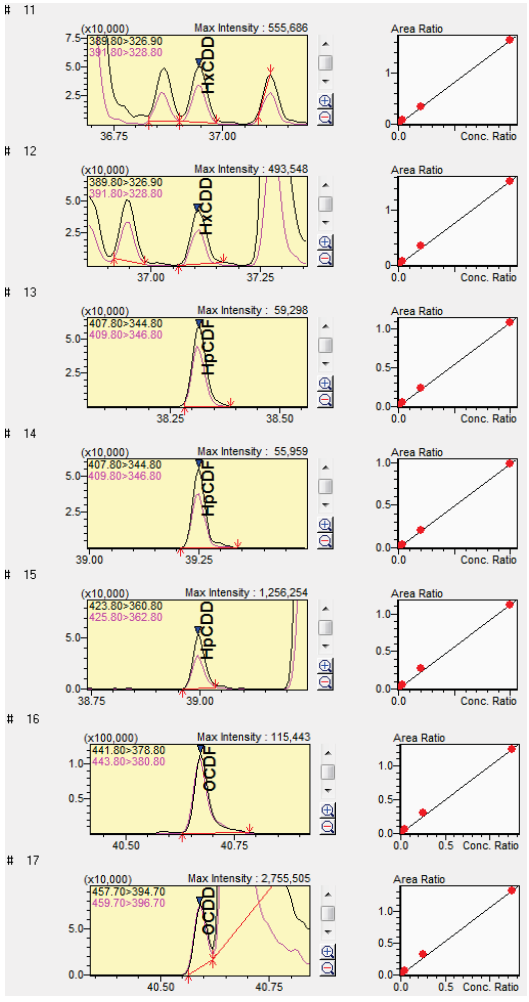


Figure 2: Native PCDD and PCDF congeners recorded with the lowest standard. Within the window of Compound No 17 partial signal from ^{13}C-OCDF is observed next to the target (see also report from standard supplier Wellington)





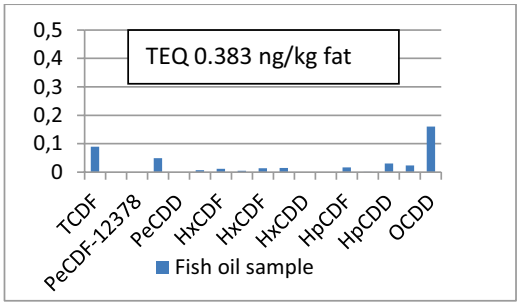
Compound	Ret.-Time	Quantifier	Qualifier
1. 2378-TCDF	25.048	303.90>240.95	305.90>242.95
2.2378-TCDD	26.023	319.90>256.90	321.90>258.90
3. 12378-PeCDF	31.099	339.90>276.90	337.90>274.90
4. 23478-PeCDF	32.715	339.90>276.90	337.90>274.90
5. 12378-PeCDD	33.192	355.90>292.90	353.90>290.90
6. 123478-HxCDF	36.064	373.80>310.90	375.80>312.90
7. 123678-HxCDF	36.176	373.80>310.90	375.80>312.90
8. 234678-HxCDF	36.704	373.80>310.90	375.80>312.90
9. 123789-HxCDF	37.286	373.80>310.90	375.80>312.90
10.123478-HxCDD	36.862	389.80>326.90	391.80>328.80
11. 123678-HxCDD	36.942	389.80>326.90	391.80>328.80
12. 123789-HxCDD	37.106	389.80>326.90	391.80>328.80
13.1234678-HpCDF	38.312	407.80>344.80	409.80>346.80
14. 1234789-HpCDF	39.247	407.80>344.80	409.80>346.80
15. 1234678-HpCDD	38.994	423.80>360.80	425.80>362.80
16. OCDF	40.671	441.80>378.80	443.80>380.80
17. OCDD	40.591	457.70>394.70	459.70>396.70
18. 13C-2378-TCDF	25.027	315.95>251.95	317.95>253.95

Compound	Ret.-Time	Quantifier	Qualifier
19. 13C-1234-TCDD	25.245	331.90>268.00	333.90>270.00
20. 13C-2378-TCDD	25.994	331.90>268.00	333.90>270.00
21. 13C-12378-PeCDF	31.073	351.90>287.90	349.90>285.90
22. 13C-23478-PeCDF	32.703	351.90>287.90	349.90>285.90
23. 13C-12378-PeCDD	33.179	365.90>301.90	367.90>303.90
24. 13C-123478-HxCDF	36.055	385.80>321.90	387.80>323.90
25. 13C-123678-HxCDF	36.169	385.80>321.90	387.80>323.90
26. 13C-234678-HxCDF	36.692	385.80>321.90	387.80>323.90
27. 13C-123789-HxCDF	37.28	385.80>321.90	387.80>323.90
28. 13C-123478-HxCDD	36.856	399.90>335.90	401.80>337.90
29. 13C-123678-HxCDD	36.935	399.90>335.90	401.80>337.90
30. 13C-123789-HxCDD	37.099	399.90>335.90	401.80>337.90
31. 13C-1234678-HpCDF	38.308	419.80>355.90	421.80>357.90
32. 13C-1234789-HpCDF	39.242	419.80>355.90	421.80>357.90
33. 13C-1234678-HpCDD	38.99	435.80>371.80	437.80>373.80
34. 13C-OCDD	40.587	469.80>405.80	471.80>407.80
35. 13C-OCDF	40.667	453.80>389.80	455.80>391.80

Table 2: Compound table list with internal standards.

Real world samples

In figure 3 the results recorded with a fish oil sample are shown.
The concentrations calculated were TCDF 0.089, PeCDF 0.049, HxCDF 0.0714, 0.012, HpCDD 0.03, OCDF 0.024, OCDD 0.16 pg/ 2 µl.



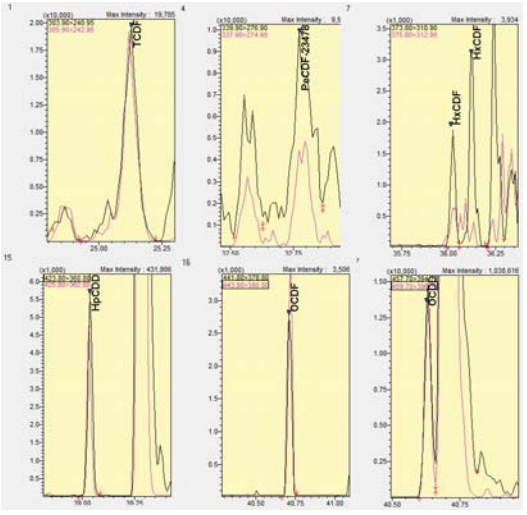


Figure 3: Results for detected compounds in a fish oil sample. Left: Concentration calculated for each congener. Right: peaks from the quantitation window

Each congener shows different toxicity which is expressed by the Toxic Equivalent Factor (TEF). This means that the analytical results relating to all the individual dioxin and dioxin-like PCB congeners of toxicological concern are expressed in terms of a quantity, namely the toxic equivalent (TEQ).

The Toxic Equivalent Factors are according to WHO from 2005 and are for the dibenzo-p-dioxins and furans: 2378-TCDD 1, 12378-Pe CDD 1, 123478-HxCDD 0.1, 123678-HxCDD 0.1, 123789-HxCDD 0.1, 1234678-HpCDD 0.01, CDD 0.0003 and 2378-TCDF 0.1, 12378-Pe CDF 0.03, 123478-HxCDF 0.1, 123678-HxCDF 0.1, 123789-HxCDF 0.1, 1234678-HpCDF 0.001, 1234789-HpCDD 0.01, and OCDF 0.0003. The main contribution to the TEQ value of 0.383 ng/kg fat in figure 3 comes from TCDF (TEQ is calculated by multiplication of the concentration).

Comparison of results with HRMS

A total number of more than 50 samples were measured with both technologies. In figure 4, a component based comparison is shown for an animal feed and fish sample. The TEQ values calculated from these samples were for the animal feed sample 0.0899 ng/kg (GCHRMS) and 0.0895 ng/kg (GCMSMS) and for the fish sample 0.307 ng/kg (GCHRMS) and 0.324 ng/kg (GCMSMS).

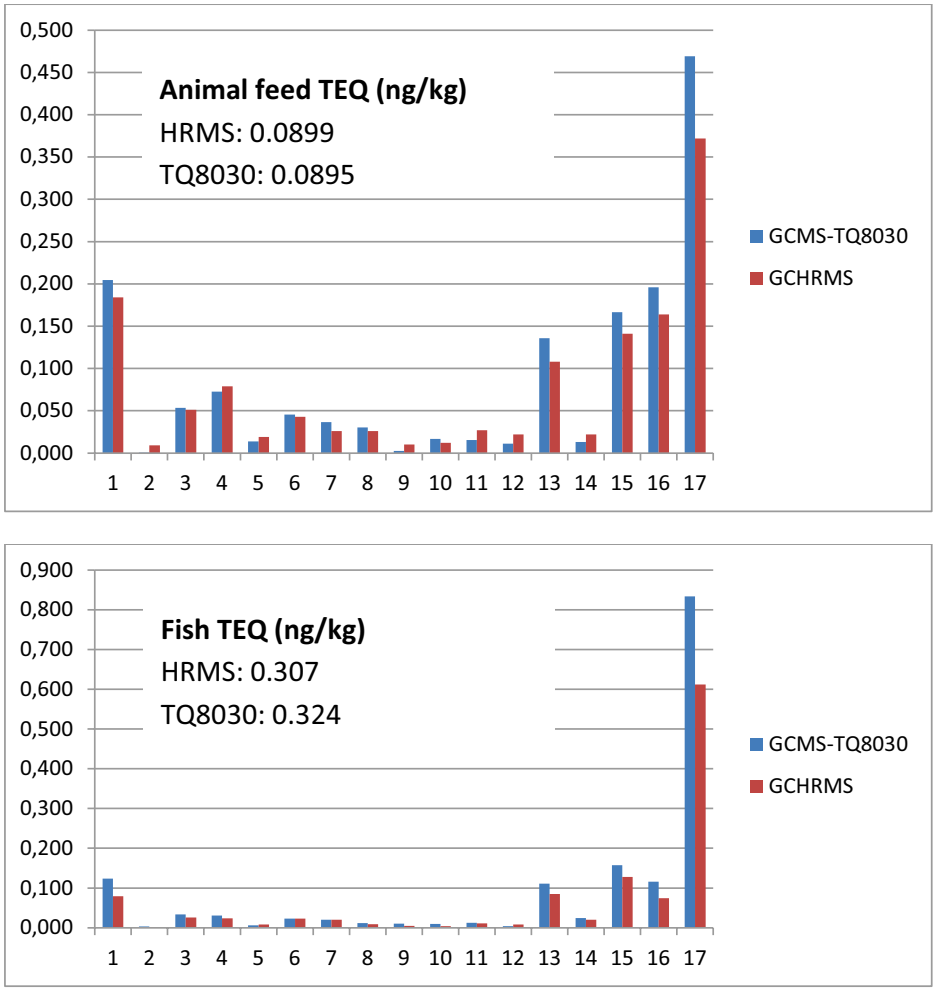


Figure 4: Comparison of concentrations (pg/µl) of individual PCDD and PCDF congeners determined with an animal feed (top) and fish sample (bottom). The x-axis numbers refer to the compounds listed in table 2.

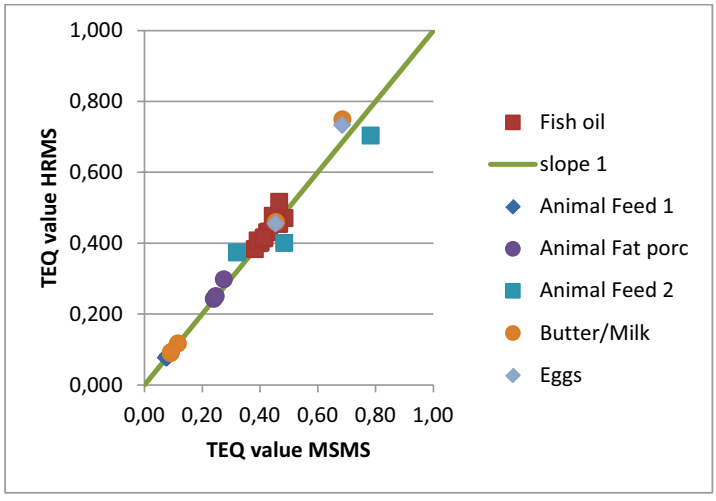


Figure 5: TEQ values (upper bound) in ng/kg calculated from GCHRMS and GCMSMS for various matrices

Then the method was applied to more than 50 samples. In figure 5 TEQ values calculated from GCHRMS and GCMSMS data are plotted against each other for various matrices. In addition the ideal curve with slope 1 is shown as well. To have a better indication on the statistics, figure 6 shows the percentage deviation of 14 fish oil samples with TEQ values (upper bound) of 0.383 to 0.477 ng/kg fat.

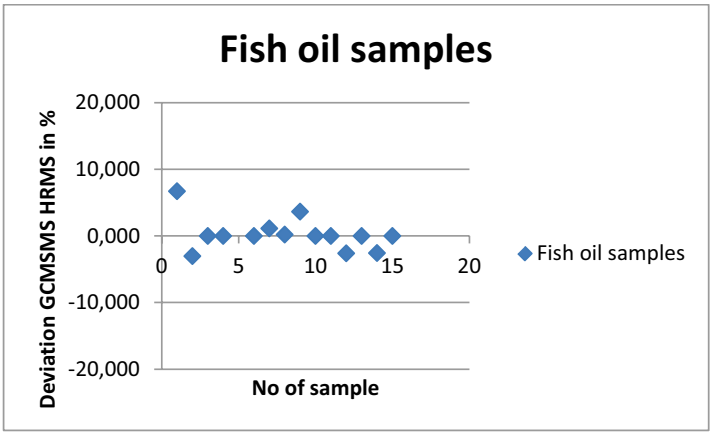


Figure 6: 14 samples (fish oil) are plotted to show the percentage deviation of the results obtained with GCMSMS relative to the ones obtained with GCHRMS.

The TEQ values derived from the GCMSMS methods shown above, indicate a very good correlation with the established HRGCMS methods. For the matrix fish, the deviation is less than 10% at TEQ levels of about 0.45 ng/kg fat. Those values are below the regulatory levels which are 1.75 ng/kg (marine oil, fish oil). The highest TEQ value observed was about 10 ng/kg. The recovery of the compounds was calculated for every sample from the recovery internal standards and the results were between 60 and 100%.

CONCLUSION

The data shown in this application news indicate that the GCMS-TQ8030 proves sufficient accuracy for quantitative screening of dioxins in food and feed samples.

The maximum deviation of TEQ values calculated from GCMSMS data compared to the one from HRMS were below 10% for many matrices measured, even for low TEQ values below 0.5 ng/kg.

[1)] Sample preparation and measurement were performed at SGS Antwerp: HRMS: Waters Autospec, MSMS: Shimadzu GCMS-TQ8030

2. Biological Contamination

PREFACE

Mycotoxins are toxic compounds produced by different types of fungi, belonging mainly to the *Aspergillus*, *Penicillium* and *Fusarium* genera. Under favourable environmental conditions, when temperature and moisture are conducive, these fungi proliferate and may produce mycotoxins. They commonly enter the food chain through contaminated food and feed crops, mainly cereals.

The presence of mycotoxins in food and feed may affect human and animal health as they may cause many different adverse health effects such as induction of cancer and mutagenicity, as well as estrogenic, gastrointestinal and kidney disorders. Some mycotoxins are also immunosuppressive, reducing resistance to infectious disease. Therefore, the removal of contaminated products from the food chain is a primary means of eliminating human exposure.

Surveillance and control of mycotoxins in food and feed has become a major objective for producers, regulatory authorities, and researchers worldwide. A large variability of food matrices and growing demands for a fast, cost-saving and accurate determination of multiple mycotoxins by a single method outlines new challenges for analytical research. Technical developments in mass spectrometry, today, has facilitated decreasing the influence of matrix effects in spite of using limited sample clean-up step.

Development of reference methods must serve several purposes: one is to confirm samples that have been determined to contain mycotoxins, based on rapid screening tests. The second is to more accurately quantitate the amount of toxin present. Reference methods for mycotoxins generally involve a chromatographic technique such as thin-layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), or liquid chromatography with mass spectrometry (LC-MS) or tandem mass spectrometry (LC/MS/MS) to further separate mycotoxins from extract impurities.

The pioneering studies on mycotoxins relied on thin-layer chromatographic (TLC) methods well before the general availability of HPLC and immunological methods. TLC is often used as a mycotoxin-screening assay. GC often is used in more technical laboratories for some of the mycotoxins and in particular for the analysis of type-A trichothecenes (T-2 toxin, HT-2 toxin, neosolaniol and diacetoxyscirpenol) that are difficult to analyse using HPLC. LC-MS or LC/MS/MS is a powerful tool for mycotoxin detection and identification. This holds true particularly for those toxins for which there is little ultraviolet/visible (UV/VIS) absorbance or native fluorescence. Though ion-trap instruments have been utilized for trace level quantification of mycotoxins, they suffer from drawbacks like lower limits of detection, poor

calibration linearity, and lower measurement repeatability when compared with triple quadrupole mass spectrometers. Not only just screening large amounts of samples for the presence of a number of mycotoxins, but LC/MS/MS also offers unprecedented performance for studying the formation of artefacts, degradation and reaction products of mycotoxins, as well as the binding of mycotoxins to matrix components during food processing.

B-1 RAPID SIMULTANEOUS ASSAY OF 23 MYCOTOXINS IN A VARIETY OF FOOD SAMPLES BY UHPLC/MS/MS USING FAST POLARITY SWITCHING

INTRODUCTION

Mycotoxins produced by fungi are common contaminants in grains. For consumer food safety, quality control of food and beverages, it is mandatory to analyse such contaminants. Rapid determination of their presence and then quantification of hazardous mycotoxins is essential. UHPLC/MS/MS offers the best combination of selectivity, sensitivity, and speed for detection of these compounds in complex matrices. In this study, a high throughput method for the quantification of 25 mycotoxins in various matrices was established.

EXPERIMENTAL

Sample preparation (modified QuEChERs)

Samples (5 g of grains or animal food or 10 g of fruits + 10 mL of water), were mixed with 10 mL of acetonitrile. After maceration, salts were added to allow phase separation. Then the supernatant was 5-fold diluted with mobile phase A and the internal standard mix was added.

Instrument parameters

System : UFLCXR with LCMS-8040

LC conditions

Column : Phenomenex Kinetix XB-C18
(50 mm L x 2 mm ID, 2.6 µm)

Column Temperature : 50 °C

Mobile Phase : A – Water + 0.5 % acetic acid
B – Isopropanol + 0.5 % acetic acid

Flow rate : 0.4 mL/min

Time program :

Time (min)	Pump B conc.
Initial	2
0.01	10
1.50	55
3.50	85
4.00	85
4.01	2
6.50	2

Injection volume : 20 µL

MS conditions

Ionisation	: ESI, Positive and Negative, MRM mode
Ion source Temperature	: Desolvation line - 250 °C; Heat Block - 500 °C
Gases	: Nebulizing - 3 L/min; Drying – 15 L/min
MRM transitions	: Two transitions per mycotxin were selected
Dwell time	: 9 msec
Loop time	: 0.27 sec (maximum)

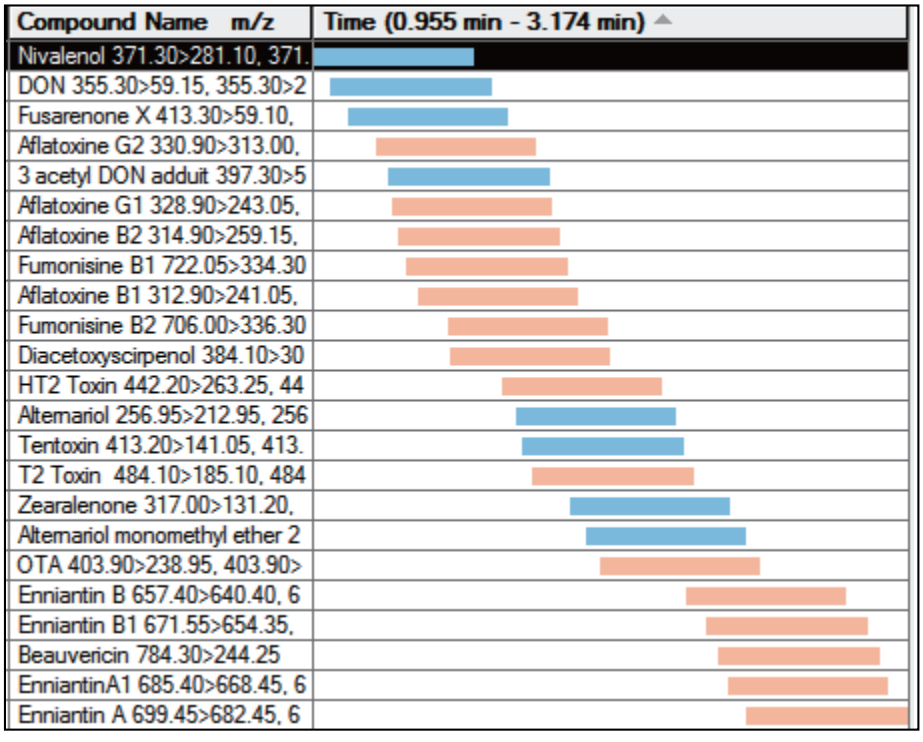


Figure 1. Staggered MRM scheduling for positive (red) and negative (blue) ionization

RESULTS AND DISCUSSION

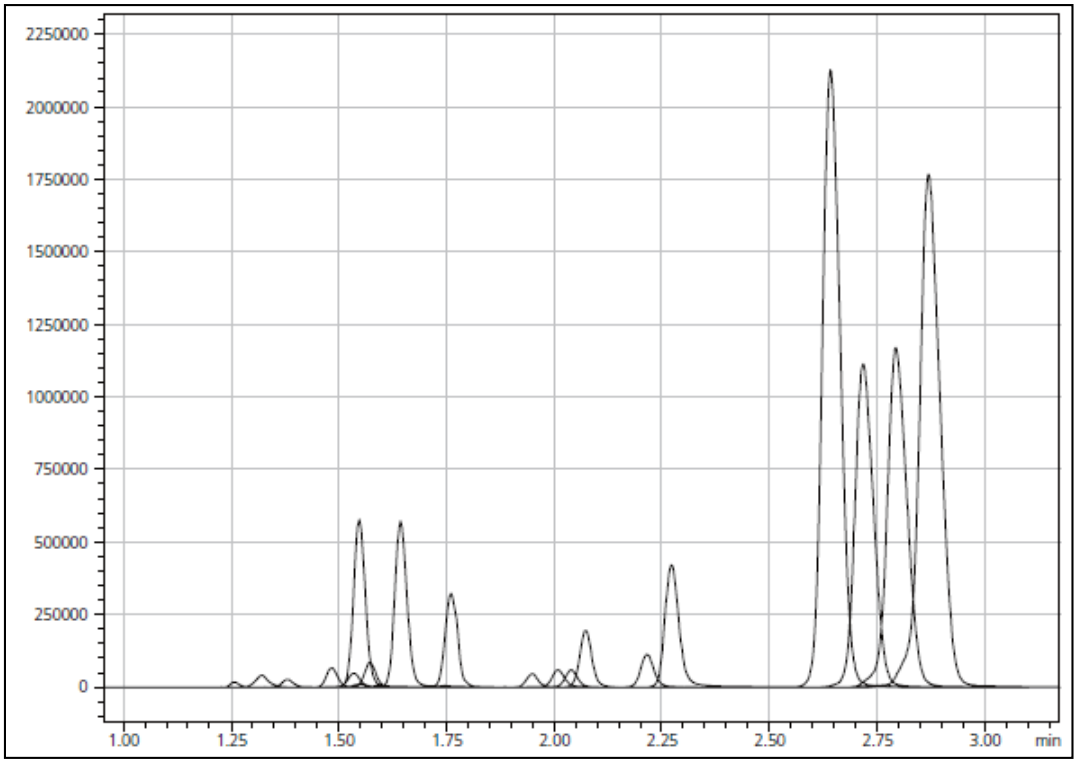


Figure 2. Typical chromatogram at 50 ppb (see Fig .1 for elution order)

Specificity and matrix effect

The specificity was studied in various matrices to show the correlation of results in neat standards with matrix standard. The concentration obtained was compared to the theoretical one. The specificity was validated in the following matrices: rice, maize, dry pastas, banana, muesli, wheat, carrot, apple compote, flour, etc. Figure 3 shows the correlation data for exemplary mycotoxins in the cited matrices at different levels. These data show that the method gives accurate concentration results of the mycotoxins whatever the matrix analyzed against neat solution standards. This suggests that the method is specific and free of significant matrix effect.

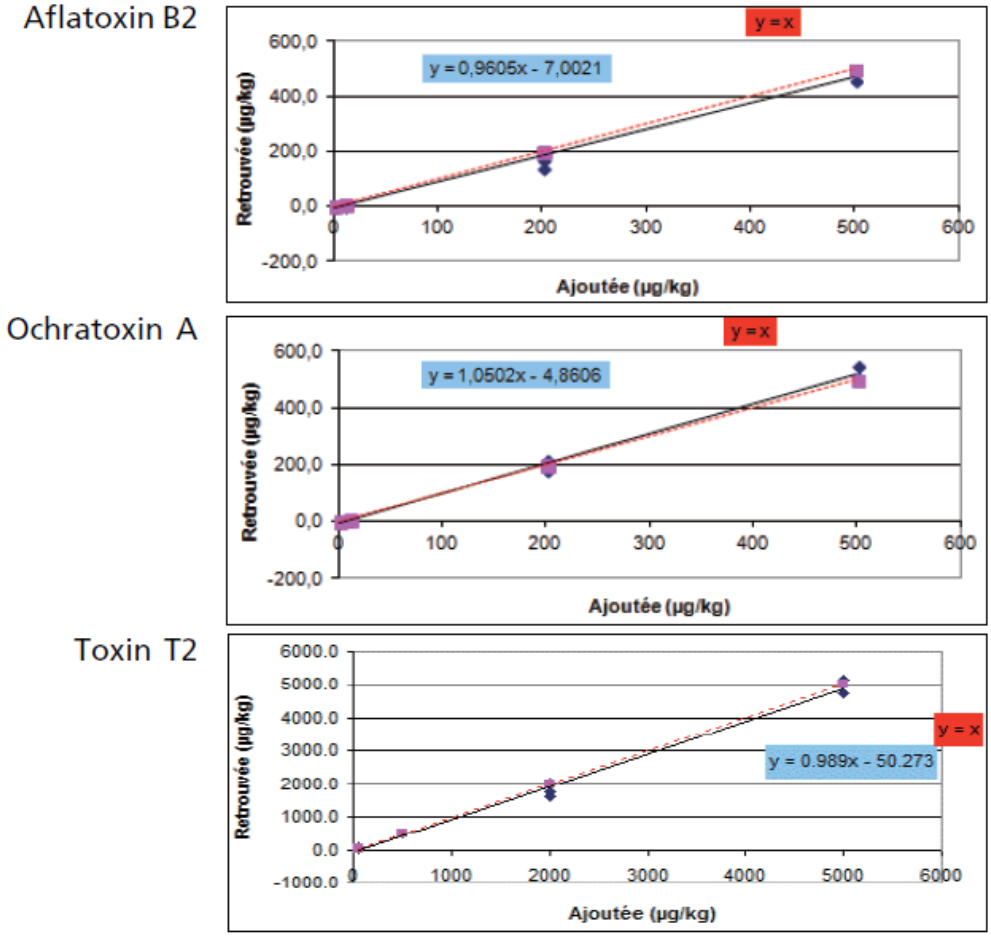


Figure 3. Correlation data

CONCLUSION

The fast polarity switching and the low electronic pause time allow simultaneous analysis of co-eluted compounds.

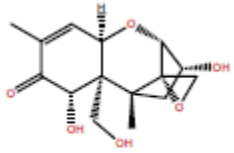
The method is fast and accurate.

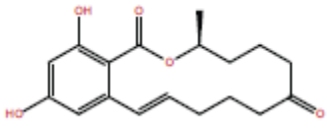
The sample preparation and selectivity of the method, lowered the matrix effect, thus neat standards can be used for a variety of different samples. This increases system productivity.

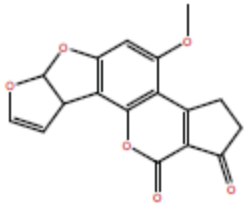
B-2 ANALYSIS OF MYCOTOXINS AND OTHER UNTARGETED CHEMICALS THREATS FROM GRAIN AND FEED SAMPLES BY UHPLC/MS/MS

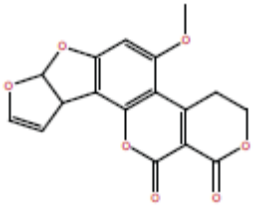
INTRODUCTION

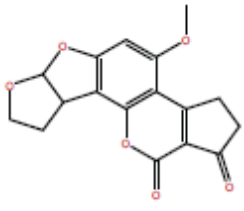
Mycotoxins are fungal poisons that threaten the world food supply. Regulatory agencies have imposed limits on levels of mycotoxins allowed in food. Food safety is ensured by testing for the presence of mycotoxins by methods such as LC/MS/MS. In addition to mycotoxins, many other known and unknown chemicals threaten the food supply. However, their presence might be missed by LC/MS/MS methods which rely exclusively on MRM settings for detection. Therefore, we have developed an extremely fast and reliable method for the detection of mycotoxins in food which has the additional advantage of collecting survey scan and data dependent MS/MS for untargeted screening for other chemical threats.

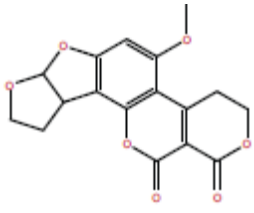

Deoxynivalenol (DON)

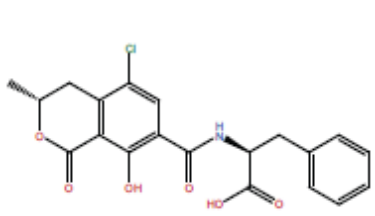

Zearalenone (ZON)


Aflatoxin B1

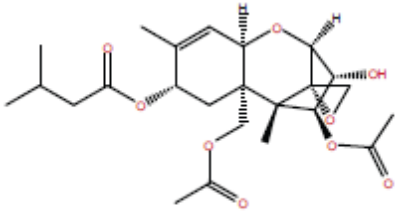

Aflatoxin G1


Aflatoxin B2

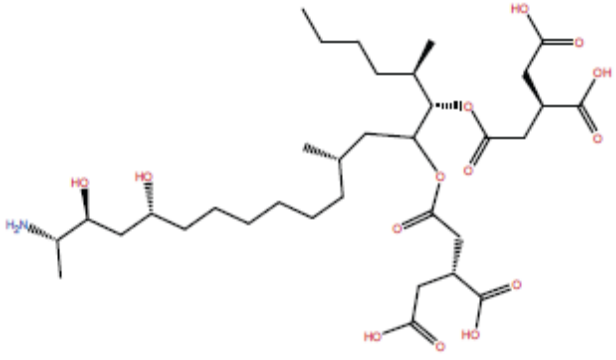

Aflatoxin G2



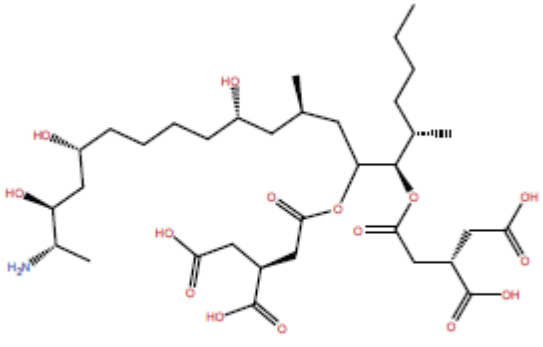
Ochratoxin A



Mycotoxin T2



Fumonisin B2



Fumonisin B1

Figure 1: Structures of selected mycotoxins

EXPERIMENTAL

Sample / Standard preparation

Reference standards were obtained for each mycotoxin. Each was diluted in an 80/20 mixture of 5 mM ammonium formate/methanol at the concentrations listed in Table 1.

Table 1: Concentrations of standards (ppb)

	L2	L3	L4	L5	L6
Aflatoxin B1	67.2	22.4	7.5	2.5	0.8
Aflatoxin B2	20.3	6.8	2.3	0.8	0.3
Aflatoxin G1	67.2	22.4	7.5	2.5	0.8
Aflatoxin G2	20.3	6.8	2.3	0.8	0.3
Fumonisin B1	833.3	277.8	92.6	30.9	10.3
Fumonisin B2	833.3	277.8	92.6	30.9	10.3
Mycotoxin T2	1666.7	555.6	185.2	61.7	20.6
DON	8100.0	2700.0	900.0	300.0	100.0
Ochratoxin	81.0	27.0	9.0	3.0	1.0
ZON	2025.0	675.0	225.0	75.0	25.0

Reference samples of feed corn and wheat with independently determined levels of various mycotoxins were prepared by solid phase extraction and analyzed by LC/MS/MS. Alternatively, oatmeal spiked with mycotoxins was prepared by reconstitution and dilution before analysis. Additionally, a feed corn sample was spiked with the pesticide Bromacil to demonstrate untargeted screening with data-dependent MS/MS and spectral pattern matching.

Mass spectra were collected at 15,000 u/sec in positive and negative mode continuously throughout the run. Data dependent MS/MS was triggered based on these survey scans at a product ion scan speed which was also set at 15,000 u/sec. To help identify any untargeted compounds of potential interest, data dependent product ion scans were compared to a database containing authentic tandem mass spectra of hundreds of substances including pesticides.

Instrument Parameters

LC conditions

System : Nexera

Column : Shim-pack XR-ODS III (50mm L x 2mm I.D., 1.6 µm)

Mobile phase : A - 5 mM ammonium formate; B - Methanol

Flow rate : 0.4 mL/min

Time program :

Time (min)	Pump B conc.
0.01	20
0.10	20
1.60	95
1.85	95
1.90	20
3.00	20

MS conditions

LC/MS/MS : LCMS-8030 :

Ionisation : ESI, Positive and Negative, MRM mode

Type	Event#	+/-	Compound Name (m/z)	Dwell Time (msec)	Q1 Pre Bias(V)	CE	Q3 Pre Bias(V)	Measurement Time
MRM	1	+	Aflatoxin B1 313>241	25	-25	-39	-25	1.25–1.75
MRM	2	+	Aflatoxin B2 315>259	25	-25	-33	-25	1.25–1.75
MRM	3	+	Aflatoxin G1 329>243	25	-25	-31	-25	1.25–1.75
MRM	4	+	Aflatoxin G2 331>245	25	-25	-33	-25	1.25–1.75
MRM	5	+	Fumonisin B1 722.4>334.4	25	-20	-40	-15	1.6–3.0
MRM	6	+	Fumonisin B2 706.4>336.2	25	-20	-40	-24	1.75–3.0
MRM	7	+	Mycotoxin T2 484.3>305.1	25	-24	-16	-21	1.75–3.0
MRM	8	-	DON 295>138	25	15	19	30	0–1.0
MRM	9	-	Ochratoxin 402.1>167	25	15	40	30	1.75–3.0
MRM	10	-	ZON 317>131	25	15	30	25	1.75–3.0

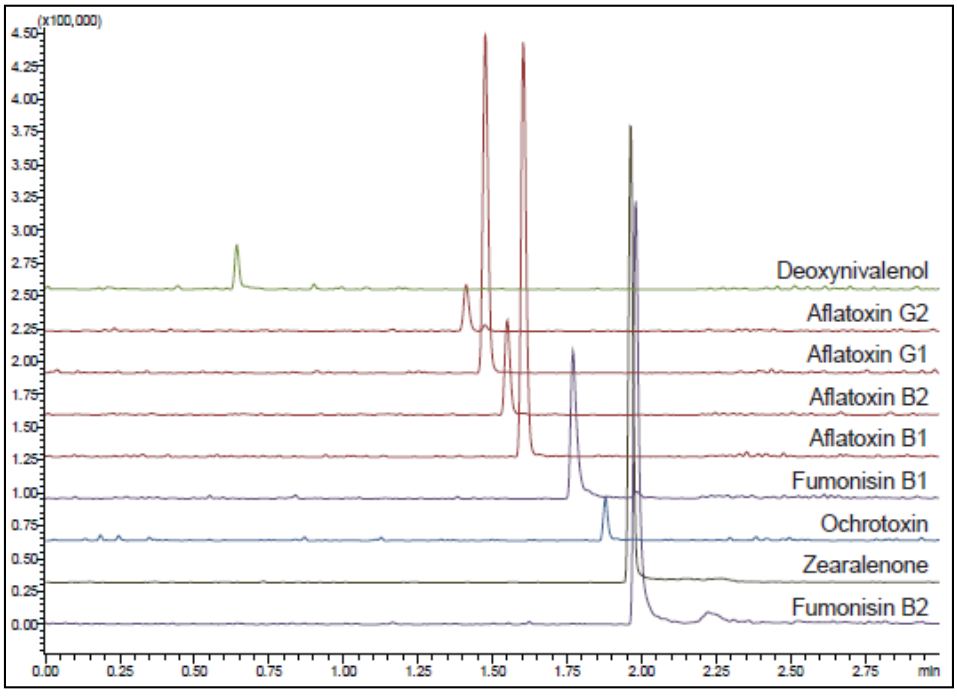


Figure 2: Chromatogram of mycotoxins standards

ESI was found to give the best overall signal intensity for the mycotoxins. The protonated molecules and sodium adducts of each of the mycotoxins were observed by ESI, APCI, and DUIS (a combination ESI and APCI source). A formic acid buffered mobile phase was found to suppress ionization of the protonated molecules, whereas ammonium formate increased their signal intensity

dramatically. Sodium adducts of the mycotoxins had the same or lower signal intensity when ionized in the presence of ammonium formate. The four aflatoxins ionized well in positive mode, while some of the other mycotoxins ionized more efficiently in negative mode.

Injection solvent was found to be critical to achieving proper peak shape. Therefore, samples were diluted in mobile-phase matched solvent (80/20 5 mM ammonium acetate/methanol).

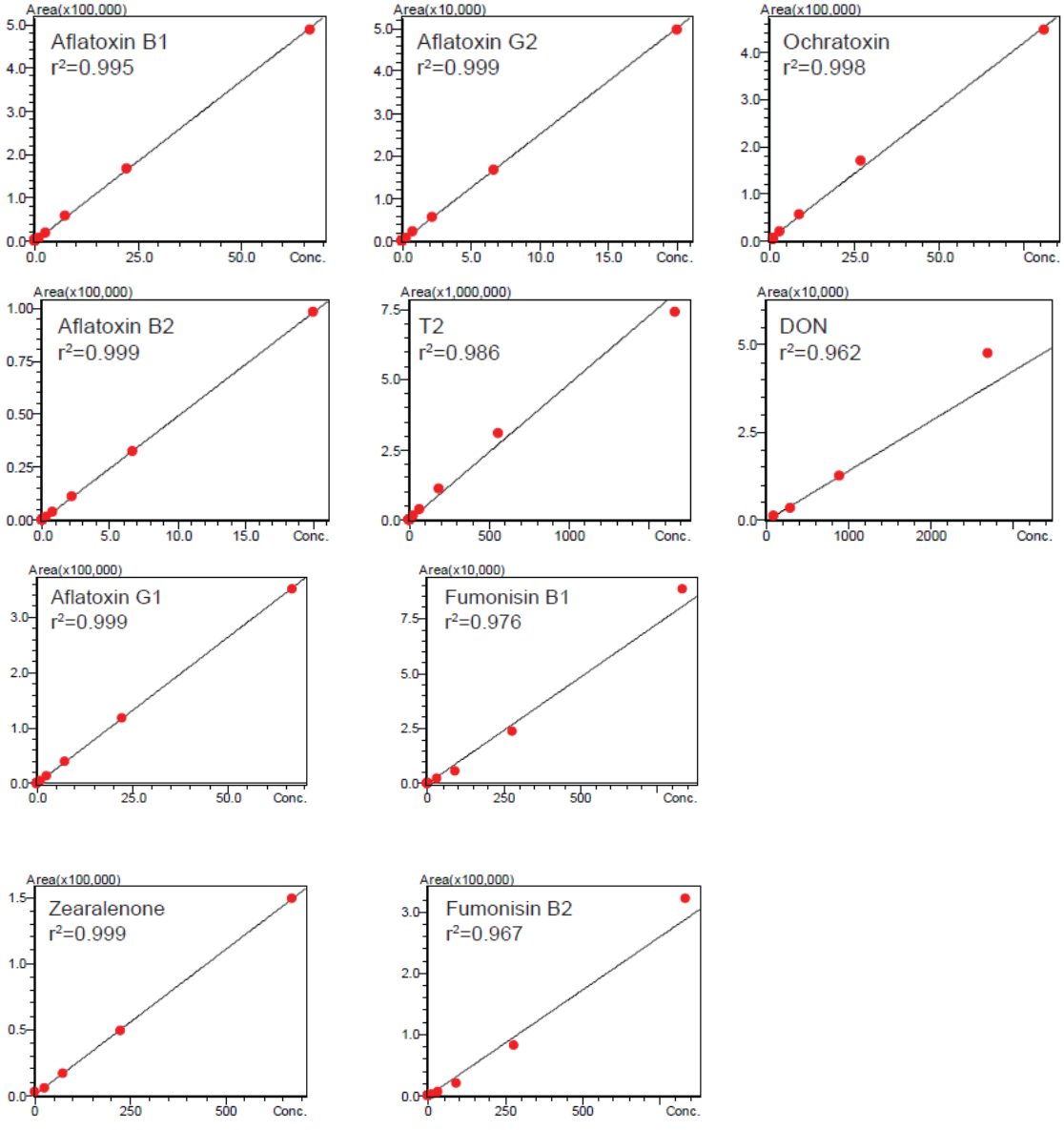


Figure 3: Calibration curves for each mycotoxin

Table 2: Percent Accuracy of mycotoxin standards in solution (*n*=5)

	L2	L3	L4	L5	L6
Aflatoxin B1	99.48	100.52	99.72	100.40	99.90
Aflatoxin B2	100.86	101.20	102.90	93.68	102.72
Aflatoxin G1	97.94	100.54	101.70	100.26	99.72
Aflatoxin G2	101.58	100.88	103.90	91.36	104.60
DON	137.40	114.40	93.42	81.68	107.58
Ochratoxin	102.58	96.40	94.30	113.40	97.10
ZON	84.30	101.86	123.68	132.42	82.62

Calibration curves were prepared for each mycotoxin with a weighting factor of 1/A^2. The curves were linear ($R^2 > 0.95$) in the tested range. The calibration curves are shown in Figure 3. Five replicates of each standard were analyzed and the accuracy reported in Table 2.

Table 3 Measured Accuracy

Fumonisin B1	Measured (ppb)	Expected (ppb)
Corn	616.8	900.0
	894.8	900.0
	5268.6	28300.0
	7669.4	6760.0
	33398.4	28300.0
	3185.0	2700.0

Fumonisin B2	Measured (ppb)	Expected (ppb)
Corn	209.0	300.0
	282.4	300.0
	1550.9	7100.0
	2167.6	1850.0
	8436.9	7100.0
	822.6	700.0

Aflatoxin B1	Measured (ppb)	Expected (ppb)
Corn	149.5	103.3
	5.7	4.2
	24.6	13.8
	27.7	18.8
Oatmeal	26.7	26.0
	11.9	15.0

Aflatoxin B2	Measured (ppb)	Expected (ppb)
Corn	13.4	7.2
	1.8	0.8
	1.9	0.9
Oatmeal	7.6	7.8
	3.4	4.5

Aflatoxin G1	Measured (ppb)	Expected (ppb)
Corn	4.6	2.5
Oatmeal	25.4	26.0
	11.1	15.0

Aflatoxin G2	Measured (ppb)	Expected (ppb)
Corn	ND	0.0
Oatmeal	7.3	7.8
	3.9	4.5

Zearalenone (ZON)	Measured (ppb)	Expected (ppb)
Corn	203.3	352.0
	112.5	105.5
	693.9	1014.7
	1252.8	1295.9
Oatmeal	91.2	40.0
	118.3	75.0

Ochratoxin	Measured (ppb)	Expected (ppb)
Corn	86.3	64.5
	45.5	61.9
	76.5	64.5

Deoxynivalenol (DON)	Measured (ppb)	Expected (ppb)
Wheat	903.9	500.0
	3271.8	5100.0
	1788.9	2500.0
	1267.2	500.0
	5707.4	6200.0
	6245.9	5100.0

Mycotoxin T-2	Measured (ppb)	Expected (ppb)
Corn	265.6	263.7
	284.7	252.5

The lower limit of quantitation (LLOQ) for each mycotoxin standard in solution corresponds approximately to the most dilute level reported in Table 1. For Fumonisin B1 and B2, the LLOQ was lower than 10 ppb and for Mycotoxin T2 the LLOQ was significantly lower than the lowest tested concentration of 20.6 ppb. Measured values for each mycotoxin are shown in Table 3. The rapid chromatographic method enables fast analysis of many samples whether the sample preparation is SPE or a simple solvent extraction.

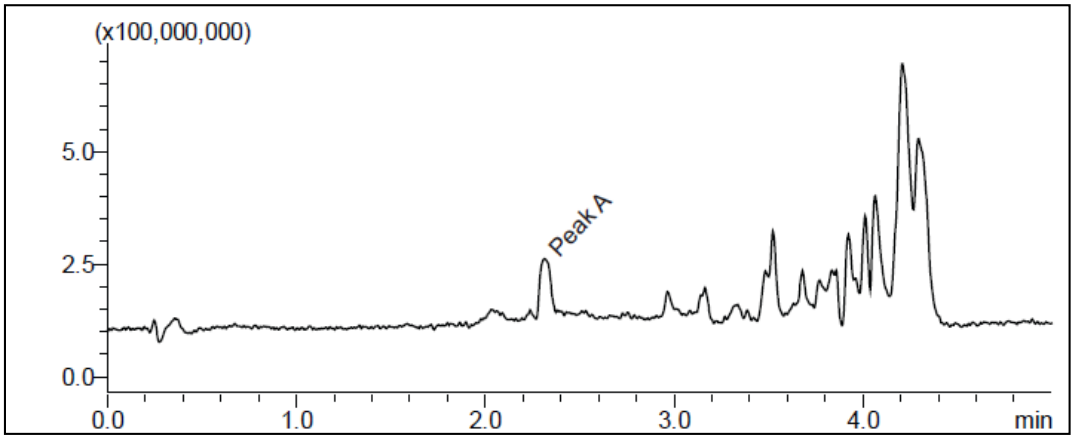


Figure 4: Total ion chromatogram from a feed-corn sample. The peak at 2.25 minutes was automatically selected for data-dependent MS/MS

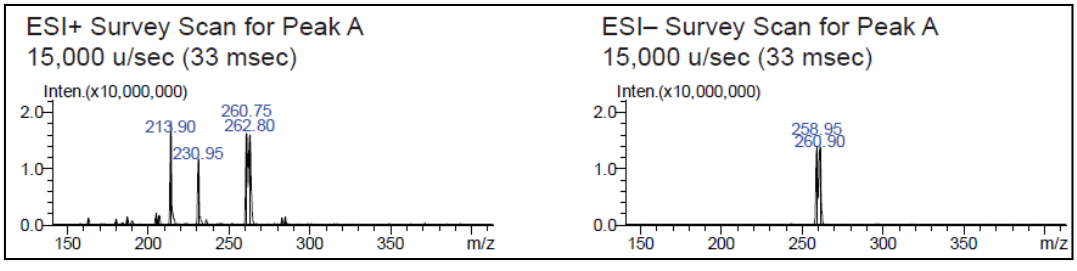


Figure 5: Survey scans of Peak A

Survey scanning at extremely fast scan speeds of 15,000 u/sec enables qualitative data to be collected without compromising quantitative performance. Automatically triggered fast tandem mass spectra were collected and used to search a library of known contaminants for untargeted screening. The mass and tandem MS spectrum of Peak A matched the pesticide Bromacil as shown in Figure 6. Even though the transitions specifically corresponding to Bromacil were not a part of the MRM method, the presence of Bromacil could be confirmed.

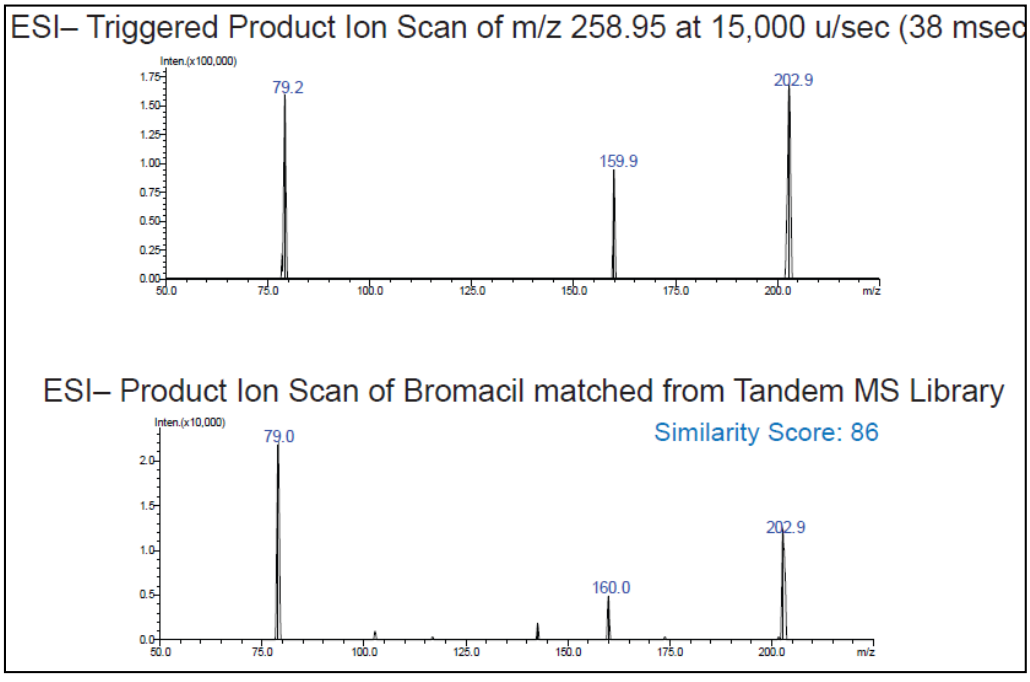


Figure 6: Data dependend product ion scan from Peak A matched to library spectrum of Bromacil.

CONCLUSION

A rapid, selective, and sensitive LC/MS/MS method for determination of mycotoxins as well as untargeted screening was developed.

B-3 HIGH THROUGHPUT QUANTITATIVE ANALYSIS OF MULTI-MYCOTOXIN IN BEER-BASED DRINKS USING UHPLC/MS/MS

INTRODUCTION

Mycotoxins often exist as contaminants in grains. To ensure consumer food safety, manufactures of food and beverages have to strictly manage risks from such contaminants. To maintain the high-quality of food standards it is essential to rapidly determine the concentrations of hazardous mycotoxins in foods or beverages. UHPLC/MS/MS offers the best combination of selectivity, sensitivity, and speed for detection of these compounds in complex matrices. In this study, a high throughput method for the quantification of 14 mycotoxins in beers was developed. Highest sensitivity of analysis is crucial to food safety. Additionally, autosampler and system carry-over need to be monitored to ensure these factors do not become a problem. In these experiments, elimination of carryover was investigated through novel rinse condition cycles of the UHPLC (Nexera) autosampler.

EXPERIMENTAL

Fourteen mycotoxins (patulin (PAT), nivalenol (NIV), deoxynivalenol (DON), aflatoxins (AF) B1, B2, G1, G2, T-2 toxin (T-2), HT-2 toxin (HT-2), zearalenone (ZON), fumonisin (FM) B1, B2, B3 and ochratoxin A (OTA)) were determined by LC/MS/MS using a UHPLC system coupled to a LCMS-8030 triple quadrupole mass spectrometer. The MRM method of 14 mycotoxins was optimized on each compound-dependent parameter and MRM transitions (Q1/Q3). As a result, all compounds were detected with high sensitivity by ESI. AFB1, B2, G1, G2, T-2, HT-2, FMB1, B2, B3 and OTA were detected in positive mode, while PAT, NIV, DON, ZON were detected in negative mode. Ultra Fast Polarity Switching of 15 msec enabled simultaneous determination of the compounds in both modes.

Instrument parameters

LC conditions	
System	: Nexera
Column	: TriartC18 (100 mm L× 2.0 mm I.D.; 1.9 µm)
Column temperature	: 40 °C
Mobile phase	: A - 10 mM Ammonium acetate in water; B - 2% Acetic acid in methanol
Flow rate	: 0.4 mL/min

Gradient program :

Time (min)	Pump B conc.
0.01	2
3.00	55
7.00	85
8.00	85
8.01	2
11.00	2

MS conditions

System : LCMS-8030
Ionization : ESI, Positive and Negative; MRM mode
Interface voltage : 3.5 kV
MRM transitions :

Mycotoxin	MRM Transition	
AF G1 (+)	329.05 > 243.05	
AF G2 (+)	331.00 > 245.00	
AF B1 (+)	313.00 > 241.05	
AF B2 (+)	315.00 > 259.00	
HT-2 (+)	442.00 > 263.05	([M+NH4]+)
T-2 (+)	483.95 > 305.00	([M+NH4]+)
OTA (+)	404.10 > 238.90	
ZON (−)	317.15 > 273.00	
NIV (−)	371.10 > 281.25	([M+CH3COO]-)
DON (−)	355.10 > 295.15	([M+CH3COO]-)
PAT (−)	153.10 > 109.20	
FM B1 (+)	722.45 > 334.30	
FM B2 (+)	706.45 > 336.25	
FM B3 (+)	706.45 > 336.25	

Sample Preparation

Mycotoxins were extracted from samples and were purified with a solid phase extraction (SPE) cartridge. Twenty commercial beers were analyzed by using this method.

RESULTS ANS DISCUSSION

For UHPLC separation, various LC mobile phase conditions were examined. Tailing of fumonisins peaks were observed when only ammonium acetate was added in mobile phase. It was found that pH of a mobile phase affected peak shape of fumonisins. In order to reduce tailing of fumonisins, acetic acid was added in mobile phase B and the gradient program was controlled to maintain high concentration of acetic acid when fumonisins were eluted. By controlling the concentration of acetic

acid and ammonium acetate with gradient program, 14 mycotoxins were separated and detected excellently in 11 minutes (refer Figure 1).

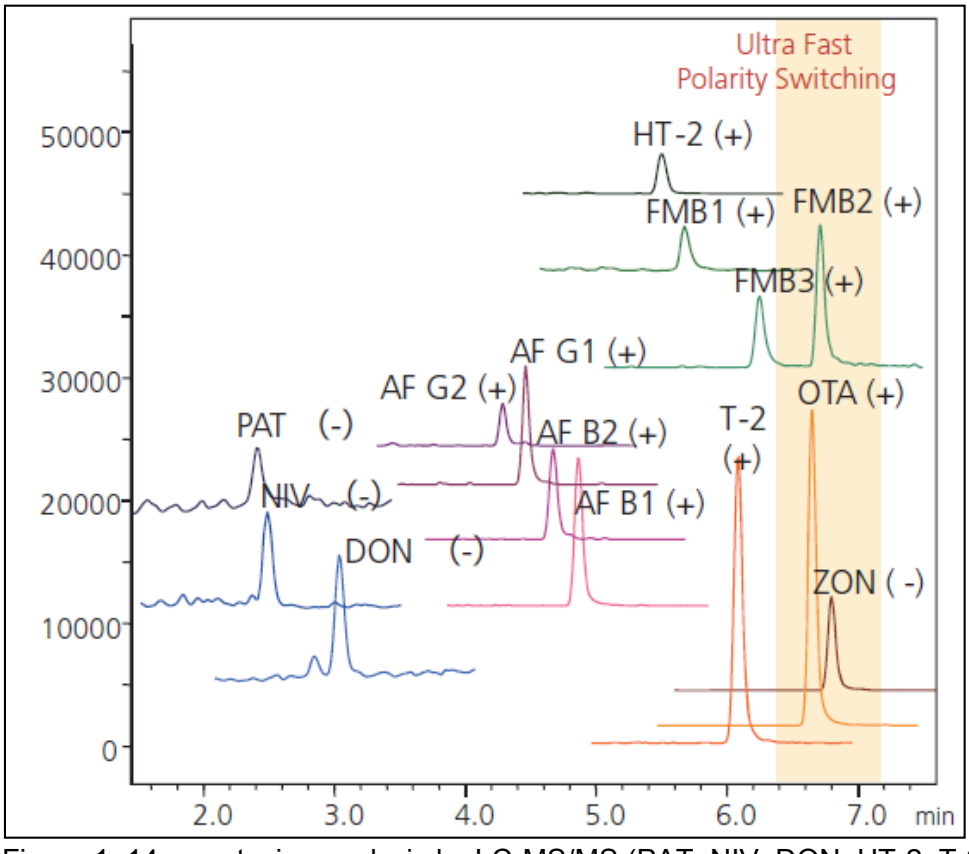


Figure 1. 14 mycotoxins analysis by LC-MS/MS (PAT, NIV, DON, HT-2, T-2, OTA, ZON, FM B1/B2/B3 50ppb; AF B1/B2/G1/G2 10 ppb)

Each mycotoxin standard was analyzed at six concentration levels. Good linearity was observed for calibration curves, and excellent sensitivity was achieved as can be seen in table 1.

Table 1. Linearity 14 mycotoxins

Mycotoxin	Calibration Range	Coefficient(R^2)
AF G1	0.4-20 ppb	0.999
AF G2	0.4-20 ppb	0.999
AF B1	0.4-20 ppb	0.999
AF B2	0.4-20 ppb	0.999
HT-2	2-100 ppb	0.998
T-2	2-100 ppb	0.999
OTA	2-100 ppb	0.999

ZON	2-100ppb	0.999
NIV	2-100ppb	0.999
DON	2-100ppb	0.997
PAT	10-100ppb	0.999
FM B1	2-100ppb	0.995
FM B2	2-100ppb	0.994
FM B3	2-100ppb	0.997

Rinse condition for eliminating carry over

Carryover of fumonisins was initially observed using the general rinse condition, because fumonisins formed complexes with trace metal ions in the sample's flow path. Probably, several carboxyl groups of fumonisins coordinated with metal ion (Figure 2).

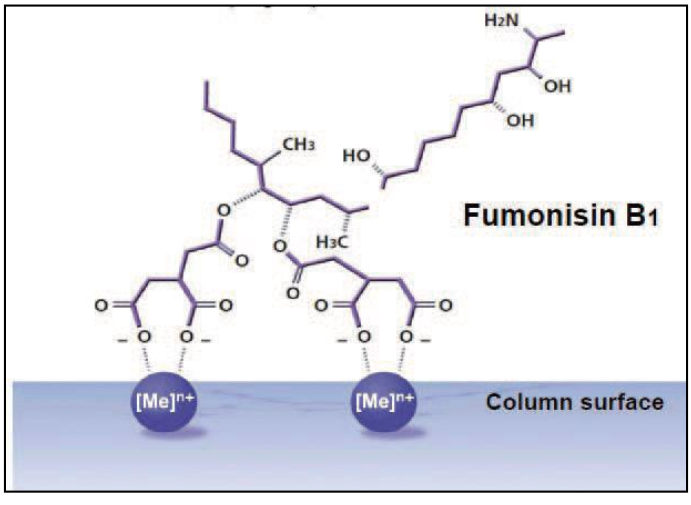


Figure 2. Possible coordination interaction with metal ion

For eliminating carry over, rinse solvent and rinse method were examined. The performance of Nexera autosampler SIL-30AC, which can wash both inner and outer needle surfaces with 4 different solvents was used. It was thought that carboxyl groups of fumonisins may preferentially pair with hydrogen ions in the presence of low pH. Therefore, formic acid was added to rinse solvent. When investigating rinse methods, it was discovered that the inner and outer rinse of needle reduced carry over more than the outer rinse of needle. Finally, the modified rinse solvent consisted of: 1% formic acid aq./methanol/acetonitrile/isopropanol (1/1/1/1). To test the modified rinse cycle method, one injection of the 100 ppb fumonisins standard solution was followed by one blank injection to check for carryover. Figure 3 shows chromatograms of the standards of FMB2 and B3, and the following blank injection. Low carry over was observed in the blank injection. It

resulted from washing fumonisins adsorbed inside needle with the needle's inner and outer rinse method and the effective rinse solvent.

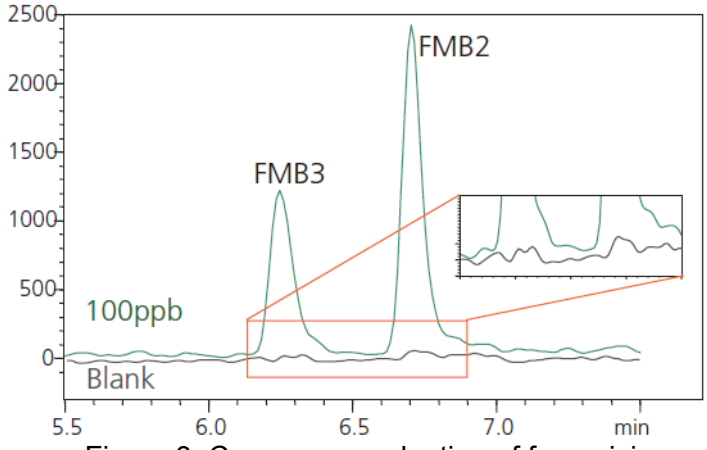


Figure 3. Carry over evaluation of fumonisins

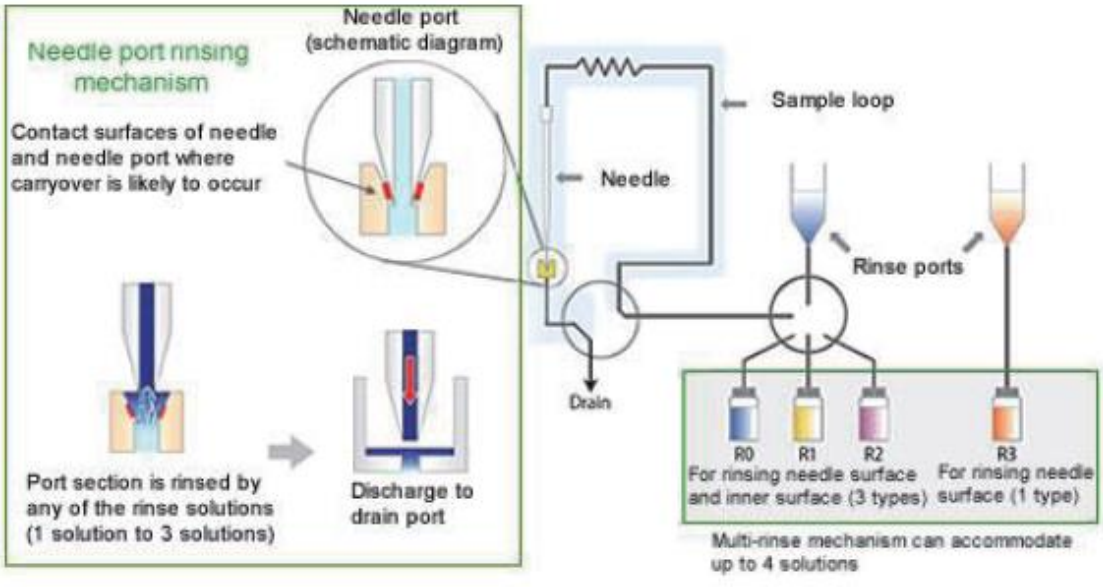


Figure 4. HPLC path of SIL-30AC

Quantitative Analysis of 14 mycotoxins in beer-based drinks

Mycotoxins were extracted from samples and were purified with a solid phase extraction (SPE) cartridge. Twenty commercial beers were analyzed by using this method. The calibration curves were assessed using beer samples spiked with mycotoxins. PAT, AFB1, B2, G1, G2, NIV, T-2 and ZON were not detected in any of the beer samples. Some of the tested samples were found to be contaminated

with DON, HT-2, OTA, FMB1, B2, and B3 at concentrations of less than their respective LOQs (each 5 ppb) as can be seen in Table 2.

Table 2. Mycotoxins detected in analyzed samples

Producing country	Concentration of mycotoxin/ppb (detected rate)					
	DON	HT-2	FMB1	FMB2	FMB3	OTA
Mexico (1sample)			< 5 (1/1)	< 5 (1/1)		
USA (1sample)	< 5 (1/1)					
China (1sample)	< 5 (1/1)					
Philippine (1sample)			< 5 (1/1)			
Australia (1sample)						
Japan (5sample)			< 5 (3/5)	< 5 (2/5)		

Producing country	Concentration of mycotoxin/ppb (detected rate)					
	DON	HT-2	FMB1	FMB2	FMB3	OTA
Hol l and (2samples)		< 5 (1/2)				< 5 (1/2)
Ireland (2sample)						
England (1sample)	< 5 (1/1)		< 5 (1/1)			< 5 (1/1)
Germany (1sample)						
Czech (1sample)						
Belguin (2samples)	6.7 (1/2)	< 5 (1/2)				
	< 5 (1/2)		< 5 (1/2)	< 5 (1/2)		

< 5 (less than 5ppb)

CONCLUSION

High throughput LC/MS/MS method for 14 mycotoxins was developed, and could be applied to the quantification of these compounds in beers. Carryover of fumonisins was eliminated by using both the needle's inner and outer rinse method with effective rinse solvent. Results from these experiments indicate that the health risk to consumers posed by intake of mycotoxins in commercial beers is relatively low.

SHIMADZU
Excellence in Science

B-4 RAPID AND HIGHLY SENSITIVE QUANTITATIVE ANALYSIS AND SCREENING OF AFLATOXINS IN FOODS USING LIQUID CHROMATOGRAPHY TRIPLE QUADRUPOLE MASS SPECTROMETRY

INTRODUCTION

Aflatoxins (AFs) are the most harmful mycotoxins produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* and can contaminate foods such as cereals and nuts. To reduce the risk of ingestion from food, analysis of AFs are carried out in many countries. It is necessary to quantitate the total aflatoxin (B1, B2, G1, G2 as shown in Figure 1) in foods by the regulation in JAPAN. The conventional LC/MS method proposed by the Ministry of Health, Labour and Welfare of Japan has a total analysis time of 30 minutes. In this study, we examined two alternative high-throughput LC/MS/MS methods. The first optimized for sensitivity & quantification; the second, a rapid screening method, using UHPLC for the purpose of increasing work flow.

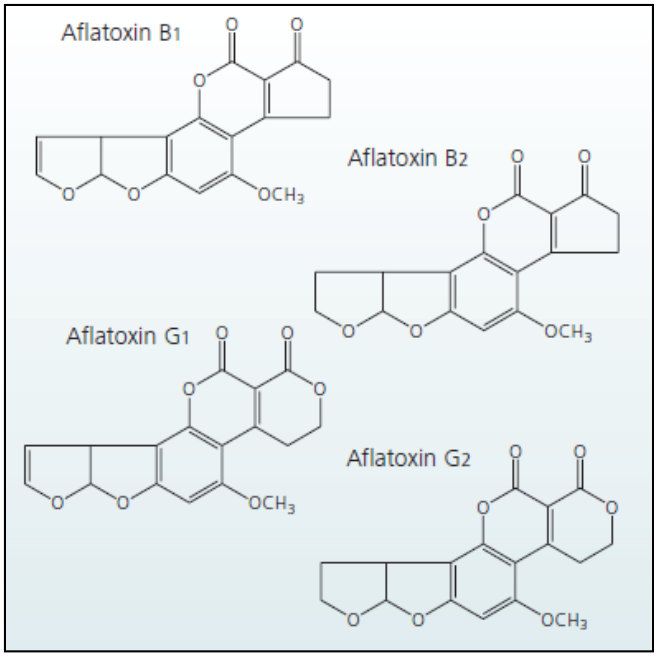


Figure 1. Structure of aflatoxins

EXPERIMENTAL

Sample preparation

AFs standard solution were obtained from Biopure, MYCOTOXIN MIX5 (AFLATOXINS) and Wako Chemicals, Aflatoxins Mixture Solution 1. Sample preparation work flow (Figure 2) shows how AFs in roast peanut was prepared by an immunoaffinity column (AFLAKING, HORIBA, JAPAN). Based on starting material 50 g (roast peanut powder) spiked Aflatoxin B1 and G1 (4 µg/Kg), B2 and G2 (1 µg/Kg) standard solution such that the final concentration of the sample solutions became: 2 µg/L B1 and G1; 0.5 µg/L B2 and G2.

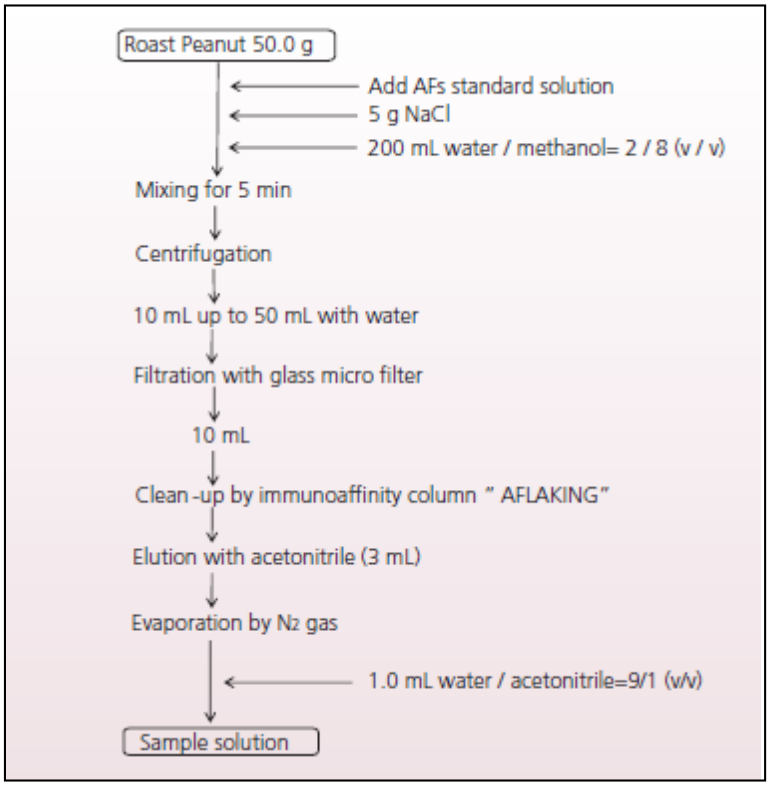


Figure 2. Sample preparation work flow

Instrument parameters

System configuration
LC : Nexera
LCMS : LCMS-8030
LC conditions
Conventional analysis
Column : Shim-pack FC-ODS (150 mm L x 2 mm I.D., 3 µm)
Mobile phase : A – 10 mM/L ammonium acetate in water
B – Methanol
Flow rate : 0.2 mL/min
Column temperature : 40 °C
Gradient program :

Time (min)	Pump B conc.
0.01	40
15.00	40
15.01	100
20.00	100
20.01	40
30.00	40

Injection volume : 6 µL

Optimized fast analysis
Column : Shim-pack XR ODS II (100 mm L x 2 mm I.D., 2.2 µm)
Mobile phase : A –10 mM/L ammonium acetate in water
B – Methanol
Flow rate : 0.45 mL/min
Column temperature : 50°C
Gradient program :

Time (min)	Pump B conc.
0.01	40
4.50	40
4.51	100
6.50	100
6.51	40
12.00	40

Injection volume : 6 µL
MS conditions (common for both)
Ionization : Electropspray Ionisation (ESI); Positive mode
Probe voltage : +4.5 kV
Nebulising gas : 3 L/min
Drying gas : 15 L/min
DL temperature : 250 °C
Heat block temperature : 400 °C

MRM conditions :

Compound	Transition	Pause time (ms)	Dwell time (ms)	CE (V)	Resolution (Q1,Q3)
Aflatoxin B1	313.10 > 240.95	3	100	-40	Unit
Aflatoxin B2	315.10 > 258.95	3	100	-33	Unit
Aflatoxin G1	328.90 > 242.95	3	100	-30	Unit
Aflatoxin G2	331.10 > 245.00	3	100	-32	Unit

Ultra High-speed method for screening analysis

LC conditions

Column : Shim-pack XR ODS III (50mm L x 2mm I.D.,1.6 µm)

Mobile phase : A –10 mM/L ammonium acetate in water
B – Methanol

Flow rate : 0.45 mL/min

Column temperature : 50 °C

Gradient program :

Time (min)	Pump B conc.
0.01	40
2.25	40
2.26	100
3.25	100
3.26	40
6.00	40

Injection volume : 6 µL

MS conditions

Ionization : Electropspray Ionisation (ESI); Positive mode

Probe voltage : +4.5 kV

Nebulising gas : 3 L/min

Drying gas : 15 L/min

DL temperature : 250 °C

Heat block temperature : 400 °C

MRM conditions :

Compound	Transition	Pause time (ms)	Dwell time (ms)	CE (V)	Resolution (Q1,Q3)
Aflatoxin B1	313.10 > 240.95	1	50	-40	Unit
Aflatoxin B2	315.10 > 258.95	1	50	-33	Unit
Aflatoxin G1	328.90 > 242.95	1	50	-30	Unit
Aflatoxin G2	331.10 > 245.00	1	50	-32	Unit

RESULTS AND DISCUSSION

The chromatogram and analytical conditions of the conventional method were compared to the high-speed method with using ultra-fast liquid chromatography. The flow rate was raised to 0.45 mL/min accelerating AFs elution to 4 min as shown in Figure 4 (operating back pressure 44-50 Mpa) as opposed to 13 min in conventional method (Figure 3).

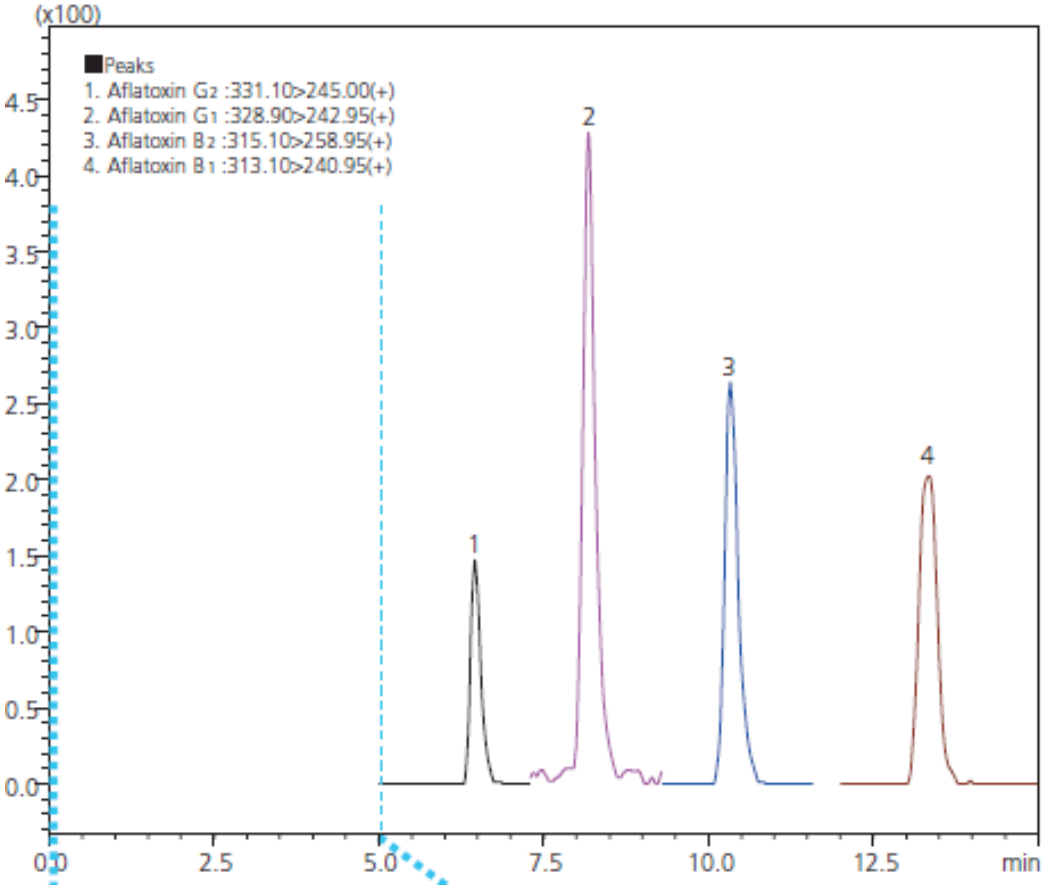


Figure 3. Chromatograms of AFs (0.5 µg/L each)

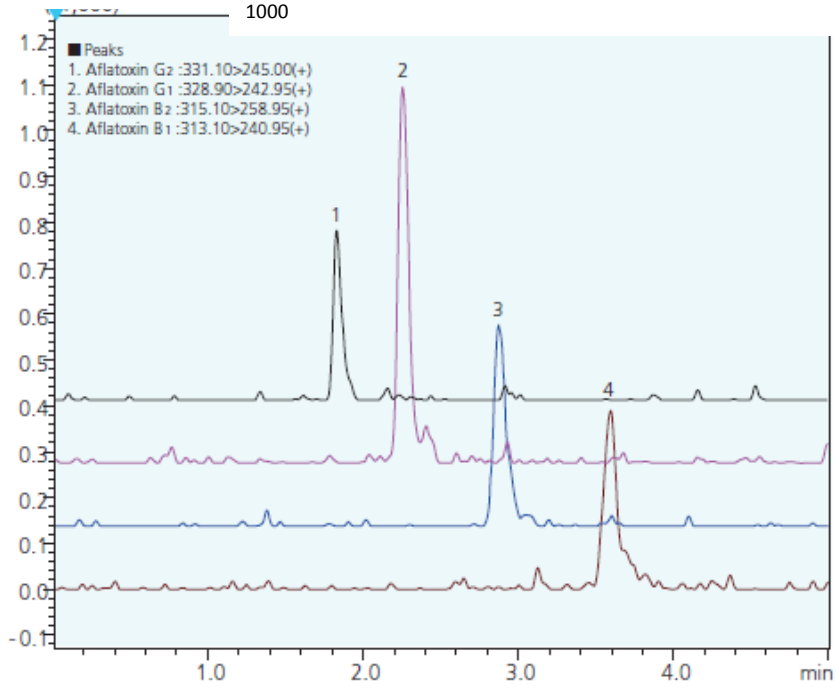


Figure 4. Chromatograms of AFs (0.5 µg/L each)

Excellent linearities with R^2=0.999 were obtained for all the AFs (Figure 5) and their respective sensitivities have been tabulated in Table 1.

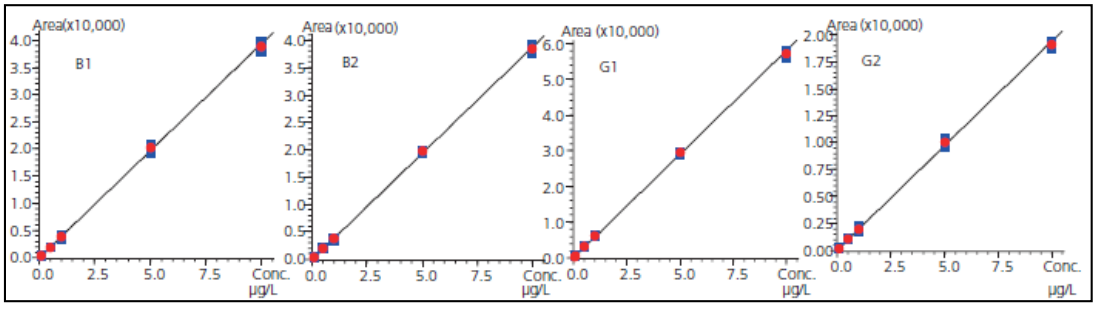


Figure 5. Calibration curves AFs (linearity beyond R^2=0.999 was acquired)

Table 1. L.O.Q. and Linearity (n=6)

Compound	L.O.Q. (µg/L)	Linearity	R^2
Aflatoxin B1	0.1	0.1-10 µg/L	0.9995372
Aflatoxin B2	0.1	0.1-10 µg/L	0.9997556
Aflatoxin G1	0.05	0.05-10 µg/L	0.9994336
Aflatoxin G2	0.1	0.1-10 µg/L	0.9992275

Effect of column temperature

Column temperature was optimized in order to accelerate compound elution without compromising peak shape or intensity. Finally a column temperature of 50 °C was chosen (Figure 6).

Effect of ESI probe position

Further optimisation was achieved through optimization of ESI probe position ranging from -2 mm to +3 mm from the central position (Figure 7). Chromatographic comparison (Figure 8) illustrates both peak intensity and level of noise are influenced by probe position. Optimization required highest S/N and minimum noise at +1 mm (Figure 9 & 10). [Noise was calculated by ASTM method with 3 blocks of 0.5 min around each peak]

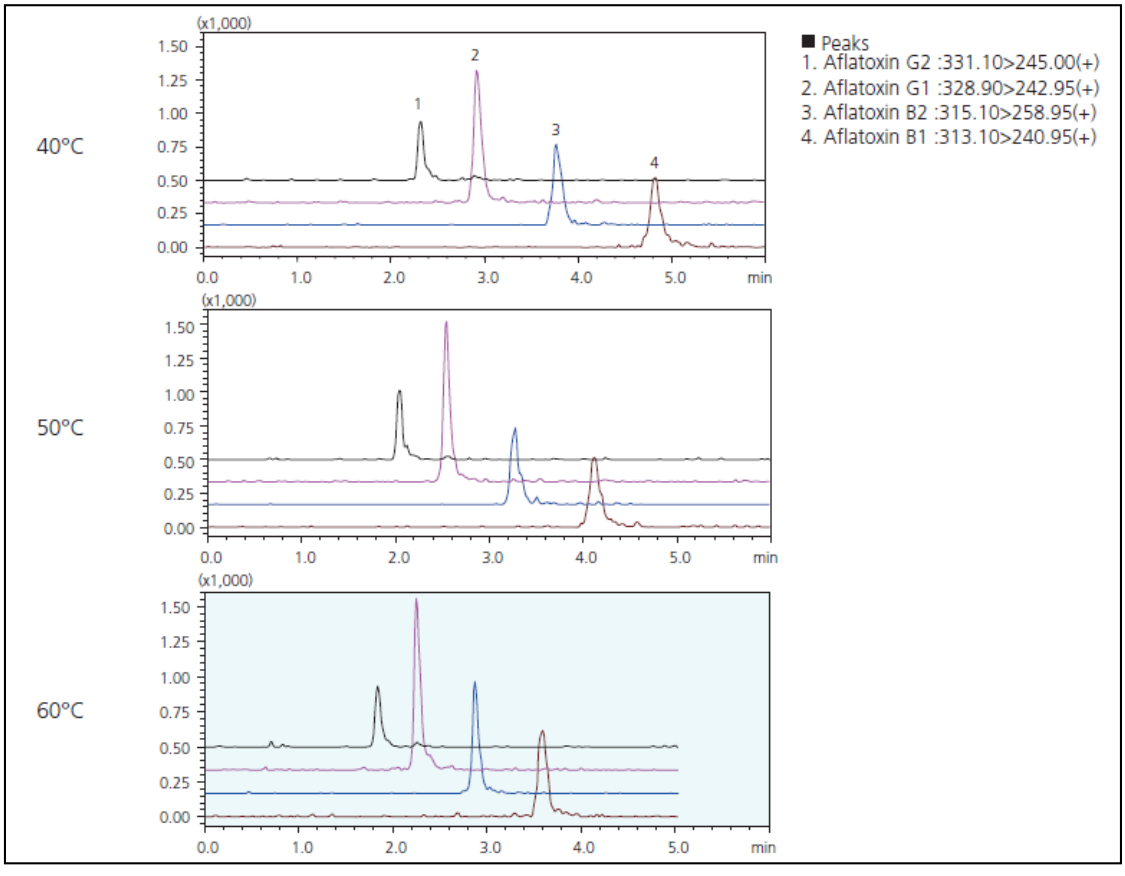


Figure 6. Chromatographic optimization of column temperature

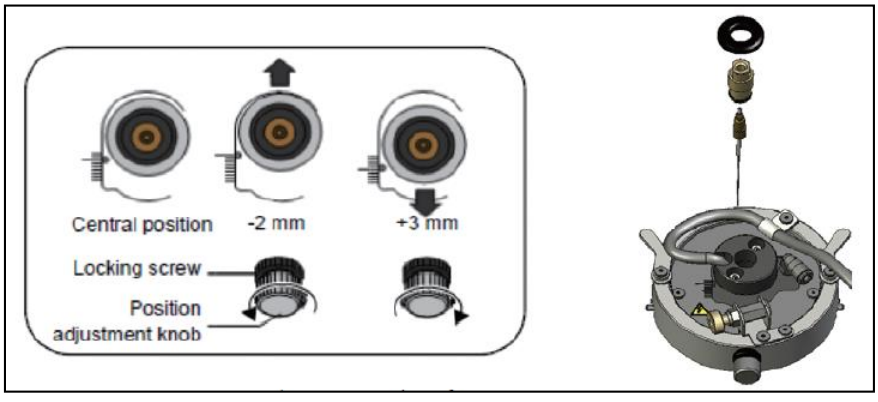


Figure 7. ESI probe of LCMS-8030

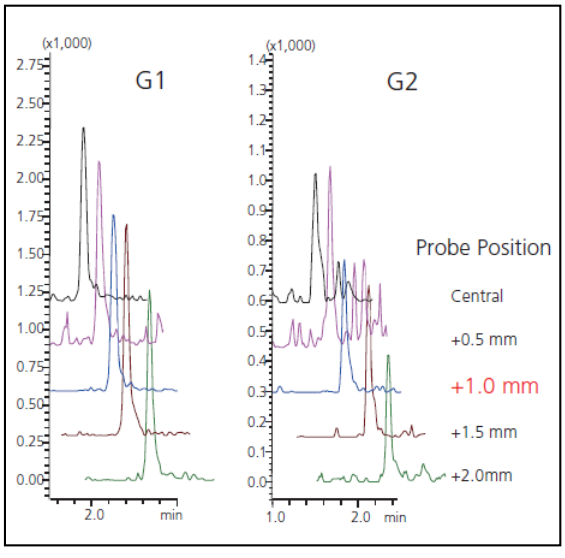


Figure 8. Chromatogram comparison of ESI probe position difference

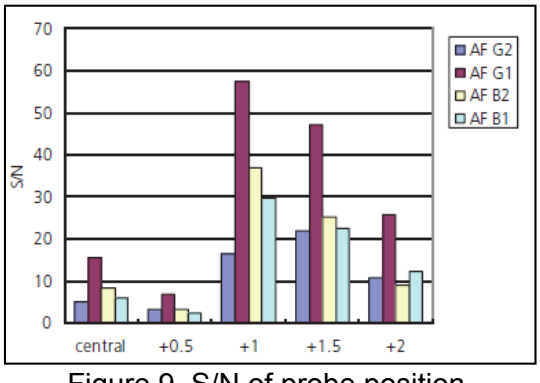


Figure 9. S/N of probe position difference

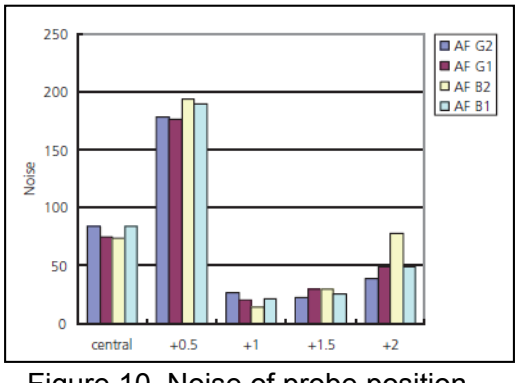


Figure 10. Noise of probe position difference

Analysis of Roast Peanut Matrix

The recovery test of AFs spiked into the roast peanut powder was performed in duplicate experiments. Overlaid chromatogram comparison of spiked AFs in roast peanut matrix with un-spiked are shown (Figure 11). Interference peaks were not detected in un-spiked samples.

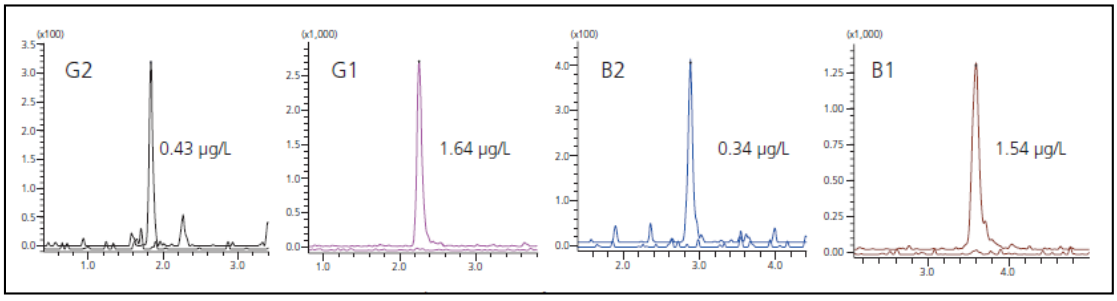


Figure 11. Chromatograms of AFs in roast peanut matrix

Quantitation results using external standard method show that the recovery rate was in the range of 69-86 % (Table 2). This relatively low recovery rate is a known problem when extracting AFs with a solvent from the powder of a roast peanut. Further method development is underway to increase recovery rate.

Table 2. Results of quantitative analysis

	Aflatoxin B1		Aflatoxin B2		Aflatoxin G1		Aflatoxin G2	
	Conc. (µg/L)	Recovery (%)	Conc. (µg/L)	Recovery (%)	Conc. (µg/L)	Recovery (%)	Conc. (µg/L)	Recovery (%)
Sample1	1.54	75	0.34	69	1.64	82	0.43	86
Sample2	1.44	70	0.34	69	1.64	82	0.37	74

Ultra High-speed Method for screening analysis

Further improvements to speed up analysis were made using a reduced column particle size and length (1.6 µm, Shim-pack XR-ODS III 50 mm L × 2.0 mm I.D.). With these conditions, AFs eluted within 2 minutes with LOQ of 0.5 µg/L. This ultra high-speed analysis could prove useful when screening many samples.

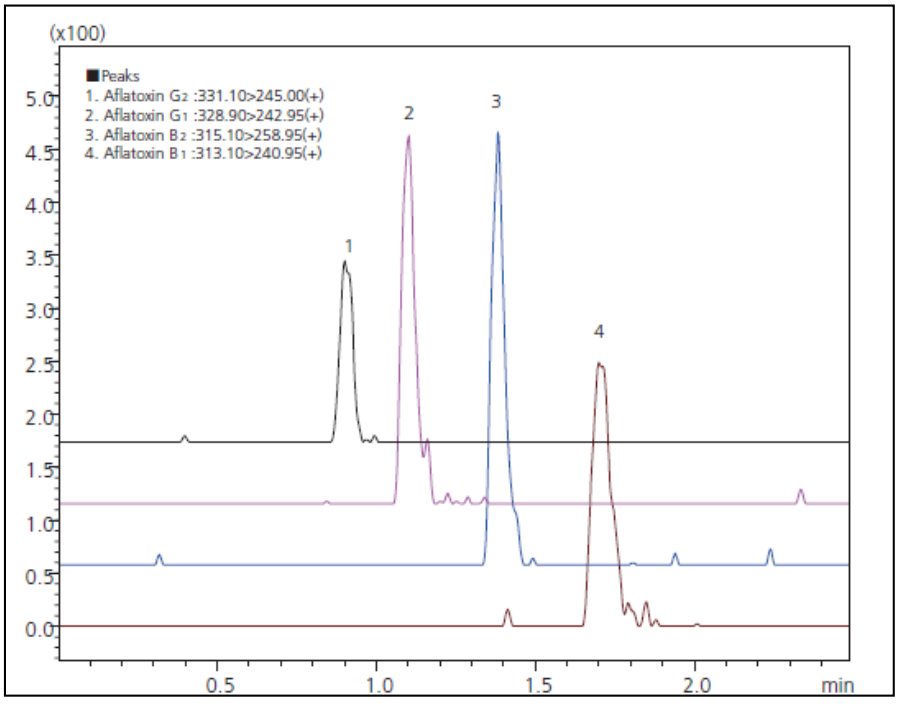


Figure 12. Chromatograms of AFs (0.5 µg/L each)

CONCLUSION

Analysis of AFs in the roast peanut was studied. Accelerated method was developed due to capacity of ultra high pressure liquid chromatography (Nexera). New choice of Shim-pack column enabled faster elution times. Two high-speed methods were developed eluting AFs within 4 and 2 min. Column temperature and ESI probe position were important conditions of AFs analysis. Immunoaffinity column was useful for cleanup from the roast peanut matrix. The results of recovery test was between 69-86%.

B-5 QUANTITATION OF AFLATOXINS FROM CHILI MATRIX USING LC/MS/MS

INTRODUCTION

Aflatoxins are the most potent carcinogens of mycotoxins and are commonly found contaminants in spices, dry fruits, edible nuts and cereals. These toxins are only produced by some strains of *Aspergillus flavus* and *Aspergillus parasiticus* moulds, if they encounter appropriate environments. The most potent aflatoxins are B1, B2, G1 and G2 all of which have been found in chili. If contamination has occurred, it is not possible to destroy aflatoxins by processing or cooking as they are chemically very stable.

India is the world's largest producer, consumer and exporter of chilies. So as to meet export standards, it becomes imperative to satisfy safety guidelines set by different countries. Few examples of the maximum limit of aflatoxins in human food are as follows:

- USA, 20 µg/kg (Food and Drug Administration, 1999)
- Australia, 5 µg/kg (Australian and New Zealand Food Authority, 1996)
- Germany, 2 µg/kg for Aflatoxin B1 and 4 µg/kg for total aflatoxin (German Aflatoxin Regulation)

The above regulations, in addition to the inherent chemical stability of aflatoxins, makes it essential to quantitate these toxins at low levels so as to ensure safety of the food consumed. Also, matrix like chili demands analytical methods to be more specific towards the analytes of interest in presence of complex interferences. This has increased the popularity of LC/MS/MS as a suitable analytical tool. The high sensitivity of Shimadzu Triple Quadrupole Liquid Chromatograph-Mass Spectrometer LCMS-8040 system alongwith its ease of maintenance ensures reliable quantitative analysis of aflatoxins from complex matrices like chili. Also, the Ultrafast MRM capabilities of LCMS-8040 (555 MRM transitions/second) alongwith minimised dwell time (1 msec) and pause time (1 msec), makes it well suited for UHPLC analysis where analysis cycle times are reduced so as to achieve high sample throughput. This application note aims at low level quantitation of aflatoxins in presence of chili matrix using LCMS-8040 system.

EXPERIMENTAL

Preparation of matrix matched standards

Commercially available red chili was powdered using mixer grinder. About 1 g of this chili powder was mixed with 20 mL acetonitrile using ultra sonicator for 10 mins. Mixture was centrifuged and supernatant was collected. This supernatant was

filtered with 0.22 µ nylon filter and used as diluent to prepare aflatoxin matrix matched mix standards (B1, B2, G1 and G2) at concentration levels of 0.05 ppb, 0.1 ppb, 0.2 ppb, 0.5 ppb, 1 ppb, 2 ppb, 5 ppb and 10 ppb.
Note: chili powder is a complex matrix and can exhibit matrix effect (either ion suppression or enhancement) during analysis. A calibration curve based on matrix matched standards can demonstrate true sensitivity of analyte in presence of matrix. Therefore, this method provides more reliable and accurate method of quantitation as compared to quantitation against neat standards.

Instrument parameters

LC conditions

System	: Nexera
Column	: Shim-pack XR-ODSII (100 mm L x 3 mm I.D.; 2.2 µ)
Guard column	: Phenomenex SecurityGuard ULTRA Cartridge
Mobile phase	: A - 10 mM ammonium formate in water B - Acetonitrile
Flow rate	: 0.4 mL/min
Column temperature	: 50 °C
Injection volume	: 5 µL
Gradient program	:

Time (min)	Pump B conc.
0.01	60
2.00	100
3.50	100
3.60	60
6.00	60

MS conditions

System	: LCMS-8040
MS interface	: Electro Spray Ionization (ESI)
Polarity	: Positive
Nebulizing gas flow	: 1.5 L/min
Drying gas flow	: 15.0 L/min
Desolvation line temperature	: 300 ºC
Heat Block temperature	: 500 ºC
MRM Transitions	:

Aflatoxin B1	313.00>241.00
Aflatoxin B2	315.00>259.00
Aflatoxin G1	328.90>243.10
Aflatoxin G2	331.00>245.00

RESULTS AND DISCUSSION

LCMS-8040 has a feature of 'Optimisation of method' in which the mass spectrometer selects the best product ion(s) and optimises voltages and collision energies for the precursor to product transition. Accordingly, the best MRM transitions were used to determine quantitation limits of aflatoxins B1, B2, G1 and G2. The results obtained are as tabulated in Table 1. Figures 1a to 1d shows the calibration curves for these aflatoxins. Figures 2a to 2d shows the chromatograms of various aflatoxins at their respective LOQ levels.

Table 1: Results for aflatoxins matrix match standards with calibration curve information

Compound name	Retention time (min)	Calibration range* (ppb)	Correlation coefficient (R^2)
Aflatoxin G2	1.37	0.2 – 10	0.9971
Aflatoxin G1	1.43	0.1 - 10	0.9995
Aflatoxin B2	1.47	0.05 – 10	0.9975
Aflatoxin B1	1.53	0.05 - 10	0.9987

* n=6 for each level

Area repeatabilty at LOQ levels for each aflatoxin showed % RSD between 11.3 – 15.2 %. For the rest of the levels, the area % RSD ranged between 0.7 to 13 % in the increasing order of linearity.

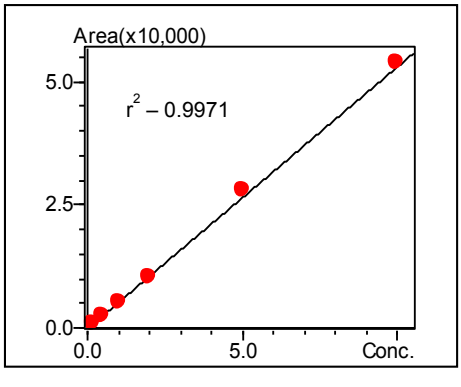


Figure 1a: Aflatoxin G2

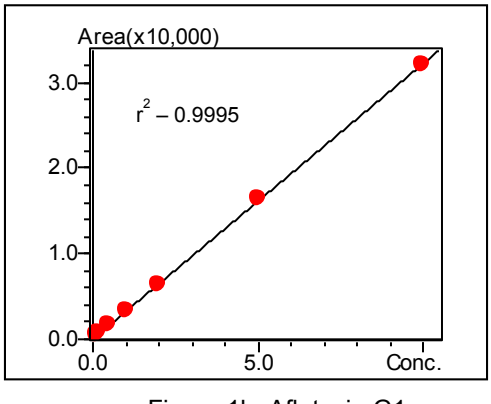


Figure 1b: Aflatoxin G1

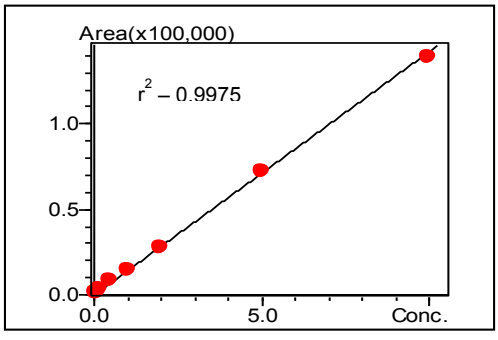


Figure 1c: Aflatoxin B2

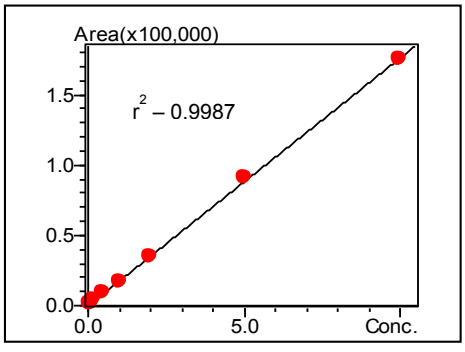


Figure 1d: Aflatoxin B1

Figures 1a to 1d: Calibration curves of Aflatoxin G2, Aflatoxin G1, Aflatoxin B2, Aflatoxin B1 are shown in Figures 1a to 1d respectively.

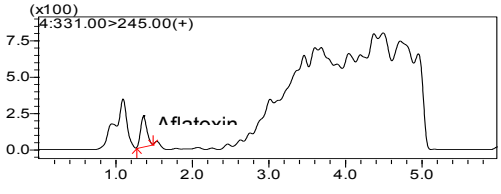


Figure 2a: Aflatoxin G2

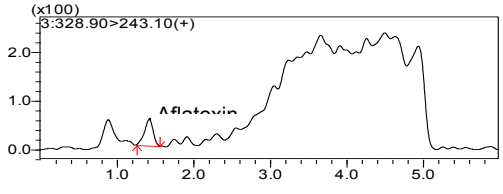


Figure 2b: Aflatoxin G1

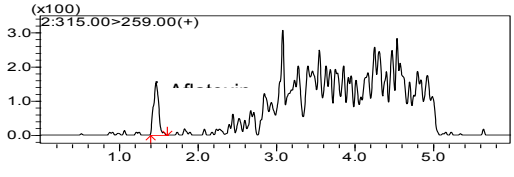


Figure 2c: Aflatoxin B2 at 0.05

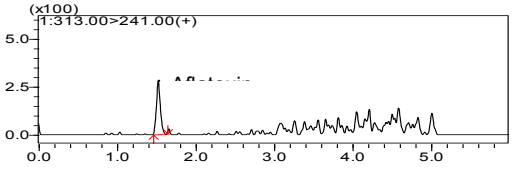


Figure 2d: Aflatoxin B1 at

Figures 2a to 2d: MRM chromatograms for matrix matched standards of Aflatoxins from chili. These concentrations correspond to the LOQ levels of the individual aflatoxins.

CONCLUSION

With the growing stringency in food safety sector, guidelines are constantly being revised so as to ensure safety of food products reaching the consumers. Accordingly, LC/MS/MS method was developed for simultaneous quantitation of aflatoxin at much lower levels in complex matrix like chili.

B-6 Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) Based Method for Routine Identification of Microorganism in Food Microbiology Lab

INTRODUCTION

MALDI-TOF MS is rapidly attracting the interest of microbiologists in food industry for routine analysis. The method allows rapid and reliable identification of microorganisms with minimal sample preparation. Cultured microorganisms can be directly analyzed using MALDI-TOF MS and automatically identified within minute. Here we present the application of the MALDI-TOF MS coupled to SARAMIS (Spectral ARchiving And Microbial Identification System, BioMérieux) for routine identification of microorganism in food microbiology. A total of 69 reference strains was subjected to evaluation, of which 66 samples were identified positively. Furthermore, 60 of the identified samples by MALDI TOF MS was in concordance to the given reference sample's identification. This demonstrates the applicability of the MALDI-TOF MS method for routine identification of microorganism samples in the Food Microbiology Lab.

EXPERIMENTAL

Fresh cells from individual colonies were transferred onto a stainless steel plate in duplicates for each specimen (Fig. 1). On the target plate, the cells were immediately extracted with 0.5 µl of matrix solution (10 mg/ml of a-cyano-4-hydroxy-cinnamic acid in acetonitrile: ethanol: water (1:1:1) with 0.3 % trifluoro acetic acid). The mixture was allowed to dry at room temperature. The prepared samples were measured on AXIMA Performance (Shimadzu Corporation). Mass spectra were acquired in positive linear mode using an acceleration voltage of 20 kV and a low mass gate of 1,500 Da. All spectra were processed with Shimadzu Biotech software and the peak lists were exported to the SARAMIS Software (BioMérieux) for identification.

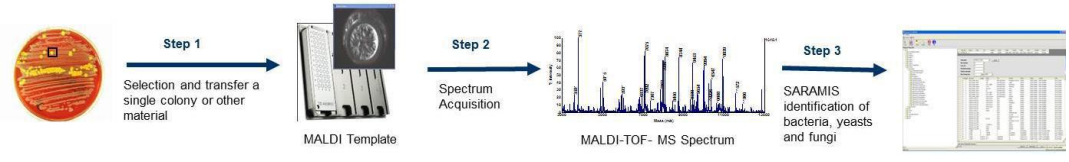


Fig.1: Workflow of MALDI TOF MS/SARAMIS Method

RESULTS AND DISCUSSION

Table 1: Table of identification results.

S/N	AVA's Reference id	SARAMIS identificaton result		
		Family	genus	species
1	9459vc-1, *Vibrio cholerae*	Vibrionaceae	Vibrio	cholerae/mimicus
2	9150-1, *Salmonella. P*	Enterobacteriace	Salmonella	enterica subsp.en
3	11555 vc-1,*V. cholerae*	Vibrionaceae	Vibrio	cholerae
4	9341-1, *Micrococcus*	Saccharomycetales	Candida	dubliniensis
5	7644-1, *Listeria. M*	Listeriaceae	Listeria	monocytogenes
6	33090-1, *Listeria. M*	Listeriaceae	Listeria	monocytogenes
7	51329-1, *Enterobacter Sakazakii*	Enterobacteriace	Citrobacter	koseri
8	35150-1, *E. coli*	Enterobacteriace		sp.
9	12759-1, *Bacillus*	Bacillaceae	Bacillus	licheniformis
10	11778-1, *Bacillus*	No ID		
11	11558 vc-1, *V. cholerae*	Vibrionaceae	Vibrio	cholerae
12	BAA-1152-1, *E. corrodens*	Neisseriaceae	Eikenella	corrodens
13	19258-1, *Streptococcus*	Streptococcacea	Streptococcus	salivarius
14	17666-1, *Staphylococcus*	Xanthomonadaceae	Stenotrophomonas	maltophilia
15	14990-1, *Staphylococcus*	Staphylococca	Staphylococcus	epidermidis
16	25923-1, *Staphylococcus*	Staphylococca	Staphylococcus	aureus
17	9290-1, *Shigella*	Enterobacteriace	Escherichia	coli
18	19585-1, *Salmonella*	Enterobacteriace	Salmonella	enterica subsp.en
19	19430-1, *Salmonella*	Enterobacteriace	Salmonella	enterica subsp.en
20	9150-1, *Salmonella*	Enterobacteriace	Salmonella	enterica subsp.en
21	8759-1, *Salmonella*	Enterobacteriace	Salmonella	enterica subsp.en
22	13076-1, *Salmonella*	Enterobacteriace	Salmonella	enterica subsp.en
23	AC1/11-1, *Bacillus*	Bacillaceae	Bacillus	subtilis
24	AC25/11-1,*Yersinia*	Enterobacteriace	Yersinia	enterocolitica
25	AC27/11-1, *Serratia*	Enterobacteriace	Serratia	liquefaciens
26	AC26/11-1, *Enterobacter*	Enterobacteriace	Enterobacter	amnigenus
27	AC6/11-1, *Bacillus*	Bacillaceae	Bacillus	cereus
28	94589-1, *V. cholerae*	Vibrionaceae	Vibrio	cholerae/mimicus
29	5/72 PV1, *V. cholerae*	Vibrionaceae	Vibrio	cholerae/mimicus
30	32/1033, *V. Para*	Vibrionaceae	Vibrio	sp.
31	51/76, *V. Vulnificus*	Vibrionaceae	Vibrio	parahaemolitycus
32	32/882, *Listeria Mono*	Listeriaceae	Listeria	monocytogenes
33	32/929, *Listeria Mono*	Listeriaceae	Listeria	monocytogenes
34	3/1368, *Ps. fluorescens*	Pseudomonadaceae	Pseudomonas	fluorescens
35	3/2292C, *Ps. fluorescens*	Pseudomonadaceae	Pseudomonas	fluorescens
36	3/1011B, *Citrobacter*	Enterobacteriace	Citrobacter	freundi
37	3/2424*, *Enterobacter*	Enterobacteriace	Enterobacter	cloacae
38	M16301 11A, *Ecoli 0157*	Enterobacteriace	Escherichia	coli
39	M16301 11B, *Ecoli*	Enterobacteriace	Escherichia	coli
40	49263, *Ecoli*	Enterobacteriace	Escherichia	coli

41	AC 107, *Aeromonas*	Aeromonadaceae	Aeromonas	hydrophila
42	4/1508A, *E.Durans*	No ID		
43	4/1508B, *E. Durans*	No ID		
44	AC100, *Enterococcus faecalis*	Enterococcaceae	Enterococcus	faecium
45	4/1508c, *Pediococcus pentosaceus*	Lactobacillaceae	Pediococcus	pentosaceus
46	4/1478. *Salmonella spp*	Enterobacteriace	Salmonella	enterica subsp.en
47	4/1477B , *Salmonella spp*	Enterobacteriace	Salmonella	enterica subsp.en
48	C16, *Campylobacter doylei*	Campylobacterace	Campylobacter	coli
49	C15, *Campylobacter jejuni 2*	Campylobacterace	Campylobacter	sp.
50	C7, *Campylobacter coli*	Campylobacterace	Campylobacter	jejuni
51	c9, *Campylobacter jejuni*	Campylobacterace	Campylobacter	coli
52	c8, *Campylobacter jejuni*	Campylobacterace	Campylobacter	jejuni
53	C3, *Campy spp*	Campylobacterace	Campylobacter	coli
54	C1, *C. jejuni 2*	Campylobacterace	Campylobacter	jejuni
55	*Ecoli*	Enterobacteriace	Escherichia	coli
56	S478, *Vibrio para*	Vibrionaceae	Vibrio	sp.
57	32/1519, *Vibrio para*	Vibrionaceae	Vibrio	sp.
58	46/450, *Myroides spp*	Vibrionaceae	Vibrio	sp.
59	46/456, *Salmonella spp*	Enterobacteriace	Salmonella	enterica subsp. en
60	46/450, *Salmonella spp*	Enterobacteriace	Salmonella	enterica subsp. en
61	Ac 96, *Bacillus cereus*	Bacilliaceae	Bacillus	cereus
62	*Ecoli*	Enterobacteriace	Escherichia	coli
63	Ac 86, *Pseudomonas fluorescens*	Bacilliaceae	Bacillus	cereus
64	Ac 82, *Pseudomonas aeroginosa*	Pseudomonadaceae	Pseudomonas	fluorescens
65	AC 94, *Pseudomonas aeroginosa*	Pseudomonadaceae	Pseudomonas	aeruginosa
66	AC 93. *Ecoli 0157*	Enterobacteriace	Escherichia	coli
67	AC 104, *Ecoli*	Enterobacteriace	Escherichia	coli
68	AC106, *Aeromonas*	Enterobacteriace	Citrobacter	sp.
69	AC107, *Aeromonas*	Enterobacteriace	Aeromonas	hydrophila

Green: Positive id and concordance (60 samples)
Yellow: Positive id but discordant (6 samples)
White blank: No id (3 samples)

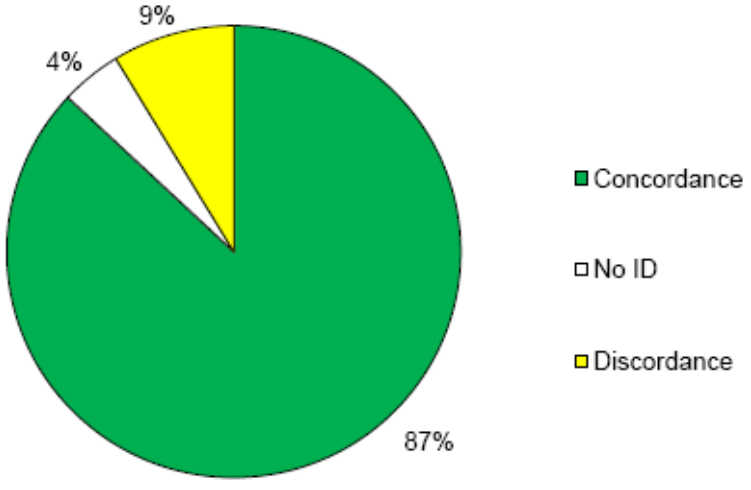


Fig. 2: Chart of identification results

Figure 2 shows the reliability of identification result by MALDI-TOF MS/SARAMIS method. Out of 69 reference samples, 66 samples (96%) were identified positively and 3 samples (4%) were not identified. In comparison to the reference id, 60 out of the 66 identified samples were in concordance (87%) and 6 were discordance (9%).

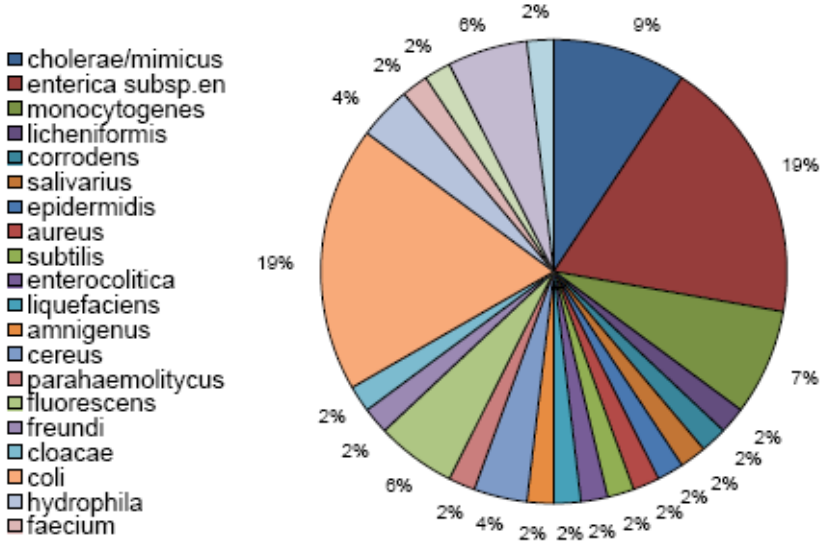


Fig. 3: Distribution chart of identified pathogens by species

Figure 3 shows the distribution chart of the 54 identified pathogens by species. Among the 60 identified isolates which are in concordance with the reference id, 54 samples were identified by MALDI-TOF MS to the species level: 5 samples (9%) were identified as *Vibrio chlorae*, 10 (19%) were identified as *Salmonella enterica subsp.en,* 4 (7%) were identified as *Listeria monocytogenes*, 1 (2%) was identified as *Bacillus licheniformis*, 1 (2%) was identified as *Eikenella corrodens*, 1 (2%) was identified as *Streptococcus salivarius*, 1 (2%) was identified as *Staphylococcus epidermidis*, 1 (2%) was identified as *Staphylococcus aureus*, 1 (2%) was identified as *Bacillus subtilis,* 1 (2%) was identified as *Yersinia enterocolitica*, 1 (2%) was identified as *Serratia liquefaciens*, 1 (2%) was identified *as Enterobacter amigenus*, 2 (4%) were identified as *Bacillus cereus*, 1 (2%) was identified as *Vibrio parahaemolitycus*, 3 (6%) were identified as *Pseudimonas fluorescens*, 1 (2%) was identified as *Citrobacter freundi*, 1 (2%) was identified as *Enterobacter cloacae,* 10 (19%) were identified as *Escherichia coli*, 2 (4%) were identified as *Aeromonas hydrophila*, 1 (2%) was identified as *Enterococcus faecium*, 1 (2%) was identified as *Pediococcus pentosaceus*, 3 (6%) were identified as *Campylobacter jejuni* and 1 (2%) was identified as *Pseudomonas aeruginosa*.

CONCLUSION

MALDI-TOF MS / SARAMIS is a rapid, reliable and easy method to use for the routine identification of food pathogens in microbiology laboratories. It offers high specificity in distinguishing samples up to the species or even subspecies level.

Liquid Chromatography Mass Spectrometry

B-7 Analysis of Diarrhetic Shellfish Toxin Using Triple Quadrupole LC/MS/MS (LCMS-8050)

The Japanese Ministry of Health, Labour and Welfare (JMHLW) specified in July, 1980 that the mouse bioassy (MBA) be used as the official method for diarrhetic shellfish toxin, and that the permissible exposure limit be 0.05 MU per gram of edible shellfish*). Shellfish in which the toxin exceeds this limit are prohibited from being sold at market according to the Japanese Food Sanitation Law Article 6, Item 2.

Due to significant technological advances since 1980, the sensitivity and accuracy obtained using the MBA method are significantly inferior compared to the high-precision, high-sensitivity possible using liquid chromatography mass spectrometry analytical instrumentation, which is currently used for this application. A complete transition to instrumental analysis for lipophilic marine biotoxins is scheduled to be implemented by January 2015 throughout the EU.

Based on this international trend, the JMHLW is currently considering migration to an instrumental analysis assay and setting new reference values to be used with instrumental analysis, in addition to the introduction of the Codex standard for okadaic acids (OA, Reference 1).

Table 1 CODEX Standard 292-2008

	Reference Value
OA Acids (OA and DTX group)	Permissible ingestion limit of 0.16 mg OA per kg of edible shellfish

Fig. 1 shows examples of LC/MS/MS high-sensitivity analysis of okadaic acid (OA), dinophysistoxin 1 (DTX1) and pectenotoxins (PTX1, 2, 6) and yessotoxin 1 (YTX1). Thus, it is possible to conduct high-sensitivity, high-separation analysis of each component.

Fig. 2 and Fig. 3 show MRM chromatograms of standard samples of OA and DTX1, respectively.

* The amount of toxin resulting in the death of two out of three mice following intraperitoneal administration of the equivalent of 20 g per edible shellfish.

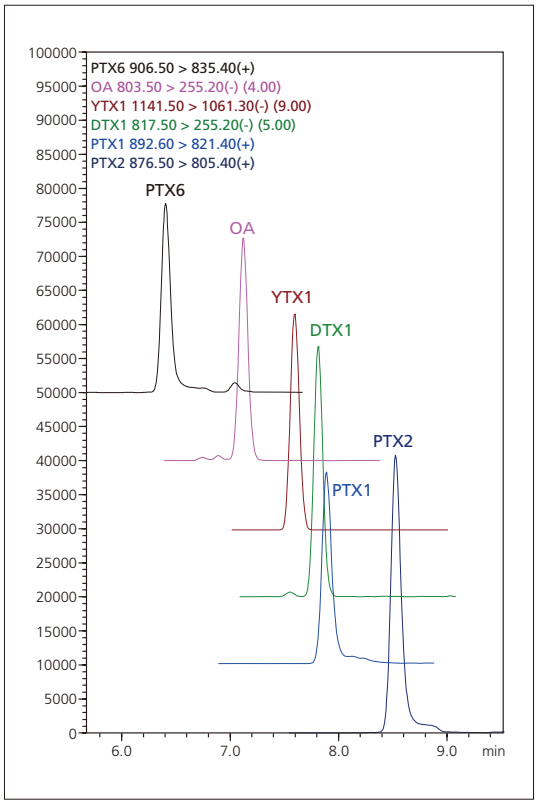


Fig. 1 MRM Chromatograms of Diarrhetic Shellfish Toxin (1 ng/mL)

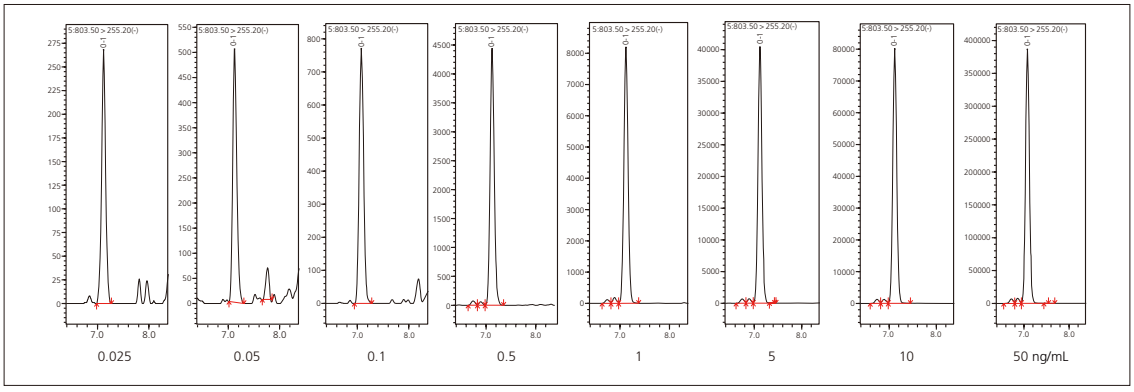


Fig. 2 MRM Chromatograms of Okadaic Acid (OA)

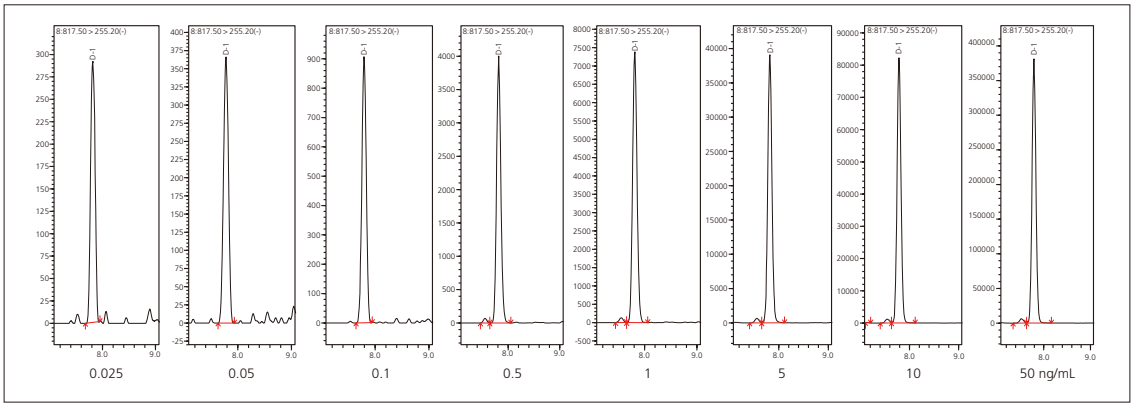


Fig. 3 MRM Chromatograms of Dinophysistoxin 1 (DTX1)

In addition, the calibration curves of OA and DTX1 are shown in Fig. 4. In both cases, the coefficient of determination R^2 was greater than 0.9999, indicating excellent linearity. Comparable linearity was also obtained for the other four substances.

Thus, instrumental analysis of shellfish by LC/MS/MS offers high sensitivity and accuracy, making it a highly effective analytical method. For this reason it is attracting attention as an alternative to the traditional MBA method.

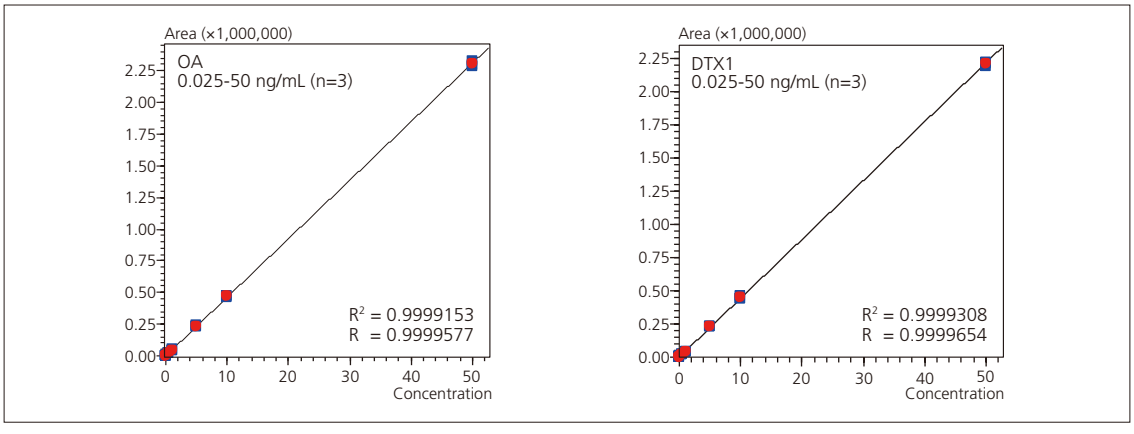


Fig. 4 Calibration Curves of OA and DTX1

Table 2 Analytical Conditions

Column	: InertSustain C8 (50 mm L. × 2.1 mm I.D., 3 µm)
Mobile Phases	: A 2 mmol/L Ammonium Formate – Water (pH adjusted to 8.5 with ammonia water)
	: B Methanol
Time Program	: 20 %B (0 min) – 100 %B (10 min) – 20 %B (10.01 min) – STOP (15 min)
Flowrate	: 0.2 mL/min
Column Temperature	: 40 ˚C
Injection Volume	: 10 µL
Probe Voltage	: +4.0 kV/-3.0 kV (ESI-positive / negative mode)
DL Temperature	: 200 ˚C
Block Heater Temperature	: 400 ˚C
Interface Temperature	: 350 ˚C
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 10 L/min
Heating Gas Flow	: 10 L/min
MRM Transition	: (+) PTX6 906.50 > 835.40, PTX1 892.60 > 821.40, PTX2 876.50 > 805.40
	: (-) OA 803.50 > 255.20, YTX1 1141.50 > 1061.30, DTX1 817.50 > 255.20

The diarrhetic shellfish toxin standards were provided courtesy of Dr. Toshiyuki Suzuki of the Japanese National Research Institute of Fisheries Science.

Access to your success
SHIMADZU

B-8

Application Note

Application Note No.4 (Lifescience)

Lifescience

Food Allergen Test
– Application of MultiNA –

1. What are Allergies?

We have immune functions that protect our bodies by creating antibodies to substances entering the body (antigens) that are foreign and hostile to the body. This is known as the "antigen-antibody reaction" or "immune response." When the same antigen subsequently enters the body, the memorized antibody activates and binds to the antigen to render it harmless.
However, if the immune functions go out of control, excess antibodies can be created or harmful antibodies produced in the body. This imbalance causes allergic diseases. Typical allergic diseases include atopic dermatitis, allergic rhinitis (hay fever, etc.), allergic conjunctivitis, allergic gastroenteritis, asthma, childhood asthma, food allergy, drug allergy, and hives.

Immunity was originally intended to protect the body from harmful substances. However, for people with a certain disposition, the immune function can activate in response to foods, pollen, dust and other substances which are usually harmless. People predisposed to such symptoms are said to have an "allergic predisposition."
When specific substances enter the body of a person with an allergic predisposition, the antibodies act abnormally to cause specific symptoms.

2-1 What are Food Allergies?

A hypersensitive immune reaction resulting from eating specific foods is called a "food allergy." Food allergies can cause a diverse range of symptoms, including skin symptoms such as hives and eczemas; gastrointestinal symptoms such as diarrhea, vomiting, and stomachache; and respiratory symptoms such as coughing and breathing difficulties. In severe cases, food allergies can lead to systemic symptoms, such as anaphylactic shock.

The substances causing food allergies and the amounts required differ from person to person. The reaction also differs according to the person's physical condition. For children, in particular, food allergies are often caused by the so-called "three major allergens": eggs, milk, and wheat. Of these, chicken eggs are the major cause of food allergies. Other causes are fish (in particular, blue-backed fish), meat (in particular, pork), shellfish, shrimp, crab, soybeans, cereals, and buckwheat.

Food allergies are mainly caused by proteins derived from the ingredients in the food. The three major allergens – eggs, milk, and wheat – are all foods with a high protein content. Normally, the proteins in foods are broken down in the stomach and intestines and absorbed as amino acids and peptides (several amino acids linked together). These small molecules do not normally cause allergies.

However, when the digestive tract and its functions are immature during infancy, inadequately digested proteins (oligopeptides) are often absorbed and are said to cause many food allergies. When the digestive functions are suppressed during illness, in particular, more undigested substances than normal pass through the digestive tract. As a result, undigested substances are more frequently absorbed and the incidence of allergies also increases.

The 2005 Ministry of Health, Labour and Welfare Science Research Report (Fig. 1) of Japan lists the following incidences of food allergies:

1. Eggs (38 % of total)
2. Dairy products (16 % of total)
3. Wheat (8 % of total)
4. Fruit (6 % of total)
5. Buckwheat (5 % of total)
6. Shrimp (4 % of total)
7. Peanuts (3 % of total)

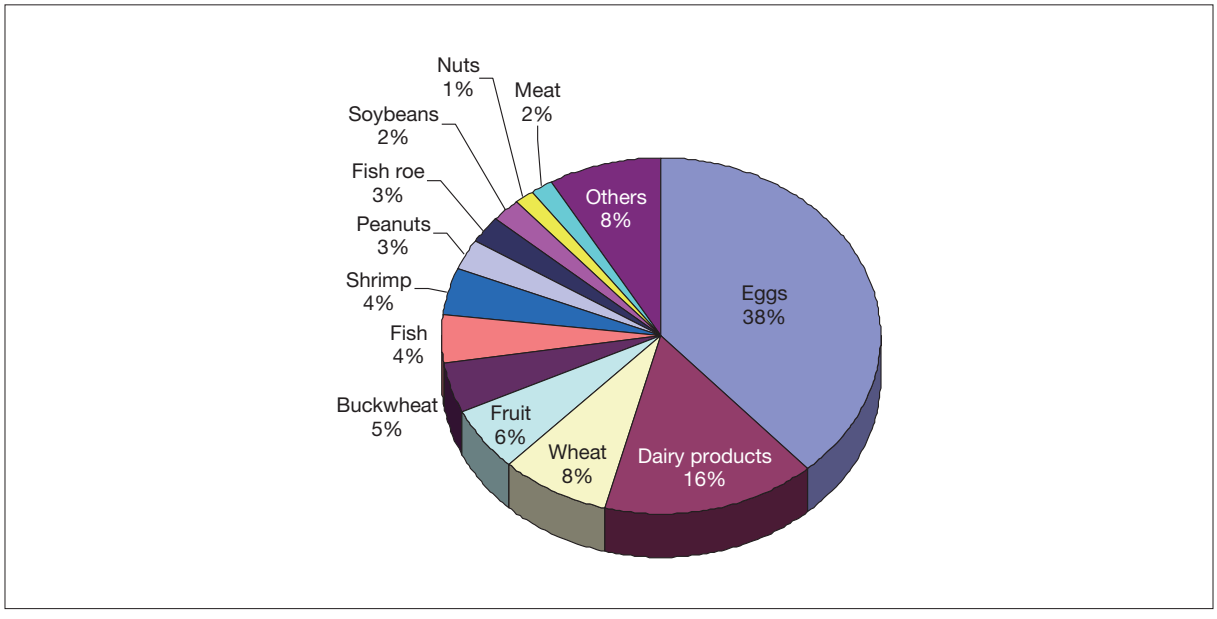
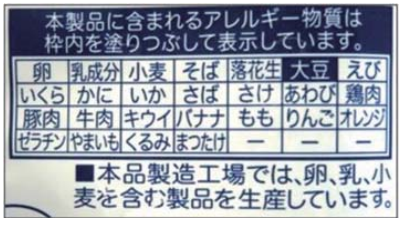


Fig. 1 Proportions of Foods Causing Allergies (source: 2005 Ministry of Health, Labour and Welfare Science Research Report of Japan)

3. Food Labeling

Japan was the world's earliest adopter of a labeling system for foods containing allergens (see Fig. 2).
The labeling of foods containing allergens is categorized into “Mandatory” (7 specified ingredients) and “Recommended” (18 specified ingredients).
Of the foods discovered to cause allergies in recent investigations, the five items with a high incidence or severity – eggs, milk, wheat, buckwheat, and peanuts – were prescribed as “specified ingredients” under the Japanese Ordinance for Enforcement of the Food Sanitation Act. Foods containing these ingredients were subject to mandatory labeling from April 2002. Two more items were added from June 2008: shrimp and crab. Labeling is required for foods containing 10 μg/g or higher of these seven specified ingredients, even if they are impurities mixed in during the manufacturing process. Labeling the possibility that the items could be included, such as "May contain xxx," is not permitted.
Eighteen other items for which labeling is recommended (items corresponding to specified ingredients) have been notified: abalone, squid, salmon roe, orange, kiwi fruit, beef, walnuts, salmon, mackerel, soybeans, chicken, banana, pork, matsutake mushroom, peach, yam, apple, and gelatin (Table 1). Labeling is intended to provide information to consumers to avoid health hazards due to allergies. Consequently, labeling must inform of even trace levels of specified ingredients contained in or mixed in a food product.
(*However, mandatory labeling of manufactured, processed, or imported food products was deferred to 3 June 2010.)

Recently, more and more food companies are producing products free of egg, milk, wheat, buckwheat, peanuts and other allergens. They implement strict product development, ingredient selection, production line cleaning (and subsequent checks), and inspections of individual production lots (according to the official method prescribed by the Japanese Ministry of Health, Labour and Welfare).



This product contains the allergens in the highlighted frames below.

Egg	Milk	Wheat	Buckwheat	Peanuts	Soybeans	Shrimp
Salmon roe	Crab	Squid	Mackerel	Salmon	Abalone	Chicken
Pork	Beef	Kiwi fruit	Banana	Peach	Apple	Orange
Gelatin	Yam	Walnuts	Matsutake mushroom	–	–	–

■ The factory that produced this product manufactures products containing egg, milk, and wheat.

Fig. 2 Example of Labeling

Table 1 Items Labeled as Allergens
(Source: March 2009 revision of "Handbook for Labeling of Processed Foods Containing Allergens," Japanese Ministry of Health, Labour and Welfare)

Labelling	Term	Name
Mandatory	Specified ingredients (7 items)	Egg, milk, wheat, buckwheat, peanuts, shrimp, crab
Recommended	Items pursuant to specified ingredients (18 items)	Abalone, squid, salmon roe, orange, kiwi fruit, beef, walnuts, salmon, mackerel, soybeans, chicken, banana, pork, matsutake mushroom, peach, yam, apple, and gelatin

*The scope of the specified ingredients is basically the range designated by the numbers in the Japan Standard Commodity Classification (JSCC). (For more details, see the March 2009 revision of "Handbook for Labeling of Processed Foods Containing Allergens," Japanese Ministry of Health, Labour and Welfare.)

4. Analysis of Allergenic Substances

Test methods have been established for 20 items: egg, milk, wheat, buckwheat, peanuts, shrimp, crab, abalone, squid, kiwi fruit, beef, walnuts, salmon, mackerel, soybeans, chicken, pork, yam, apple, and banana.
These test methods are included in the Japanese Ministry of Health, Labour and Welfare Notification "Regarding the testing method for foods containing allergenic substances," No. 0622003 issued by the Dept. of Food Safety, June 22, 2006.
Test methods for proteins derived from specified ingredients in foods include the ELISA method (*1) based on antigen-antibody reactions for quantitative analysis, Western blotting method (*2) for qualitative analysis, and PCR method (*3) (Table 2).
The ELISA quantitative test method is used for the screening of the seven specified ingredients – egg, milk, wheat, buckwheat, peanuts, shrimp, crab – as well as soybeans, which are listed as items corresponding to specified ingredients.
Western blotting method is generally used for the qualitative analysis of egg and milk.
The polymerase chain reaction (PCR) method uses specific sequences for the confirmation testing of the specified ingredients wheat, buckwheat, peanuts, shrimp, and crab (excluding egg and milk) and for soybeans, beef, pork, chicken, salmon, mackerel, abalone, squid, kiwi fruit, walnuts, yam, apple, and banana that are items corresponding to specified ingredients.

Table 2 Test Methods of Allergen

Test Method	Application
ELISA	Screening (quantitative)
Western blotting	Confirmation testing (qualitative)
PCR	Confirmation testing (qualitative)

Table 3 summarizes the test methods applicable for each item.

Table 3 Test Methods for Each Item

	Item	Test Method
Specified Ingredients Mandatory Labeling	Egg	ELISA, Western blotting
	Milk	ELISA, Western blotting
	Wheat	ELISA, PCR
	Buckwheat	ELISA, PCR
	Peanuts	ELISA, PCR
	Shrimp	ELISA, PCR
	Crab	ELISA, PCR
Items Corresponding to Specified Ingredients Recommended Labeling	Soybeans	ELISA, PCR
	Beef	PCR
	Pork	PCR
	Chicken	PCR
	Salmon	PCR
	Mackerel	PCR
	Abalone	PCR
	Squid	PCR
	Kiwi fruit	PCR
	Walnuts	PCR
	Yam	PCR
	Apple	PCR
	Banana	PCR

(*1) ELISA (Enzyme-Linked ImmunoSorbent Assay) Method
The Enzyme-Linked ImmunoSorbent Assay is an analysis method that combines an immunoreaction (antigen-antibody reaction) and an enzyme-substrate reaction. This method is used to detect and quantify the concentration of antibodies and antigens contained in the sample. This method is known as ELISA.

(*2) Western Blotting Method
After separating a sample by electrophoresis, it is transferred and bound to a membrane. It is reacted with an antibody (primary antibody) for the protein of interest. A secondary enzyme-marked antibody is reacted with the primary antibody and the target substance is detected through luminescence or fluorescence.

(*3) Polymerase Chain Reaction (PCR) Method
This method selectively amplifies part of the DNA, using the sample DNA as a template. Cycle reactions (separation of double-stranded DNA → primer binding → DNA synthesis) are performed using a primer (short sequence-specific single-stranded DNA with each end of the region to be amplified) and DNA polymerase to amplify the required DNA region. In principle, even a single DNA molecule can be amplified in multiples of the number of reaction cycles. The presence of the substance of interest can be evaluated from whether the regions straddling the primer are amplified.

5. Analysis by PCR

1) Extracting and Purifying DNA from Food Samples
The extraction and purification of DNA can be performed by the cetyltrimethylammonium bromide surfactant (CTAB) method or methods using a silica gel membrane or ion-exchange resin. Each method has its own characteristics. The CTAB method makes it difficult for PCR inhibitors to remain in the food. Commercial kits are available for extraction and purification methods using a silica gel membrane or ion-exchange resin, making them relatively simple to perform.
The CTAB method is applicable to test samples with a low degree of processing, such as wheat flour or buckwheat flour. Methods using a silica gel membrane or ion-exchange resin are applicable to test samples subjected to a high degree of processing, including sweetening, oil treatment, hot mixing, or fermentation.

2) Confirming DNA Purification and Quantitation
The extracted and purified DNA sample solution is diluted ten times and the absorbance measured at 230 nm, 260 nm, and 280 nm. In principle, the DNA sample solution is prepared at 20 ng/μL concentration.

3) PCR
The base sequence region of interest contained in the extracted and purified DNA is amplified by performing polymerase chain reaction (PCR) using the appropriate organism-specific primer (Table 4). These amplification products are separated and detected by electrophoresis to determine the absence or presence of the specified ingredient in the inspected sample. Fig. 3 shows the detection procedure.

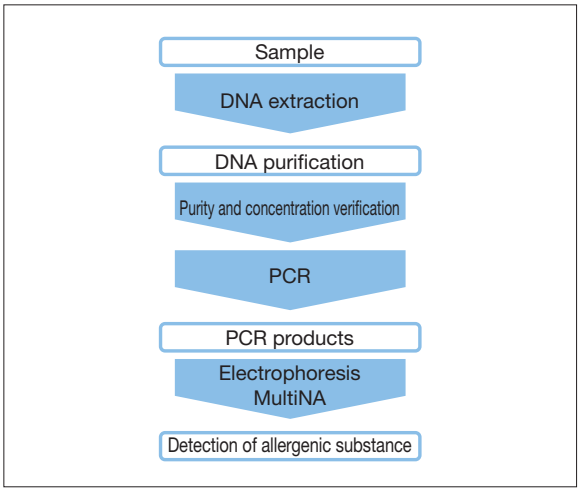


Fig. 3 Experimental Procedure for Detection of Allergenic Substances

Table 4 Primers for Enzyme Detection
Japanese Ministry of Health, Labour and Welfare Notification "Regarding the testing method for foods containing allergenic substances," No. 0724, Publication No. 1 issued by the Dept. of Food Safety, July 24, 2009 (See Note)

	Plant DNA	Animal DNA	Wheat	Buckwheat	Peanuts	Shrimp	Crab
PCR Amplification Product Size (bp)	124	370-470	141	127	95	187	62

	F-Primer	R-Prime
Plant DNA	CP03-5' : 5'-CGG ACG AGA ATA AAG ATA GAG T-3'	CP03-3' : 5'-TTT TGG GGA TAG AGG GAC TTG A-3'
Animal DNA	AN1-5': 5'-TGA CCG TGC GAA GGT AGC-3' AN2-5': 5'-TAA CTG TGC TAA GGT AGC-3' Use 1:1 mixture of AN1-5' and AN2-5'.	AN-3' : 5'-CTT AAT TCA ACA TCG AGG TC-3'
Wheat	Wtr01-5' : 5'-CAT CAC AAT CAA CTT ATG GTG G-3'	Wtr10-3' : 5'-TTT GGG AGT TGA GAC GGG TTA-3'
Buckwheat	FAG19-5' : 5'-AAC GCC ATA ACC AGC CCG ATT-3'	FAG22-3' : 5'-CCT CCT GCC TCC CAT TCT TC-3'
Peanuts	agg04-5' : 5'-CGA AGG AAA CCC CGC AAT AAA T-3	agg05-3' : 5'-CGA CGC TAT TTA CCT TGT TGA G-3'
Shrimp	ShH12-05' : 5'-TTA TAT AAA GTC TRG CCT GCC-3' ShH12-05' is synthesized as A and G mixed bases (R) to the 8th base from the 3' terminal.	ShH13-03'-1: 5'-GTC CCT CTA GAA CAT TTA AGC CTT TTC-3' ShH13-03'-2: 5'-GTC CCT TTA TAC TAT TTA AGC CTT TTC-3' ShH13-03'-3: 5'-GTC CCC CCA AAT TAT TTA AGC CTT TTC-3 Use a 1:1:1 mixture of ShH13-03'-1, ShH13-03'-2, and ShH13-03'-3.
Crab	CrH16-05'-1: 5'-GCG TTA TTT TTT TTG AGA GTT CWT ATC GTA-3' CrH16-05'-2: 5'-GCG TAA TTT TTT CTG AGA GTT CTT ATC ATA-3' CrH16-05'-3: 5'-GCG TTA TTT TTT TTA AGA GTA CWT ATC GTA-3' CrH16-05'-4: 5'-GCG TTA TTT CTT TTG AGA GCT CAT ATC GTA-3' CrH16-05'-1 and CrH16-05'-3 are synthesized as A and T mixed bases (W) to the 8th base from the 3' terminal. Use a 10:1:6:3 mixture of CrH16-05'-1, CrH16-05'-2, CrH16-05'-3, and CrH16-05'-4.	CrH11-03' : 5'-TTT AAT TCA ACA TCG AGG TCG CAA AGT-3'

* The amplification products of Shanghai hairy crab, Dungeness crab, giant spider crab, red queen crab, deep sea red crab, or swimming crab may be detected by PCR for shrimp. If it is unknown whether the amplification products obtained are derived from shrimp or crab, they can be identified by performing restriction enzyme digestion on the PCR products. For details, see the Japanese Ministry of Health, Labour and Welfare Notification "Regarding the testing method for foods containing allergenic substances."
Note) See rear cover for details.

6. MCE-202 MultiNA Microchip Electrophoresis System

The long series of operations required for agarose gel electrophoresis – reagent preparation, gel preparation, electrophoresis, acquiring result images, and clean-up – requires a lot of time and effort. Moreover, the data obtained is objectively poor in terms of sensitivity, separation, reproducibility, and quantitativeness.

The MCE-202 MultiNA Microchip Electrophoresis System overcomes the problems with agarose gel electrophoresis.

Fig. 4 MultiNA Microchip

Fig. 5 MultiNA Regent Kit

Features of MultiNA

- Microchip electrophoresis by MultiNA offers superior sensitivity, separation, reproducibility, and quantitativeness to agarose gel electrophoresis.
- Simply load the samples and reagents for automated, unmanned analysis of up to 120 samples. Pretreatment and electrophoresis proceed in parallel to achieve an analysis time of just 80 s (*) per sample.
- MultiNA offers extremely easy analysis operation. Once the analysis schedule is created, simply load the samples and reagents and click the Start button.
- Reusable high-performance microchip achieves running costs equal to or lower than agarose gel electrophoresis.

(*) DNA standard analysis (DNA-100 kit/Pre-Mix mode) using four microchips.
However, this time does not include the times for initial and subsequent rinsing or the time for initial analysis.

Fig. 6 MultiNA Operation Screen

7. Detection of Allergenic Substances Using MCE-202 MultiNA Microchip Electrophoresis System

7. Detection of Allergenic Substances Using MCE-202 MultiNA Microchip Electrophoresis System

The results of analysis of the PCR amplification products of DNA derived from wheat, buckwheat, peanuts, shrimp and crab, respectively, using the MultiNA are shown in Fig. 7. The PCR amplification products derived from the wheat, buckwheat, peanuts, shrimp and crab substances were all clearly detected using the MultiNA. (The estimated sizes shown in the figure were obtained in this experiment.)

The results of analysis by agarose gel electrophoresis of the same PCR amplification products are shown in Fig.8 [Reference]. The sizes of the PCR amplification products are imprecise, resulting in the lack of objectivity in interpreting the gel electrophoresis. However, the results obtained using the MultiNA consist of an electropherogram (Fig. 7-b) in addition to a gel image (Fig. 7-a), ensuring a high level of accuracy. Despite the proximity of the wheat and buckwheat amplification products, they could be separated. Compared to agarose gel electrophoresis, the MultiNA's excellent resolution and sensitivity allow these to be clearly detected.

Fig. 9 shows a photograph of the MCE-202 MultiNA Microchip Electrophoresis System.

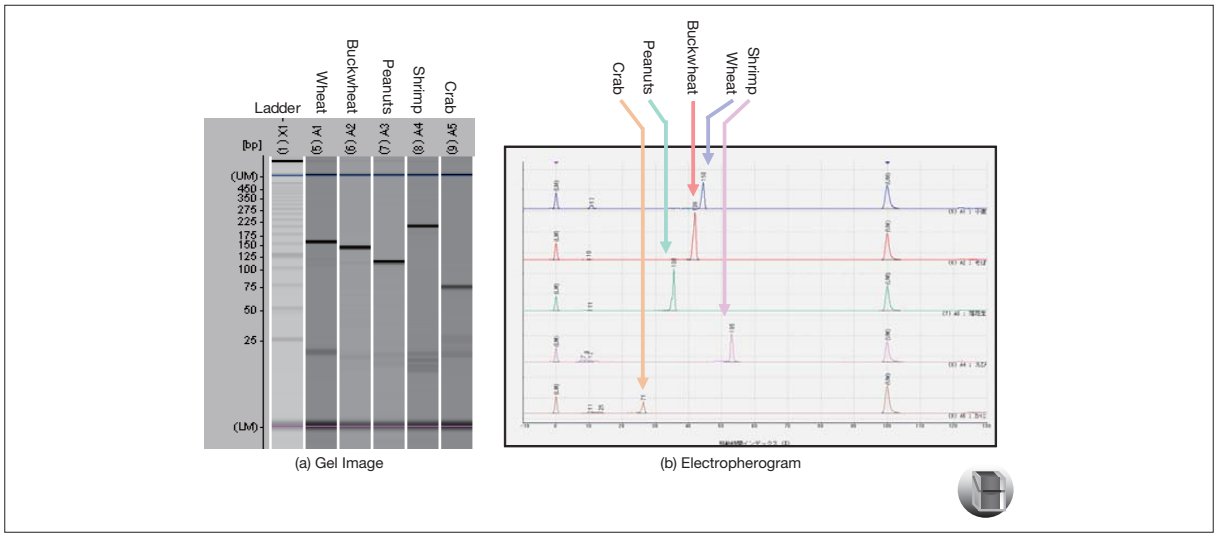


Fig. 7 Analytical Results for PCR Products from Allergenic Substances

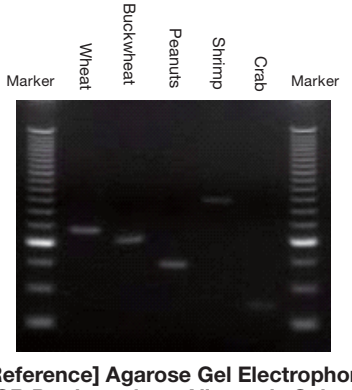


Fig. 8 [Reference] Agarose Gel Electrophoresis of PCR Products from Allergenic Substances

Fig. 9 MCE-202 MultiNA Microchip Electrophoresis System

References:

"Regarding the testing method for foods containing allergenic substances," No. 0724, Publication No. 1, the Dept. of Food Safety, Ministry of Health, Labour and Welfare of Japan, July 24, 2009

"Handbook for Labeling of Processed Foods Containing Allergens," Japanese Ministry of Health, Labour and Welfare, March 2009 revision

"What You Need to Know About Food Labeling," Japanese Ministry of Health, Labour and Welfare, Japanese Ministry of Agriculture, Forestry and Fisheries, Japan Fair Trade Commission, March 2009

Note)
Separate arrangements are required for contract testing using the primers described above for commercial applications on behalf of analytical laboratories, with the exception of public institutions.
Contact the appropriate company below. The synthesis and application of these primers for research applications is unrestricted.

- Animal: Nissin Food Products Co., Ltd.
- Wheat, buckwheat, soybeans: Nisshin Seifun Group Inc.
- Shrimp, crab: House Foods Corporation

SHIMADZU
Excellence in Science

B-9 Testing and Analysis of Genetically Modified Food – Application of MultiNA –

LifeScience

(Photo Above: Natural product, unrelated to text contents)

1. Introduction

Genetically modified organism (GMO) has burgeoned over the years in order to satiate the global appetite or to add value to natural agriculture products. Technology to increase crop yields has been a constant demand, and the introduction and success of increased agricultural yield by using gene recombinant technology has indeed increased productivity in crop yields. On the other hand, the question arises as to whether these genetically modified food sources safe to eat, and nutritionally beneficial compared to natural products? The necessity to protect consumers while assisting agricultural food producers is a challenge for regulatory agencies globally, requiring that they keep up with quickly evolving technology and increasing genetically modified foods. In this environment, various regulations are conducted in many countries. For example, in Japan, only genetically modified foods that have received approval through safety assessment are permitted to be circulated in the domestic food market.

Domestic consumers tend to avoid such genetically modified foods though many GMO food have been approved by the safety assessment, the cultivation and circulation of foods developed using gene recombinant technology are currently rare in Japan. However, genetically modified organisms are actively cultivated globally, and large quantities of genetically modified organisms and their processed foods spread all over the world. Thus, genetically modified may penetrate gradually into the Japanese market in future.

2. Genetically Modified Organism (GMO)

Genetically modified organisms are farm products that breed improvement is introduced by gene recombinant technology. Comparing to conventional methods of hybridization and artificial mutation, the gene recombinant technology transforms plants artificially and enables the introduction of genes from other species and a wider variety of breed improvements in a much shorter period of time.
Various characteristic forms including insect resistance, virus resistance and herbicide tolerance for the improvement of cultivation, high lysine for the increment of nutritive value and high oleic for health promotion are introduced to genetically modified organisms distributed globally.
According to ISAAA[1] 2009 statistics, the cultivation area for genetically modified organisms has reached as much as 134 million hectares. The proportion of genetically modified organisms to whole organisms has reached 77 % for soybeans, 26 % for corn, 21 % for rapeseed, and 49 % for cotton respectively. Genetically modified organisms and their processed food are referred to as genetically modified food.
According to the Food Sanitation Act in Japan, the safety assessment of genetically modified foods is mandatory and only foods approved in the assessment are permitted to be imported and circulated.
As of July 2010, genetically modified foods corresponding to 126 varieties of genetically modified organisms have been approved through safety assessments. These include 8 varieties of potatoes, 7 varieties of soybeans, 3 varieties of sugar beets, 70 varieties of corn, 15 varieties of rapeseed, 20 varieties of cotton, and 3 varieties of alfalfa[2].

3. Labeling of Genetically Modified Foods in Japan

According to Food Sanitation Act and Japanese Agricultural Standard (JAS) Law[2, 3] (Law Concerning Standardization and Proper Labeling of Agricultural and Forestry Products ~ Law No. 175, 1950), the genetically modified foods which are specified to be labeled as such are shown in Table 1[2, 3]. The items for which food labeling is obligatory include the 7 types of agricultural products along with the 32 types of processed foods containing those products listed in (1) of 1 of Table 1, as well as high oleic soybean and high lysine corn of category 2 in Table 1. The labeling of processed foods (corresponding to (2) of 1 in Table 1) in which genetically modified DNA or resulting protein does not remain after processing, is voluntary.The main raw ingredients of processed foods (those among the top 3 ingredients in terms of weight ratio of all ingredients, and where the weight ratio is at least 5 %) must be included in the label. The labeling of genetically modified foods is summarized in Table 2[2, 3].

The segregation of genetically modified and non-genetically modified agricultural products (foods) is extremely important for labeling of genetically modified products . Whether or not identity preserved handling (IP) has been conducted is specified on the label.
Identity preserved handling refers to the management system in which genetically modified and non-genetically modified foods are segregated through every stage including production, distribution (truck, silo, container ship, etc.), and processing (at processing companies) under the greatest care. Further, its strict management should be confirmed by documents.
The implementation of Identity preserved handling cannot necessarily prevent the mixing of genetically modified foods into non-genetically modified foods.
If Identity preserved handling for soybean or corn is conducted and its mixing rate of genetically modified organism into non-genetically modified organism (GMO content) is less than 5 %, this Identity preserved handling is regarded as proper. The subject of the GMO content is described further on page 7.

[References]
1) ISAAA (International Service for the Acquisition of Agri-biotech Applications), http://www.isaaa.org
2) Department of Food Safety, The Ministry of Health, Labour and Welfare, Japan, http://www.mhlw.go.jp/english/topics/foodsafety/dna/index.html
3) Ministry of Agriculture, Forestry and Fisheries, Japan, "Food Labeling for Processed Foods", http://www.maff.go.jp/e/jas/labeling/modified.html

Table 1 Foods to Be Labeled as Genetically Modified Food in Japan

1	Produce whose composition and nutritional value are similar to that of conventional produce		
	(1) Agricultural products and processed foods containing genetically modified DNA or protein even after processing		
	Agricultural Products	Soybean (including green soybeans and soybean sprouts), Corn, Potato, Rapeseed, Cottonseed, Alfalfa, and Sugar beet	
	Processed Foods	Items subject to labeling	Ingredient to be labeled
		1. *Tofu* (soybean curd) and fried *tofu*	Soybean
		2. Dried soybean curd, soybean refuse, *yuba*	
		3. *Natto* (fermented soy beans)	
		4. *To-nyu* (Soy milk)	
		5. *Miso* (soybean paste)	
		6. Cooked soy bean	
		7. Canned or bottled soybean	
		8. *Kinako* (roasted soybean fluor)	
		9. Roasted soybean	
		10. Item containing food of items 1 to 9 as a main ingredient	
		11. Item containing soybeans (for cooking) as a main ingredient	
		12. Item containing soybean flour as a main ingredient	
		13. Item containing soybean protein as a main ingredient	
		14. Item containing *edamame* (green soybean) as a main ingredient	*Edamame*
		15. Item containing soybean sprouts as a main ingredient	Soybean sprouts
		16. Corn snacks	Corn
		17. Corn starch	
		18. Popcorn	
		19. Frozen corn	
		20. Canned corn or bottled corn	
		21. Item containing corn flour as a main ingredient	
		22. Item containing corn grits as a main ingredient (except corn flakes)	
		23. Item containing corn (for cooking) as a main ingredient	
		24. Item containing food of items 16 to 20 as a main ingredient	
		25. Frozen potato	Potato
		26. Dried potato	
		27. Potato starch	
		28. Potato snacks	
		29. Item containing food of items 25 to 28 as a main ingredient	
		30. Item containing potatoes (for cooking) as a main ingredient	
		31. Item containing alfalfa as a main ingredient	Alfalfa
		32. Item containing sugar beet as a main ingredient	Sugar beet
	(2) Processed foods in which genetically modified DNA or resulting protein does not remain after processing (e.g., soybean oil, soy source, corn oil, isomerized liquid sugar)		
2	Produce whose composition or nutritional value is markedly different from that of conventional produce (high oleic acid soybeans, high lysine corn)		

Table 2 Labeling of Genetically Modified Foods in Japan

	Classification		Labeling Example	Labeling
1	Produce whose composition and nutritional value are similar to that of conventional produce			
	(1) Agricultural products and processed foods containing genetically modified DNA or resulting protein even after processing (corresponding to 7 agricultural products and 32 processed food categories in Table 1)	GM agricultural products under the identity preserved handling or processed foods made form those	"GMO segregated from non-GMO", "GMO"	Mandatory
		Agricultural products, not segregated GM products and non-GM products, or processed foods made from those the identity preserved handling2 or processed foods made form those	"Not segregated from GMO"	Mandatory
		non-GM agricultural products under the identity preserved handling or processed foods made from those	"Non-GMO segregated from GMO", "Non-GMO"	Voluntary
	(2) Processed foods in which genetically modified DNA or resulting protein does not remain after processing (e.g., soybean oil, soy source, corn oil, isomerized liquid sugar)		"Non-GMO segregated from GMO", "Non-GMO"	Voluntary
2	Produce whose composition or nutritional value is markedly different from that of conventional produce(high oleic acid soybeans, high lysine corn)		"soybeans (high oleic, genetically modified)"	Mandatory

4. Testing and Analysis of Genetically Modified Foods in Japan

The standard tests and methods used for analysis of genetically modified foods are specified in "Testing for Foods Produced by Recombinant DNA Techniques"[1], notifications concerning inspection and guidance of imported foods[2) – 5)] by The Ministry of Health, Labour and Welfare, and "JAS analytical test handbook"[6] by Food and Agricultural Materials Inspection Center.
Table 3 shows the genetically modified foods that are subject to testing, and test methods to be used. Both approved and unapproved genetically modified foods based on safety assessment are subject to the testing.
As of July, 2010, papaya (55-1), corn (CBH351), corn (Bt10), corn (DAS59132), rice (LLRICE601), rice (Bt), and rapeseed (RT73 B.rapa) have not been unapproved by the safety assessment.
The tests can be classified to qualitative testing to determine the presence or absence of genetically modified organisms (GMO) and quantitative testing to determine the ratio of genetically modified organisms to non-genetically modified organisms (GMO content). The methods adopted for qualitative testing include lateral flow immunoassay, qualitative PCR, and the GUS gene test, while quantitative PCR and ELISA (enzyme-linked immunosorbent assay) are adopted for quantitative testing.

Table 3 Testing Methods for Genetically Modified Foods in Japan

Food Product	Genetically Modified Gene	Test Type	Test Method	References
Papaya (raw or processed)	Papaya (55-1)	Qualitative test	Qualitative PCR, GUS gene test	1)
Corn (grain)	Corn (CBH351)		Lateral flow immunoassay	
Corn (partially processed)			Lateral flow immunoassay, Qualitative PCR	
Corn (processed)			Qualitative PCR	
Corn (grain)	Corn (Bt10)			
Corn (partially processed)				
Corn (grain)	Corn (DAS59132)			
Corn	Corn (GA21)	Quantitative test or Qualitative test / Quantitative test	Quantitative PCR Qualitative PCR / Quantitative PCR	1) 6)
	Corn (Event176)			
	Corn (Bt11)			
	Corn (T25)			
	Corn (Mon810)			
Soybean	Soybean (Roundup Ready Soybean)	Quantitative test or Qualitative test / Quantitative test	Quantitative PCR Qualitative PCR / Quantitative PCR	1) 6)
Soybean	CP4EPSPS protein	Quantitative test	ELISA	1)
Rice	Rice (LLRICE601)	Qualitative test	Qualitative PCR	3)
Rice	Rice (Bt)			4)
Rapeseed	Rapeseed (RT73 B.rapa)			5)
Potato	Potato (New Leaf)			6)
	Potato (New Leaf Plus)			

[References]
1) The Ministry of Health, Labour and Welfare, Japan, "Testing for Foods Produced by Recombinant DNA Techniques", http://www.mhlw.go.jp/english/topics/food/sec05-1a.html
2) http://www.mhlw.go.jp/english/topics/importedfoods/index.html
3) Department of Food Safety, The Ministry of Health, Labour and Welfare, Japan, Notification No. 0915002, September 15, 2006.
4) Department of Food Safety, The Ministry of Health, Labour and Welfare, Japan, Notification No. 0220002, February 20, 2007.
5) Department of Food Safety, The Ministry of Health, Labour and Welfare, Japan Notification No. 0914 -5, September 14, 2009.
6) "Japanese Agricultural Standard (JAS) analytical test handbook: genetically modified food quality, labeling analysis manual for individual products, 2nd revision (2002)", Food and Agricultural Materials Inspection Center, Japan.

Table 4 presents an overview of analysis methods for testing genetically modified foods. Since the ELISA and lateral flow immunoassay methods are based on antigen-antibody reactions, they are not applicable to testing for processed foods because antigenicity is lost due to protein denaturation during heat processing, etc. DNA exhibits superior stability to protein because DNA has better thermal stability and is more tolerant to decomposition and denaturation upon heating or other processes.

On the other hand, qualitative PCR is applicable for testing of both agricultural products and processed foods due to the high possibility of target gene amplification by PCR. However, quantitative PCR cannot be applied to determination of recombinant gene content in processed foods, as discussed later in this document.

Table 4 Analysis Methods Used for Testing Genetically Modified Foods

Analysis Method	Overview
ELISA (Enzyme-Linked Immunosorbent Assay)	It is used for quantitative analysis or qualitative analysis (detection) of antigens and antibodies in a sample. It utilizes the high specificity of the antigen-antibody reaction and the high sensitivity of enzymatic reactions.
Lateral Flow Immunoassay	This is one type of immunochromatography that uses the antigen-antibody reaction as in the ELISA method. Here, a drop of sample is applied to a test strip, and as it migrates along the strip by capillary action, the presence or absence of an antigen in the sample is determined based on comparison of the color patterns in the test line and a control line. When the a sample including the target antigen passes through a zone including dyed antigen-specific antibodies, complex between antigen in the sample and dyed antibody (antigen- dyed antibody complex) is formed. Antigen-specific antibody is fixed in the test line zone, and it binds to the antigen- dyed antibody complex. The control line includes an antibody that binds dyed-antibody. If antigen is present in the sample, both the test line and control line are colored. If there are no antigens present in the sample, only the control line is colored.
GUS Gene Test	A β-glucuronidase (GUS) gene might be introduced along with an exogenous gene for use as an indicator (reporter) of genetic recombination. In this type of gene recombination, the GUS gene is expressed along with the recombinant gene, making it possible to confirm the success of recombination process based on the presence or absence of GUS activity. In the GUS gene test, the reagent containing the substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Gluc) is added, and GUS activity is confirmed by the appearance of blue coloring.
Qualitative PCR	PCR (Polymerase Chain Reaction) is a technique in which a specific region of a DNA from template DNA is selectively amplified. In PCR, two single chain DNA fragments (primer pair) which are complimentary to both ends of the specific region to be amplified are used. An enzyme for DNA synthesis (DNA polymerase) is also added to reaction tube and a cycle reaction (dissociation of double-strand DNA to single-strand DNA (Denaturation) → primers binding to each strand (Annealing) → DNA synthesis (Elongation) is repeated to amplify the specific gene region selectively. Thus, in principle, the specific gene region is amplified by a factor of 2 during each reaction cycle. In qualitative PCR, PCR is conducted to detect a target gene region included in a DNA template extracted from the sample, and the obtained amplification product (PCR product) is subjected to electrophoretic analysis. If the target gene region is included in the extracted DNA, the PCR product corresponding to the target gene region will be detected.
Quantitative PCR	In quantitative PCR, PCR is conducted to amplify the specific gene region using a template DNA extracted from a sample and its amplification process is monitored just-timely. A fluorescent compound (intercalator) which can bind with double-stranded DNA, or a fluorescent marker probe to enable recognition of a specific part of the amplification region is added to allow monitoring of the amplification products at each cycle. Analysis of the obtained amplification curve allows determination of the quantity (number of copies) of the target genes.

Fig. 1 shows an example of the procedure used for analysis of genetically modified food using qualitative PCR. The sample is pulverized, and the DNA is extracted using an extraction kit. The DNA concentration of the extracted DNA is determined using the BioSpec-nano Ultraviolet-Visible spectrophotometer for life sciences, and PCR is conducted using a specified quantity of extracted DNA as the template. Electrophoretic analysis of the obtained PCR products is conducted using the 'MCE-202' MultiNA microchip electrophoresis system, and the presence or absence of PCR products corresponding to the target region is confirmed.

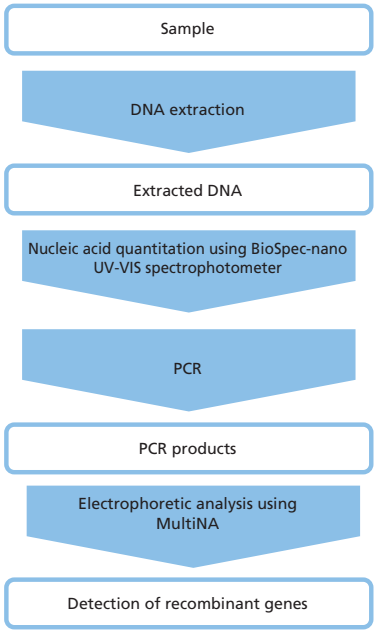


Fig. 1 Example of Procedure for Analysis of Genetically Modified Food by Qualitative PCR

Due to extremely high analysis sensitivity, qualitative PCR can detect even minute levels of modified genes in extracted DNA.When Identity preserved handling is implemented, the permissible genetically modified organism (GMO) content to non-GMO is 5 %.
However, recombinant gene is often detected even though the GMO content is below 5 %. When recombinant DNA is detected by qualitative PCR, quantitative PCR testing will be conducted to determine the GMO content. DNA extracted from the sample is used as the template in quantitative PCR, and PCR is conducted using a primer to detect recombinant and endogenous genes.
The number of copies of recombinant and endogenous genes in the extracted DNA can be determined by analysis of the quantitative PCR amplification curve. The GMO content and internal standard ratio are defined according to Equation 1 and 2, respectively. When the content of genetically modified species exceeds 5 % in identity preserved handling foods labeled as "non-GM (non-Genetically Modified)" or "Non-GM segregated from GM", a close inspection of the identity preserved handling is necessary. It should be mentioned that since the degradation rates of recombinant genes and endogenous genes are not necessarily same in processed food, obtaining the GMO content accurately by quantitative PCR method is impossible.

GMO content (%) = {(number of copies of the recombinant gene) / (number of copies of internal standard genes)} × (1 / Internal standard ratio) × 100 (Equation 1)
Internal standard ratio = (Number of recombinant gene in pure genetically modified agriculture product) / (Number of internal gene in pure genetically modified agriculture product) (Equation 2)

[Reference]
"Japanese Agricultural Standard (JAS) analytical test handbook:
genetically modified food quality, labeling analysis manual for individual products, 2nd revision(2002)", Food and Agricultural Materials Inspection Center, Japan.

5. Introduction of Tools for Optimizing Inspection and Analysis of Foods by Qualitative PCR

MCE-202 MultiNA Microchip Electrophoresis System

Agarose gel electrophoretic analysis requires a great deal of time and effort to conduct a series of operations including the mixing of reagents, preparing the gel, conducting electrophoresis, acquiring an image of the results, and post-cleanup. In addition, its data has a tendency to show inferior sensitivity, resolution and quantitation performance.
The MCE-202 MultiNA microchip electrophoresis system solves these problems of agarose gel electrophoresis all at once since the system is based on brand-new, superior platform and fully automated.

Fig. 2 MultiNA

MultiNA Features

High Analysis Performance
Compared with agarose gel electrophoresis, the microchip electrophoretic analysis with the MultiNA delivers excellent sensitivity, separation, repeatability and quantitation performance.

Automated Operation for up to 120 Analyses
Simply set up the samples and the separation buffer for automated analysis of up to 120 analyses. The parallel processing for analysis pretreatment and electrophoresis permits a processing speed of just 80 seconds per analysis.[1)]

Maximum Ease of Use
Analysis operation with the MultiNA is extremely simple. Just set up the analysis schedule, and then simply load the reagents and samples and click the [Start] button.

Reduce Analysis Costs
The reusable, high-performance microchip achieves lower running costs per analysis than agarose gel electrophoresis.

[1)] When 4 microchips are used in DNA standard analysis (for example, DNA-1000 Kit / Premix mode), this does not include the time required for the initial rinse and final rinse, and the initial analysis.

BioSpec-nano UV-VIS Spectrophotometer for Life Sciences

To successfully guide the PCR reaction to completion, confirming the DNA concentration of the extracted DNA and checking of DNA purity using OD ratio (OD260/280) are indispensable. Conducting analysis with UV-VIS spectrophotometers which use conventional cuvettes is both labor intensive and time consuming due to the required rinsing and drying of the cuvettes.
The BioSpec-nano, which incorporates a cuvette-free optical system, an innovative, automatic mounting mechanism, and an automatic wiping mechanism, offers simple fast and simple nucleic acid analysis of 1 to 2 µL samples. High throughput analysis is achieved, requiring a mere 15 seconds to complete one analysis.

Fig. 3 BioSpec-nano

BioSpec-nano Features

Drop and Click Analysis
DNA concentration and purity can be checked by just dropping the sample on the target, and clicking the button. Measurement and wiping are both handled automatically by the instrument.

Nucleic Acid Quantitation of 1 to 2 µL Samples
Sample volumes of 1 µL (0.2 mm optical path length) and 2 µL (0.7 mm optical path length) can be measured.

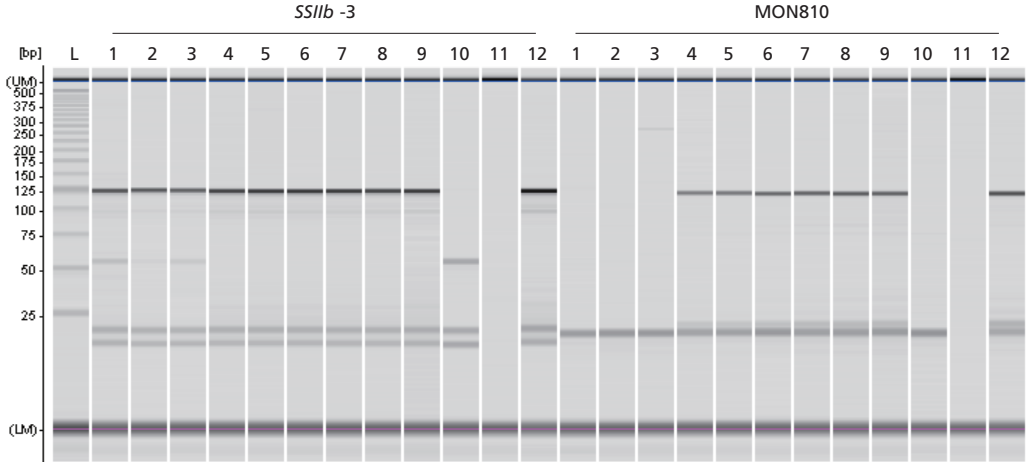
Simple and Quick Analysis
Blank measurement, sample measurement, report output in PDF or CSV format, and other basic operations are conducted simply and quickly just by clicking a button.

Support for Wide Range of Analyses
Nucleic acid quantitation, quantitation of nucleic acid labeled for micro-array, protein quantitation by OD280, and labeled protein quantitation are all supported.

6. Examples of Qualitative PCR Analysis of Genetically Modified Foods Using the MultiNA
6.1 Analysis of Genetically Modified Corn (MON810)

Here we introduce an analysis of genetically modified corn (MON810) as an example of genetically modified food analysis. After extracting DNA from 3 powdered samples consisting of genetically modified corn (MON810) having GMO content of 0 %, 1 % and 5 %, respectively, the extracted DNA from each of the samples was used as a template. PCR was then conducted using a primer for endogenous gene *SSIIb*-3 detection[2] and a primer for the genetically modified MON810 detection[3]. The electrophoretic analysis of PCR product using the MultiNA are shown in Fig. 4. In analysis of the PCR products using the primer for the endogenous gene *SSIIb*-3 detection, the PCR product (114 bp) corresponding to *SSIIb*-3 was detected in all of the samples except for the negative control. The endogenous gene *SSIIb*-3 is a gene that is specific to corn, and detection of the endogenous gene in a sample means PCR testing of the recombinant gene in that sample is effective. On the other hand, in analysis of the PCR products using the primer for the genetically modified MON810 detection, the PCR product (113 bp) corresponding to MON810 was detected in the 1 % and 5 % GMO content samples, as well as in the positive control sample.

Gel Image



L : 25 bp DNA Ladder
1-3 : MON810 0 % GMO content sample
4-6 : MON810 1 % GMO content sample
7-9 : MON810 5 % GMO content sample
10 : Negative control (without template DNA)
11 : Negative control (without primer)
12 : Positive control plasmid

Fig. 4 Analysis of Genetically Modified Corn (MON810) Using MultiNA

[References]
1) Shimadzu Application News No. B29, Qualitative Analysis of Genetically Modified Corn by Standard Method with MCE-202 "MultiNA"
2) The Ministry of Health, Labour and Welfare, Japan, "Testing for Foods Produced by Recombinant DNA Techniques", http://www.mhlw.go.jp/english/topics/food/sec05-1a.html
3) "Japanese Agricultural Standard (JAS) analytical test handbook: genetically modified food quality, labeling analysis manual for individual products, 2nd revision (2002)", Food and Agricultural Materials Inspection Center, Japan.

6.2 Detection of Genetically Modified DNA (GA21) in Processed Corn

Here we introduce an example of analysis of genetically modified DNA in processed corn using qualitative PCR. DNA was extracted from 4 types of processed corn products (2 types of canned corn, 1 type of popcorn, 1 type of corn starch), and the DNA extracted from each of the samples was used as a template. PCR was conducted using a primer for the endogenous gene *SSIIb* detection and a primer for the genetically modified GA21 detection. Next, the obtained PCR products were analyzed using the MultiNA. The analysis results are shown in Fig. 5. In PCR using the primer for the endogenous gene *SSIIb* detection, the PCR product (151 bp) corresponding to *SSIIb* was detected in all of the processed food samples and in the positive control plasmid. If damage to DNA derived from processed food is considerable due to heating during processing,the endogenous genes will not be detected. In samples where the endogenous gene is not detected, qualitative testing for genetically modified genes in these samples is regarded as invalid. On the other hand, in analysis of the PCR products using the primer for the genetically modified GA21 detection, the PCR product (133 bp) corresponding to GA21 was detected only in the positive control plasmid, and was not detected in the 4 types of processed food samples, which were labeled as " Non-GMO"

As shown in the electropherograms of the negative and positive controls, the *SSIIb* gene (151 bp) and GA21 gene (133 bp) are clearly detected using only 20 copies of the positive control plasmid. Thus, high-sensitivity qualitative PCR analysis of genetically modified food is clearly achieved using the MultiNA.

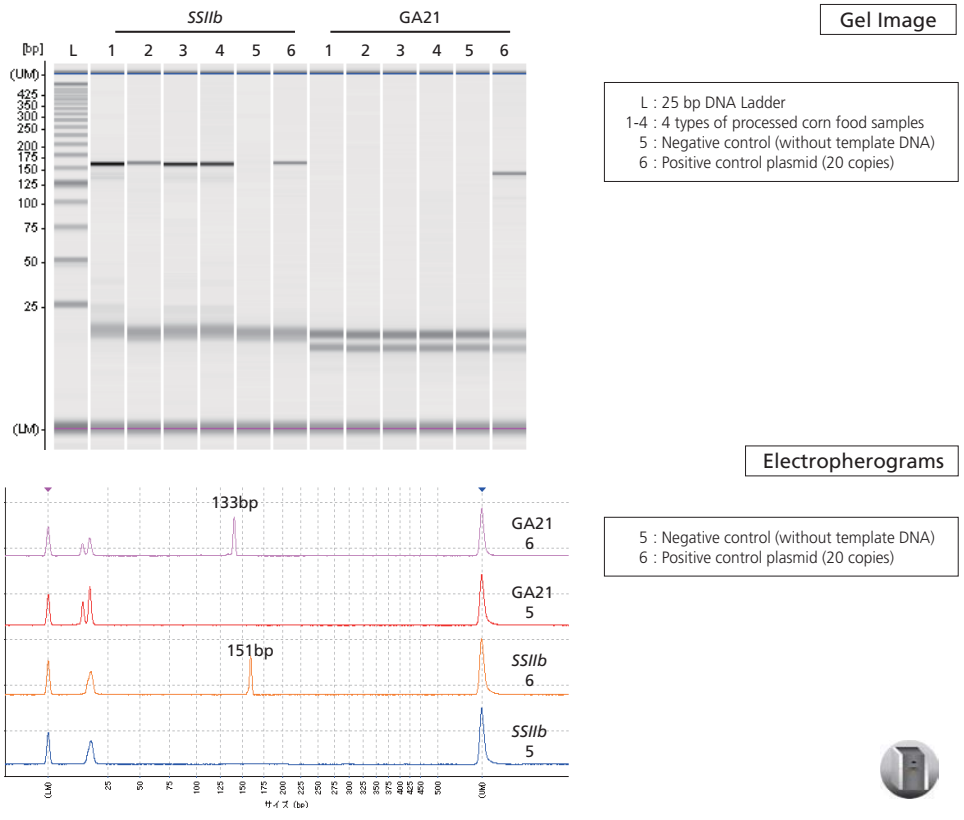


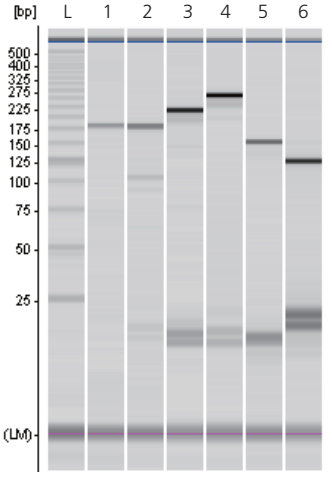
Fig. 5 Analysis of Genetically Modified Gene (GA21) in Processed Corn Food Products Using MultiNA

[Reference]
"Japanese Agricultural Standard (JAS) analytical test handbook:
genetically modified food quality, labeling analysis manual for individual products, 2nd revision (2002)", Food and Agricultural Materials Inspection Center, Japan.

6.3 Analysis of Genetically Modified Foods Unapproved by Safety Assessment in Japan

Some genetically modified foods that are unapproved by Japanese safety assessment, are permitted to be circulated in global market.
These particular foods are subject to qualitative inspection in Japan (Table 3). Positive control plasmids and primers according to official inspection methods are commercially available, making it possible to conduct analysis for modified DNA in these foods by the qualitative PCR method.

Examples of analysis of 3 types of modified DNA that were unapproved by the safety inspection as of July, 2010, including corn (CBH351)[1)], papaya (55-1)[1)], and rice (Bt)[2)], are shown below. PCR was conducted using the respective positive control plasmids as templates, and the primers specified in the respective test methods for detection and identification. The obtained PCR products were analyzed using the MultiNA, and the results shown in Fig. 6 were obtained. The PCR products corresponding to the respective target genes and the primers can be verified.



Gel Image

L : 25 bp DNA Ladder
1 : Corn (CBH351) positive control plasmid, detection primer, target PCR product (170 bp)
2 : Corn (CBH351) positive control plasmid, identification primer, target PCR product (171 bp)
3 : Papaya (55-1) positive control plasmid, detection primer, target PCR product (207 bp)
4 : Papaya (55-1) positive control plasmid, identification primer, target PCR product (250 bp)
5 : Rice (Bt) positive control plasmid, detection primer, target PCR product (147 bp)
6 : Rice (Bt) positive control plasmid, identification primer, target PCR product (120 bp)

Fig. 6 Analysis of Genetically Modified Corn (CBH351), Papaya (55-1), Rice (Bt) using MultiNA

[References]
1) The Ministry of Health, Labour and Welfare, Japan, "Testing for Foods Produced by Recombinant DNA Techniques", http://www.mhlw.go.jp/english/topics/food/sec05-1a.html
2) Department of Food Safety, The Ministry of Health, Labour and Welfare, Japan, Notification No. 0220002 of February 20, 2007.

3. Veterinary Drug

PREFACE

Veterinary drugs are used in livestock to serve a variety of purposes viz. for disease treatment and maintain herd and flock health, promote growth, improve meat quality in a sense of reducing fat and increasing lean meat yield, and otherwise reducing production costs. Veterinary drug residues are the very small amounts of veterinary medicines that remain in animal products and therefore, make their way into the food chain. These include any degradation products, which are the result of the medicine breaking down into its component parts. The chemical group of veterinary drugs available is very diverse as is their application. Generally speaking, there are two big groups of veterinary drugs, antibiotics and hormones.

An antibiotic is a substance or compound that kills bacteria or inhibits their growth and are used to treat infections caused by microorganisms, including fungi and protozoa. Hormones are used for breeding to enhance body protein accretion, metabolize fat stores and increase lean growth rate. Government regulatory authorities control the use of veterinary drugs by approving or registering safe uses and monitoring food for unsafe or prohibited residues. There are several regulations in place which veterinary drugs are allowed to be used for food production and which are banned.

A maximum residue limit (MRL) is an amount of drug residue that — if present in the tissue of a food animal or a food product derived from a food-producing animal that has been treated with a veterinary drug — will not pose an unacceptable risk to the safety of the food. This residue, at this level, is considered to pose no adverse health effects if ingested daily by humans over a lifetime.

While efficient methods for residue extraction coupled with immunoassay determination provide very useful methods for routine analysis for β-agonists, confirmatory procedures are required, also, to support the routine methods. Mass spectrometry (MS), because it gives structural information on the analyte, can supply unequivocal identification of the substance detected and, because MS can be interfaced with chromatographic systems such as GC

and HPLC, the hyphenated techniques of GC/MS and LC/MS are increasingly becoming the confirmatory techniques of choice for residue analysis.

V-1 Determination of Malachite Green and Crystal Violet in Aquatic Products by UFLC/Triple Quadrupole Mass Spectrometry

INTRODUCTION

In this paper, a method was proposed for fast determination of malachite green, leuco-malachite green, crystal violet and leuco-crystal violet in aquatic products with Shimadzu LC-30A ultra fast liquid chromatograph (UFLC) and LCMS-8030 triple quadrupole mass spectrometer. Samples were extracted, separated by LC-30A ultra fast liquid chromatograph, and then quantitatively determined using LCMS-8030 triple quadrupole mass spectrometer with internal standard. The samples got separated and detected rapidly within 2 minutes. The method was of good linearity for malachite green and leuco-malachite green at the concentration range of 0.5~200 µg/L, for crystal violet at the concentration range of 0.5~500 µg/L, and for leuco-crystal violet at the concentration range of 0.1~200 µg/L. All calibration curves had a correlation coefficient higher than 0.999. Precision test was performed on multi-standard solutions at concentrations of 1 µg/L, 50 µg/L and 200 µg/L by 6 successive injections, the %RSDs of retention time and peak area were below 0.18% and 3.0%, respectively, suggesting that the system's precision is satisfactory. The method's LOQ was 0.1 µg/kg, better than 0.5 µg/kg, the required LOQ regulated in GB/T 19857-2005 Determination of malachite green and crystal violet residues in aquatic product.

Malachite green can remain in the body of fish for a long time and that malachite green is a highly poisonous, persistent, carcinogenic, teratogenic, and mutagenic substance. Therefore, many countries have listed malachite green as a banned drug in aquaculture. China also included malachite green in the *List of veterinary drugs and their compounds forbidden to use in animal food* in May 2002. However, malachite green is inexpensive and its efficacy in the treatment of saprolegniasis is "irreplaceable" by other drugs, and as a

result some profit-seeking people may be reluctant to restrain from using this substance. In this paper, a method was proposed in reference with GB/T 19857-2005 *Determination of malachite green and crystal violet residues in aquatic product* for fast determination of malachite green and crystal violet in aquatic products with Shimadzu LC-30A UFLC and LCMS-8030 triple quadrupole mass spectrometer.

EXPERIMENTAL

A combined system of Shimadzu LC-30A UFLC and LCMS-8030 triple quadrupole mass spectrometer was used in the experiment. The configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communication bus module, LCMS-8030 triple quadrupole mass spectrometer and LabSolutions Ver. 5.41 chromatography workstation.

Conditions of Analysis

UFLC Conditions

Analytical apparatus: LC-30A system

Column : Shim-pack XR-ODS III 2.0 mm I.D.×50 mm L., 1.6 μm

Mobile phase : A- 5 mmol/L ammonium acetate+0.05% formic acid aqueous solution: B-Acetonitrile

Flow rate : 0.4 mL/minute

Injection volume : 10 μL

Column temperature: 40 °C

Elution mode : Binary gradient with an initial concentration of 50%B,

Table 1 Binary Gradient

Time(min)	Module	Command	Value
0.50	Pumps	Pump B Conc.	95
1.80	Pumps	Pump B Conc.	95
1.81	Pumps	Pump B Conc.	50
3.50	Controller	Stop	

MS conditions

Analytical apparatus : LCMS-8030
Ion source : ESI(+)
Ionization voltage : 4.5 kV
Nebulizing gas : Nitrogen 3.0 L/min
Drying gas : Nitrogen 15 L/min
Collision gas : Argon
DL temperature : 250 °C
Heater block temperature : 400 °C
Scan mode : Multiple reaction monitoring (MRM)
Pause time : 10 ms
Dwell time : 3 ms
MRM parameters : Listed in Table 2

Table 2 MRM Parameters

No.	Name	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
1	Malachite green	329.20	313.15	-20.0	-40.0	-23.0
			208.10*	-30.0	-35.0	-16.0
2	Leuco-malachite green	331.20	316.20	-10.0	-20.0	-24.0
			239.15*	-16.0	-30.0	-29.0
3	Crystal violet	372.25	356.20	19.0	40.0	27.0
			251.20*	11.0	35.0	19.0
4	Leuco-crystal violet	374.25	359.20	11.0	25.0	28.0
			238.15*	11.0	30.0	18.0
5	D5-malachite green	334.25	318.20	30.0	40.0	23.0
6	D6-leuco-malachite green	337.25	322.25	10.0	20.0	25.0

* refers to qualitative ion.

Sample Preparation

Preparation of standard solution:

100 μg/L multi-standard intermediate solution was prepared using acetonitrile as solvent, and then diluted with acetonitrile : 5mmol/L ammonium acetate aqueous solution (1:1, v/v) into a series of multi-standard working solutions of concentrations of 0.1, 0.5, 1.0, 2.0, 5.0, 10, 20, 50, 100, 200 and 500 μg/L. Each milliliter of the multi-standard working solution contains 2 ng deuterated malachite green and 2 ng deuterated leuco-malachite green.

Sample pretreatment method:

The same as the method specified in China national standard GB/T 19857-2005 *Determination of malachite green and crystal violet residues in aquatic product*.

RESULTS AND DISCUSSION

MRM Chromatogram of Standard Samples

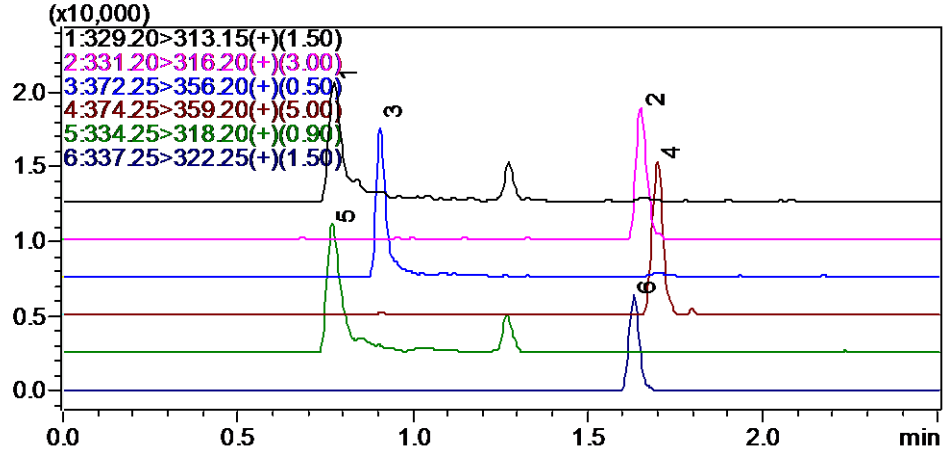


Fig. 1 MRM chromatogram of 1 μg/L standard mixture

（1: Malachite green; 2: Leuco-malachite green; 3: Crystal violet; 4: Leuco-crystal violet ; 5: D5-malachite green; 6: D6-leuco-malachite green;)

Linearity

A series of multi-standard working solutions of concentrations of 0.1, 0.5, 1.0, 2.0, 5.0, 10, 20, 50, 100, 200 and 500 μg/L were determined according to the conditions of analysis specified in 1.2. The contents of malachite green and crystal violet were calculated using deuterated malachite green as internal standard, the contents of leuco-malachite green and leuco-crystal violet were calculated using deuterated leuco-malachite green as internal

standard; calibration curves were plotted as shown in Figs. 2~5 with concentration ratio as abscissa and peak area ratio as ordinate. The resulted calibration curves were of good linearity and their linear equations and correlation coefficients were listed in Table 3.

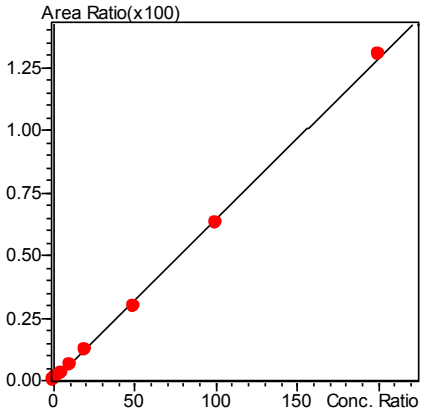


Figure 2

Calibration curve of malachite green

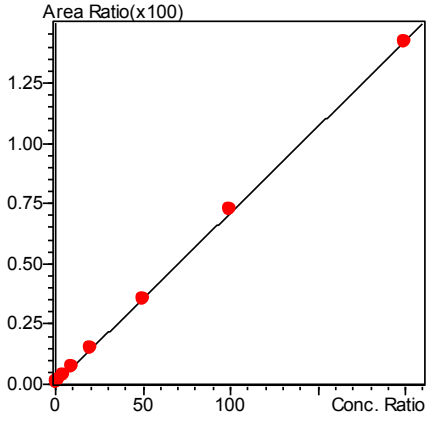


Figure 3

Calibration curve of leuco-malachite green

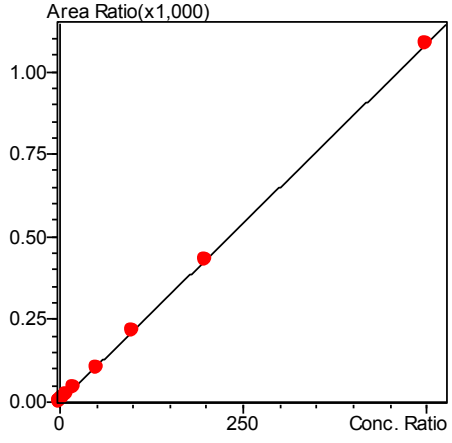


Figure 4

Calibration curve of crystal violet

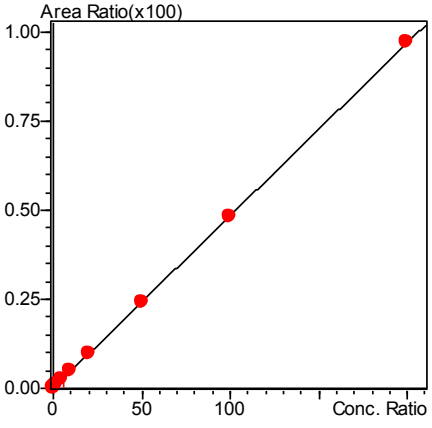


Figure 5

Calibration curve of leuco-crystal violet

Table 3 Parameters of calibration curves

No	Name	Calibration Curve	Linear Range (mg/L)	Correlation Coefficient (r)
1	Malachite green	Y = (0.659584)X	0.5~200	0.9997
2	Leuco-malachite green	Y = (0.71228)X	0.5~200	0.9996
3	Crystal violet	Y = (2.16652)X	0.5~500	0.9999
4	Leuco-crystal violet	Y = (0.484806)X	0.1~200	0.9999

Precision test

Table 4 Repeatability - retention time and peak area (n=6)

Sample name	%RSD (1 μg/L)		%RSD (50 μg/L)		%RSD (200 μg/L)	
	R.T.	Area	R.T.	Area	R.T.	Area
Malachite green	0.177	1.709	0.083	0.747	0.16	2.373
Leuco-malachite green	0.088	2.925	0.095	1.293	0.097	1.504
Crystal violet	0.055	1.385	0.039	0.680	0.084	0.825
Leuco-crystal violet	0.153	2.113	0.096	1.832	0.071	1.351

Multi-standard working solutions of concentrations of 1, 50, and 200 μg/L were determined for 6 times in succession to determine the precision. The repeatability results of retention time and peak area data were as shown in Table 4. The results showed that the %RSD of retention time and peak area data of standard solutions of the 3 concentrations were in the range of 0.039%~0.160% and 0.680%~2.925%, respectively, indicating that the precision was satisfactory.

Sensitivity test

In order to determine the method's sensitivity, 0.1 μg/kg multi-standard solution was spiked into blank matrix samples of shrimp. Chromatograms were as shown in Fig. 6 and Fig. 7. The method's LOQ was 0.1 μg/kg, better than 0.5 μg/kg, which is the required LOQ in GB/T 19857-2005

Determination of malachite green and crystal violet residues in aquatic product.

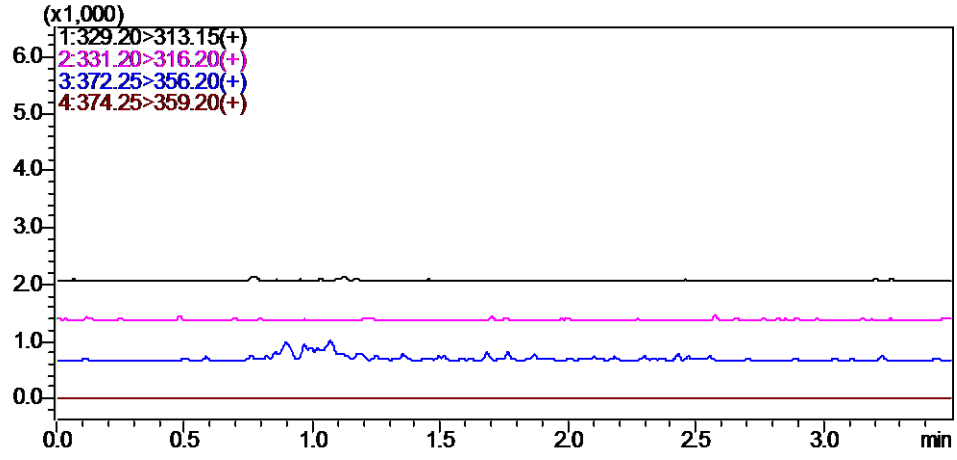


Figure 6 MRM chromatograms of shrimp sample

(1: Malachite green; 2: Leuco-malachite green; 3: Crystal violet;

4: Leuco-crystal violet)

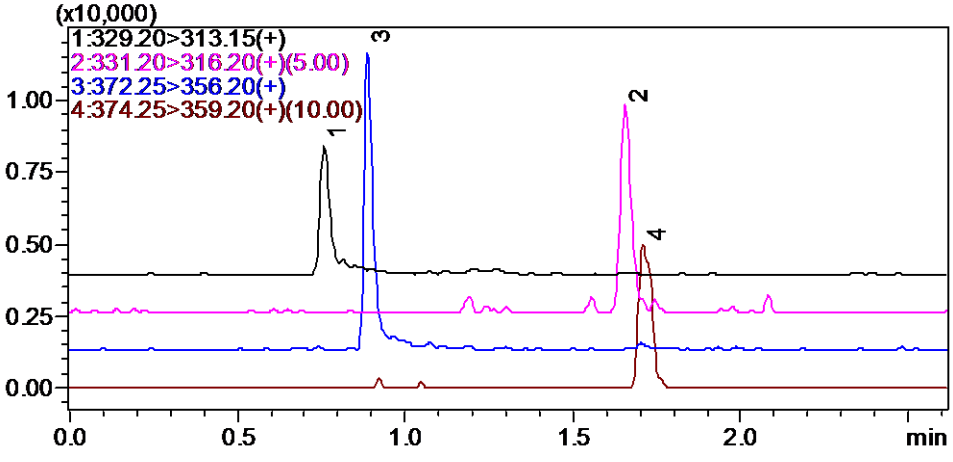


Figure 7 MRM chromatograms of shrimp sample spiked with 0.1 μg/L standards

(1: Malachite green; 2: Leuco-malachite green; 3: Crystal violet;

4: Leuco-crystal violet)

CONCLUSION

A method was established for the determination of malachite green and crystal violet in aquatic products with Shimadzu LC-30A UFLC and LCMS-8030 triple quadrupole mass spectrometer. The method has the merits of fast analysis speed and good precision. The correlation coefficients of all calibration curves were greater than 0.999. The method's LOQ is 0.1 µg/kg, better than 0.5 µg/kg, which is the required LOQ in GB/T 19857-2005 *Determination of malachite green and crystal violet residues in aquatic product.*

V-2 Determination of *N*-nitrosamines in Drinking Water by GC/MS/MS

INTRODUCTION

A method was proposed in this paper for the determination of *N*-nitrosamines in drinking water with triple quadrupole GC-MS. The results showed that GCMS-TQ8030 when used to analyze *N*-nitrosamines demonstrated good linearity in the range 0.5~100 µg/L, peak area RSDs lower than 2% (n=5) for 0.5 µg/L standard solutions, and excellent sensitivity to 0.1 µg/L standard mixture. *N*-nitrosamines are a class of compounds with the N-N=O structure. Of the 130 odd *N*-nitrosamines that have been found, more than 80% are strong carcinogens. In recent years, chloramine has been used in place of chlorine gas to disinfect drinking water. However, studies have shown that disinfection with chloramine may generate *N*-nitrosamines.

As early as in 1994, *N*-nitrosodimethylamine (NDMA) was first detected in drinking water from Lake Ontario. Later on, other nitrosamines including *N*-Nitrosodiethylamine (NDEA) and *N*-Nitrosodiphenylamine (NDPHA) were also identified in drinking water. In 2005, EPA listed 6 genetoxic *N*-nitrosamines as unregulated contaminants that need to be detected in drinking water. The maximum contaminant level (MCL) in drinking water that EPA set for NDMA, *N*-nitroso-methyl-ethylamine (NMEA) and NDEA were 7, 20 and 2 ng/L, respectively.

In this paper, a method was proposed for determination of *N*-nitrosamines in drinking water by triple quadrupole GC-MS. In the method, *N*-nitrosamines in drinking water were enriched by means of solid-phase extraction (SPE). The results showed that GCMS-TQ8030 when used to analyze *N*-nitrosamines standard solutions, demonstrated good linearity in the range 0.5~100 µg/L and excellent sensitivity to 0.1 µg/L.

EXPERIMENTAL

GC-MS/MS: GCMS-TQ8030

Conditions of Analysis

Column	: Stabilwax, 30 m × 0.25 mm × 0.25 µm
Injector temperature	: 230 °C
Injection mode	: splitless injection (1 min)

Column temperature program : 60 °C (2 min)→@8 °C/min→140 °C (8 min)
→@40°C/min→240 °C (10 min)
CLV : 36.5 cm/sec
High pressure injection : 250 kPa (1 min)
Injection volume : 2 µL
Ionization mode : EI
Temperature of ion source : 200 °C
Temperature of GC-MS : 240 °C
Interface
Solvent dwell time : 5.5 min
Acquisition mode :Multiple Reaction Monitoring (MRM)
Characteristic ions were as listed in Table 1

Sample Preparation

100 mL water was sampled and filtered with a 0.45 µm nylon filter membrane. A Lichrolut® EN columnella was fitted to a solid-phase extraction device and eluted with 10 mL dichlormethane, 10 mL methanol and 10 mL distilled water; then the water sample was loaded to the column and allowed to percolate at the flowrate of 3 mL/min; at the end of the percolation, the column was suctioned dry, then eluted with 10 mL dichlormethane; the eluant was brought to the volume of 1 mL and used for analysis.

RESULTS AND DISCUSSION

Chromatogram

Standard solutions at concentrations of 100 µg/L were prepared and collected in reference with the above-mentioned conditions. MRM chromatograms were shown in Fig. 1.

The standard solutions of *N*-nitrosamines were diluted to 0.1 µg/L and subject to analysis once again, yielding chromatograms of the *N*-nitrosamines as shown in Fig. 2.The results indicated that GCMS-TQ8030 has excellent response to 0.1 µg/L standard solutions.

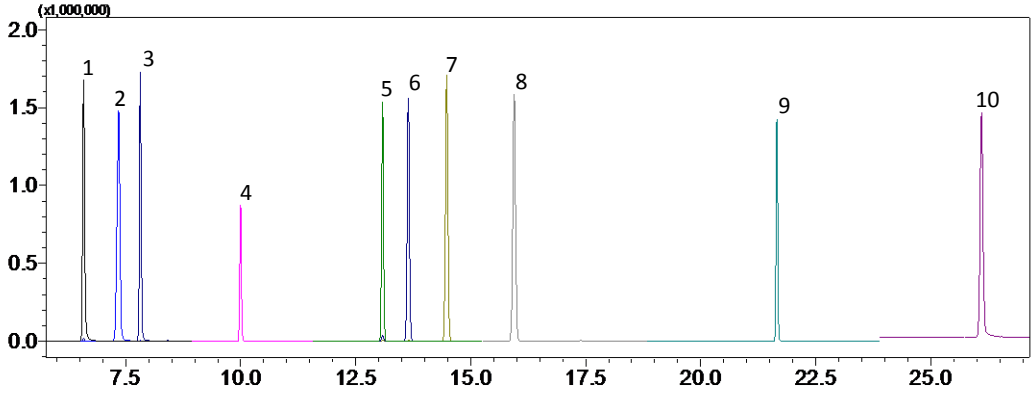


Figure 1 MRM chromatograms of *N*-nitrosamines
(At concentration of 100 µg/L)

Table 1 Retention time and MRM parameters of *N*-nitrosamines

ID	Compound	Retention Time (min)	Precursor Ion>Product Ion (m/z)	Collision Energy (V)
1	NDMA	7.350	74.00>42.00	21
			74.00>44.00	7
2	NMEA	8.158	88.00>71.00	5
			88.00>57.00	10
3	NDEA	8.658	102.00>85.00	5
			102.00>57.00	13
4	NDPA	10.942	130.00>113.00	5
			130.00>102.00	5
5	NDBA	14.075	158.00>141.00	5
			158.00>99.00	9
6	NPIP	14.258	114.00>84.00	9
			114.00>55.00	20
7	NPYR	14.350	100.00>70.00	7
			100.00>68.00	9
8	NMOR	14.625	116.00>86.00	5
			116.00>56.00	12
9	NDPhA	15.467	169.00>141.00	26
			169.00>115.00	30
10	NDBzA	16.942	226.00>181.00	20
			226.00>166.00	5

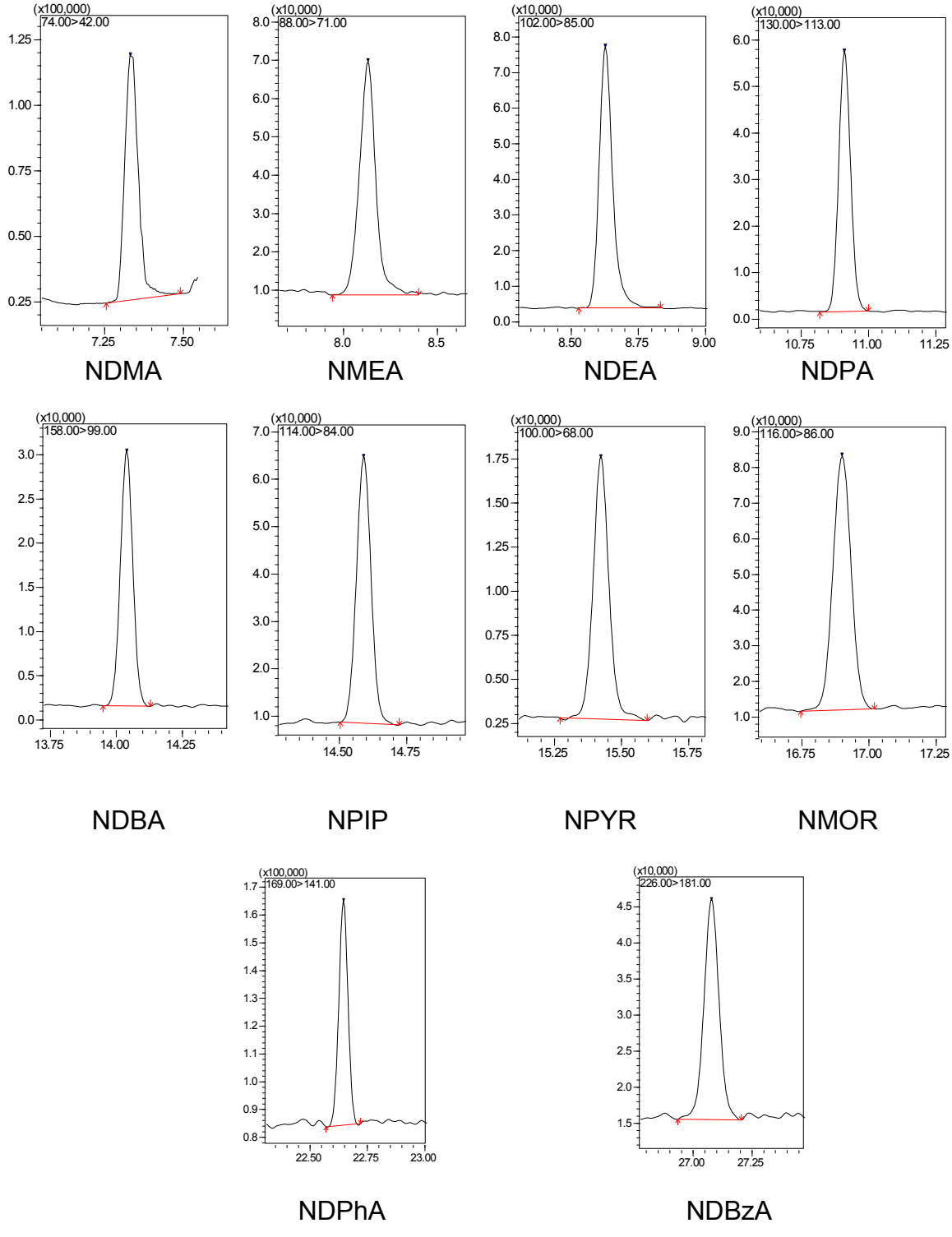
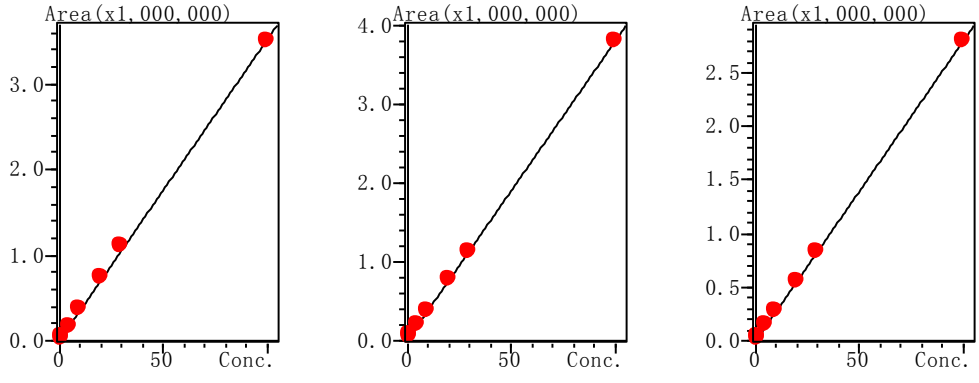


Figure 2. 10 Chromatogram of 10 *N*-nitrosamines (at concentration of 0.1 µg/L)

Calibration curve, repeatability, recovery and LOD

Multi-standard solutions of *N*-nitrosamines were prepared at concentrations of 0.5, 1, 5, 10, 20, 30 and 100 µg/L. MRM mode was used for analysis. The calibration curves and repeatability test results of the compositions are shown in Fig. 3. Three representative calibration curves of NDMA, NMEA and NDEA were listed here.

A 20 µg/L *N*-nitrosamine standard solution was spiked at spike concentration of 10 pg/L into 3 blank water samples. The samples were then subject to the above-mentioned pretreatment procedures and their recoveries were calculated. The LODs were calculated and are listed in Table 2.



NDMA NMEA NDEA

Figure 3.Calibration curves of NDMA, NMEA and NDEA

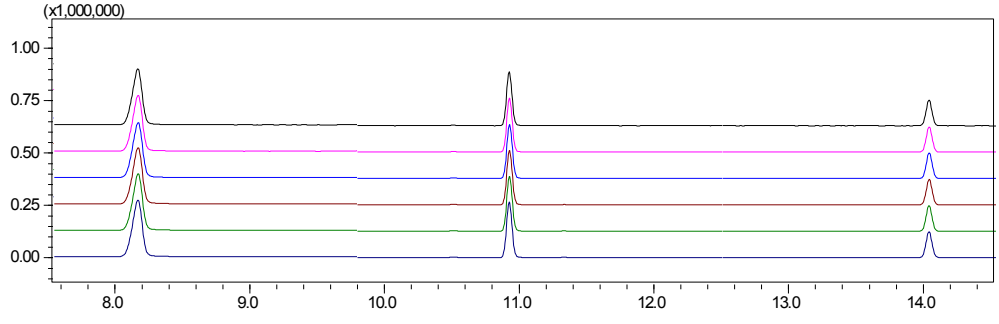


Figure 4.Repeatability of NMEA, NDPA and NDBA (at concentration of 0.5 µg/L)

Table 2. Correlation coefficient of calibration curves, repeatability, recovery and LOD results

No.	Compound	Correlation Coefficient (r)	Peak area %RSD (0.5µg/L) (n=5)	%Average Recovery (n=3)	LOD (pg/L)
1	NDMA	0.9998	1.37	83.51	0.31
2	NMEA	0.9998	1.19	74.96	0.01
3	NDEA	0.9999	1.17	88.78	0.17
4	NDPA	0.9999	1.65	78.73	0.03
5	NDBA	0.9999	1.35	82.62	0.03
6	NPIP	0.9999	1.18	73.12	0.03
7	NPYR	0.9999	1.55	78.52	0.09
8	NMOR	0.9999	1.37	81.71	0.04
9	NDPhA	0.998	5.89	74.69	0.09
10	NDBzA	0.9999	2.21	97.57	0.06

CONCLUSION

A method was proposed for analysis of *N*-nitrosamines in drinking water with Shimadzu Triple Quadrupole GCMS-TQ8030. The method had the merits of simple sample treatment, high sensitivity and demonstrated good linearity in the range of 0.5~100 µg/L. The RSD% of peak areas of compounds in 5 successive injections of 0.5 µg/L standard solution was less than 2 %, suggesting that the method had good reproducibility.

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V-3

DETERMINATION OF 68 VETERINARY DRUGS IN MARINE PRODUCTS BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TRIPLE QUADRUPOLE MASS SPECTROMETRY

INTRODUCTION

Veterinary drugs are widely used in breeding of marine products. However, residual veterinary drugs could enter human body and harm human health. Therefore, those veterinary drugs in marine products have been strictly regulated in the world. In recent years, the China government continues to strengthen supervision and is developing quicker and highly sensitive analytical method. Usually, the qualitative method of LC/MS/MS is based on the ratio of intensities between qualitative ion and quantitative ion. This paper describes ultra high performance liquid chromatography-triple quadrupole mass spectrometry for rapid screening of 68 veterinary drugs which belong to 12 categories.

EXPERIMENTAL

Sample Preparation

Samples of marine products were extracted with acetonitrile. After centrifugation, concentration and filtration, the final extract was injected to the LC/MS/MS instrument.

Instrument parameters

System configuration

HPLC	: Nexera
Pumping uint	: LC-30AD
Column oven	: CTO-30A
Degassing unit	: DGU-30A$_3$
Autosampler	: SIL-30AC
LC/MS/MS	: LCMS-8040

LC Conditions

Column	: Shim-pack XR-ODSIII (50 mmL. × 2.0 mmi.d., 1.6 µm)
Mobile phase	: A - 0.1% formic acid ; B - Acetonitrile
Flow rate	: 0.4 mL/min
Elution mode	: Gradient
Column temperature	: 40 °C
Injection volume	: 10 µL

MS conditions

Ionization	: ESI ; Positive & Negative polarity
Probe voltage	: +4.5 kV (positive), -3.5 kV (negative)
Nebulizing gas flow	: 1.5 L/min
Drying gas pressure	: 10 L/min
DL temperature	: 250 ºC
BH temperature	: 400 ºC

Antibiotics analysed in this paper have been classified into respective groups and tabulated in Table 1.

Table 1. List of veterinary drugs

Sulfonamides [12]
Sulfacetamide
Sulfadiazine
Sulfathiazole
Sulfapyridine
Sulfamerazine
Sulfamethazine
Sulfamethoxypyridazine
Sulfchloropyridazine
Sulfamethoxazole
Sulfisoxazole
Sulfadimethoxine
Sulfachinoxalin

Quinolones [10]
Pipemidic acid
Enoxacin sesquihydrate
Ofloxacin
Norfloxacin
Ciprofloxacin hydrochloride
Lomefloxacin
Danofloxain
Enrofloxacin
Sarafloxacin hydrochloride
Cinoxacin

Hormones [17]
19-nor-4-androstene-3,17-dione
1-Dehydrotestosterone Sulfate
Danazol
Fluoxymesterone
Testosterone
17-alpha-methyltestosterone
Methadrostenolone
Nandrolone
19-nor-4-androstene-3,17-dione
Trenbolone
Megestrol-17-acetate
Medroxyprogesterone
Medroxyprogesterone-17-acetate
Norgestrel
Chloromadinone 17-acetate
Norethindrone
Progesterone

Macrolides [5]
Spiramycin
leucomycin hydrate
Erythromycin
Tilmicosin
Acetylisovaleryltylosin Tartrate

Chloramphenicols [3]
Thiamphenicol
Florfenicol
Chloramphenicol

Nitro imidazoles [5]
Ronidazole
2-methyl-5-nitroimidazole
Metronidazole
4-Nitroimidazole
Ipronidazole

Nitrofuran metabolites [4]
Furazolidone
Furaltadone
Nitrofurantion
Furacilinum

Tetracyclines [5]
Tetracycline hydrochloride
Oxytetracycline
Demeclocycline hydrochloride
Chlorotetrachclie hydrochloride
Doxycycline

Lincosamides [2]
Lincocin Hydrochloride
Clindamycin

Other drug [5]
Trimethoprin
Malachite green oxalate
Leucomalachite green
Basic violet 3
Leucocrystal violet

RESULTS AND DISCUSSION

Using a polarity switching speed of 15 msec and a scan speed of 15,000 u/sec, MRM spectra were generated in both positive and negative ionization. Fast polarity switching helps to provide informative product ion spectra subsequently resulting in better detection and identification.
Figure 1 shows the representative calibration curves of sulfonamides. Excellent linearity was demonstrated in the range of 1 to 200 µg/L for sulfadiazine, sulfamethoxypyridazine, sulfamethoxazole and sulfisoxazole, with correlation coefficients greater than 0.998.

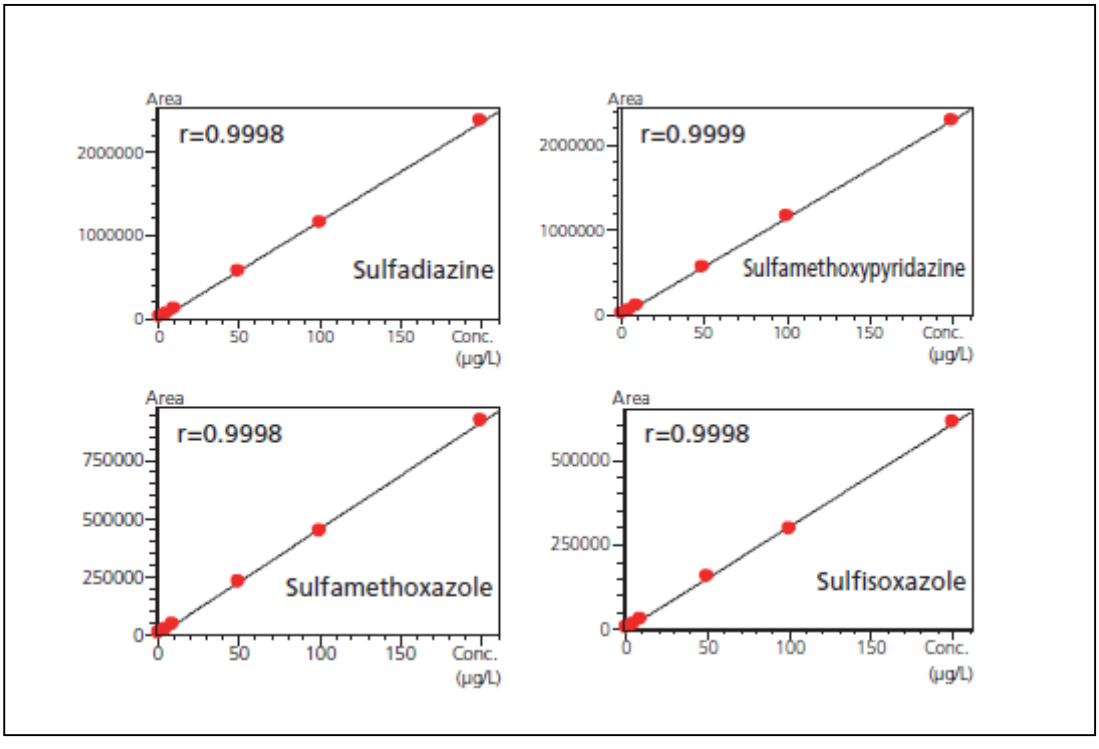

Figure. 1 Representative calibration curves of sulfonamides

The repeatabilities of 68 drugs (1-10 µg/L) were investigated, and the % RSDs of peak area were less than 5 %, and those for retention time were less than 0.9%, as shown in Table 2.

Table 2. Repeatability of 68 drugs (n=6)

No.	Compound	%RSD		No.	Compound	%RSD	
		R.T	Area			R.T	Area
1	Sulfacetamide	0.40	4.43	35	Medroxyprogesterone-17-acetate	0.14	3.33
2	Sulfadiazine	0.27	3.91	36	Norgestrel	0.21	4.13
3	Sulfathiazole	0.21	3.13	37	Chloromadinone-17-acetate	0.14	4.09
4	Sulfapyridine	0.32	2.85	38	Norethindrone	0.27	3.46
5	Sulfamerazine	0.28	3.86	39	Progesterone	0.17	3.28
6	Sulfamethazine	0.18	2.40	40	Spiramycin	0.67	1.37
7	Sulfamethoxypyridazine	0.21	4.02	41	leucomycin hydrate	0.11	0.85
8	Sulfchloropyridazine	0.11	3.47	42	Erythromycin	0.08	1.88
9	Sulfamethoxazole	0.08	4.46	43	Tilmicosin	0.05	2.01
10	Sulfisoxazole	0.07	4.41	44	Acetylisovaleryltylosin tartrate	0.07	1.28
11	Sulfadimethoxine	0.10	2.06	45	Thiamphenicol	0.15	3.34
12	Sulfachinoxalin	0.09	4.00	46	Florfenicol	0.09	2.39
13	Pipemidic acid	0.23	2.82	47	Chloramphenicol	0.16	1.68
14	Enoxacin sesquihydrate	0.50	1.68	48	Ronidazole	0.82	1.25
15	Ofloxacin	0.14	2.88	49	2-methyl-5-nitroimidazole	0.69	1.34
16	Norfloxacin	0.09	2.61	50	Metronidazole	0.76	1.37
17	Ciprofloxacin hydrochloride	0.08	2.18	51	4-Nitroimidazole	0.51	1.10
18	Lomefloxacin	0.04	2.14	52	Ipronidazole	0.07	0.91
19	Danofloxain	0.02	2.42	53	Furazolidone	0.15	2.36
20	Enrofloxacin	0.04	2.04	54	Furaltadone	0.09	2.13
21	Sarafloxacin hydrochloride	0.03	2.27	55	Nitrofurantion	0.27	3.49
22	Cinoxacin	0.04	1.94	56	Furacilinum	0.28	4.66
23	19-nor-4-androstene-3,17-dione	0.19	3.71	57	Tetracycline hydrochloride	0.65	1.60
24	1-Dehydrotestosterone sulfate	0.19	2.57	58	Oxytetracycline	0.65	1.60
25	Danazol	0.23	3.51	59	Demeclocycline hydrochloride	0.64	1.80
26	Fluoxymesterone	0.21	4.78	60	Chlorotetrachclie hydrochloride	0.65	1.60
27	Testosterone	0.22	3.05	61	Doxycycline	0.65	2.00
28	17-alpha-methyltestosterone	0.12	3.67	62	Lincocin hydrochloride	0.11	2.37
29	Methadrostenolone	0.20	1.44	63	Clindamycin	0.19	3.26
30	Nandrolone	0.19	2.89	64	Trimethoprin	0.29	1.72
31	19-nor-4-androstene-3,17-dione	0.18	3.83	65	Malachite green oxalate	0.08	0.75
32	Trenbolone	0.23	2.94	66	Leucomalachite green	0.10	1.29
33	Megestrol-17-acetate	0.10	3.02	67	Basic violet 3	0.04	0.68
34	Medroxyprogesterone	0.25	3.17	68	Leucocrystal violet	0.10	1.83

In this study, different marine products were studied. The recoveries of drugs in fish samples (the concentration of spiked drugs ranged from 0.1 to 2 µg/kg) are as summarized in Table 3. The average recovery range of 52 compounds was from 74 to 120%.

Table 3. Recovery test of 52 compounds

	Compound	Recovery (%)
Sulfonamides	Sulfacetamide	88.6
	Sulfadiazine	96.4
	Sulfathiazole	91.1
	Sulfapyridine	106.3
	Sulfamerazine	94.0
	Sulfamethazine	97.2
	Sulfamethoxypyridazine	91.9
	Sulfchloropyridazine	95.3
	Sulfamethoxazole	103.1
	Sulfisoxazole	102.2
	Sulfadimethoxine	94.1
	Sulfachinoxalin	93.9

Quinolones	Pipemidic acid	81.0
	Enoxacin sesquihydrate	68.0
	Ofloxacin	88.0
	Norfloxacin	77.0
	Ciprofloxacin hydrochloride	95.5
	Lomefloxacin	92.0
	Danofloxain	83.0
	Enrofloxacin	74.0
	Sarafloxacin hydrochloride	76.0
	Cinoxacin	82.5
Tetracyclines	Tetracycline hydrochloride	105.0
	Oxytetracycline	120.0
	Demeclocycline hydrochloride	110.0
	Chlorotetrachclie hydrochloride	100.0
	Doxycycline	115.0
Hormones	19-nor-4-androstene-3,17-dione	106.0
	1-Dehydrotestosterone Sulfate	99.0
	Danazol	93.0
	Fluoxymesterone	113.5
	Testosterone	106.0
	17-alpha-methyltestosterone	94.7
	Methadrostenolone	90.5
	Nandrolone	95.0
	19-nor-4-androstene-3,17-dione	80.0
	Trenbolone	96.1
	Megestrol-17-acetate	95.0
	Medroxyprogesterone	94.1
	Medroxyprogesterone-17-acetate	114.5
	Norgestrel	115.0
	Chloromadinone 17-acetate	110.5
	Norethindrone	111.6
	Progesterone	78.0
Macrolides	Spiramycin	89.0
	leucomycin hydrate	91.0
	Erythromycin	84.0
	Tilmicosin	104.0
	Acetylisovaleryltylosin Tartrate	93.0

Chloramphenicols	Thiamphenicol	105.0
	Florfenicol	81.5
	Chloramphenicol	93.3

CONCLUSION

A simultaneous and cost-effective method of identification and quantification of 68 veterinary drugs in marine products was developed. Improved selectivity and sensitivity of the instrumental analysis was achieved by LC/MS/MS technique.

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GC-MS/MS

V-4 High Sensitivity Detection and Quantification of Trace Levels of *N*-Nitrosamines in Beers by GC-MS/MS Method

INTRODUCTION

Most of *N*-nitrosamines are known to be carcinogenic and mutagenic. Consumption of nitrosamines, e.g., *N*-nitrosodimethylamine (NDMA), was reported to be a cause of gastric cancer, liver cancer, glioma and blood disorder. *N*-nitrosamines could be formed by reaction between amine and nitrite under heating conditions in food processing. For example, they were found in nitrite-treated meat and malt-derived beverages like beer at trace level. The presence of NDMA in malt and beer was first reported in 1974 [1]. Its concentration in malt depends on the drying techniques used. According to the US and EU regulation, the amount of nitrosamines in beer must be controlled to the acceptable levels, typically at 0.2 ~ 5.0 ppb depending on the country [2]. The main *N*-nitrosamine that is monitored in malt and beer is NDMA. Here, we report a new GC-MS/MS method using Multiple Reaction Monitoring (MRM) mode for simultaneous detection and quantification of six *N*-nitrosamines including NDMA for enhanced selectivity and sensitivity from the potential matrix interferences in beer samples.

EXPERIMENTAL

Preparation of Calibrants and Samples

Six *N*-nitrosamines, namely *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodi-butylamine (NDBA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosomorpholine (NMOR) and *N*-nitrosopiperidine (NPIP) were selected in this study. *N*-nitrosodi-propylamine (NDPA) was used as internal standard (IS). A nitrosamines mixed standard stock solution (10 ppm) was prepared using dichloromethane as solvent. Subsequently, a series of calibrants each spiked with 50.0 ppb IS (NDPA) ranging from 0.1 to 50.0 ppb was prepared. The beer samples were prepared based on the modified AOAC Official Method 982.11, 2000 [3].

Instrument and Analytical Conditions

GC-MS: GCMS-TQ8030
Auto injector: AOC-20i+s
Column: Stabilwax DB (Restek Corp.)
30 m L. x 0.25 mm I.D. x 0.25µm δf
Workstation: GCMS solution Version 4.01

Table 1: GC-MS/MS analytical conditions

GC	
Injection Temp.	200°C
Column Temp.	50°C (2 min), 20°C/min ~210°C (15 min)
Injection Mode	Pulse splitless (300 kPa for 1 min)
Carrier Gas	He
Linear Velocity	40.0 cm/sec
Purge Flow	3 mL/min
Injection Volume	4 mL

MS/MS	
Ion Source Temp.	200°C
Interface Temp.	210°C
CID gas	Ar
Solvent cut Time	4 min
Event time	0.30 sec
Acquisition Mode	MRM

RESULTS AND DISCUSSION

Method Development

Figure 1 shows the Total Ion Chromatogram (TIC) of the six *N*-nitrosamines and internal standard (IS) in full scan mode. Based on the spectra of eluted peaks, precursor ions were selected for MS/MS product ion scan analysis as well as MRM optimization. As an example, figure 2 shows the full scan spectrum of NDEA and its product ion scans (m/z 102) with two different collision energies (4 and 14 V).

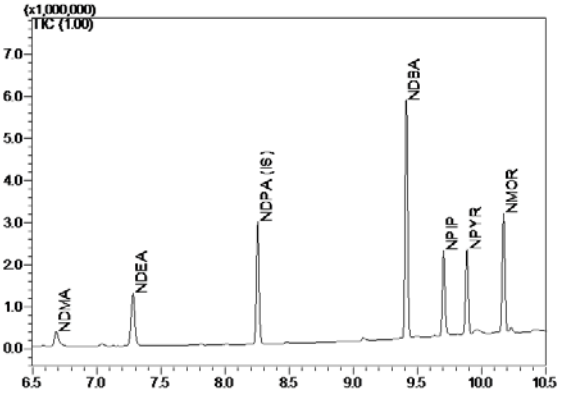

Figure 1: TIC of scan data for N-nitrosamines and internal standard NDPA in GC-MS mode

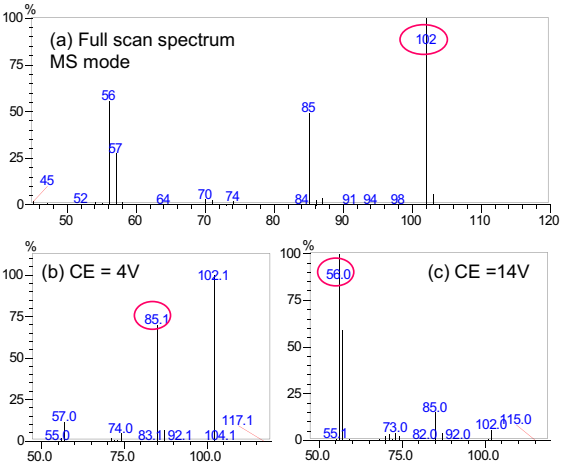

Figure 2: NDE full scan MS spectrum (a) and product ion scan of precursor ion m/z102 with different collision energy (b, c).

MRM optimization of the six *N*-nitrosamines studied were carried out systemically to obtain the optimized collision energy (CE) for two transitions. The MRM transitions and CE are compiled into Table 2. The MRM transition with higher intensity was used as the quantitative ion and the other one as qualitative ion for confirmation.

Method Performance Evaluation

A MRM quantification method was set up based on the MRM transitions in Table 2. Linear calibration curves with internal standard (IS) were established for the six *N*-nitrosamines as shown in Figure 3. The linearity with correlation coefficient (R^2) greater than 0.999 across the calibration range of 0.1 ppb – 50.0 ppb was obtained.

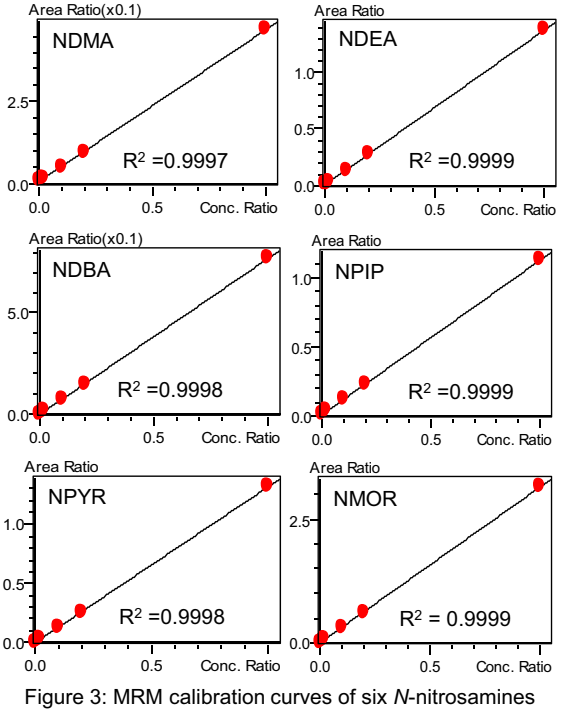

Figure 3: MRM calibration curves of six *N*-nitrosamines from 0.1, 0.5,1.0,5.0,10.0 and 50.0 ppb with IS.

Table 2: MRM parameters of six *N*-nitrosamines and IS on GCMS-TQ8030 triple quadrupole

ID	Name	RT (min)	Quantitative Ion		Qualitative Ion	
			Transition	CE	Transition	CE
1	NDMA	6.685	74.1>42.0	11	74.1>30.0	11
2	NDE	7.282	102.1>85.1	4	102.1>56.0	14
3	NDPA (IS)	8.257	130.2>113.1	4	130.2>88.1	4
4	NDBA	9.412	158.2>99.1	8		
5	NPIP	9.708	114.1>97.1	8	114.1>84.1	8
6	NPYR	9.886	100.1>70.0	7	100.1>55.0	8
7	NMOR	10.172	116.1>86.0	6	116.1>56.0	11

Figure 4 shows the MRM peaks of the *N*-nitrosamines at 0.1 ppb level. The repeatability, LOD and LOQ were evaluated at the concentration level 0.5 ppb and tabulated in Table 3. The peak area %RSD for all the target analytes were below 5%, except for NPYR. The overlay mass chromatograms in Figure 5 demonstrated the peak area consistency of NMOR graphically for five consecutive injections.

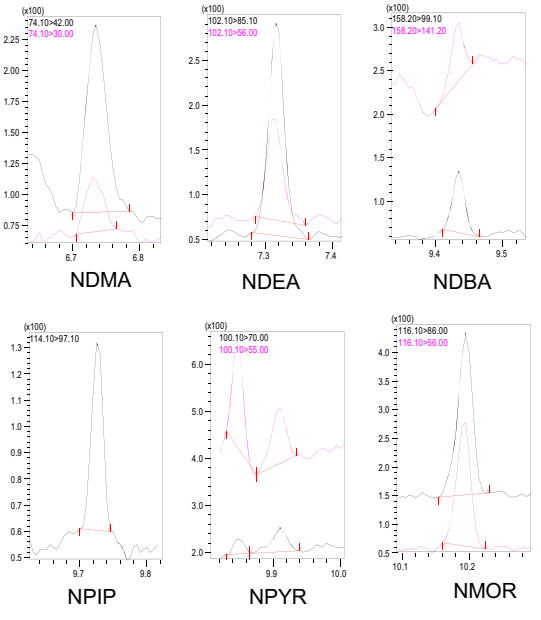

Figure 4: MRM peaks of six *N*-nitrosamines of 0.1 ppb on GCMS-TQ8030

Table 3: Performance evaluation of MRM quantification method of *N*-nitrosamines with 0.5 ppb level (n=5)

ID	Compound Name	%RSD	S/N	LOD* (ppb)	LOQ** (ppb)
1	NDMA	4.63	3.12	0.48	1.6
2	NDEA	2.76	33.77	0.04	0.15
4	NDBA	2.78	19.17	0.08	0.26
5	NPIP	4.77	16.58	0.09	0.3
6	NPYR	6.33	3.22	0.47	1.55
7	NMOR	1.5	62.44	0.02	0.08

* S/N = 3; ** S/N = 10

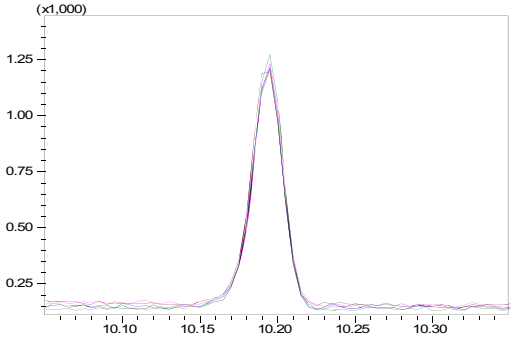

Figure 5: MRM chromatograms of NMOR at 0.5 ppb, five consecutive injections (n=5)

***N*-nitrosamines in Beer Sample**

The GC-MS/MS method was established for the application of beer samples. The investigated beer samples were pre-concentrated 25 times based on preparation method described earlier. Figure 6 shows the TICs of MRM data for the spiked beer extract. It was found that some trace amount of NDBA, NPYR and NMOR were present in the sample blank (un-spiked beer extract) as summarized in Table 3. However, there is no safety concern as it is far below the regulation limit. The recovery of the *N*-nitrosamines was calculated with the post spiked beer sample extract to be within ±12 %.

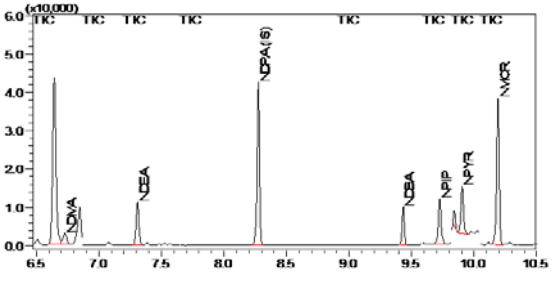

Figure 6: TICs of MRM data for spiked beer extract with added 50.0 ppb IS

Table 4: Analysis result and recovery of *N*-nitrosamines in beer sample

Compound	[1]Spiked beer extract (ppb)	[2]Recovery (%)	[3]Concentration in beer (ppb)
NDMA	9.6	96	Not Detected
NDEA	8.1	81	Not Detected
NDBA	9.6	93	0.012
NPIP	10.4	104	Not Detected
NPYR	10.6	102	0.016
NMOR	11.3	111	0.008

Notes:
[1] Mixed standard 10.0 ppb was spiked in beer extract
[2] Recovery = (Spiked beer extract – Sample blank /10) * 100
[3] Concentration in beer (ppb) = Sample blank / 25

CONCLUSION

A highly sensitive and selective GC-MS/MS method using Shimadzu GCMS-TQ8030 was developed for *N*-nitrosamines analysis. With proper sample preparation and MRM data acquisition mode, the system has been used successfully to determine trace-level of six *N*-nitrosamines in beer sample which are lower than the limit maximum required by regulation.

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V-5 QUANTITATIVE ANALYSIS OF NITROFURAN PARENTS USING LCMS-8040

INTRODUCTION

Nitrofurans are Schiff's base derivatives of nitrofuraldehyde and are known to have a broad-spectrum of antimicrobial activity. These are widely used in animal feed in food-producing animals like poultry, swine, cultured fish as they serve to be efficient growth promoters as well as for treatment and prevention of various gastrointestinal infections caused by bacteria or protozoa. These were banned from use in the European Union (EU) in 1995 due to concerns about the carcinogenicity of their residues in edible tissue. The most common nitrofurans are furazolidone (FZD), nitrofurantoin (NFT), nitrofurazone (NFZ) and furaltadone (FTD). These compounds are rapidly metabolized in vivo, leading to a significant decrease of their parent compounds levels in plasma. Animal feeds must, hence, be analyzed with analytical procedures capable of measuring very low concentrations of nitrofuran parents to assure its "fit-for-use". Efforts have been made here to develop a sensitive LC/MS/MS method using standard nitrofuran parent compounds and establish their LOQs on LCMS-8040 system.

EXPERIMENTAL

Instrument parameters

System configuration

HPLC	: Nexera (UHPLC)
Degassing unit	: DGU-20A$_5$
Pumping uint	: LC-30AD
Autosampler	: SIL-30AC
Column oven	: CTO-30A
Communication bus module	: CBM-20A

MS : LCMS-8040

LC conditions
Column : Shim-packXR-ODS (75mmLx3mmI.D.x2.2 µm)
Mobile phase : A–5mM ammonium formate in water : methanol (80:20)
B-5 mM ammonium formate in water : methanol (10:90)
Flow rate : 0.25 mL/min
Oven temperature : 40 °C
Diluent : Water
Injection volume : 50 µL
Gradient program :

Time (min)	Pump B conc.
0.01	10
5.00	100
8.00	100
9.00	10
12.00	10

MS conditions
Ionisation : Electrospray solution (ESI); positive & negative
Mode : MRM
Nebulising gas flow : 3 L/min
Drying gas flow : 10 L/min
DL temperature : 280 °C
Heat block temperature : 480 °C
MRM transitions :

Compound Name	MRM Transition (Quantifier)	MRM Transition (Qualifier)	Polarity	Q1 Resolution	Q3 Resolution	Calibration Method Used	Calibration range (ppb)
Furaltadone	325.10>281.10	325.20>100.10	Positive	Unit	Unit	External Standard	0.05 - 10
Furazolidone	242.80>226.00	243.20>139.00	Positive	Unit	Unit	External Standard	0.01 -10
Nitrofurazone	197.10>123.90	-	Negative	Low	Low	External Standard	0.05 - 5
Nitrofurantoin	237.00>152.00	237.00>124.10	Negative	Unit	Unit	External Standard	0.01 - 2

RESULTS AND DISCUSSION

Table 1 gives a list of the quantitative results of the four nitrofuran parent compounds and chromatograms at LOQ levels and calibrations graphs have been shown in Figures 1 & 2a to 2d respectively.

Table 1. LOQs of nitrofuran parent molecules and s/n at LOQ levels

Compound Name	Retention Time (min)	Correlation coefficient (r2)	LOQ (ppb)	S/N at LOQ Level
Furaltadone	3.99	0.9990	0.05	170.95
Furazolidone	3.37	0.9991	0.01	69.53
Nitrofurazone	3.49	0.9965	0.05	17.62
Nitrofurantoin	3.17	0.9957	0.01	190.20

Note: LOQs were determined based on following criteria:-
1. S/N > 10 (calculated by RMS); 2. % RSD< 16% for (n=3);
3 Accuracy = 80-120%

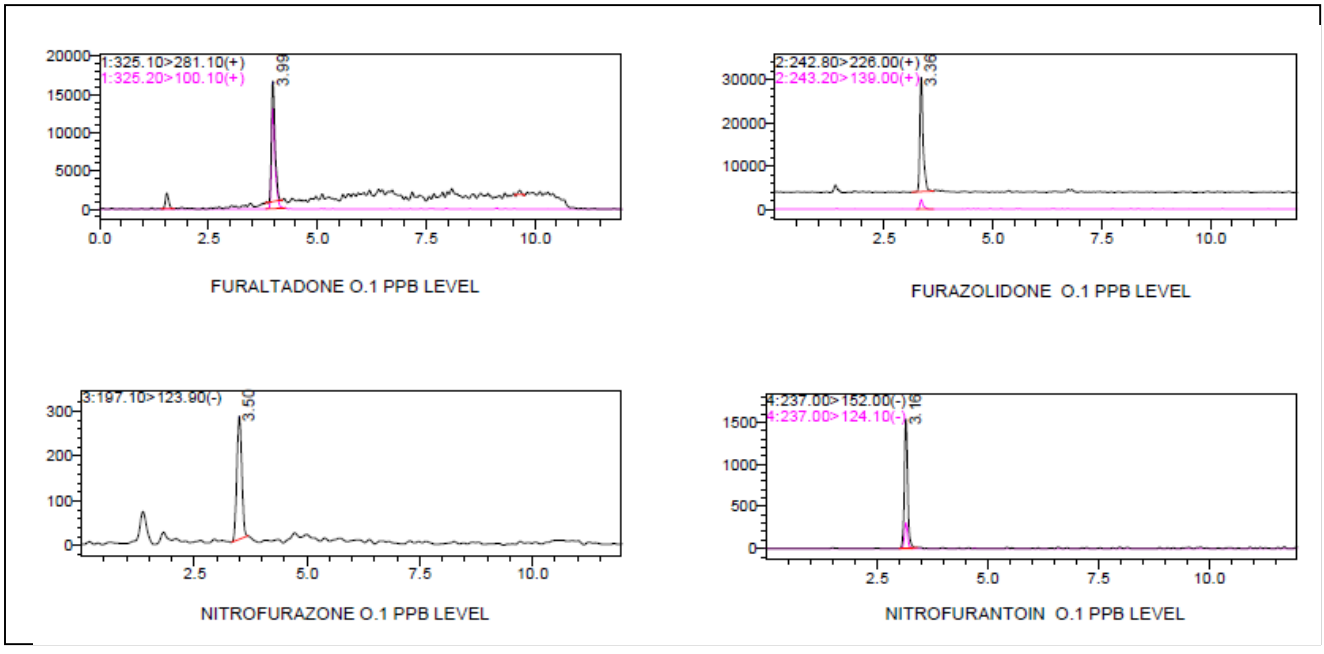
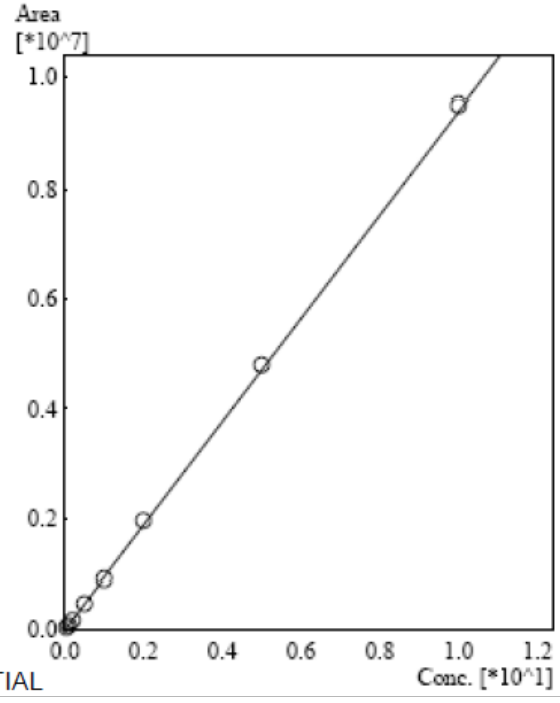


Figure 1. Chromatograms of nitrofurans at 0.1 ppb

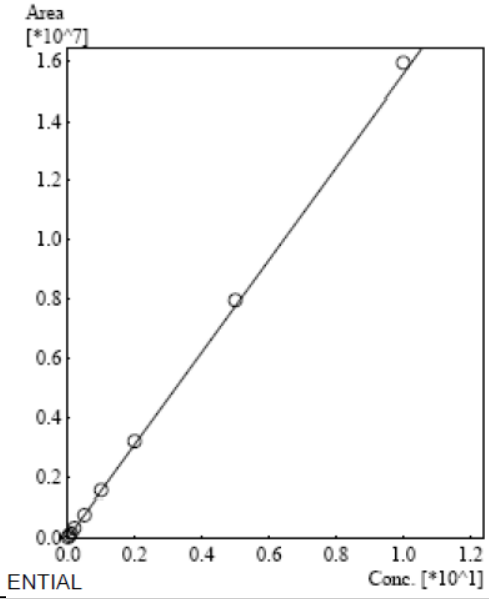
ID# : 1 m/z : 325.10>281.10
Name : FURALTADONE
Quantitative Method : External Standard
Function : f(x)=942102*x-711.559
Rr1=0.9990816 Rr2=0.9981640
MeanRF: 9.408230e+005 RF SD: 3.505702e+004 RF %RSD: 3.726207
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/A^2



#	Conc.(Ratio)	MeanArea	Area	Area%RSD
2	0.05	48305	47167	2.136049
			48571	
			49179	
3	0.1	87834	87838	1.846221
			89453	
			86210	
4	0.2	184008	184361	3.041923
			189420	
			178242	
5	0.5	478047	480432	1.451905
			470228	
			483481	
6	1	912849	925531	1.355578
			900807	
			912208	
7	2	1958484	1933187	1.587611
			1993196	
			1949068	
8	5	4803442	4793757	0.542654
			4783605	
			4832964	
9	10	9532282	9487506	0.496516
			9527533	
			9581806	

Figure 2a. Calibration graph and quantitative information for Furaltadone

ID# : 2 m/z : 242.80>226.00
Name : FURAZOLIDONE
Quantitative Method : External Standard
Function : f(x)=1.55396e+006*x-961.673
Rr1=0.9991184 Rr2=0.9982377
MeanRF: 1.543402e+006 RF SD: 8.148225e+004 RF %RSD: 5.279391
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/A^2

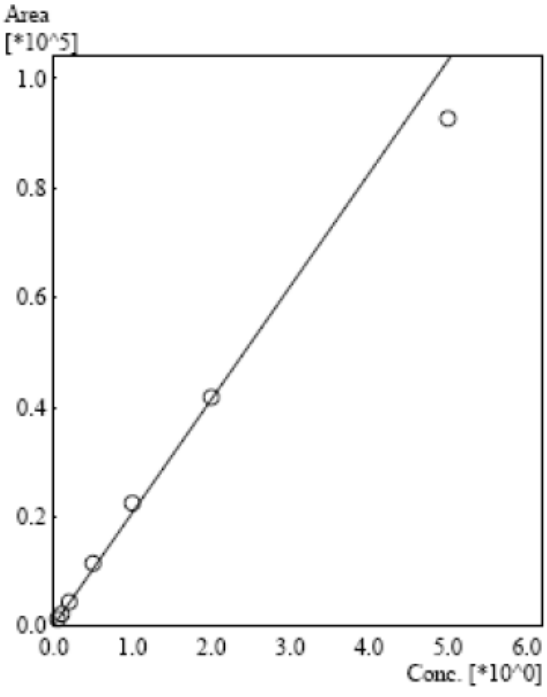


ENTIAL

#	Conc.(Ratio)	MeanArea	Area	Area%RSD
1	0.01	14742	16312	9.770099
			13481	
			14435	
2	0.05	75235	73284	2.507338
			77050	
			75369	
3	0.1	141831	138340	3.196412
			146955	
			140198	
4	0.2	324473	309472	4.084682
			334598	
			329348	
5	0.5	765734	764212	0.256549
			767952	
			765039	
6	1	1554775	1573784	1.069019
			1547565	
			1542977	
7	2	3202350	3240471	2.599518
			3259709	
			3106870	
8	5	7960565	7992387	1.782996
			8083890	
			7805419	
9	10	15914994	15803887	0.826239
			16060173	
			15880922	

Figure 2b. Calibration graph and quantitative information for Furazolidone

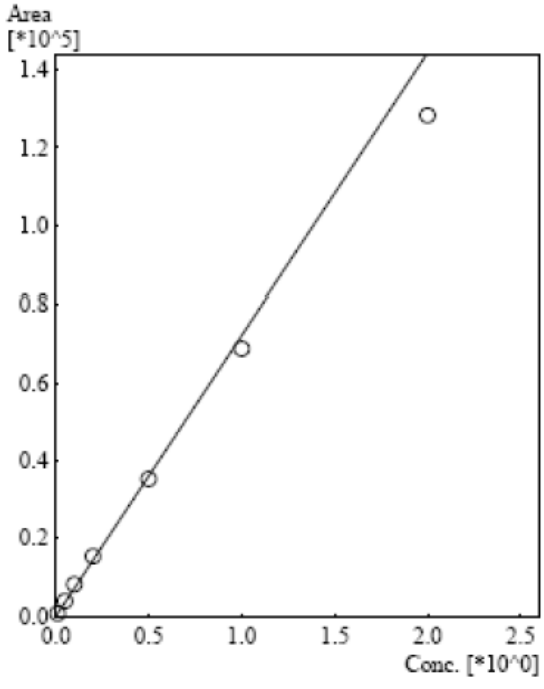
ID# : 3 m/z : 197.10>123.90
Name : NITROFURAZONE
Quantitative Method : External Standard
Function : f(x)=20643.5*x+167.756
Rr1=0.9965766 Rr2=0.9931648
MeanRF: 2.173456e+004 RF SD: 2.122379e+003 RF %RSD: 9.764996
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/A^2



#	Conc.(Ratio)	MeanArea	Area	Area%RSD
2	0.05	1211	1181	2.310547
			1216	
			1236	
3	0.1	2138	2101	1.532669
			2159	
			2155	
4	0.2	4362	4871	10.954383
			3922	
			4294	
5	0.5	11446	11412	2.810433
			11784	
			11143	
6	1	22420	19567	11.309700
			23279	
			24415	
7	2	41729	40141	5.358362
			40759	
			44286	
8	5	92739	100794	8.099308
			91495	
			85927	

Figure 2c. Calibration graph and quantitative information for Nitrofurazone

ID# : 4 m/z : 237.00>152.00
Name : NITROFURANTOIN
Quantitative Method : External Standard
Function : f(x)=72267.0*x-66.9797
Rr1=0.9957678 Rr2=0.9915534
MeanRF: 7.187089e+004 RF SD: 7.336973e+003 RF %RSD: 10.208546
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/A^2



#	Conc.(Ratio)	MeanArea	Area	Area%RSD
1	0.01	640	610	5.893341
			628	
			682	
2	0.05	3864	3874	0.661742
			3884	
			3835	
3	0.1	8153	7886	4.162861
			8535	
			8039	
4	0.2	15349	15984	8.676427
			16244	
			13818	
5	0.5	35292	33123	6.947233
			37952	
			34800	
6	1	68782	65978	3.577954
			69785	
			70584	
7	2	128308	125231	6.876642
			121435	
			138258	

Figure 2d. Calibration graph and quantitative information for Nitrofurantoin

CONCLUSION

An LC/MS/MS method was successfully developed for simultaneous analysis of four nitrofuran parent compounds. LOQs were established at sub-ppb levels on LCMS-8040 system. With the development of proper extraction procedures, this method can be extended to quantitate nitrofuran parents from samples such as animal feed.

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V-6 MRM METHOD FOR QUANTITATION OF NITROFURAZONE AND FURALTADONE USING LCMS-8040

INTRODUCTION

Nitrofuran antibiotics, are employed for the treatment of bacterial diseases in livestock production. A great advantage of these compounds in comparison with other antimicrobial agents, is the slow development and only to a limited extent of the in vivo bacterial resistance. Based on the evidence of carcinogenic and genotoxic effects of these nitrofuran bound metabolites, European Union (EU) has forbidden the use of nitrofuran drugs in food producing animals for more than a decade now. Hence, it becomes imperative to monitor these parents in animal feeds so as to ensure these are not fed to animals. In this note, LC/MS/MS method has been developed for two nitrofurans namely nitrofurazone and furaltadone.

EXPERIMENTAL

Instrument Parameters

System configuration

HPLC	: Nexera (UHPLC)
Degassing unit	: DGU-20A$_5$
Pumping uint	: LC-30AD
Autosampler	: SIL-30AC
Column oven	: CTO-30AC
Communication bus module	: CBM-20A
MS	: LCMS-8040
Software	: Labsolution ver 5.42SP4

LC conditions

Column	: Shim-pack XR-ODS III (50 mm L x 2 mm I.D., 1.6 µm)
Mobile phase	: A–Water (Millipore), B– Acetonitrile; A : B – 30:70 v/v

Flow rate : 0.4 mL/min
Oven temperature : 40 °C
Diluent : Water
Injection volume : 1 µL
Diluent : Acetonitrile

MS conditions
Ionisation : Electrospray solution (ESI); positive & negative
Mode : MRM
Nebulising gas flow : 3 L/min
Drying gas flow : 15 L/min
DL temperature : 250 °C
Heat block temperature : 400 °C
MRM Transitions :
Nitrofurazone (-ve)

MRM transitions	Dwell time (ms)	Q1 PreBias (V)	CE (V)	Q3 PreBias (V)
(-)197.0>124.0	5	12	8	25
(-)197.0>150.0	5	12	9	25
(-)197.0>80.0	5	12	10	30

Furaltadone (+ve)

MRM transitions	Dwell time (ms)	Q1 PreBias (V)	CE (V)	Q3 PreBias (V)
325.0>100.0	5	-15	-28	-17
325.0>252.1	5	-15	-17	-26
325.0>281.0	5	-15	-12	-12

Standard Preparation

Separate stock solutions of 100 mg/L (ppm) of the individual compounds were prepared from solid in pure acetonitrile. All standard samples were prepared using pure acetonitrile (100%) without any acid or buffer.

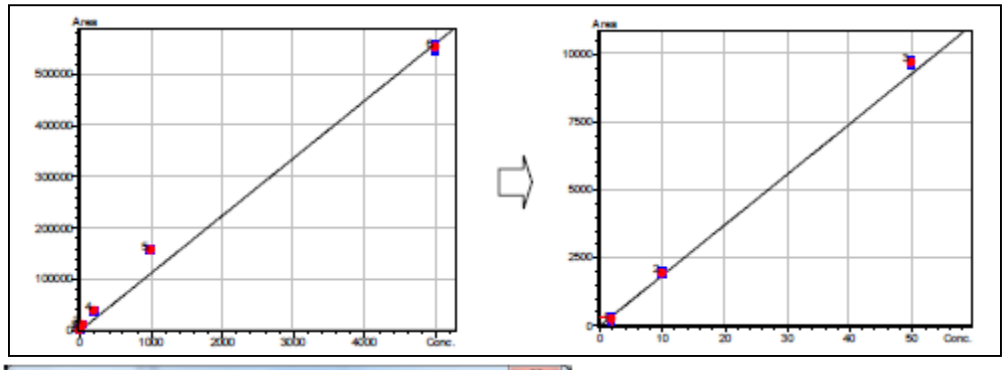
Standard series (in pure ACN) pg/uL	0.5	2.0	10	50	200	1000	5000
Abs (pg)	0.5	2.0	10	50	200	1000	5000

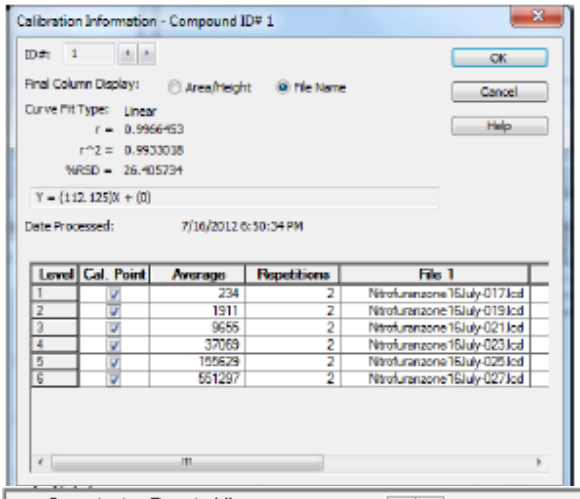
0.5 pg/uL x 1 µL = 0.5 pg = 500 fg

RESULTS AND DISCUSSION

Calibration curve of the two nitrofuran parent comounds have been represented in Figures 1a to 1c and 2a to 2c. Figure 3a and 3b shows representative chromatogram at 2 pg and 0.5 pg levels respectively.

Nitrofurazone, parent ion (-) m/z 197

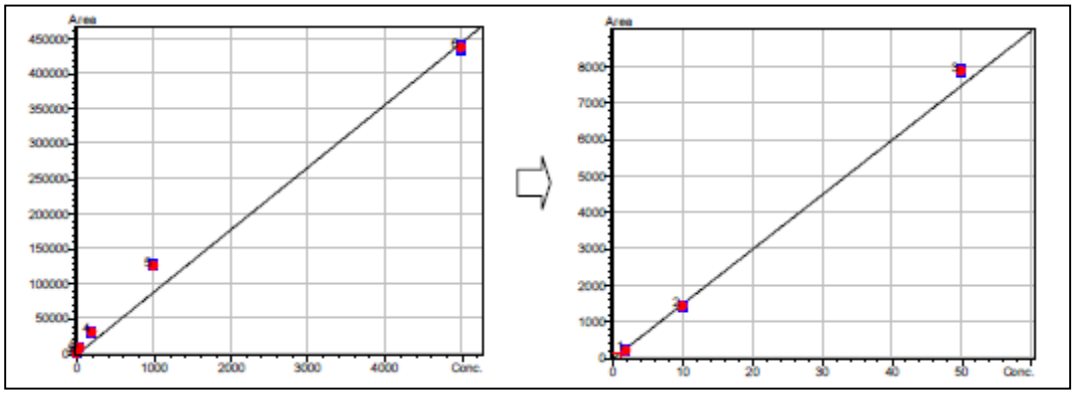


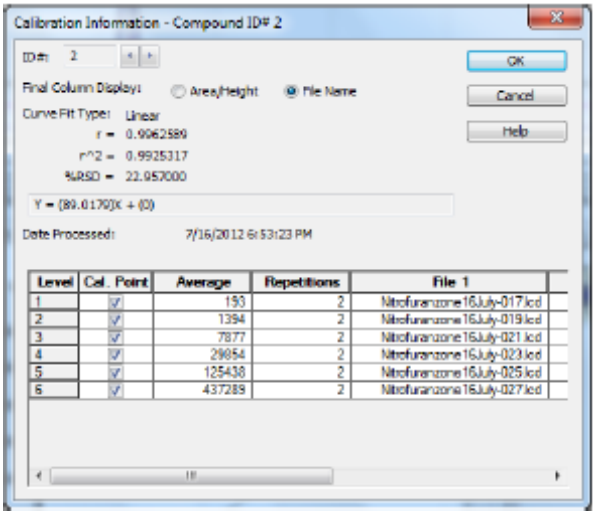


Quantitative Results View ID# 1 Nitrofurazone

	Data Filename	Sample Name	Ret. Time	Area	Conc. (ppb)
1	Nitrofurantoin16July-043.lcd	Nitrofrazone009	0.311	2,124	18.94
2	Nitrofurantoin16July-042.lcd	Nitrofrazone008	0.311	1,822	16.25
3	Nitrofurantoin16July-041.lcd	Nitrofrazone007	0.308	2,099	18.72
4	Nitrofurantoin16July-040.lcd	Nitrofrazone006	0.312	2,211	19.72
5	Nitrofurantoin16July-039.lcd	Nitrofrazone005	0.311	3,050	27.21
6	Nitrofurantoin16July-038.lcd	Nitrofrazone004	0.310	2,207	19.68
	Average		0.311	2,252	20.09
	%RSD		0.446117	18.478142	18.486573
	Maximum		0.312	3,050	27.21
	Minimum		0.308	1,822	16.25
	Std. Dev.		0.001386	416.149193	3.713336

Figure 1a. Calibration curve for nitrofurazone (-) 197 > 124 (2-5000 pg) and repetability data at 10 pg conc.

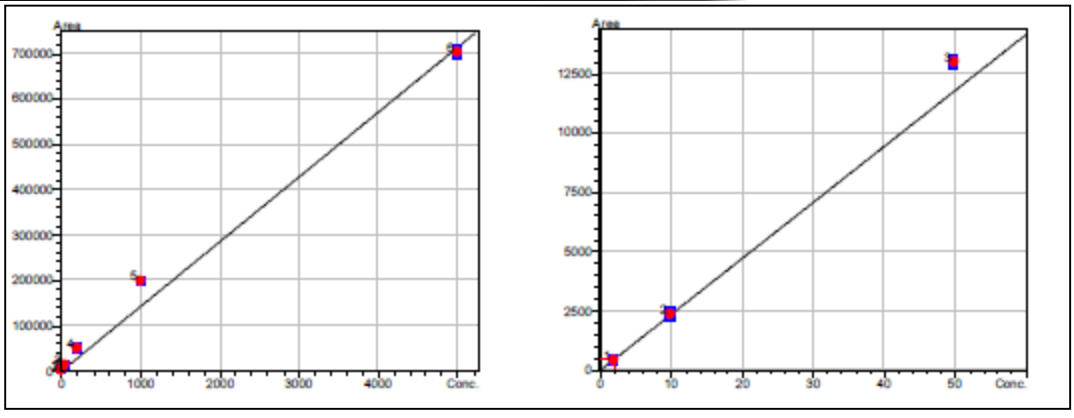


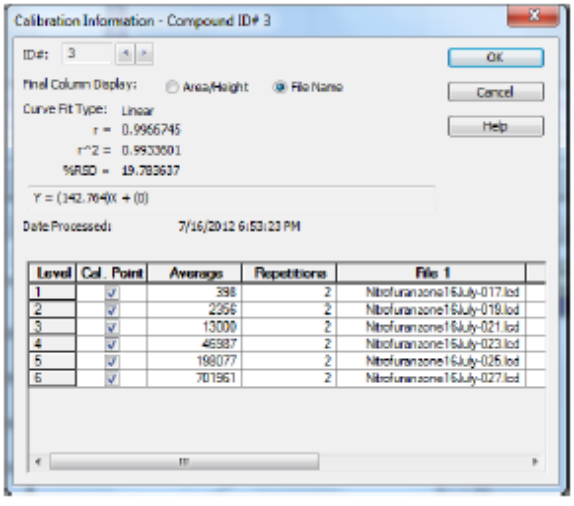


Quantitative Results View ID# 2 Nitrofurazone

	Data Filename	Sample Name	Ret. Time	Area	Conc. (ppb)
1	Nitrofuranzone16July-043.lcd	Nitrofrazone009	0.313	1,461	16.41
2	Nitrofuranzone16July-042.lcd	Nitrofrazone008	0.312	1,853	20.82
3	Nitrofuranzone16July-041.lcd	Nitrofrazone007	0.313	1,625	18.25
4	Nitrofuranzone16July-040.lcd	Nitrofrazone006	0.311	1,668	18.74
5	Nitrofuranzone16July-039.lcd	Nitrofrazone005	0.312	2,346	26.35
6	Nitrofuranzone16July-038.lcd	Nitrofrazone004	0.310	1,882	21.14
	Average		0.312	1,806	20.29
	%RSD		0.317893	16.974962	16.983740
	Maximum		0.313	2,346	26.35
	Minimum		0.310	1,461	16.41
	Std. Dev.		0.000991	306.531487	3.445152

Figure 1b. Calibration curve for nitrofurazone (-) 197 > 150 (2-5000 pg) and repetability data at 10 pg conc.



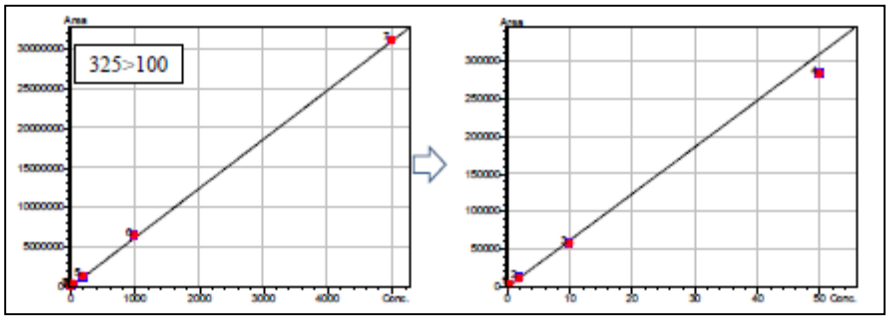


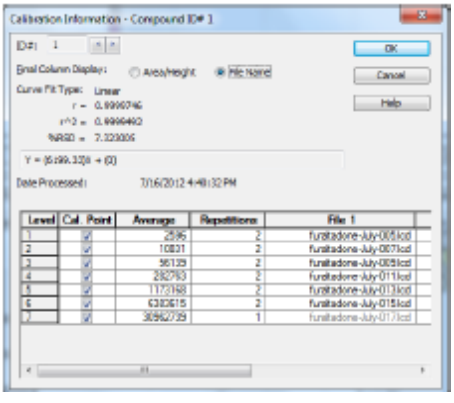
Quantitative Results View ID# 3 Nitrofurazone

	Data Filename	Sample Name	Ret. Time	Area	Conc. (ppb)
	Nitrofuranzone16July-043.lcd	Nitrofrazone009	0.311	3,157	22.12
	Nitrofuranzone16July-042.lcd	Nitrofrazone008	0.311	3,060	21.43
	Nitrofuranzone16July-041.lcd	Nitrofrazone007	0.313	2,994	20.97
	Nitrofuranzone16July-040.lcd	Nitrofrazone006	0.311	3,106	21.76
	Nitrofuranzone16July-039.lcd	Nitrofrazone005	0.311	4,417	30.94
	Nitrofuranzone16July-038.lcd	Nitrofrazone004	0.312	2,380	16.67
	Average		0.312	3,186	22.32
	%RSD		0.187842	20.944080	20.942059
	Maximum		0.313	4,417	30.94
	Minimum		0.311	2,380	16.67
	Std. Dev.		0.000585	667.203825	4.673221

Figure 1c. Calibration curve for nitrofurazone (-) 197 > 80 (2-5000pg) and repetability data at 10 pg conc.

Furaltadone, parent ion (+) m/z 325

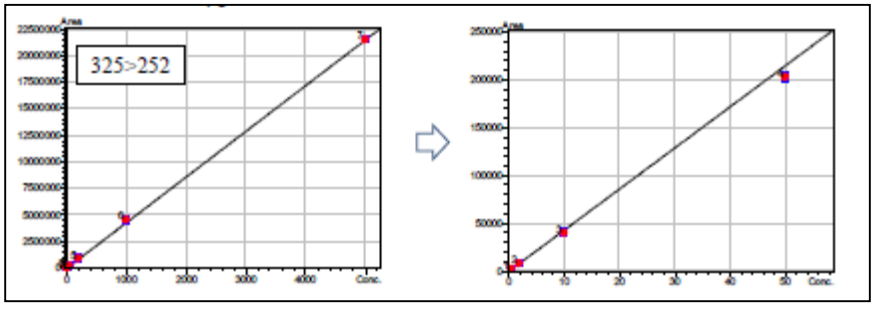


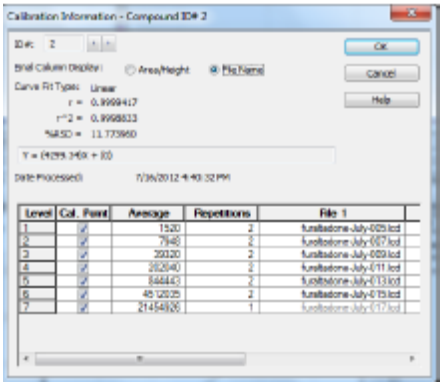


Quantitative Results View ID# 1 furaltadone

	Data Filename	Sample Name	Ret. Time	Area	Conc. (pg)
1	furaltadone-July-06.lcd	Furaltadone	0.337	11,542	1.86
2	furaltadone-July-05.lcd	Furaltadone	0.337	11,243	1.81
3	furaltadone-July-04.lcd	Furaltadone	0.336	10,005	1.61
4	furaltadone-July-03.lcd	Furaltadone	0.337	10,442	1.68
5	furaltadone-July-02.lcd	Furaltadone	0.336	10,271	1.66
6	furaltadone-July-01.lcd	Furaltadone	0.336	10,551	1.70
	Average		0.337	10,676	1.72
	%RSD		0.161269	5.549587	5.540060
	Maximum		0.337	11,542	1.86
	Minimum		0.336	10,005	1.61
	Std. Dev.		0.000543	592.458244	0.095289

Figure 2a. Calibration curve for furaltadone (+) 325 > 100 (0.5-5000 pg) and repetability data at 2 pg conc.

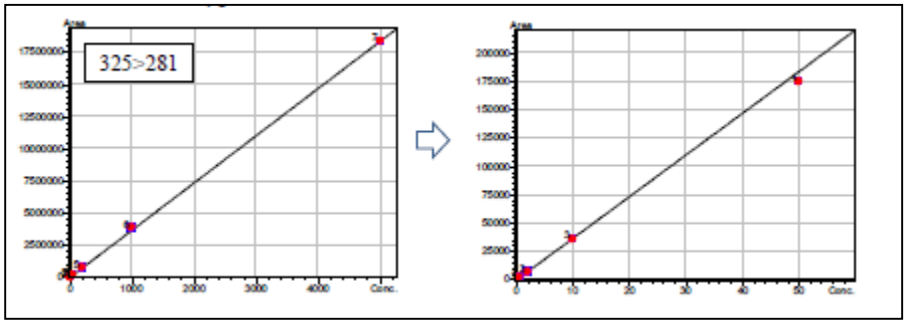


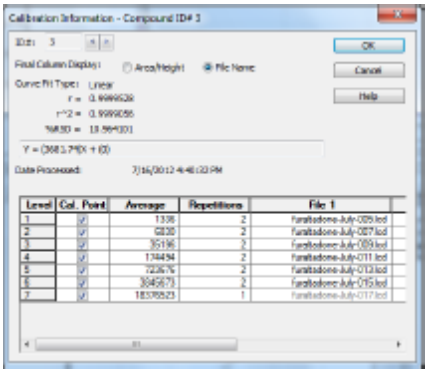


Quantitative Results View ID# 2 furaltadone

	Data Filename	Sample Name	Ret. Time	Area	Conc. (pg)
1	furaltadone-July-06.lcd	Furaltadone	0.336	7,701	1.79
2	furaltadone-July-05.lcd	Furaltadone	0.338	7,862	1.83
3	furaltadone-July-04.lcd	Furaltadone	0.336	7,680	1.79
4	furaltadone-July-03.lcd	Furaltadone	0.337	8,192	1.91
5	furaltadone-July-02.lcd	Furaltadone	0.336	8,134	1.89
6	furaltadone-July-01.lcd	Furaltadone	0.336	7,957	1.85
	Average		0.336	7,923	1.84
	%RSD		0.294141	2.716186	2.716092
	Maximum		0.338	8,192	1.91
	Minimum		0.336	7,680	1.79
	Std. Dev.		0.000989	215.195557	0.050067

Figure 2b. Calibration curve for furaltadone (+) 325 > 252 (0.5-5000 pg) and repetability data at 2 pg conc.





Quantitative Results View ID# 3 furaltadone

	Data Filename	Sample Name	Ret. Time	Area	Conc. (pg)
1	furaltadone-July-06.lcd	Furaltadone	0.337	6,422	1.74
2	furaltadone-July-05.lcd	Furaltadone	0.336	6,700	1.82
3	furaltadone-July-04.lcd	Furaltadone	0.338	6,241	1.70
4	furaltadone-July-03.lcd	Furaltadone	0.337	6,725	1.83
5	furaltadone-July-02.lcd	Furaltadone	0.338	6,282	1.71
6	furaltadone-July-01.lcd	Furaltadone	0.335	6,309	1.71
	Average		0.337	6,446	1.75
	%RSD		0.349773	3.329319	3.336951
	Maximum		0.338	6,725	1.83
	Minimum		0.335	6,241	1.70
	Std. Dev.		0.001178	214.621066	0.058452

Figure 2c. Calibration curve for furaltadone (+) 325 > 281 (0.5-5000 pg) and repetability data at 2 pg conc.

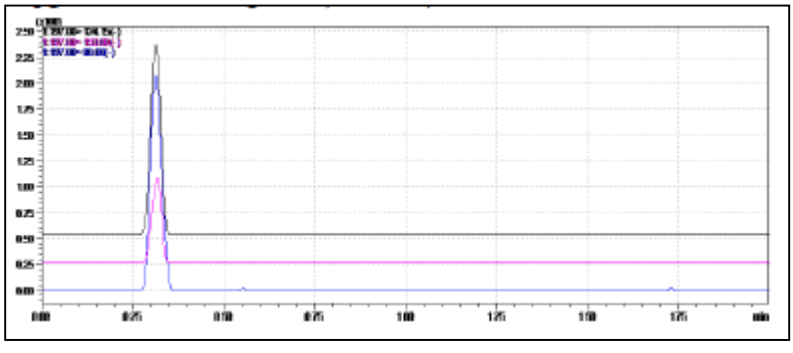


Figure 3a. Chromatogram at 2 pg concentration for nitrofurazone parent

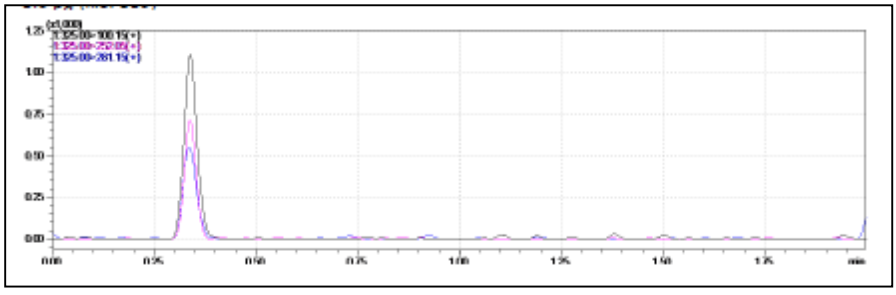


Figure 3b. Chromatogram at 0.5 pg concentration for Furaltadone parent

CONCLUSION

This preliminary analysis of two nitrofurans, nitrofurazone (MW 198) and furaltadone (MW 324) were carried out on LCMS-8040 under neutral mobile phase and solvent conditions. The sensitivity and repeatability results may be not the best values in actual sample analysis conditions.
Under such conditions, the sensitivity LOD (S/N > 3) is better than 2 pg and 0.5 pg (absolute amount) on-column for nitrofurazone and furaltadone, respectively. The repeatability (RSD) for nitrofurazone at 10 pg level is better than 21 % and for furaltadone at 2 pg level is better than 5.5 %.

V-7 QUANTITATIVE ANALYSIS OF NITROFURAN METABOLITES USING LCMS-8040

INTRODUCTION

The use of nitrofurans has been banned due to their carcinogenic and genotoxic effects on humans. Prior to the prohibition of nitrofurans, furazolidone was broadly used in European countries and hence, residual control was based on the measurement of furazolidone concentration in blood and tissues of animals. However, studies concerning the metabolism and toxicity of furazolidone and other nitrofurans revealed that the monitoring of residues based only on the detection of parent nitrofuran structures did not provide adequate data for the evaluation of real tissue contamination and their health risk since nitrofuran parent drugs are very quickly metabolised. In order to control the illegal use of nitrofuran antibiotics by measurement of residue levels in tissues, defined metabolic structures of the drugs were established as marker residues. A sensitive LC/MS/MS method for quantitation of these nitrofuran metabolites has been reported below.

EXPERIMENTAL

Instrument parameters

System configuration

HPLC	: Nexera (UHPLC)
Degassing unit	: DGU-20A$_5$
Pumping uint	: LC-30AD
Autosampler	: SIL-30AC
Column oven	: CTO-30A
Communication bus module	: CBM-20A
MS	: LCMS-8040

LC conditions

Column	: Shim-pack XR-ODS (75 mmLx3mm ID; 2.2 µm)

Mobile phase :A-5 mM ammonium formate in water:methanol (80:20)
B-5 mM ammonium formate in water:methanol (10:90)
Flow rate : 0.2 mL/min
Oven temperature : 40 °C
Diluent : water
Injection volume : 50 µL
Gradient program :

Time (min)	Pump B conc.
0.01	10
5.00	100
8.00	100
9.00	10
12.00	10

MS conditions
Ionisation : Electrospray solution (ESI); positive
Mode : MRM
Nebulising gas flow : 1.5 L/min
Drying gas flow : 10 L/min
DL temperature : 120 °C
Heat block temperature : 200 °C
MRM conditions :

Analyte name	Analyte type	MRM transition Quantifier	MRM transition Quanlifier	Calibration method	Q1 Resolution	Q3 Resolution	Cal. range (ppb)
AMOZ	Target	335.35>291.15	335.35>100.05	IS*	Unit	Unit	0.05 - 5
AOZ	Target	253.30>134.05	253.30>104.05	IS*	Unit	Unit	0.01 - 10
AMOZ D5	ISTD	340.35>296.15	-	-	Unit	Unit	0.5
AOZ D4	ISTD	257.30>134.00	-	-	Unit	Unit	0.5
AHD	Target	266.10>249.00	266.10>134.00	ES**	Unit	Unit	0.05 - 10
SCA	Target	226.20>209.00	226.20>192.00	ES**	Low	Low	0.1 - 10

*IS-Internal Standard; **ES-External Standard

RESULTS AND DISCUSSION

Representative chromatograms and calibration curves for the four nitrofuran metabolites namely AMOZ, AOZ, AHD, SCA have been shown in Figure 1, Figure 2, Figure 3 and Figure 4 respectively.

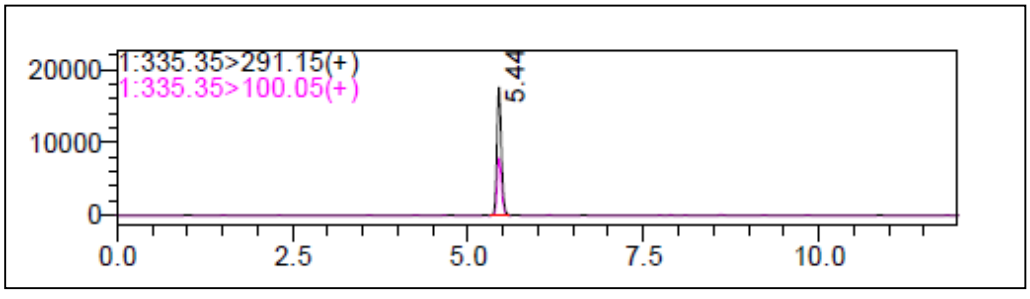


Figure 1a. Chromatogram of target AMOZ at 0.1 ppb level

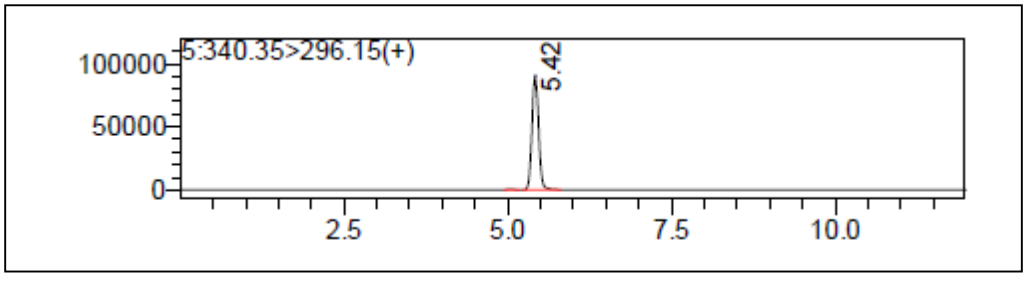
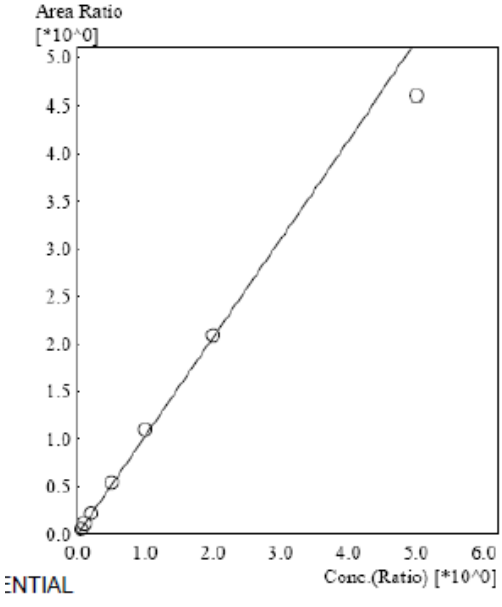


Figure 1b. Chromatogram of ISTD AMOZ D5 at 0.5 ppb

ID# : 1 m/z : 335.35>291.15
Name : AMOZ
Quantitative Method : Internal Standard
Function : f(x)=1.03105*x+0.00789244
Rr1=0.9969294 Rr2=0.9938683
MeanRF: 1.082012e+000 RF SD: 8.314409e-002 RF %RSD: 7.684215
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/A^2



#	Conc.(Ratio)	MeanArea Ratio	Area	rea Ratio%RS
2	0.05	0.0574134	35911	2.771014
			37671	
			36392	
3	0.1	0.116406	77537	6.140499
			68800	
			73276	
4	0.2	0.218766	140942	2.108107
			138748	
			144248	
5	0.5	0.550512	373795	1.699404
			364239	
			362334	
6	1	1.10244	734669	1.935027
			748857	
			785816	
7	2	2.09287	1519172	1.971837
			1504810	
			1470968	
8	5	4.59009	3884666	3.349465
			3816473	
			3583714	

ENTIAL

Figure 1c. Calibration Curve of AMOZ from 0.05 – 5 ppb.

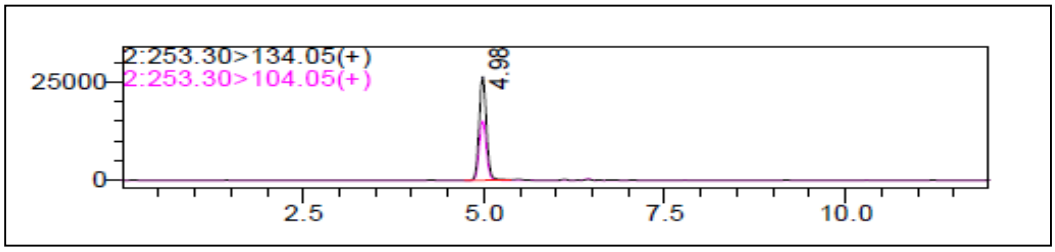


Figure 2a. Chromatogram of target AOZ at 0.1 ppb level

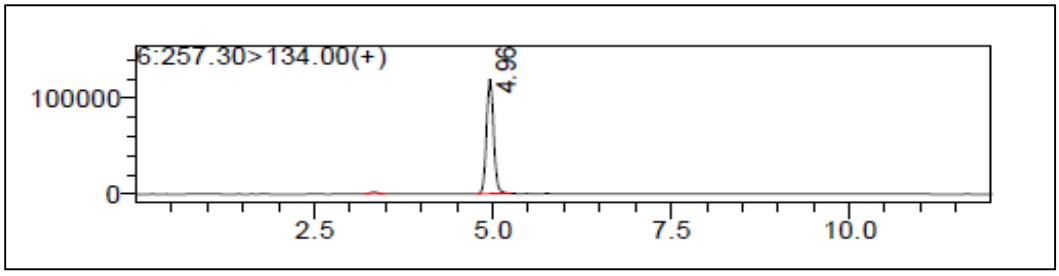
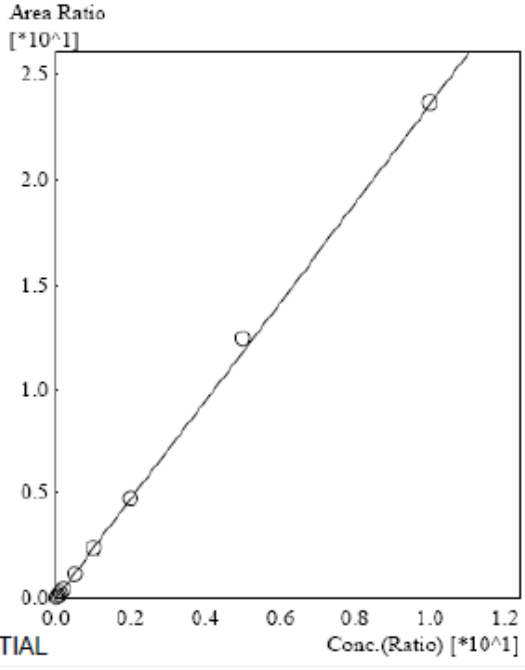


Figure 2b. Chromatogram of ISTD AOZ D4 at 0.5 ppb level

ID# : 2 m/z : 253.30>134.05
Name : AOZ
Quantitative Method : Internal Standard
Function : f(x)=2.35724*x-0.000574986
Rr1=0.9996924 Rr2=0.9993849
MeanRF: 2.350706e+000 RF SD: 1.002360e-001 RF %RSD: 4.264080
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/A^2



DENTIAL

#	Conc.(Ratio)	MeanArea Ratio	Area	rea Ratio%RS
1	0.01	0.0230823	16904	10.383316
			20605	
			18499	
2	0.05	0.117509	94797	1.128174
			97939	
			92226	
3	0.1	0.229430	184090	4.571661
			192146	
			182512	
4	0.2	0.456257	380600	4.733731
			403317	
			380793	
5	0.5	1.17598	956222	0.974862
			953834	
			968435	
6	1	2.35594	2009444	2.223788
			2091518	
			1959274	
7	2	4.73133	3938019	1.576617
			3989477	
			4014292	
8	5	12.4353	9921270	0.536730
			10043541	
			9851791	
9	10	23.6176	19381829	1.801334
			19302151	
			19267622	

Figure 2c. Calibration Curve of AOZ from 0.01 – 10 ppb

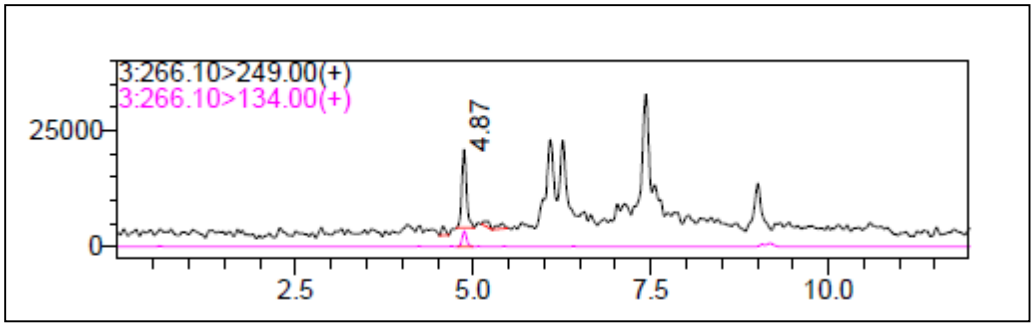
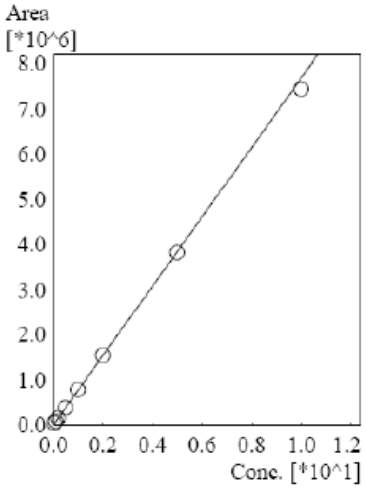


Figure 3a. Chromatogram of AHD at 0.1 ppb level

ID# : 1 m/z : 266.10>249.00
Name : AHD
Quantitative Method : External Standard
Function : f(x)=769522*x-1633.95
Rr1=0.9991915 Rr2=0.9983836
MeanRF: 7.632427e+005 RF SD: 4.680612e+004 RF %RSD: 6.132534
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/A^2



#	Conc.(Ratio)	MeanArea	Area	Area%RSD
2	0.05	35744	35519	7.503060
			38531	
			33181	
3	0.1	81590	78083	6.268081
			79229	
			87458	
4	0.2	149742	170614	12.556713
			144482	
			134129	
5	0.5	386404	399933	3.039178
			380434	
			378845	
6	1	777415	778429	0.115865
			776708	
			777108	
7	2	1535100	1503782	3.796672
			1602346	
			1499171	
8	5	3821424	3713612	2.585995
			3907705	
			3842956	

NFIDENTIAL

Figure 3b. Calibration Curve of AHD from 0.05 – 5 ppb

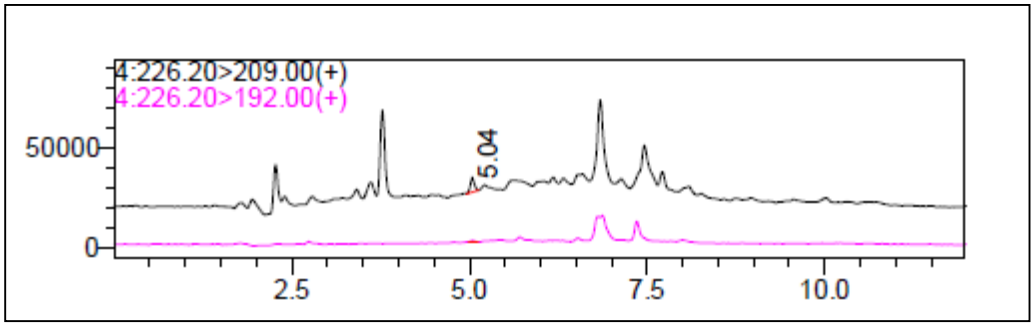
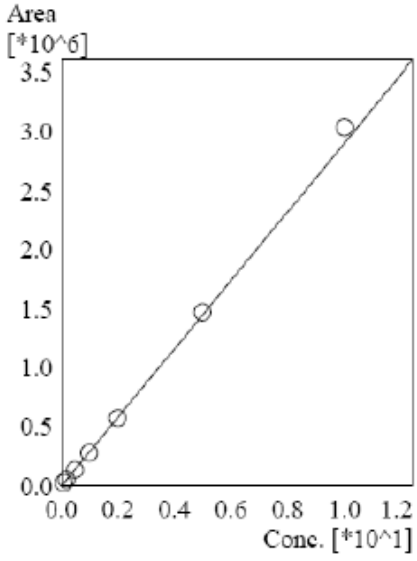


Figure 4a. Chromatogram of SCA at 0.1 ppb level

ID# : 2 m/z : 226.20>209.00
Name : SCA
Quantitative Method : External Standard
Function : f(x)=290880*x-2013.15
Rr1=0.9995571 Rr2=0.9991143
MeanRF: 2.857923e+005 RF SD: 1.288388e+004 RF %RSD: 4.508129
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/A^2



#	Conc.(Ratio)	MeanArea	Area	Area%RSD
3	0.1	27386	26738	8.682053
			30020	
			25399	
4	0.2	55777	54301	2.302496
			56384	
			56644	
5	0.5	139080	138711	0.726846
			138306	
			140224	
6	1	282222	281907	1.109024
			279261	
			285497	
7	2	577487	577440	0.925001
			582852	
			572169	
8	5	1473925	1456276	1.255283
			1472322	
			1493176	
9	10	3038937	3049143	0.346443
			3039554	
			3028114	

IDENTIAL

Figure 4b. Calibration Curve of SCA from 0.1 – 10 ppb

The list of calibration curve information with correlation coefficients has been tabulated in Table 1 below.

Table 1. Quantitative results of nitrofuran metabolite analysis

Compound name	Retention time (min)	Correlation coefficient (r2)	LOQ (ppb)	S/N at LOQ
AMOZ	5.44	0.9969	0.05	579.07
AOZ	4.98	0.9996	0.01	34.8
AHD	4.86	0.9991	0.05	401.92
SCA	5.04	0.9995	0.1	12.58

Note :
LOQs were determined based on following criteria:-

1) S/N > 10 (calculated by RMS)
2) % RSD < 16 % for (n=3)
3) Accuracy = 80 – 120 %

CONCLUSION

Four nitrofuran metabolite standards (received as derivatised standards) were analysed on LCMS-8040 system. Based on the availability of internal standards, two metabolites namely AMOZ and AOZ were analysed by internal standard methods and AHD and SCA by external standard method of quantitation. Accordingly, sub ppb LOQ levels were achieved for these analytes. For SCA, however, low Q1 and Q3 resolutions had to be used to achieve the aforesaid sensitivity.

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V-8

DETERMINATION OF THE DERIVATIVES OF NITROFURAN METABOLITES IN MARINE PRODUCTS BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY / TRIPLE QUADRUPOLE MASS SPECTROMETRY

INTRODUCTION

This paper describes a method by ultrahigh performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS) to analyze 4 derivatives of nitrofuran metabolites in marine products. The high sensitivity and selectivity of UHPLC/MS/MS method made determination of these compounds successful. The limit of quantitation (LOQ) of AMOZ was 0.4 µg / kg, which is lower than the guideline value (0.5 µg / kg) proposed by the USFDA. The derivatives of nitrofuran metabolites (structures as shown in Figure 1) used in breeding of marine products are known to have a carcinogenic effect. Quantities of these compounds in marine products were regulated by the USFDA. This paper describes a UHPLC/ESI-MS/MS method to determine 4 derivatives of nitrofuran metabolites. The method is simple, rapid and highly sensitive and meets the regulatory requirements.

EXPERIMENTAL

Sample Preparation

(1) Weigh and transfer 2 g of the sample in a 50 mL centrifuge tube.
(2) Add 10 mL of methanol:water (1:1) to the centrifuge tube, shake for 10 minutes.
(3) Centrifuge at 4000 rpm for 5 minutes.
(4) Discard the remaining solution in the centrifuge tube, add 10 mL of 0.2 mol/L hydrochloric acid and homogenize in a refiner.
(5) Further add 100 µL of o-nitrobenzaldehyde, centrifuge and vortex for 30 seconds, shake for 30 minutes. Incubate the reaction mixture for 16 hours at 37 °C.
(6) Remove the samples by adding 2 mL of 0.3 mol/L potassium phosphate. Adjust the pH to 7.4 using 2 mol/L sodium hydroxide.

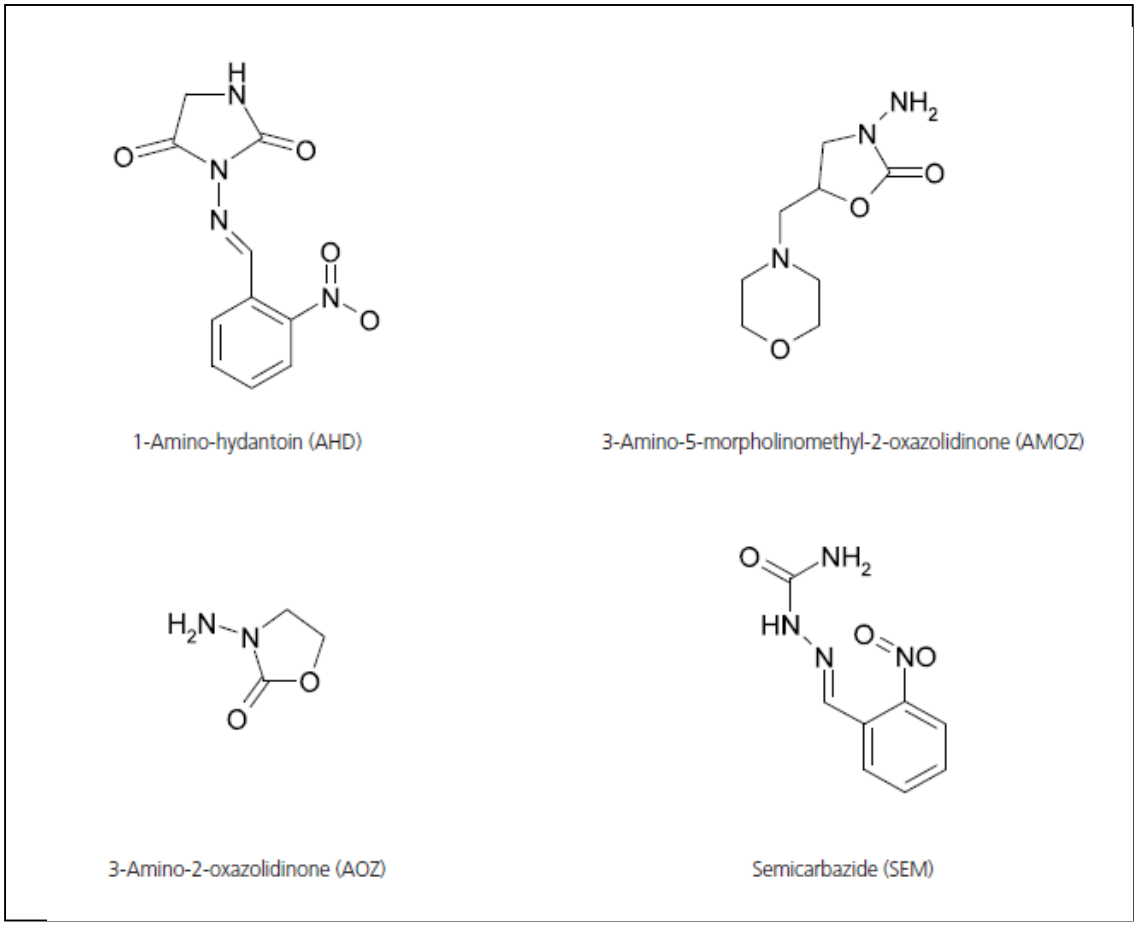

Figure 1. Structure of Nitrofuran metabolites

(7) Add 10 mL of ethyl acetate to the sample. Shake for 10 minutes and centrifuge at 10000 rpm for 10 minutes for collecting the ethyl acetate solution. Repeat this process twice.
(8) Dry the ethyl acetate solution at 40 °C with nitrogen. Dissolve the residue with 1.0 mL of 0.1% aqueous formic acid solution. Remove fat with n-hexane. Filter the lower water phase with a microporous membrane.

Instrument parameters

System configuration

HPLC	: Nexera (UHPLC)
Degassing unit	: DGU-20A$_3$
Pumping uint	: LC-30AD
Autosampler	: SIL-30AC
Column oven	: CTO-30A

MS : LCMS-8030

LC conditions
Column : Shim-pack XR-ODS III (150 mm L x 2 mm ID; 2.2 µm)
Mobile phase : A-0.02 % formic acid in water
B-Acetonitrile
Flow rate : 0.4 mL/min
Oven temperature : 40 °C
Injection volume : 20 µL
Gradient program :

Time (min)	Pump B conc.
0.01	40
1.50	95
1.51	40
4.00	40

MS conditions
Ionisation : Electrospray solution (ESI); positive (+4.5kV)
Mode : MRM
Nebulising gas flow : 3 L/min
Drying gas flow : 20 L/min
DL temperature : 300 °C
Heat block temperature : 500 °C
MRM transitions :

No.	Compound	Procursor Ion (*m/z*)	Product Ion (*m/z*)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	AOZ	236	134.10* 104.05	-18.0 -18.0	-12.0 -24.0	-29.0 -22.0
2	AMOZ	335	291.00* 262.00	-13.0 -18.0	-11.0 -17.0	-22.0 -19.0
3	AHD	249	134.00* 103.95	-13.0 -13.0	-13.0 -22.0	-15.0 -11.0
4	SEM	209	166.10* 192.00	-11.0 -11.0	-11.0 -12.0	-18.0 -14.0

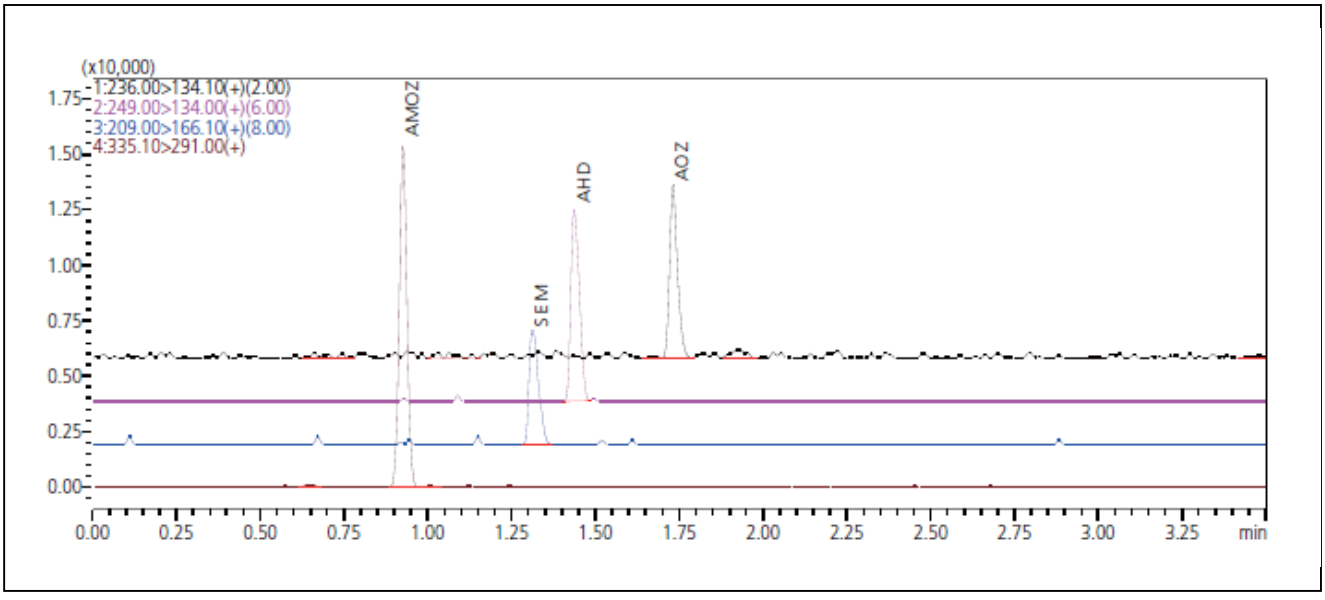

Figure 2. MRM chromatograms of 4 derivatives of nitrofuran metabolites

RESULTS AND DISCUSSION

The MRM chromatograms in the positive ion modes of 4 derivatives of nitrofuran metabolites are shown in Figure 2. Figure 3 shows the calibration curves of 4 derivatives of nitrofuran metabolites. Linearity was demonstrated in the range of 1.0 to 200 µg/L for AOZ, AMOZ, AHD and SEM, with correlation coefficients greater than 0.999. The repeatability of those compounds in different concentration were investigated, and the RSDs of peak area were less than 4.8%, and RSDs of retention time were less than 0.3%, as shown in Table 1.

Table 1. Repeatability of 4 marine toxins at different concentrations (n = 6)

No.	Compound	RSD% (1 µg/L)		RSD% (10 µg/L)		RSD% (50 µg/L)	
		R.T. (min)	Area	R.T. (min)	Area	R.T. (min)	Area
1	AOZ	0.13	3.84	0.15	2.36	0.09	2.16
2	AMOZ	0.11	4.24	0.09	2.13	0.07	1.69
3	AHD	0.14	3.56	0.27	3.49	0.12	2.24
4	SEM	0.27	4.76	0.28	4.66	0.12	1.68

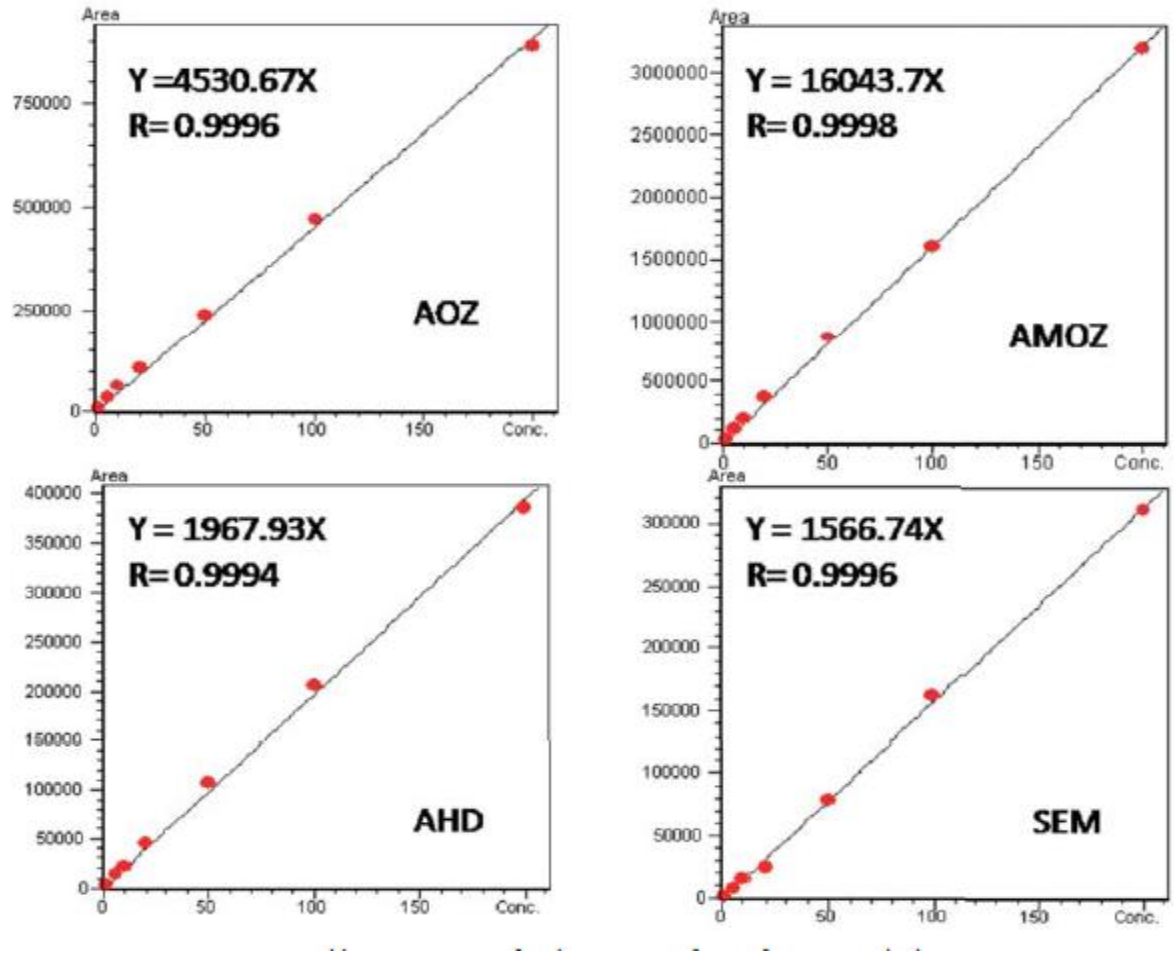

Figure 3. Calibration curve of 4 derivatives of nitrofuran metabolites

CONCLUSION

A LC/MS/MS method has been developed for quantitative analysis of 4 derivatives of nitrofuran metabolites in marine products using Shimadzu Nexera UHPLC and LCMS-8030 triple quadrupole mass spectrometer. All of them were separated in 4 minutes, and analyzed in positive mode. The calibration curves were linear well between peak area of the selected MRM transitions and the concentration of target compounds with the correlation coefficient over 0.999. Thus method was established for fast quantitative determination of 4 derivatives of nitrofuran metabolites.

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V-9 LC/MS/MS METHOD FOR THE QUANTIFICATION OF EPIMERS OF TETRACYCLINE (E-TC), CHLOROTETRACYCLINE (E-CTC) AND OXYTETRACYCLINE (E-OTC)

INTRODUCTION

Tetracyclines rank among the antimicrobial substances most frequently used in the animal food production. The presence of residues at much higher levels in foods may constitute a variety of public health hazards including toxicological, microbiological, immunological, and pharmacological hazards. Most important health aspects which should be taken into account are possible impact on the emergence of antimicrobial resistance for antimicrobials administered in human therapy, disorders in the intestinal flora, and possible occurrence of allergic symptoms. To prevent these health hazards, stringent regulations have been set by Food and Agricultural Organization, World Health Organization and European Union (EU); for example, EU legislation has set maximum residual limit of 0.1 mg/kg (100 ng/g) for tertracycline, oxytetracycline as well as chlortetracycline in raw cow milk.

LC/MS/MS analysis provides a platform for high sensitive analysis with selectivity even in the presence of complex matrices. Here, a method of analysis has been established for analysis of tratracycline standards with a view of determining LOQ levels for these compounds on LCMS-8040 system.

EXPERIMENTAL

Instrument parameters

HPLC	: Nexera (UHPLC)
Degassing unit	: DGU-20A$_5$
Pumping uint	: LC-30AD
Autosampler	: SIL-30ACMP
Column oven	: CTO-30A
Communication bus module	: CBM-20A

MS : LCMS-8040

LC conditions
Column : Waters 5C18-MS-II (50 mm L x 4.6 mm ID)
Mobile phase : A–0.1 % formic acid in water
B-Methanol
Flow rate : 1 mL/min
Oven temperature : 40 °C
Injection volume : 10 µL
Gradient program :

Time (min)	Pump B conc.
0.01	0
5	40
6	40
7	100
8	100
9	0
12	0

MS conditions
Ionisation : Electrospray solution (ESI); positive
Mode : MRM
Nebulising gas flow : 2 L/min
Drying gas flow : 15 L/min
DL temperature : 250 °C
Heat block temperature : 400 °C
MRM transitions : 1. e-TC : 445.20 > 410.00
2. e-CTC : 479.00 > 444.00
3. e-OTC : 461.20 > 426.10

Standard Preparation

1000 ppm stock of each standard was diluted using water:methanol (90:10 v/v) to make mixed standard having concentration range from 1 ppb to 500 ppb. Concentration levels of 1 ppb, 5 ppb, 10 ppb, 50 ppb, 100 ppb and 500 ppb used to plot a calibration graph.

RESULTS AND DISCUSSION

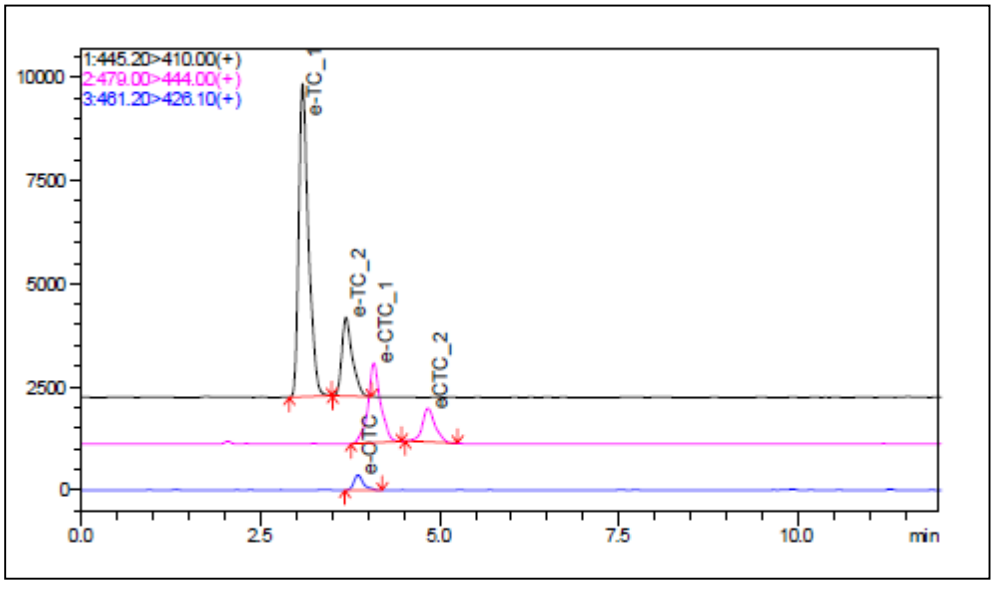


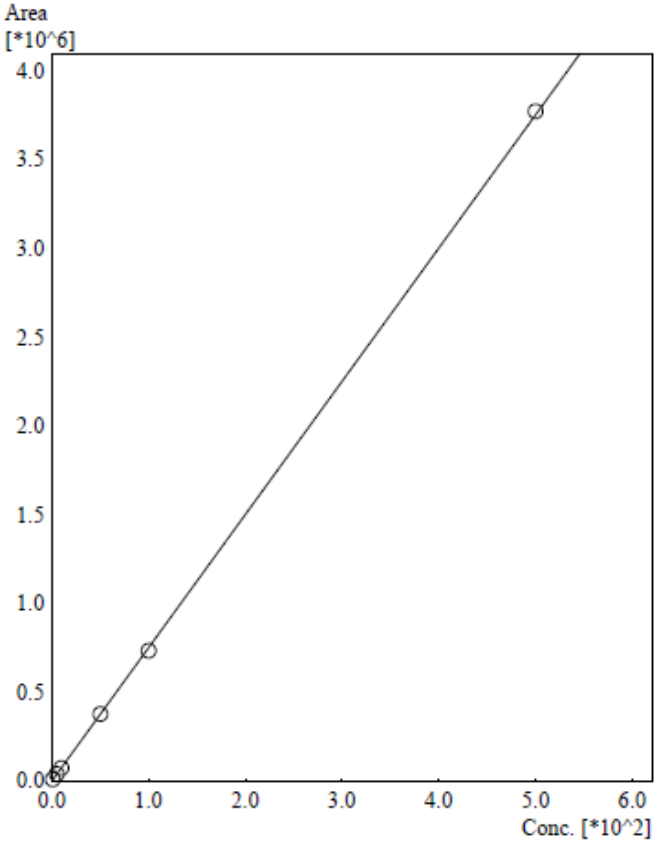
Figure 1. Representative chromatogram of 5ppb Mix Standard

Linear calibration curves were obtained for all compounds with regression coefficients (r2) > 0.99. % RSD was within 10 % and accuracy was within 80-120 % for all calibration levels. Figure 1 represents chromatographic separation of the epimers and table 1 gives LOQ levels of each epimer. Figure 2 shows calibration curves of all analytes.

Table 1. Quanitative results of tetracycline epimers

Sr.No:	Compound name	Retention time (min)	LOQ (ppb)
1	e-TC_1	3.07	1
2	e-TC_2	3.67	1
3	e-CTC_1	4.06	1
4	e-CTC_2	4.83	5
5	e-OTC	3.86	5

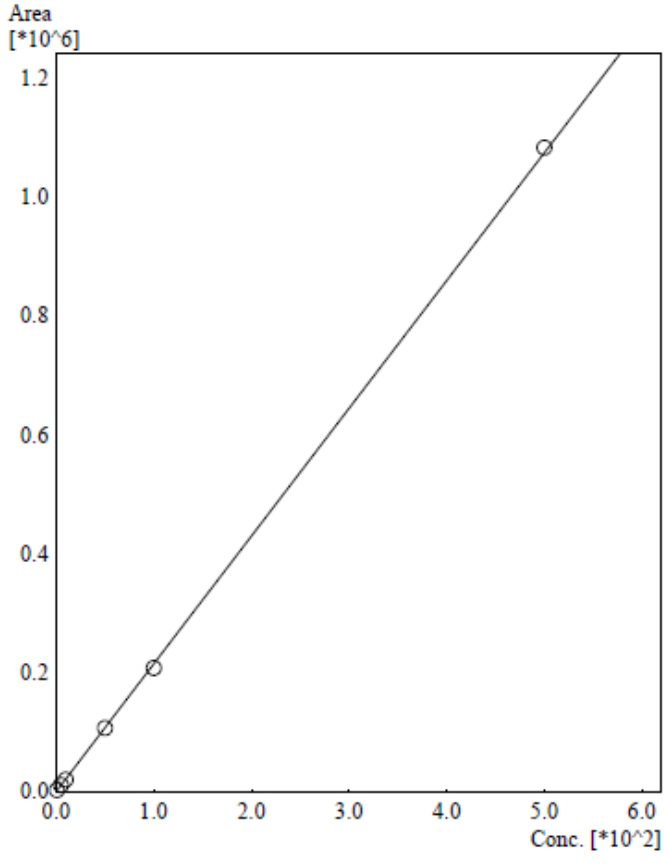
ID# : 1 m/z : 445.20>410.00
Name : e-TC 1
Quantitative Method : External Standard
Function : f(x)=7506.09*x-767.337
Rr1=0.9999291 Rr2=0.9998582
MeanRF: 7.335918e+003 RF SD: 2.602773e+002 RF %RSD: 3.547985
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/A



#	Conc.(Ratio)	MeanArea	Area
1	1	7136	7185
			7360
			6518
			7739
			7369
			6643
2	5	37024	36800
			38320
			37992
			36485
			36625
			35922
3	10	71085	71290
			71783
			71930
			70782
			69835
			70888
4	50	374754	376289
			376421
			366167
			383389
			371907
			374352
5	100	732648	732941
			738541
			725504
			735167
			730431
			733303
6	500	3772488	3783810
			3785180
			3783854
			3773301
			3751510
			3757271

Figure 2a. Calibration curve of e-TC_1

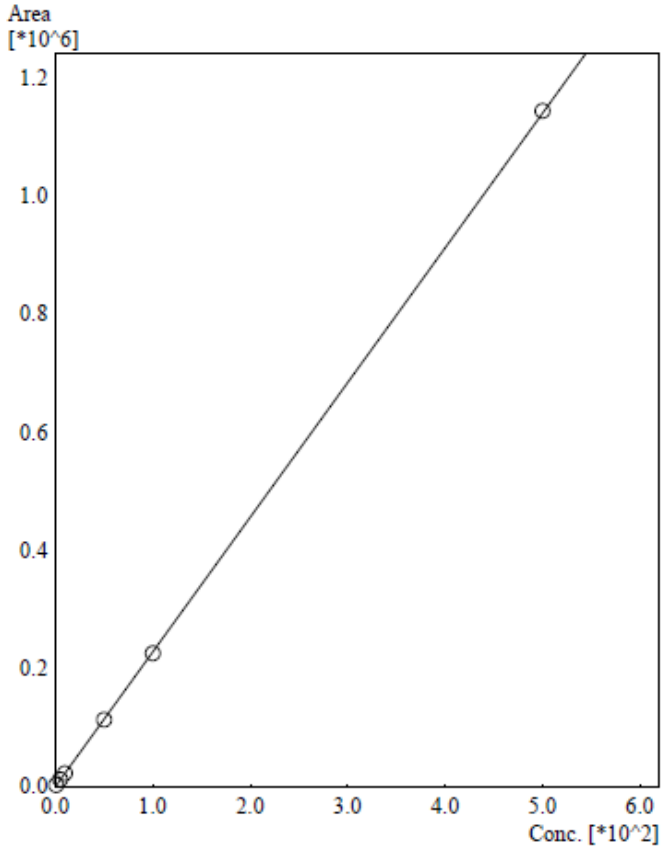
ID# : 2 m/z : 445.20>410.00
Name : e-TC 2
Quantitative Method : External Standard
Function : f(x)=2148.82*x-346.360
Rr1=0.9998592 Rr2=0.9997183
MeanRF: 2.071363e+003 RF SD: 1.059043e+002 RF %RSD: 5.112782
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/A



#	Conc.(Ratio)	MeanArea	Area
1	1	1956	2215
			1709
			1881
			1831
			2050
			2047
2	5	10543	10691
			10608
			10645
			10514
			10164
			10638
3	10	19916	19407
			19848
			20249
			20359
			19402
			20234
4	50	106477	104898
			105164
			104560
			108990
			107059
			108189
5	100	207718	208760
			205677
			207969
			208927
			207651
			207320
6	500	1082816	1084866
			1085823
			1085991
			1074458
			1084482
			1081277

Figure 2b. Calibration curve of e-TC_2

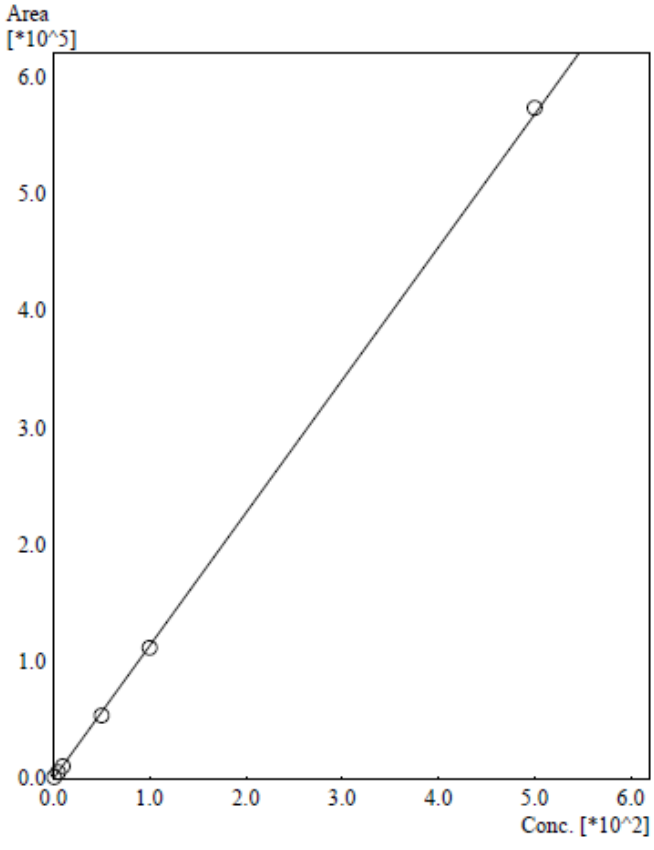
ID# : 3 m/z : 479.00>444.00
Name : e-CTC 1
Quantitative Method : External Standard
Function : f(x)=2280.84*x-238.715
Rr1=0.9999734 Rr2=0.9999467
MeanRF: 2.228234e+003 RF SD: 9.722215e+001 RF %RSD: 4.363193
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/A



#	Conc.(Ratio)	MeanArea	Area
1	1	2048	1985
			2007
			1995
			2086
			2078
			2136
2	5	11560	11671
			11534
			11481
			11325
			11454
			11893
3	10	22132	23666
			22296
			21668
			21964
			21831
			21365
4	50	112920	113516
			114050
			112257
			113506
			112171
			112020
5	100	225004	225054
			226274
			223970
			222870
			226591
			225262
6	500	1144021	1140227
			1147229
			1142364
			1147375
			1137996
			1148936

Figure 2c. Calibration curve of e-CTC_1

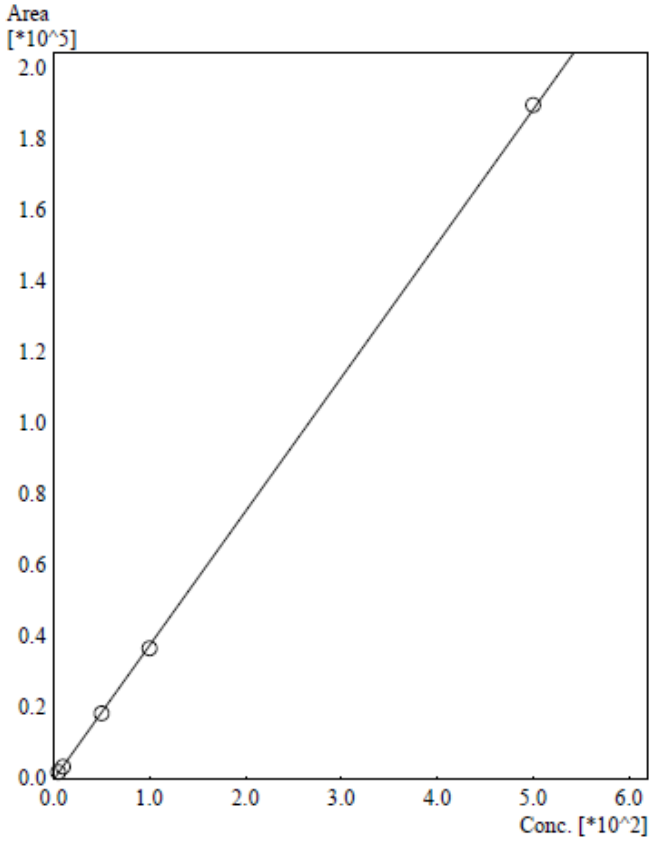
ID# : 4 m/z : 479.00>444.00
Name : eCTC 2
Quantitative Method : External Standard
Function : f(x)=1137.32*x-306.629
Rr1=0.9998001 Rr2=0.9996003
MeanRF: 1.083354e+003 RF SD: 5.498691e+001 RF %RSD: 5.075617
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/A



#	Conc.(Ratio)	MeanArea	Area
1	1	967	967
2	5	5308	5390
			5684
			4941
			5370
			5244
			5221
3	10	10337	10049
			9750
			10164
			11204
			10215
			10638
4	50	53737	54267
			54606
			53220
			52664
			53561
			54103
5	100	111867	111763
			110538
			110349
			112876
			112933
			112743
6	500	573692	574691
			574624
			577093
			577118
			569342
			569285

Figure 2d. Calibration curve of e-CTC_2 epimer

ID# : 5 m/z : 461.20>426.10
Name : e-OTC
Quantitative Method : External Standard
Function : f(x)=377.288*x-323.610
Rr1=0.9998840 Rr2=0.9997680
MeanRF: 3.558069e+002 RF SD: 2.257105e+001 RF %RSD: 6.343623
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/A



#	Conc.(Ratio)	MeanArea	Area
2	5	1706	1686
			1699
			1698
			1821
			1502
			1829
3	10	3277	3282
			3409
			3369
			3202
			3235
			3164
4	50	18279	18177
			18952
			18557
			18428
			17173
			18385
5	100	36558	37857
			36897
			36848
			35635
			35857
			36256
6	500	189511	191060
			189015
			188204
			188613
			189691
			190482

Figure 2e. Calibration curve of OTC

CONCLUSION

A robust and highly sensitive LC/MS/MS method was developed for simultaneous quantification of epimers of tetracycline, chlorotetracycline and oxytetracycline.

V-10 QUANTITATIVE ANALYSIS OF CHLORAMPHENICOL USING LCMS-8040

INTRODUCTION

Chloramphenicol is a potent, broad-spectrum antibiotic drug and a potential carcinogen used only at therapeutic doses for treatment of serious infections in humans. Due to the unpredictable effects of dose on different patient populations, a safe level of human exposure to chloramphenicol has not yet been established. Federal regulations in the United States, Canada and the European Union, hence, prohibit its use in food producing animals and animal-feed products, including honey bees.
In this note, a sensitive method for analysis of chloramphenicol on LC/MS/MS system was developed on LCMS-8040 system reaching an LOQ of 50 ppt using standard.

EXPERIMENTAL

Instrument parameters

System configuration

HPLC	: Nexera (UHPLC)
Degassing unit	: DGU-20A$_5$
Pumping uint	: LC-30AD
Autosampler	: SIL-30AC
Column oven	: CTO-30A
Communication bus module	: CBM-20A
MS	: LCMS-8040

LC conditions

Column	: Shim-pack XR-ODS (50 mmLx3mmI.D.;2.2 µm)
Mobile phase	: A - Water B - Methanol
Flow rate	: 0.3 mL/min
Oven temperature	: 40 °C
Injection volume	: 5 µL

Gradient program :

Time (min)	Pump B conc.
0.01	30
5	100
6	100
7	30
8	30

MS conditions

Ionisation : Electrospray solution (ESI); negative
Mode : MRM
Nebulising gas flow : 3 L/min
Drying gas flow : 10 L/min
DL temperature : 250 °C
Heat block temperature : 400 °C
MRM transitions : 321.10 > 152.00

RESULTS AND DISCUSSION

Calibration curve was linear for the concentration range of 50 ppt to 10 ppb. Representative chromatogram and calibration graph are shown in Figures 1 and 2 respectively.

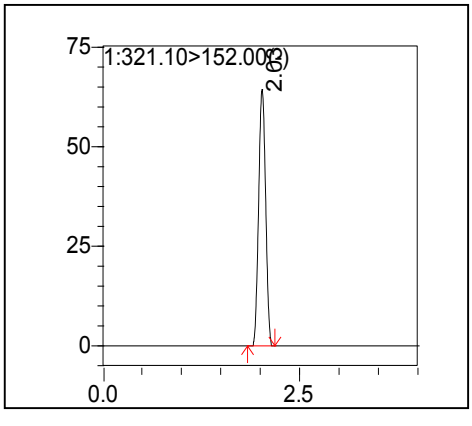
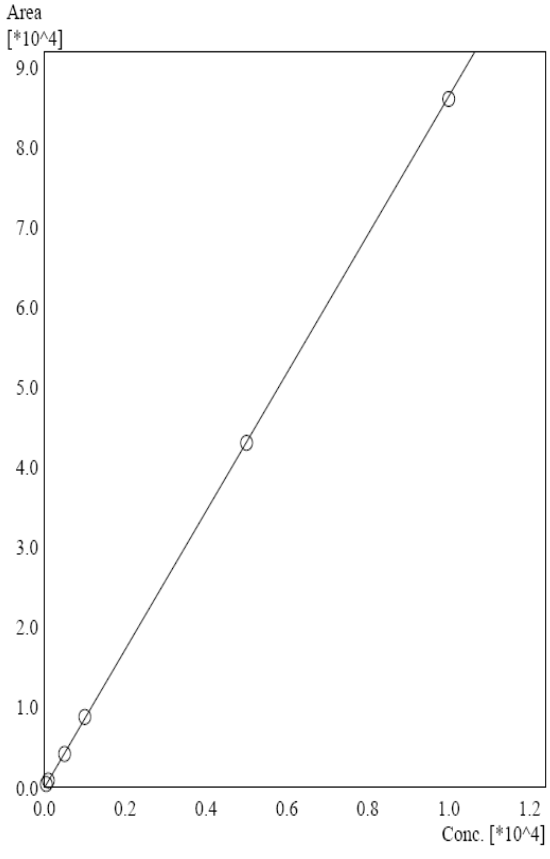


Figure 1. MRM chromatogram of chloramphenicol at 50 ppt

ID# : 1 m/z : 321.10>152.00
Name : Chloramphenicol
Quantitative Method : External Standard
Function : f(x)=8.64126*x+1.82939
Rr1=0.9997068 Rr2=0.9994136
MeanRF: 8.657887e+000 RF SD: 5.187403e-001 RF %RSD: 5.991535
FitType : Linear
ZeroThrough : Not Through
Weighted Regression : 1/A^2



#	Conc.(Ratio	MeanArea	Area	Area%RSD
1	50	429	397	13.600352
			378	
			464	
			471	
			359	
			503	
2	100	891	932	3.384397
			867	
			863	
			922	
			868	
			894	
3	500	4202	4116	2.620823
			4369	
			4154	
			4294	
			4081	
			4197	
4	1000	8831	8418	3.841823
			9406	
			8767	
			9007	
			8723	
			8665	
5	5000	43088	42926	2.384547
			43453	
			42486	
			41913	
			42844	
			44909	
6	10000	86060	85392	1.722867
			86997	
			87276	
			86305	
			87017	
			83374	

Figure 2. Calibration curve of chloramphenicol from 50 ppt to 10 ppb.

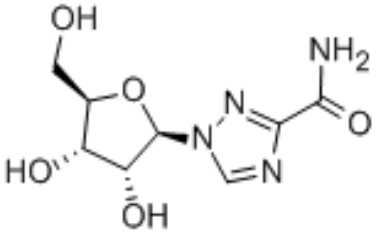
CONCLUSION

A fast and simple method was developed on LCMS-8040 system for sub ppb level quantitation of chloramphenicol.

V-11 DETERMINATION OF RIBAVIRIN IN CHICKEN USING ULTRA FAST LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

INTRODUCTION

Chickens allegedly raised on large quantities of hormones and other drugs have been supplied to fast-food restaurants. These drugs are banned by china's food and drug administration. Within just 40 days, they could grow to weigh up to 3 kilograms. The case has prompted great public to question the safety of food production. Ribavirin (as shown in following figure) is a broad-spectrum anti-viral drug used off-label for severe respiratory syncytial virus pneumonia infection, hepatitis c infection and some other viral infections. It is a guanosine analog which could stop viral rna synthesis for dna and rna virus. As a feed additive, ribavirin not only decreased the flavor and quality of chicken, but also caused the occurrence of bacterial resistance and drug residue, which pose potential threats to the human health. In this study, a quick and sensitive analytical method was established for ribavirin determination in chicken.



RIBAVIRIN ($C_8H_{12}N_4O_5$)

EXPERIMENTAL

Sample preparation

All chicken samples (2.0 g) were homogenized for 1 min with 10 ml acetonitrile: 1% trichloroacetic acid solution(7/3; v/v). After centrifugation for 10 min at 10000 rpm, the supernatant pH value was adjusted into 8.5 with ammonium hydroxide. After centrifugation for 10 min at 10000 rpm once again, the supernatant was purified by SPE. Initially, the PBA cartridges were activated with 2 ml of acetonitrile, followed by 5 ml 0.1% formic acid-acetonitrile solution and 8 ml ammonium acetate (pH 8.5) solution. Then, 5 ml supernatant was loaded to the cartridge manually and washed with 10 ml acetonitrile - pH 8.5 ammonium acetate solution(1/9; v/v). After drying, the cartridge was eluted by 5 ml of 0.1m formic acid water solution. The eluate was filtered through 0.22 μm filter membrane. After that, the sample solution was analyzed by lc-ms/ms.

LC-MS/MS Analysis

The analyses were performed on a Shimadzu Nexera UFLC instrument (Kyoto, Japan) equipped with LC-30AD pump, CTO-30A column oven, DGA-30A5 degasser, and SIL-30AC autosampler. The separation was carried out on a Shim-pack XR-ODS iii (2.0 mm i.d. x 150 mm l., 2.2 μm) with the column temperature at 30 ºc. The mobile phase consisted of (a) 5 mmol/l ammonium acetate and 0.1% formic acid-water and (b) acetonitrile using a gradient elution, shown as table 1.The initial b concentration was 3%. The flow rate was 0.4 ml/min. The injection volume was 2 μl.

Table 1 Time program

TIME (MIN)	MODULE	COMMAND	VALUE
1.60	PUMPS	PUMP B CONC.	3
1.90	PUMPS	PUMP B CONC.	92
2.90	PUMPS	PUMP B CONC.	92
3.00	PUMPS	PUMP B CONC.	3
4.5	CONTROLLER	STOP	

A triple quadrupole mass spectrometer (Shimadzu lcms-8050, Kyoto, Japan) was connected to the Shimadzu fast analytical UHPLC instrument via an ESI interface. The mass spectra were acquired in positive ion mode with a dl temperature at 200 ºc, heat block temperature at 400 ºc, interface temperature at 360 ºc. The heating gas flow rate was 3 l/min. The dwell time was 100 ms and pause time was 3 ms. The MRM parameters are shown in table 2.

Table 2 MRM mode parameters

COMPOUND	PRECURSOR ION (*M/Z*)	PRODUCT ION(*M/Z*)	Q1 BIAS (V)	CE(V)	Q3 BIAS (V)
RIBAVIRIN	245.3	113.0*	-11	-8	-21
		96.0	-11	-28	-17

* for quantitation

RESULTS AND DISCUSSION

Standard sample results
All samples were analyzed in 4.5 min. the MRM chromatograms in positive ion mode of ribavirin was given in figure 1.

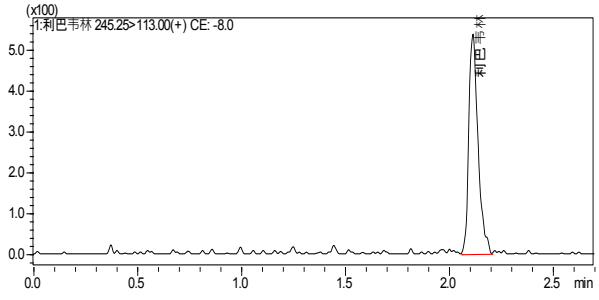


Fig. 1 MRM chromatograms of ribavirin (0.1 μg/L)

Linearity and Limit of Quantification(LOQ)

Fig.2 shows the calibration curve of ribavirin. A linear relationship was found between peak area and different sample concentrations of ribavirin within 0.02, 0.1, 0.5, 1, 5 and 10 µg/L. An excellent linear relationship was obtained. The calibration curves of ribavirin was constructed with correlation coefficients (r) more than 0.999. The limits of detection (LOD) and the limits of quantitation (LOQ) for ribavirin was calculated by standard deviation of 7 samples (0.02 µg/L), shown in Table 3.

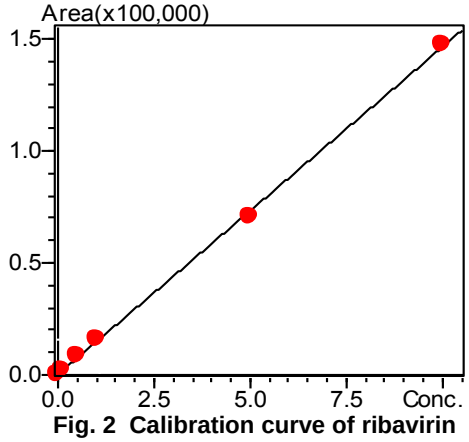


Fig. 2 Calibration curve of ribavirin

Table 3 The LOD and LOQ of ribavirin

Compound	Standard Deviation	LOD (µg/L)	LOQ (µg/L)
Ribavirin	0.0016	0.005	0.02

Precision and Recovery

In this study, the repeatability of ribavirin in different concentrations were investigated. The %RSDs of retention time were better than 0.39 and %RSDs of peak area were less than 5.38, as shown in Table 4.

Table 4 Repeatability result of ribavirin (n=6)

Conc.(µg/L)	%RSD (R.T.)	%RSD (Area)
0.1	0.39	5.38
0.5	0.25	3.06
1	0.37	1,05

The standard sample was spiked into the blank chicken sample at levels of 1 μg/kg to evaluate the recovery of this method developed in this study. The analyse was performed using above UFLC and mass spectrometry analytical conditions. A recovery rate of 94.5% was obtained for ribavirin.

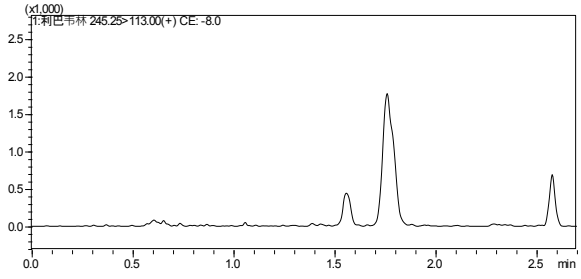


Fig. 3 MRM chromatogram for the blank sample

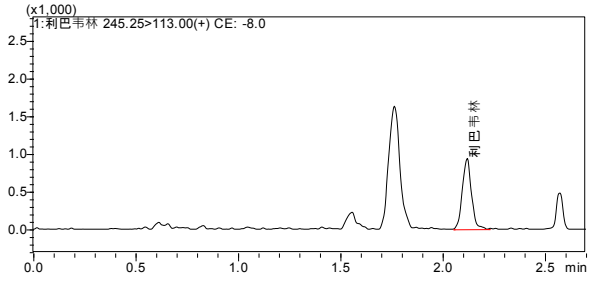


Fig. 4 MRM chromatogram for the spiked sample (1 μg/kg)

CONCLUSION

An UFLC-MS/MS method has been developed for the analysis of ribavirin in chicken samples using Shimadzu Nexera UFLC and LCMS-8050 triple quadrupole mass spectrometer. All samples were analyzed within 4.5 min., and the calibration curve was linear well between peak area of the selected ions and different concentrations of ribavirin with the correlation coefficient over 0.999. This method was established for fast and simultaneously qualitative confirmation and quantitative determination of ribavirin in chicken.

V-12 Determination of lean meat powder in pork by UFLC-triple quadrupole mass spectrometry

INTRODUCTION

A method was proposed for determination of clenbuterol, ractopamine and salbutamol in pork using Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer. Extracted samples were separated by LC-30A ultra fast liquid chromatograph, and then quantitatively assayed with LCMS-8030 triple quadrupole mass spectrometer. The calibration curves of clenbuterol, ractopamine and salbutamol were of good linearity in the concentration range of 0.05~100 μg/L with correlation coefficients higher than 0.999. Precision tests were conducted on standard solutions at concentrations of 0.1 μg/L, 0.5 μg/L, 1 μg/L, 5 μg/L and 10 μg/L. The %RSDs of retention time and peak areas in 6 successive injections were below 0.42% and 5.47%, respectively, suggesting that the system was of good precision. The method's LOQs met the requirement of 0.5 μg/kg (for clenbuterol, the LOQ requirement was 0.05 μg/kg) stipulated in SNT 1924-2007.

Lean meat powder refers to a group of veterinary drugs which, when added into feedstuffs, can increase the lean meat rate of livestocks, reduce feedstuff consumption, and cut costs by marketing meat products ahead of schedule. When people refer to "lean meat powder" in China, most of the time they mean clenbuterol, a drug that was used for treatment of bronchial asthma but was later banned because of its serious side effects. At present, a category of drugs called β-receptor agonists are used for this function. Examples of these drugs include ractopamine and salbutamol. However, these drugs also pose potential safety hazard to human health because of their health-impairing effect in spite of their "lean meat rate promoting" action. For this reason, they are also banned globally. In China, β-receptor agonists were listed on the *Catalog of Drugs Prohibited from Use in Feed or Drinking Water for Animals* issued in 2002. It is stipulated in SNT 1924-2007, a standard issued by China Entry-Exit Inspection and Quarantine Bureau, that an LOQ requirement of 0.5 μg/kg applies to all methods for analysis of lean meat powder. The Positive List System of

Japan has a stricter MRL, i.e. 0.05 μg/kg, for clenbuterol. Therefore, the LOQ requirement for product exported to Japan is even higher. In this paper, a method that meets the requirements for the assay of export food was proposed for determination of clenbuterol, ractopamine and salbutamol in pork with Shimadzu ultra-high performance liquid chromatograph-tandem mass spectrometer.

EXPERIMENTAL

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The detailed configuration included two LC-30AD pumps, DGU-20A5 online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, LabSolutions ver. 5.41 chromatography workstation.

Analytical conditions

LC conditions

Column : Shim-pack XR-ODSIII 2.0 mm I.D.×50 mm L., 1.6 μm

Mobile phase : A-0.1% aqueous solution of Formic acid; B-0.1% Acetonitrile solution of formic acid

Flow rate : 0.4 mL/min

Column temperature : 40 °C

Injection volume : 20 μL

Time program :

Time (min)	Module	Command	Value
0.56	Pump	B Conc.	50%
0.90	Pump	B Conc.	50%
0.95	Pump	B Conc.	10%
1.80	Controller	Stop	

MS condition

Ionization mode	: ESI (+)
Ionization voltage	: +4.5 kV
Nebulizing gas	: Nitrogen 3.0 L/min
Drying gas	: Nitrogen 15 L/min
Collision gas	: Argon
DL temperature	: 250 °C
Heater block temperature	: 400 °C
Mode	: Multiple reaction monitoring (MRM)
Dwell time	: 20 ms
Pause time	: 3 ms

MRM parameters: see Table1

Preparation of standard solutions and pretreatment of samples

1 μg/mL multi-standard intermediate solution and 100 ng/mL multi-standard intermediate solution of isotope internal standards were prepared using methanol as solvent. The multi-standard intermediate solution was diluted with water into a series of multi-standard working solutions at concentrations of 0.05, 0.1, 0.5, 1, 5, 10, 50 and 100 ng/mL, which were then spiked with isotope internal standards at spiked level of 1.0 ng/mL.

Sample pretreatment was carried out in reference with SNT 1924-2007 *Determination of Clenbuterol, Ractopamine, Salbutamol, and Terbutalin residues in Animal Derived Food for Import and Export - HPLC-MS/MS Method,* with a modification that 5 g of sample that had been subjected to the pretreatment procedures was brought to the volume of 1 mL in the end.

Table 1 MRM parameters

Name	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
Salbutamol	239.95	148.10	-13.0	-20.0	-18.0
		222.10*	-13.0	-10.0	-18.0
D3-salbutamol	243.20	151.10	-13.0	-20.0	-18.0
Ractopamine	302.15	164.15	-12.0	-15.0	-13.0
		107.15*	-12.0	-30.0	-23.0
D6-ractopamine	307.90	168.15	-25.0	-15.0	-13.0
Clenbuterol	277.10	203.00	-11.0	-15.0	-16.0
		259.05*	-11.0	-10.0	-20.0
D9-clenbuterol	286.10	204.00	-15.0	-20.0	-16.0

* refers to qualitative ion.

RESULTS AND DISCUSSION

Chromatograms of multi-standard working solutions

The MRM chromatograms of 1 ng/mL multi-standard working solution are shown in Figs. 1-6. The retention time data of salbutamol, D3-salbutamol, ractopamine, D6-ractopamine, clenbuterol and D9-clenbuterol were 0.476, 0.475, 0.869, 0.867, 0.911 and 0.910 minutes, respectively.

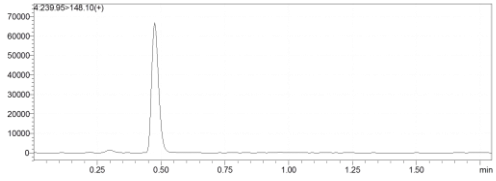


Fig.1 MRM chromatogram of

1 ng/mL salbutamol (239.95>148.10)

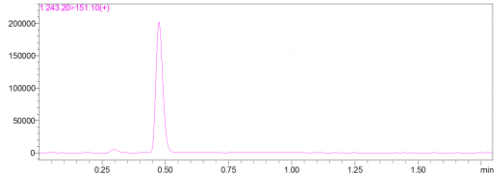


Fig.2 MRM chromatogram of
1 ng/mL D3-salbutamol (243.20>151.10)

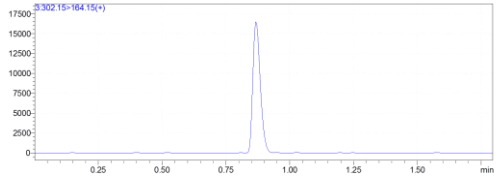


Fig.3 MRM chromatogram of
1 ng/mL ractopamine (302.15>164.15)

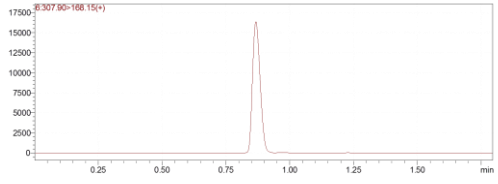


Fig.4 MRM chromatogram of
1 ng/mL D6-ractopamine (307.90>168.15)

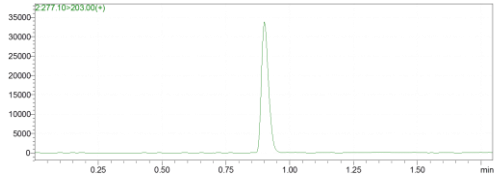


Fig.5 MRM chromatogram of
1 ng/mL clenbuterol (277.10>203.00)

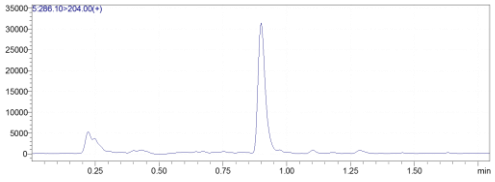


Fig.6 MRM chromatogram of

1 ng/mL D9-clenbuterol (286.10>204.00)

Linearity

Multi-standard working solutions at concentrations of 0.05, 0.1, 0.5, 1, 5, 10, 50 and 100 ng/mL were analyzed using the aforesaid analytical conditions. Calibration curves were plotted using concentration ratio as abscissa and peak area ratio as ordinate. The plotted calibration curves were of satisfactory linear relation and relevant information was shown in Table 2.

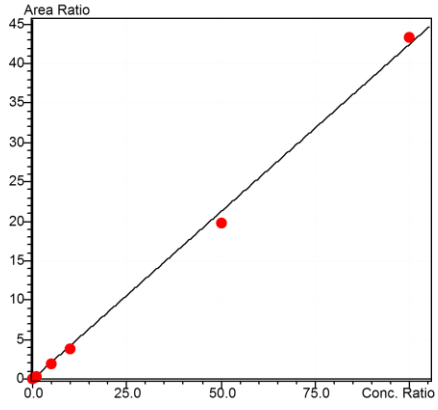


Fig.7 Calibration curve of salbutamol

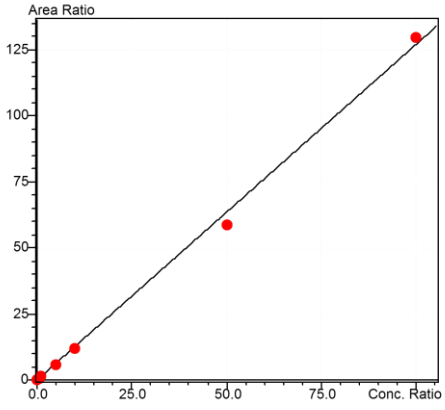


Fig.8 Calibration curve of ractopamine

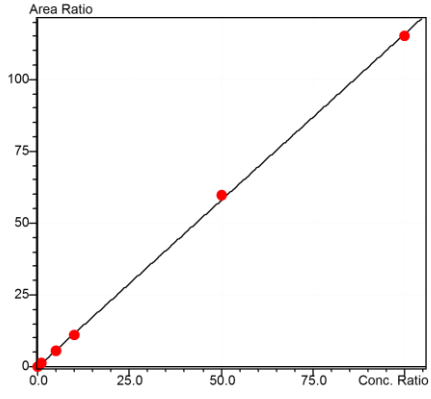


Fig.9 Calibration curve of clenbuterol

Table 2. Calibration curves of 3 β-receptor agonists

Name	Calibration Curve	Correlation Coefficient (r)
Salbutamol	Y = (0.424827)X	0.9991
Ractopamine	Y = (1.27146)X	0.9991
Clenbuterol	Y = (1.15869)X	0.9998

Precision test

Multi-standard working solutions at concentrations of 0.1, 0.5,1, 5 and 10 ng/mL were assayed with 6 consecutive injections to assess the method's precision. The repeatability of retention time and peak area is shown in Table 3.

Table 3 Repeatability of salbutamol (n=6)

Conc. (ng/mL)	%RSD (RT)	%RSD (Area)
0.1	0.12	2.43
0.5	0.18	2.98
1	0.12	0.81
5	0.07	1.26
10	0.14	1.05

Table 4 Repeatability of ractopamine (n=6)

Conc. (ng/mL)	%RSD (RT)	%RSD (Area)
0.1	0.15	5.47
0.5	0.13	2.08
1	0.10	1.89
5	0.08	1.70
10	0.06	1.91

Table 5 Repeatability of clenbuterol (n=6)

Conc.(ng/mL)	%RSD (RT)	%RSD (Area)
0.1	0.15	4.55
0.5	0.04	3.05
1	0.11	1.50
5	0.04	1.16
10	0.05	1.14

Sensitivity test

In order to evaluate the method's sensitivity, matrix blank samples of pork were spiked with 0.05 µg/kg clenbuterol, the resulted chromatograms are shown in Figs. 10 and 11. The method's LOQ for clenbuterol was determined to be 0.05 µg/kg.

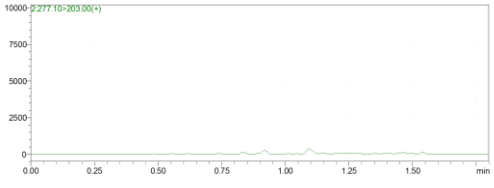


Fig.10 MRM chromatogram of pork matrix blank sample (277.10>203.00)

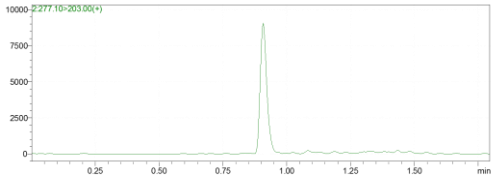


Fig.11 MRM chromatogram of pork matrix spiked with 0.05 μg/kg clenbuterol standard (277.10>203.00)

CONCLUSION

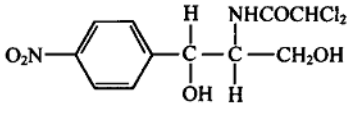
A method was proposed for the assay of 3 β-receptor agonists in pork with Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer. The method had the merits of fast analysis speed, good precision, and wide linear range (0.05-100 ng/mL). The correlation coefficients of all calibration curves were higher than 0.999. The method's LOQ met the requirement stipulated in China's national standard (0.5 μg/kg; for clenbuterol in pork, 0.05 μg/kg). It was concluded that Shimadzu ultra fast liquid chromatograph-tandem mass spectrometer can meet the requirements for the assay of lean meat powder in food for import and export.

V-13 Determination of chloromycetin (chloramphenicol) by ultra fast liquid chromatography-triple quadrupole mass spectrometry

INTRODUCTION

Chloromycetin (chloramphenicol) is an abroad-spectrum antibiotic which is banned for animal derived food in many countries because of its hematopoietic function inhibiting action. In this paper, a method is proposed for fast and sensitive determination of chloromycetin with Shimadzu LC-30A ultra fast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. The method demonstrated good linearity for chloromycetin in the concentration range of 0.05-50 μg/L, with a correlation coefficient of calibration curve higher than 0.9999. Results of precision test on 6 successive injections showed that the %RSDs of retention time were 0.14-0.35% and RSDs of peak area ratio were 2.96-4.16%, suggesting that the system was of good precision. Moreover, the method was highly sensitive and achieved an LOQ of 0.005 ng/mL.

Chloromycetin, also called chloramphenicol, is an abroad-spectrum antibiotic which is commonly used for the treatment of bacterial infectious diseases in fishery and poultry husbandry production. Its chemical structural formula is as follows.



Chlorampenicol(CAP)

Because of the hematopoietic function inhibiting action of chloromycetin, its application in food of animal origin is banned in many countries and an MRL of zero is set for chloromycetin in edible tissues of all food animals. It is stipulated by the Ministry of Agriculture of China (in No. 227 announcement of the year 2002) that the aforementioned ban was also applied in China and chloromycetin was included in the *List of Food Additives That May Be Illegally Added into Food and Abused (the fifth batch)*.

Therefore, it is absolutely necessary to develop simple, fast, and sensitive analytical methods for chloromycetin. Ultra fast liquid chromatography (UFLC) comprehensively improves separation efficiency, peak capacity and sensitivity with its 1.6 μm sized filler. The UFLC system was used in conjunction with Shimadzu LCMS-8030 triple quadrupole mass spectrometer to develop a fast and sensitive analytical method which was capable of completing analysis of samples within 1.5 min. The proposed method had an LOQ of 0.05 ng/mL for chloromycetin and met the requirements for analysis of banned veterinary drug residues.

EXPERIMENTAL

Apparatus

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The detailed configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, LabSolutions ver. 5.41 chromatography workstation.

Analytical conditions

LC conditions

Column :Shim-pack XR-ODS III 2.0 mm I.D.× 50 mmL., 1.6 μm

Mobile phase :A-water

Mobile phase :B-acetonitrile

Time program

Time	B Conc
0 min	30%
0.35-0.75 min	90%
0.76-1.5 min	30%

Flow rate : 0.4 mL/min

Column temperature : 40 °C

Injection volume : 20 μL

MS condition

Ionization mode	: ESI (-)
Ionspray voltage	: -3.0 kV
Nebulizing gas	: Nitrogen 3.0 L/min
Drying gas	: Nitrogen 15 L/min
Collision gas	: Argon
DL temperature	: 250 °C
Heater block temperature	: 400 °C
Mode	: multiple reaction monitoring (MRM)
Dwell time	: 30 ms
Pause time	: 3 ms
MRM parameters	: see Table 1

Preparation of standard solutions

1 mg/mL standard stock solution was prepared using methanol as solvent. The standard stock solution was then diluted with water into a series of standard working solutions at concentrations of 0.05, 0.1, 0.5, 1, 5, 10 and 50 ng/mL, into which deuterated (d5) chloromycetin was added to serve as internal standard. The spiked level was 1 ng/mL.

Table 1 MRM parameters

Name	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
Chloromycetin	321.20	152.20	12.0	20.0	29.0
D5- chloromycetin (IS)	326.20	157.20	12.0	15.0	29.0

RESULTS AND DISCUSSION

Mass spectrum and MRM chromatogram of chloromycetin

Fig. 1 shows the MS/MS spectrum and Fig. 2 and Fig. 3 shows MRM chromatograms of 1 ng/mL chloromycetin and 1 ng/mL deuterated chloromycetin standard working solutions. In this experiment, chloromycetin was quantitatively assayed using the peak area of product ion at *m/z* 152.20 while deuterated chloromycetin was quantitatively assayed using the peak area of product ion at *m/z* 157.20.

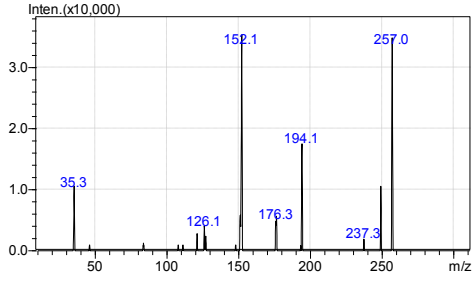


Fig.1 MS/MS spectrum of 1 ng/mL chloromycetin.

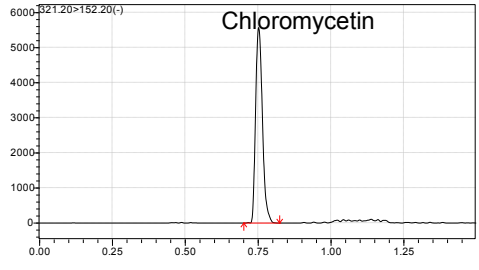


Fig.2 MRM chromatogram of 1 ng/mL chloromycetin (321.20>152.20).

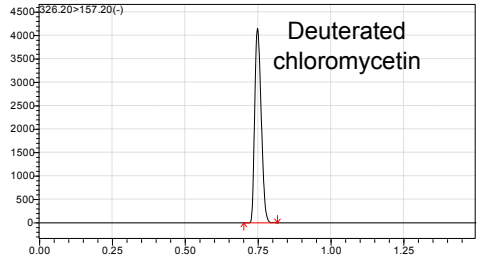


Fig.3 MRM chromatogram of 1 ng/mL deuterated chloromycetin (326.20>157.20).

Linear range

Multi-standard working solutions at concentrations of 0.05, 0.1, 0.5, 1, 5, 10, and 50 ng/mL were analyzed using above mentioned analytical conditions. Calibration curves were plotted with concentration ratio as abscissa and peak area ratio as ordinate. The calibration curve was of good linearity and had a linear equation of Y=(1.19739)X+(0.195016) and a linear correlation coefficient r=0.99996.

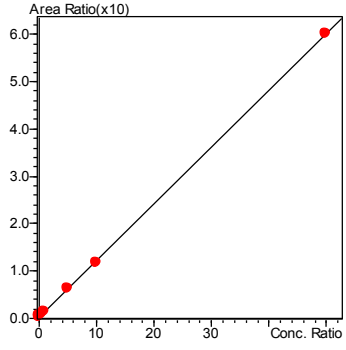


Fig.4 Calibration curve of chloromycetin

Precision test

Multi-standard working solution was analyzed for 6 times in succession to evaluate the method's precision. The repeatability results of retention time and peak area are shown in Table 2. The method demonstrated good repeatability.

Table 2 Repeatability of chloromycetin(n=6)

Conc. (ng/mL)	%RSD (RT)	%RSD (Area Ratio)
0.05	0.35%	3.64%
0.1	0.18%	2.96%
0.5	0.33%	3.80%
1	0.23%	3.14%
5	0.20%	4.16%
10	0.29%	3.73%
50	0.14%	4.14%

Sensitivity test

MRM chromatogram of 0.05 ng/mL chloromycetin standard working solution is shown in Fig.4. Its S/N ratio results are shown in Table 3. The LOQ was calculated to be 0.005 ng/mL.

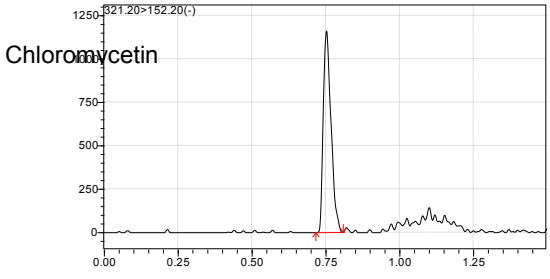


Fig.5 Chromatogram of 0.05 ng/mL chloromycetin(321.20>152.20)

Table 3 S/N ratio of chloromycetin

(321.20>152.20, 0.05 ng/mL)

n	S/N ratio
1	90.4
2	79.1
3	137.6
4	141.2
5	89.7
6	101.4
Mean	106.5

CONCLUSION

A method was developed for the assay of chloromycetin with Shimadzu LC-30A ultrafast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. The method was rapid and was having high precision. The %RSDs of retention time and peak area ratio in 6 successive injections were 0.14-0.35% and 2.96-4.16%, respectively. The proposed method had a wide linear range (0.05-50 ng/mL), in which range the correlation coefficient of calibration curve was higher than 0.9999. Moreover, the method was highly sensitive and achieved an LOQ of 0.005 ng/mL. The method demonstrated that Shimadzu LCMS-8030 can be used for highly sensitive quantitative analysis of chloromycetin.

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V-14 Determination of Glucocorticoids in Milk Powder by UFLC/Triple Quadrupole Mass Spectrometry

INTRODUCTION

In this paper, a method was developed for the determination of glucocorticoids in milk powder with Shimadzu LC-30A ultra fast liquid chromatograph (UFLC) and Shimadzu LCMS-8030 triple quadrupole mass spectrometer. Samples were extracted, separated with LC-30A ultra fast liquid chromatograph, and then quantitatively analyzed with LCMS-8030 triple quadrupole mass spectrometer. Seven glucocorticoids were separated and analyzed rapidly within 2 minutes. For these compounds, the proposed method was of good linearity in the concentration range of 0.5~40 μg/L, the correlation coefficients of calibration curves were all greater than 0.999; precision test was performed on multi-standard solutions at concentrations of 2 μg/L, 10 μg/L and 40 μg/L by 6 replicate injections, the retention time and %RSD of peak area were below 3.860% and 0.583%, respectively, suggesting that the system's performance is satisfactory. When used for determination of glucocorticoids in milk powder, the method's LOQ was 0.4 μg/kg for prednisone, prednisolone, hydrocortisone, dexamethasone, beclometasone and methyl prednisone and 1.0 μg/kg for fludrocortisone acetate.

Glucocorticoids, also referred to as adrenal cortex hormone (ACH), are a group of steroid hormones secreted by adrenal cortex that have regulation actions on the biosynthesis and metabolism of carbohydrates, fats, and proteins. Glucocorticoids also have anti-inflammatory actions and can be used for the treatment of diseases such as SARS and septicemia when the effects of common antibiotics or anti-inflammatory agents are less satisfactory. They are called "glucocorticoids" because of their carbohydrate metabolism regulating activity, which is the first of their actions known by people. Recently in China, the report of domestic milk powder suspected of causing precocious puberty in babies is yet another food safety scandal after the "melamine-tainted milk powder" scandal in 2008. The No. 235

Announcement of the Ministry of Agriculture of the People Republic of China issued in 2002 has banned the use of trenbolone and other chemically synthesized hormones and has stipulated that these hormones shall not be detected in foods of animal origin. EU Directive 96/22/EC, FDA, and Japan's positive list system have also banned the use of hormonal drugs in animal-derived foods. In this paper, a method was proposed in reference with GB/T 21981-2008 Determination of hormone multi-residues in foodstuffs of animal origin -- LC-MS/MS method. Fast and accurate determination of glucocorticoids in milk powder with Shimadzu LC-30A UFLC- LCMS-8030 triple quadrupole mass spectrometer is described for the reference of relevant laboratories.

EXPERIMENTAL

A combined system of Shimadzu LC-30A UFLC and LCMS-8030 triple quadrupole mass spectrometer was used in the experiment. The configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communication bus module, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutionsVer. 5.41 chromatography workstation.

Conditions of Analysis

UFLC Conditions

Analytical apparatus : LC-30A system

Column :Shimadzu Shim-pack XR-ODS III 2.0 mm I.D.×50 mm L., 1.6 μm

Mobile phase :A－0.1% formic acid aqueous solution; B－acetonitrile

Flow rate : 0.4 mL/min

Injection volumn : 10 μL

Column temperature : 40 °C

Elution mode : Binary gradient with an initial concentration of 60%B,

See Table 1 for time sequence

Table 1 Binary Gradient

Time(min)	Module	Command	Value
2.00	Pumps	Pump B Conc.	70
2.01	Pumps	Pump B Conc.	90
2.50	Pumps	Pump B Conc.	90
2.51	Pumps	Pump B Conc.	60
3.50	Controller	Stop	

MS conditions

Analytical apparatus : LCMS-8030
Ion source : ESI(-)
Interface voltage of ion source : -3.5 kV
Nebulizing gas : Nitrogen 3.0 L/min
Drying gas : Nitrogen 15 L/min
Collision gas : Argon
DL temperature : 250 °C

Heater block temperature : 400 °C
Scan mode : Multiple reaction monitoring(MRM)
Pasue time : 10 ms
Dwell time : 3 ms
MRM parameters : Listed in Table 2

Table 2 MRM Parameters

No.	Name	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
1	Prednisone	403.15	327.20	14.0	15.0	21.0
			357.20*	14.0	10.0	25.0
2	Prednisolone	405.20	329.15	14.0	20.0	22.0
			359.25*	14.0	15.0	24.0
3	Hydrocortisone	407.20	331.20	15.0	20.0	22.0
			361.15*	15.0	10.0	25.0
4	Dexamethasone	437.20	361.20	16.0	20.0	23.0
			391.25*	16.0	15.0	27.0
5	Beclometasone	453.20	377.15	10.0	15.0	25.0
			407.15*	10.0	15.0	28.0
6	Fludrocortisone acetate	467.25	421.25	17.0	15.0	28.0
			349.20*	17.0	25.0	24.0
7	Methyl prednisone	419.30	343.15	15.0	16.0	23.0
			294.50*	15.0	44.0	29.0

* refers to qualitative ion.

Sample Preparation

Preparation of standard solution:

100 μg/L standard mixture was prepared using acetonitrile as solvent, and diluted with 50% acetonitrile aqueous solution into standard working solutions at concentrations of 0.5, 1, 2, 5, 10, 20, and 40 μg/L.

Sample pretreatment method:

The same as specified in GB/T 21981-2008 *Determination of hormone multi-residues in foodstuffs of animal origin—LC-MS/MS method*.

RESULTS AND DISCUSSION

MRM Chromatogram of Standard Samples

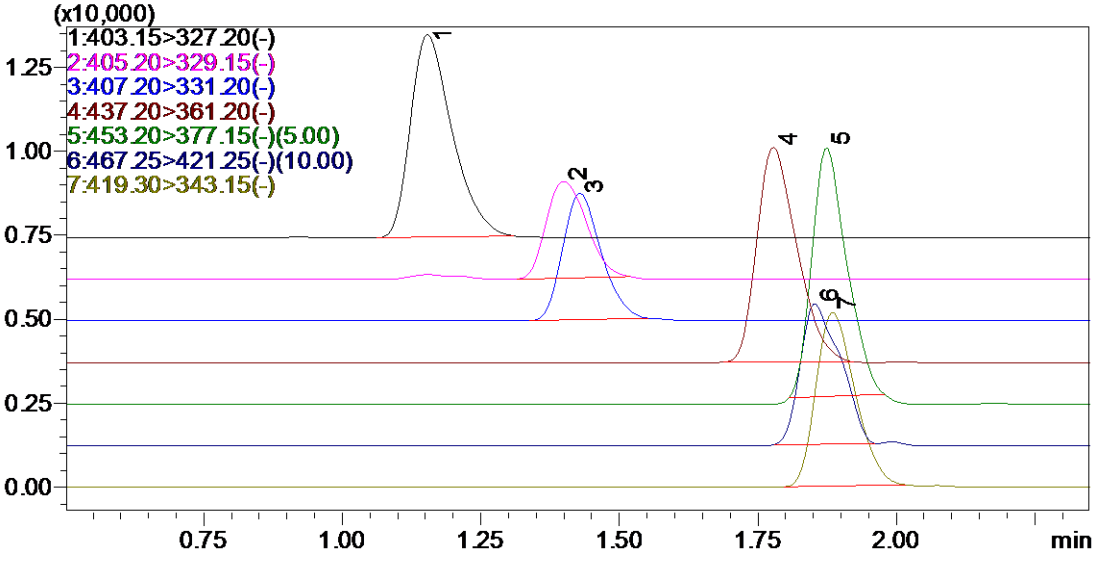


Fig. 1 MRM chromatograms of 10 μg/L standard mixture

(1: Prednisone; 2: Prednisolone; 3: Hydrocortisone; 4: Dexamethasone; 5: Beclometasone; 6: Fludrocortisone acetate; 7: Methyl prednisone)

Linearity

Standard working solutions of concentrations of 0.5, 1.0, 2.0, 5, 10, 20, 40 μg/L were determined under the analysis conditions as specified in 1.2 and calibration curves were plotted as shown in Figures 2~8 with concentration as abscissa and peak area as ordinate; the resulted calibration curves were of good linearity and their linear equations and correlation coefficients were listed in Table 3.

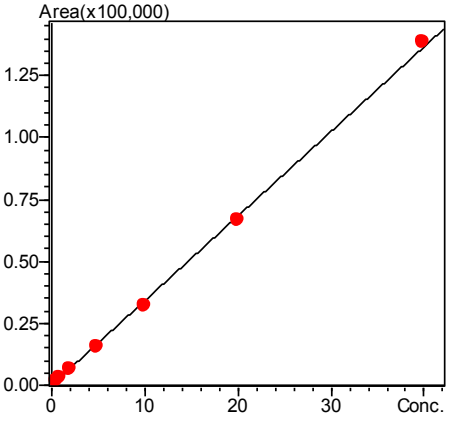


Fig. 2 Calibration curve of prednisone

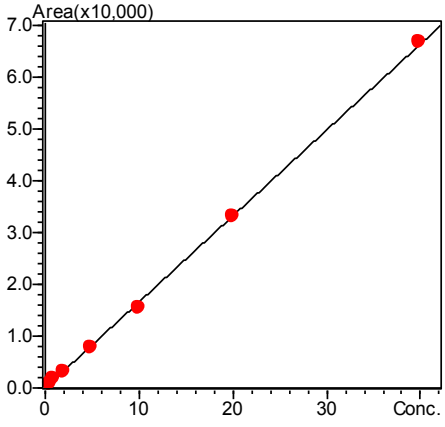


Fig.3 Calibration curve of prednisolone

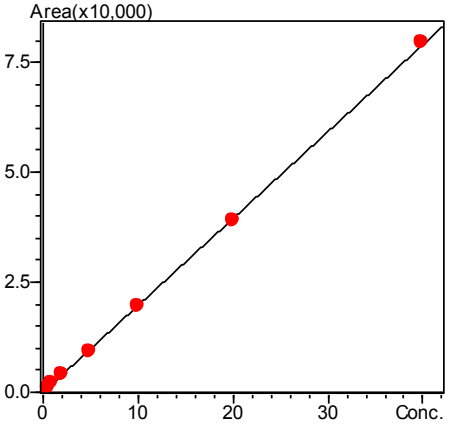


Fig. 4 Calibration curve of hydrocortisone

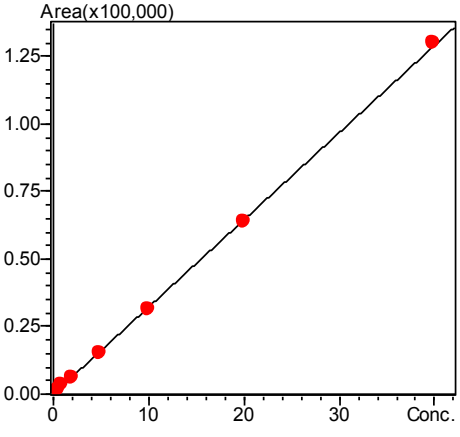


Fig. 5 Calibration curve of dexamethasone

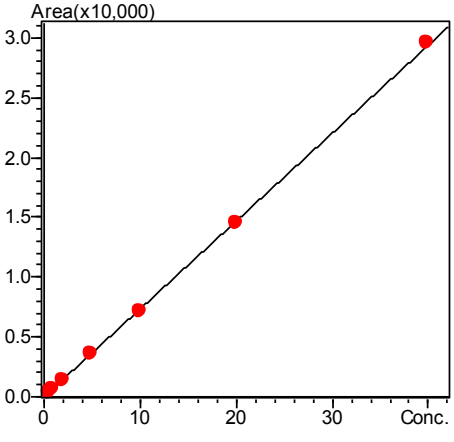


Fig. 6 Calibration curve of beclometasone

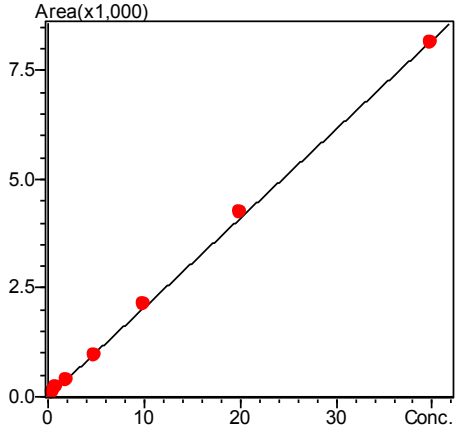


Fig.7 Calibration curve of fludrocortisone acetate

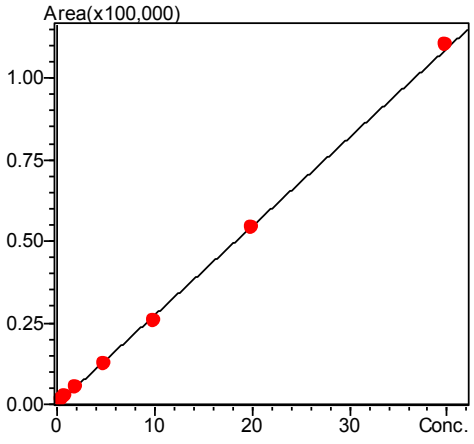


Fig. 8 Calibration curve of methyl prednisone

Table 3 Parameters of calibration curves of the 7 glucocorticoids

No.	Name	Calibration Curve	Correlation Coefficient (r)
1	Prednisone	Y = (3416.53)X + (0)	0.9996
2	Prednisolone	Y = (1657.36)X + (0)	0.9998
3	Hydrocortisone	Y = (1975.28)X + (0)	0.9998
4	Dexamethasone	Y = (3228.82)X + (0)	0.9999
5	Beclometasone	Y = (734.315)X + (0)	0.9999
6	Fludrocortisone acetate	Y = (205.149)X + (0)	0.9996
7	Methyl prednisone	Y = (2729.61)X + (0)	0.9998

Precision test

Multi-standard working solutions at concentrations of 2, 10, and 40 μg/L were determined for 6 times in succession to evaluate the precision of the method. The repeatability results of retention time and peak area data were as shown in Table 4. The results showed that the %RSDs of peak area and retention time data of standard solutions of the 3 concentrations ranged

between 1.496% and 3.860% and between 0.102% and 0.583%, respectively, indicating that the method's precision was satisfactory.

Table 4 Repeatability - retention time and peak area (n=6)

Sample name	%RSD (2 µg/L)		%RSD (10 µg/L)		%RSD (40 µg/L)	
	Area	R.T.	Area	R.T.	Area	R.T.
Prednisone	2.699	0.426	1.615	0.140	1.496	0.209
Prednisolone	2.652	0.535	1.932	0.105	2.389	0.130
Hydrocortisone	3.317	0.183	2.619	0.204	2.028	0.130
Dexamethasone	2.343	0.224	2.404	0.030	2.646	0.115
Beclometasone	3.826	0.139	2.509	0.137	3.704	0.154
Fludrocortisone acetate	3.860	0.583	2.095	0.364	2.832	0.212
Methyl prednisone	2.690	0.209	2.826	0.417	2.165	0.102

Sensitivity test

In order to determine the method's sensitivity, multi-standard solution were spiked into blank samples of milk powder at spiked level of 0.4 µg/kg and 1.0 µg/kg, the resulted chromatograms were as shown in Figures 9~11. As can be seen in Fig. 9, trace amount of hydrocortisone was detected in the milk powder sample and its concentration was about 0.03 µg/kg. The method's LOQ was 0.4 µg/kg for prednisone, prednisolone, hydrocortisone, dexamethasone, beclometasone and methyl prednisone and 1.0 µg/kg for fludrocortisone acetate, all meeting the requirements regulated in GB/T 21981-2008 *Determination of hormone multi-residues in foodstuffs of animal origin—LC-MS/MS method*.

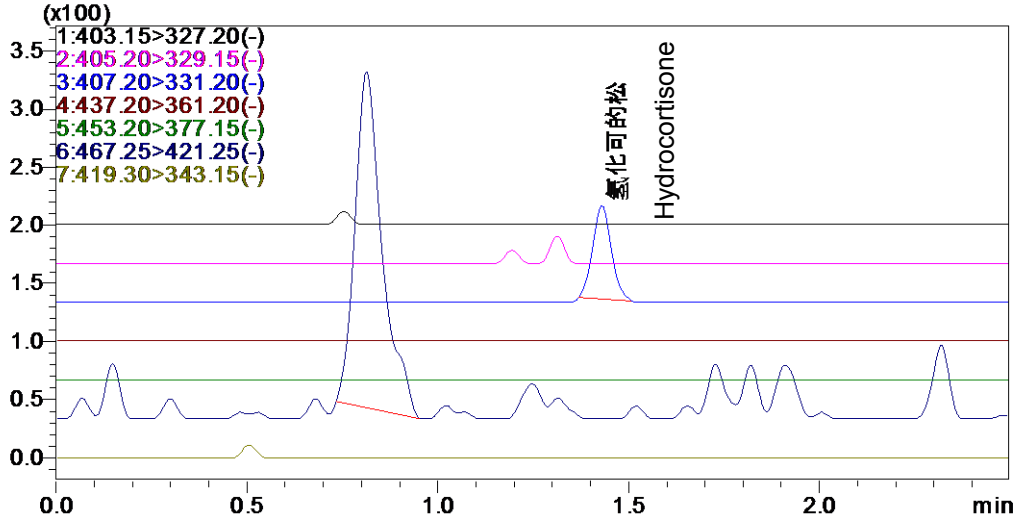


Fig. 9 MRM chromatogram of milk powder sample

（1: Prednisone; 2: Prednisolone; 3: Hydrocortisone; 4: Dexamethasone; 5: Beclometasone; 6: Fludrocortisone acetate; 7: Methyl prednisone)

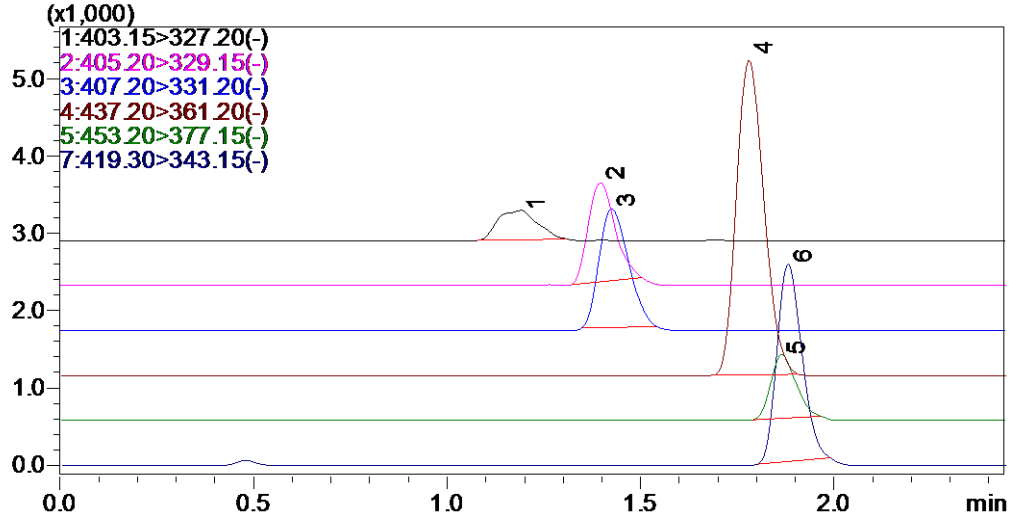


Fig. 10 MRM chromatogram of milk powder sample spiked with 0.4 μg/L standards

（1: Prednisone; 2: Prednisolone; 3: Hydrocortisone; 4: Dexamethasone; 5: Beclometasone; 6: Methyl prednisone)

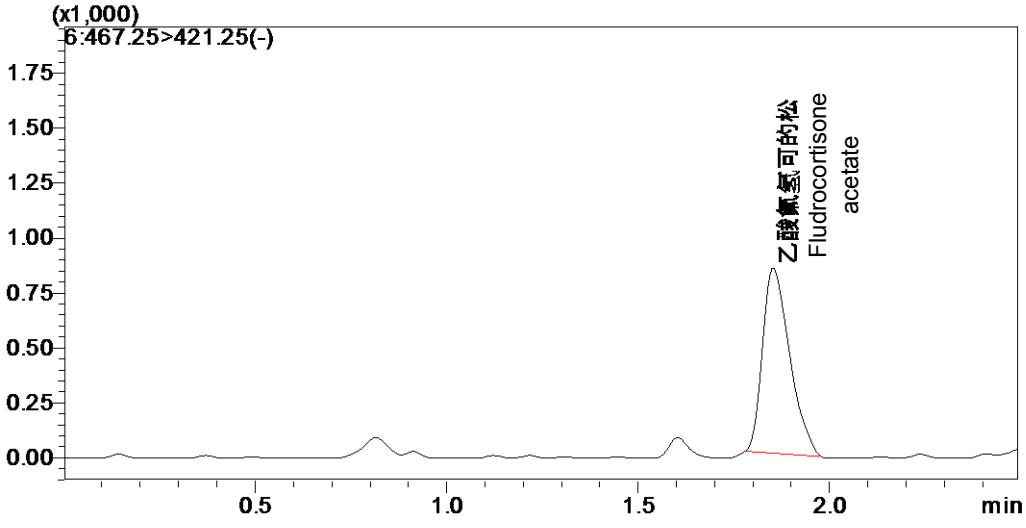


Fig.11 MRM chromatogram of a sample spiked with 1.0 µg/kg fludrocortisone acetate

CONCLUSION

A method was established for the determination of 7 glucocorticoids in milk powder with Shimadzu LC-30A UFLC-LCMS-8030 triple quadrupole mass spectrometer. The method was rapid and was having high precision. All correlation coefficients of the calibration curves in the concentration range of 0.5~40 µg/L were greater than 0.999. When used to analyze glucocorticoids in milk powder, the method's LOQ was 0.4 µg/kg for prednisone, prednisolone, hydrocortisone, dexamethasone, beclometasone and methyl prednisone and 1.0 µg/kg for fludrocortisone acetate, all meeting the requirements regulated in GB/T 21981-2008 *Determination of hormone multi-residues in foodstuffs of animal origin—LC-MS/MS method*.

V-15 Determination of Sulfonamides in Pork by UFLC/MS/MS

INTRODUCTION

In this paper, a method was proposed for determination of 9 sulfonamides, i.e. sulfadiazine, sulfamerazine, sulfadimidine, sulfamethoxypyridazine, sulfamethoxazole, sulfamonomethoxine, sulfisoxazole, sulfadimethoxypyrimidine, and sulfaquinoxaline, using the combination of Shimadzu LC-30A ultra fast liquid chromatograph (UFLC) and LCMS-8030 triple quadrupole mass spectrometer. The 9 sulfonamides were of good linearity in the concentration range of 0.5~1000 ng/mL and the correlation coefficients of their calibration curves were all greater than 0.999. Precision test was performed by 6 successive injections of standard solutions at concentrations of 5 ng/mL, 50 ng/mL and 500 ng/mL, the resulted retention time and %RSD of peak area were below 0.19% and 2.46%, respectively, suggesting that the system is having good precision. The method is of high sensitivity, its LOQs ranged from 0.04 to 0.31 μg/kg, meeting the detection limit requirements in No. 1025 Announcement -23-2008 of the Ministry of Agriculture of the People's Republic of China well.

Sulfonamides (sulfa drugs) are a group of artificially synthesized antibacterial agents. Because of their broad antibacterial spectrum, convenient administration methods, and inexpensive prices, sulfonamides are widely used as feedstuff additives and during the breeding of animals in order to promote husbandry output. Over dosage of sulfonamides may cause gastrointestinal irritation, renal damage, hypersensitive, drug resistance and other side effects. Residues of sulfonamides in food may subject consumers of the food to hypersensitive responses. If accumulated in human body for a prolonged period, these drugs can lead to allergic responses or even induce cancers. Codex Alimentarius Commission (CAC) and most European or American countries have set up maximum residue limits (MRLs) for sulfonamides in food or feedstuffs. In China, No. 235 Announcement of the Ministry of Agriculture of the People's Republic of China promulgated an administrative regulation, *MRLs of veterinary drugs in foodstuffs of animal origin*, in which a total MRL of 100 μg/kg was set up for sulfonamides in target tissues. Veterinary drug residue monitoring is an important measure to safeguard food safety and people's health.

In this paper, a method was developed in reference with the sample extraction and purification process and analytical method specified in No.

1025 Announcement-23-2008 *Determination of sulfonamide residues in foodstuffs of animal origin—LC-MS/MS method*, a guidance document promulgated by the Ministry of Agriculture of the People's Republic of China, for determination of 9 sulfonamides in pork, i.e. sulfadiazine(SD), sulfamerazine(SM1), sulfadimidine(SM2), sulfamethoxypyridazine(SMP), sulfamethoxazole(SMZ), sulfamonomethoxine(SMM), sulfisoxazole(SIZ), sulfadimethoxypyrimidine (SDM), and sulfaquinoxaline(SQX), with Shimadzu LC-30A UFLC and LCMS-8030 triple quadrupole mass spectrometer. The proposed method has the merits of fast analysis speed, good system precision and high sensitivity.

EXPERIMENTAL

A combined system of Shimadzu LC-30A UFLC and LCMS-8030 triple quadrupole mass spectrometer was used in the experiment. The configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC auto-sampler, CTO-30A column oven, CBM-20A communication bus module, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.41 chromatography workstation.

Conditions of Analysis

UFLC conditions

Column	:Shim-pack XR-ODS III 2.0mmI.D.×50mmL.,1.6 μm
Mobile phase	:A-5mM ammonium acetate-0.1 % formic acid aqueous solution B-acetonitrile
Flow rate	: 0.4 mL/min
Column temperature	: 40 °C
Injection volume	: 10 μL
Elution mode	: binary gradient, see Table 1 for time program

Table 1 Binary Gradient

Time	Module	Command	Value(%)
0.01	Pumps	B.Conc	20
3.50	Pumps	B.Conc	30
4.00	Pumps	B.Conc	30
4.01	Pumps	B.Conc	55
4.50	Pumps	B.Conc	55
4.51	Pumps	B.Conc	20
5.00	Controller	Stop	

MS conditions

Ionization mode	: ESI(+)
Ionspray voltage	: 4.5 kV
Nebulizing gas	: Nitrogen 3.0 L/min
Drying gas	: Nitrogen 15 L/min
Collision gas	: Argon
DL temperature	: 250 °C
Heater block temperature	: 400 °C
Mode	: multiple reaction monitoring (MRM)
Pause time	: 10 ms
Dwell time	: 3 ms
MRM parameters	: See Table 2

Preparation of standard solutions and samples

Preparation of standard solution:

Nine sulfonamide standards were accurately weighed and prepared into 10 μg/mL multi-standard solution using acetonitrile as solvent, then diluted with 20% acetonitrile aqueous solution to prepare a series of multi-standard working solutions of concentrations of 0.5, 1, 2, 5, 10, 50, 100, 500 and 1000 ng/mL.

Sample pretreatment method:

Refer to the sample extraction and purification methods specified in *Determination of sulfonamide residues in foodstuffs of animal origin—LC-MS/MS method*, a guidance document promulgated by No. 1025 Announcement -23-2008 of the Ministry of Agriculture of the People's Republic of China.

Table 2 MRM parameters

Name	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
SD	215.15	156.05	-10	-15	-19
		108.10*	-10	-25	-22
SM1	265.10	156.00	-14	-20	-18
		172.05*	-14	-15	-20
SM2	279.15	186.05	-30	-20	-19
		156.05*	-30	-20	-22
SMP	281.10	156.05	-30	-20	-18
		108.10*	-30	-30	-23
SMM	281.10	156.05	-15	-20	-19
		108.10*	-15	-25	-22
SMZ	254.15	156.05	-13	-15	-18
		108.10*	-13	-25	-24
SIZ	268.15	156.05	-14	-15	-13
		113.15*	-14	-15	-12
SDM	311.10	156.05	-30	-20	-18
		108.10*	-30	-30	-23
SQX	301.10	156.05	-16	-20	-18
		108.10*	-16	-25	-23

Note: * refers to qualitative ion

RESULTS AND DISCUSSION

MRM Chromatogram of Standard Working Solution

The MRM chromatograms of 10 ng/mL standard working solutions were as shown in Fig. 1-Fig. 9.

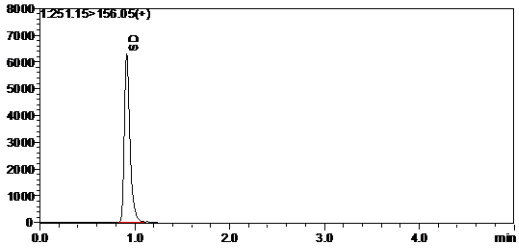


Fig. 1. Chromatogram of sulfadiazine (215.15>156.05)

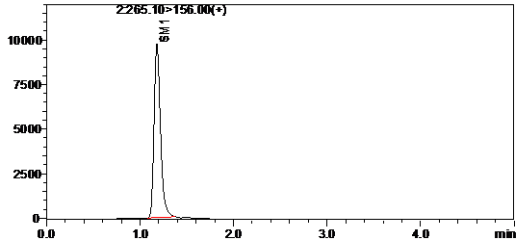


Fig. 2. Chromatogram of sulfamerazine (265.10>156.00)

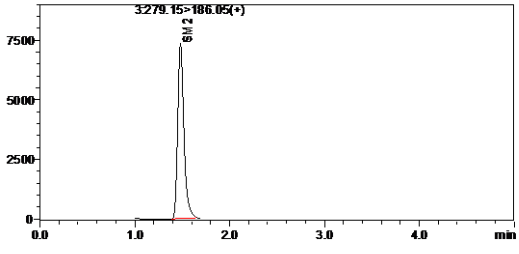


Fig. 3. Chromatogram of sulfadimidine (279.15>186.05)

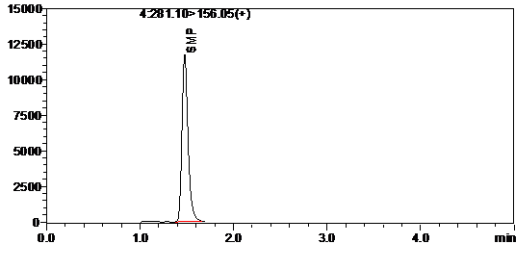


Fig. 4. Chromatogram of sulfamethoxypyridazine (281.10>156.05)

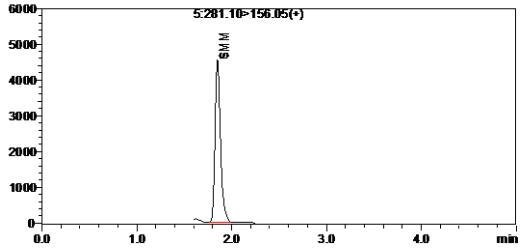


Fig. 5. Chromatogram of sulfamonomethoxine (281.10>156.05)

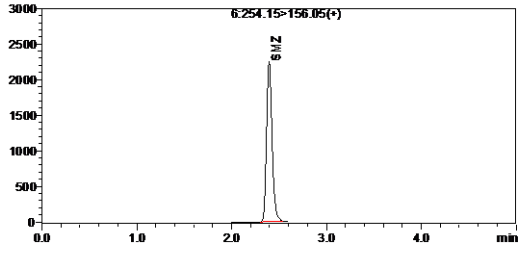


Fig. 6. Chromatogram of sulfamethoxazole (254.15>156.05)

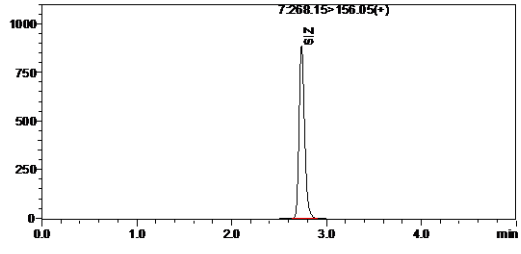


Fig. 7. Chromatogram of sulfisoxazole (268.15>156.05)

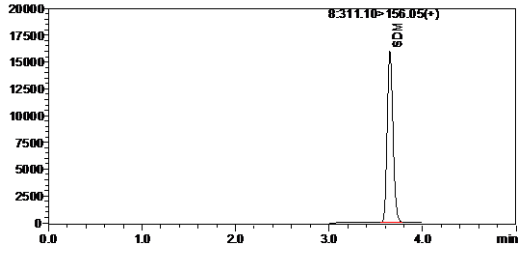


Fig. 8. Chromatogram of sulfadimethoxypyrimidine (311.10>156.05)

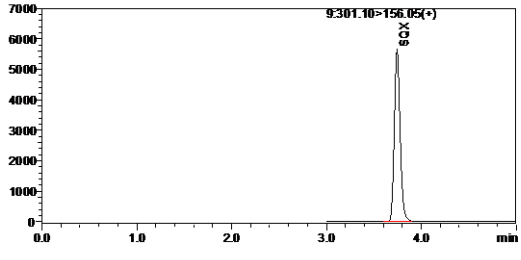


Fig. 9. Chromatogram of sulfaquinoxaline (301.10>156.05)

Linearity

Multi-standard working solutions of concentrations of 0.5, 1, 5, 10, 50, 100, 500 and 1000 ng/mL were determined using the analysis conditions mentioned above. Calibration curves were plotted using concentration as abscissa and peak area as ordinate. Relevant information was listed in Table 3.

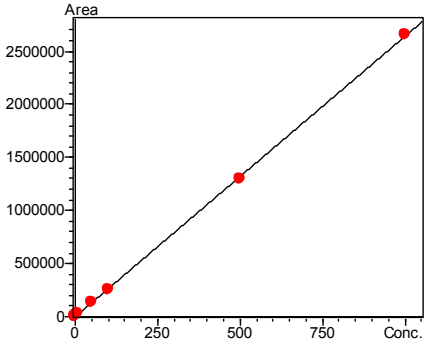


Fig. 10. Calibration curve of sulfadiazine

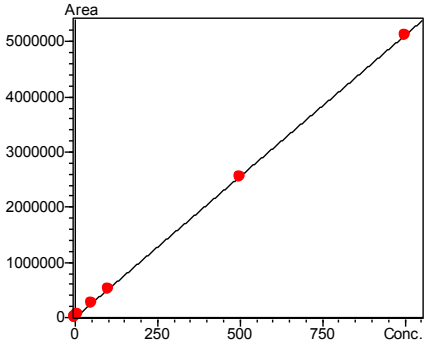


Fig. 11. Calibration curve of sulfamerazine

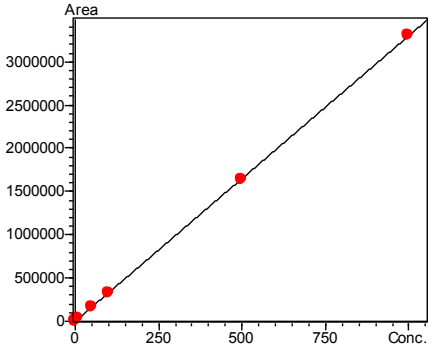


Fig. 12. Calibration curve of sulfadimidine

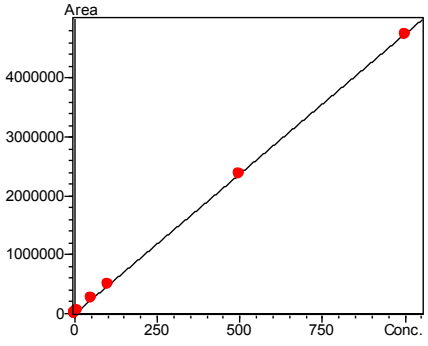


Fig. 13. Calibration curve of sulfamethoxypyridazine

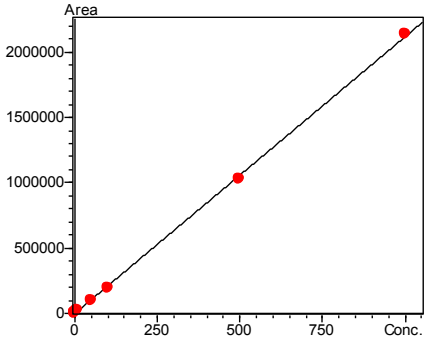


Fig. 14. Calibration curve of sulfamonomethoxine

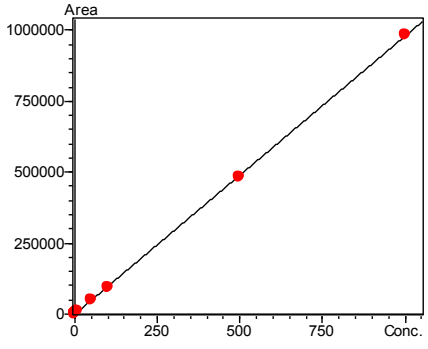


Fig. 15. Calibration curve of sulfamethoxazole

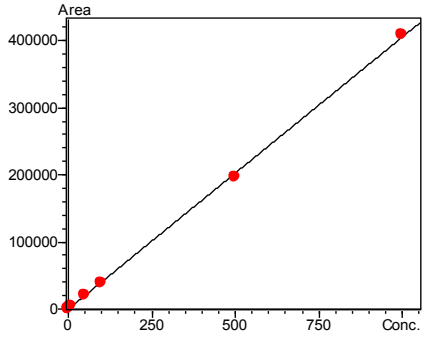


Fig. 16. Calibration curve of sulfisoxazole

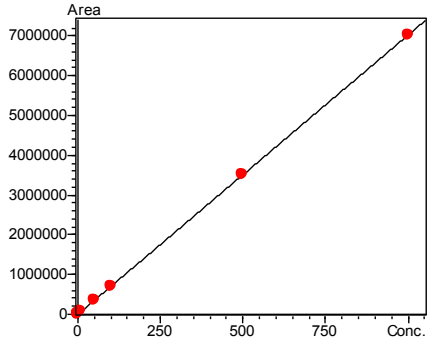


Fig. 17. Calibration curve of sulfadimethoxypyrimidine

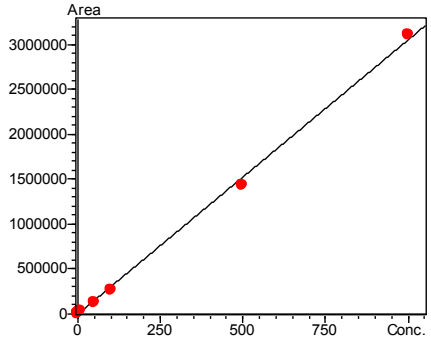


Fig. 18. Calibration curve of sulfaquinoxaline

Table 3. Information on calibration curves of the 9 sulfonamides

Name	Calibration Curve	Correlation Coefficient (r)
Sulfadiazine	Y= 5118.114 X	0.9999
Sulfamerazine	Y= 5118.114 X	0.9999
Sulfadimidine	Y= 3302.206 X	0.9999
Sulfamethoxypyridazine	Y= 4750.240 X	0.9999
Sulfamonomethoxine	Y= 2117.916 X	0.9996
Sulfamethoxazole	Y= 979.5841 X	0.9999
Sulfisoxazole	Y= 405.7352 X	0.9998
Sulfadimethoxypyrimidine	Y= 7023.682 X	0.9999
Sulfaquinoxaline	Y= 3048.031 X	0.9992

Precision test

The method's precision was evaluated by 6 successive determinations of standard working solutions of the three concentrations of 5 ng/mL, 50 ng/mL and 500 ng/mL. The resulted repeatability data of retention time and peak area were as listed in Table 4. The results showed that the %RSDs of retention time and peak area of standard solutions at low, intermediate and high concentration falling in the range of 0.03%~0.19% and 0.49%~2.46%, respectively, suggesting that the method is having good precision.

Table 4. Repeatability data of sulfonamides (n=6)

Drug Name	Conc. 5 ng/mL		Conc. 50 ng/mL		Conc. 500 ng/mL	
	R.T. (%RSD)	Area (%RSD)	R.T. (%RSD)	Area (%RSD)	R.T. (%RSD)	Area (%RSD)
Sulfadiazine	0.16	1.72	0.19	0.56	0.05	0.70
Sulfamerazine	0.07	0.85	0.15	0.95	0.07	0.49
Sulfadimidine	0.11	1.31	0.11	1.44	0.08	0.84
Sulfamethoxypyridazine	0.04	2.07	0.12	0.79	0.08	0.59
Sulfamonomethoxine	0.03	1.49	0.10	1.00	0.09	0.88
Sulfamethoxazole	0.08	2.24	0.14	0.84	0.07	1.11
Sulfisoxazole	0.05	2.46	0.15	1.56	0.06	1.11
Sulfadimethoxypyrimidine	0.05	1.62	0.11	0.57	0.06	0.70
Sulfaquinoxaline	0.07	0.99	0.11	1.31	0.06	0.58

Sensitivity test

In order to assess the method's sensitivity, 9 sulfonamides were spiked into blank samples of pork at spiked level of 0.5 μg/kg, the resulted chromatograms were as shown in Figures 19~27.The results showed that the proposed method's LOD fell in the range of 0.04~0.31 μg/kg, lower than 0.5 μg/kg, the required LOD in national standard *Determination of sulfonamide residues in foodstuffs of animal origin—LC-MS/MS* promulgated by No. 1025 Announcement -23-2008 of the Ministry of Agriculture of the People's Republic of China.

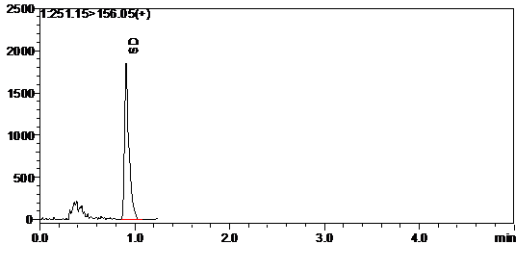


Fig. 19. Chromatogram of 0.5 μg/kg sulfadiazine (215.15>156.05)

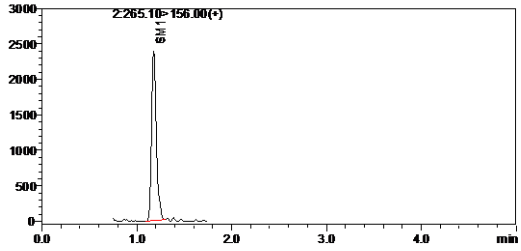


Fig. 20. Chromatogram of 0.5 µg/kg sulfamerazine (265.10>156.00)

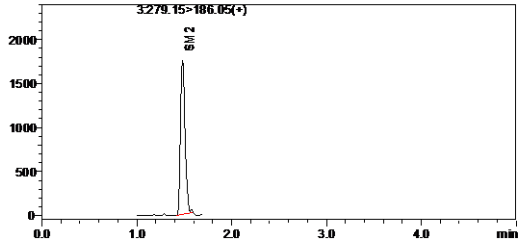


Fig. 21. Chromatogram of 0.5 µg/kg sulfadimidine (279.15>186.05)

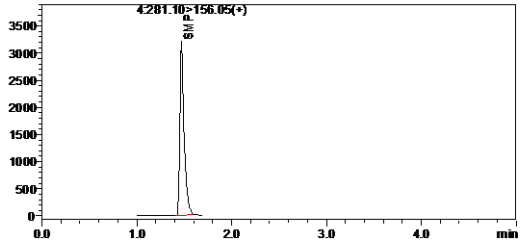


Fig. 22. Chromatogram of 0.5 µg/kg sulfamethoxypyridazine (281.10>156.05)

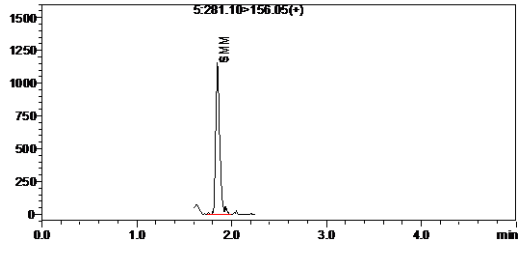


Fig. 23. Chromatogram of 0.5 µg/kg sulfamonomethoxine (281.10>156.05)

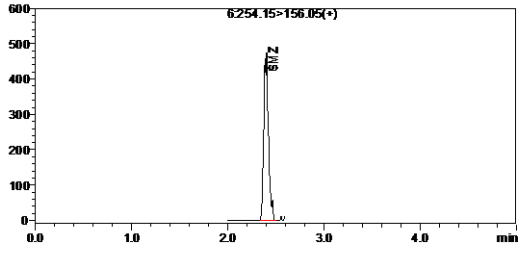


Fig. 24. Chromatogram of 0.5 µg/kg sulfamethoxazole (254.15>156.05)

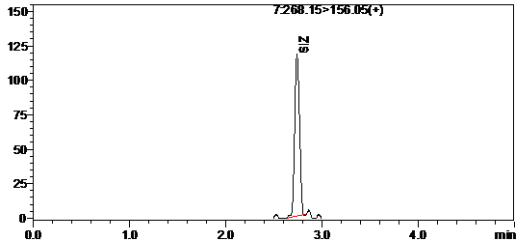


Fig. 25. Chromatogram of 0.5 µg/kg sulfisoxazole (268.15>156.05)

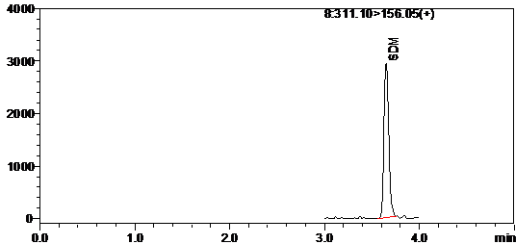


Fig. 26. Chromatogram of 0.5 µg/kg sulfadimethoxypyrimidine (311.10>156.05)

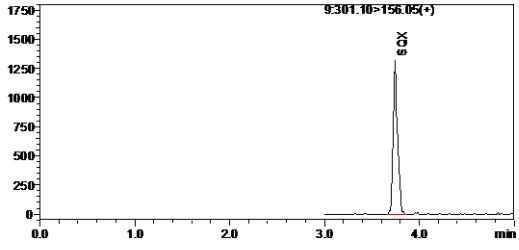


Fig. 27. Chromatogram of 0.5 µg/kg sulfaquinoxaline (301.10>156.05)

CONCLUSION

A method was established for the detection of sulfonamides in pork with Shimadzu LC-30A UFLC-LCMS-8030 triple quadrupole mass spectrometer. The proposed method has the merits of fast analysis speed, high precision, broad linear range (0.5~1000 ng/mL) and high sensitivity. The correlation coefficients of calibration curves were greater than 0.999. The method's LOQ for the 9 sulfonamides fell in the range of 0.04~0.31 µg/kg, better than 0.5 µg/kg, which is the required LOQ in a standard of the Ministry of Agriculture of the People's Republic of China. It was concluded that Shimadzu UFLC-tandem mass spectrometer can meet the requirements for the detection of sulfonamides in pork.

V-16 Determination of Lean Meat Powder in Pork with GC-MS/MS

INTRODUCTION

A method was proposed in this paper for detection of clenbuterol hydrochloride, salbutamol and ractopamine in pork with GC-MS/MS. In the method, extracted samples were back-extracted with diluted hydrochloric acid, and the extract was subjected to purification with SCX solid-phase extraction column after adjusted to pH 5.2; the separated residue was converted to derivatives with derivation reagent BSTFA:TMCS(99:1) and then subjected to determination with GC-MS/MS. The method showed good linearity in the concentration range of 0.2~10 μg/L with a correlation coefficient greater than 0.999. The method had a recovery of spiked samples greater than 70% at both spike concentrations of 1 μg/kg and 5 μg/kg and provided LODs within 0.003~0.008 μg/kg for the three compositions, meeting the requirements for daily food safety supervision.

Clenbuterol hydrochloride, salbutamol and ractopamine are β-receptor agonists which, though of certain medicine significance, may produce serious side effects if used at excessively high dosage. Nowadays, outlaw livestock breeding companies in China use these substances at 5~10 times of medical dosage and prolong their use to speed up livestock growth and improve lean mean ratio in order to gain extra profits. However, β-receptor agonists are hard to decompose and, if consumed by people, may produce evident poisonous effects. Therefore, it is an important task for food safety supervision authorities to detect β-receptor agonists in animal tissues.

Commonly available detection methods of β-receptor agonists include ELISA, HPLC, LC/MS, and GC/MS. In this paper, a method was proposed for determination of clenbuterol hydrochloride, salbutamol and ractopamine in pork by means of GC/MS/MS, a method with significantly higher sensitivity than GC/MS, aiming to reduce the impact of sample matrix on determination results. The proposed method is sensitive, accurate, and stable and can be used conveniently in daily supervision of food safety.

EXPERIMENTAL

GC-MS/MS : GCMS-TQ8030

Conditions of Analysis
GC-MS/MS conditions

Column : Rxi-5 Sil MS, 30 m × 0.25 mm × 0.25 µm
Injector temperature : 250 °C
Column temperature Programme : 70 °C (1 min)→@(25 °C/min)→-230 °C (5min)→@ (25 °C/min)→280 °C(5 min)

Carrier gas control mode : Constant Linear Velocity
Linear velocity of carrier gas : 35 cm/sec
Injection mode : Splitless injection (1 min)
High pressure injection : 250kPa (1 min)
Ion source : 230 °C
Interface temperature : 280 °C
MRM acquisition conditions: See Table 1

Table 1. Retention time and MRM parameters

No.	Retention Time	Compound Name	Quantitative Ion (CE)	Qualitative Ion (CE)
1	9.545	Clenbuterol hydrochloride	86>30 (7)	86>57 (12)
2	9.615	Salbutamol	86>30 (7)	86>57 (12)
3	17.145	Ractopamine	250>58 (15)	267>73 (20)

Sample Preparation

A sample pretreatment process shown in Fig. 1 was developed in reference with NY/T 468-2006.

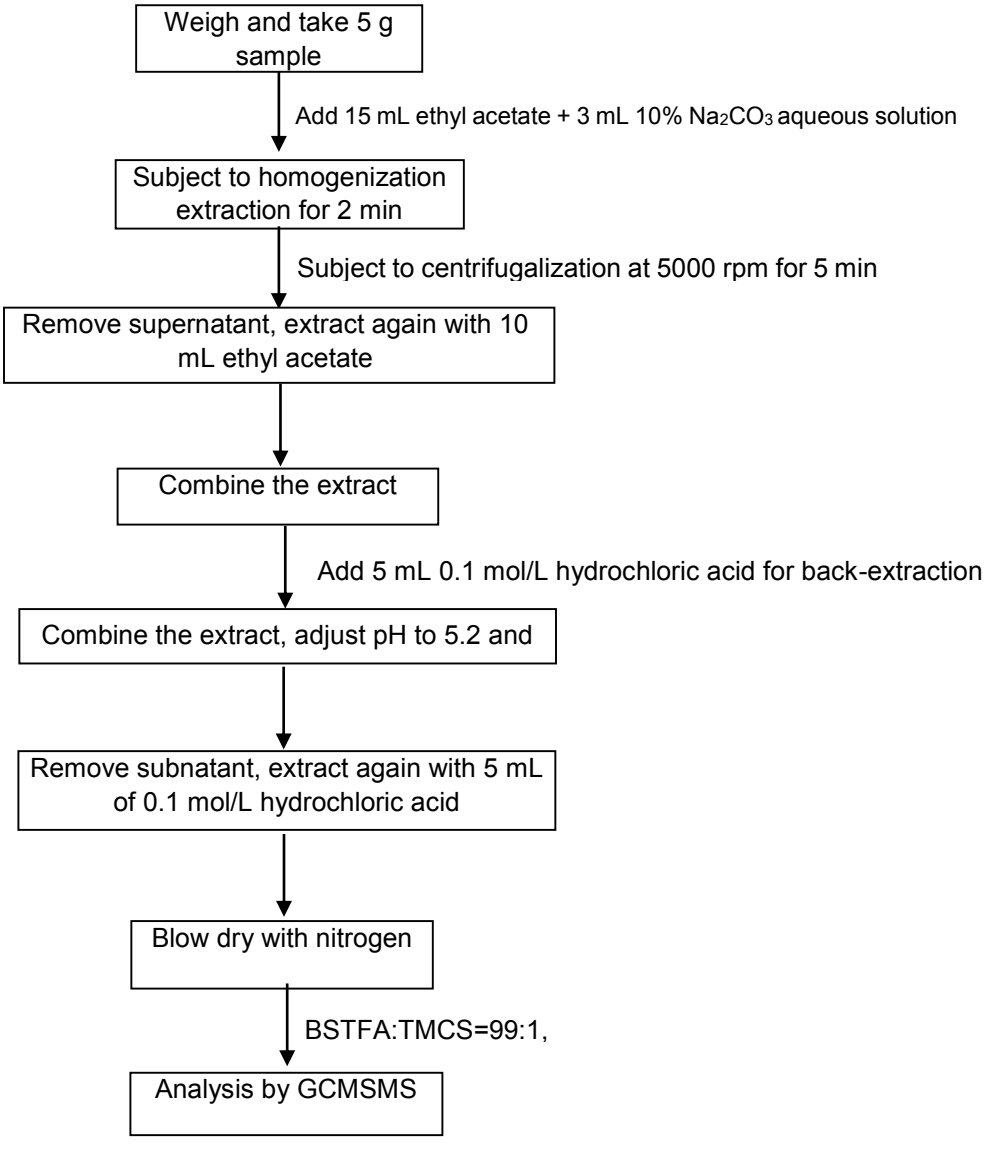


Fig. 1 Sample pretreatment

Note: BSTFA:TMCS refers to N,O-bis (trimethylsilyl)trifluoroacetamide:trimethylchlorosilane

RESULTS AND DISCUSSION

Chromatogram of standard sample

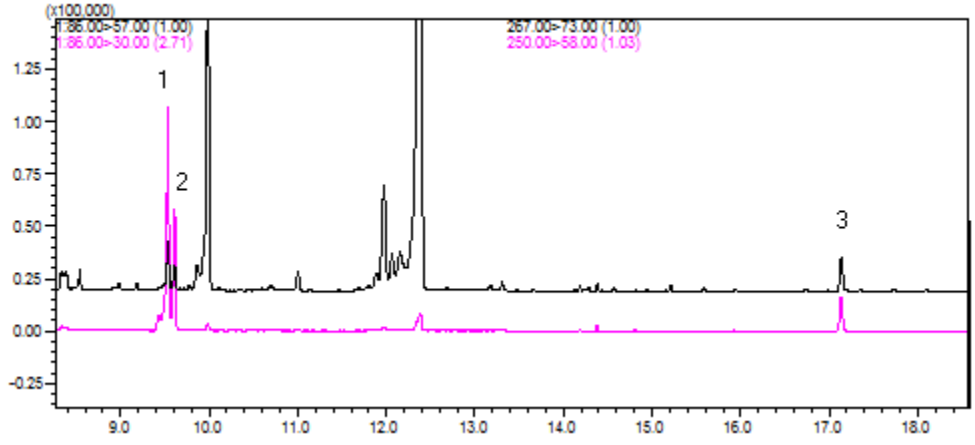


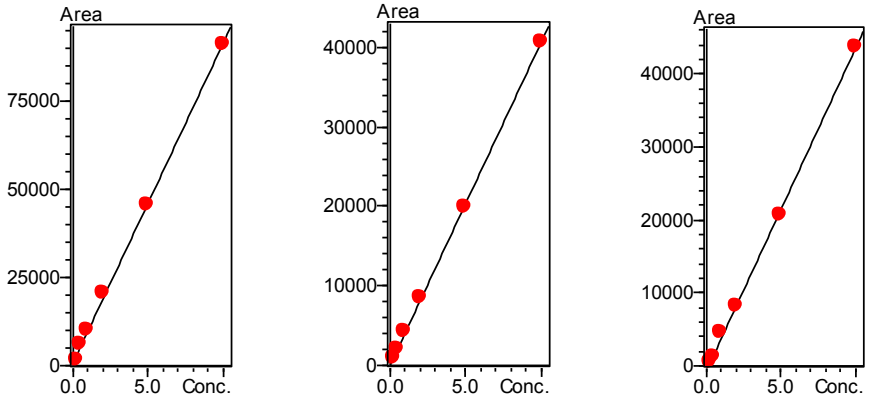
Fig. 2 MRM chromatograms of standard mixture (10 µg/L each)

1 Clenbuterol hydrochloride 2 Salbutamol 3 Ractopamine

Calibration curve

A series of standard mixture at concentrations of 0.2, 0.5, 1, 2, 5, 10 µg/L were prepared using n-hexane as solvent. They were subjected to drying under nitrogen blow at 40 °C until dried, added 100 µL toluene and 100µL BSTFA:TMCA=99:1, then subjected to derivatization for 1 h in a 80 °C oven; at the end of the derivatization, the derivatives were allowed to cool down, added 0.3 mL toluene, transferred to an sample vial to be injected for analysis with GC-MS/MS. Calibration curves were plotted as shown in Fig. 3, using concentration as abscissa and peak area as ordinate. LODs were calculated at S/N ratio of 3. To assess the repeatability of peak area, 10 µg/L

standard samples were injected 6 times in succession and the %RSDs were calculated. The correlation coefficients of calibration curves, LODs and %RSDs of peak areas are shown in Table 2.



Clenbuterol hydrochloride Salbutamol Ractopamine

Fig. 3 Calibration curves

Table 2. Correlation coefficients of calibration curves, LODs and %RSDs of peak areas

No.	Compound Name	Correlation Coefficient (r)	LOD (µg/kg)	%RSD (n=6)
1	Clenbuterol hydrochloride	0.9996	0.008	2.29
2	Salbutamol	0.9997	0.006	1.67
3	Ractopamine	0.9995	0.003	2.07

Recovery

Mixed standard solutions of the three components were spiked into blank pork matrices at concentrations of 1 µg/kg and 5 µg/kg. 3 parallel samples were processed and the recoveries (average recovery and %RSDs of the 3 parallel samples) of the components are shown in Table 3.

Table 3 Recoveries of spiked pork

No.	Compound Name	Spiked Amount 1 µg/kg		Spiked Amount 5 µg/kg	
		(%)Average recovery	%RSD	(%)Average recovery	%RSD
1	Clenbuterol hydrochloride	72.4	7.89	82.3	5.26
2	Salbutamol	71.6	5.24	85.2	6.18
3	Ractopamine	78.9	4.18	89.7	2.46

CONCLUSION

A method was proposed for the quantitative analysis of 3 β-receptor agonists in pork with Shimadzu GCMS-TQ8030. The proposed method has the merits of low LOD, good reproducibility, and high recovery. Moreover, it can reduce the false positive phenomenon caused by interference of pork matrix. It provided a spike recovery higher than 70.0% for the three β-receptor agonists at concentrations of 1 µg/kg and 5 µg/kg, meeting the requirements for daily food safety supervision. It is, therefore, suitable for qualitative and quantitative determination of these substances in pork and other animal tissues.

V-17 Determination of Illegally added 10 Sildenafils in Anti-fatigue Health Food by UFLC/Triple Quadrupole Mass Spectrometry

INTRODUCTION

A method was proposed for detection of illegally-added sildenafils in anti-fatigue health products with Shimadzu LC-30A liquid chromatograph-tandem LCMS-8030 triple quadrupole mass spectrometer. Extracted samples was quickly separated by LC-30A ultra fast liquid chromatograph (UFLC), and then quantitatively assayed with LCMS-8030 triple quadrupole mass spectrometer. For 8 compounds, the correlation coefficients of their calibration curves were greater than 0.999; for the rest 2 compounds, the correlation coefficients were greater than 0.99. Precision test was performed on 12 matrix solutions spiked with standards at LOQ concentration, the RSDs of retention time and peak area were better than 0.22% and 12.7%, respectively, suggesting that the method was of good precision. The method's LOQs were in the range of 0.4 μg/g~4.0 μg/g.

The addition of drugs into health products, especially health foods, is strictly prohibited. Some enterprises, however, illegally add drugs into health foods without authorization to "boost" the functionality of their products. The consumption of such health products by consumers who know nothing about the illegally adulteration of drugs may lead to drug abuse or even drug dependence. In consideration of the particularity of health foods and TCM (traditional Chinese medicine) health products, CFDA (China Food and Drug Administration) promulgated an announcement on May 25, 2011 regarding the crackdown of illegally adulteration of drugs into health foods and cosmetics. The announcement emphasized the sampling inspection of 1) health foods with alleged sleep improving, blood glucose reducing, fatigue relieving, and weight losing actions; 2) skincare products with alleged whitening, acne-removing, wrinkle-reducing, and anti-aging actions; 3) special cosmetics for speckle-elimination, hair growth, and hair dyeing; and 4) bathing products. With regard to substances and compositions apt to be added into anti-fatigue health products and the inspection bases for these substances and compositions, the (first batch) list included vardenafils, sildenafils and tadalafils drugs. In this paper, a method for fast and accurate determination of 10 illegally-added sildenafils with Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer was proposed.

EXPERIMENTAL

A combined system of Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer was used in the experiment. The configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.41 chromatography workstation.

Conditions of Analysis

LC Conditions

Column :Shim-pack XR-ODS II 2.0 mm × 100 mm, 2.2 µm

Mobile phase A :0.01% acetic acid-12 mM ammonium formate aqueous solution

Mobile phase B :Acetonitrile

Flow rate :0.4 mL/min

Injection volume :5 µL

Column temperature:40 °C

Elution mode : Binary gradient with an initial concentration of 15%B, See Table 1 for time program.

Table 1 Time program

Time(min)	Module	Command	Value
1.00	Pumps	Pump B Conc.	15
3.00	Pumps	Pump B Conc.	30
5.00	Pumps	Pump B Conc.	50
10.00	Pumps	Pump B Conc.	50
12.00	Pumps	Pump B Conc.	60
12.10	Pumps	Pump B Conc.	100
14.00	Pumps	Pump B Conc.	100
14.10	Pumps	Pump B Conc.	15
18.00	Controller	Stop	

MS conditions

Ionization	: ESI (+)
Ionization voltage	: 4.5 kV
Nebulizing gas	:Nitrogen 3.0 L/min
Drying gas	: Nitrogen 15 L/min
Collision gas	: Argon
DL temperature	: 250 °C
Block heater temperature	:400 °C
Mode	:MRM
Pause time	:30 ms
Dwell time	:30 ms
MRM parameters	: Listed in Table 2

Table 2. MRM parameter

No.	Name	Precursor ion (*m/z*)	Product ion (*m/z*)	Q1 Pre Bias (V)	CE (V)	Q1 Pre Bias (V)
1	*N*-desmethylsildenafil	461.20	85.20	-23.0	-40.0	-18.0
			283.10*	-23.0	-35.0	-19.0
2	Dimethylsildenafil	489.00	113.20	-24.0	-30.0	-24.0
			99.20*	-24.0	-35.0	-19.0
3	Hydroxyhomosildenafil	505.20	99.20	-26.0	-40.0	-21.0
			112.10*	-26.0	-30.0	-22.0
4	Sildenafil	475.00	100.20	-23.0	-30.0	-19.0
			283.10*	-23.0	-40.0	-20.0
5	Homosildenafil	489.20	113.20	-25.0	-35.0	-23.0
			99.20*	-24.0	-35.0	-19.0
6	Gliclazide	324.10	127.15	-16.0	-20.0	-13.0
			110.15*	-16.0	-20.0	-11.0
7	Thiodimethylsildenafil	505.10	99.15	-26.0	-35.0	-21.0
			113.15*	-26.0	-35.0	-23.0

8	Hydroxythiohomosildenafil	521.10	99.15	-26.0	-45.0	-19.0
			129.15*	-26.0	-35.0	-25.0
9	Thiosildenafil	491.20	100.20	-25.0	-30.0	-19.0
			299.10*	-25.0	-40.0	-22.0
10	Norneosildenafil	460.10	283.10	-23.0	-40.0	-20.0
			299.10*	-23.0	-35.0	-21.0
11	Thiohomosildenafil	505.10	99.20	-26.0	-45.0	-20.0
			113.20*	-26.0	-30.0	-23.0

Note: * refers to qualitative ion, gliclazide is internal standard

Sample Preparation

Preparation of standard solution:

2 mg of hydroxyhomosildenafil, thiodimethylsildenafil, dimethylsildenafil, and homosildenafil were accurately weighed (with a precision of 0.01 mg), 2.81 mg sildenafil citrate (equivalent to 2 mg sildenafil) was accurately weighed, and transferred to a 10 mL volumetric flask, respectively, dissolved and brought to metered volume with methanol, shaken evenly to get 200 μg/mL concentrated standard stock solutions.

2 mg controls of hydroxythiohomosildenafil, *N*-desmethylsildenafil, thiosildenafil, norneosildenafil, and thiohomosildenafil were accurately weighed (with a precision of 0.01 mg), respectively, transferred to a 5 mL volumetric flask, dissolved and brought to metered volume with methanol, shaken evenly to get 400 μg/mL concentrated standard stock solutions.

Working solutions were prepared by diluting the above-mentioned stock solutions with 80% methanol solution in specified proportion. Eventually the following solutions were prepared: (1) a series of standard solutions of hydroxyhomosildenafil, sildenafil, thiodimethylsildenafil, dimethylsildenafil, and homosildenafil at concentrations of 4, 10, 20, 40, 100, 200 , 400, 800, and 1000 ng/mL; (2) a series of standard solutions of hydroxythiohomosildenafil, *N*-desmethylsildenafil, thiosildenafil, norneosildenafil, and thiohomosildenafil at concentrations of 8, 20, 40, 80, 200, 400, 800, 1600, and 2000 ng/mL;

Sample pretreatment method:

For each of the 12 products, about 0.2 g blank sample was taken, accurately weighed (with a precision of 1 mg), and transferred to a 10 mL plastic centrifuge tube. 5 mL of methanol was added, and weighed. It was subjected to vortex mixer for 60 s and ultrasonic treatment for 15 min, allowed to cool

down, weighed again. It was replenished with methanol to make up for the lost weight. 50 µL of sample solution was pipetted, 20 µL of 5 µg/mL internal standard solution was spiked, and diluted with methanol to the volume of 0.4 mL. After that the solution was subjected to vortex mixer for 30 s and centrifugation at 13000 rpm for 10 min. The supernatant was transferred to a sample vial.

Method for preparation of solutions for calibration curves:

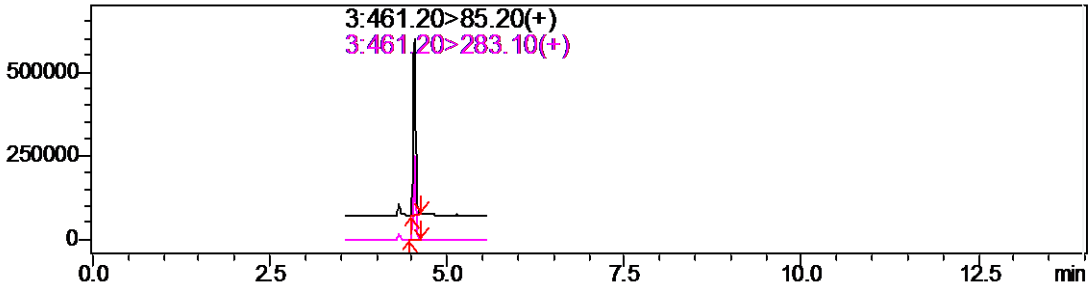
Each of the above-mentioned standard solutions was pipetted 0.1 mL. 0.1 mL of 100 ng/mL internal standard solution was added, subjected to vortex mixer for 30 s and centrifugation at 13000 rpm for 10 min. The resulted supernatant was transferred to a sample vial.

RESULTS AND DISCUSSION

MRM Chromatogram of Standard Samples

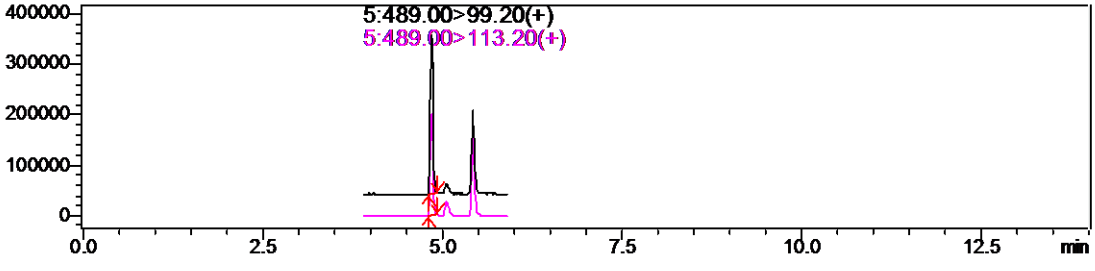
MRM chromatograms of sildenafil standards are shown in Fig. 1.

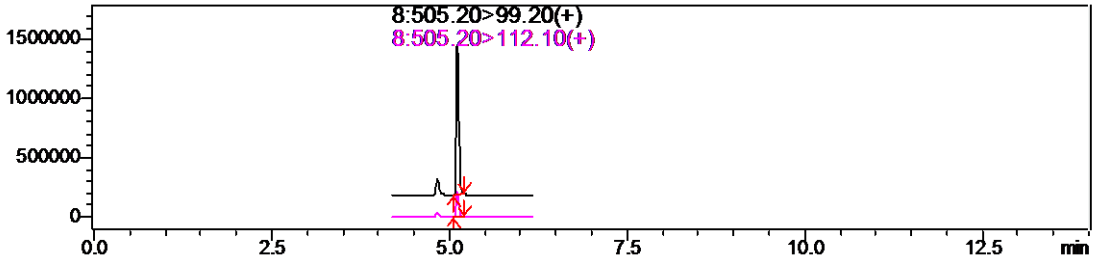
1 µg/mL *N*-desmethylsildenafil



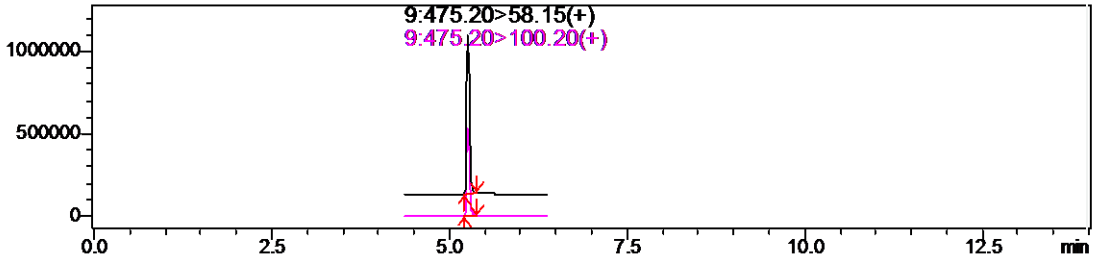
0.5 µg/mL dimethylsildenafil

0.5 µg/mL hydroxyhomosildenafil

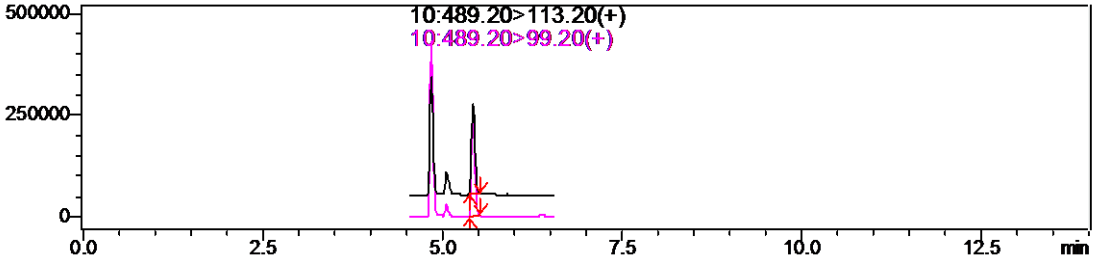




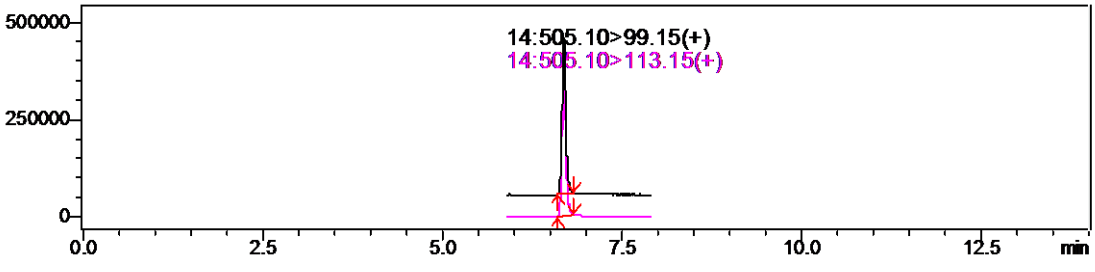
0.5 µg/mL sildenafil



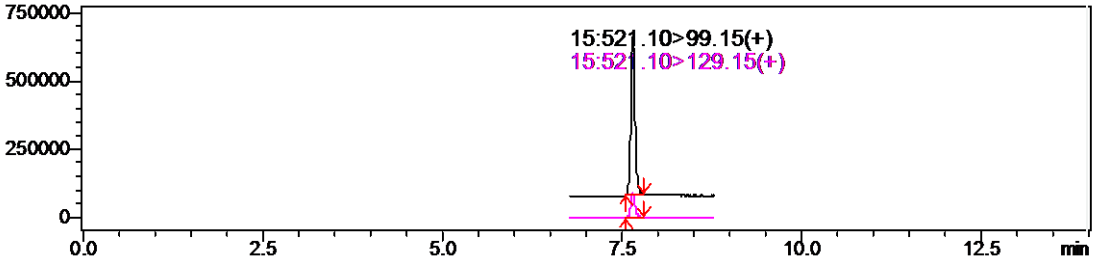
0.5 µg/mL homosildenafil



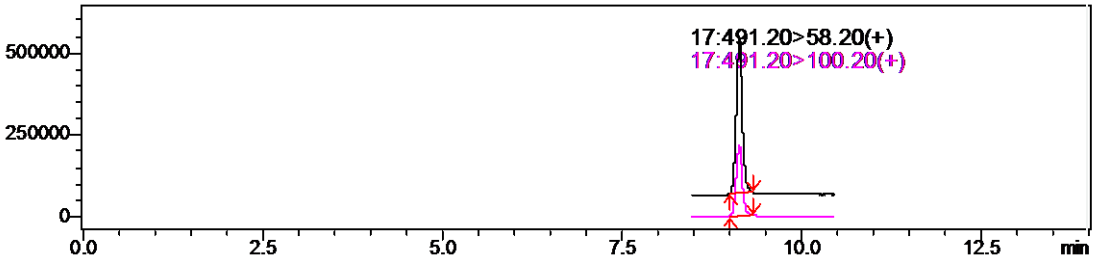
0.5 µg/mL thiodimethylsildenafil



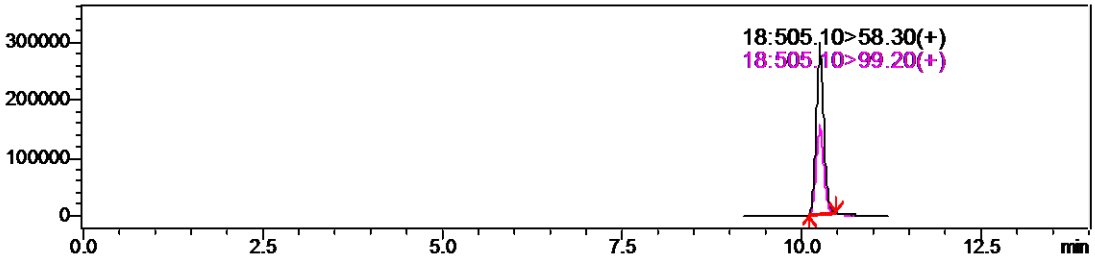
1 µg/mL hydroxythiohomosildenafil



1 µg/mL thiosildenafil



1 µg/mL thiohomosildenafil



1 µg/mL norneosildenafil

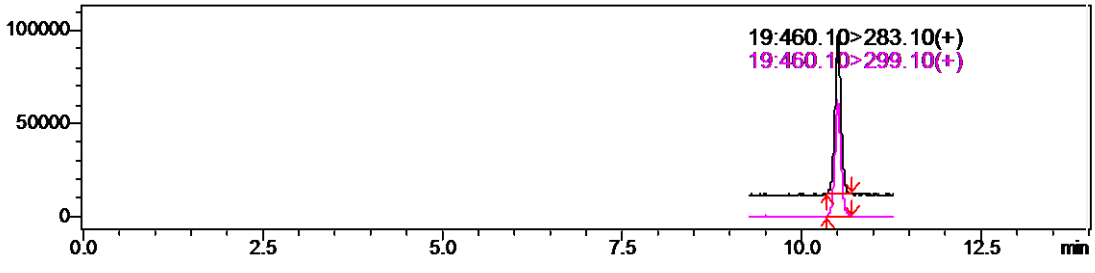
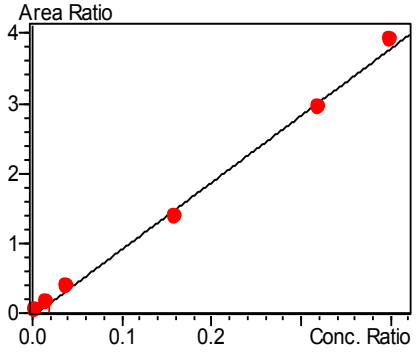


Fig. 1 MRM chromatograms of sildenafils

Linearity

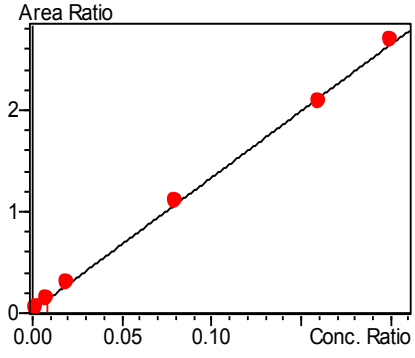
The standard working solutions prepared per section 1.3 were subject to determination according to the conditions of analysis specified above, with gliclazide as internal standard; calibration curves were plotted with concentration ratio as abscissa and peak area ratio as ordinate. The resulted calibration curves were of good linearity: 8 out of the 10 sildenafils has a linear correlation coefficient greater than 0.999.



N-desmethylsildenafil,

Y = (9.56234)X + (-0.0443543)

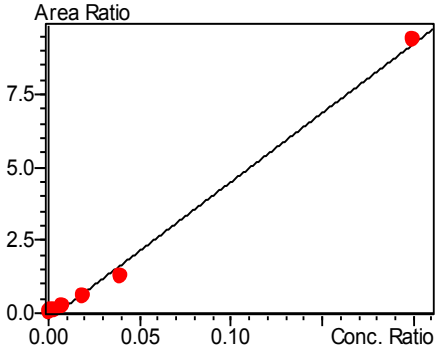
R=0.9986



Dimethylsildenafil,

Y = (13.12)X + (0.0235184)

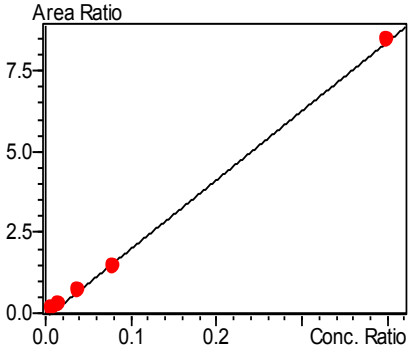
R=0.9996



Hydroxyhomosildenafil

Y = (47.319)X + (-0.223636)

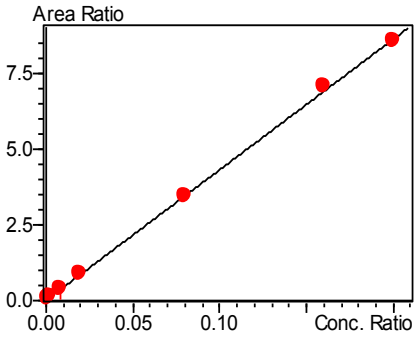
R=0.9978



Thiosildenafil,

Y = (21.3593)X + (-0.147992)

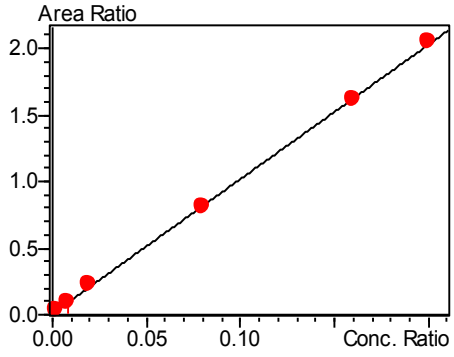
R=0.9997



Sildenafil

Y = (43.0576)X + (0.011666)

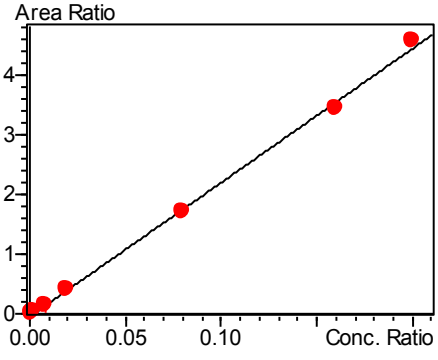
R=0.9998



Homosildenafil,

Y = (10.0914)X + (0.01023)

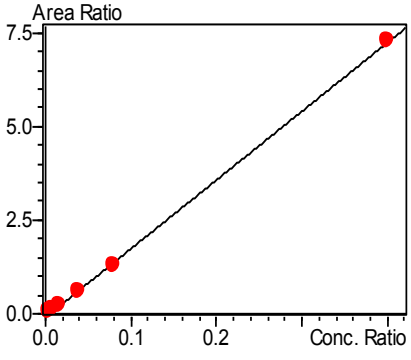
R=0.9999



Thiodimethylsildenafil

Y = (22.5015)X + (-0.0461142)

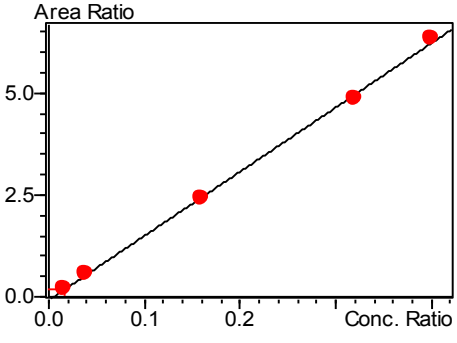
R=0.9992



Hydroxythiohomosildenafil

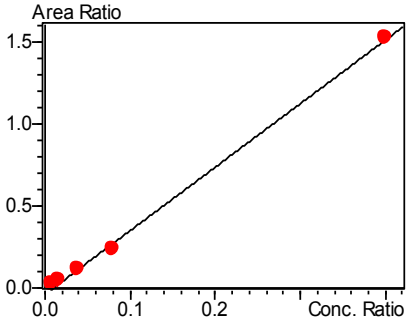
Y = (18.3356)X + (-0.0953574)

R=0.9997



Thiohomosildenafil R=0.9996

Y = (15.7576)X + (-0.0794382)



Norneosildenafil, R=0.9991

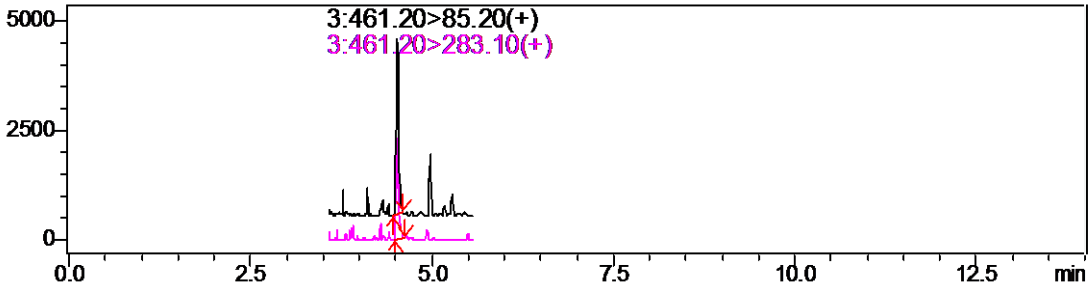
Y = (3.88236)X + (-0.0378209)

Fig. 2 Calibration curves of sildenafils

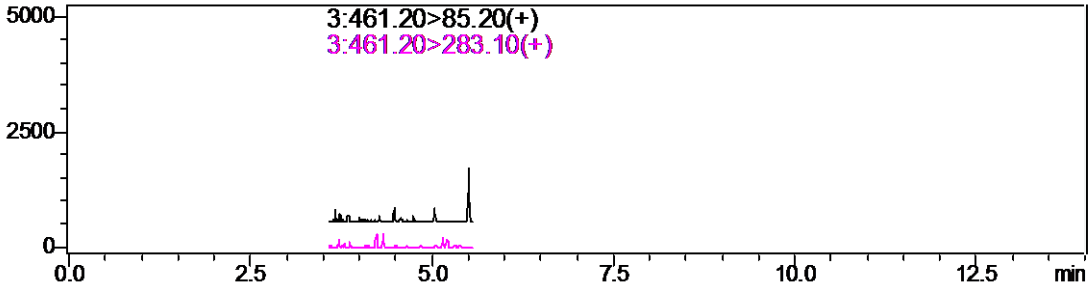
Sensitivity test

MRM chromatograms of every matrix spiked with standard and MRM chromatograms of corresponding blank matrix are shown in Fig. 3.

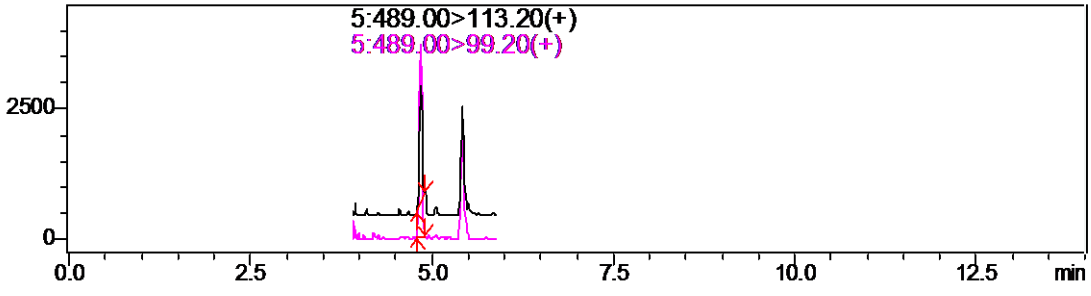
N-desmethylsildenafil spiked in matrix (2.0 µg/g)



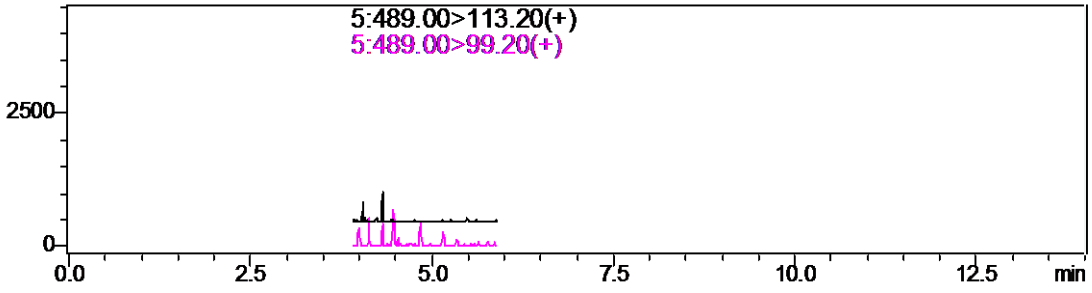
N-desmethylsildenafil: blank matrix



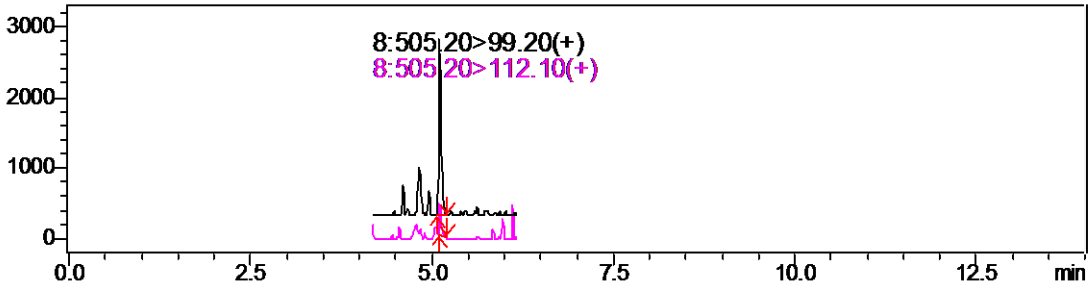
Dimethylsildenafil spiked in matrix (1.0 µg/g)



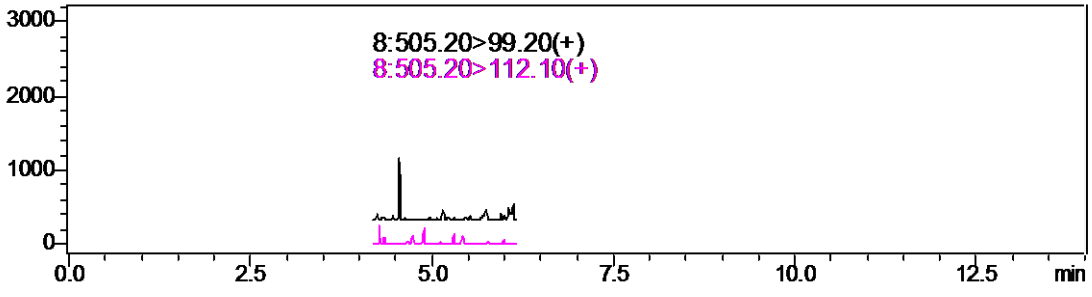
Dimethylsildenafil: blank matrix



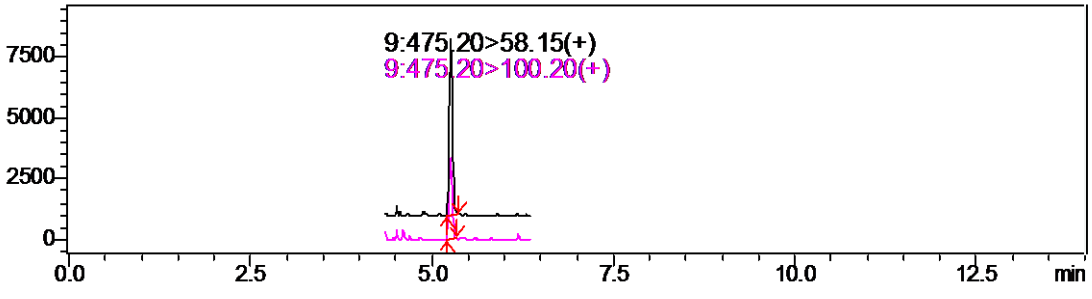
Hydroxyhomosildenafil spiked in matrix (0.4 µg/g)



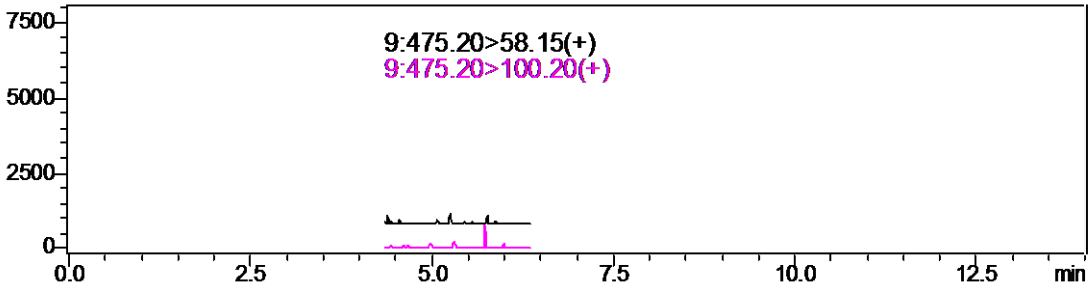
Hydroxyhomosildenafil: blank matrix



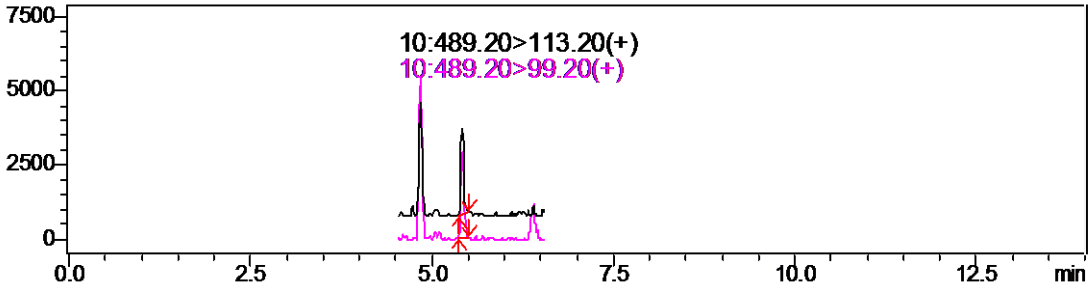
Sildenafil spiked in matrix (0.4 µg/g)



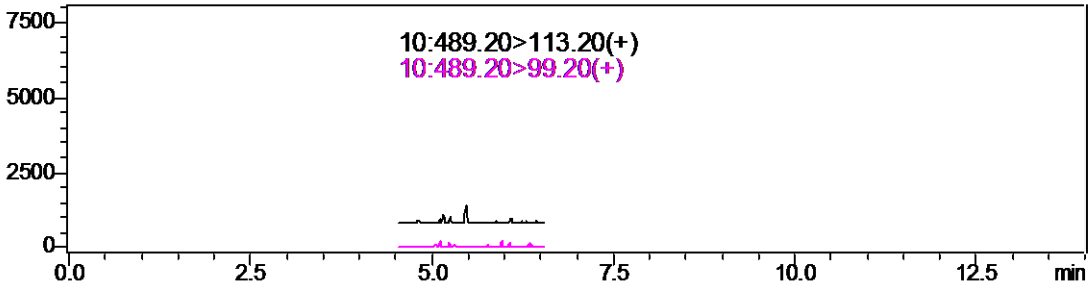
Sildenafil :blankmatrix



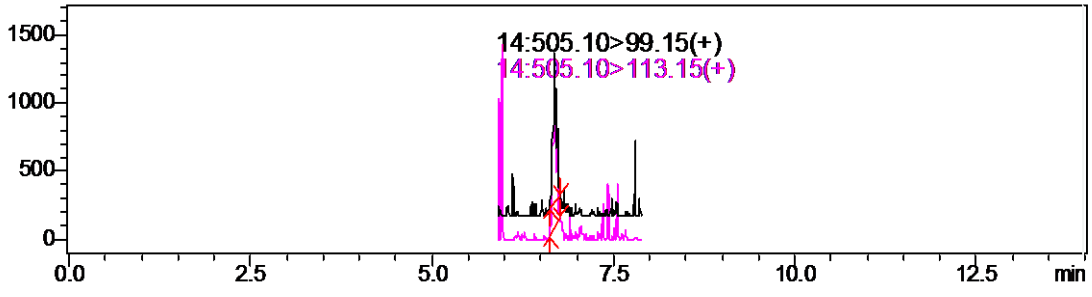
Homosildenafil spiked in matrix (0.9 µg/g)



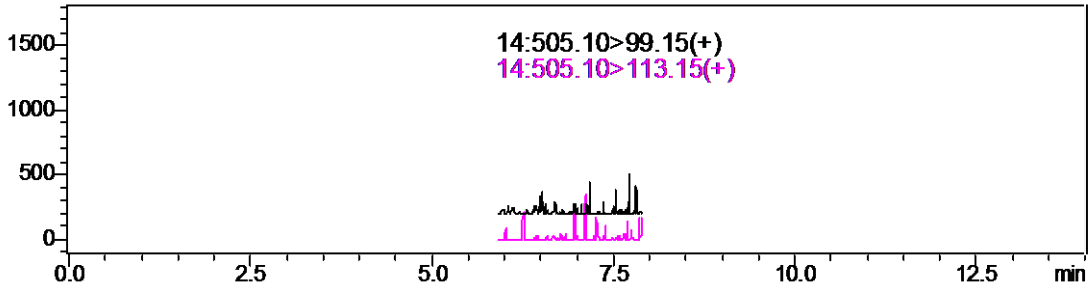
Homosildenafil: blank matrix



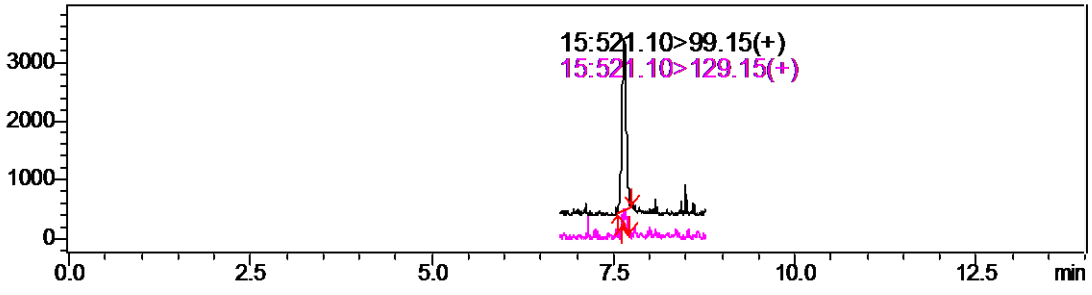
Thiodimethylsildenafil spiked in matrix (0.4 µg/g)



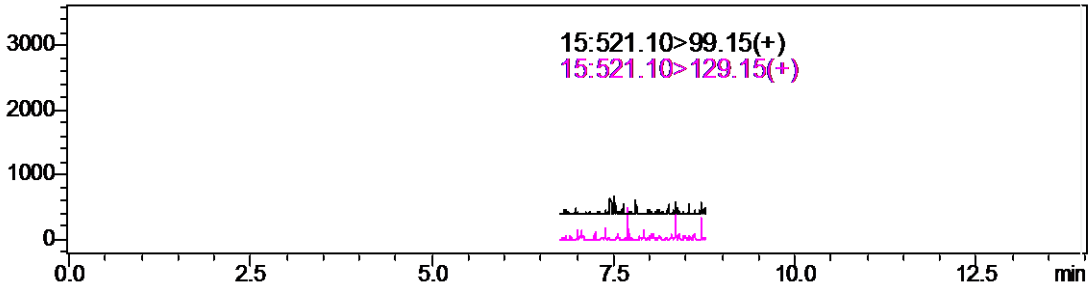
Thiodimethylsildenafil: blank matrix



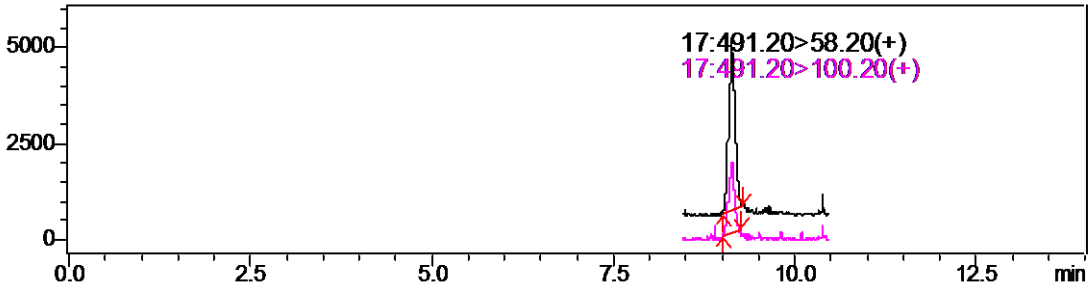
Hydroxythiohomosildenafil spiked in matrix (1.9 µg/g)



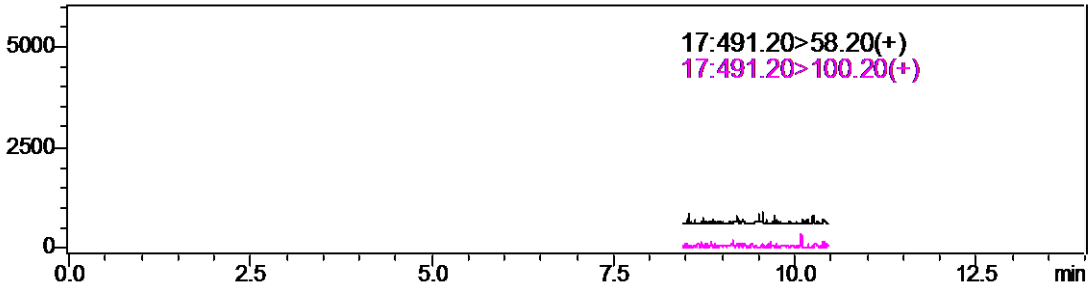
Hydroxythiohomosildenafil: blank matrix



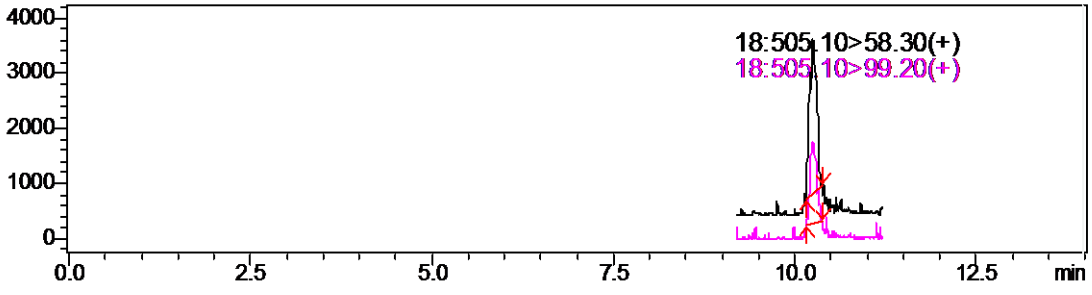
Thiosildenafil spiked in matrix (4.0 µg/g)



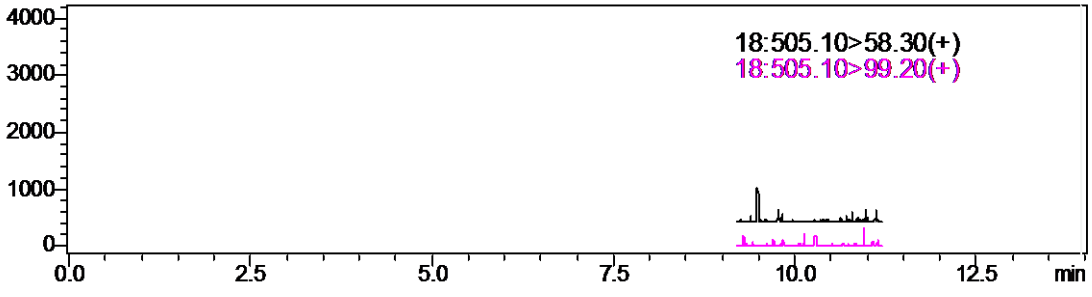
Thiosildenafil: blank matrix



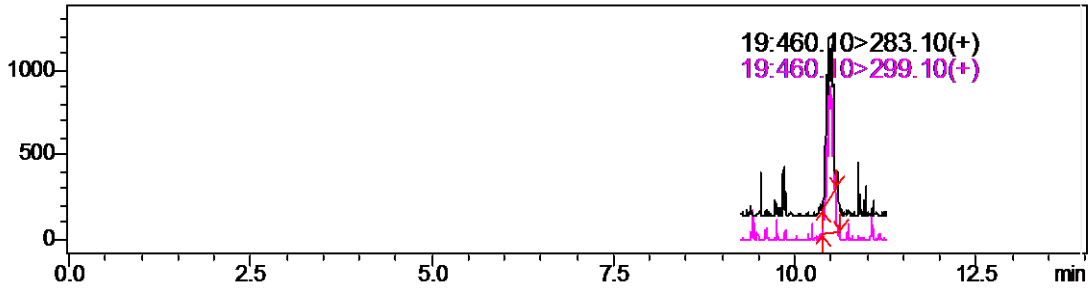
Thiohomosildenafil spiked in matrix (4.0 µg/g)



Thiohomosildenafil: blank matrix



Norneosildenafil spiked in matrix (3.9 µg/g)



Norneosildenafil: blank matrix

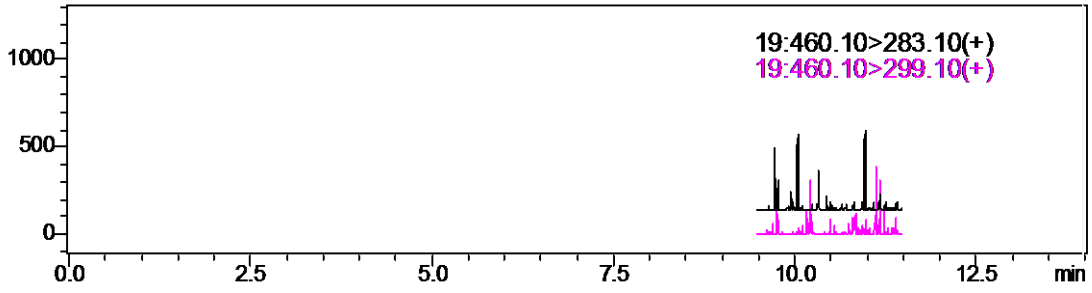


Fig. 3 MRM chromatograms of sildenafils spiked in matrices

Precision test

The method's precision was evaluated by analysis of 12 solutions of matrix spiked with standards. The results of repeatability test on peak area and retention time is listed in Table 3. The results showed that the %RSDs of peak area and retention time of the 12 solutions of matrix spiked with standards were better than 12.7% and 0.22% respectively, indicating that the method's precision was satisfactory.

Table 3 Repeatability – peak area and retention time (n=12)

No.	*N*-desmethylsildenafil		Dimethyl sildenafil		Hydroxy homosildenafil	
	Area	R.T.	Area	R.T.	Area	R.T.
1	11,506	4.531	10,327	4.844	22,237	10.261
2	12,495	4.529	10,990	4.842	22,267	10.263
3	11,164	4.528	11,371	4.841	23,073	10.244
4	11,381	4.532	10,767	4.846	26,156	10.234
5	12,420	4.532	10,483	4.846	22,593	10.242
6	11,740	4.531	10,583	4.844	21,640	10.254
7	11,901	4.532	10,129	4.845	24,045	10.280
8	12,392	4.530	10,173	4.843	22,494	10.254
9	12,553	4.529	11,189	4.842	22,197	10.231
10	12,445	4.529	12,028	4.843	19,678	10.253

11	12,291	4.532	9,307	4.846	21,722	10.267
12	12,674	4.529	9,723	4.845	17,283	10.259
Average	12,080	4.530	10,589	4.844	22,115	10.254
%RSD	4.000	0.036	7.027	0.036	9.741	0.135

No.	Sildenafil		Homosildenafil		Norneosildenafil	
	Area	R.T.	Area	R.T.	Area	R.T.
1	18,753	5.262	8,428	5.429	7,801	10.482
2	17,356	5.258	10,246	5.426	8,204	10.471
3	17,637	5.263	8,509	5.428	6,756	10.489
4	16,387	5.264	9,636	5.427	7,026	10.483
5	16,335	5.261	9,577	5.425	6,703	10.486
6	17,661	5.261	8,902	5.424	8,122	10.493
7	18,357	5.264	9,628	5.425	5,180	10.465
8	18,246	5.260	8,826	5.427	6,977	10.457
9	18,414	5.259	11,409	5.423	7,077	10.492
10	17,118	5.261	9,401	5.425	6,625	10.485
11	18,552	5.263	10,161	5.43	6,435	10.486
12	16,475	5.262	9,408	5.427	8,282	10.486
Average	17,608	5.261	9,511	5.426	7,099	10.481
%RSD	4.999	0.034	8.761	0.037	12.603	0.105

No.	Thiosildenafil		Thio homosildenafil		Thio dimethylsildenafil	
	Area	R.T.	Area	R.T.	Area	R.T.
1	29,033	9.125	22,237	10.261	3,814	6.701
2	28,197	9.137	22,267	10.263	4,454	6.682
3	28,343	9.127	23,073	10.244	3,908	6.698
4	26,897	9.134	26,156	10.234	4,345	6.693

5	27,739	9.122	22,593	10.242	4,038	6.693
6	27,463	9.118	21,640	10.254	3,911	6.700
7	25,130	9.132	24,045	10.280	2,969	6.700
8	26,726	9.139	22,494	10.254	3,613	6.696
9	26,361	9.125	22,197	10.231	3,484	6.698
10	28,261	9.122	19,678	10.253	3,407	6.650
11	26,901	9.126	21,722	10.267	4,013	6.702
12	26,170	9.128	17,283	10.259	3,805	6.684
Average	27,269	9.128	22,115	10.254	3,813	6.691
%RSD	4.069	0.069	9.741	0.135	10.691	0.218

No.	Hydroxy thiohomosildenafil	
	Area	R.T.
1	13,756	7.653
2	13,981	7.640
3	13,655	7.647
4	14,040	7.643
5	13,271	7.640
6	11,947	7.649
7	12,312	7.642
8	13,517	7.643
9	13,428	7.650
10	11,885	7.645
11	12,618	7.648
12	12,374	7.642
Average	13,065	7.645
%RSD	6.053	0.056

CONCLUSION

A method was established for the determination of 10 sildenafils illegally-added into anti-fatigue health food using Shimadzu LC-30A ultra fast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. The method had wide linear range and yielded calibration curves with correlation coefficient greater than 0.999 for 8 sildenafils. Shimadzu UFLC-triple quadrupole mass spectrometer can meet the requirements for detection of sildenafils illegally added into anti-fatigue health products.

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V-18 Determination of Illegally added 4 Vardenafils in Anti-fatigue Health Food by UFLC/Triple Quadrupole Mass Spectrometry

INTRODUCTION

A method was proposed for detection of illegally-added drugs in anti-fatigue health products with Shimadzu LC-30A liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. Extracted samples were quickly separated by LC-30A ultra fast liquid chromatograph (UFLC), and then quantitatively determined with LCMS-8030 triple quadrupole mass spectrometer. The correlation coefficients of the 4 vardenafils' calibration curves were greater than 0.999. Precision test was performed on matrix solutions spiked with standards at LOQ concentration, the RSDs of peak area and retention time were better than 12.2% and 0.08%, respectively, suggesting that the method was of good precision. The method's LOQ was 0.4 μg/g for all of the 4 vardenafils.

The addition of drugs into health products, especially health foods, is strictly prohibited. Some enterprises, however, illegally add drugs into health foods without authorization to "boost" the functionality of their products. The consumption of such health products by consumers who know nothing about the illegally adulteration of drugs may lead to drug abuse or even drug dependence. In consideration of the particularity of health foods and traditional Chinese medicine (TCM) health products, China Food and Drug Administration (CFDA) promulgated an announcement on May 25, 2011 regarding the crackdown of illegally adulteration of drugs into health foods and cosmetics. The announcement emphasized the sampling inspection of 1) health foods with alleged sleep improving, blood glucose reducing, fatigue relieving, and slimming actions; 2) skincare products with alleged whitening, acne-removing, wrinkle-reducing, and anti-aging actions; 3) special cosmetics for speckle-elimination, hair growth, and hair dyeing; and 4) bathing products. With regard to substances and compositions apt to be added into anti-fatigue health products and the inspection bases for these substances and compositions, the (first batch) list included vardenafils, sildenafils and tadalafils drugs. In this paper, a method for fast and accurate determination of vardenafils with Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer was proposed for the reference of relevant laboratorians.

EXPERIMENTAL

A combined system of Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer was used in the experiment. The configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A system controller, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.41 chromatography workstation.

Conditions of Analysis
LC Conditions

Column :Shim-pack XR-ODS II (2.0 mm × 100 mm, 2.2 µm)

Mobile phase :A:0.01 % acetic acid, 12 mM ammonium formate aqueous solution

Mobile phase :B:Acetonitrile

Flow rate : 0.4 mL/min

Injection volume : 5 µL

Column temperature: 40 °C

Elution mode :Binary gradient with an initial concentration of 15%B, See Table 1 for time program.

Table 1 Time program

Time(min)	Module	Command	Value
1.00	Pumps	Pump B Conc.	15
3.00	Pumps	Pump B Conc.	30
5.00	Pumps	Pump B Conc.	50
10.00	Pumps	Pump B Conc.	50
12.00	Pumps	Pump B Conc.	60
12.10	Pumps	Pump B Conc.	100
14.00	Pumps	Pump B Conc.	100
14.10	Pumps	Pump B Conc.	15
18.00	Controller	Stop	

MS conditions

Ionization :ESI(+)
Ionization voltage :+4.5 kV
Nebulizing gas :Nitrogen 3.0 L/min
Drying gas :Nitrogen 15 L/min
Collision gas :Argon
DL temperature :50 °C
Block heater temperature :400 °C
Mode :MRM
Pause time :30 ms
Dwell time :3 ms
MRM parameters :Listed in Table 2

Table 2. MRM parameters

No.	Name	Precursor ion (*m/z*)	Product ion (*m/z*)	Q1 Pre Bias (V)	CE (V)	Q1 Pre Bias (V)
1	Desethylvardenafil	461.00	151.05	-23.0	-40.0	-15.0
			312.05*	-23.0	-40.0	-22.0
2	Hydroxyvardenafil	505.20	151.05	-26.0	-50.0	-16.0
			99.20*	-26.0	-40.0	-21.0
3	Vardenafil	489.20	151.15	-25.0	-45.0	-16.0
			312.20*	-25.0	-40.0	-22.0
4	Gliclazide	324.10	127.15	-16.0	-20.0	-13.0
			110.15*	-16.0	-20.0	-11.0
5	Pseudo vardenafil	460.20	151.10	-17.0	-45.0	-30.0
			312.15*	-23.0	-40.0	-22.0

Note: * refers to qualitative ion, gliclazide is internal standard

Sample Preparation

Preparation of standard solution:

2mg of desethylvardenafil, hydroxyvardenafil, vardenafil, and pseudovardenafil were accurately weighed [with a precision of 0.01 mg; for vardenafil, 2.15 mg vardenafil hydrochloride (equivalent to 2 mg vardenafil)

was accurately weighed], and transferred to a 10 mL volumetric flask, respectively. Those were dissolved and diluted up to the mark with methanol. The solution was shaken evenly to get 200 μg/mL concentrated standard stock solutions.

0.5 mL of concentrated standard stock solutions of the above-mentioned vardenafils were precisely pipetted and transferred to a 10 mL volumetric flask. The solution was diluted and brought to volume with 80% methanol solution, shaken evenly to get 10 μg/mL multi-standard stock solution. The solution was diluted with 80% methanol solution in specified proportions to get standard working solutions of a series of concentrations. A series of standard solutions at concentrations of 4, 10, 20, 40, 100, 200, 400, 800 and 1000 ng/mL were prepared.

Sample pretreatment method

For each of the 6 products, about 0.2 g blank sample was taken, accurately weighed (with a precision of 1 mg), and transferred to a 10 mL plastic centrifuge tube, respectively. 5 mL of methanol was added, and weighed. That was subjected to vortex mixer for 60s and ultrasonic treatment for 15 min, allowed to cool down, weighed again, and replenished with methanol to make up for the lost weight. 50 μL of sample solution was pipetted, spiked with 20 μL of 5 μg/mL internal standard solution, diluted with methanol to the volume of 0.4 mL. The solution was subjected to vortex mixer for 30 s and centrifugalization at 13000 rpm for 10 min. The resulted supernatant was transferred to a sample vial.

Method for preparation of solutions for calibration curves:

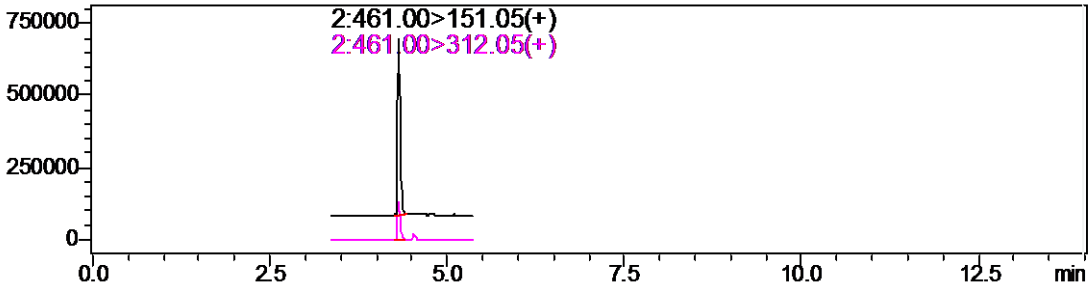
Each of the above-mentioned standard solutions was pipetted 0.1 mL. 0.1 mL of 100 ng/mL internal standard solution was added, and subjected to vortex mixer for 30 s and centrifugation at 13000 rpm for 10 min. The supernatant was transferred to a sample vial.

RESULTS AND DISCUSSION

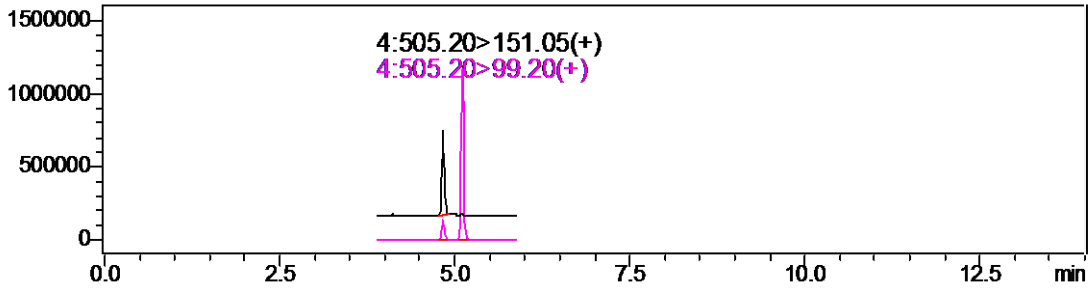
MRM Chromatogram of Standard Samples

MRM chromatograms of vardenafils are shown in Fig. 1.

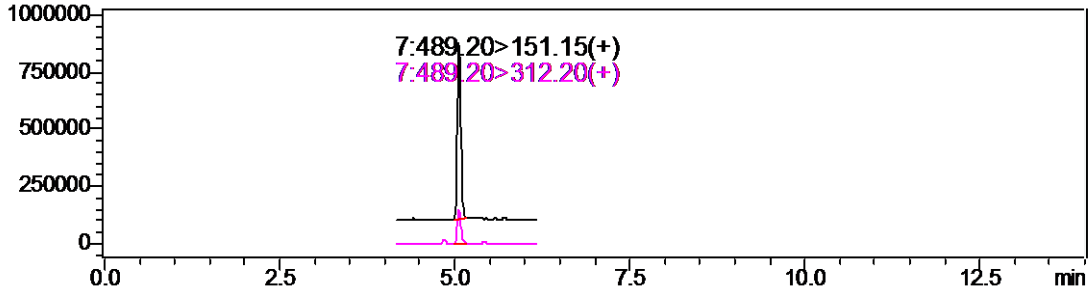
0.5 µg/mL desethylvardenafil



0.5 µg/mL hydroxyvardenafil



0.5 µg/mL vardenafil



0.5 µg/mL pseudovardenafil

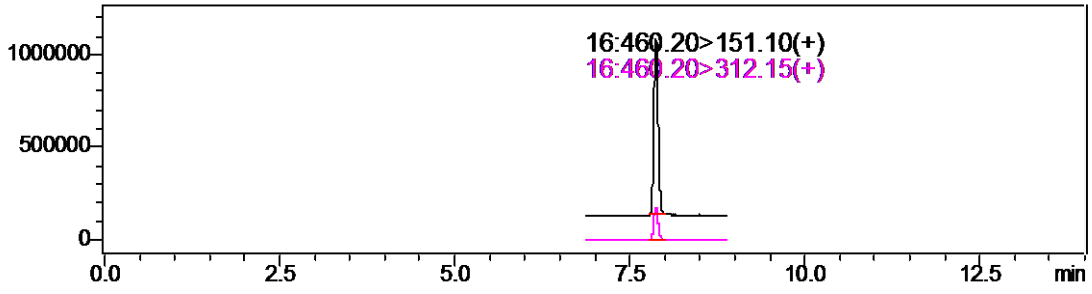
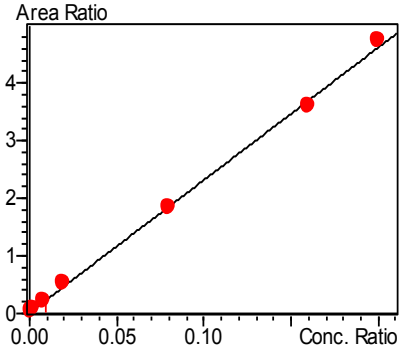


Fig. 1 MRM chromatograms of vardenafils

Linearity

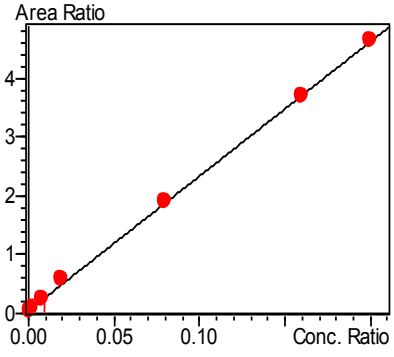
The standard working solutions prepared as mentioned above at concentrations of 2, 5, 10, 50, 100 and 200 ng/mL were subjected to determination under analytical conditions specified above, with gliclazide as internal standard. Calibration curves were plotted with concentration ratio as abscissa and peak area ratio as ordinate. The resulted calibration curves were of good linearity. All 4 vardenafils have correlation coefficients greater than 0.999.



Desethylvardenafil

Y = (23.0089)X + (0.0171359)

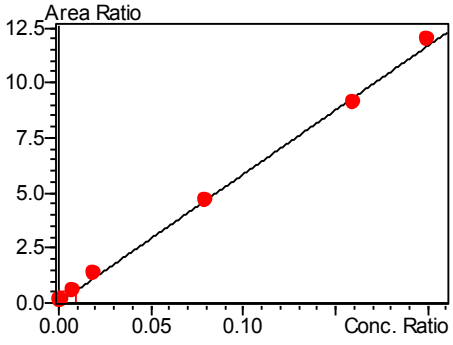
R=0.9993



Hydroxyvardenafil,

Y = (22.8477)X + (0.049665)

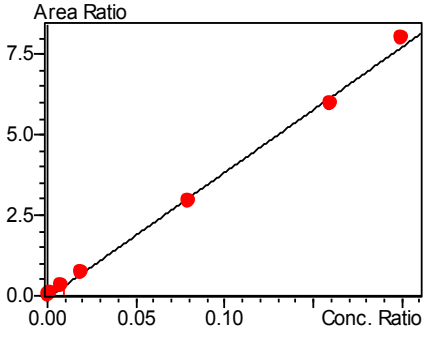
R=0.9998



Pseudo vardenafil

Y = (58.3348)X + (0.0160524)

R=0.9995



Vardenafil,

Y = (38.9278)X + (-0.0648331)

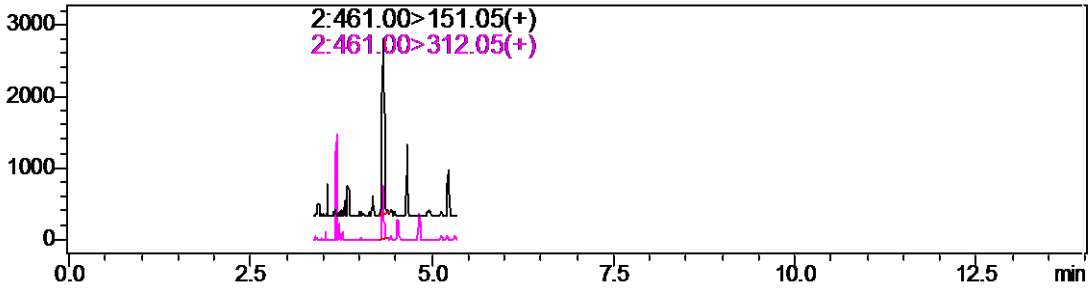
R=0.9991

Fig. 2 Calibration curves of vardenafils

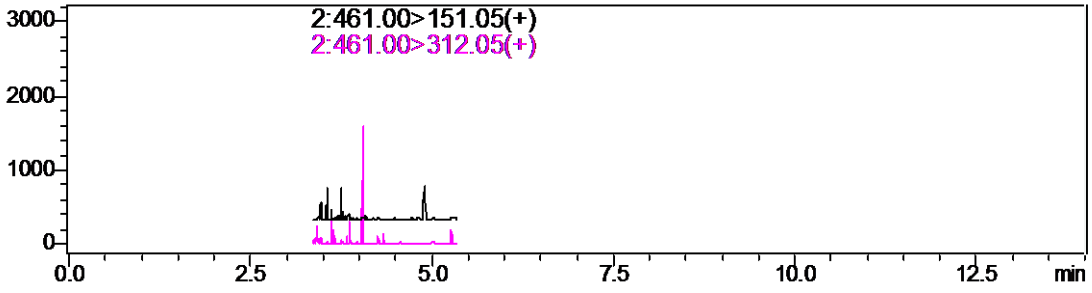
Sensitivity test

The MRM chromatograms of every composition's matrix spiked with standard and the MRM chromatograms of corresponding blank matrix were shown in Fig. 3.

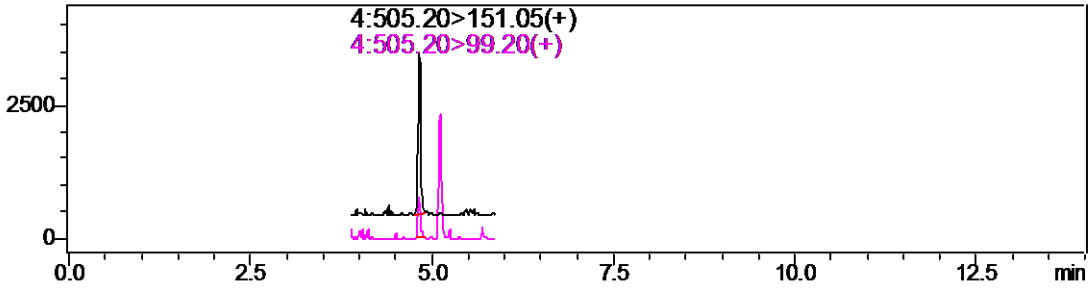
Desethylvardenafil spiked in matrix (0.4 µg/g)



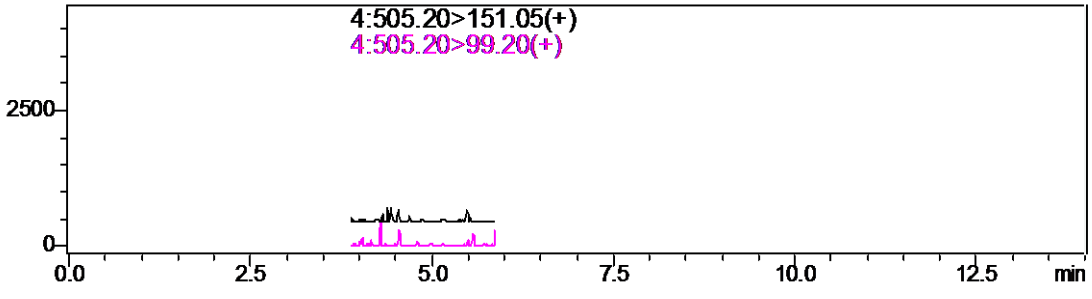
Desethylvardenafil: blank matrix



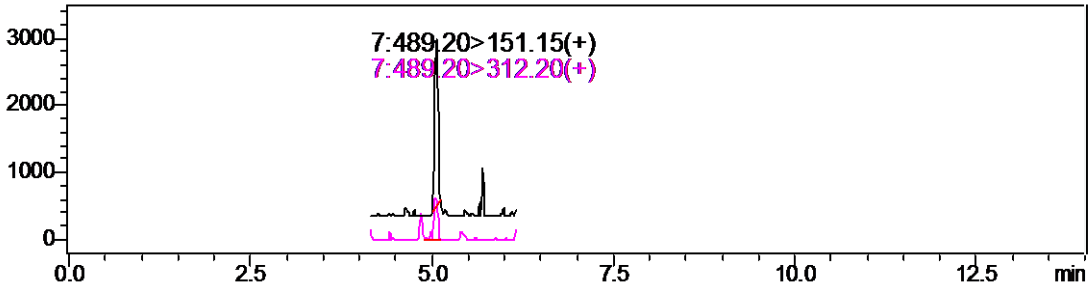
Hydroxyvardenafil spiked in matrix (0.4 µg/g)



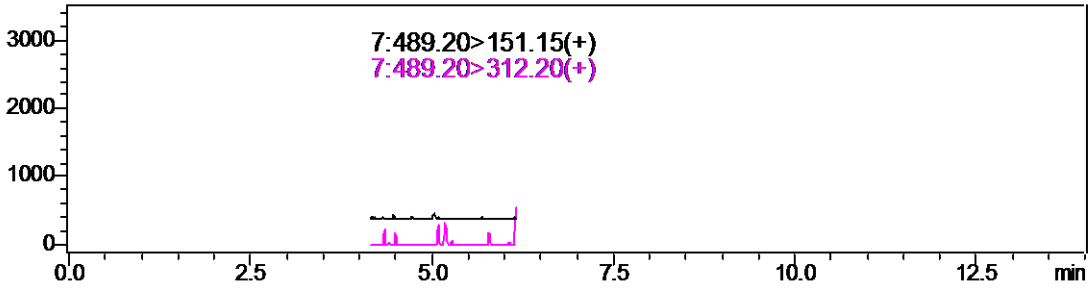
Hydroxyvardenafil: blank matrix



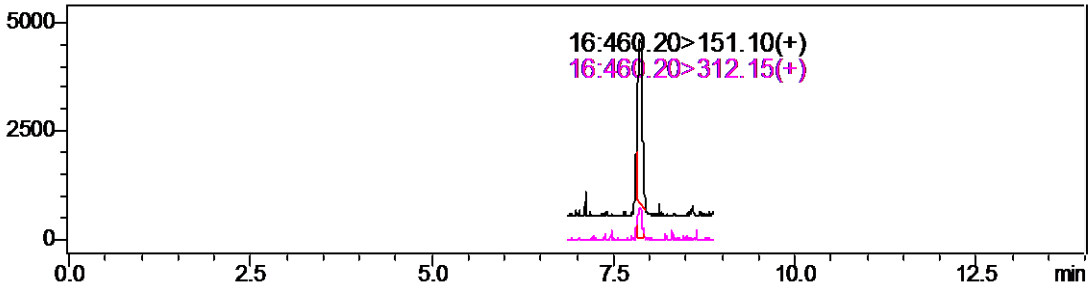
Vardenafil spiked in matrix (0.4 µg/g)



Vardenafil: blank matrix



Pseudovardenafil spiked in matrix (0.4 µg/g)



Pseudo vardenafil: blank matrix

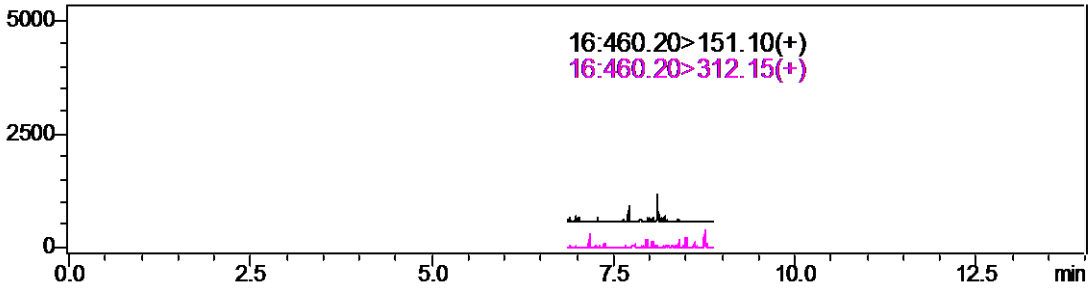


Fig. 3 MRM chromatograms of vardenafils spiked in matrices

Precision test

The method's precision was evaluated with the help of 12 multi-standard solutions, which were prepared as mentioned above for determination of LOQs. The resulted repeatability of peak area and retention time is listed in Table 3. The results showed that the RSDs of peak area and retention time of the 12 solutions of matrix spiked with standards were better than 12.2% and 0.08% respectively, indicating that the method's precision was satisfactory.

Table 3 Repeatability - retention time and peak area (n=12)

No.	Desethylvardenafil		Hydroxyvardenafil		Vardenafil	
	Area	R.T. (min)	Area	R.T. (min)	Area	R.T. (min)
1	6,728	4.326	6,052	4.828	8,883	5.060
2	7,444	4.317	7,447	4.823	9,176	5.056
3	6,852	4.319	5,708	4.824	8,793	5.055
4	6,771	4.324	6,628	4.826	9,767	5.060
5	7,377	4.318	7,045	4.822	7,865	5.058
6	6,435	4.318	6,772	4.826	9,575	5.054
7	7,573	4.320	6,988	4.826	8,888	5.058
8	6,550	4.320	6,577	4.825	7,118	5.057
9	5,613	4.319	7,247	4.824	8,462	5.058

10	7,560	4.326	7,140	4.824	8,817	5.058
11	7,293	4.319	6,977	4.825	7,386	5.062
12	6,803	4.318	6,426	4.825	6,416	5.057
Average	6,917	4.320	6,751	4.825	8,429	5.058
%RSD	8.29	0.073	7.48	0.033	12.15	0.044

No.	Pseudovardenafil	
	Area	R.T (min)
1	16,423	7.868
2	14,857	7.860
3	14,536	7.866
4	15,910	7.858
5	16,404	7.868
6	16,427	7.861
7	15,781	7.872
8	16,621	7.867
9	16,335	7.853
10	15,783	7.860
11	15,957	7.858
12	15,783	7.864
Average	15,901	7.863
%RSD	4.026	0.070

CONCLUSION

A method was established for the determination of 4 vardenafils illegally-added into anti-fatigue health food using Shimadzu LC-30A ultra fast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. The method had wide linear range and yielded calibration curves with correlation coefficient greater than 0.999 for the 4 vardenafils. Shimadzu UFLC-triple quadrupole mass spectrometer can meet the requirements for detection of illegally-added vardenafils in anti-fatigue health products.

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V-19 Determination of 11 illegally added drugs in hypoglycemic health food by UFLC-MS/MS

INTRODUCTION

A method was proposed for determination of 11 illegally-added drugs in hypoglycemic health food with a Shimadzu LC-30A ultra fast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer system. Extracted samples were separated by LC-30A ultra fast liquid chromatograph, and then quantitatively assayed with LCMS-8030 triple quadrupole mass spectrometer. The correlation coefficients of calibration curves of the 11 analytes were all greater than 0.994. Recovery and precision tests were performed on matrix spiked with standard solutions at the four levels of LOQ, LQC, MQC, HQC by 5 successive injections, and the precision and recovery data fell in the ranges of 0.25%-7.31% and 78%-110.7%, respectively, suggesting that the method was of good precision. The method's LODs of the analytes fell in the range of 0.963~26.2 μg/g and LODs fell in the range of 0.0400 μg/g~9.89 μg/g. 3 off-the-shelf hypoglycemic health products were randomly sampled and subjected to analysis with this method, and the results showed that the proposed method can be used for fast screening of illegally-added drugs.

The addition of drugs into health products, especially health foods, is strictly prohibited. Some enterprises, however, illegally add drugs into health foods without authorization to "boost" the functionality of their products. The consumption of such health foods by consumers who know nothing about the illegal adulteration of drugs may lead to drug abuse or even drug dependence. In consideration of the particularity of health foods and TCM health products, CFDA promulgated an announcement on May 25, 2011 regarding the crackdown of illegal adulteration of drugs into health foods and cosmetics. The announcement emphasized the sampling inspection of 1) health foods with alleged sleep improving, blood glucose reducing, fatigue relieving, and weight losing actions; 2) skincare products with alleged whitening, acne-removing, wrinkle-reducing, and anti-aging actions; 3) special cosmetics for speckle-elimination, hair growth, and hair dyeing; and 4) bathing products. With regard to substances and compositions apt to be added into anti-fatigue health products and the inspection bases for these substances and compositions, the (first batch) list included vardenafils, sildenafils and tadalafils drugs. A method was proposed in this paper for fast and accurate determination of 11 illegally-added

hypoglycemic drugs with a Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer system for reference of relevant laboratorians.

EXPERIMENTAL

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The specific configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.41 chromatography workstation.

Analytical Conditions
LC Conditions

Apparatus	: LC-30A
Column	:Shim-pack XR-ODS II 2.0mm×100 mm,2.2 μm
Mobile phase	:A-0.1 % acetic acid, 10 mM ammonium formate aqueous solution
Mobile phase	:B-Acetonitrile
Flow rate	: 0.4 mL/min
Injection volume	:5 μL
Column temperature	:40 °C
Elution mode	:Gradient elution with an initial concentration of 5% Mobile phase B

Table 1 for time program

Time(min)	Module	Command	Value
2.00	Pump	B Conc.	45
6.00	Pump	B Conc.	45
7.00	Pump	B Conc.	55
8.00	Pump	B Conc.	90
10.00	Pump	B Conc.	90
10.10	Pump	B Conc.	5
14.00	Controller	Stop	

MS conditions

Apparatus :LCMS-8030
Ion source :ESI-positive, negative
Ionization voltage :4.5 kV
Nebulizing gas :Nitrogen 3.0 L/min
Drying gas :Nitrogen 15 L/min
Collision gas :Argon
DL temperature :250 °C
Heater block temperature :400 °C
Mode: Multiple reaction monitoring (MRM)
Dwell time: 20 ms
Pause time: 2 ms
MRM parameters :Listed in Table2

Ion pairs and relevant voltage parameter settings were as listed in Table 2

Table 2 MRM transitions

No.	Name	Precursor ion (*m/z*)	Product ion (*m/z*)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Dimethyl biguanide	130.10	60.20*	-14.0	-15.0	-22.0
			71.15	-14.0	-20.0	-14.0
2	Phenethyl biguanide	206.10	60.20*	-23.0	-20.0	-23.0
			105.20	-23.0	-25.0	-20.0
3	Chlorpropamide	274.80	190.00*	29.0	15.0	13.0
			126.20	29.0	30.0	24.0
4	Glipizide	446.00	103.15*	-22.0	-45.0	-20.0
			167.00	-22.0	-30.0	-17.0

No.	Name	Precursor ion (*m/z*)	Product ion (*m/z*)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
5	Tolbutamide	271.10	74.20*	-19.0	-15.0	-14.0
			91.15	-19.0	-30.0	-17.0
6	Tolazamide	312.00	115.15*	-16.0	-20.0	-24.0
			91.10	-16.0	-35.0	-17.0
7	Gliclazide	324.10	127.15*	-16.0	-20.0	-13.0
			110.15	-16.0	-20.0	-23.0
8	Nateglinide	318.20	69.15*	-16.0	-30.0	-13.0
			120.15	-16.0	-20.0	-12.0
9	Glibenclamide	494.00	169.00*	-25.0	-35.0	-18.0
			304.05	-25.0	-25.0	-22.0
10	Glimepirid	491.10	126.10*	-25.0	-30.0	-13.0
			181.10	-25.0	-30.0	-12.0
11	Repaglinide	453.10	230.20*	-22.0	-30.0	-16.0
			86.20	-23.0	-30.0	-17.0

*: quantitative ion

Sample Preparation

Preparation of standard solution:

Dimethyl biguanide, phenethyl biguanide, glimepirid, and repaglinide were accurately weighed at 2 mg (with a precision of 0.01 mg; in the experiment, 2.56 mg of biguanide hydrochloride (equivalent to 2 mg of dimethyl biguanide) and 2.36 mg of phenethyl biguanide hydrocholoride (equivalent to 2 mg of phenethyl biguanide) were weighed) and transferred to 50 mL volumetric flasks, respectively. The drugs were then dissolved, brought to metered volume with methanol and shaken evenly to get 40 μg/mL standard concentrated stock solutions of dimethyl biguanide, phenethyl biguanide, glimepirid, and repaglinide.

Glipizide, tolazamide, gliclazide, and glibenclamide were accurately weighed at 2 mg and transferred to 10 mL volumetric flasks, respectively. The drugs were then dissolved, brought to metered volume with methanol, and shaken evenly to get 200 μg/mL standard concentrated stock solutions of glipizide, tolazamide, gliclazide, and glibenclamide.

Chlorpropamide, tolbutamide, and nateglinide were accurately weighed at 2 mg (with a precision of 0.01 mg) and transferred to 2 mL volumetric flasks. The drugs were then dissolved, brought to metered volume with methanol, and shaken evenly to get 1000 μg/mL standard concentrated stock solutions of chlorpropamide, tolbutamide, and nateglinide.

Standard concentrated stock solutions of the 11 illegally-added hypoglycemics drugs were accurately pipetted at 0.5 mL and transferred to a 10 mL volumetric flask, diluted and brought to metered volume with 80% methanol solution, and shaken evenly to get a multi-standard concentrated stock solution which contained 2 μg/mL of dimethyl biguanide hydrochloride, phenethyl biguanide hydrochloride, glimepirid, and repaglinide, 10 μg/mL of glipizide, tolazamide, gliclazide, and glibenclamide, and 50 μg/mL of chlorpropamide, tolbutamide, and nateglinide. The multi-standard stock solution was diluted with 80% methanol solution in specified proportions to get control working solutions of a series of concentrations. The following solutions were prepared in the end: (1) a series of standard solutions of dimethyl biguanide, phenethyl biguanide, glimepirid, and repaglinide at concentrations of 0.8, 1.6, 3.2, 8, 16, 20, 32, 40, 64, 80, 200, 400, 800 ng/mL, 1.6, and 2 μg/mL; (2) a series of standard solutions of glipizide, tolazamide, gliclazide, and glibenclamide of concentrations at 4, 8, 16, 40, 80, 100, 160, 200, 320, 400 ng/mL, 1, 2, 4, 8, and 10 μg/mL; and (3) a series of standard solutions of

chlorpropamide, tolbutamide, and nateglinide at concentrations of 20, 40, 80, 200, 400, 500, 800 ng/mL, 1, 1.6, 2, 5, 10, 20, 40, and 50 µg/mL.

Sample pretreatment method

5 pills/tablets/sachets of sample were taken, pulverized, and mixed evenly. About 0.2 g (with a precision of 0.01 mg) of sample was transferred to a 10 mL plastic centrifuge tube, added with 5 mL of methanol, and then weighed; the sample was then subjected to vortex mixer for 60s and ultrasonication for 15 min, allowed to cool down, weighed again, and replenished with methanol to make up for the lost weight, after which it was centrifuged at 4500 rpm for 10 min. 20 µL of sample solution was pipetted, diluted to 1 mL with 80% methanol, subjected to vortex mixer for 30 s followed by centrifugalization at 13000 rpm for 10 min. The supernatant was taken out and transferred to a sampler vial. 5 µL was injected for analysis by UFLC-MS/MS. Sample solutions of concentration beyond linear range were diluted before UFLC-MS/MS analysis.

RESULT AND DISCUSSION

MRM Chromatogram of Standard Samples

As can be seen in Fig. 1, which shows the chromatogram of 10 ng/mL multi-standard solution of the 11 analytes, the peaks of the analytes were of good shape and well separated.

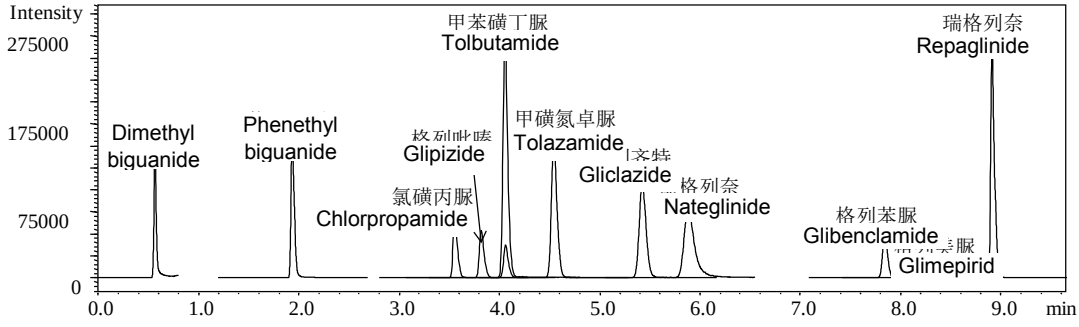

Fig. 1 MRM chromatograms of drugs

Linearity and LOD

The results showed that under the set analysis conditions, the calibration curves of all 11 compounds were in their linear range. The peak areas of these drugs were of good linearity, with correlation coefficients (r) greater than 0.994. The method's LOQs of dimethyl biguanide, phenethyl biguanide, chlorpropamide, glipizide, tolbutamide, tolazamide, gliclazide, nateglinide, glibenclamide, glimepirid, and repaglinide were 0.963, 0.998,

24.7, 5.00, 26.2, 5.00, 5.02, 24.9, 5.02, 1.04, and 1.00 µg/g, respectively, showing that the method met the requirements for determination of actual samples. By calculation of S/N ratio, the method's LODs of dimethyl biguanide, phenethyl biguanide, chlorpropamide, glipizide, tolbutamide, tolazamide, gliclazide, nateglinide, glibenclamide, glimepirid, and repaglinide were 0.0770, 0.0798, 9.89, 2.00, 2.10, 1.00, 1.00, 4.98, 1.01, 0.414, and 0.0400 µg/g, respectively.

Table 3 Regression equations, correlation coefficients, linear ranges, and LOQs (matrix calibration curve)

Compound	Regression Equation	Correlation Coefficient (r)	Linear Range	LOD (ng/mL)	Determined concentration (µg/g)
Dimethyl biguanide	Y=1932.007+20977.014X	1.000	0.963-48.2 µg/g	0.0616	0.0770
Phenethyl biguanide	Y=3055.323+13252.99X	0.998	0.998-49.9 µg/g	0.0639	0.0798
Chlorpropamide	Y=-606.422+259.047X	0.994	24.7-1236 µg/g	7.92	9.89
Glipizide	Y=-166.785+772.715X	0.998	5.00-250 µg/g	1.60	2.00
Tolbutamide	Y=-3176.184+857.126X	0.995	26.2-1312 µg/g	1.68	2.10
Tolazamide	Y=510.439+2555.127X	1.000	5.00-250 µg/g	0.800	1.00
Gliclazide	Y=952.949+2093.000X	0.999	5.02-504 µg/g	0.803	1.00
Nateglinide	Y=-301.193+541.488X	0.999	24.9-1243 µg/g	3.98	4.98
Glibenclamide	Y=-125.412+1310.823X	0.997	5.02-251 µg/g	0.804	1.01
Glimepirid	Y=-28.644+1537.736X	0.999	1.04-51.8 µg/g	0.331	0.414
Repaglinide	Y=1927.581+20218.295X	1.000	1.00-50.0 µg/g	0.0320	0.0400

Precision test

Recovery and precision tests were performed on matrix spiked with standard solutions at the four levels of LOQ, LQC, MQC, HQC by 5 successive injections, and the resulted precision and recovery data falling in the ranges of 0.25%~7.31% and 78%~110.7%, respectively, suggesting that the method was of good precision.

Table 4 Absolute recoveries of the illegally-added hypoglycemic drugs (n=5)

Category	Actual concentration (μg/g)	Determined concentration (μg/g)	RSD(%)	Accuracy (%)
	0.963	0.818±0.013	1.59	84.9
Dimethyl biguanide	1.93	1.80±0.03	1.52	93.4
	9.63	9.10±0.16	1.73	94.5
	38.5	34.7±0.3	0.77	90.1
	0.998	0.663±0.025	3.78	85.4
Phenethyl biguanide	2.00	1.71±0.03	1.87	105.7
	9.98	9.45±0.17	1.84	94.6
	39.9	34.1±0.2	0.66	85.5
Chlorpropamide	24.7	24.9±0.7	2.76	100.8
	49.5	48.1±1.1	2.20	97.3
	247	243±4	1.57	98.5
	989	1055±16	1.52	106.6
Glipizide	5.00	3.94±0.18	4.64	78.7
	10.0	8.33±0.36	4.32	83.3
	50.0	44.3±0.6	1.28	88.6
	200	188±2	1.19	93.9
Tolbutamide	26.2	27.7±0.8	2.99	105.6
	52.5	51.9±1.2	2.30	98.9
	262	256±2	0.80	97.5
	1050	1162±12	1.00	110.7
Tolazamide	5.00	4.05±0.14	3.42	81.1
	10.0	8.71±0.12	1.42	87.1
	50.0	46.1±1.2	2.59	92.2
	200	179±2	1.11	89.6
Gliclazide	5.02	3.92±0.15	3.73	78.0
	10.0	8.75±0.15	1.69	87.5
	50.2	46.4±0.1	0.25	92.4

Category	Actual concentration (μg/g)	Determined concentration (μg/g)	RSD(%)	Accuracy (%)
Nateglinide	201	182±1	0.53	90.7
	24.9	21.4±0.6	2.61	86.0
	49.8	44.3±0.6	1.38	89.0
	249	224±3	1.34	89.9
	995	928±15	1.61	93.2
Glibenclamide	5.02	4.45±0.25	5.66	88.6
	10.0	9.02±0.23	2.51	90.2
	50.2	45.6±1.0	2.27	90.8
Glimepirid	201	193±2	1.23	96.1
	1.04	0.855±0.052	6.10	82.2
	2.07	1.87±0.14	7.31	90.3
	10.4	9.40±0.30	3.16	90.4
	41.4	38.0±0.2	0.42	91.8
Repaglinide	1.00	0.848±0.013	1.59	84.8
	2.00	1.87±0.03	1.52	93.5
	10.0	9.40±0.08	0.88	94.0
	40.0	36.0±0.3	0.77	90.0

Determination of actual samples

Samples with positive results in screening test were subjected to quantitative determination using the calibration curves of matrix, and the results were shown in Table 5:

Table 5 Results of hypoglycemic health foods

Name	Dimethyl biguanide (mg/g)	Phenethyl biguanide (mg/g)	Glibenclamide (mg/g)	Glimepirid (mg/g)
Sample A	75.9	0.00975	2.64	ND
Sample B	ND	9.75	2.27	0.00617
Sample C	0.0435	ND	5.91	ND

ND: not detected

CONCLUSION

A method was proposed for determination of 11 illegally-added hypoglycemic drugs in health foods with a Shimadzu LC-30A ultra fast liquid chromatograph-tandem LCMS-8030 triple quadrupole mass spectrometer system. The proposed method was of wide linear range and demonstrated correlation coefficients greater than 0.994 for all 11 hypoglycemic drugs, meeting the requirements for actual screening satisfactorily. The UFLC-MS/MS method was used for determination of illegally-added hypoglycemic drugs in health foods in the study. As can be inferred from the sample results, the practice of illegal addition of drugs into hypoglycemic health foods is quite prevalent and the spiked level of drugs can be several milligrams or even hundreds of milligrams. The practice of illegal addition of several drugs into a health food product is also identified. In light of this, it is necessary to intensify supervisory monitoring of illegal addition of chemical drugs into hypoglycemic health foods. In the meantime, it is also necessary to develop methods for daily analysis in order to safeguard food safety of health products.

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V-20 Determination of estrogen in milk powder by triple quadrupole mass spectrometry

INTRODUCTION

A method is proposed for determination of 8 estrogens in the milk powder using Shimadzu LC-30A ultra fast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. Samples were extracted, separated by LC-30A ultra fast liquid chromatograph, and then quantitatively assayed using LCMS-8030 triple quadrupole mass spectrometer with the internal standard method. The method demonstrated good linearity for estriol, 17-α-estradiol, ethinyloestradiol and estrone in the concentration range of 1 ~ 100 μg/L, for 17-β-estradiol in the concentration range of 2 ~ 100 μg/L, and for stilboestrol, hexestrol and dienestrol in the concentration range of 0.5 ~ 500 μg/L. Precision tests were performed by 6 successive injections of multi-standard solutions at concentrations of 5 μg/L,10 μg/L and 100μg/L, the %RSDs of retention time and peak area of the multi-standard solutions at 3 concentration levels were 0.034%~0.638% and 0.864%~4.843%, respectively, suggesting that the method's precision was good. Spiked blank matrices of the milk powder were assessed, and the results showed that all of the 8 compounds had good response at LOQ level. The method's LOQs met the requirements in *GB/T 21981-2008 Determination of Hormone Multi-residues in Foodstuffs of Animal Origin—LC-MS/MS Method.*Estrogens are a category of steroid compounds which have such physiological actions as promoting and maintaining female reproductive organs and secondary sex characters. When used as veterinary drugs, estrogens can make dams give milk or can treat infertility of dams. The Ministry of Agriculture of the People's Republic of China issued on December 24, 2002 the No. 235 Announcement promulgating the *MRLs for veterinary drugs in food of animal origin* which set forth clear regulations on the use of estrogen drugs in animals. According to the regulations, benzestrofol shall only be used as a therapeutic drug, and the use of diethylstilbestrol and its salts, esters and depo-medroxy progesterone acetate must be banned. It is also stipulated that the above-mentioned substances shall not be detected in food of animal origin.

EU Directive 96/22/EC, FDA, and Japan's Positive List System have also banned the use of hormonal drugs in foods of animal origin. In this paper, a

method is proposed in reference with GB/T 21981-2008 Determination of Hormone Multi-residues in Foodstuffs of Animal Origin—LC-MS/MS Method for fast and accurate determination of glucocorticoids in milk powder with Shimadzu LC-30A ultra fast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer for reference of relevant laboratorians.

EXPERIMENTAL

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The specific configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A system controller, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.41 chromatography workstation.

Conditions of Analysis
LC Conditions

Apparatus	:LC-30A system
Chromatographic column	:Shim-pack XR-ODS III2.0mmI.D.×75 mmL,1.6 μm
Mobile phase	:A:water
Mobile phase	:B:methanol/acetonitrile=1:1 (v/v)
Flow rate	:0.4 mL/min
Injection volume	:10 μL
Column temperature	:40 °C
Elution mode	:Gradient elution with initial concentration of phase B of 45%

Table 1 Time program

Time(min)	Module	Command	Value
1.50	Pumps	Pump B Conc.	55
4.00	Pumps	Pump B Conc.	60
4.50	Pumps	Pump B Conc.	100
5.00	Pumps	Pump B Conc.	100
5.10	Pumps	Pump B Conc.	45
7.00	Controller	Stop	

MS conditions

Apparatus :LCMS-8030
Ionization :ESI, negative
Ionization voltage :-3.5 kV
Nebulizing gas :Nitrogen, 3.0 L/min
Drying gas :Nitrogen, 20 L/min
Collision gas :Argon
DL temperature :250 °C
Heater block Temperature :400°C
Acquisition Mode :multiple reaction monitoring (MRM)
Dwell time :50 ms
Pause time :3 ms
MRM parameters :See Table 2

Table 2 MRM Parameters

No.:	Name	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
1	Estriol	287.30	171.20*	22.0	40.0	16.0
			145.10	22.0	40.0	27.0
2	17-α-estradiol	271.30	145.20*	21.0	50.0	13.0
			183.05	21.0	45.0	18.0
3	17-β-estradiol	271.30	145.10*	20.0	45.0	24.0
			183.00	20.0	45.0	19.0
4	Ethinyloestradiol	295.30	145.20*	11.0	50.0	26.0
			227.50	11.0	25.0	23.0
5	Estrone	269.30	145.15*	20.0	40.0	28.0
			183.05	20.0	40.0	20.0
6	Stilboestrol	267.25	251.15*	20.0	25.0	28.0
			237.10	20.0	30.0	25.0

7	Hexestrol	269.25	134.20*	10.0	15.0	26.0
			119.05	10.0	40.0	21.0
8	Dienestrol	265.25	93.10*	20.0	25.0	17.0
			249.15	20.0	25.0	28.0
9	Estradiol-13C2	273.30	147.15	20.0	45.0	28.0
10	Stilboestrol-d8	275.30	245.15	21.0	30.0	27.0

* refers to quantitative ion.

Sample Preparation

Preparation of standard solution:

A multi-standard solution at concentration of 10 mg/L was prepared with methanol and progressively diluted into standard working solutions at concentrations of 100, 40, 20, 10, 5, 2, 1 and 0.5 μg/L, which contained 10 μg/L of internal standard substance.

Sample pretreatment method

The same as specified in GB/T 21981-2008 *Determination of Hormone Multi-residues in Foodstuffs of Animal Origin—LC-MS/MS Method .*

RESULT AND DISCUSSION

MRM chromatogram of standard samples

MRM chromatograms of 100 μg/L multi-standard sample are shown in Fig.1

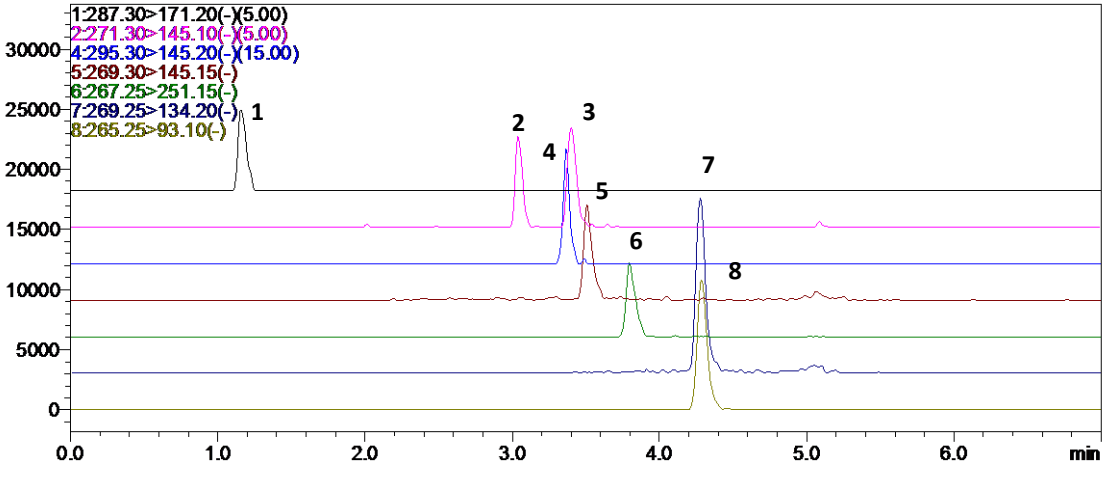


Fig. 1 MRM chromatograms of 100 μg/L multi-standard mixture

(1. estriol; 2. 17-β-estradiol; 3. 17-α-estradiol; 4. ethinyloestradiol ; 5. estrone ; 6.stilboestrol; 7. hexestrol; 8. dienestrol)

Scan of standard samples and mass scan spectrogram of product ions

The mass spectra and MS/MS spectra of 8 estrogen standards are shown below.

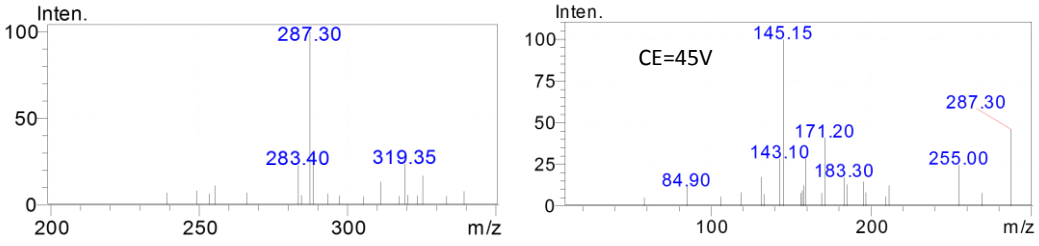


Fig. 2 Mass spectrum (left) and MS/MS spectrum (right) of estriol

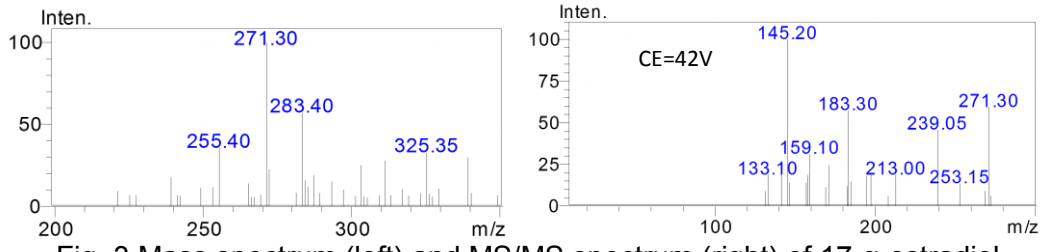


Fig. 3 Mass spectrum (left) and MS/MS spectrum (right) of 17-α-estradiol

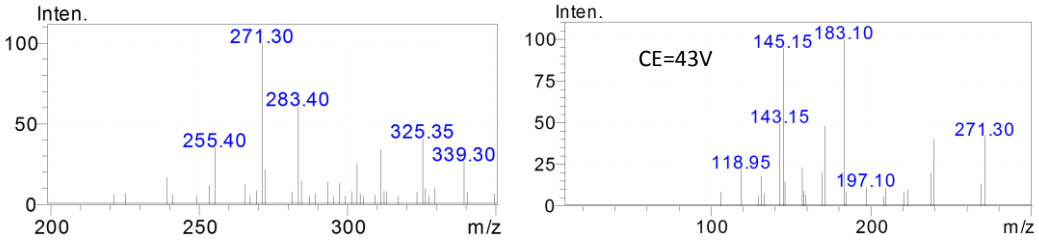


Fig. 4 Mass spectrum (left) and MS/MS spectrum (right) of 17-β-estradiol

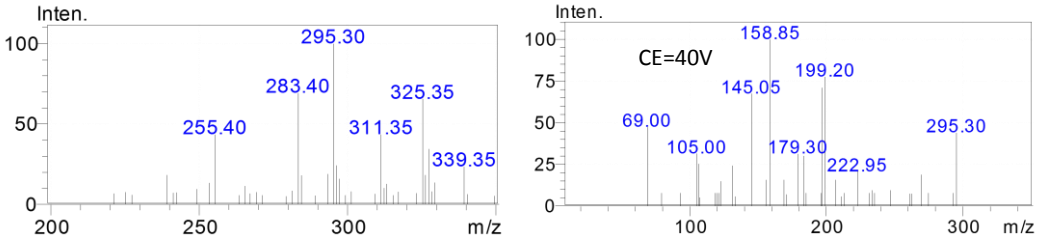


Fig. 5 Mass spectrum (left) and MS/MS spectrum (right) of ethinyloestradiol

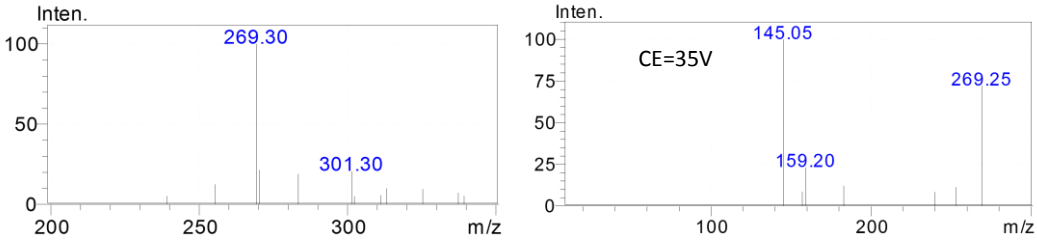


Fig. 6 Mass spectrum (left) and MS/MS spectrum (right) of estrone

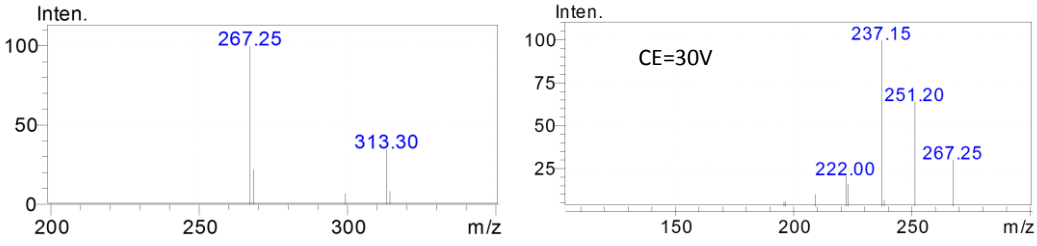


Fig. 7 Mass spectrum (left) and MS/MS spectrum (right) of stilboestrol

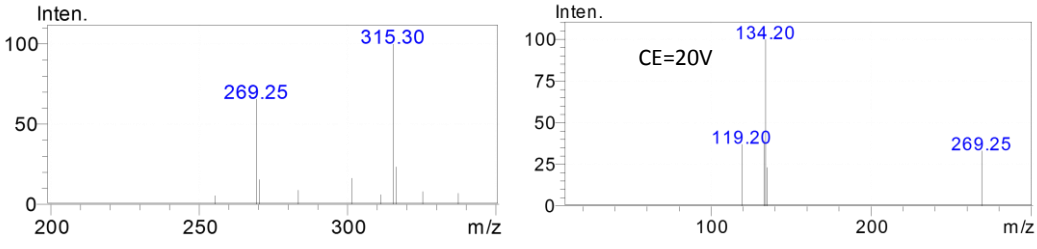


Fig. 8 Mass spectrum (left) and MS/MS spectrum (right) of hexestrol

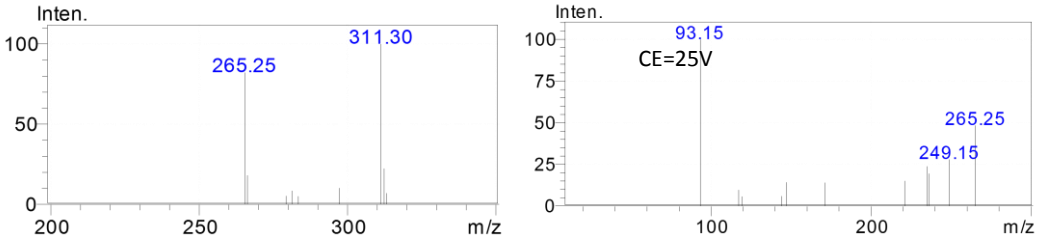


Fig. 9 Mass spectrum (left) and MS/MS spectrum (right) of dienestrol

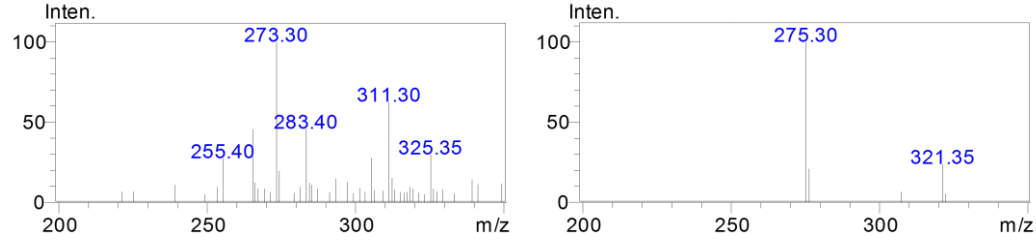


Fig. 10 Mass spectrum of estradiol-13C2 (left) and mass spectrum of stilboestrol-d8 (right)

Linearity

Multi-standard working solutions (containing 10 μg/L of internal standard substance) at concentrations of 100, 40, 20, 10, 5, 2, 1 and 0.5 μg/L were assayed using the analysis conditions specified above. Calibration curves were plotted by the internal standard method as shown in Figs.11 - 18 with concentration ratio as X-axis and peak area ratio as Y-axis. The calibration curves of the 8 hormones were of good linearity in certain concentration ranges. Relevant linear equations, linear ranges, correlation coefficients and calculated LODs and LOQs were listed in Table 3.

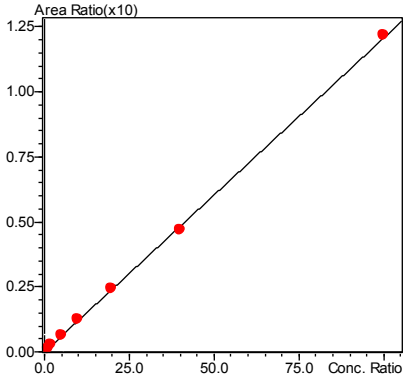


Fig.11 Calibration curve of estriol.

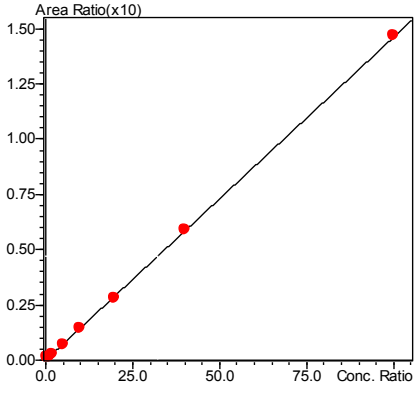


Fig.12 Calibration curve of 17-α-estradiol

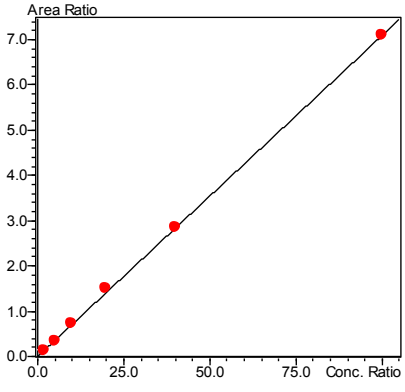


Fig.13 Calibration curve of 17-β-estradiol

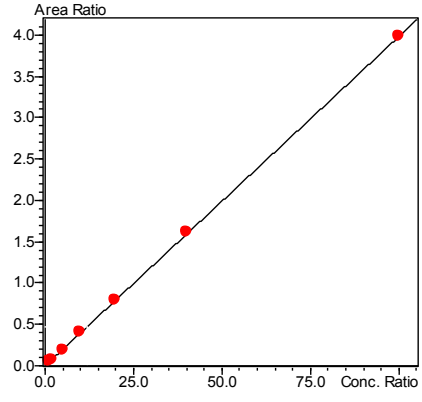


Fig.14 Calibration curve of ethinyloestradiol

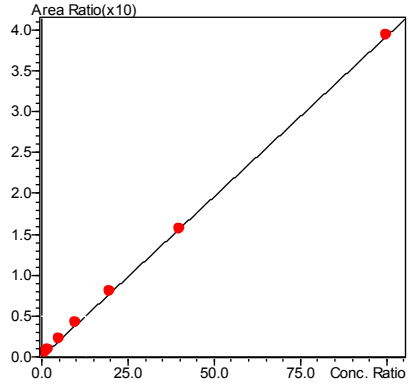


Fig. 15 Calibration curve of estrone.

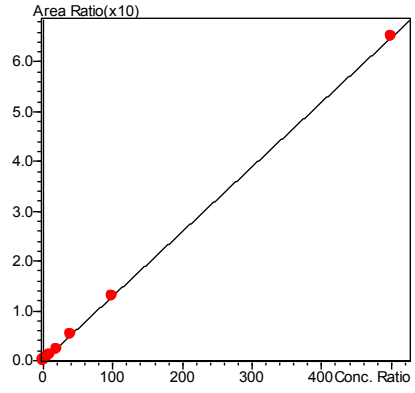


Fig. 16 Calibration curve of stilboestrol

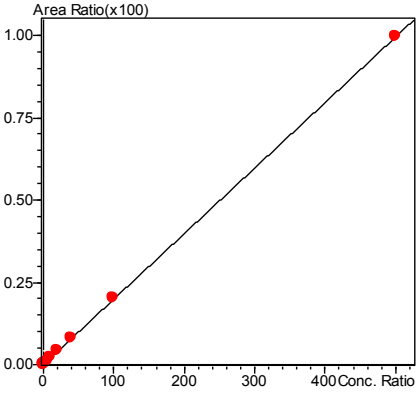


Fig. 17 Calibration curve of hexestrol

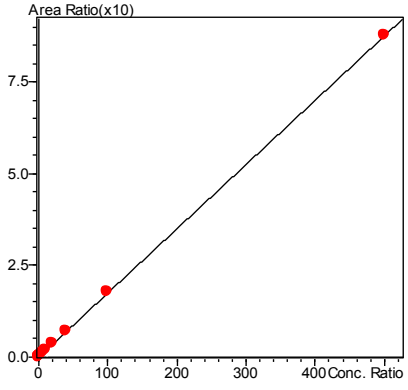


Fig. 18 Calibration curve of dienestrol

Table 3 Parameters of calibration curves of the 8 estrogens

No.	Name	Calibration Curve	Correlation Coefficient (r)	Linear range (μg/L)	LOD (μg/kg)	LOQ (μg/kg)
1	Estriol	Y = (0.120659)X + (0)	0.9998	1-100	0.01	0.03
2	17-α-estradiol	Y = (0.146104)X + (0)	0.9999	1-100	0.05	0.15
3	17-β-estradiol	Y = (0.0709176)X + (0)	0.9998	2-100	0.13	0.39
4	Ethinyloestradiol	Y = (0.039812)X + (0)	0.9999	1-100	0.12	0.36
5	Estrone	Y = (0.392514)X + (0)	0.9999	1-100	0.06	0.18
6	Stilboestrol	Y = (0.129798)X + (0)	0.9999	0.5-500	0.03	0.09
7	Hexestrol	Y = (0.199119)X + (0)	0.9999	0.5-500	0.04	0.12
8	Dienestrol	Y = (0.175211)X + (0)	0.9998	0.5-500	0.03	0.09

Precision test

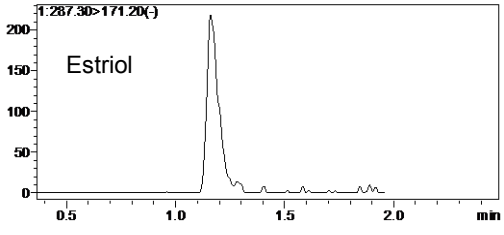
Precision tests were performed by 6 successive injections of multi-standard solutions at concentrations of 5 μg/L, 10 μg/L and 100 μg/L. The %RSDs of peak area and retention time of standards of the 3 concentration levels fell in the ranges of 0.864~4.843% and 0.034~0.638%, respectively, suggesting that the method's precision was satisfactory.

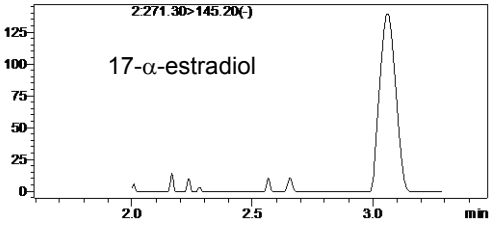
Table 4 Repeatability - retention time and peak area (n=6)

Sample name	%RSD (5 μg/L)		%RSD (20 μg/L)		%RSD (100 μg/L)	
	Area	R.T	Area	R.T	Area	R.T
Estriol	3.160	0.638	2.448	0.135	1.801	0.122
17-α-estradiol	4.236	0.323	1.318	0.084	1.852	0.053
17-β-estradiol	4.843	0.240	4.354	0.080	1.816	0.057
Ethinyloestradiol	4.401	0.454	4.675	0.084	2.846	0.097
Estrone	4.007	0.241	1.818	0.051	1.146	0.051
Stilboestrol	4.753	0.271	0.929	0.079	0.864	0.059
Hexestrol	2.372	0.034	0.868	0.080	1.463	0.057
Dienestrol	1.159	0.194	1.079	0.058	0.981	0.095

Assessment of the apparatus' sensitivity

In order to assess the method's sensitivity, treated blank milk powder samples were spiked with multi-standard solution. The spiked level of 17-β-estradiol and ethinyloestradiol was 0.4 μg/kg; the spiked level of other samples was 0.2 μg/kg. The spiked samples yielded MRM chromatograms as shown in Fig.19. As can be seen in the figure, matrix samples spiked with standards demonstrated good response at LOQ level, meeting the requirements in GB/T 21981-2008 *Determination of Hormone Multi-residues in Foodstuffs of Animal Origin—LC-MS/MS Method*.





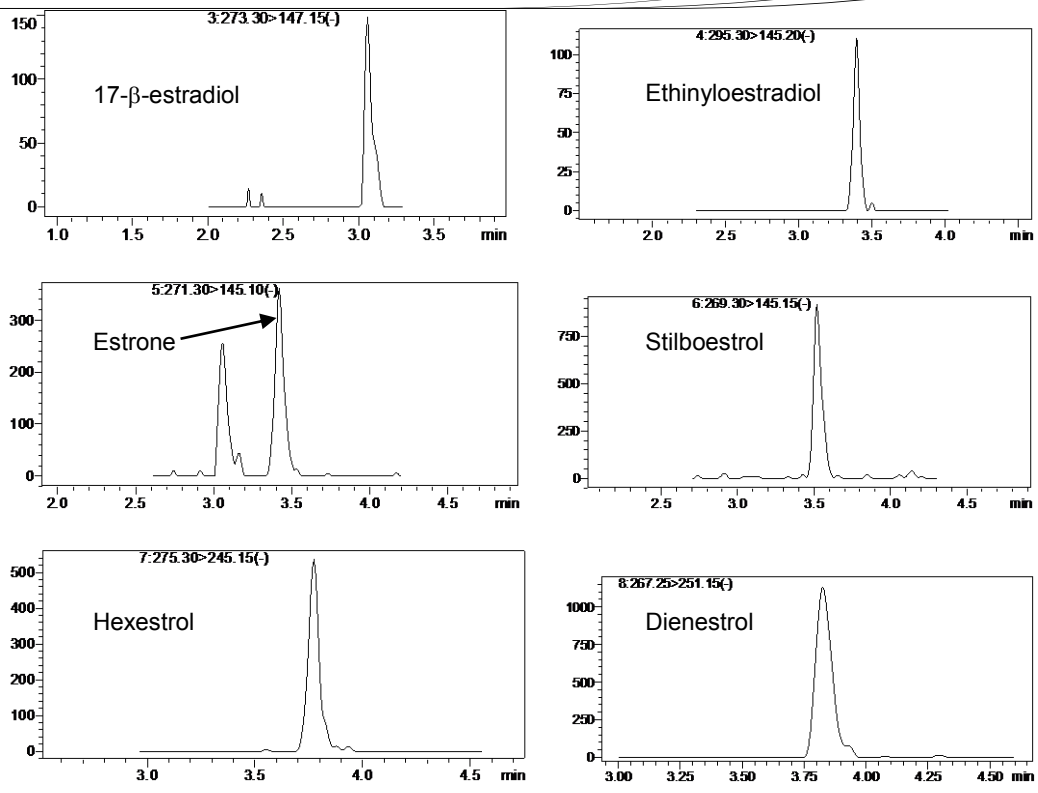


Fig. 19 Chromatograms of milk powder blank matrix samples spiked with standards

CONCLUSION

A method was established for the assay of 8 estrogens in the milk powder with Shimadzu LC-30A ultra fast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. The method was rapidd, good repeatability, and satisfactory precision and demonstrated good linearity for estriol, 17-α-estradiol, ethinyloestradiol and estrone in the concentration range of 1 ~ 100 μg/L, for 17-β-estradiol in the concentration range of 2 ~ 100 μg/L, and for stilboestrol, hexestrol and dienestrol in the concentration range of 0.5 ~ 500 μg/L. The method yielded the calibration curves with correlation coefficients greater than 0.999 for all samples and demonstrated LOQs meeting the requirements in GB/T 21981-2008 *Determination of Hormone Multi-residues in Foodstuffs of Animal Origin—LC-MS/MS Method.*

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V-21 Determination of 6 zearanols in pork by triple quadrupole mass spectrometry

INTRODUCTION

In this paper, a method is proposed for determination of 6 zearanols in pork with Shimadzu LC-30A ultra fast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. The method was of fast analysis speed, good repeatability and satisfactory precision and demonstrated good linearity for the 6 zearanols in a concentration range covering two orders of magnitude. The calibration curves of all samples were with correlation coefficients greater than 0.999. Precision tests were performed by 6 successive injections of multi-standard solutions at concentrations of 5 μg/L, 10 μg/L and 100 μg/L, the %RSDs of peak area and retention time of standard solutions at 3 concentration levels in the ranges of 1.86 ~ 4.61% and 0.04 ~ 0.29%, respectively, suggesting that the method was of good precision.

The method demonstrated good response to pork blank samples spiked with 1 μg/kg multi-standard solution. The method's LOQs meet the requirements in *GB/T 21982-2008 Determination of Residues of Zearalanol, β-zearalanol, α-zearalenol, β-zearalenol, Zearalanone and Zearalenone in Foodstuffs of Animal Origin--LC-MS/MS Method.* Zearanols are a group of non-steroids, non-hormonal compounds which frequently serve as growth promoter of cattle and sheep because of their growth hormone and insulin promoting, protein synthesis promoting, lean meat rate enhancing, and feed conversion rate improving actions. However, zearanols can give rise to sexual function disorders and affect normal development of secondary sex character because of their weak estrogen-like actions and may be carcinogenic under the induction of external environment. Moreover, zearanols discharged from animals can also cause secondary pollution and environmental pollution to drinking water and food.

In 1998, EU banned the application of hormonal drugs including zearanols in poultry and livestock husbandry. The Ministry of Agriculture of China also issued the No. 235 Announcement in which the use of zearanols-contaminated foodstuffs for edible animals was banned. Therefore, no zearanols shall be detected in edible animals. In this paper, a method is proposed in accordance with *GBT 21982-2008 Determination of*

Residues of Zearalanol, β-zearalanol, α-zearalenol, β-zearalenol, Zearalanone and Zearalenone in Foodstuffs of Animal Origin--LC-MS/MS Method for fast and accurate determination of zearanols in pork with Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer for the reference of relevant laboratorians.

EXPERIMENTAL

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The specific configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.41 chromatography workstation.

Analytical conditions

LC Conditions

Apparatus :LC-30A system

Column :Shim-pack XR-ODS III 2.0 mmI.D.×50 mmL., 1.6 μm

Mobile phase :A:Water

Mobile phase :B:Acetonitrile

Flow rate :0.4 mL/min

Injection volume :10 μL

Column Temperature :40 °C

Elution mode :Gradient elution with initial concentration of phase B of 25%.See Table 1

Table 1 Time program

Time(min)	Module	Command	Value
3.00	Pumps	B Conc.	70
4.00	Pumps	B Conc.	70
4.20	Pumps	B Conc.	25
5.00	Controller	Stop	

MS conditions

Apparatus :LCMS-8030
Ionization :ESI, positive
Ionization voltage :+4.5 kV
Nebulizing gas :Nitrogen, 3.0 L/min
Drying gas :Nitrogen, 20 L/min
Collision gas :Argon
DL temperature :250 °C
Heater block temperature :400°C
Mode :multiple reaction monitoring (MRM)
Dwell time :50 ms
Pause time :3 ms
MRM parameters :See Table 2

Table 2 MRM Parameters

No	Name	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
1	β-zearanol	323.20	305.15*	13.0	10.0	23.0
			282.30	13.0	10.0	22.0
2	β-zearalenol	321.20	303.15*	13.0	10.0	23.0
			285.15	13.0	10.0	22.0
3	α-zearanol	323.20	305.15*	13.0	10.0	23.0
			123.15	13.0	20.0	26.0
4	α-zearalenol	321.20	303.15*	17.0	10.0	23.0
			285.15	17.0	10.0	22.0
5	Zearelone	321.20	303.15*	13.0	15.0	23.0
			69.10	13.0	30.0	28.0
6	Zearalenone	319.20	301.15*	16.0	10.0	23.0
			283.15	16.0	15.0	22.0

* refers to quantitative ion.

Sample Preparation

Preparation of standard solution:

A multi-standard solution at concentration of 10 mg/L was prepared with acetonitrile, and then progressively diluted into standard working solutions at concentrations of 500, 250, 100, 50, 25, 10, 5, 2 and 1 μg/L.

Sample pretreatment method

Identical with that in *GB/T 21982-2008 Determination of Residues of Zearalanol, β-zearalanol, α-zearalenol, β-zearalenol, Zearalanone and Zearalenone in Foodstuffs of Animal Origin--LC-MS/MS Method .*

RESULT AND DISCUSSION

MRM chromatogram of standard samples

MRM chromatograms of 100 μg/L multi-standard mixture are shown in Fig. 1.

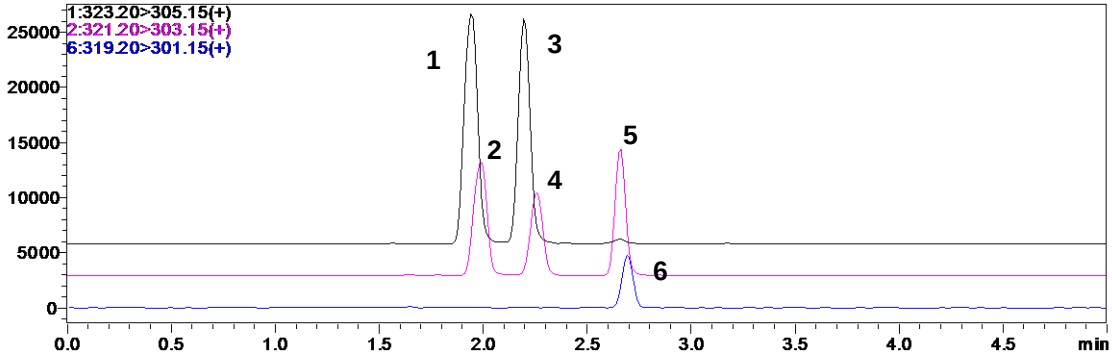


Fig. 1 MRM chromatograms of 100 μg/L multi-standard sample

(1. β-zearanol; 2. β-zearalenol; 3. α-zearanol; 4. α-zearalenol; 5. zearelone; 6. zearalenone)

Mass spectrum and MS/MS spectrum

Mass spectra and MS/MS spectra of 6 zearanols standards are shown below.

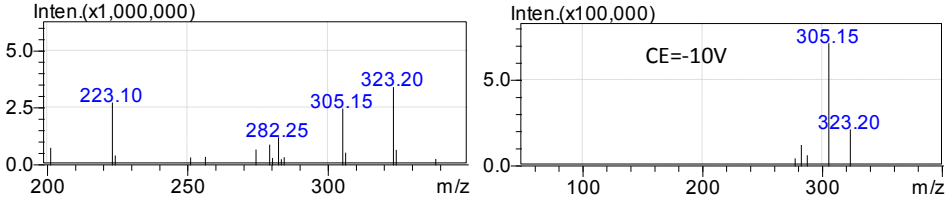


Fig. 2 Mass spectrum (left) and MS/MS spectrum (right) of β-zearanol

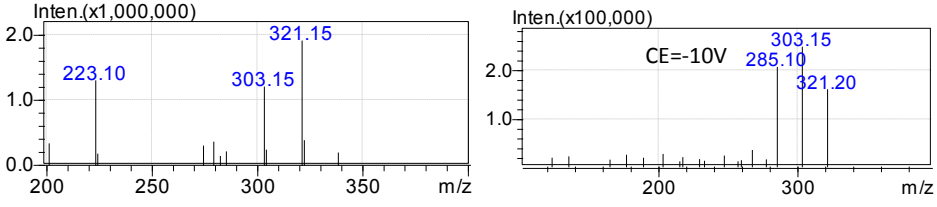


Fig. 3 Mass spectrum (left) and MS/MS spectrum (right) of β-zearalenol

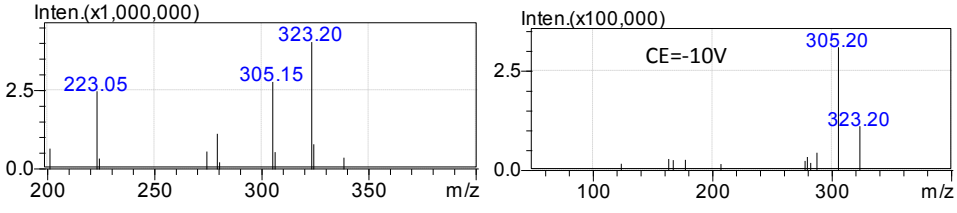


Fig. 4 Mass spectrum (left) and MS/MS spectrum (right) of α-zearanol

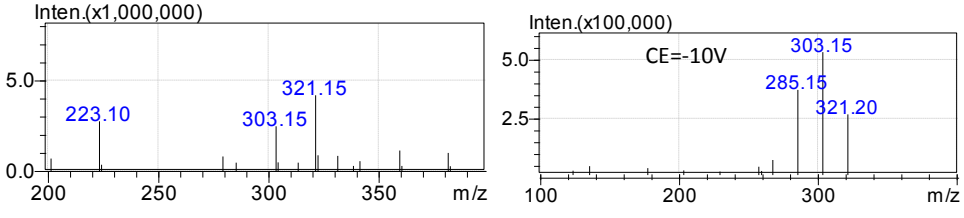


Fig. 5 Mass spectrum (left) and MS/MS spectrum (right) of α-zearalenol

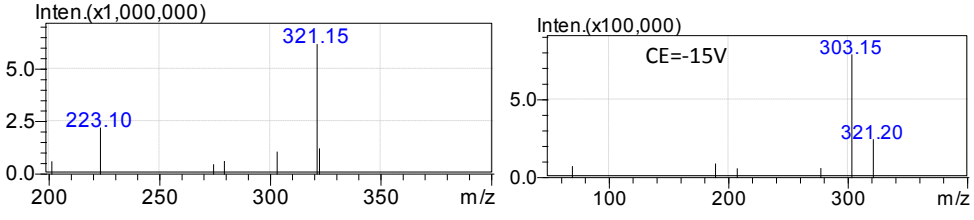


Fig. 6 Mass spectrum (left) and MS/MS spectrum (right) of zearelone

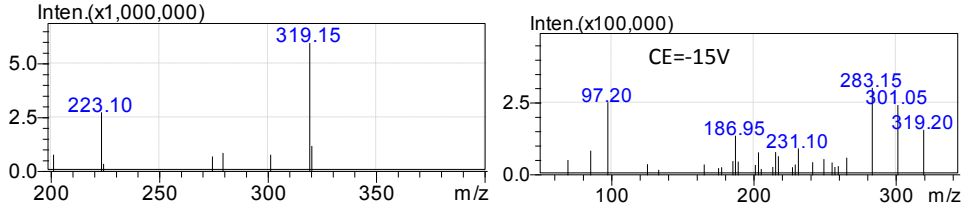


Fig. 7 Mass spectrum (left) and MS/MS spectrum (right) of zearalenone

Linearity

Multi-standard working solutions at concentrations of 500, 250, 100, 50, 25, 10, 5, 2 and 1 μg/L were assayed using the analysis conditions specified above. Calibration curves as shown in Figs.8 – 13 were plotted by the external standard method with concentration as X-axis and peak area as Y-axis. The calibration curves of the 6 zearanols were of good linearity in certain concentration ranges. Relevant linear equations, linear ranges, correlation coefficients and calculated LODs and LOQs are listed in Table 3.

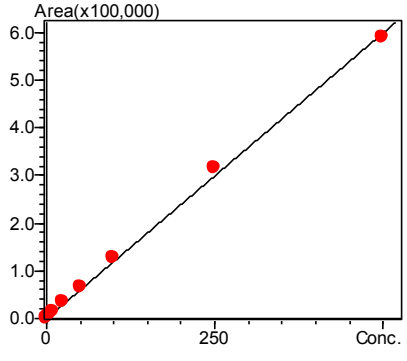


Fig.8 Calibration curve of β–zearanol

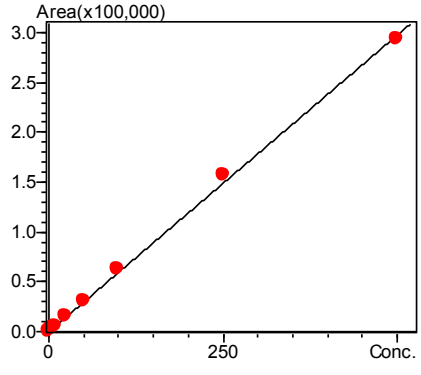


Fig.9 Calibration curve of β-zearalenol

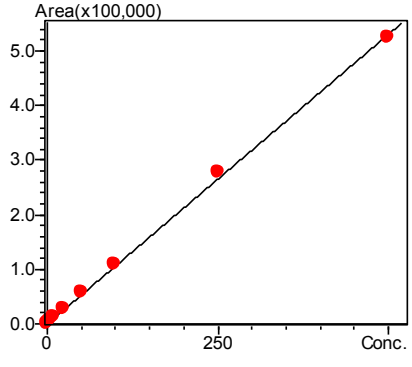


Fig.10 Calibration curve of zearanol

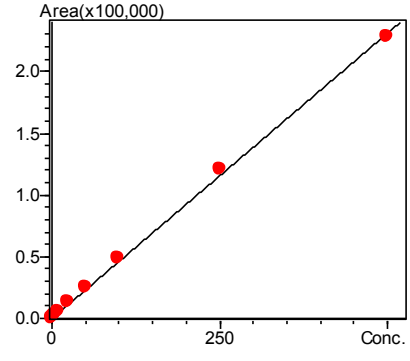


Fig.11 Calibration curve of α-zearalenol

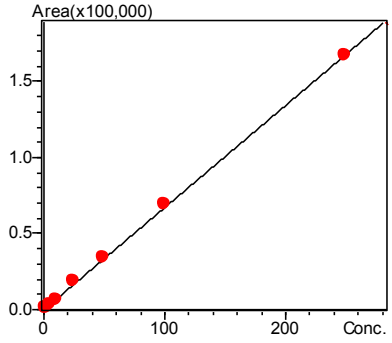


Fig.12 Calibration curve of zearelone

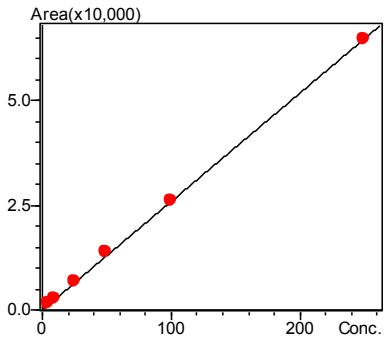


Fig.13 Calibration curve of zearalenone

Table 3 Parameters of calibration curves of the 6 zearanols

No	Name	Calibration Curve	Correlation Coefficient (r)	Linear range (µg/L)	LOD (µg/kg)	LOQ (µg/kg)
1	β-zearanol	Y = (1197.30)X	0.9994	1-500	0.31	1.05
2	β-zearalenol	Y = (595.740)X	0.9994	2-500	0.58	1.92
3	α-zearanol	Y = (1061.59)X	0.9996	1-500	0.32	1.05
4	α-zearalenol	Y = (462.248)X	0.9995	2-500	0.55	1.85
5	Zearelone	Y = (670.969)X	0.9998	1-250	0.31	1.02
6	Zearalenone	Y = (258.828)X	0.9999	5-250	1.47	4.90

Precision test

Precision tests were performed by 6 successive injections of multi-standard solutions at concentrations of 5 µg/L, 10 µg/L and 100 µg/L. The %RSDs of peak area and retention time of standards at the 3 concentration levels fell in the ranges of 1.86~4.61% and 0.04~0.29%, respectively, suggesting that the method's precision was satisfactory.

Table 4 Repeatability - retention time and peak area (n=6)

Compound	%RSD (5 μg/L)		%RSD (10 μg/L)		%RSD (100 μg/L)	
	Area	R.T	Area	R.T	Area	R.T
	4.42	0.28	3.96	0.23	2.26	0.17
β-zearalenol	4.15	0.25	3.93	0.27	2.70	0.16
Zearanol	3.70	0.28	3.29	0.18	1.90	0.15
α-zearalenol	2.80	0.29	2.23	0.22	2.27	0.04
Zearelone	4.61	0.26	3.08	0.11	1.86	0.05
Zearalenone	3.07	0.29	3.03	0.08	3.03	0.07

Sensitivity

Pork samples were subjected to pretreatment and assay in accordance with *GB/T 21982-2008 Determination of Residues of Zearalanol, β-zearalanol, α-zearalenol, β-zearalenol, Zearalanone and Zearalenone in Foodstuffs of Animal Origin--LC-MS/MS Method* and the assay results of blank pork samples are shown in Fig. 14. None of the 6 zearanols was detected in the blank pork sample. In order to assess the method's sensitivity, multi-standard solution was spiked into blank samples of pork at spiked level of 1 μg/kg and chromatograms are shown in Fig. 15. As can be seen from the chromatograms, the system responded well to all spiked matrix samples at LOQ levels, meeting the requirements in GB/T 21982-2008.

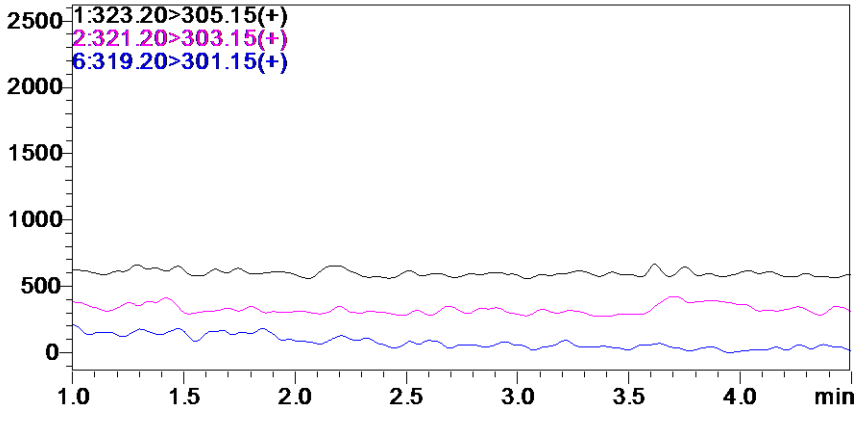
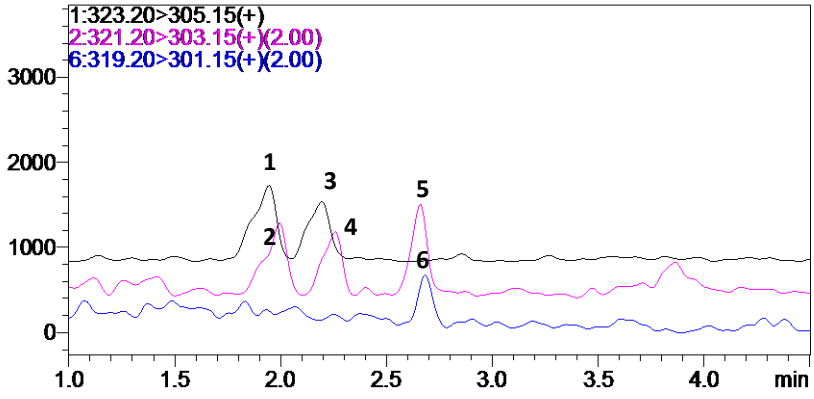


Fig. 14 Chromatogram of a blank pork sample



CONCLUSION

A method is proposed for determination of 6 zearanols in pork with Shimadzu LC-30A ultra fast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. The method was of fast analysis speed, good repeatability and satisfactory precision and demonstrated good linearity for zearanol and β-zearanol in the concentration range of 1 ~ 500 μg/L, α-zearalenol and β-zearalenol in the concentration range of 2 ~ 500 μg/L, zearelone in the concentration range of 2~ 250 μg/L, and zearalenone in the concentration range of 5 ~ 250 μg/L. The method also yielded calibration curves with correlation coefficients greater than 0.999 for all compounds. In order to assess the method's sensitivity, multi-standard solution was spiked into blank samples of pork at spiked level of 1 μg/kg and the system responded well to all spiked matrix samples at LOQ levels, meeting the requirements in GB/T 21982-2008 *Determination of Residues of Zearalanol, β-zearalanol, α-zearalenol, β-zearalenol, Zearalanone and Zearalenone in Foodstuffs of Animal Origin--LC-MS/MS Method*.

SHIMADZU
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V-22 Determination of macrolide antibiotics in pork by triple quadrupole mass spectrometry

INTRODUCTION

A method is proposed for determination of macrolide antibiotics in pork with Shimadzu LC-30A ultra fast liquid chromatograph in conjunction with Shimadzu LCMS-8030 triple quadrupole mass spectrometer. Pork samples were, after having been processed, separated by the LC-30A ultra fast liquid chromatograph, and then assayed with the LCMS-8030 triple quadrupole mass spectrometer. 8 macrolide antibiotics were separated and assayed rapidly within 4 minutes. The method demonstrated good linearity for spiramycin and tilmicosin in the concentration range of 5 ~ 200 µg/L and oleandomycin, tylosin, kitasamycin, erythromycin, josamycin, and roxithromycin in the concentration range of 1-500 µg/L. The calibration curves of these antibiotics were all with correlation coefficients greater than 0.9996. Precision tests were performed on multi-standard solutions of concentrations of 5 µg/L, 20 µg/L and 200 µg/L, and the %RSDs of retention time and peak area in 6 successive injections were below 1.87% and 5.04%, respectively, suggesting that the method's precision was good.

Macrolides (MALs) are a category of antibiotics generated by actinobacillus or micromonosporaceae. MALs have become one of the antibiotic categories that witness fastest growth in demand and sales worldwide. Because of their broad-spectrum antibacterial actions which enable them to fight Gram-positive bacteria, mycoplasma and some Gram-negative bacteria, MALs are widely used for the treatment of respiratory and intestinal infectious diseases in pigs, cattle, sheep, shrimps, and poultry or used at low dosage as feed additives to promote animal growth and development. However, residues of macrolide antibiotics in food are apt to give rise to hypersensitiveness and the spread of drug resistant bacterial strains. Just like many other veterinary drugs, the monitoring and control of macrolide drugs residues in food of animal origin have been attached much importance by governments in many countries including China. It is stipulated in No. 235 Announcement issued by the Ministry of Agriculture of the P.R.C. that the MRL of erythromycin in animal tissues, milk and eggs is 40-200 µg/kg; the MRL of tilmicosin in animal tissues and milk is 50-1500 µg/kg; the MRL of tylosin in animal tissues, milk and eggs is 50-200 µg/kg. In this paper, a method is proposed for fast determination of 8 macrolide

antibiotics (spiramycin, tilmicosin, oleandomycin, tylosin, kitasamycin, erythromycin, josamycin, and roxithromycin) in pork with Shimadzu LC-30A ultra fast liquid chromatograph-tandem LCMS-8030 triple quadrupole mass spectrometer for the reference of relevant laboratorians. The method has the merits of fast analysis speed, good repeatability and high sensitivity.

EXPERIMENTAL

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.41 chromatography workstation.

Analytical conditions

LC Conditions

Apparatus :LC-30A system

Column :Shim-pack XR-ODS III(2.0mmI.D.×50 mm L,1.6 μm)

Mobile phase :A:0.1 % formic acid aqueous solution

Mobile phase :B:Acetonitrile

Flow rate :0.4 mL/min

Injection volume :20 μL

Column temperature:40 °C

Elution mode :Binary gradient with an initial concentration of 15% of mobile phase B

Table 1 Time program

Time (min)	Module	Command	Value
2.00	Pumps	B Conc.	40
2.01	Pumps	B Conc.	80
2.20	Pumps	B Conc.	80
2.30	Pumps	B Conc.	15
4.00	Controller	Stop	

MS conditions

Apparatus	:LCMS-8030
Ionization	:ESI, positive
Ionization voltage	:ESI (+), +4.5 kV
Nebulizing gas	:Nitrogen 3.0 L/min
Drying gas	:Nitrogen 15 L/min
Collision gas	:Argon
DL temperature	:250 °C
Heater block temperature	:400 °C
Mode	:Multiple reaction monitoring (MRM)
Dwell time	:20 ms
Pause time	:2 ms
MRM parameters	:Listed in Table 2

Table 2 MRM Parameters

No.:	Compound	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
1	Spiramycin	843.5	174.14*	-26	-45	-19
			540.25	-26	-35	-20
2	Tilmicosin	869.5	174.10*	-28	-50	-20
			696.55	-28	-40	-28
3	Oleandomycin	688.4	158.15*	-22	-30	-18
			544.35	-22	-15	-30
4	Tylosin	916.5	174.15*	-30	-45	-20
			722.45	-30	-30	-30
5	Kitasamycin	772.3	174.10*	-24	-35	-21
			109.05	-24	-45	-23
6	Erythromycin	734.3	158.15*	-40	-35	-18
			576.35	-40	-20	-32
7	Josamycin	828.4	174.15*	-26	-35	-20
			229.15	-26	-30	-17
8	Roxithromycin	837.5	158.15*	-26	-40	-18
			679.40	-26	-25	-36

* refers to quantitative ion.

Sample Preparation

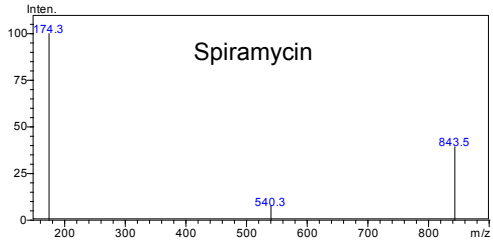
Preparation of standard solution:

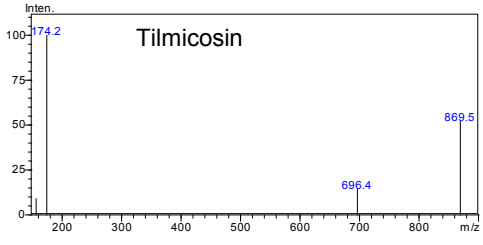
Sufficient quantity of 8 standard substances (spiramycin, tilmicosin, oleandomycin, tylosin, kitasamycin, erythromycin, josamycin, and roxithromycin) was accurately weighed and prepared with methanol into 1000 mg/L of multi-standard stock solution, which was then diluted with the initial mobile phase into standard working solutions of concentrations of 1, 2, 5, 10, 20, 50, 100, 200, and 500 μg/L.

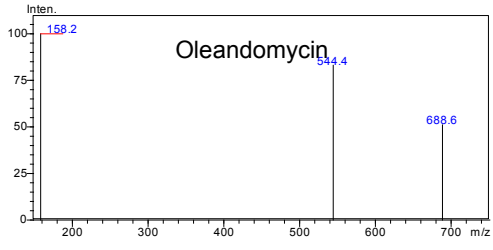
Pretreatment method of samples: refer to *SN/T1777.2-2007 Determination of macrolide antibiotic residues in food of animal origin—Part 2: LC-MS/MS method for the preparation of pork samples and purification and extraction of analytes*.

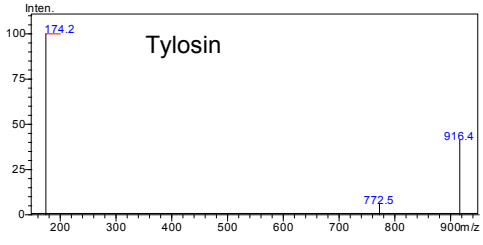
RESULT AND DISCUSSION

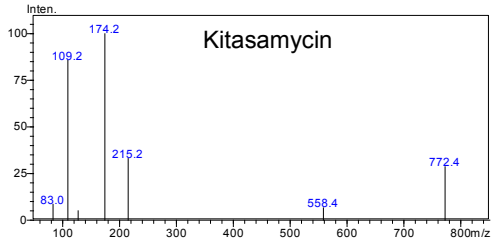
MS/MS spectra

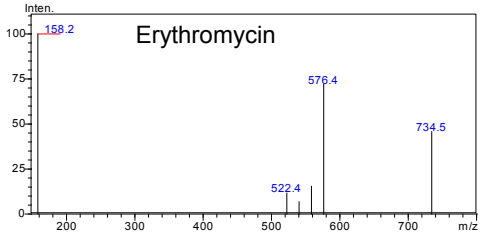












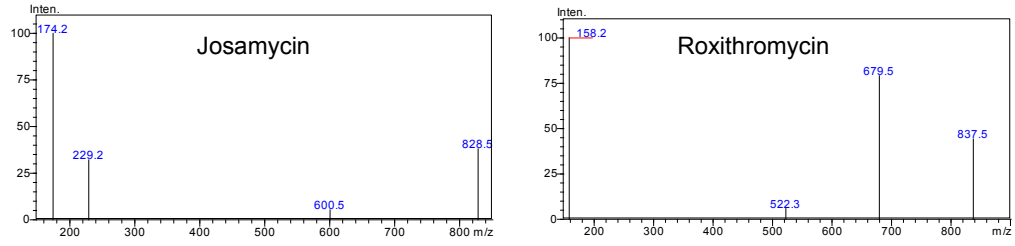


Fig. 1 MS/MS spectra of standard macloride antibiotics

MRM chromatogram of standard mixture

Fig. 2 shows MRM chromatograms of 500 µg/L standard mixture. The 8 macrolide antibiotics were fast assayed within 4 minutes.

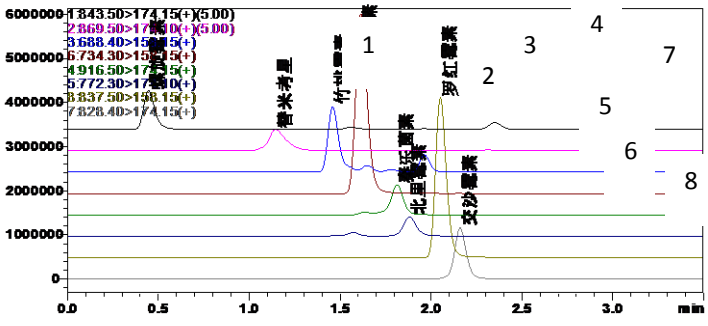
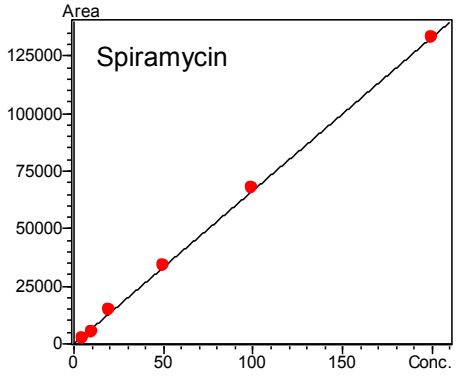


Fig. 2 MRM chromatograms of standard macrolide antibiotics

Linearity

Multi-standard working solutions of concentrations of 1, 2, 5, 10, 20, 50, 100, 200, and 500 µg/L were assayed using the analytical conditions specified above. Calibration curves were plotted as shown in Fig. 3 with concentration as X-axis and peak area as Y-axis. The method demonstrated satisfactory linearity for spiramycin and tilmicosin in the concentration range of 5 ~ 200 µg/L and oleandomycin, tylosin, kitasamycin, erythromycin, josamycin, and roxithromycin in the concentration range of 1-500 µg/L. Relevant linear equations, correlation coefficients and calculated LODs and LOQs are listed in Table 3.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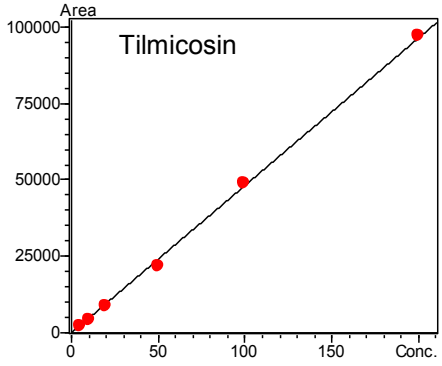
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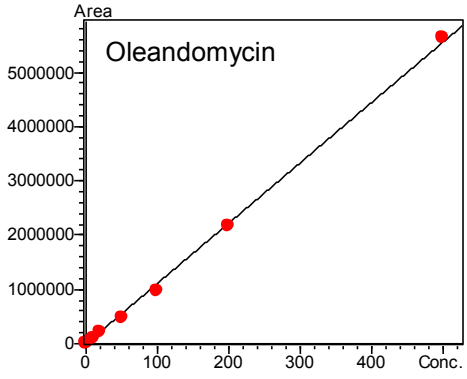
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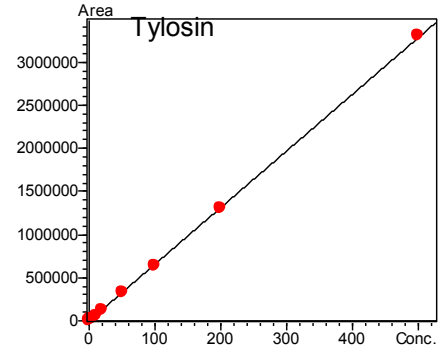
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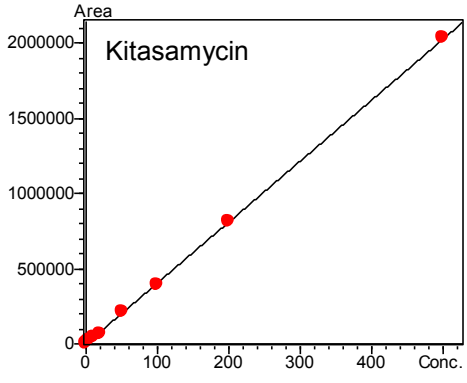
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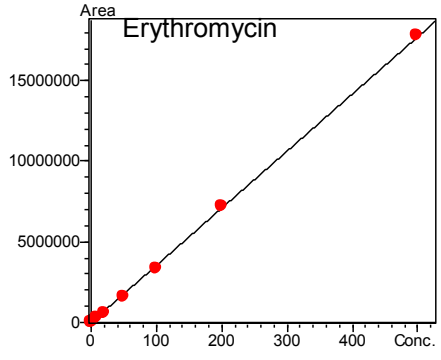
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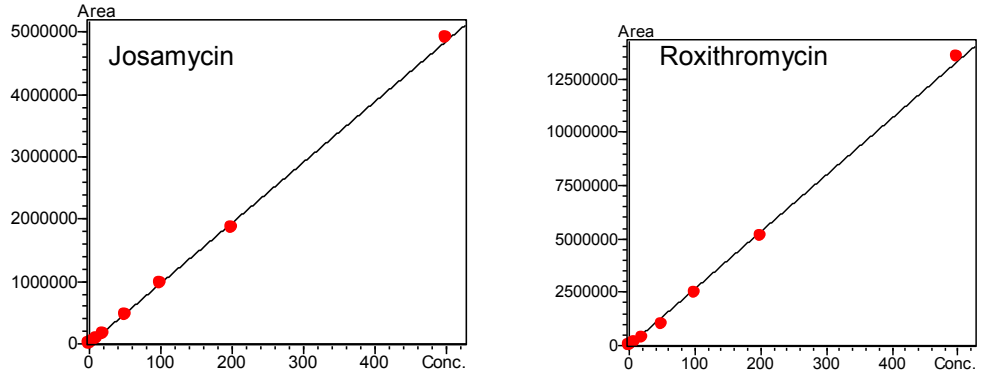


Fig. 3 Calibration curves of the 8 macrolide antibiotics

Table 3 Parameters of the calibration curves of 8 macrolide antibiotics

No.	Compound	Calibration Curve	Correlation Coefficient (r)	Linear Range	LOD (μg/L)	LOQ (μg/L)
1	Spiramycin	Y= 665.9713 X	0.9997	5-200	1.24	3.76
2	Tilmicosin	Y= 482.1126 X	0.9997	5-200	1.07	3.24
3	Oleandomycin	Y= 11126.75 X	0.9996	1-500	0.16	0.48
4	Tylosin	Y= 6573.865 X	0.9999	1-500	0.11	0.35
5	Kitasamycin	Y= 4055.260 X	0.9999	1-500	0.19	0.57
6	Erythromycin	Y= 35452.73 X	0.9999	1-500	0.13	0.39
7	Josamycin	Y= 9725.036 X	0.9998	1-500	0.18	0.54
8	Roxithromycin	Y= 26698.20 X	0.9996	1-500	0.13	0.40

Precision test

Multi-standard solutions of concentrations of 5 μg/L, 20 μg/L and 200 μg/L were injected for 6 successive times to assess the precision of the method. The repeatability results of retention time and peak area are shown in Table 4. The %RSDs of retention time and peak area data of standard solutions of 3 concentrations fell in the ranges of 0.02% ~1.87% and 0.97% ~5.04 %, respectively, suggesting that the method's precision was satisfactory.

Table 4 Repeatability - retention time and peak area (n=6)

Sample name	%RSD (5 µg/L)		%RSD (20 µg/L)		%RSD (200 µg/L)	
	R.T	Area	R.T	Area	R.T	Area
Spiramycin	1.87	3.82	0.17	1.67	0.20	1.22
Tilmicosin	1.50	4.06	0.36	3.53	0.42	2.04
Oleandomycin	0.11	1.83	0.13	2.55	0.08	1.51
Tylosin	0.06	3.40	0.05	2.23	0.05	0.97
Kitasamycin	0.07	4.52	0.09	3.81	0.03	1.68
Erythromycin	0.10	5.04	0.08	4.41	0.08	1.24
Josamycin	0.10	2.97	0.04	3.74	0.03	1.81
Roxithromycin	0.08	5.00	0.05	4.16	0.02	1.06

Spiked matrix test

In order to assess the method's sensitivity, blank pork matrix samples that had been subjected to the sample preparation method as specified in section 1.3 for extraction and purification of analytes were spiked with multi-standard solution at the spiked level of 1 µg/L. MRM chromatograms of blank pork matrix are shown in Fig. 4 and MRM chromatograms of pork matrix spiked with standards are shown in Fig. 5. As can be seen from the chromatograms, the system responded well to the spiked matrix sample at LOQ levels.

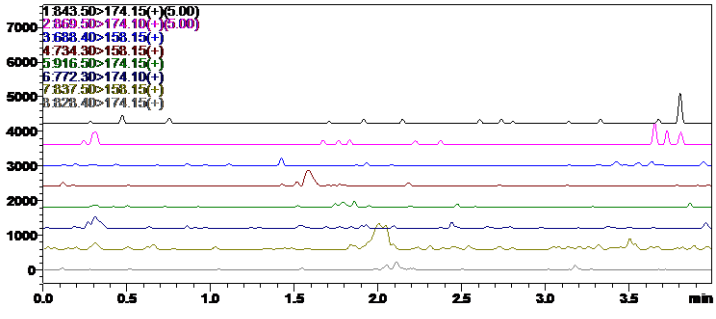


Fig. 4. MRM chromatograms of pork blank matrix sample

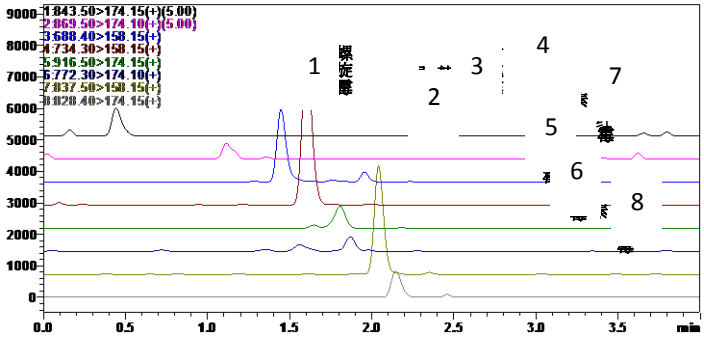


Fig. 5 MRM Chromatogram of pork matrix sample spiked with standards

CONCLUSION

A method was established for the assay of 8 macrolide antibiotics in pork with Shimadzu LC-30A ultra fast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. The method had the merits of fast analysis speed, high sensitivity, and satisfactory precision and demonstrated good linearity for spiramycin and tilmicosin in the concentration range of 5~200 µg/L and oleandomycin, tylosin, kitasamycin, erythromycin, josamycin, and roxithromycin in the concentration range of 1-500 µg/L. The correlation coefficients of calibration curves of all samples were greater than 0.9996. Blank pork matrices that had been subjected to the pretreatment procedures were spiked with multi-standard solution and the matrix sample spiked with standards responded well at LOQ levels.

V-23 Determination of quinolone antibiotics residues in milk by ultra fast liquid chromatography-tandem triple quadrupole mass spectrometry

INTRODUCTION

A method was developed for determination of 14 quinolone antibiotics residues in food of animal origin using Shimadzu ultra fast liquid chromatograph-triple quadrupole mass spectrometer. Samples were, after having been processed, fast separated by the LC-30A ultra fast liquid chromatograph within 7 minutes, and then quantitatively assayed with the LCMS-8030 triple quadrupole mass spectrometer. Calibration curves of the 14 quinolone antibiotics were plotted using the external standard method. The plotted calibration curves were of satisfactory linearity with correlation coefficients higher than 0.999. Standard solutions of various concentrations were used for precision test. The %RSDs of retention time and peak area data of 6 successive injections were below 0.437% and 4.937%, respectively, showing that the method had satisfactory precision.

Quinolones are a category of chemically synthesized antibacterial agents featuring 4-quinolones parent nucleus. They have broad antibacterial spectrum and potent antibacterial activity and are extensively used in livestock husbandry and aquaculture. However, quinolone drugs have potential carcinogenic and genetoxic actions and tend to induce drug resistance of germs. Therefore, people have become more and more concerned by the issue of quinolone drug residues. FDA had imposed a ban on the sales and applications of antibacterial agent enrofloxacin for the treatment of bacterial infection in poultry in 2005. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) and EU has stipulated MRLs in animal tissues for many quinolone drugs.

High performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) technique is developed rapidly in recent years. With such merits as high selectivity and sensitivity, strong capacity for identification of antibiotics residues in complex matrices with high accuracy, it has become the first-choice method for analysis of ultra-trace level of antibiotics residues. A method was developed for the determination of 14 quinolone antibiotics residues in milk using Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer.

EXPERIMENTAL

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The configuration included two LC-30AD pumps, DGU-20A5 online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, LabSolutions Ver. 5.41 chromatography workstation.

Analytical conditions

LC conditions

Column :Shim-pack XR-ODS III 2.0 mm I.D.×150 mm L., 2.2 µm

Mobile phase :A-0.2% formic acid aqueous solution

Mobile phase :B:-methanol/acetonitrile (40/60, v/v)

Flow rate :0.3 mL/min

Column Temperature :40 °C

Injection volume :20 µL

Elution mode :Binary gradient with initial concentration of 20% of mobile phase B, see Table 1 for time program

Table 1 Time program

Time(min)	Module	Command	Value
4.5	Pumps	B Conc.	40
4.6	Pumps	B Conc.	95
5.5	Pumps	B Conc.	95
5.6	Pumps	B Conc.	20
7	Controller	Stop	

MS conditions

Ionization :ESI (+)

Ionization voltage :+4.5 kV

Nebulizing gas :Nitrogen 3.0 L/min

Drying gas	:Nitrogen 15 L/min
Collision gas	:Argon
DL temperature	:250 °C
Heater block Temperature	:400 °C
Mode	:multiple reaction monitoring (MRM)
Dwell time	:20 ms
Pause time	:3 ms
MRM parameters	:See Table 3

Preparation of standard solutions

A total of 14 standard substances, including enoxacin, ofloxacin, norfloxacin, pefloxacin, ciprofloxacin, lomefloxacin, danofloxacin, enrofloxacin, sarafloxacin, oxolinic acid, flumequine, pipemedic acid, nalidixic acid and cinoxacin, were used in the study.

Preparation of standard working solutions: A multi-standard intermediate solution of concentration of 20 mg/L was prepared using methanol as solvent, then diluted into multi-standard working solutions of various concentrations with methanol aqueous solution containing 0.2 % formic acid (20/80, v/v). The detailed concentrations of standard antibiotics are shown in Table 2.

Table 2 Concentrations of standard antibiotics in multi-standard working solution (μg/L)

Compound	Conc.1	Conc.2	Conc.3	Conc.4	Conc.5	Conc.6
Enoxacin	1	5	10	50	100	500
Ofloxacin	0.5	2.5	5	25	50	250
Norfloxacin	5	25	50	250	500	2500
Pefloxacin	1	5	10	50	100	500
Ciprofloxacin	1	5	10	50	100	500
Lomefloxacin	0.5	2.5	5	25	50	250
Danofloxacin	1	5	10	50	100	500
Enrofloxacin	1	5	10	50	100	500
Sarafloxacin	1	5	10	50	100	500
Oxolinic acid	0.5	2.5	5	25	50	250

Flumequine	0.5	2.5	5	25	50	250
Pipemedic acid	0.5	2.5	5	25	50	250
Nalidixic acid	0.5	2.5	5	25	50	250
Cinoxacin	5	25	50	250	500	2500

Sample pretreatment method

Refer to *GB/T 21312-2007 Analysis of fourteen quinolones in food of animal origin by high performance liquid chromatograph tandem mass spectrometry* for the pretreatment procedures of milk samples.

Table 3 MRM parameters

Compound	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
Enoxacin	321.2	303.1*	-16.0	-20.0	-23.0
		203.9	-16.0	-48.0	-23.0
Ofloxacin	362.2	318.2*	-30.0	-18.0	-24.0
		261.1	-30.0	-28.0	-19.0
Norfloxacin	320.2	302.1*	-16.0	-20.0	-23.0
		231.1	-16.0	-46.0	-28.0
Pefloxacin	334.2	316.2*	-13.0	-20.0	-24.0
		290.1	-13.0	-16.0	-22.0
Ciprofloxacin	332.2	314.1*	-13.0	-16.0	-24.0
		231.0	-13.0	-44.0	-18.0
Lomefloxacin	352.2	265.0*	-18.0	-22.0	-20.0
		308.2	-18.0	-16.0	-23.0
Danofloxacin	358.2	340.1*	-14.0	-20.0	-26.0
		255.0	-14.0	-42.0	-29.0
Enrofloxacin	360.3	342.2*	-29.0	-20.0	-26.0
		316.2	-29.0	-20.0	-24.0
Sarafloxacin	386.2	368.1*	-15.0	-20.0	-28.0
		299.1	-15.0	-25.0	-23.0
Oxolinic acid	262.1	244.1*	-30.0	-18.0	-30.0
		216.0	-30.0	-32.0	-26.0
Flumequine	262.1	244.1*	-30.0	-16.0	-18.0

		202.0	-30.0	-36.0	-23.0
Pipemedic acid	304.2	286.1*	-30.0	-18.0	-22.0
		215.1	-30.0	-38.0	-24.0
Nalidixic acid	233.1	215.1*	-30.0	-14.0	-26.0
		187.0	-30.0	-28.0	-22.0
Cinoxacin	263.1	245.1*	-21.0	-16.0	-19.0
		189.0	-21.0	-30.0	-22.0

Note: *refers to quantitative ion

RESULTS AND DISCUSSION

Mass spectra and MS/MS spectra

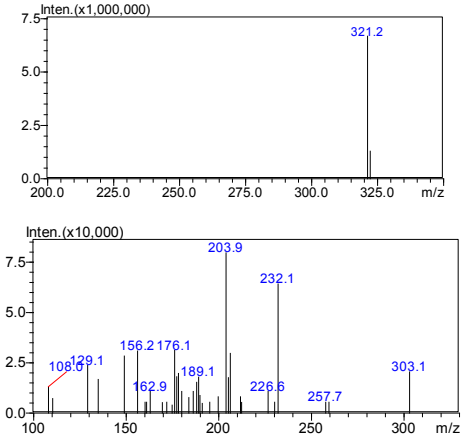


Fig. 1 Mass spectrum (upper) and MS/MS spectrum (lower, CE -48V) of enoxacin

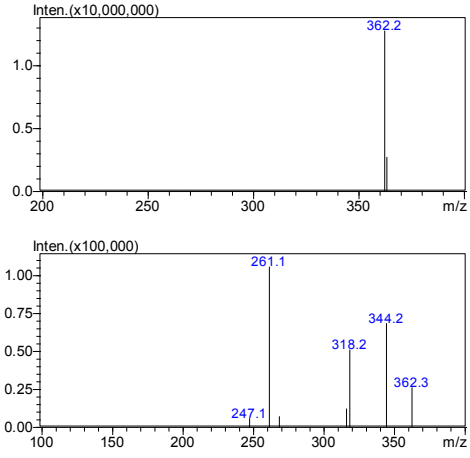


Fig. 2 Mass spectrum (upper) and MS/MS spectrum (lower, CE -25V) of ofloxacin

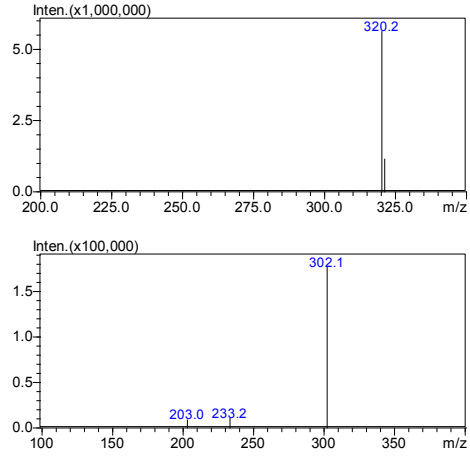


Fig. 3 Mass spectrum (upper) and MS/MS spectrum (lower, CE -25V) of norfloxacin

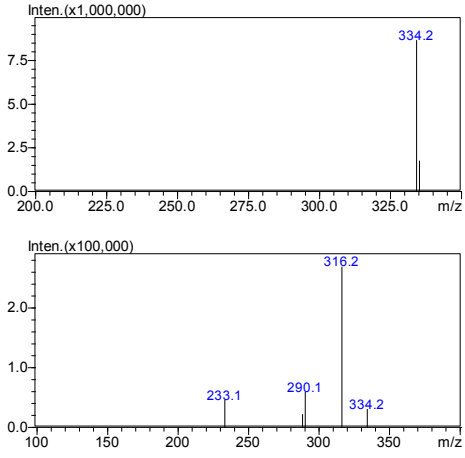


Fig. 4 Mass spectrum (upper) and MS/MS spectrum (lower, CE -25V) of pefloxacin

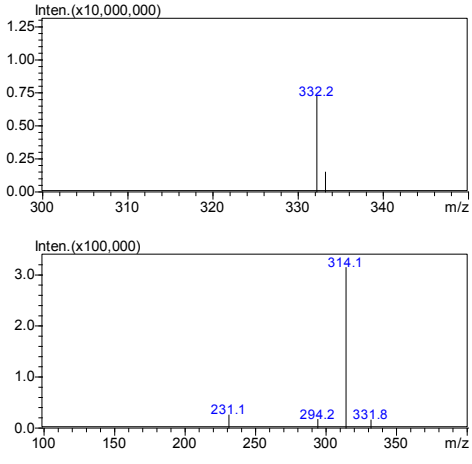


Fig. 5 Mass spectrum (upper) and MS/MS spectrum (lower, CE -25V) of ciprofloxacin

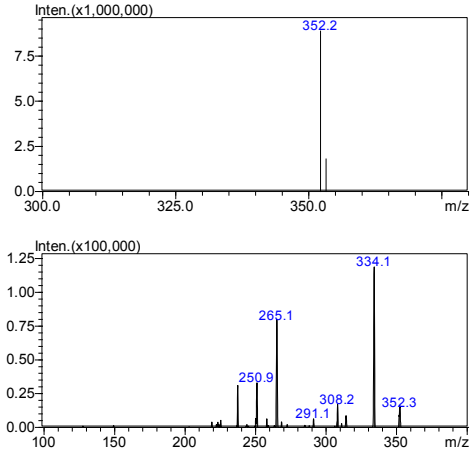


Fig. 6 Mass spectrum (upper) and MS/MS spectrum (lower, CE -25V) of lomefloxacin

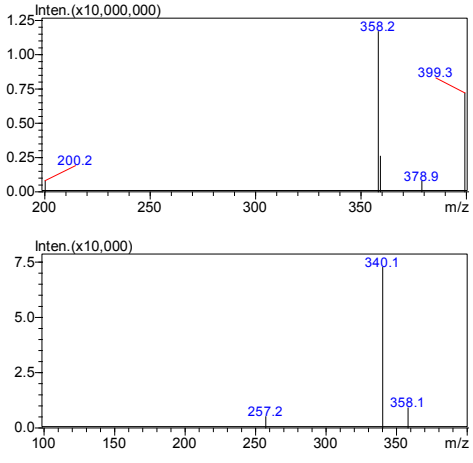


Fig. 7 Mass spectrum (upper) and MS/MS spectrum (lower, CE -25V) of danofloxacin

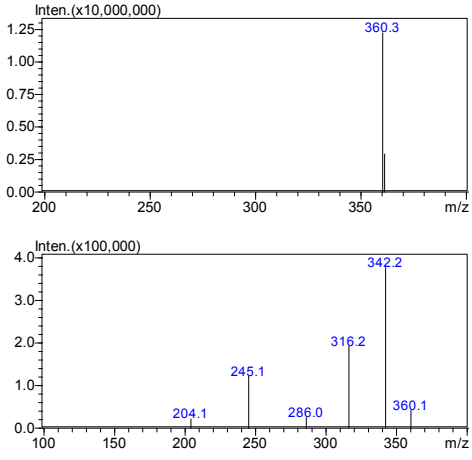


Fig. 8 Mass spectrum (upper) and MS/MS spectrum (lower, CE -25V) of enrofloxacin

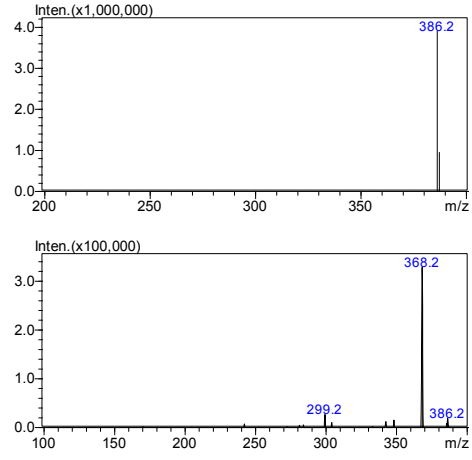


Fig. 9 Mass spectrum (upper) and MS/MS spectrum (lower, CE -25V) of sarafloxacin

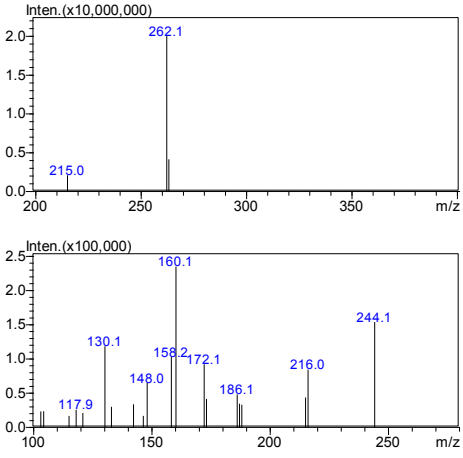


Fig. 10 Mass spectrum (upper) and MS/MS spectrum (lower, CE - 25V) of oxolinic acid

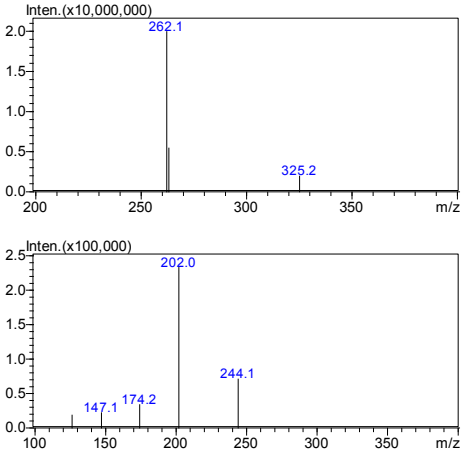


Fig. 11 Mass spectrum (upper) and MS/MS spectrum (lower, CE - 25V) of flumequine

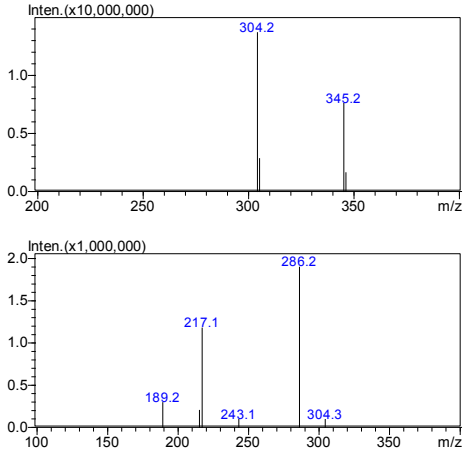


Fig. 12 Mass spectrum (upper) and MS/MS spectrum (lower, CE - 25V) of pipemedic acid

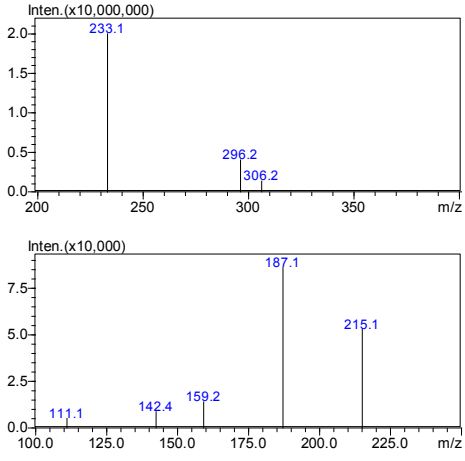


Fig. 13 Mass spectrum (upper) and MS/MS spectrum (lower, CE - 25V) of nalidixic acid

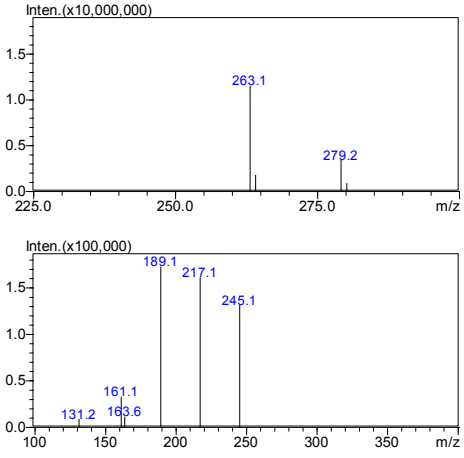


Fig. 14 Mass spectrum (upper) and MS/MS spectrum (lower, CE - 25V) of cinoxacin)

MRM chromatogram of standard mixture

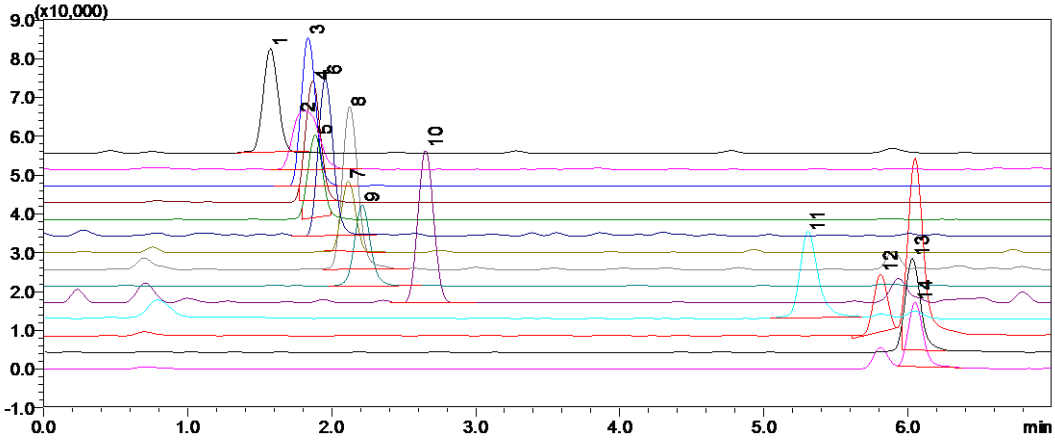


Fig. 15 MRM chromatograms of standard mixture

(1. 2.5 μg/L pipemedic acid; 2. 5 μg/L enoxacin; 3. 2.5 μg/L ofloxacin; 4. 25 μg/L norfloxacin; 5. 5 μg/L pefloxacin; 6. 5 μg/L ciprofloxacin; 7. 2.5 μg/L lomefloxacin; 8. 5 μg/L danofloxacin; 9. 5 μg/L enrofloxacin;10. 5 μg/L sarafloxacin; 11. 25 μg/L cinoxacin; 12. 2.5 μg/L oxolinic acid; 13. 2.5 μg/L nalidixic acid;14. 2.5 μg/L flumequine)

Linear range

Multi-standard working solutions of various concentrations were assayed and quantitatively determined by the external standard method under the analysis conditions as specified above. Calibration curves were plotted as shown in Fig. 16 to Fig. 29 with concentration as abscissa and peak area as ordinate; the calibration curves were of satisfactory linearity

and their linear equations and correlation coefficients are shown inTable 4.

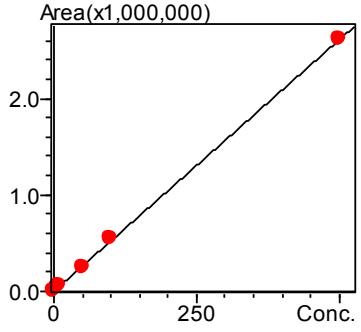


Fig. 16 Calibration curve of enoxacin

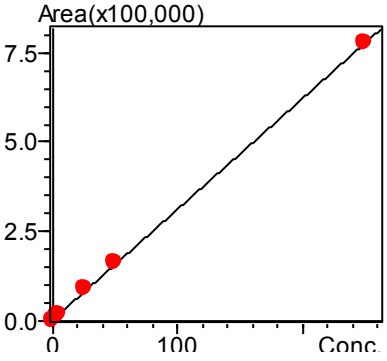


Fig. 17 Calibration curve of ofloxacin

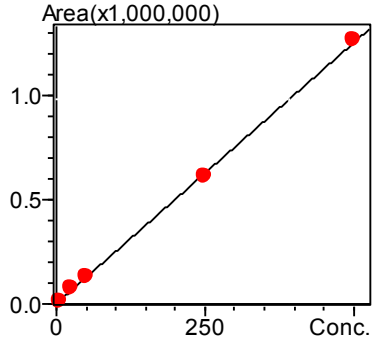


Fig. 18 Calibration curve of norfloxacin

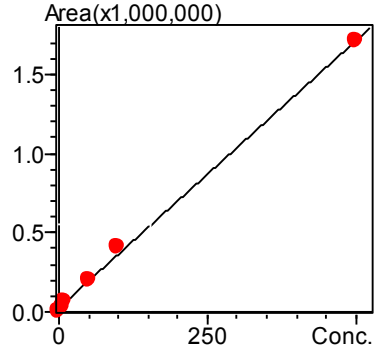


Fig. 19 Calibration curve of pefloxacin

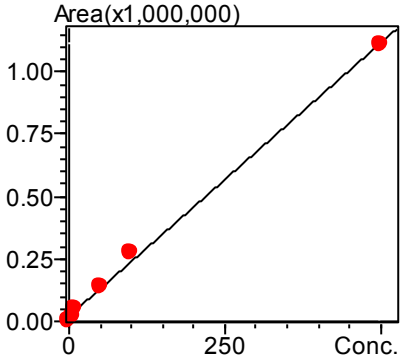


Fig. 20 Calibration curve of ciprofloxacin

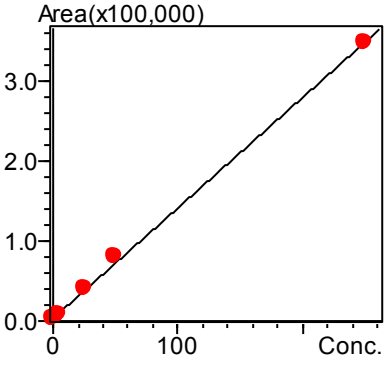


Fig. 21 Calibration curve of lomefloxacin

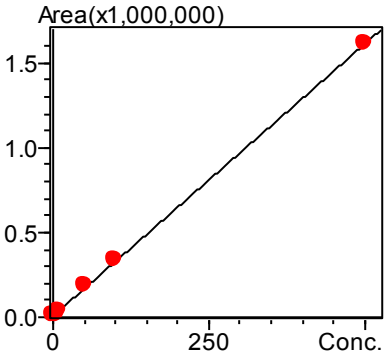


Fig. 22 Calibration curve of danofloxacin

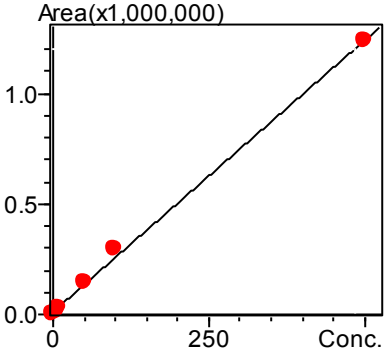


Fig. 23 Calibration curve of enrofloxacin

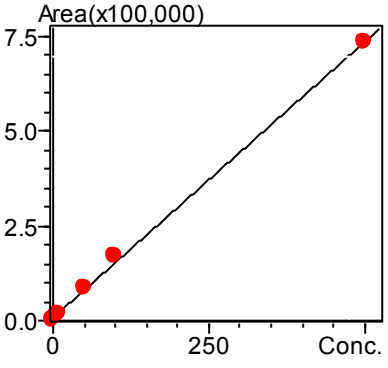


Fig. 24 Calibration curve of sarafloxacin

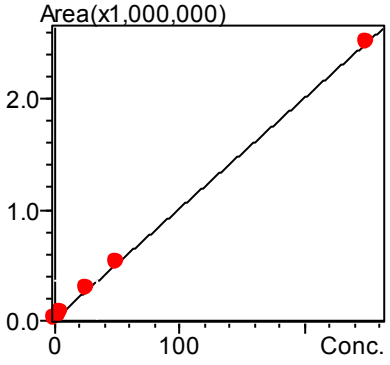


Fig. 25 Calibration curve of oxolinic acid

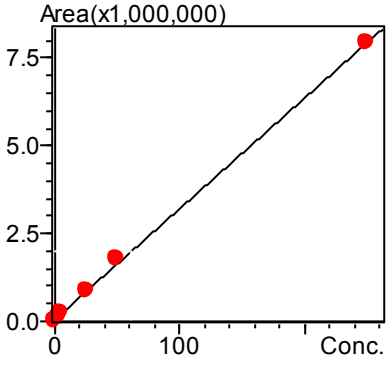


Fig. 26 Calibration curve of flumequine

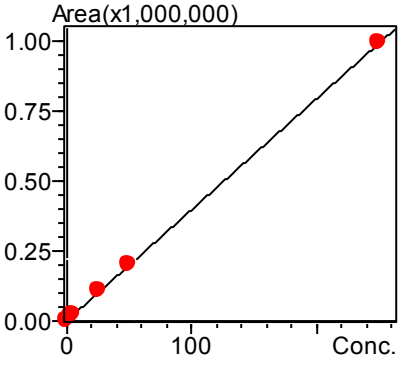


Fig. 27 Calibration curve of pipemedic acid

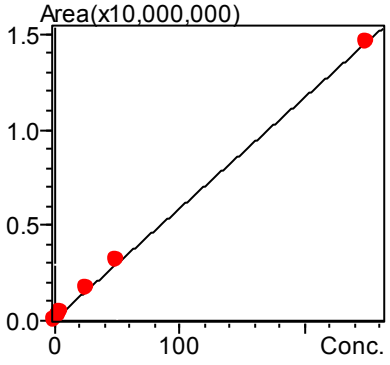


Fig. 28 Calibration curve of pipemedic acid

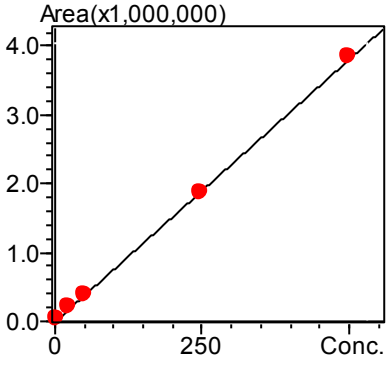


Fig. 29 Calibration curve of cinoxacin

Table 4 Parameters of calibration curves

No.	Compound	Calibration Curve	Linear Range (mg/L)	Correlation Coefficient (r)
1	Enoxacin	Y = (5210.26)X + (3090.70)	1.0~500	1.0000
2	Ofloxacin	Y = (3088.28)X + (4327.79)	0.5~250	0.9998
3	Norfloxacin	Y = (2499.58)X + (1672.78)	5~500	0.9998
4	Pefloxacin	Y = (3370.81)X + (26856.7)	1.0~500	0.9993
5	Ciprofloxacin	Y = (2189.90)X + (21093.5)	1.0~500	0.9992
6	Lomefloxacin	Y = (1382.09)X + (3417.15)	0.5~250	0.9995
7	Danofloxacin	Y = (3207.19)X + (7670.04)	1.0~500	0.9999
8	Enrofloxacin	Y = (2457.58)X + (12362.3)	1.0~500	0.9992
9	Sarafloxacin	Y = (1453.99)X + (7474.59)	1.0~500	0.9994
10	Oxolinic acid	Y = (9946.96)X + (18523.3)	0.5~250	0.9999
11	Flumequine	Y = (31449.4)X + (66461.2)	0.5~250	0.9998
12	Pipemedic acid	Y = (3955.87)X + (2805.99)	0.5~250	1.0000
13	Nalidixic acid	Y = (57834.6)X + (105320)	0.5~250	0.9999
14	Cinoxacin	Y = (7624.15)X + (-8145.69)	5~500	0.9998

Precision test

Multi-standard solutions of various concentrations were assayed for 6 times in succession to evaluate the method's precision. Reproducibility of retention time and peak area data is shown in Table 5. The result showed that the %RSDs of retention time and peak area data of standard solutions of various concentrations fell in the ranges of 0.014 %~0.437 % and 1.309 %~4.937% respectively, suggesting that the method had satisfactory precision.

Table 5 Repeatability - retention time and peak area (n=6)

Compound	%RSD (5 µg/L)		%RSD (10 µg/L)		%RSD (50 µg/L)	
	R.T.	Area	R.T.	Area	R.T.	Area
Enoxacin	0.437	4.576	0.272	3.315	0.152	1.309
Pefloxacin	0.158	4.419	0.248	3.974	0.065	1.762
Ciprofloxacin	0.273	4.706	0.163	3.438	0.085	2.810
Danofloxacin	0.143	3.545	0.186	3.172	0.079	2.468

Enrofloxacin	0.212	4.756	0.225	3.750	0.099	2.719
Sarafloxacin	0.238	4.353	0.060	1.918	0.119	2.622
	%RSD (2.5 µg/L)		**%RSD (5 µg/L)**		**%RSD (25 µg/L)**	
	R.T.	**Area**	**R.T.**	**Area**	**R.T.**	**Area**
Ofloxacin	0.245	4.625	0.300	4.556	0.145	2.719
Lomefloxacin	0.188	4.937	0.140	4.052	0.119	2.916
Oxolinic acid	0.023	4.790	0.023	3.308	0.014	2.450
Flumequine	0.018	2.934	0.014	2.184	0.019	2.251
Pipemedic acid	0.202	3.765	0.268	3.762	0.036	2.645
Nalidixic acid	0.057	4.633	0.010	2.592	0.046	2.738
	%RSD (25 µg/L)		**%RSD (50 µg/L)**		**%RSD (250 µg/L)**	
	R.T.	**Area**	**R.T.**	**Area**	**R.T.**	**Area**
Norfloxacin	0.200	3.989	0.252	2.864	0.069	2.706
Cinoxacin	0.046	4.384	0.075	1.782	0.040	1.344

Sensitivity test

In order to assess the method's sensitivity, a multi-standard solution of low concentration was subjected to assay under the analytical conditions as specified above. S/N ratios and LODs (S/N=3) were calculated with LabSolutionsVer. 5.41. The S/N ratios and LODs of enoxacin, ofloxacin, norfloxacin, pefloxacin, ciprofloxacin, lomefloxacin, danofloxacin, enrofloxacin, sarafloxacin, oxolinic acid, flumequine, pipemedic acid, nalidixic acid and cinoxacin are listed in Table 6.

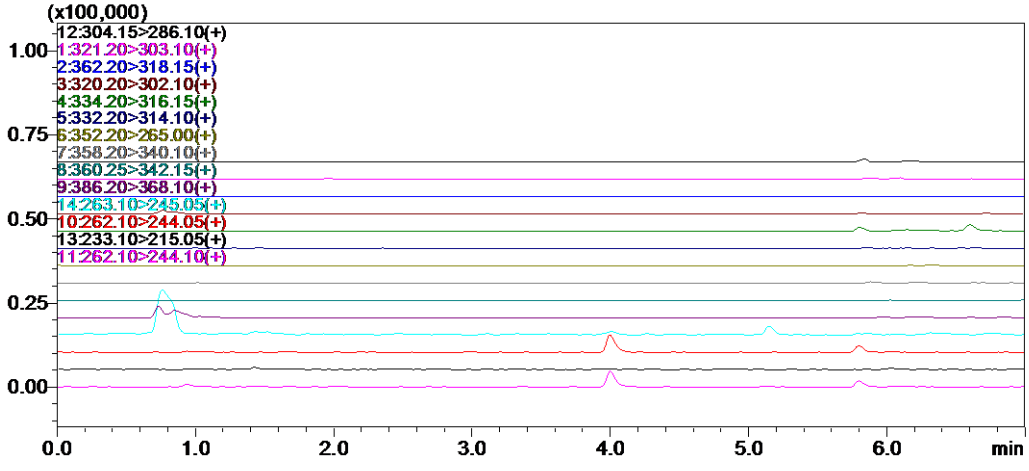
Table 6 S/N ratios and LODs

Compound	Concentration level (µg/L)	S/N	LOD (µg/L)
Enoxacin	1.00	14	0.24
Ofloxacin	0.50	31	0.05
Norfloxacin	5.00	39	0.43
Pefloxacin	1.00	47	0.07
Ciprofloxacin	1.00	8	0.41
Lomefloxacin	0.50	51	0.03
Danofloxacin	1.00	10	0.35
Enrofloxacin	1.00	32	0.10

Sarafloxacin	1.00	9	3.60
Oxolinic acid	0.50	40	0.04
Flumequine	0.50	70	0.02
Pipemedic acid	0.50	27	0.06
Nalidixic acid	0.50	44	0.04
Cinoxacin	5.00	30	0.56

Spiked matrix test

Milk containing none of the 14 quinolone antibiotics was taken as a blank sample for the spiked matrix test. The spiked level of enoxacin, pefloxacin, ciprofloxacin, danofloxacin, enrofloxacin and sarafloxacin was 2 µg/kg, the spiked level of ofloxacin, lomefloxacin, oxolinic acid, flumequine, pipemedic acid and nalidixic acid was 1 µg/kg, and the spiked level of norfloxacin and cinoxacin was 10 µg/kg. MRM chromatograms of a milk sample and a spiked sample are shown in Fig. 30 and Fig. 31, respectively. As can be inferred from the results in Table 7, the method's sensitivity was high enough to meet the LOQ requirements for the assay of quinolone antibiotics in milk samples specified in GB/T 21312-2007 *Analysis of fourteen quinolones in food of animal origin by high performance liquid chromatograph tandem mass spectrometry*.



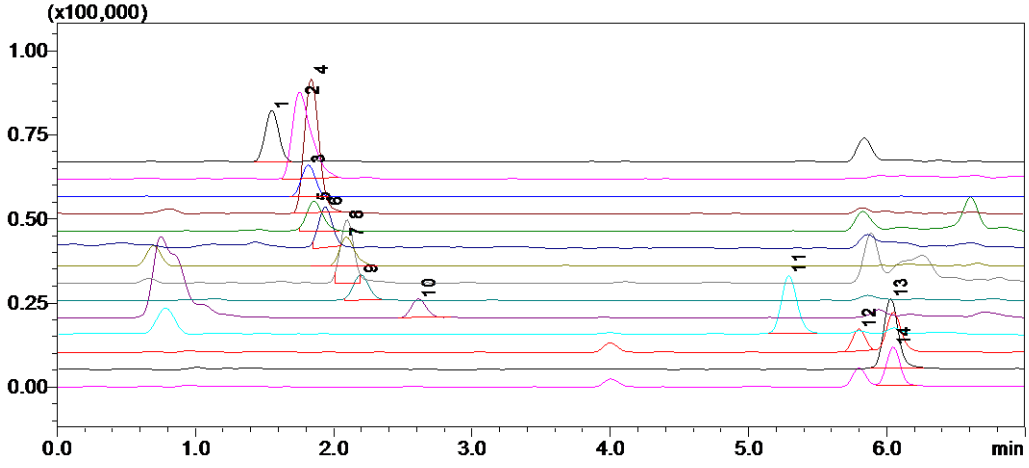


Fig. 31 MRM chromatograms of spiked milk sample

(1. pipemedic acid; 2. enoxacin; 3. ofloxacin; 4. norfloxacin; 5. pefloxacin; 6. ciprofloxacin; 7. lomefloxacin; 8. danofloxacin; 9. enrofloxacin; 10. sarafloxacin; 11. cinoxacin; 12. oxolinic acid; 13. nalidixic acid; 14. flumequine)

Table 7 S/N ratios of antibiotics spiked in milk

Compound	Spiked level (μg/kg)	S/N
Enoxacin	2.00	40
Ofloxacin	1.00	79
Norfloxacin	10.00	45
Pefloxacin	2.00	10
Ciprofloxacin	2.00	11
Lomefloxacin	1.00	7
Danofloxacin	2.00	9
Enrofloxacin	2.00	17
Sarafloxacin	2.00	10
Oxolinic acid	1.00	16
Flumequine	1.00	15
Pipemedic acid	1.00	11
Nalidixic acid	1.00	58
Cinoxacin	10.00	11

CONCLUSION

A method was developed for the determination of 14 quinolone antibiotics residues in milk using Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer. The method completed the separation and analysis of 14 antibiotics within 7.0 minutes with high precision. It demonstrated that the linear range was wide and the correlation coefficients of all calibration curves were greater than 0.999. Precision test was performed on standard solutions of various concentrations. The %RSDs of retention time and peak area data of 6 successive injections were below 0.437% and 4.937%, respectively, showing that the method had satisfactory precision. The method has such merits as ultra fast speed and high sensitivity, making it suitable for fast determination of quinolone antibiotic residues in food of animal origin.

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V-24 Determination of 5-nitroimidazoles residues in fishery products with LCMS-8030

INTRODUCTION

In this paper, a method is proposed for determination of 5-nitroimidazoles residues in fishery products using Shimadzu LC-30A ultra fast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. Analytes in samples that had been processed were fast separated by the LC-30A ultra fast liquid chromatograph within 6 minutes, and then quantitatively assayed with the LCMS-8030 triple quadrupole mass spectrometer. Linearity, precision, LODs, and LOQs of the method in determination of five 5-nitroimidazoles were evaluated. The method demonstrated good linearity for ronidazole, 2-methyl-5-nitromidazol, arilin, 4-nitroimidazole, and ipronidazole in the concentration range of 2~100 μg/L with correlation coefficients all greater than 0.999. Precision test was performed on multi-standard solutions of concentrations of 5 μg/L, 20 μg/L and 100 μg/L. The experiment results showed that the %RSDs of retention time and peak area in 6 successive injections fell in the ranges of 0.07~1.37% and 0.66~2.52%, respectively, suggesting that the method's precision was good. The LODs were 0.17~0.40 μg/L and LOQs were 0.59~1.21 μg/L. 5-nitroimidazoles drugs, a category of antibiotics featuring a 5-nitroimidazole ring, consist of ronidazole, 2-methyl-5-nitromidazol, arilin, 4-nitroimidazole, ipronidazole, etc. 5-nitroimidazoles are commonly used in poultry and livestock feedstuffs to treat and control protozoons and bacteria infections or promote growth. However, these drugs and some of their metabolites can be carcinogenic, teratogenic, mutagenic and genetoxic to mammals. Abuse of 5-nitroimidazoles in feedstuffs may cause the problem of drugs residues in edible animal tissues which jeopardizes people's food safety.

Many assay methods of 5-nitroimidazoles residues in fishery products and livestock products have been reported in China or abroad, of which methods liquid chromatography (LC) and gas chromatography (GC) fail to meet the requirements stipulated in EU Commission Decision 2002/657/EC, gas chromatography-tandem mass spectrometry (GC-MS/MS) requires complicated derivatization pretreatment. Compared to them, liquid chromatography-tandem mass spectrometry (LC-MS/MS) can effectively reduce background interference and improve sensitivity and

therefore will be the first choice for the assay of 5-nitroimidazoles residues. China also promulgated relevant standards on the assay of 5-nitroimidazoles residues, such as *GB/T 21318-2007 Determination of Nitroimidazoles Residues in Foodstuffs of Animal Origin*. In this paper, a method is proposed in reference with GB/T 21318-2007 for determination of residues of 5-nitroimidazoles (ronidazole, 2-methyl-5-nitromidazol, arilin, 4-nitroimidazole and ipronidazole) with Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer for the reference of relevant laboratorians.

EXPERIMENTAL

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.42 chromatography workstation.

Analytical conditions

LC Conditions

Apparatus :LC-30A system

Column :Shim-pack XR-ODS II 2.0 mmI.D.×75 mmL., 2.2 μm

Mobile phase :A:0.1% formic acid

Mobile phase :B:Methanol

Flow rate :0.25 mL/min

Injection volume :10 μL

Column temperature :40 °C

Elution mode: Binary gradient with initial concentration of 15% of phase B.

Table 1 Time program

Time (min)	Module	Command	Value
4.50	Pumps	B Conc.	100
5.00	Pumps	B Conc.	100
5.01	Pumps	B Conc.	15
9.50	Controller	Stop	

MS conditions

Apparatus :LCMS-8030
Ionization :ESI, positive
Ionization voltage :+4.5 kV
Nebulizing gas :Nitrogen, 3.0 L/min
Drying gas :Nitrogen, 15 L/min
Collision gas :Argon
DL temperature :250 °C
Heater block temperature :400 °C
Mode :multiple reaction monitoring (MRM)
Dwell time :35 ms
Pause time :3 ms
MRM parameters:see Table 2

Table 2 MRM parameters

No.	Compound	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
1	Ronidazole	201.0	139.7*	-22.0	-10	-29.0
			54.6	-11.0	-25	-24.0
2	2-methyl-5-nitroimidazole	128.0	41.9*	-20.0	-30	-19.0
			81.9	-10.0	-20	-16.0
3	Arilin	172.0	81.8*	-14.0	-25	-16.0
			127.7	-14.0	-15	-26.0
4	4-nitroimidazole	114.0	67.9*	-14.0	-20	-27.0
			41.1	-21.0	-15	-16.0
5	Ipronidazole	170.1	124.1*	-14.0	-20	-27.0
			109.1	-14.0	-25	-22.0

* refers to quantitative ion.

Sample Preparation

Preparation of standard solution

Standard substances: ronidazole, 2-methyl-5-nitromidazol, arilin, 4-nitroimidazole, ipronidazole.

10 mg of each of the above standard substances was taken and weighed, and then dissolved with methanol and brought to metered volume to get standard intermediate solutions of concentration of 1000 μg/L.

A multi-standard intermediate solution of concentration of 10 mg/L was prepared with methanol as solvent, and then progressively diluted into standard working solutions of concentrations of 100, 50, 20, 10, 5 and 2 μg/L with methanol and water (15/85).

Sample pretreatment method:

Refer to *GB/T 21318-2007 Determination of Nitroimidazoles Residues in Foodstuffs of Animal Origin*.

RESULTS AND DISCUSSION

Mass spectra and MS/MS spectra

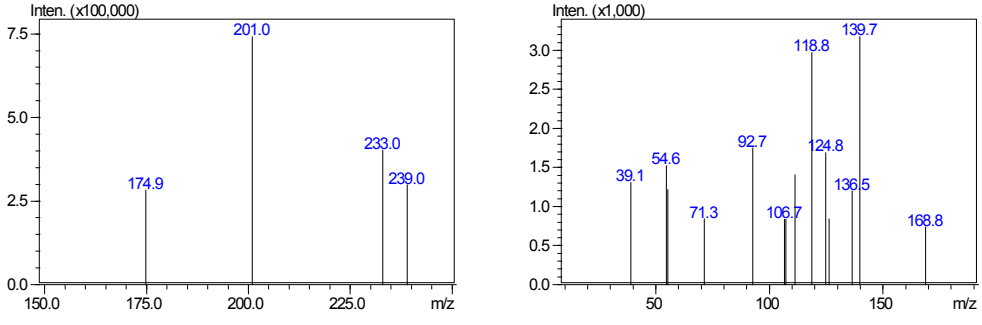

Fig.1 Mass spectrum (left) and MS/MS spectrum (right) of ronidazole

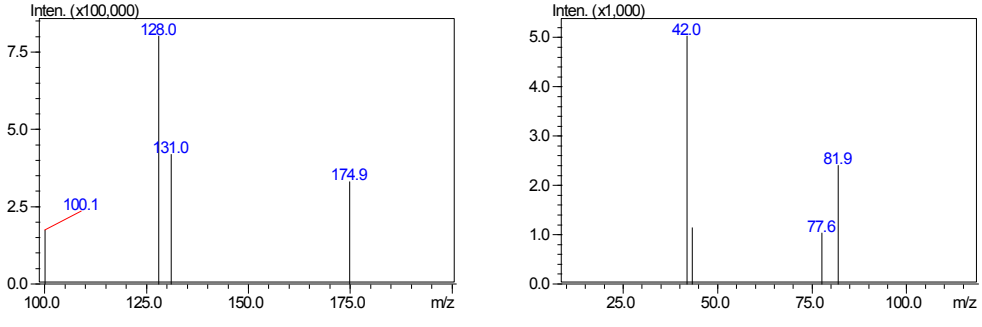

Fig.2 Mass spectrum (left) and MS/MS spectrum (right) of 2-methyl-5-nitroimidazole

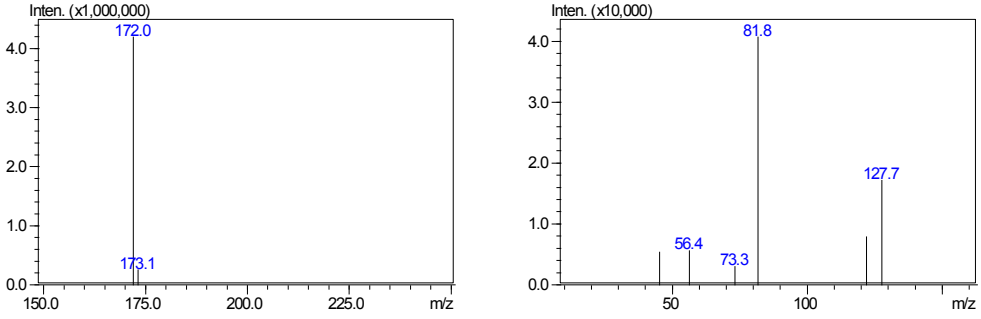

Fig.3 Mass spectrum (left) and MS/MS spectrum (right) of arilin

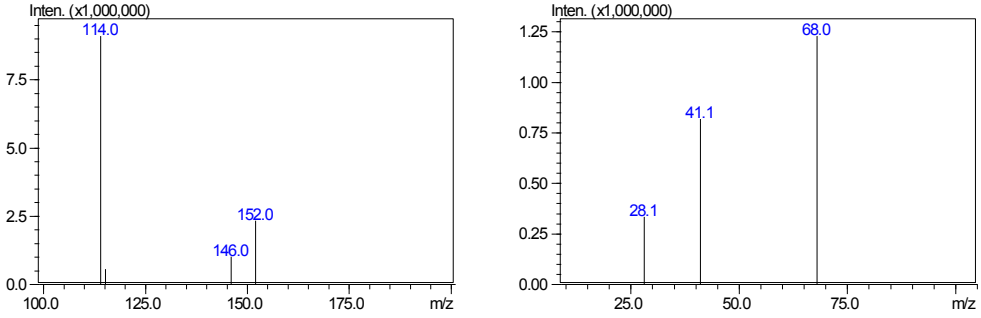

Fig.4 Mass spectrum (left) and MS/MS spectrum (right) of 4-nitroimidazole

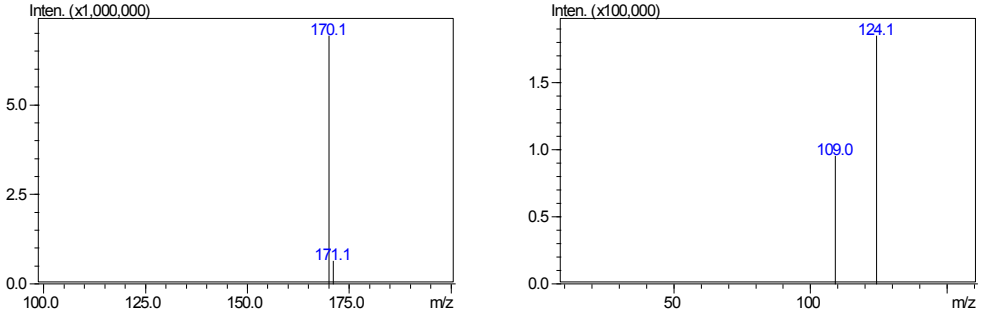

Fig.5 Mass spectrum (left) and MS/MS spectrum (right) of ipronidazole

MRM chromatogram of standard mixture

MRM chromatograms of 50 μg/L multi-standard mixture are shown in Fig. 6.

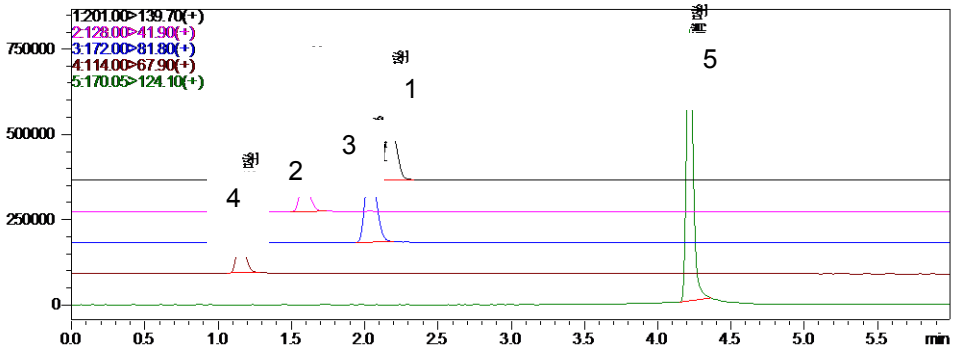

Fig. 6 MRM chromatograms of 50 μg/L multi-standard mixture

Linearity

Multi-standard working solutions of concentrations of 2, 5, 10, 20, 50 and100 μg/L were assayed using the analytical conditions specified above. Calibration curves as shown in Figs. 7 ~ 11 were plotted by the external standard method with concentration as X-axis and peak area as Y-axis. The calibration curves of the 5-nitroimidazoles were of good linearity in the concentration range of 2~100 μg/L. Their linear equations and correlation coefficients are listed in Table3.

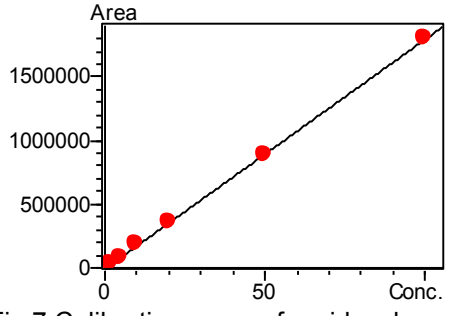

Fig.7 Calibration curve of ronidazole

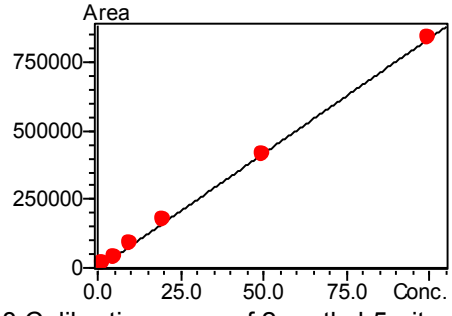

Fig.8 Calibration curve of 2-methyl-5-nitromidazol

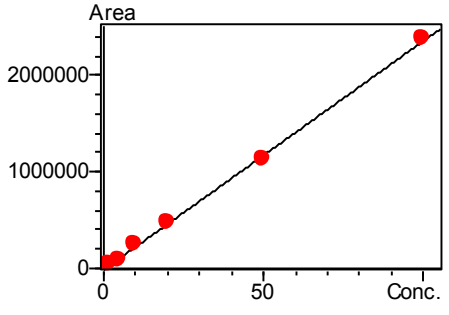

Fig. 9 Calibration curve of arilin

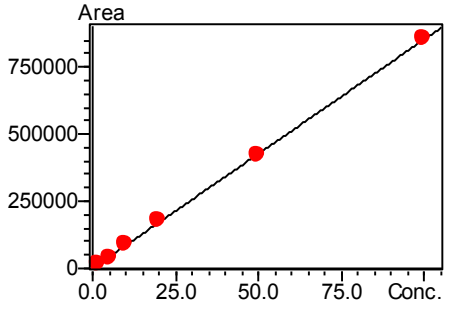

Fig. 10 Calibration curve of 4-nitroimidazole

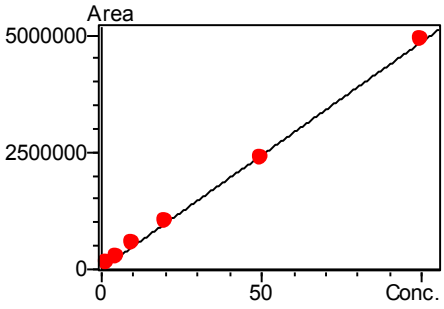

Fig. 11 Calibration curve of ipronidazole

Table 3 Parameters of calibration curves of the 5-nitroimidazoles

No.	Compound	Calibration Curve	Correlation Coefficient (r)
1	Ronidazole	Y = (18033.6)X + (-7926.56)	0.9999
2	2-methyl-5-nitroimidazole	Y = (8405.32)X + (-3629.04)	0.9999
3	Arilin	Y = (23695.8)X + (-13207.9)	0.9996
4	4-nitroimidazole	Y = (8511.96)X + (1085.90)	0.9999
5	Ipronidazole	Y = (48457.1)X + (16721.6)	0.9998

LODs and LOQs

7 standard samples of concentration of 2 μg/L were prepared and then directly injected for analysis. Standard deviation (S) of the results of the 7 assays were calculated after excluding outlier(s), and limits of detection (LODs) were calculated as 3.3S and limits of quantification (LOQs) were calculated as 10S. The assay results are shown in Table 4.

Table 4 LODs and LOQs of the 5 nitroimidazoles

No.	Compound	Standard Deviation (S)	LOD (μg/L)	LOQ (μg/L)
1	Ronidazole	0.06	0.19	0.59
2	2-methyl-5-nitroimidazole	0.09	0.29	0.87
3	Arilin	0.08	0.25	0.77
4	4-nitroimidazole	0.12	0.40	1.21
5	Ipronidazole	0.05	0.17	0.52

Precision test

6 replicate samples of concentrations of 5 µg/L, 20 µg/L and 100µg/L were prepared and injected for analysis in succession. The %RSDs of retention time and peak area data of standard solutions of the 5-nitroimidazoles fell in the ranges of 0.07-1.37% and 0.66 ~ 2.52%, respectively, showing that the method's precision was satisfactory.

Table 5 Repeatability - retention time and peak area (n=6)

No.	Compound	%RSD (5 µg/L)		%RSD (20 µg/L)		%RSD (100 µg/L)	
		R.T	Area	R.T	Area	R.T	Area
1	Ronidazole	1.23	1.53	0.82	1.25	1.16	0.64
2	2-methyl-5-nitroimidazole	1.03	2.52	0.69	1.34	0.93	1.22
3	Arilin	1.14	1.72	0.76	1.37	1.08	0.76
4	4-nitroimidazole	0.75	2.16	0.51	1.10	0.97	0.86
5	Ipronidazole	0.15	1.85	0.07	0.91	0.10	0.66

Spiked matrix test

In order to assess the method's sensitivity, blank fish meat matrix samples that had been subjected to the sample preparation method as specified above for extraction and purification of analytes were spiked with multi-standard solution at the spiked level of 2 µg/L (0.1 µg/kg). MRM chromatograms of the blank fish meat matrix are shown in Fig. 12 and MRM chromatograms of the fish meat matrix spiked with standards are shown in Fig. 13. As can be seen from the chromatograms, the system responded well to the matrix samples spiked with 2 µg/L standards.

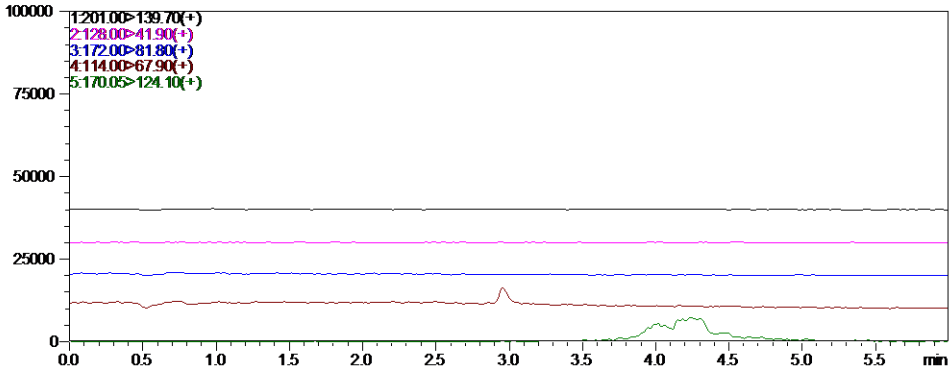

Fig. 12 Chromatogram of fish meat blank matrix samples

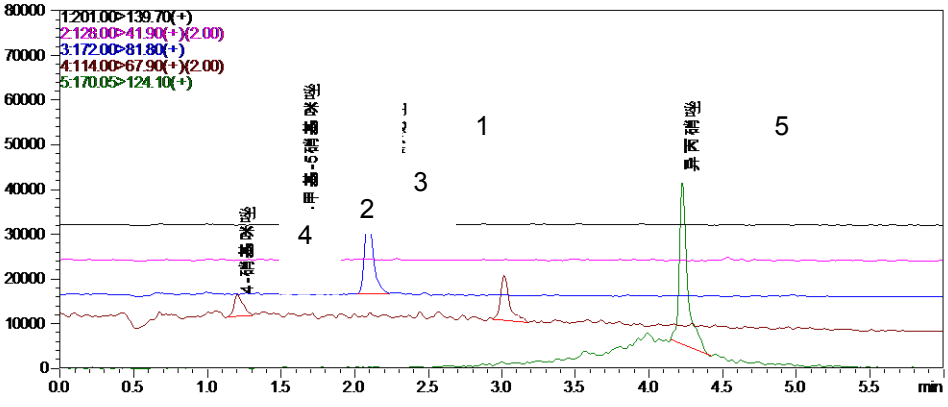

Fig.13 Chromatogram of matrix samples spiked with standards

CONCLUSION

A method was developed in reference with GB/T 21318-2007 for the determination of 5 nitroimidazoles residues in fish products using Shimadzu LC-30A ultra-performance liquid chromatograph in conjunction with LCMS-8030 triple quadrupole mass spectrometer. The method demonstrated good linearity for 5 nitroimidazoles, including ronidazole, 2-methyl-5-nitromidazol, arilin, 4-nitroimidazole, and ipronidazole, in the concentration range of 2~100 μg/L with all correlation coefficients greater than 0.999. The method's LODs were 0.17-0.40 μg/L and LOQs were 0.59~1.21 μg/L. Fish meat matrix that had been subjected to sample pretreatment procedures was spiked with standards at the level of 0.1 μg/kg, and the spiked matrix samples responded well to the method. The method's LODs are lower than the required LOD of 0.5 μg/kg specified in *GB/T 21318-2007 Determination of Nitroimidazoles Residues in Foodstuffs of Animal Origin*.

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V-25 Determination of lincosamides residues in fishery products by triple quadrupole mass spectrometry

INTRODUCTION

This paper intends to propose a method for determination of lincosamides residues in fishery products using Shimadzu LC-30A ultra fast liquid chromatograph combined with Shimadzu LCMS-8030 triple quadrupole mass spectrometer. Fishery product samples after pretreatment were separated by the LC-30A ultra fast liquid chromatograph, and then assayed with the LCMS-8030 triple quadrupole mass spectrometer. The proposed method was of good linearity for lincomycin hydrochloride in the concentration range of 1-100 μg/L and clindamycin hydrochloride in the concentration range of 1-100 μg/L, the correlation coefficients of calibration curves were all greater than 0.9996; precision test was carried out on multi-standard solutions of lincomycin and clindamycin at concentrations of 1 μg/L, 5 μg/L and 10 μg/L by 6 replicate injections, and the %RSDs of retention time and peak area were below 0.31% and 3.95%, respectively, suggesting that the method had satisfactory precision.

Lincosamides, including lincomycin, are category of antibiotics generated by actinobacillus or micromonosporaceae. They demonstrate strong antibacterial action against Gram-positive bacteria, certain anaerobic bacteria and mycoplasma but their antibacterial spectrum is narrower than erythromycin. Staphylococcus aureus, hemolytic streptococcus, pneumonococcus and porcine mycoplasma hyopneumoniae, and mycoplasma gallisepticum are sensitive to this category of antibiotics; anaerobic bacteria such as bacteroids, tetanus bacillus, clostridia, clostridieum welchii, peptococcus are also sensitive to them. Enterococci, however, are generally resistant to these drugs. Lincosamides are mainly used for the treatment of various infections caused by Gram-positive bacteria, especially penicillin-resistant ones, including poultry chronic respiratory diseases and porcine enzootic pneumonia caused by mycoplasma, anaerobic bacteria infections such as necrotic enteritis in chicken, swine dysentery, toxoplasmosis, and actinomycosis in dogs and cats. It is stipulated in GB/T 20762-2006 Method for the determination of lincomycin, oleandomycin, erythromycin, tilmicosin, tylosin, clindamycin, spiramycin, kitasamycin, and josamycin residues in livestock and poultry muscles—LC-MS-MS method that the maximum residue limits (MRLs) for lincomycin and clindamycin are both 1.0 μg/kg. In this paper, a method was

proposed for the determination of lincomycin and clindamycin residues in fishery products using Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer for the reference to relevant laboratorians.

EXPERIMENTAL

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The specific configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.41 chromatography workstation.

Conditions of Analysis

LC Conditions

Apparatus	:LC-30A system
Column	:Shim-pack XR-ODS III
	(2.0mm ID×75 mmL, 2.2 μm)
Mobile phase	:0.1 %formic acid aqueous solution/methanol
	(20/80)
Flow rate	:0.2 mL/min
Injection volume	:10 μL
Column temperature	:40 °C

MS conditions

Apparatus	:LCMS-8030
Ionization	:ESI, positive mode scan
Ionization voltage	:+4.5kV
Nebulizing gas	:Nitrogen, 3.0 L/min
Drying gas	:Nitrogen, 15.0 L/min
Collision gas	:Argon
DL temperature	:250 °C
Heater block temperature	:400 °C
Scan mode	:Multiple Reaction Monitoring (MRM)

Dwell time :100 ms

Delay time :3 ms

MRM parameters: See Table 1

Table 1 MRM parameters

No.	Compound	Precursor ion	Product ion	Q1 Pre bias (V)	CE (V)	Q2 Pre bias (V)
1	Lincomycin	407.3	126.1*	-20	-40	-17
			359.3	-20	-20	-27
2	Clindamycin	425.3	126.1*	-21	-50	-10
			377.3	-21	-20	-28

* refers to quantitative ion.

Sample Preparation

Preparation of standard solutions: Standard substances of lincomycin and clindamycin were taken in sufficient quantity, accurately weighed, and dissolved in methanol to prepare a multi-standard stock solution of the concentration of 1000 mg/L, which was then diluted with the mobile phase to get a series of standard working solutions of concentrations of 1, 5, 10, 20, 50, and 100 μg/L.

Method for sample pretreatment: The preparation of fishery products samples and extraction and purification of analytes were done in reference with GB/T 20762-2006 Method for the determination of lincomycin, oleandomycin, erythromycin, tilmicosin, tylosin, clindamycin, spiramycin, kitasamycin, and josamycin residues in livestock and poultry muscles—LC-MS-MS method.

RESULT AND DISCUSSION

Mass spectrum and MS/MS spectrum

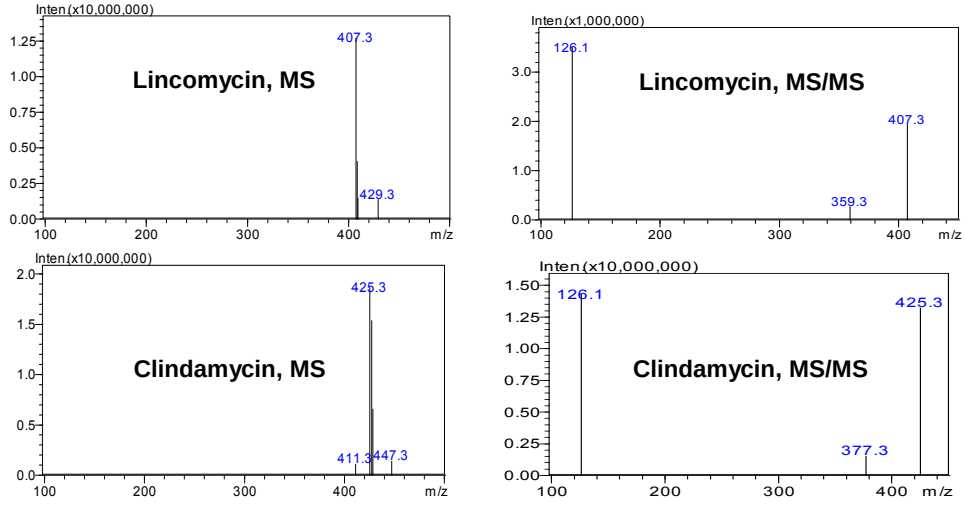

Fig. 1 Mass spectrum and MS/MS spectrum

MRM chromatogram of standard mixture

Fig. 2 shows the MRM chromatograms of 50 μg/L standard mixture. Lincomycin and clindamycin were assayed within 2 minutes.

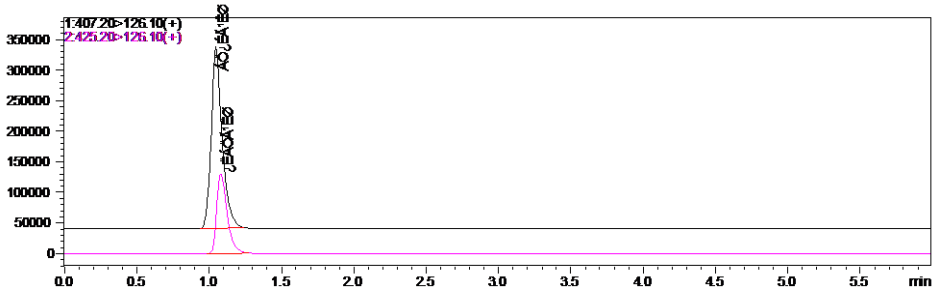

Fig. 2.MRM chromatograms of 50 μg/L standard sample of the lincosamides

2.3 Linearity

Multi-standard working solutions of concentrations of 1, 5, 10, 20, 50, and 100 μg/L were assayed under the analysis conditions specified above. Calibration curves were plotted as shown in Fig. 3 with concentration as X-axis and peak area as Y-axis. The plotted calibration curves were of good linearity for lincomycin in the concentration range of 1-100 μg/L and clindamycin in the concentration range of 1-100 μg/L. The linear equations and correlation coefficients are listed in Table 2.

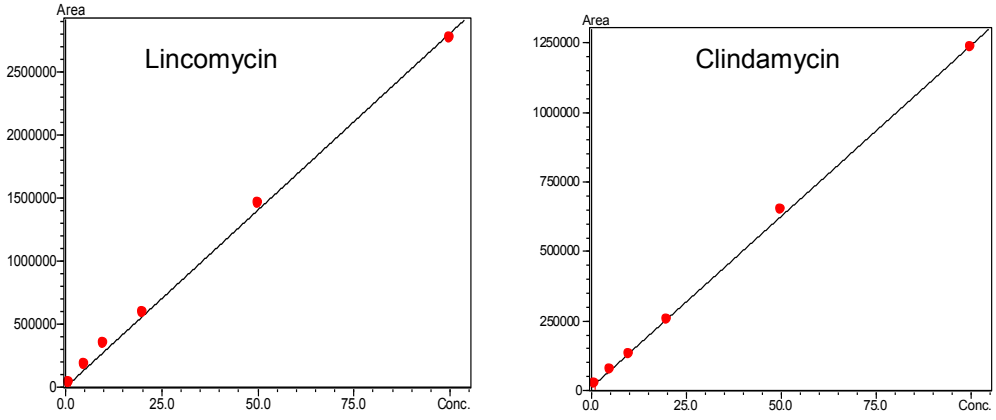

Fig. 3. Calibration curves of lincomycin and clindamycin

Table 2. Parameters of the calibration curves of lincomycin and clindamycin

No.	Compound	Calibration Curve	Correlation Coefficient (r)	Linear Range (µg/L)
1	Lincomycin	Y= 28070.0 X	0.9996	1-100
2	Clindamycin	Y= 12478.9 X	0.9997	1-100

LODs and LOQs

7 standard samples of concentration of 1 µg/L were prepared and then directly injected for analysis. Standard deviation (S) of the results of the 7 assays were calculated after excluding outlier(s), wherein limits of detection (LODs) = 3.3S and limits of quantification (LOQs) = 10S. The assay results are shown in Table 3.

Table 3 LODs and LOQs of lincomycin and clindamycin

No.	Compound	Standard Deviation (S)	LOD (µg/L)	LOQ (µg/L)
1	Lincomycin	0.07	0.22	0.68
2	Clindamycin	0.04	0.13	0.39

Precision test

Multi-standard solutions of lincomycin and clindamycin at concentrations of 1 µg/L, 5 µg/L and 10 µg/L were injected 6 consecutive times for evaluating the method's precision. The repeatability results of retention time and peak area are shown in Table 4. The %RSDs of retention time and peak area data of standard solutions of 3 concentrations fell in the ranges of 0.02% -

1.87% and 0.97% - 5.04 %, respectively, suggesting that the method's precision was satisfactory.

Table 4 Repeatability - retention time and peak area (n=6)

Compound	%RSD (1 µg/L)		%RSD (5 µg/L)		%RSD (10 µg/L)	
	R.T.	Area	R.T.	Area	R.T.	Area
Lincomycin	0.31	3.01	0.11	2.37	0.17	2.82
Clindamycin	0.26	3.95	0.19	3.26	0.16	3.21

Spiked matrix test

In order to evaluate the method's sensitivity, blank pork matrix samples that had been subjected to the sample preparation method as specified above for extraction and purification of analytes were spiked with multi-standard solution of lincomycin and clindamycin at the spiked level of 1 µg/L. The MRM chromatograms of the blank fishery product matrix are shown in Fig. 4 and the MRM chromatograms of the fishery product matrix spiked with standards are shown in Fig. 5. As can be seen from the MRM chromatograms, the system responded well to all spiked matrix samples at LOQ levels.

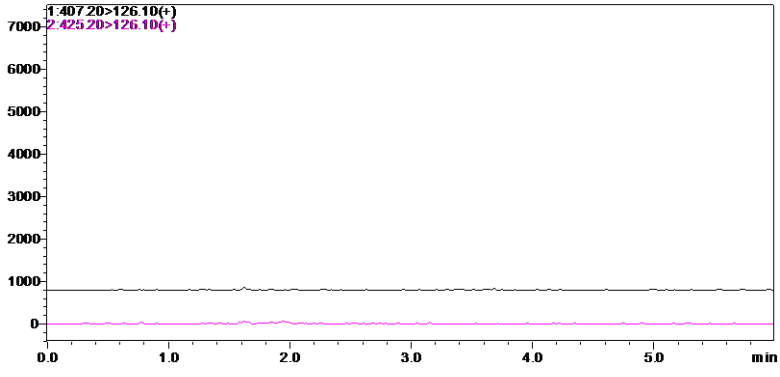

Fig. 4. MRM chromatograms of fishery product blank matrix

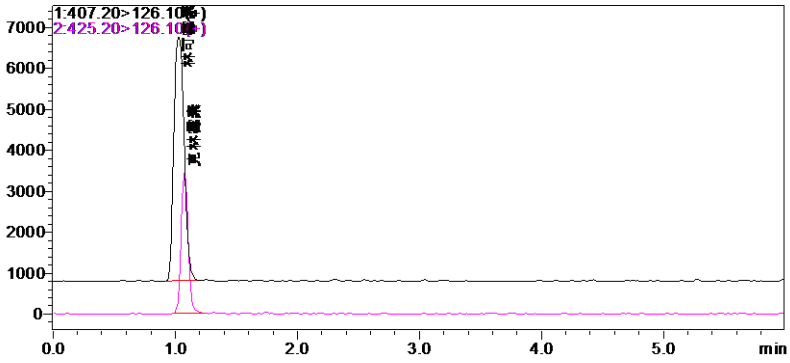

Fig. 5. MRM chromatograms of the fishery product blank matrix sample spiked with standards

CONCLUSION

We established a method for the determination of lincomycin and clindamycin in fishery products using Shimadzu LC-30A UFLC and LCMS-8030 triple quadrupole mass spectrometer. The method demonstrated high sensitivity and satisfactory precision and good linearity for the assay of lincomycin and clindamycin in the concentration range of 1-100 µg/L with correlation coefficients greater than 0.9996. It responded well to the samples of matrix spiked with standards which were prepared by subjecting fishery product blank matrices to the specified pretreatment procedures.

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V-26 Determination of Sulfonamide Residues in Aquatic Products with LCMS-8040

INTRODUCTION

A method is proposed for determination of sulfonamide residues in aquatic products using Shimadzu UFLCXR ultra fast liquid chromatograph and LCMS-8040 triple quadrupole mass spectrometer. Sulfonamide residues were separated by UFLCXR ultra fast liquid chromatograph within 5 minutes, and then subjected to quantitative analysis with an LCMS-8040 triple quadrupole mass spectrometer. The method is capable of fast and accurate determination of 12 sulfonamide residues. The method showed good linearity for the 12 sulfonamides, all of which had a correlation coefficient greater than 0.999; the results of precision test at various concentrations demonstrated that the %RSDs of retention time and peak area fell in the ranges of 0.05 ~ 0.40% and 1.00 ~ 5.57%, respectively. The LODs were 0.10 ~ 0.24 μg/L, the LOQs were 0.41 ~ 0.97 μg/L. The recoveries of spiked samples were 85.7 ~ 116.5%, LOQ was 0.20 μg/kgChina's aquiculture has developed rapidly in recent years. With increased intensification of aquiculture, aquiculture diseases are becoming a tougher challenge. Various drugs are widely used in the production of aquatic products, and the problem of drug residue is becoming increasingly prominent. Sulfonamides are a group of synthesized antibacterial agents and antipathogens in common use which can lead to various side effects such as allergic reactions and drug resistance. Therefore, many countries around the world have set up strict MRLs for them. The monitoring of sulfonamide residues is of positive significance for the promotion of aquatic products export and the guaranty of food safety of products of animal origin.

So far, China has established a number of standards on the determination of sulfonamide residues, including national standard GB/T 22951-2008, commodity inspection standard SN 0208-93 and agricultural standard NY 1077-1-2008. All of these standards use LC or LC-MS/MS for determination of sulfonamide residues. In this paper, a method for determination of sulfonamide residues in aquatic products using Shimadzu UFLCXR ultra fast liquid chromatograph and LCMS-8040 triple quadrupole mass spectrometer is proposed in reference with *Simultaneous*

determination of 17 sulfonamides and 15 quinolones residues in aquatic products by LC-MS/MS method, a national standard promulgated by Ministry of Agriculture of the People's Republic of China in No. 1077 Announcement-1-2008, for the reference of laboratory analysts. The method has the merits of fast speed, simple operation, good selectivity and high sensitivity.

EXPERIMENTAL

Apparatus

A combined system of Shimadzu UFLCXR ultra fast liquid chromatograph and LCMS-8040 triple quadrupole mass spectrometer was used in the experiment. The configuration included two LC-20AD$_{XR}$ pumps, DGU-20A$_3$ online degasser, SIL-20AC$_{XR}$ autosampler, CTO-20A column oven, CBM-20A communications bus module, LCMS-8040 triple quadrupole mass spectrometer, LabSolutions Ver. 5.53.

Analytical conditions

LC Conditions

Instrument	:UFLCXR system
Column	:Shim-pack XR-ODSIII (2.0 mmI.D.×50 mmL. 1.6 μm)
Mobile phase	:A:5 mM ammonium acetate + 0.1% formic acid aqueous solution
Mobile phase	:B:acetonitrile
Flow rate	:0.4 mL/min
Injection volume	:10 μL
Column temperature	:40 ºC
Elution mode	:Binary gradient with initial concentration of 15% of mobile phase B

See Table 1 for time program.

Table 1Time program

Time (min)	Module	Command	Value
2.50	Pumps	B Conc.	30
3.50	Pumps	B Conc.	30
3.60	Pumps	B Conc.	55
4.00	Pumps	B Conc.	55
4.01	Pumps	B Conc.	15
5.50	Controller	Stop	

MS conditions

Instrument :LCMS-8040
Ionization :ESI, positive
Ionization voltage :+4.5 kV
Nebulizing gas :Nitrogen, 3.0 L/min
Drying gas :Nitrogen, 15 L/min
Collision gas :Argon
DL temperature :250 ºC
Heater block temperature :400 ºC
Mode :MRM
Dwell time :10 ms
Pause time :3 ms
MRM parameters :see Table 2

Table 2 MRM parameters

No.	Name	Precursor ion	Product ion	Q1 Pre bias(V)	CE (V)	Q3 Pre bias(V)
1	Sulfacetimide	215.1	156.1*	-14	-9	-16
			92.2	-14	-23	-18
2	Sulfadiazine	251.1	156.0*	-17	-14	-16
			108.1	-17	-23	-20
3	Sulfathiazole	256.1	156.0*	-17	-14	-16
			108.2	-17	-24	-21
4	Sulfapyridine	250.2	156.0*	-17	-15	-16
			92.1	-17	-26	-10
5	Sulfamerazine	265.1	156.0*	-18	-17	-16
			92.1	-18	-29	-18
6	Sulfadimidine	279.2	186.1*	-19	-16	-20
			92.1	-19	-30	-18
7	Sulfamethoxypyridazine	280.9	156.1*	-18	-17	-16
			92.1	-18	-30	-18
8	Sulfapyridine	284.9	156.1*	-19	-14	-16
			92.1	-19	-31	-17
9	Sulfamethoxazole	254.2	156.1*	-17	-14	-16
			92.1	-17	-27	-10
10	Sulfisoxazole	268.0	156.0*	-18	-12	-16
			92.2	-18	-26	-19
11	Sulfadimethoxypyrimidine	311.0	156.1*	-21	-21	-16
			92.1	-21	-31	-18
12	Sulfaquinoxaline	301.0	156.0*	-20	-15	-16
			92.1	-20	-31	-19

* refers to quantitative ion.

Sample Preparation

12 standard substances, i.e. sulfacetimide, sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfadimidine, sulfamethoxypyridazine, sulfapyridine, sulfamethoxazole, sulfisoxazole, sulfadimethoxypyrimidine and sulfaquinoxaline, were used in the experiment.

Preparation of standard solutions: 1 mg/L multi-standard intermediate solution was prepared using methanol as solvent, and then diluted with methanol aqueous solution (v/v, 15:85) into multi-standard working solutions of concentrations of 1 μg/L, 5 μg/L, 10 μg/L, 50 μg/L, 100 μg/L and 200 μg/L.

Sample pretreatment method: Refer to *Simultaneous determination of 17 sulfonamides and 15 quinolones residues in aquatic products by LC-MS/MS method*, which is a national standard promulgated by Ministry of Agriculture of the People's Republic of China in No. 1077 Announcement-1-2008, for details.

RESULTS AND DISCUSSION

Mass spectrum and MS/MS spectrum

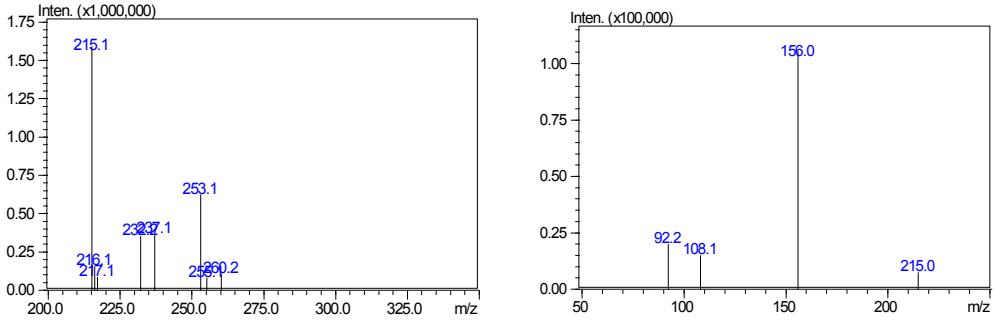

Fig. 1 Mass spectrum (left) and MS/MS spectrum (right, CE -12V) of sulfacetimide

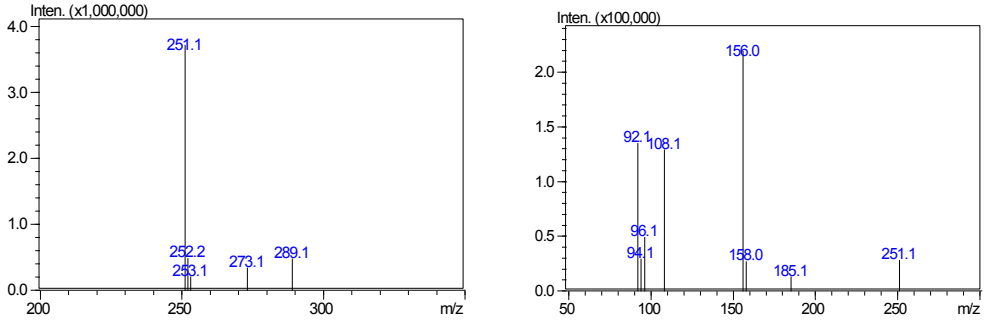

Fig. 2 Mass spectrum (left) and MS/MS spectrum (right, CE -18V) of sulfadiazine

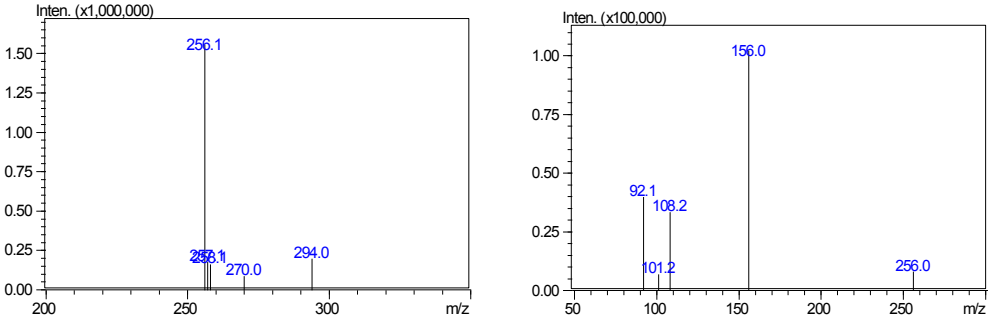

Fig. 3 Mass spectrum (left) and MS/MS spectrum (right, CE -17V) of sulfathiazole

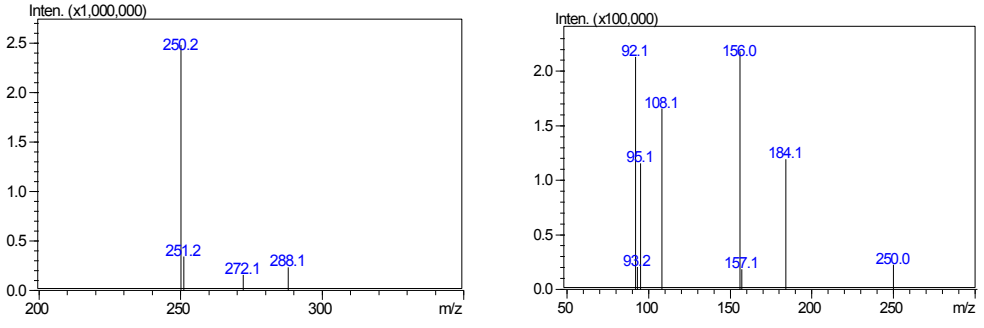

Fig. 4 Mass spectrum (left) and MS/MS spectrum (right, CE -21 V) of sulfapyridine

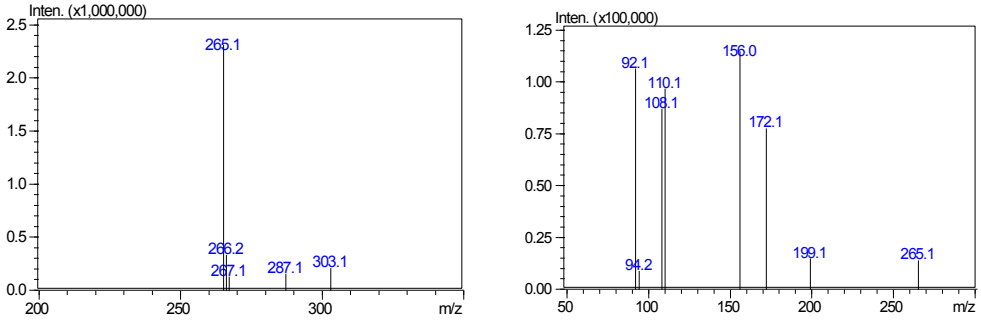

Fig. 5 Mass spectrum (left) and MS/MS spectrum (right, CE -20 V) of sulfamerazine

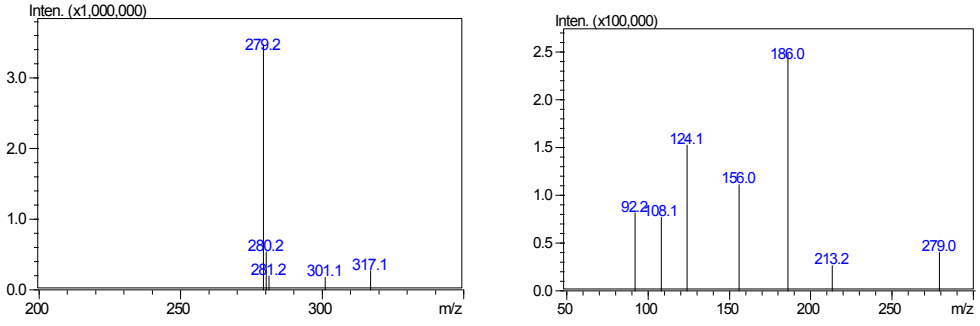

Fig.6 Mass spectrum (left) and MS/MS spectrum (right, CE -20 V) of sulfadimidine

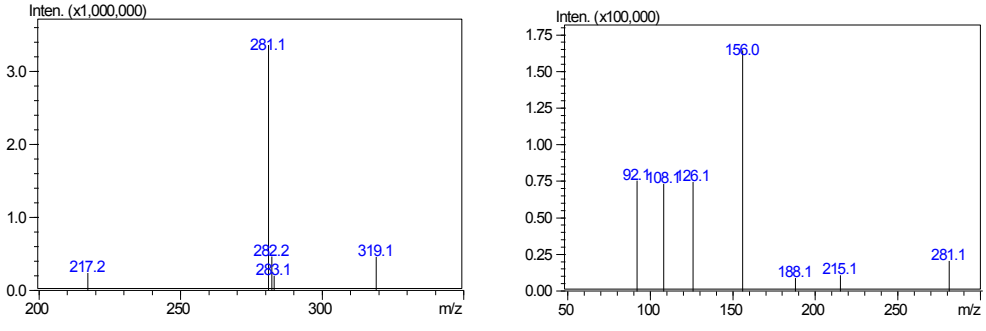

Fig.7 Mass spectrum (left) and MS/MS spectrum (right, CE -20 V) of sulfamethoxypyridazine

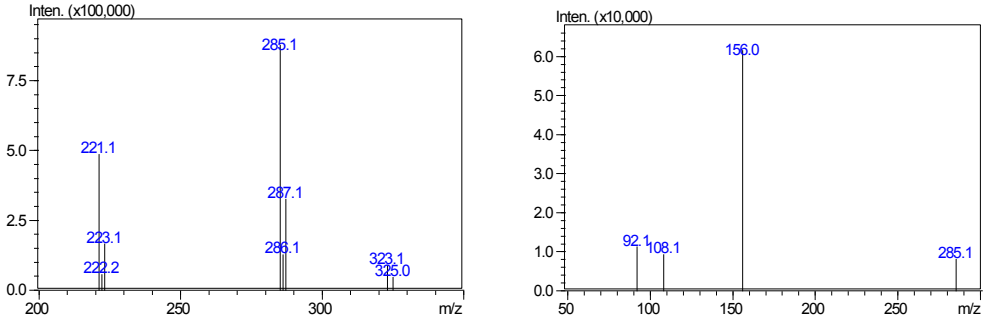

Fig.8 Mass spectrum (left) and MS/MS spectrum (right, CE -16 V) of sulfapyridine

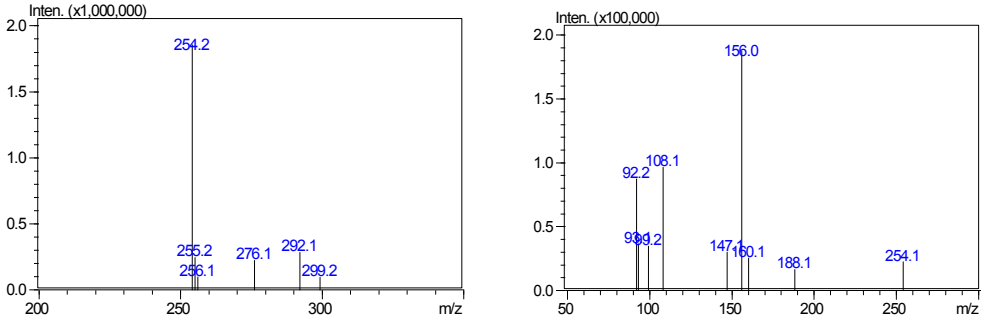

Fig.9 Mass spectrum (left) and MS/MS spectrum (right, CE -18 V) of sulfamethoxazole

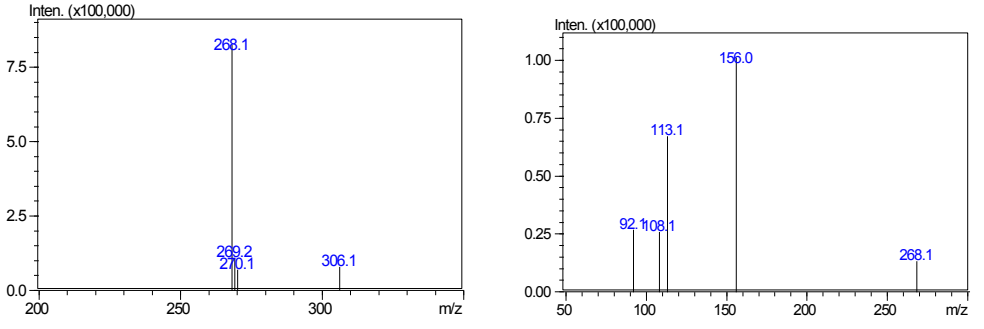

Fig.10 Mass spectrum (left) and MS/MS spectrum (right, CE -16 V) of sulfisoxazole

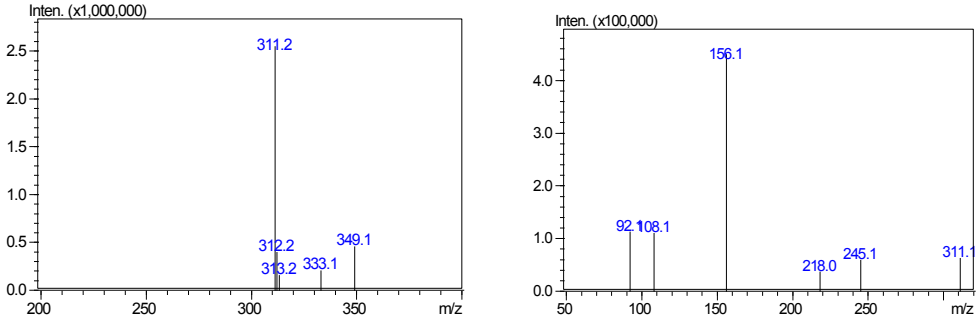

Fig.11 Mass spectrum (left) and MS/MS spectrum (right, CE -22 V) of sulfadimethoxypyrimidine

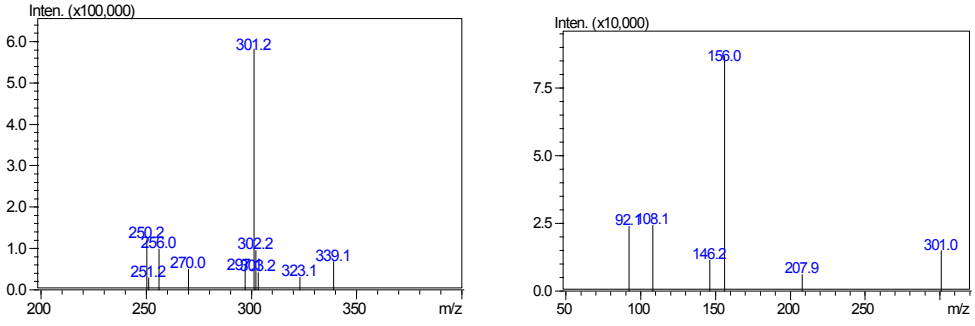

Fig.12 Mass spectrum (left) and MS/MS spectrum (right, CE -19 V) of sulfaquinoxaline

MRM Chromatogram of standard mixture

The MRM chromatograms of multi-standard sample is as shown in Fig. 13.

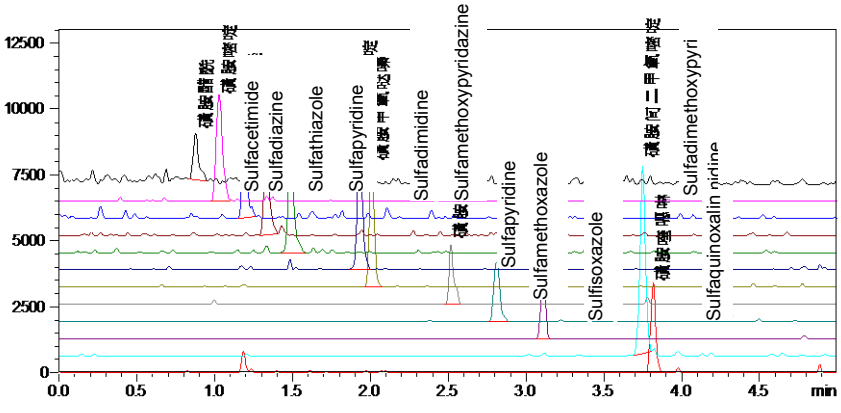

Fig. 13 MRM chromatograms of 1 µg/L multi-standard mixture

Linearity

Multi-standard working solutions of concentrations of 1, 5, 10, 50, 100 and 200 µg/L were assayed using the analysis conditions specified in section 1.2. Calibration curves were plotted as shown in Figs.14 - 25 using external standard method with concentration as X-axis and peak area as Y-axis. The calibration curves were of good linearity in the concentration range of 1~200 µg/L. Their linear equations, linear ranges and correlation coefficients are listed in Table 3.

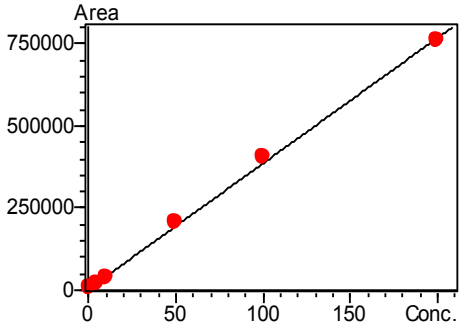

Fig.14 Calibration curve of sulfacetimide

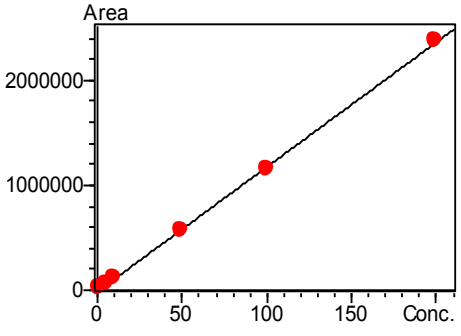

Fig. 15 Calibration curve of sulfadiazine

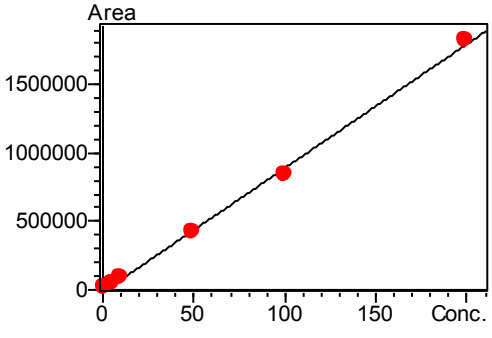

Fig.16 Calibration curve of sulfathiazole

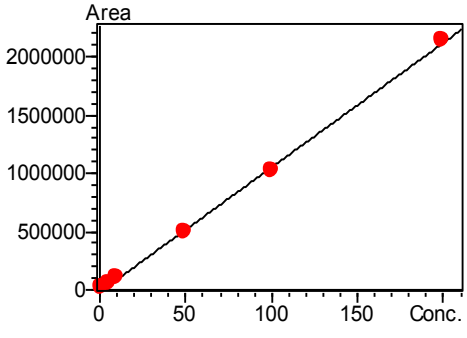

Fig. 17 Calibration curve of sulfapyridine

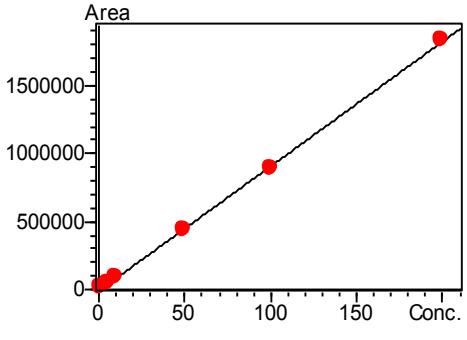

Fig.18 Calibration curve of sulfamerazine

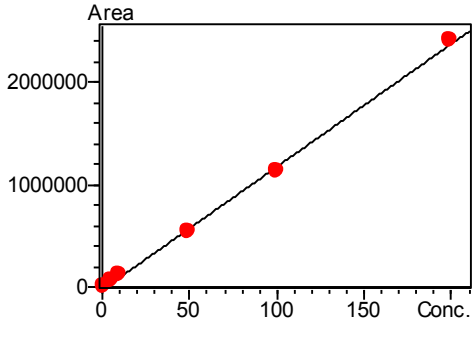

Fig. 19 Calibration curve of sulfadimidine

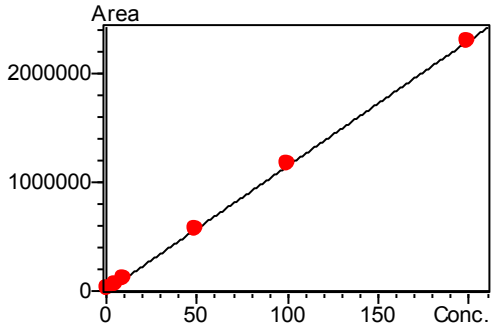

Fig.20 Calibration curve of sulfamethoxypyridazine

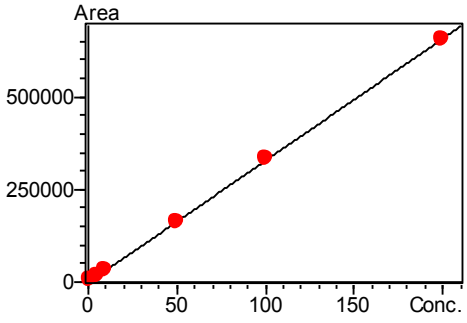

Fig. 21 Calibration curve of sulfapyridine

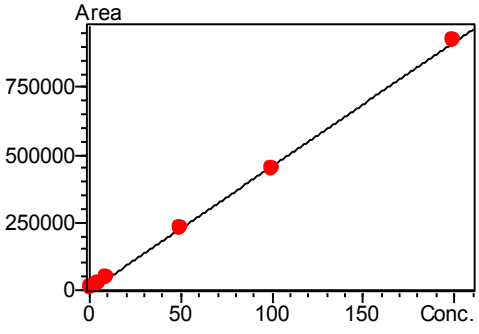

Fig.22 Calibration curve of sulfamethoxazole

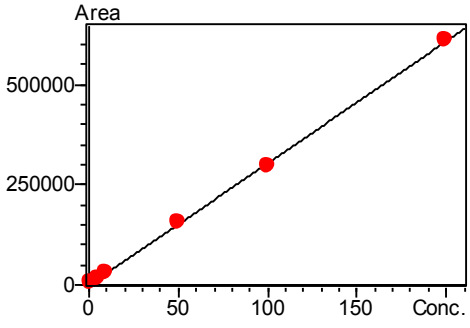

Fig. 23 Calibration curve of sulfisoxazole

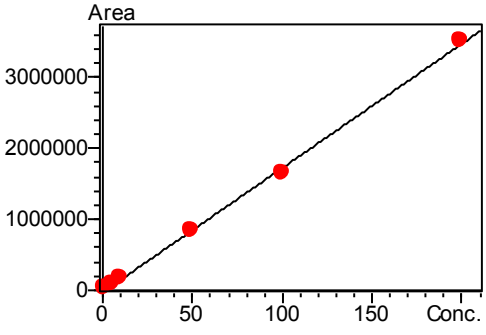

Fig.24 Calibration curve of sulfadimethoxypyrimidine

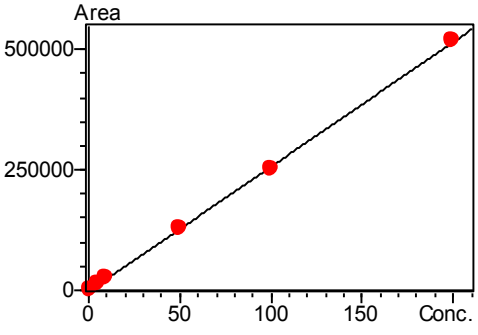

Fig.25 Calibration curve of sulfaquinoxaline

Table 3 Parameters of calibration curves

No.	Compound	Calibration Curve	Correlation Coefficient (R)
1	Sulfacetimide	Y = (3826.48)X + (4169.68)	0.9995
2	Sulfadiazine	Y = (11871.8)X + (-11818.8)	0.9998
3	Sulfathiazole	Y = (9060.06)X + (-15028.4)	0.9991
4	Sulfapyridine	Y = (10690.2)X + (-17268.2)	0.9996
5	Sulfamerazine	Y = (9172.93)X + (-10178.5)	0.9998
6	Sulfadimidine	Y = (12027.5)X + (-27177.9)	0.9993
7	Sulfamethoxypyridazine	Y = (11505.3)X + (-2363.67)	0.9999
8	Sulfapyridine	Y = (3292.91)X + (-1440.52)	0.9999
9	Sulfamethoxazole	Y = (4594.02)X + (-1854.49)	0.9998
10	Sulfisoxazole	Y = (3040.69)X + (-1135.20)	0.9998
11	Sulfadimethoxypyrimidine	Y = (17525.0)X + (-25256.2)	0.9994
12	Sulfaquinoxaline	Y = (2587.97)X + (-2408.19)	0.9999

LODs and LOQs

Seven 1 µg/L multi-standard samples were prepared and injected for analysis. After culling out the outliers from the results, the standard derivation (S) of these 7 assay results was calculated. The minimum limit of detection (MDL) and limit of quantification (LOQ) were calculated using the formulae MDL= 3.14×S, LOQ=4×MDL. The assay results are shown in Table 4.

Table 4. MDLs and LOQs of the 12 substances (n=7)

No.	Compound	Standard deviation (S)	MDL (µg/L)	LOQ (µg/L)
1	Sulfacetimide	0.07	0.21	0.84
2	Sulfadiazine	0.03	0.10	0.41
3	Sulfathiazole	0.07	0.21	0.85
4	Sulfapyridine	0.07	0.23	0.94
5	Sulfamerazine	0.06	0.18	0.70
6	Sulfadimidine	0.04	0.13	0.51

7	Sulfamethoxypyridazine	0.04	0.13	0.52
8	Sulfapyridine	0.07	0.20	0.82
9	Sulfamethoxazole	0.06	0.20	0.81
10	Sulfisoxazole	0.08	0.24	0.95
11	Sulfadimethoxypyrimidine	0.04	0.13	0.50
12	Sulfaquinoxaline	0.08	0.24	0.97

Precision test

6 replicate multi-standard solutions of concentrations as shown in Table 5 were prepared and injected for analysis in succession. The %RSDs of retention time and peak area of the 12 target compounds fell in the ranges of 0.05 ~ 0.40% and 1.00 ~ 5.57%, respectively, indicating that the method was of satisfactory precision.

Table 5 Repeatability - retention time and peak area (n=6)

No.	Compound	%RSD (1 µg/L)		%RSD (10 µg/L)		%RSD (100 µg/L)	
		R.T.	Area	R.T.	Area	R.T.	Area
1	Sulfacetimide	0.38	4.71	0.40	4.43	0.17	2.34
2	Sulfadiazine	0.32	3.26	0.27	3.91	0.22	2.21
3	Sulfathiazole	0.22	5.22	0.21	3.13	0.22	1.82
4	Sulfapyridine	0.38	5.57	0.32	2.85	0.26	1.16
5	Sulfamerazine	0.13	5.28	0.28	3.86	0.19	1.00
6	Sulfadimidine	0.06	4.32	0.18	2.40	0.16	2.64
7	Sulfamethoxypyridazine	0.19	5.49	0.21	4.02	0.18	1.69
8	Sulfapyridine	0.24	5.39	0.11	3.47	0.13	2.49
9	Sulfamethoxazole	0.15	5.18	0.08	4.46	0.13	3.33
10	Sulfisoxazole	0.19	5.25	0.07	4.41	0.12	1.91
11	Sulfadimethoxypyrimidine	0.12	4.85	0.10	2.06	0.05	1.83
12	Sulfaquinoxaline	0.29	4.93	0.09	4.00	0.05	2.53

Spiked matrix test

4 replicate assays were performed on samples which, prepared according to the sample preparation method specified in section 1.3, were spiked with multi-standard solution at spiked levels as shown in Table 6. The test results indicated that the spiked recoveries of fish samples fell in the range of 85.7~ 116.5%. Details of the results are listed in Table 6. MRM chromatograms of blank fish matrix are shown in Fig. 26 and MRM chromatograms of a spiked fish sample are shown in Fig. 27.

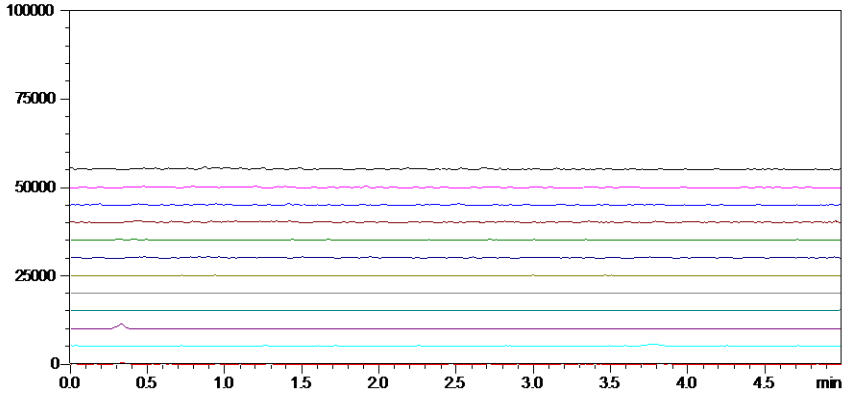

Fig.26 MRM chromatograms of blank fish matrix

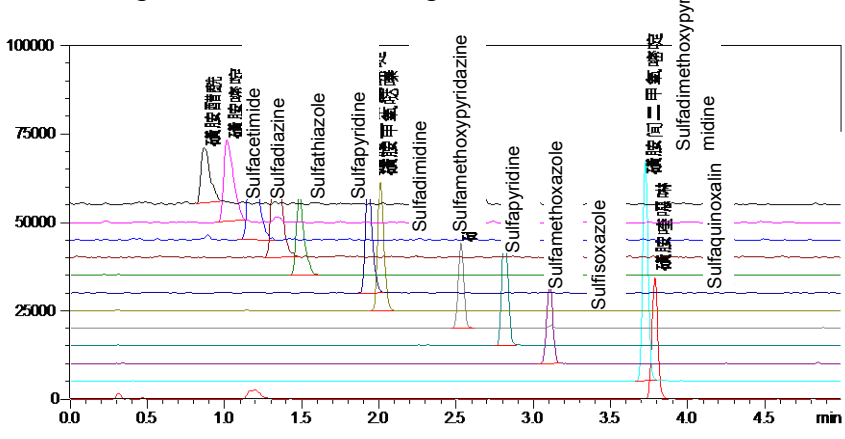

Fig.27 MRM chromatograms of sample matrix spiked with 2 μg/kg standards

Table 6 Recoveries of spiked samples (n=4)

No.	Compound	Recovery (%)		
		0.2 µg/kg	2 µg/kg	20 µg/kg
1	Sulfacetimide	110.4	88.6	91.2
2	Sulfadiazine	97.9	96.4	89.6
3	Sulfathiazole	91.2	91.1	90.0
4	Sulfapyridine	107.7	106.3	101.6
5	Sulfamerazine	85.7	94.0	88.8
6	Sulfadimidine	116.3	97.2	87.4
7	Sulfamethoxypyridazine	106.5	91.9	90.5
8	Sulfapyridine	98.7	95.3	91.3
9	Sulfamethoxazole	89.7	103.1	104.8
10	Sulfisoxazole	98.3	102.2	106.8
11	Sulfadimethoxypyrimidine	93.6	94.1	93.5
12	Sulfaquinoxaline	113.9	93.9	95.0

CONCLUSION

Sulfonamide residues in aquatic products were assayed using Shimadzu UFLCXR ultra fast liquid chromatograph and LCMS-8040 triple quadrupole mass spectrometer. The method demonstrated good linearity for the 12 sulfonamides, all of which had a correlation coefficient greater than 0.999; the method's LODs fell in the range of 0.10 ~ 0.24 µg/L, LOQs were 0.41 ~ 0.97 µg/L. Spiked matrix test was performed on matrices spiked with multi-standard solutions of the 12 sulfonamides at spiked levels of 0.2 µg/kg, 2 µg/kg and 20 µg/kg, yielding spiked recoveries in the range of 85.7 ~ 116.5%. The proposed method had an LOQ of 0.2 µg/kg, which is lower than the required MDL of 1 µg/kg and LOQ of 2 µg/kg in *Simultaneous determination of 17 sulfonamides and 15 quinolones residues in aquatic products by LC-MS/MS method*, a national standard promulgated by Ministry of Agriculture of the People's Republic of China in No. 1077 Announcement-1-2008.

The method has the merits of fast speed, simple operation, good selectivity and high sensitivity and can serve as an effective analysis method for determination of sulfonamide residues in aquatic products

V-27 Determination of Residues of Five Tetracyclines in Honey with UFLC-Triple Quadrupole Mass Spectrometry

INTRODUCTION

In this paper, a method is proposed for the determination of 5 tetracyclines residues in honey using Shimadzu ultra fast liquid chromatograph (UFLC) and triple quadrupole mass spectrometer. Tetracyclines in honey sample were first enriched by solid-phase extraction, then fast separated with LC-30A UFLC, and finally quantitatively assayed with LCMS-8040 triple quadrupole mass spectrometer. The calibration curves of 5 tetracyclines were plotted by an external standard method and all demonstrated a wide linear range and correlation coefficients greater than 0.9996. Precision tests were performed on 5 μg/L, 10 μg/L and 50 μg/L multi-standard solutions and the %RSDs of retention time and peak area of 6 successive injections fell in the ranges of 0.20%~1.14% and 0.62%~3.79%, respectively, suggesting that the method's precision was good. LODs fell in the range of 31.9~63.4 ng/L and LOQs were 127~254 ng/L. The recovery of spiked samples fell in the range of 86.9~ 98.1%.

Tetracyclines (TCs) are a category of broad-spectrum antibiotics that is widely used clinically. However, irrational use of such drugs, such as excessive use of agents, prolonged drug use, drug abuse and non-compliance with withdrawal period to slaughter ahead, causes such drugs and their metabolites residual in animal muscle, eggs, milk, organ tissues and secretions. Tetracyclines cannot be completely absorbed by animals and a considerable part enters the food chain and the environment in the primary form or metabolite form, indirectly affecting human health.

The honey industry has developed rapidly in recent years. Our bees are mostly imported from abroad with relatively high incidence rate. Though China advocates biological control, some people still use chemical drugs and antibiotics to treat the bees, resulting in higher level of antibiotics in honey. Therefore, there is an urgent need to establish an effective and sensitive method to detect tetracylines in honey.

High performance liquid chromatography (HPLC)-tandem mass spectrometry has been developed rapidly in recent years. It has such merits as high selectivity and sensitivity and accurate quantitation of drug residues in complex matrices. A method was proposed for determination

of five tetracyclines in honey with Shimadzu LC-30A UFLC and LCMS-8040 triple quadrupole mass spectrometer.

EXPERIMENTAL

Apparatus

A combined system of Shimadzu UFLC LC-30A and triple quadrupole mass spectrometer LCMS-8040 was used in the experiment. The configuration included two LC-30AD pumps, a DGU-20A$_5$ online degasser, a SIL-30AC autosampler, a CTO-30A column oven, a CBM-20A communication bus module, a LCMS-8040 triple quadrupole mass spectrometer, and a LabSolutions ver. 5.53 chromatography workstation.

Conditions of Analysis

LC conditions

Column	:Shim-pack XR-ODS II 2.0 mm I.D.× 100 mm L., 2.2 µm
Mobile phase	:A:0.1% formic acid aqueous solution
Mobile phase	:B:methanol
Flow rate	:0.25 mL/min
Column temperature	:room temperature
Injection volume	:20 µL
Elution mode	:Gradient elution with initial concentration of mobile phase B of 20%

See Table 1 for the time program.

Table 1 Time program

Time (min)	Module	Command	Value
0.00	Pumps	B Conc.	20
5.00	Pumps	B Conc.	95
6.00	Pumps	B Conc.	95
6.01	Pumps	B Conc.	20
8.00	Pumps	B Conc.	20
8.00	Controller	Stop	

MS conditions

Ionization mode	:ESI(+)
Ionization voltage	:4.5 kV
Nebulizing gas	:Nitrogen, 3.0 L/min
Drying gas	:Nitrogen, 15 L/min
Collision gas	:Argon
DL temperature	:250 ºC
Heater block temperature	:400 ºC
Scan mode	:Multiple Reaction Monitoring (MRM)
Dwell time	:10 ms
Pause time	:3 ms
MRM parameters	:See Table 2

Sample Preparation

Preparation of standard solution: take appropriate standard substances of tetracycline, terramycin, ledermycin, aureomycin and doxycycline, and prepare 1000 mg/L multi-standard stock solutions with them and methanol, and then dilute the stock solutions with methanol and 0.1% formic acid aqueous solution (1:4, v/v) to get multi-standard working solutions of various concentrations.

Sample pretreatment method: refer to “GB/T 23409-2009 Determination of residues of oxytetracycline, tetracycline, chlortetracycline, and doxycycline in royal jelly - LC-MS/MS method” for the preparation of honey samples and purification and extraction of analytes.

Table 2 Optimized MRM parameters

Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Tetracycline	445.20	410.10*	-22	-20	-29
		427.15	-22	-14	-30
Terramycin	461.20	426.10*	-23	-19	-30
		443.20	-23	-14	-21
Ledermycin	465.10	448.10*	-23	-19	-30
		430.10	-23	-22	-30
Aureomycin	479.15	444.20*	-24	-22	-30
		462.15	-24	-18	-22
Doxycycline	445.15	428.25*	-22	-19	-30
		154.20	-22	-34	-28

Note: * refers to quantitative ion

RESULTS AND DISCUSSION

Mass spectrum and MS/MS spectrum

The mass spectrum of tetracycline is shown in Fig. 1 and the MS/MS spectrum is shown in Fig. 2.

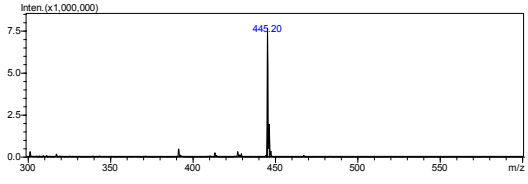

Fig. 1 Mass spectrum of tetracycline

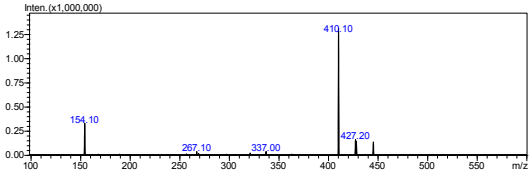

Fig. 2 MS/MS spectrum of tetracycline (CE -20V)

The mass spectrum of terramycin is shown in Fig. 3 and the MS/MS spectrum is shown in Fig. 4.

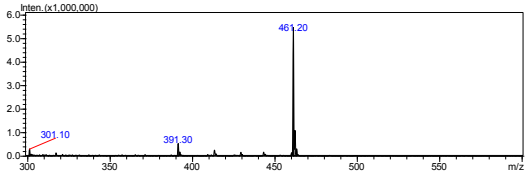

Fig.3 Mass spectrum of terramycin

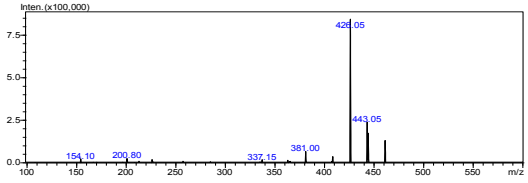

Fig.4 MS/MS spectrum of terramycin (CE -19V)

The mass spectrum of ledermycin is shown in Fig. 5 and the MS/MS spectrum is shown in Fig. 6.

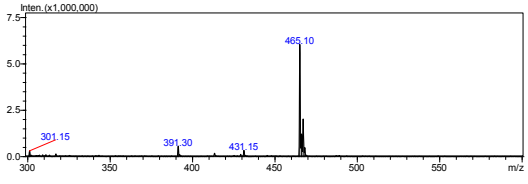

Fig. 5 Mass spectrum of ledermycin

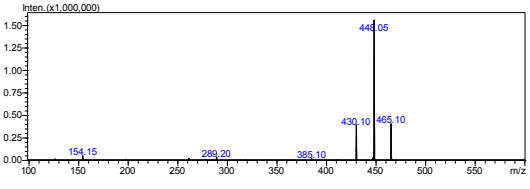

Fig. 6 MS/MS spectrum of ledermycin (CE -19V)

The mass spectrum of aureomycin is shown in Fig. 7 and the MS/MS spectrum is shown in Fig. 8.

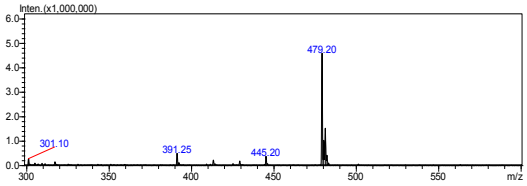

Fig. 7 Mass spectrum of aureomycin

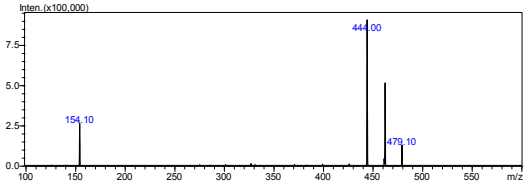

Fig. 8 MS/MS spectrum of aureomycin (CE -22V)

The mass spectrum of doxycycline is shown in Fig. 9 and the MS/MS spectrum is shown in Fig. 10.

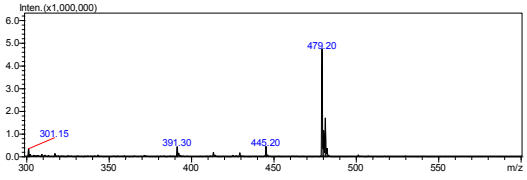

Fig. 9 Mass spectrum of doxycycline

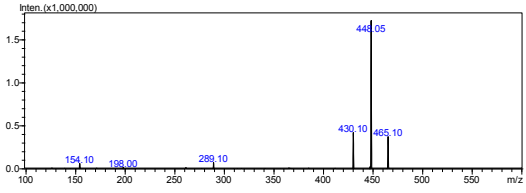

Fig. 10 MS/MS spectrum of doxycycline (CE -19V)

MRM chromatogram of standard mixture

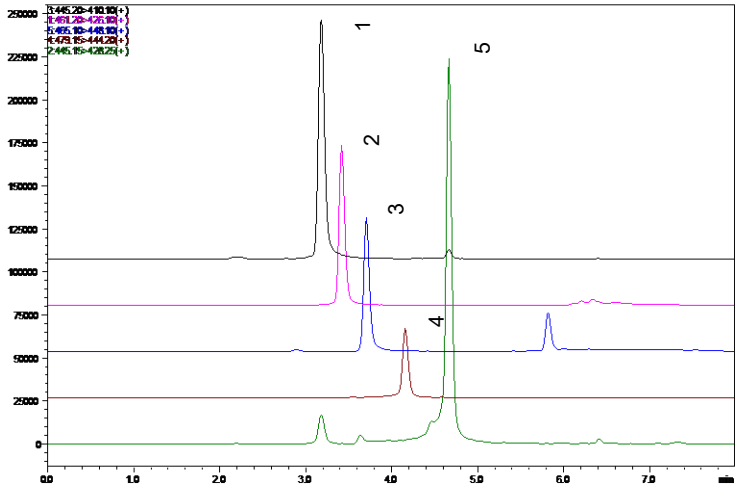

Fig. 11. MRM chromatograms of standard mixture (100 μg/L)

(1. Tetracycline; 2. Terramycin; 3. Ledermycin; 4. Aureomycin; 5. Doxycycline)

Linear range

Multi-standard solutions at concentrations of 0.2 μg/L, 0.5 μg/L, 1 μg/L, 2.5 μg/L, 5 μg/L, 10 μg/L, 50 μg/L, 100 μg/L and 200 μg/L were subjected to quantitative assay by external calibration method under the analysis conditions as specified in section 1.2. Calibration curves were plotted as shown in Fig. 12 to Fig. 16 with concentration as abscissa and peak area as ordinate; the calibration curves were of satisfactory linearity and their linear equations and correlation coefficients are shown in Table 3.

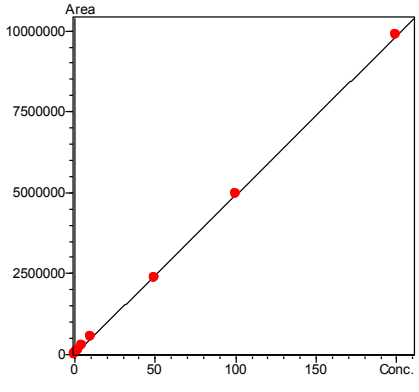

Fig. 12 Calibration curve of tetracycline

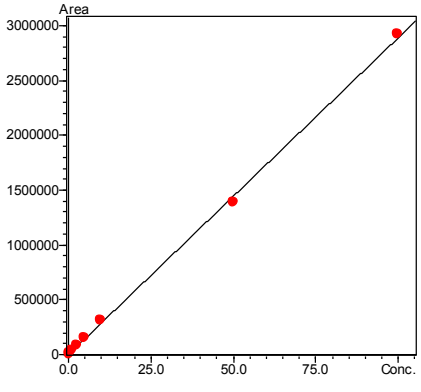

Fig.13 Calibration curve of terramycin

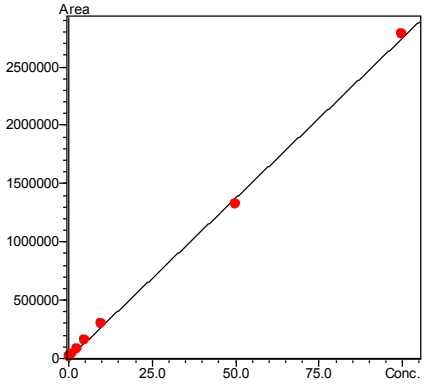

Fig. 14 Calibration curve of ledermycin

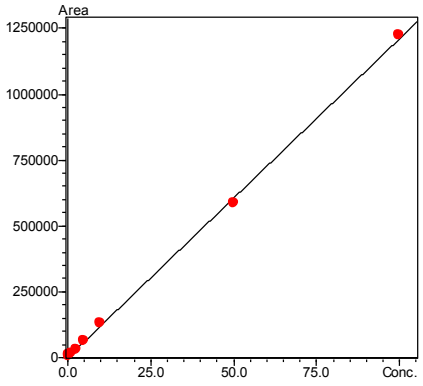

Fig. 15 Calibration curve of aureomycin

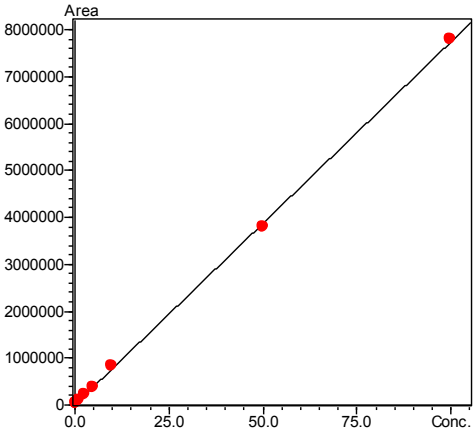

Fig. 16 Calibration curve of doxycycline

Table 3 Parameters of calibration curves

No.	Compound	Calibration Curve	Linear Range (μg/L)	Correlation Coefficient (r)
1	Tetracycline	Y = (49259.9)X + (-1866.27)	0.2~200	0.9999
2	Terramycin	Y = (28905.9)X + (-348.296)	0.5~100	0.9997
3	Ledermycin	Y = (27468.4)X + (1698.49)	0.5~100	0.9996
4	Aureomycin	Y = (12102.7)X + (571.906)	0.5~100	0.9997
5	Doxycycline	Y = (77333.8)X + (5973.94)	0.2~100	0.9998

Precision test

Multi-standard working solutions of various concentrations were determined for 6 times in succession to assess the method's precision. Repeatability of retention time and peak area was shown in Table 4. The results showed that the %RSDs of retention time and peak area data of standard solutions of various concentrations fell in the ranges of 0.20%~1.14% and 0.62%~3.79% respectively, suggesting the method had satisfactory precision.

Table 4 Repeatability - retention time and peak area (n=6)

Compound	%RSD (5 μg/L)		%RSD (10 μg/L)		%RSD (50 μg/L)	
	R.T.	Area	R.T.	Area	R.T.	Area
Tetracycline	1.14	3.21	0.93	2.65	1.10	1.42
Terramycin	0.82	2.76	0.91	2.87	0.79	0.62
Ledermycin	0.88	3.27	0.78	3.04	0.70	2.90
Aureomycin	0.48	3.79	0.46	2.98	0.38	1.80
Doxycycline	0.20	2.71	0.22	1.72	0.20	1.20

LOD

Seven standard samples at 200 ng/L were prepared and directly injected for analysis. After discounting the outliers from the results, the standard deviation S of these 7 measurements was calculated. The limit of detection (LOD) and the lower limit of quantitation (LLOQ) were calculated using these formulae LOD=3.14×S, LOQ=4×MDL. The assay results are shown in Table 5.

Table 5 LODs and LLOQs of tetracyclines

No.	Compound	Standard deviation (S)	MDL (ng/L)	LLOQ (ng/L)
1	Tetracycline	14.9	46.8	187
2	Terramycin	20.2	63.4	254
3	Ledermycin	17.8	55.9	224
4	Aureomycin	18.4	57.8	231
5	Doxycycline	10.2	31.9	127

Recovery test

Honey samples were analyzed for the 5 tetracyclines in honey. Tetracycline was detected in 2 g of honey samples at concentration of 0.249 µg/kg. The resulted chromatograms are shown in Fig. 17. In order to assess the method's actual detection effect of tetracyclines in honey samples, honey samples were spiked with five tetracyclines standard substances at concentration of 2 µg/kg. The chromatograms of a spiked sample are shown in Fig. 18 and recoveries of a spiked sample are shown in Table 6.

MRM Chromatogram of Actual Samples

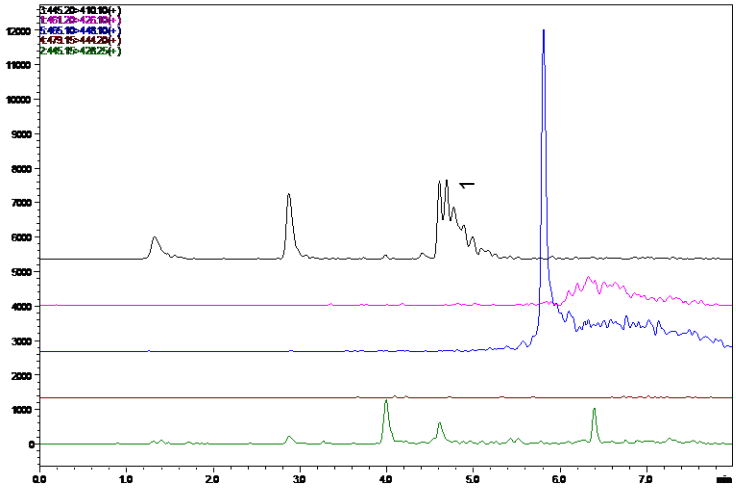

Fig.17 MRM chromatograms of honey sample (1 tetracycline detected)

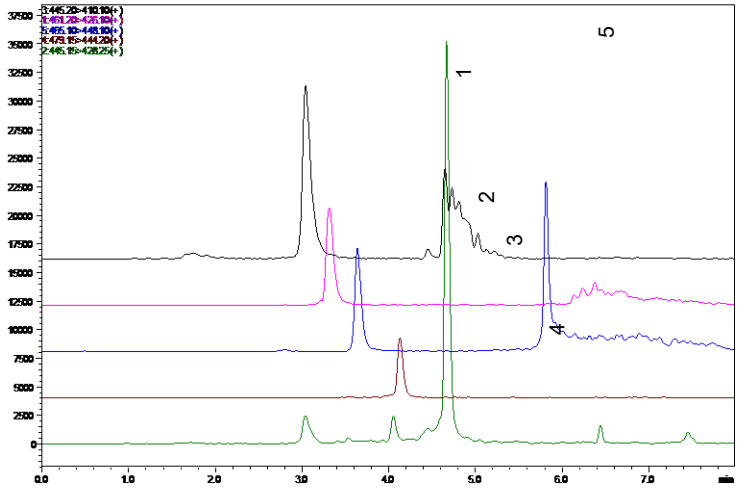

Fig. 18 MRM Chromatograms of a spiked honey sample (2 μg/kg)

(1.Tetracycline; 2.Terramycin; 3.Ledermycin; 4.Aureomycin; 5. Doxycycline)

Table 6. Spike recoveries of tetracyclines

No.	Compound	Tested concentration of Sample 1 (μg/kg)	Tested concentration of Sample 2 (μg/kg)	Average Recovery (%)
1	Tetracycline	2.18	2.25	98.1
2	Terramycin	1.93	1.87	95.2
3	Ledermycin	1.67	1.81	86.9
4	Aureomycin	1.78	1.76	88.3
5	Doxycycline	1.89	1.92	98.1

CONCLUSION

A method was proposed for detection of tetracyclines residues in honey using Shimadzu LC-30A UFLC and LCMS-8040 triple quadrupole mass spectrometer. The method is of high sensitivity, good precision and wide linear range with the correlation coefficient greater than 0.9996. Detection of trace tetracycline was realized by determining commercial honey samples. The recoveries of spiked samples were in the range of 86.9~98.1% by spiked analysis at high, medium and low levels for the reagent samples, proving that the method is suitable for analysis and detection of tetracycline in honey samples.

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V-28 Determination of chloramphenicol analog residues in fishery products using LCMS-8030

INTRODUCTION

In this paper, a method is proposed for determination of chloramphenicol analog residues in fishery products using Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer. Analytes were fast separated within 2.5 minutes with LC-30A ultra fast liquid chromatograph and quantitatively assayed with LCMS-8030 triple quadrupole mass spectrometer. The proposed method was capable of fast and accurate determination of thiamphenicol, florfenicol and chloramphenicol. It demonstrated good linearity and correlation coefficients greater than 0.999 for all of the 3 substances; precision test was performed at various concentrations and the results showed the %RSDs of retention time and peak area of the substances were in the ranges of 0.13 ~ 0.54% and 1.68 ~ 4.31%, respectively, suggesting that the system has good precision. The method's LODs were 0.10, 0.04 and 0.04 µg/L and LOQs were 0.41, 0.17 and 0.15 µg/L for thiamphenicol, florfenicol and chloramphenicol, respectively. Recoveries of analytes from spiked samples were in the range of 81.5~ 105.5%.

Chloramphenicol, thiamphenicol and florfenicol(Nuflor) are antibiotics in the category of chloramphenicols (CAPs). With chemical structures and pharmacological actions similar to those of chloramphenicol, thiamphenicol and florfenicol are new chloramphenicol antibiotics that have been widely used in livestock husbandry and aquaculture for their broad antibacterial spectrum. However, the application of chloramphenicols in edible animals is liable to give rise to excessive residues in food, which may lead to side effects including aregenerative anemia and hemolytic anemia that seriously endanger human health. In light of this, the abuse of chloramphenicols has become an issue of concern in China and/or foreign countries.

To date, the application of chloramphenicols in edible animals has been subjected to strict MRL restrictions in most countries. In EU, it is stipulated that no chloramphenicol and no more than 50 µg/kg thiamphenicol shall be detected in edible animals; and the MRL stipulated for florfenicol in finfish is 1000 µg/kg; in China, the applicable MRLs stipulated in GB/T 20756-2006 are 0.1 µg/kg for chloramphenicol and 1 µg/kg for thiamphenicol and florfenicol.

According to available Chinese and foreign research literature on the assay methods of chloramphenicols, most of the published methods are based on GC, GC-MS and LC-MS and for the assay of a single chloramphenicols antibiotic only and few has been reported on methods for concurrently assay of the 3 chloramphenicols. In China, most analytical methods for the detection of the 3 chloramphenicols are based on the LC-MS/MS method stipulated in GB/T 20756-2006 and the GC-MS method stipulated in Announcement No. 958-14-2007 issued by the Ministry of Agriculture of the P.R.C. But the first choice world-wide for precise assay of thiamphenicol, florfenicol, and chloramphenicol is LC-MS/MS. In the light of this, a fast, highly selective and sensitive analytical method was proposed in this paper for the assay of chloramphenicol analog residues in fishery products with Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer for the reference of relevant laboratorians.

EXPERIMENTAL

Apparatus

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The configuration included two LC-30AD pumps, DGU-20A$_5$ online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-

20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, and LabSolutions Ver. 5.42 chromatography workstation.

Conditions of Analysis

LC Conditions

Apparatus	:LC-30A system
Column	:Shim-pack XR-ODSIII 2.0 mmI.D.×50 mmL., 1.6 μm
Mobile phase	:A: water
Mobile phase	:B: acetonitrile
Flow rate	:0.4 mL/min
Injection volume	:10 μL
Column temperature	: 40 °C

Elution mode :Binary gradient with initial concentration of 30% of B Conc. See Table 1 for time program.

Table 1Time program

Time (min)	Module	Command	Value
0.50	Pumps	Pump B Conc.	30
1.50	Pumps	Pump B Conc.	60
2.00	Pumps	Pump B Conc.	60
2.01	Pumps	Pump B Conc.	30
4.00	Controller	Stop	

MS conditions

Analytical apparatus :LCMS-8030
Ionization :ESI, negative mode
Interface voltage of ion source :-3.5 kV
Nebulizing gas :Nitrogen, 3.0 L/min
Drying gas :Nitrogen, 15.0 L/min
Collision gas :Argon
DL temperature :250 °C
Heater block temperature :400 °C
Mode :Multiple Reaction Monitoring (MRM)
Dwell time :40 ms
Pause time :3 ms
MRM parameters :See Table 2

Table 2 MRM parameters

No.	Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Thiamphenicol	354.0	185.0*	27	22	19
			289.9	27	12	20
2	Florfenicol	356.0	336.0*	13	10	23
			185.0	13	20	19
3	Chloramphenicol	321.0	152.1*	25	18	15
			257.0*	25	12	18

* refers to quantitative ion.

Sample Preparation

A total of 3 standard substances were used, i.e. thiamphenicol, florfenicol and chloramphenicol.

Preparation of standard solutions: A 10 mg/L multi-standard intermediate solution was prepared using methanol as solvent, and then diluted with methanol aqueous solution (50/50, v/v) into multi-standard working solutions of concentrations of 0.2 μg/L, 0.5 μg/L, 1 μg/L, 5 μg/L, 10 μg/L, 50 μg/L and 100 μg/L.

Sample pretreatment method: 5.0 g of sample was weighed (with a precision of 0.01 g) and transferred to a 50 mL centrifuge tube, 25 mL of ethyl acetate was added, subjected to homogenization at 14000 rpm for 30 seconds in a homogenizer followed by centrifugation at 4000 rpm for 5 minutes, the resulted supernatant of ethyl acetate extract was pipetted into a 50 mL flat bottom flask; the residue was added 20 mL ethyl acetate and subjected to the above-mentioned procedures, and the resulted supernatant was combined with the first supernatant; the combined supernatant was condensed to dry under reduced pressure in a rotary evaporator, the residue was dissolved with 1 mL methanol-water solution (50/50, v/v), 3.0mL of n-hexane was added, mixed well and transferred to a 10 mL colorimetric cylinder, subjected to vortex mixer for 30 seconds followed by centrifugation at 4000 rpm for 2 minutes, the resulted n-hexane supernatant was discarded; the bottom layer was transferred and filtered through 0.22 μm filter membrane for analysis with LC-MS/MS.

RESULTS AND DISCUSSION

Mass and MS/MS spectra of standard samples

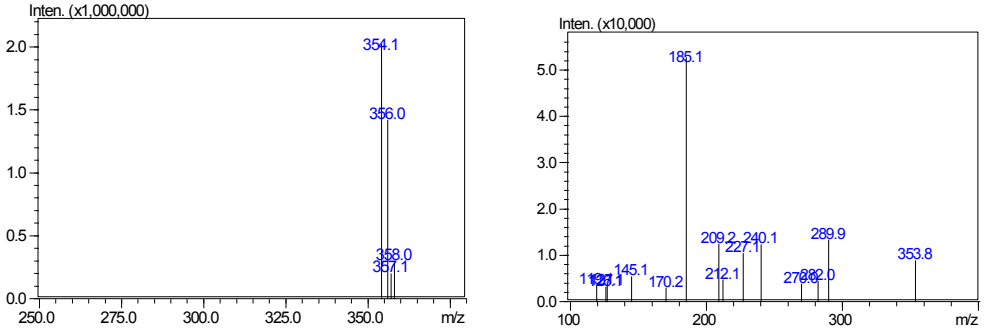


Fig. 1 Mass spectrum (left) and MS/MS spectrum (CE 20V, right) of thiamphenicol

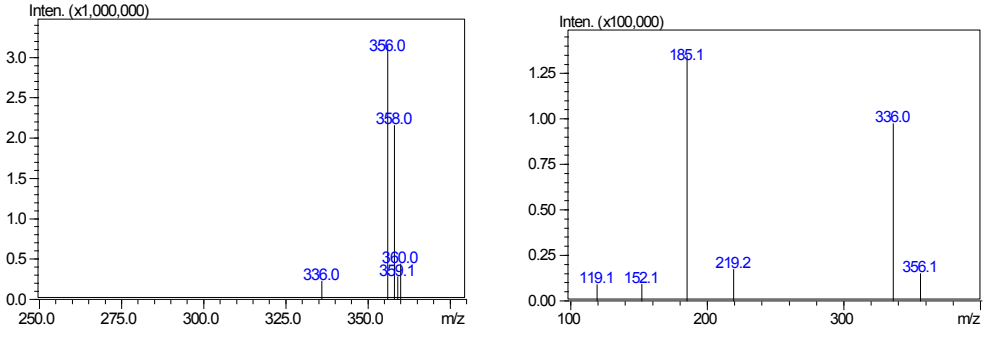


Fig. 2 Mass spectrum (left) and MS/MS spectrum (CE 16V, right) of florfenicol

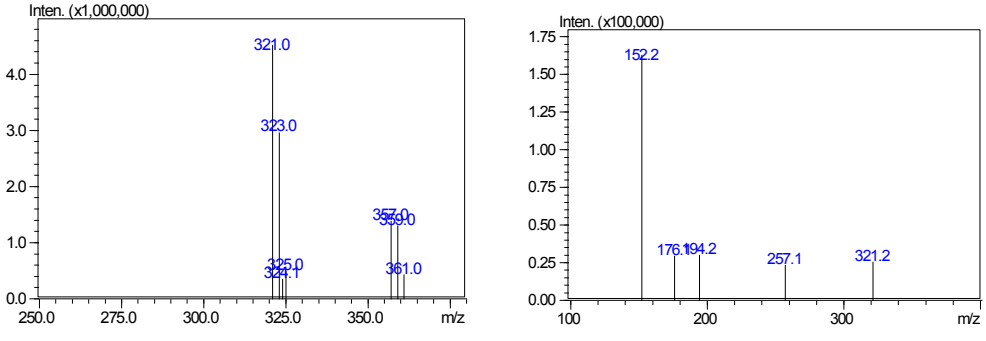


Fig. 3 Mass spectrum (left) and MS/MS spectrum (CE 18V, right) of chloramphenicol

MRM Chromatogram of Standard Samples

The MRM chromatograms of standard mixture are shown in Fig. 4.

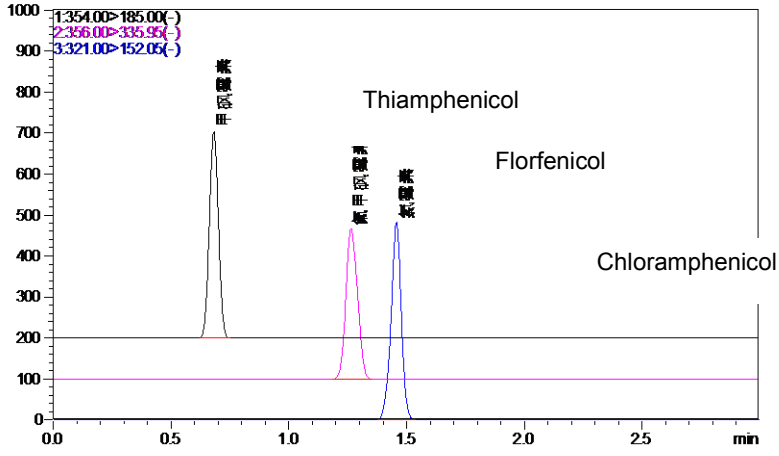
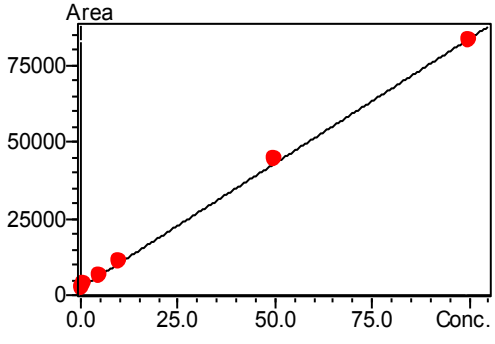


Fig. 4 MRM chromatograms of standard mixture (1 μg/L each)

Linearity

Multi-standard working solutions of thiamphenicol, florfenicol and chloramphenicol at concentrations of 0.2, 0.5, 1, 5, 10, 50 and 100 μg/L were assayed using the analysis conditions specified in section 1.2. Calibration curves as shown in Figs.5 ~ 7 were plotted by the external standard method with concentration as X-axis and peak area as Y-axis. The resulted calibration curves of thiamphenicol, florfenicol and chloramphenicol were of good linearity. Their linear equations, linear ranges and correlation coefficients are listed in Table 3.



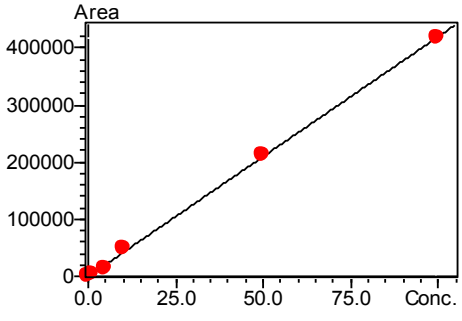


Fig. 5 Calibration curve of thiamphenicol; Fig. 6 Calibration curve of florfenicol

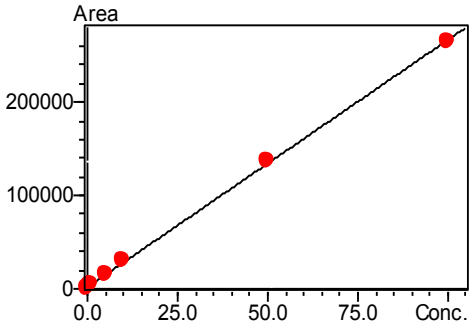


Fig. 7 Calibration curve of chloramphenicol

Table 3 Parameters of the 3 substances' calibration curves

No.:	Compound	Linear Range	Calibration Curve	Correlation Coefficient (r)
1	Thiamphenicol	0.5~100 μg/L	Y = (813.877)X + (2393.00)	0.9998
2	Florfenicol	0.2~100 μg/L	Y = (4198.77)X + (-157.491)	0.9996
3	Chloramphenicol	0.2~100 μg/L	Y = (2653.64)X + (1739.35)	0.9998

LODs and LOQs

Seven multi-standard samples were prepared and injected for analysis. The standard deviation (S) of these 7 assay results was calculated. LODs and LOQs were calculated using the formulae LOD= 3.14×S, LOQ=4×LOD. The assay results are shown in Table 4:

Table 4 LODs and LOQs of the 3 analytes

No.	Compound	Concentration (μg/L)	Standard Deviation (S)	LOD (μg/L)	LOQ(μg/L)
1	Thiamphenicol	0.50	0.033	0.102	0.41
2	Florfenicol	0.20	0.014	0.043	0.17
3	Chloramphenicol	0.20	0.011	0.038	0.15

Precision test

Multi-standard solutions as listed in Table 5 were prepared and subjected to 6 replicate assays in succession. The relative standard deviations (RSD) of the retention time data and the peak area data of these 3 target analytes were 0.13 ~ 0.54% and 1.68 ~ 4.31%, respectively, suggesting a satisfactory instrument precision.

Table 5 Repeatability - retention time and peak area (n=6)

No.	Compound	%RSD (0.5 µg/L)		%RSD (10 µg/L)		%RSD (100 µg/L)	
		R.T.	Area	R.T.	Area	R.T.	Area
1	Thiamphenicol	0.54	4.31	0.15	3.34	0.25	1.51
2	Florfenicol	0.40	2.66	0.09	2.39	0.16	1.87
3	Chloramphenicol	0.38	2.26	0.16	1.68	0.13	1.98

Spiked matrix test

4 replicate assays were performed on samples which, prepared according to the sample preparation method specified in section 1.3, were spiked with multi-standard solution at spiked levels as shown in Table 6. The test results indicated that the recoveries of analytes from shrimp spiked samples fell in the range of 81.5.7~ 105.5%. Details of the results are listed in Table 6. The chromatograms of blank shrimp matrix are shown in Fig. 8 and the chromatograms of shrimp spiked samples are shown Fig. 9.

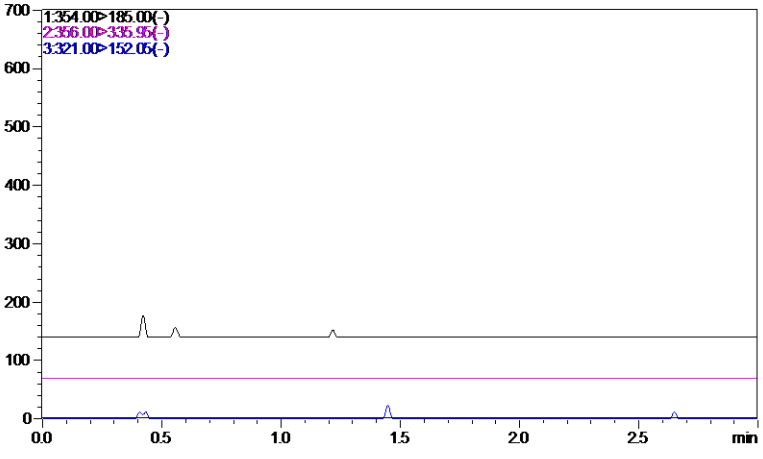


Fig. 8 Chromatograms of blank shrimp matrix

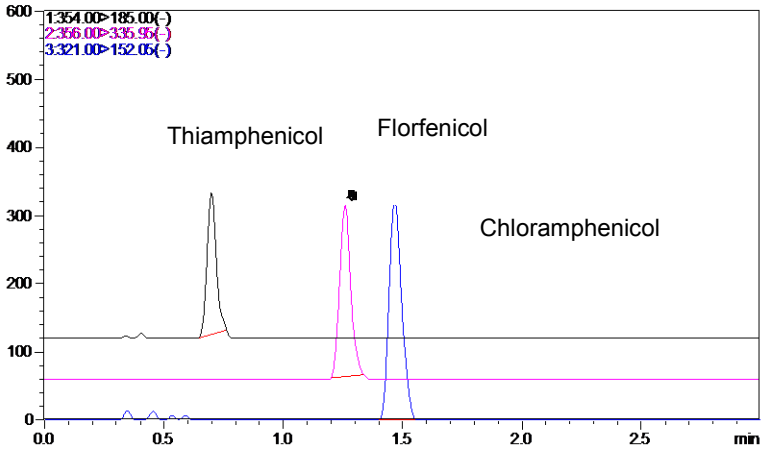


Fig. 9 Chromatograms of sample matrix spiked with 0.1 µg/kg standards

Table 6 Recoveries of spiked samples (%)

No.	Compound	Recovery (%)		
		0.1 µg/kg	1 µg/kg	20 µg/kg
1	Thiamphenicol	84.8	90.3	105.5
2	Florfenicol	89.8	83.7	81.5
3	Chloramphenicol	96.9	99.8	93.3

CONCLUSION

Residues of thiamphenicol, florfenicol and chloramphenicol in fishery products were assayed with the proposed method using Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer. The method demonstrated good linearity for the 3 analytes with correlation coefficients all greater than 0.999. The method's LODs were 0.10, 0.04 and 0.04 µg/L and LOQs were 0.41, 0.17 and 0.15 µg/L for thiamphenicol, florfenicol and chloramphenicol, respectively. Recoveries of analytes spiked into matrix were in the range of 81.5~ 105.5%. As shown in Fig. 9, the proposed method responded well to matrix spiked with chloramphenicols at 0.1 µg/kg and yielded satisfactory recoveries. It sufficed for MRL assay of thiamphenicol, florfenicol and chloramphenicol in accordance with GB/T 20756-2006 and EU standards and demonstrated

LOQs lower than regulatory requirements for the assay of thiamphenicol and florfenicol.

With the merits of fast speed, good selectivity and high sensitivity, the proposed method can be used for daily assay of chloramphenicols residues in fishery products.

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V-29 Fast determination of multiple hormones residues in fishery products by triple quadrupole mass spectrometry

INTRODUCTION

In this paper, a method is proposed for determination of multiple hormones residues in fishery products using Shimadzu LC-30A ultra fast liquid chromatograph- LCMS-8030 triple quadrupole mass spectrometer. Analytes in fish meat samples that had been subjected to pretreatment procedures were separated by the LC-30A ultra fast liquid chromatograph, and then assayed with the LCMS-8030 triple quadrupole mass spectrometer. Calibration curves were plotted for 17 hormones using external standard method, all plotted calibration curves had good linearity with a correlation coefficient in the range of 0.9987~0.9999. Precision test was performed on 6 successive assays of multi-standard solutions at low, medium and high concentrations. The RSDs of retention time and peak area of 6 successive injections were below 0.322% and 5.82%, respectively, suggesting that the system had satisfactory precision. The method's LODs for the hormone residues were in the range of 0.07~1.06 µg/L. Methodological verification was performed by calculating the recoveries of standards spiked into fish meat matrix; the resulted recoveries of the 17 hormones fell in the range of 78.0%~115%, suggesting that the proposed method is sufficient for the assay of hormone residues in fish meat.

Hormones, such as androgen, estrogen and progesterone, have been playing a very important role in living organisms. Androgen, a major male steroid hormone and the primary decisive factor of embryonic development and masculine feature emergence of males in adolescence, is usually used for restoration of muscle shape and force. It is a general knowledge that estrogen has direct influence on the brain region that controls emotion and cognition. Estrogen is usually used in conjunction with progesterone for the treatment of climacteric syndrome. Hormones compounds are extensively used in aquaculture for their ability to enhance feed conversion ratio and promote growth. For example, growth hormones can promote the growth and development of fish; estradiol, testosterone, and progesterone can regulate the reproductive behavior of fish. In aquaculture, these hormones are commonly used for sex reversal of fish. Generally, hormones are rather stable and hard to degrade, as a result, residues of natural and synthetic hormones in animal tissues can enter human body via the food chain and, because of their potent bioactivity and potential carcinogenicity,

induce central obesity, immune deficiency, osteoporosis and other diseases. Therefore, many countries have restricted or banned the use of hormones in the breeding of edible animals. However, these compounds are still abused out of profit driven motivation. Because of this, it is necessary to develop reliable analytical methods for the supervision of hormones residues in fishery products. Generally, HPLC is intended for general-purpose analysis and inadequate for more specific assays; GC-MS frequently requires derivatization of analytes before chromatographic separation because most analytes are unstable to heat, non-volatile, and/or of disadvantageous polarity. UHPLC-MS/MS, on the other hand, has become the first choice for the assay of hormones because the method's high separation efficiency and identification accuracy.

In this paper, a method is proposed in reference with GB/T 21981-2008 *Determination of hormone multi-residues in foodstuffs of animal origin - LC-MS/MS method* for accurate determination of multiple hormones residues in fishery products by ultra fast liquid chromatography and triple quadrupole mass spectrometry.

EXPERIMENTAL

Apparatus

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The configuration included two LC-30AD pumps, a DGU-20A$_5$ online degasser, a SIL-30AC autosampler, a CTO-30A column oven, a CBM-20A communications bus module, a LCMS-8030 triple quadrupole mass spectrometer, and a LabSolutions Ver. 5.53 chromatography workstation.

Conditions of Analysis

LC Conditions

Apparatus	:LC-30A system
Column	:Shim-pack XR-ODS III 2.1 mmI.D.×50 mmL.,1.6 µm
Mobile phase	:0.1% formic acid aqueous solution/methanol (35/65)
Flow rate	:0.3 mL/min
Elution mode	:isocratic elution
Injection volume	:10 µL
Column temperature	:40 °C

MS conditions

Analytical apparatus	:LCMS-8030
Ionization	:ESI, positive mode
Ionization voltage	:+4.5 kV
Nebulizing gas	:Nitrogen, 3.0 L/min
Drying gas	:Nitrogen, 15.0 L/min
Collision gas	:Argon
DL temperature	:250 °C
Heater block temperature	:400 °C
Mode	:Multiple Reaction Monitoring (MRM)
Dwell time	:30 ms
Pause time	:3 ms
MRM parameters	:See Table 1

Table 1 MRM Parameters

Compound	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
Androstenedione	287.05	97.00*	-15.0	-25.0	-20.0
	287.05	109.15	-15.0	-25.0	-23.0
Boldenone	287.10	121.05*	-26.0	-25.0	-26.0
	287.10	135.05	-26.0	-15.0	-15.0
Danazol	338.25	148.15*	-26.0	-30.0	-16.0
	338.25	120.00	-26.0	-35.0	-24.0
Fluoxymesterone	337.15	241.15*	-13.0	-25.0	-18.0
	337.15	131.00	-13.0	-35.0	-28.0
Testosterone	289.25	97.00*	-25.0	-25.0	-20.0
	289.25	109.05	-25.0	-25.0	-22.0
Methyltestosterone	303.25	109.15*	-25.0	-30.0	-21.0
	303.25	97.15	-25.0	-25.0	-19.0
Metandienone	301.25	121.15*	-26.0	-25.0	-13.0
	301.25	149.15	-26.0	-15.0	-16.0

Norandrostenolone	275.10	109.10*	-13.0	-30.0	-22.0
	275.10	257.20	-13.0	-15.0	-20.0
Norandrostenedione	273.10	109.15*	-10.0	-25.0	-23.0
	273.10	197.10	-10.0	-20.0	-15.0
Trenbolone	271.20	253.15*	-27.0	-20.0	-19.0
	271.20	199.05	-27.0	-25.0	-15.0
Megestrol acetate	385.10	267.15*	-23.0	-20.0	-20.0
	385.10	325.20	-23.0	-15.0	-25.0
Medroxyprogesterone	345.30	123.00*	-13.0	-25.0	-13.0
	345.30	97.20	-13.0	-25.0	-19.0
Medroxyprogesterone acetate	387.30	123.00*	-15.0	-30.0	-13.0
	387.30	327.25	-15.0	-10.0	-25.0
Methylnorethindrone	313.25	109.15*	-12.0	-35.0	-24.0
	313.25	245.20	-12.0	-25.0	-18.0
Chlormadinone acetate	405.05	345.20*	-24.0	-15.0	-18.0
	405.05	309.15	-24.0	-20.0	-23.0
Norethindrone	299.05	109.05*	-19.0	-35.0	-22.0
	299.05	91.15	-19.0	-25.0	-11.0
Progesterone	315.15	97.15*	-12.0	-25.0	-20.0
	315.15	109.00	-12.0	-30.0	-21.0

* refers to quantitative ion.

Sample Preparation

Preparation of standard solution

Standard solutions of the 17 hormones of concentration of 100 mg/L were progressively diluted with ultrapure water to prepare a 1000 µg/L multi-standard solution. The 1000 µg/L multi-standard solution of the 17 hormones was then progressively diluted into a series of standard working solutions of various concentrations (see Table 2) for plotting calibration curves for the hormones.

Table 2 Concentrations of standard substances in multi-standard working solution (μg/L)

No	Compound	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6
1	Norandrostenedione	1	2	10	20	50	100
2	Trenbolone	2	5	10	20	50	100
3	Boldenone	2	5	10	20	50	100
4	Norandrostenolone	1	2	5	20	50	100
5	Androstenedione	1	5	10	20	50	100
6	Metandienone	2	5	10	20	50	100
7	Testosterone	1	2	10	20	50	100
8	Methyltestosterone	1	2	10	20	50	100
9	Medroxyprogesterone	1	2	10	20	50	100
10	Megestrol acetate	1	2	10	20	50	100
11	Medroxyprogesterone acetate	0.5	2	10	20	50	100
12	Progesterone	1	2	10	20	50	100
13	Norethindrone	2	5	10	20	50	100
14	Methylnorethindrone	1	5	10	20	50	100
15	Chlormadinone acetate	0.5	2	10	20	50	100
16	Fluoxymesterone	2	5	10	20	50	100
17	Danazol	1	5	10	20	50	100

Sample pretreatment method

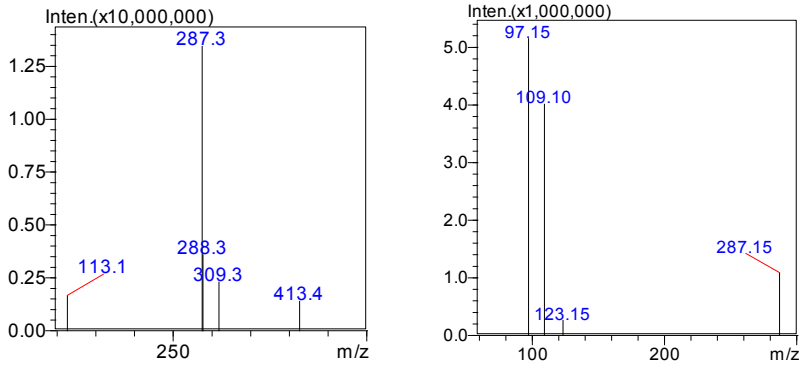
5.0 g sample was weighed and transferred to a 50 mL polytetrafluoroethylene centrifuge tube, added 20 mL acetonitrile - formic acid (0.1%) solution, subjected to homogenizer for 1 min followed by centrifugation at 4000 rpm for 5 min, the resulted supernatant was pipetted into another 60 mL polytetrafluoroethylene centrifuge tube and the centrifuged residue was subjected to extraction with 20 mL acetonitrile - formic acid (0.1%) solution another time, the resulted supernatant was combined with the previous supernatant.

The combined supernatant was transferred to a 125 mL separating funnel, added 25 mL acetonitrile saturated n-hexane, shaken for 2 min, the resulted upper layer was discarded, and the underlayer was transferred to a 100 mL brown pear-shaped bottle, subjected to evaporation in a rotary evaporator on 40 °C water bath to almost dry, flush dried with nitrogen; 1.0 mL acetonitrile - formic acid (0.1%) solution was accurately added to dissolve the residue; the resulted solution was subjected to vortex mixer

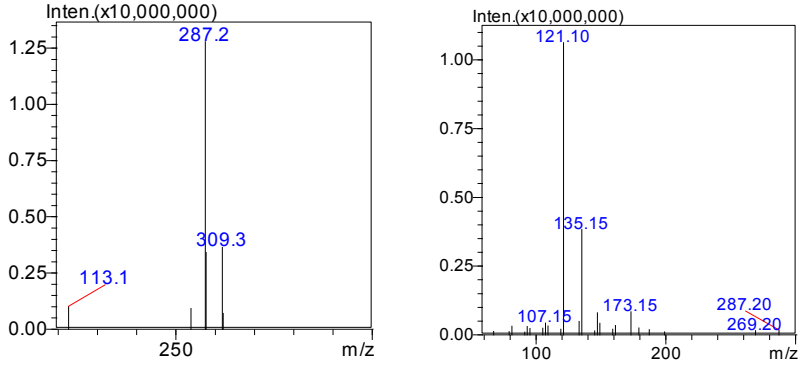
and then filtered through a disposable syringe filter into a sample bottle for assay by LC –MS/MS.

RESULTS AND DISCUSSION

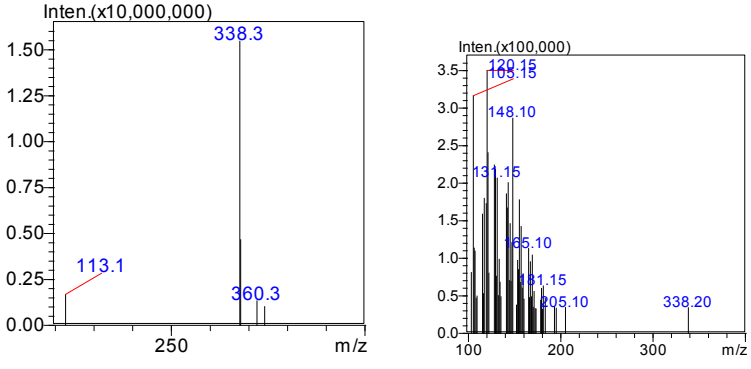
Mass spectra and MS/MS spectra of standards



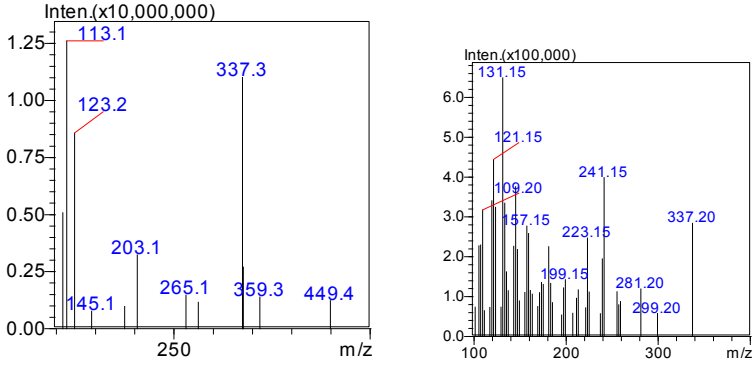
1. Mass spectrum (left) and MS/MS spectrum (right, CE value: -22V) of androstenedione



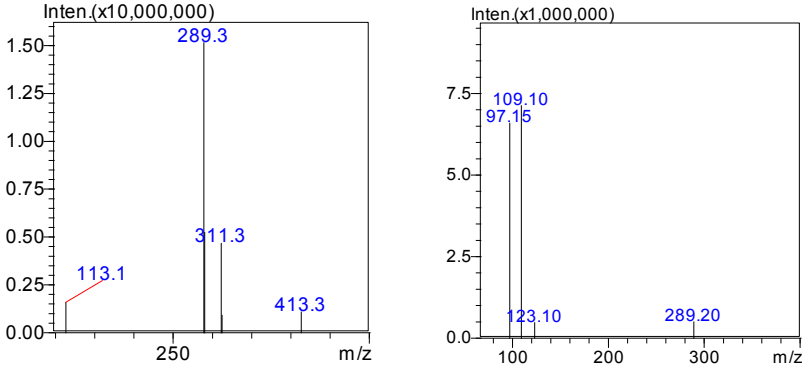
2. Mass spectrum (left) and MS/MS spectrum (right, CE value: -21V) of boldenone



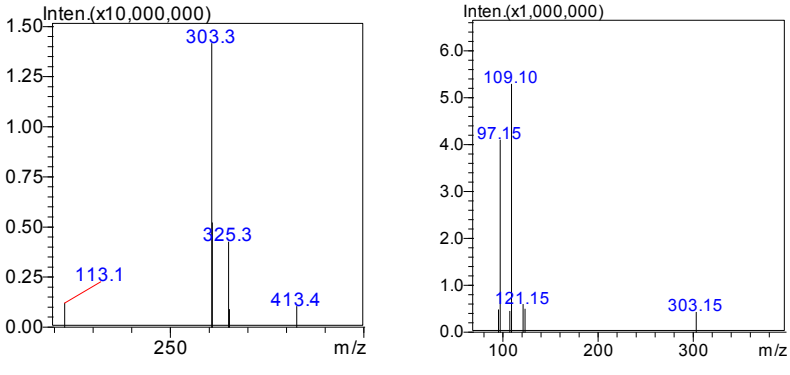
3. Mass spectrum (left) and MS/MS spectrum (right, CE value: -40V) of danazol



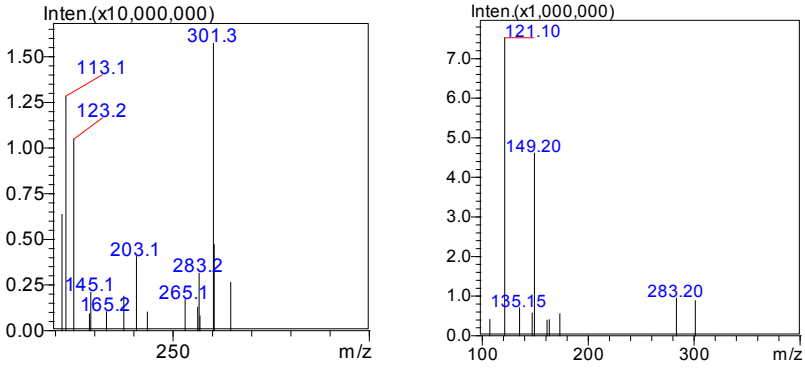
4. Mass spectrum (left) and MS/MS spectrum (right, CE value: -32V) of fluoxymesterone



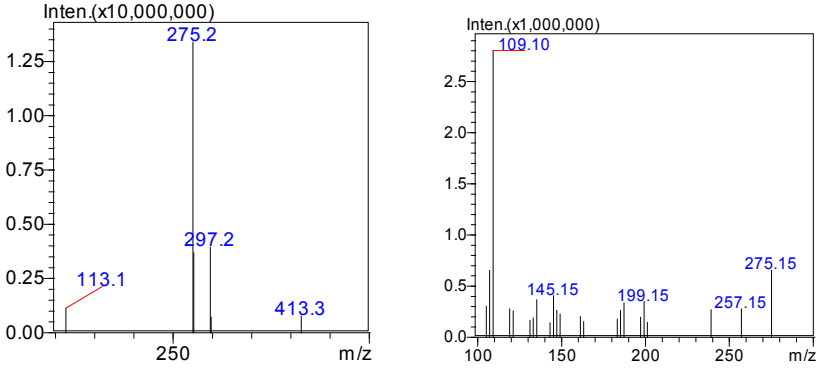
5. Mass spectrum (left) and MS/MS spectrum (right, CE value: -25V) of testosterone



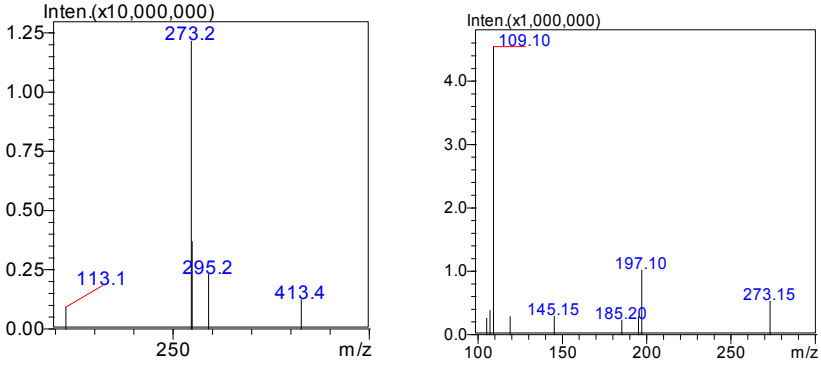
6. Mass spectrum (left) and MS/MS spectrum (right, CE value: -26V) of methyltestosterone



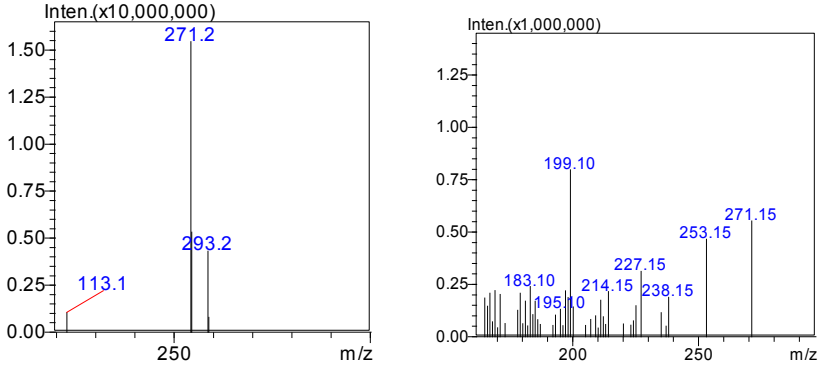
7. Mass spectrum (left) and MS/MS spectrum (right, CE value: -18V) of metandienone



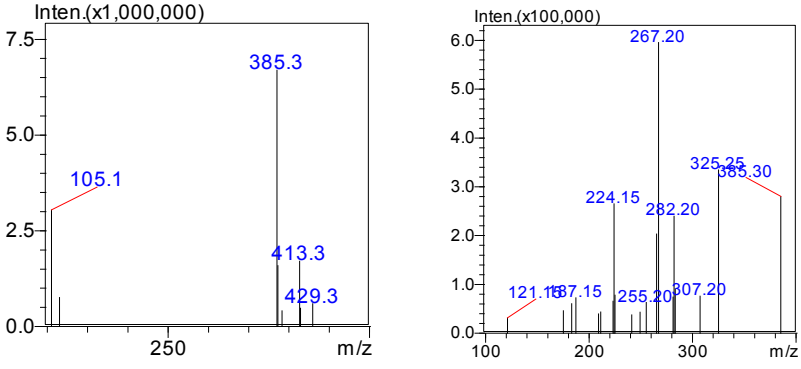
8. Mass spectrum (left) and MS/MS spectrum (right, CE value: -24V) of norandrostenolone



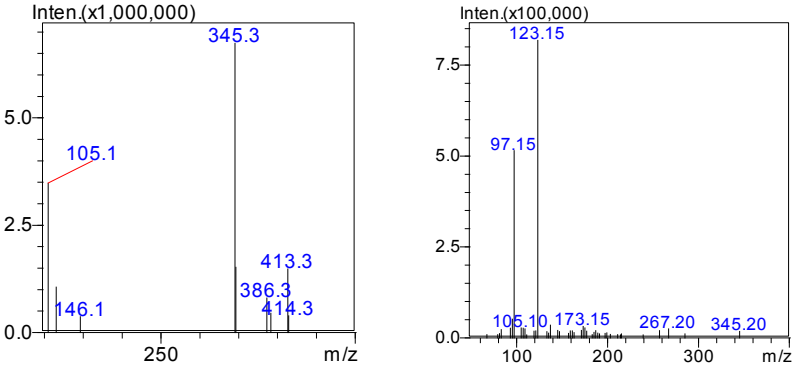
9. Mass spectrum (left) and MS/MS spectrum (right, CE value: -25V) of norandrostenedione



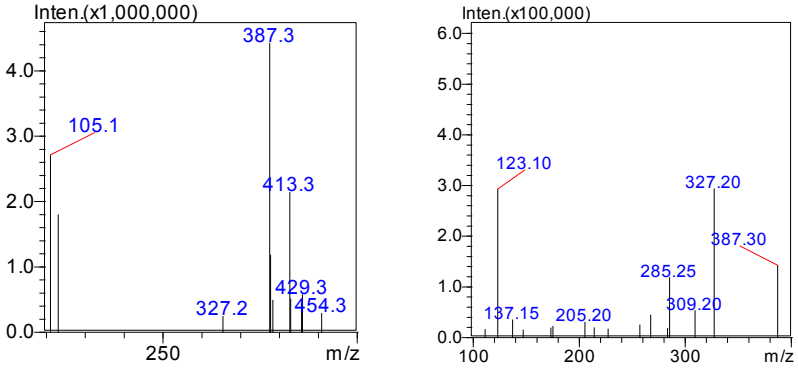
10. Mass spectrum (left) and MS/MS spectrum (right, CE value: -30V) of trenbolone



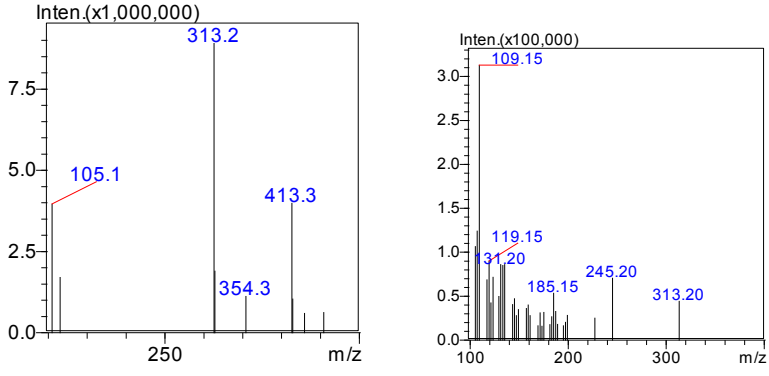
11. Mass spectrum (left) and MS/MS spectrum (right, CE value: -19V) of megestrol acetate



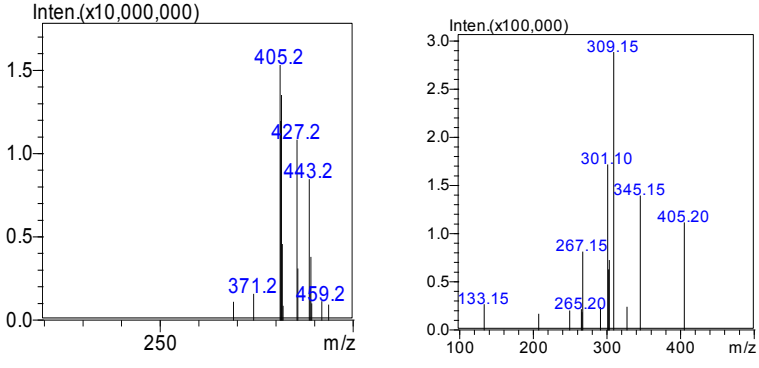
12. Mass spectrum (left) and MS/MS spectrum (right, CE value: -29V) of medroxyprogesterone



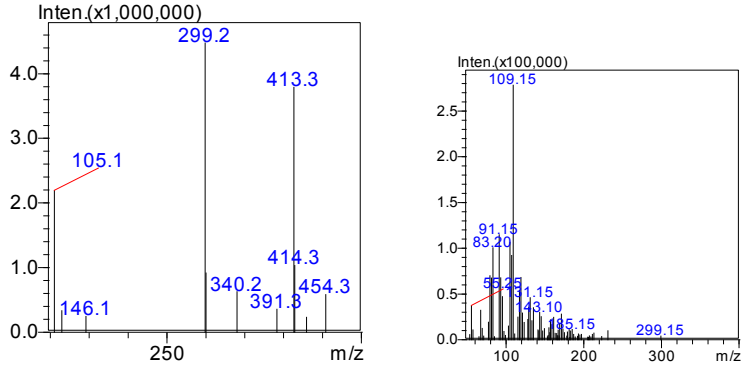
13. Mass spectrum (left) and MS/MS spectrum (right, CE value: -20V) of medroxyprogesterone acetate



14. Mass spectrum (left) and MS/MS spectrum (right, CE value: -26V) of methylnorethindrone



15. Mass spectrum (left) and MS/MS spectrum (right, CE value: -18V) of chlormadinone acetate



16. Mass spectrum (left) and MS/MS spectrum (right, CE value: -32V) of norethindrone

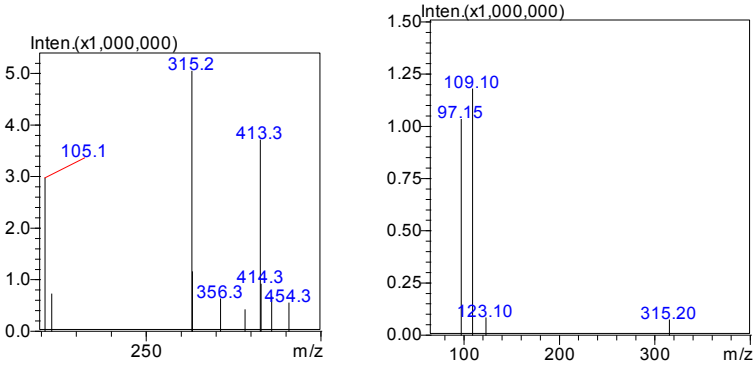


Fig. 1 Mass spectra and MS/MS spectra of 17 hormones

MRM Chromatogram of Standards

The MRM chromatograms of the multi-standard solution are shown in Fig.2.

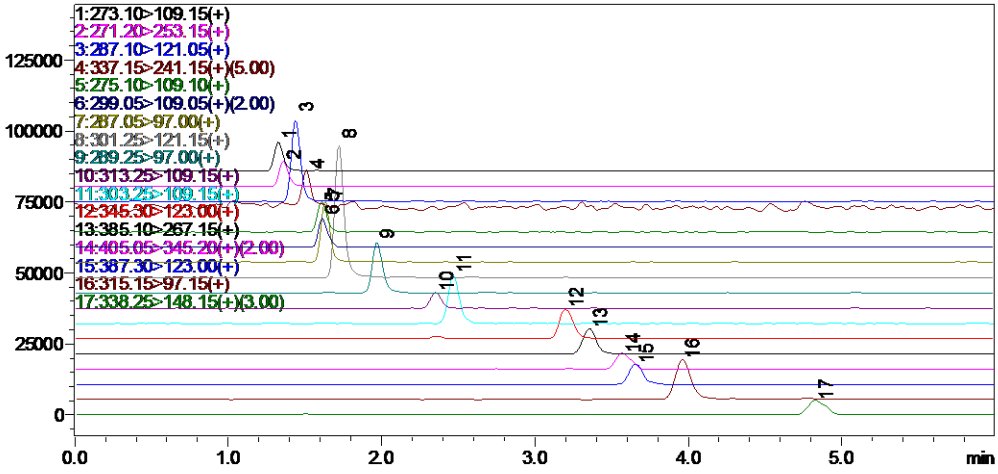


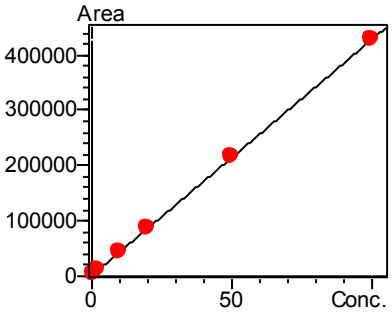
Fig.2 MRM Chromatograms of multi-standard sample (10 µg/L)

Assignment of peaks:1.Norandrostenedione; 2.Trenbolone;3..Boldenone; 4.Fluoxymesterone; 5. Norandrostenolone;6.Norethindrone; 7.Androstenedione;8.Metandienone; 9.Testosterone; 10. Methylnorethindrone; 11. Methyltestosterone; 12. Medroxyprogesterone; 13. Megestrol acetate; 14.Chlormadinone acetate;15. Medroxyprogesterone acetate; 16.Progesterone; 17.Danazol

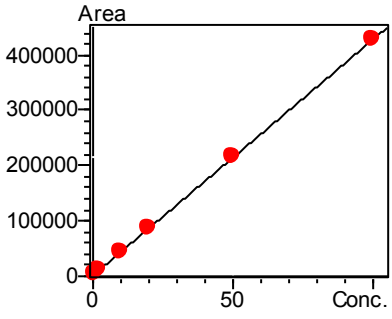
Linearity

Samples at concentration points within the linearity range as shown in Table 2 were subjected to assay under the analysis conditions specified in 1.2 and calibrations curves were plotted as shown in Fig. 3 by 6-point external standard method with concentration as abscissa and peak area as ordinate. The peak area and concentration of the calibration curves of the 17 hormones were of good linear dependence in the hormones' respective linear concentration range, and all calibration curves had a correlation coefficient in the range of 0.9987~0.9999 (See Table 3).

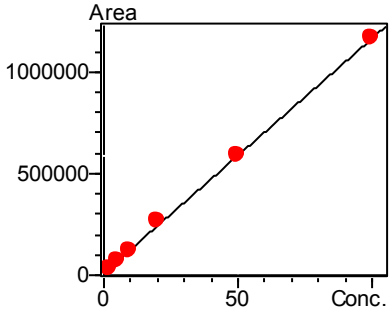
1. Norandrostenedione



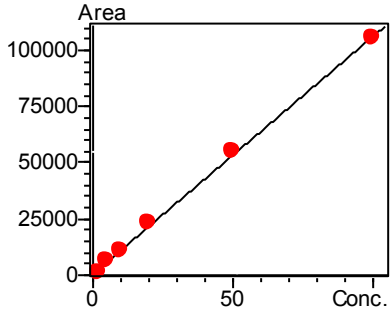
2. Trenbolone



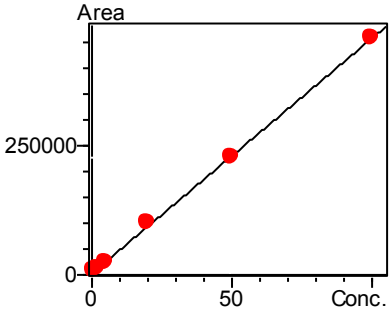
3. Boldenone



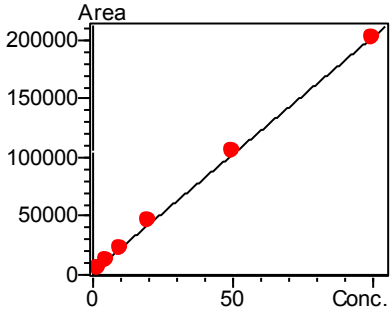
4.Fluoxymesterone



5. Norandrostenolone

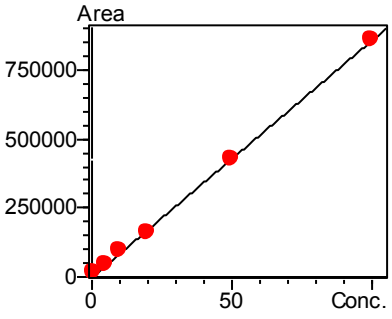


6.Norethindrone

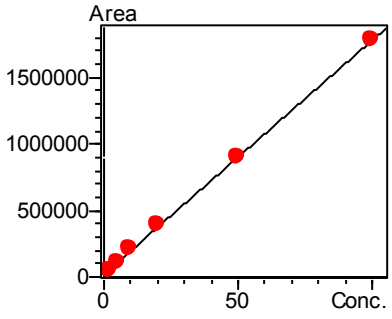


7.Androstenedione

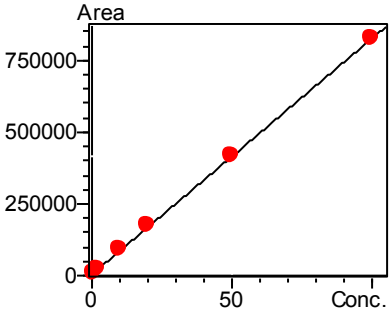
8.Metandienone



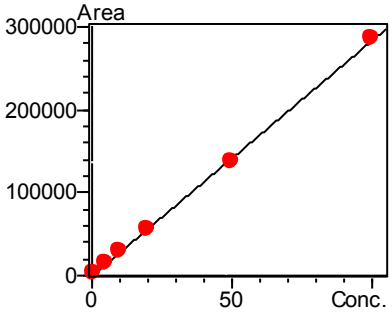
9. Testosterone



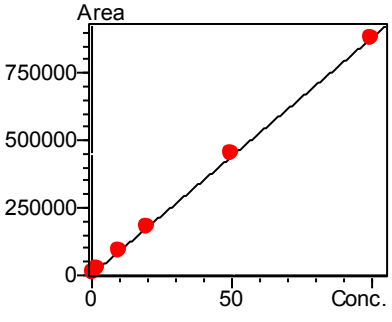
10. Methylnorethindrone



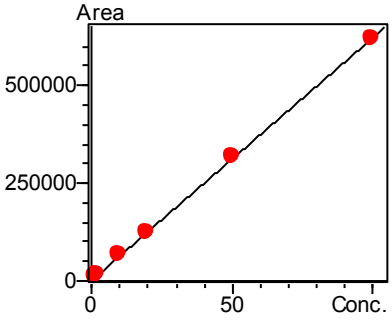
11. Methyltestosterone



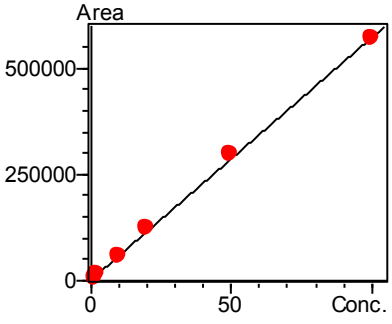
12. Medroxyprogesterone

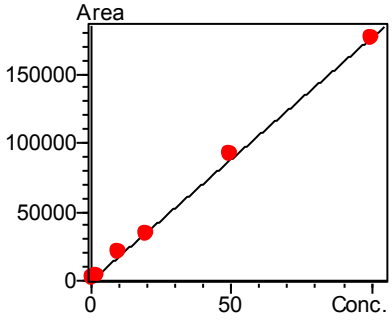


13. Megestrol acetate

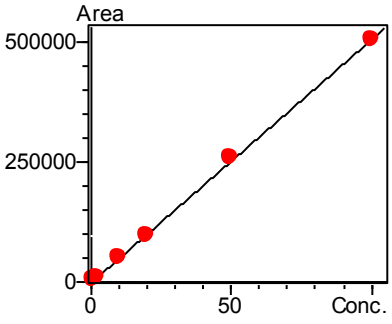


14.Chlormadinone acetate

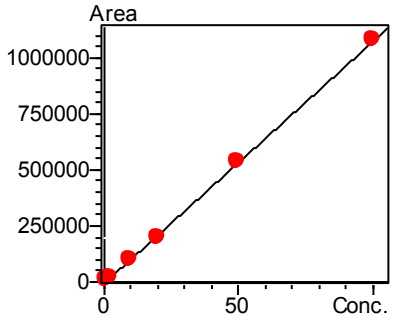




15. Medroxyprogesterone acetate



16.Progesterone



17. Danazol

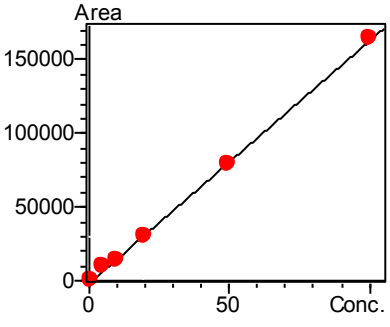


Fig. 3 Calibration curves of the 17 hormones

Table 3 Parameters of the calibration curves of the 17 hormones

No.:	Compound	Calibration Curve	Linear Range (µg/L)	Correlation Coefficient (R)
1	Norandrostenedione	Y = (4254.64)X + (1493.63)	1~100	0.9999
2	Trenbolone	Y = (3379.96)X + (6893.91)	2~100	0.9987
3	Boldenone	Y = (11511.7)X + (12943.8)	2~100	0.9998
4	Fluoxymesterone	Y = (1053.25)X + (632.811)	2~100	0.9996
5	Norandrostenolone	Y = (4550.24)X + (2929.70)	1~100	0.9998
6	Norethindrone	Y = (1992.13)X + (3128.01)	2~100	0.9997
7	Androstenedione	Y = (8551.73)X + (-384.882)	1~100	0.9998
8	Metandienone	Y = (17580.0)X + (21981.1)	2~100	0.9999
9	Testosterone	Y = (8203.99)X + (4305.48)	1~100	0.9999
10	Methylnorethindrone	Y = (2832.40)X + (-276.314)	1~100	0.9997
11	Methyltestosterone	Y = (8743.59)X + (3603.80)	1~100	0.9999
12	Medroxyprogesterone	Y = (6163.55)X + (3450.24)	1~100	0.9999

13	Megestrol acetate	Y = (5674.36)X + (3494.83)	1~100	0.9997
14	Chlormadinone acetate	Y = (1761.35)X + (153.117)	0.5~100	0.9994
15	Medroxyprogesterone acetate	Y = (5053.94)X + (-1205.06)	0.5~100	0.9998
16	Progesterone	Y = (10770.8)X + (-5643.77)	1~100	0.9998
17	Danazol	Y = (1640.93)X + (-1559.86)	1~100	0.9997

Precision test

Precision test was carried out on 6 replicate assays of multi-standard solutions at low, medium and high concentrations(as shown in Table 5), the resulted RSDs of the retention time and peak area of the 17 analytes were in the ranges of 0.063%~0.322% and 0.555%~5.82%, respectively, suggesting the system had satisfactory precision.

Table 4 Repeatability - retention time and peak area (n=6)

No.	Compound	RSD% (2 µg/L)		RSD% (20 µg/L)		RSD% (100 µg/L)	
		R.T.	Area	R.T.	Area	R.T.	Area
1	Norandrostenedione	0.135	5.23	0.178	3.83	0.115	2.70
2	Trenbolone	0.096	5.35	0.227	2.94	0.085	1.23
3	Boldenone	0.230	4.53	0.187	2.57	0.118	1.37
4	Fluoxymesterone	0.242	4.98	0.205	4.78	0.105	3.69
5	Norandrostenolone	0.192	3.27	0.186	2.89	0.120	2.52
6	Norethindrone	0.310	5.12	0.269	3.46	0.117	2.13
7	Androstenedione	0.199	2.83	0.186	3.71	0.117	1.71
8	Metandienone	0.176	4.97	0.201	1.44	0.133	0.555
9	Testosterone	0.212	4.98	0.222	3.05	0.115	1.00
10	Methylnorethindrone	0.322	5.82	0.205	4.13	0.105	1.93
11	Methyltestosterone	0.220	5.42	0.120	3.67	0.122	1.52
12	Medroxyprogesterone	0.273	5.32	0.250	3.17	0.095	2.36
13	Megestrol acetate	0.167	4.85	0.104	3.02	0.068	1.99
14	Chlormadinone acetate	0.282	5.66	0.136	4.09	0.063	1.44
15	Medroxyprogesterone acetate	0.268	3.69	0.137	3.33	0.073	2.14
16	Progesterone	0.243	5.13	0.169	3.28	0.081	1.61
17	Danazol	0.285	5.63	0.227	3.51	0.098	2.13

LODs and LOQs

In order to evaluate the method's sensitivity, 1 multi-standard sample solution was prepared at the concentration of 2 µg/L (see Table 5) and subjected to 7 replicate injections for analysis. LODs and LOQs were calculated for the analytes as 3 times of S/N ratio and 10 times of S/N ratio, respectively, based on the average S/N ratios of the 7 assays. The assay results were as shown in Table 5.

Table 5 LODs and LOQs of the 17 hormones

No.	Compound	S/N	LOD (µg/L)	LOQ (µg/L)
1	Norandrostenedione	30.7	0.20	0.67
2	Trenbolone	48.7	0.12	0.40
3	Boldenone	65.1	0.09	0.31
4	Fluoxymesterone	18.9	0.32	1.06
5	Norandrostenolone	27.6	0.22	0.72
6	Norethindrone	30.3	0.20	0.67
7	Androstenedione	22.2	0.27	0.90
8	Metandienone	86.7	0.07	0.23
9	Testosterone	56.3	0.11	0.37
10	Methylnorethindrone	19.8	0.30	1.00
11	Methyltestosterone	45.5	0.13	0.44
12	Medroxyprogesterone	58.1	0.10	0.35
13	Megestrol acetate	261.3	0.023	0.08
14	Chlormadinone acetate	200	0.03	0.10
15	Medroxyprogesterone acetate	277	0.022	0.07
16	Progesterone	19.3	0.31	1.03
17	Danazol	110.4	0.05	0.18

Spiked matrix test

Fig. 5 shows the MRM chromatograms of fish meat matrix that had been subjected to the sample preparation method specified in 1.3. The MRM chromatograms of fish meat matrix spiked with standards of the 17 hormones are shown in Fig. 6. The detection results of the 17 hormones in

fish meat and the recoveries of the 17 hormones from spiked samples were as shown in Table 6.

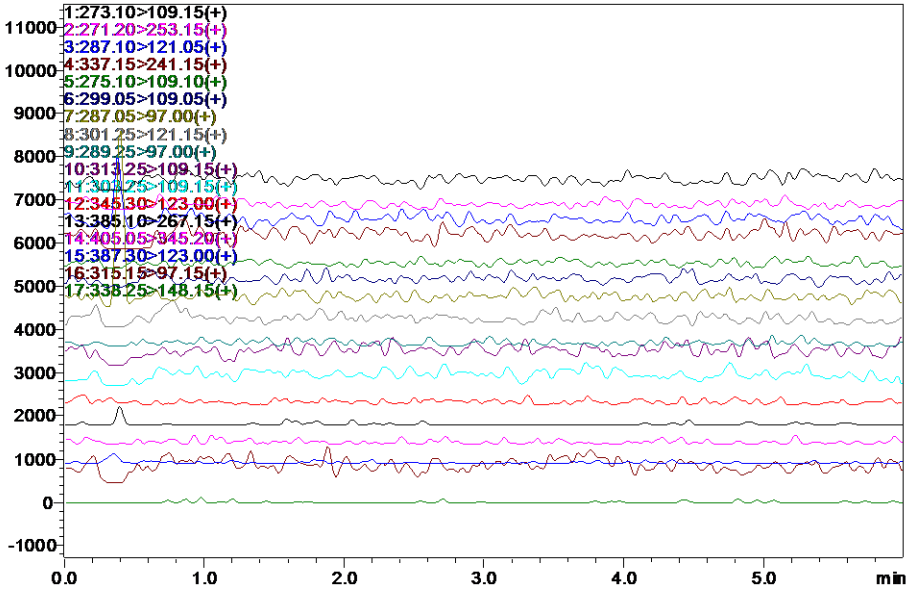


Fig. 5 MRM chromatograms of fish meat matrix

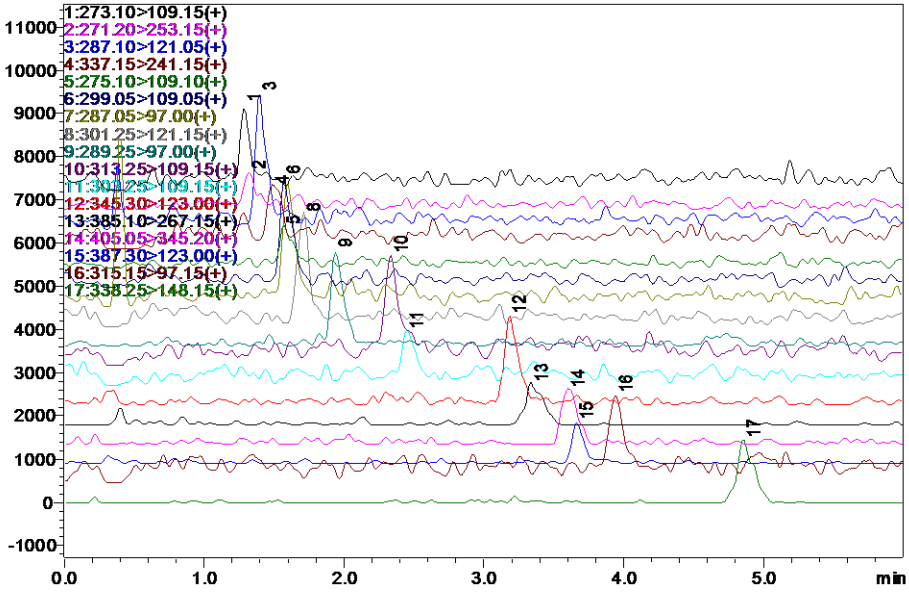


Fig. 6. MRM chromatograms of fish meat matrix spiked with standards (see Table 6 for the spiked levels)

Assignment of peaks: 1. Norandrostenedione; 2.Trenbolone; 3. Boldenone; 4.Fluoxymesterone;5. Norandrostenolone; 6.Androstenedione; 7.Norethindrone; 8.Metandienone; 9.Testosterone; 10. Methylnorethindrone; 11.Methyltestosterone;12.Medroxyprogesterone, 13. Megestrol acetate;14.Chlormadinone acetate; 15. Medroxyprogesterone acetate; 16.Progesterone; 17.Danazol

Table 6 Detection results and recoveries of the 17 hormones from fish meat

No.	Compound	Detected concentration in fish meat (μg/kg)	Spiked level in fish meat (μg/kg)	Tested concentration (μg/kg)	Recovery (%)
1	Norandrostenedione	N.D.	0.4	0.424	106.0
2	Trenbolone	N.D.	0.4	0.396	99.0
3	Boldenone	N.D.	0.4	0.372	93.0
4	Fluoxymesterone	N.D.	2	2.27	113.5
5	Norandrostenolone	N.D.	0.4	0.424	106
6	Androstenedione	N.D.	1	0.947	94.7
7	Norethindrone	N.D.	0.4	0.362	90.5
8	Metandienone	N.D.	0.4	0.380	95.0
9	Testosterone	N.D.	0.4	0.320	80.0
10	Methylnorethindrone	N.D.	1	0.961	96.1
11	Methyltestosterone	N.D.	0.4	0.380	95.0
12	Medroxyprogesterone Megestrol acetate	N.D.	0.4	0.376	94.1
13	Chlormadinone acetate	N.D.	0.4	0.458	114.5
14		N.D.	1	1.15	115
15	Medroxyprogesterone acetate	N.D.	0.4	0.442	110.5
16	Progesterone	N.D.	0.4	0.446	111.6
17	Danazol	N.D.	2	1.46	78.0

Note: N.D. means not detected

CONCLUSION

A method is proposed in this paper for determination of 17 hormones residues in fishery products using Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8030 triple quadrupole mass spectrometer. The proposed method demonstrated a wide linear range for the 17 hormones with correlation coefficients all falling in the range of 0.9987~0.9999. Precision test was conducted on 6 successive assays of multi-standard solutions at low, medium and high concentrations. The RSDs of retention

time and peak area of 6 successive injections were in the ranges of 0.063%~0.322% and 0.555%~5.82%, respectively, showing that the method had satisfactory precision. Spike recoveries of the analytes from spiked fishery products matrix were in the range of 78.0%~115%. The study results showed the method had high sensitivity and LODs lower than the requirements stipulated in GB/T 21981-2008 *Determination of hormone multi-residues in foodstuffs of animal origin -- LC-MS/MS method*, suggesting that the method is sufficient for detecting hormones residues in fishery products.

V-30 Fast determination of quinolones residues in fishery products by UHPLC-MS/MS

INTRODUCTION

An analytical method is proposed in this paper for the determination of 10 quinolones residues in fishery products using Shimadzu ultra fast liquid chromatograph (UFLC) and triple quadrupole mass spectrometer. Quinolones in samples that had been processed were fast separated by the LC-30A ultra fast liquid chromatograph within 6 minutes, and then quantitatively assayed with the LCMS-8040 triple quadrupole mass spectrometer. Calibration curves of the 10 quinolone antibiotics were plotted using the external standard method. The plotted calibration curves were of satisfactory linearity with correlation coefficients in the range of 0.9989~0.9999. Standard solutions of various concentrations were used for precision test. The RSDs% of retention time and peak area data of 6 successive injections were below 0.50% and 5.30%, respectively, showing that the method had satisfactory precision. Spike recoveries of analytes from matrices of various concentrations were in the range of 68.0%~95.5%.

Quinolones, a category of artificially synthesized antibacterial agents, are extensively used for the prevention and treatment of a variety of infectious diseases of human and animals for their broad antibacterial spectrum, strong antibacterial action, excellent tissue penetration, low cost, and scarce cross tolerance with other antibacterial agents. This category of drugs is frequently used for the prevention and treatment of diseases in fish. They even demonstrated certain growth promoting actions when used at small dosage. However, they may also remain in the tissues of edible animals and give rise to the problem of drugs residues if used excessively or inappropriately. Prolonged consumption of food contaminated with the residues of these drugs may induce drug tolerance and resistance problems and, in serious cases, the

problems of long-term toxicity and/or carcinogenesis/teratogensis/mutagenesis in consumers. Therefore, people have become more and more concerned by the issue of quinolone drug residues. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) and EU have stipulated MRLs in animal tissues for many quinolone drugs including enrofloxacin, ciprofloxacin, ofloxacin, norfloxacin, sxolinic acid, and flumequine; FDA had imposed a ban on the sales and applications of the antibacterial agent enrofloxacin for the treatment of bacterial infection in poultry and fish in 2005. Japan's Positive List System had also stipulated MRLs for enrofloxacin, ciprofloxacin, sxolinic acid, and flumequine.

HPLC is of poor sensitivity when used for determination of quinolones residues in fishery products because these products are featured by high protein and fat content and complicated matrix. UHPLC-MS/MS, on the other hand, has been frequently used in the confirmatory analysis of quinolones for their good selectivity, high sensitivity, and ability to detect multiple quinolones residues simultaneously. In this paper, a method was developed for the determination of 10 quinolones residues in fishery products using Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8040 triple quadrupole mass spectrometer.

EXPERIMENTAL

Apparatus

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8040 was used in the experiment. The configuration included two LC-20AD$_{XR}$ pumps, a DGU-20A$_5$ online degasser, a SIL-20AC autosampler, a CTO-20AC column oven, a CBM-20A communications bus module, a LCMS-8040 triple quadrupole mass spectrometer, and a LabSolutions Ver.5.53 chromatography workstation.

Conditions of Analysis

LC conditions

Column	:Shim-pack XR-ODS II 2.0 mm I.D.× 75 mm L., 2.2 µm
Mobile phase	:A: 5mM ammonium acetate (containing 0.1% formic acid)
Mobile phase	:B:acetonitrile
Flow rate	:0.4 mL/min
Column temperature	:40 °C
Injection volume	:20 µL
Elution mode	:Binary gradient with initial concentration of 10% of mobile phase B., see Table 1 for time program.

Table 1 Time program

Time (min)	Module	Command	Value
1.0	Pumps	Pump BConc.	10%
4.0	Pumps	Pump B Conc.	60%
4.5	Pumps	Pump B Conc.	60%
4.6	Pumps	Pump B Conc.	10%
6.0	Controller	Stop	

MS conditions

Ionization mode	:ESI(+)
Ionization voltage	:+4.5 kV
Nebulizing gas	:Nitrogen, 3.0 L/min
Drying gas	:Nitrogen, 15.0 L/min
Collision gas	:Argon
DL temperature	:250 °C
Heater block temperature	:400 °C
Mode	:Multiple Reaction Monitoring (MRM)
Dwell time	:10 ms

Pause time :3 ms

MRM parameters: see Table 2

Table 2 Optimized MRM parameters

Compound	Precursor Ion	Product Ion	Q_1 Pre Bias(V)	CE(V)	Q_3 Pre Bias(V)
Pipemedic acid	304.2	286.1*	-30.0	-19.0	-20.0
		217.1	-30.0	-21.0	-23.0
Enoxacin	321.2	303.2*	-15.0	-18.0	-22.0
		204.1	-15.0	-41.0	-21.0
Ofloxacin	362.2	318.2*	-30.0	-18.0	-22.0
		261.1	-30.0	-26.0	-27.0
Pefloxacin	334.2	316.1*	-22.0	-21.0	-16.0
		290.2	-22.0	-16.0	-30.0
Ciprofloxacin	332.2	314.1*	-15.0	-20.0	-22.0
		231.1	-15.0	-39.0	-25.0
Lomefloxacin	352.2	265.1*	-12.0	-23.0	-29.0
		308.2	-12.0	-16.0	-24.0
Danofloxacin	358.2	340.2*	-17.0	-19.0	-17.0
		82.2	-17.0	-44.0	-30.0
Enrofloxacin	360.3	342.2*	-17.0	-21.0	-25.0
		316.2	-17.0	-18.0	-22.0
Sarafloxacin	386.2	368.3*	-26.0	-20.0	-25.0
		270.2	-26.0	-44.0	-26.0
Cinoxacin	263.1	245.1*	-12.0	-14.0	-12.0
		189.0	-12.0	-27.0	-19.0

Note: *refers to quantitative ion

Sample Preparation

Preparation of standard solution

A total of 10 standard substances, i.e. enoxacin, ofloxacin, pefloxacin, ciprofloxacin, lomefloxacin, danofloxacin, enrofloxacin, sarafloxacin, pipemedic acid, and cinoxacin, were used in the study.

Preparation of standard working solutions: A multi-standard intermediate solution of concentration of 10 mg/L was prepared using acetonitrile as solvent, then diluted into a series of multi-standard working solutions of various concentrations with acetonitrile and methanol aqueous solution containing 0.1% formic acid (1/9, v/v). The detailed concentrations of standard substances were as shown in Table 3.

Table 3 Concentrations of standard substances in multi-standard working solution (μg/L)

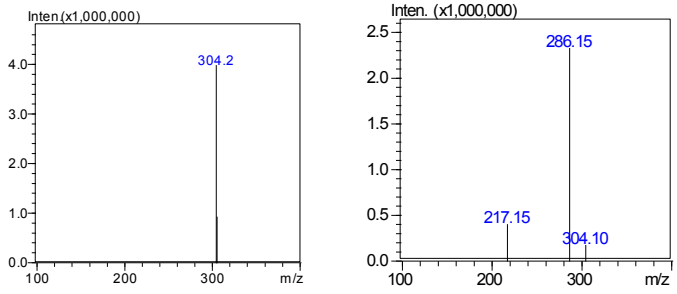
Compound	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6
Pipemedic acid	2	4	10	40	200	500
Enoxacin	2	4	10	40	200	500
Ofloxacin	2	4	10	40	200	500
Pefloxacin	1	2	5	20	100	250
Ciprofloxacin	0.5	1	2.5	10	50	125
Lomefloxacin	2	4	10	40	200	500
Danofloxacin	1	2	5	20	100	250
Enrofloxacin	0.5	1	2.5	10	50	125
Sarafloxacin	0.4	0.8	2	8	40	100
Cinoxacin	2	4	10	40	200	500

Sample pretreatment method

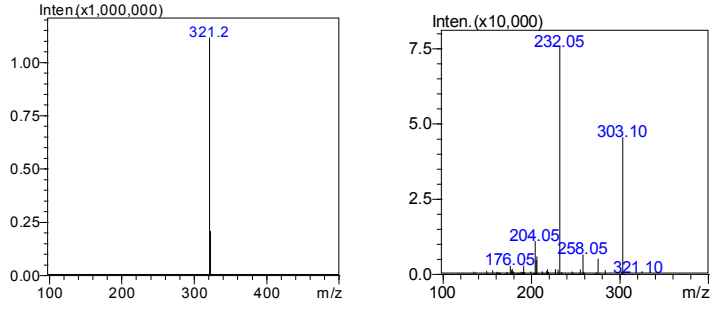
Fishery products matrices were subjected to the pretreatment procedures specified in GB/T 20366-2006 *Method for the determination of quinolones in animal tissues--LC-MS/MS method*.

Results and Discussion

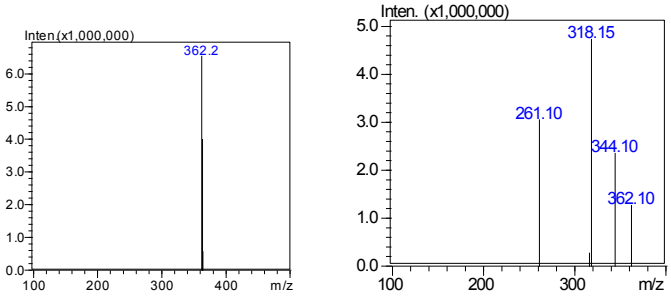
Mass and MS/MS spectra of standard samples



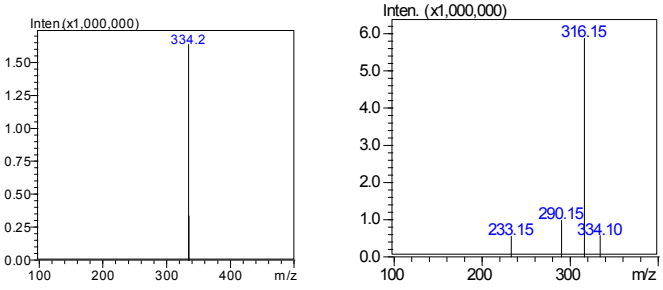
1 Mass spectrum (left) and MS/MS spectrum (right, CE value: -20V) of pipemedic acid



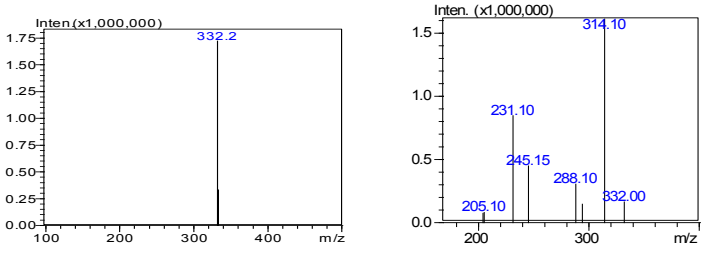
2 Mass spectrum (left) and MS/MS spectrum (right, CE value: -20V) of enoxacin



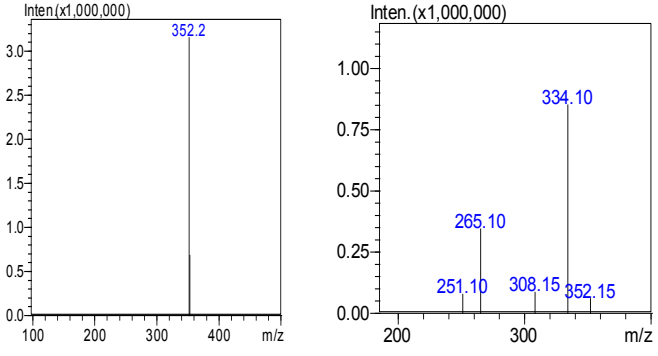
3 Mass spectrum (left) and MS/MS spectrum (right, CE value: -22V) of ofloxacin



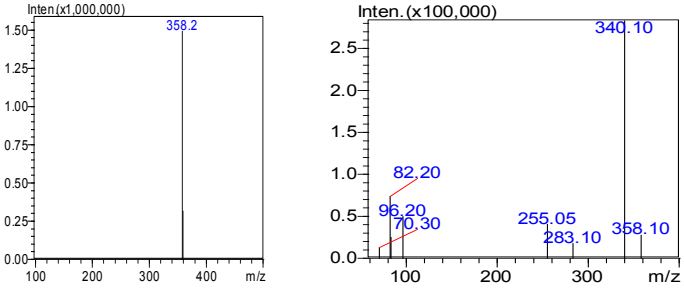
4 Mass spectrum (left) and MS/MS spectrum (right, CE value: -21V) of pefloxacin



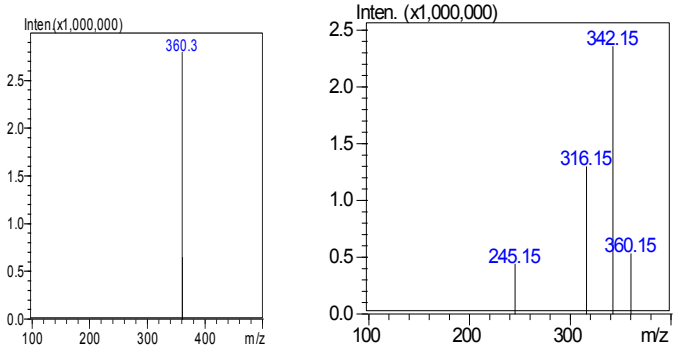
5 Mass spectrum (left) and MS/MS spectrum (right, CE value: -24V) of ciprofloxacin



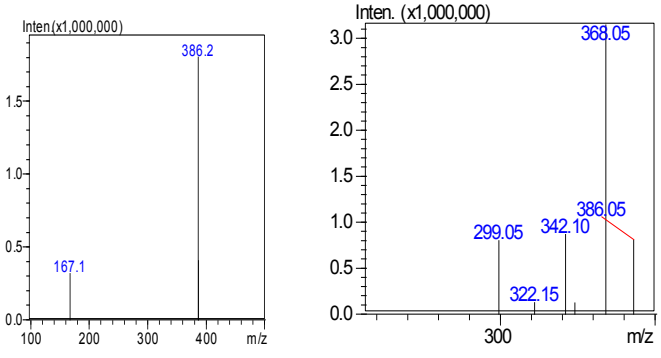
6 Mass spectrum (left) and MS/MS spectrum (right, CE value: -23V) of lomefloxacin



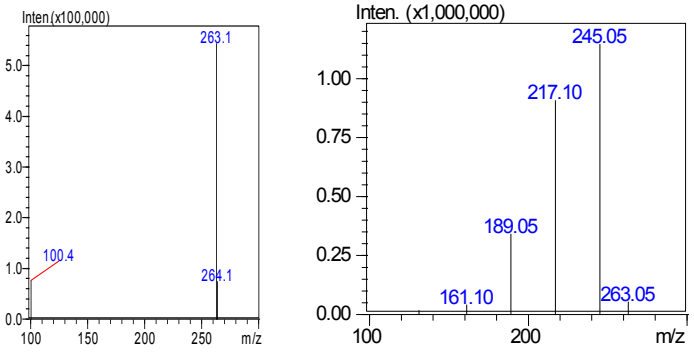
7 Mass spectrum (left) and MS/MS spectrum (right, CE value: -30V) of danofloxacin



8 Mass spectrum (left) and MS/MS spectrum (right, CE value: -21V) of enrofloxacin



9 Mass spectrum (left) and MS/MS spectrum (right, CE value: -22V) of sarafloxacin



10. Mass spectrum (left) and MS/MS spectrum (right, CE value: -20V) of cinoxacin

Fig. 1 Mass spectra and MS/MS spectra of 10 quinolones

MRM Chromatogram of Standards

The MRM chromatograms of the 10 quinolones are shown in Figure 2.

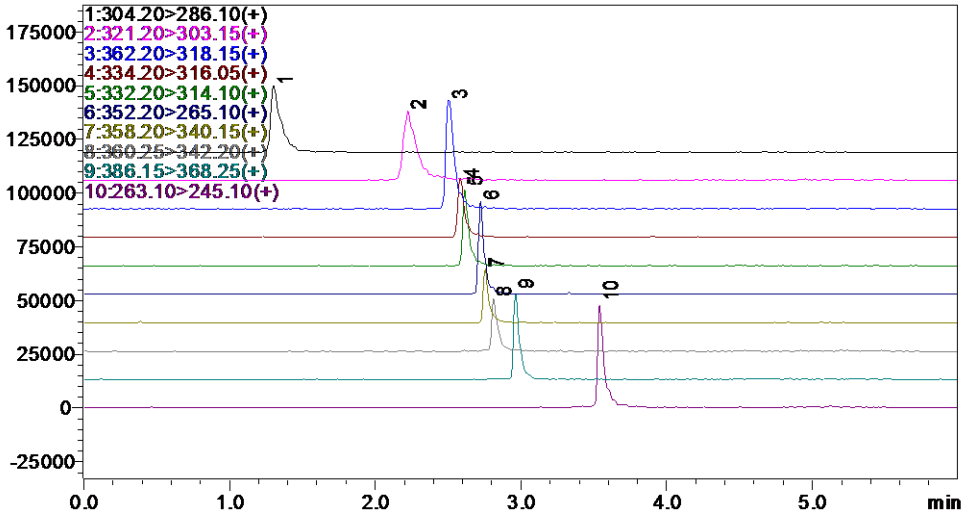


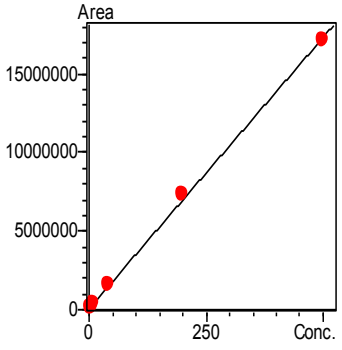
Fig. 2 MRM chromatogram of 10 quinolones standards

Assignment of peaks: 1. pipemedic acid (4 μg/L); 2. enoxacin (4 μg/L); 3. ofloxacin (4 μg/L); (2 μg/L); 5. ciprofloxacin (1 μg/L); 6. lomefloxacin (4 μg/L); 7. danofloxacin (2 μg/L); 8. enrofloxacin (1 μg/L); 9. sarafloxacin (0.8 μg/L); 10. cinoxacin (4 μg/L)

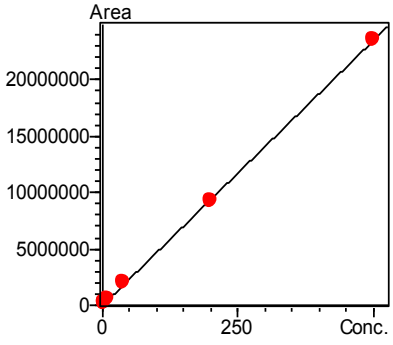
Linearity

Multi-standard working solutions of various concentrations (see Table 3) were determined under the analysis conditions as specified in Section 1.2 and calibration curves were plotted as shown in Fig. 3 using external standard method with concentration as abscissa and peak area as ordinate; the resulted calibration curves were of good linearity and their linear equations and correlation coefficients are listed in Table 4.

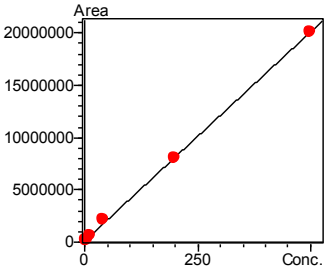
1. pipemedic acid



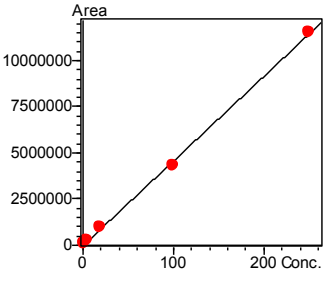
2. enoxacin



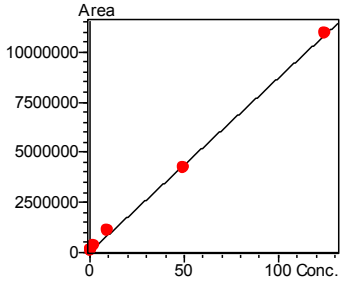
3. ofloxacin



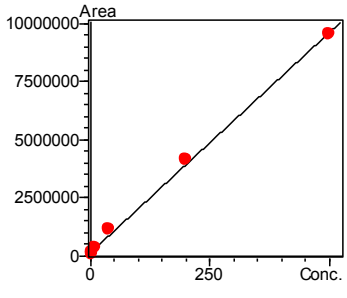
4. pefloxacin



5. ciprofloxacin

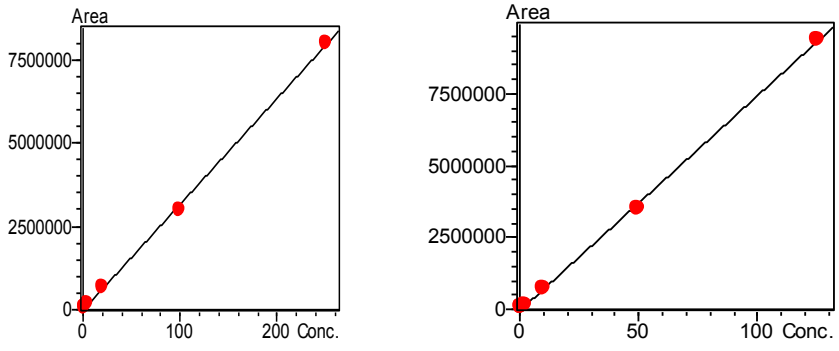


6. lomefloxacin



7. danofloxacin

8. enrofloxacin



9. sarafloxacin 10. cinoxacin

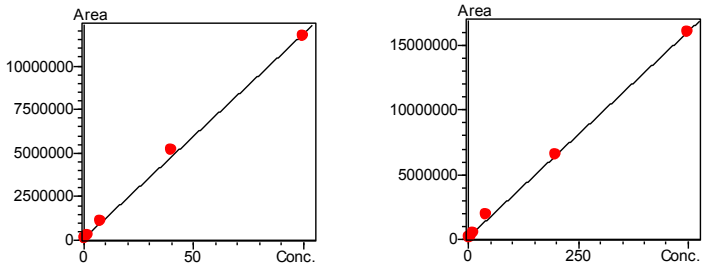


Fig. 3 Calibration curves of the 10 quinolones standards

Table 4 Parameters of the calibration curves of the 10 quinolones

No.	Compound	Calibration Curve	Linear Range (μg/L)	Correlation Coefficient (r)
1	Pipemedic acid	Y = (36578.1)X + (-5519.45)	2~500	0.9999
2	Enoxacin	Y = (46525.9)X + (19258.1)	2~500	0.9999
3	Ofloxacin	Y = (44869.6)X + (66854.4)	2~500	0.9994
4	Pefloxacin	Y = (45846.3)X + (-22086.0)	1~250	0.9996
5	Ciprofloxacin	Y = (95160.9)X + (-3949.78)	0.5~125	0.9991
6	Lomefloxacin	Y = (19564.3)X + (168771)	2~500	0.9989
7	Danofloxacin	Y = (31820.2)X + (-19813.1)	1~250	0.9997
8	Enrofloxacin	Y = (74899.2)X + (-49109.1)	0.5~125	0.9997
9	Sarafloxacin	Y = (117672)X + (120403)	0.4~100	0.9993
10	Cinoxacin	Y = (31726.8)X + (200913)	2~500	0.9994

Precision test

Multi-standard solutions of various concentrations were assayed for 6 times in succession to evaluate the method's precision. The resulted repeatability of retention time and peak area data was as shown in Table 5. The result showed that the %RSDs of retention time and peak area of standard solutions of various concentrations fell in the ranges of 0.02%~ 0.50 % and 0.87%~5.30% respectively, suggesting the method had satisfactory precision.

Table 5 Repeatability - retention time and peak area (n=6)

Compound	RSD% (2 µg/L)		RSD% (40 µg/L)		RSD% (500 µg/L)	
	R.T.	Area	R.T.	Area	R.T.	Area
Pipemedic acid	0.39	3.64	0.23	2.82	0.34	1.42
Enoxacin	0.26	1.67	0.50	1.68	0.37	2.01
Ofloxacin	0.14	3.60	0.14	2.88	0.09	0.96
Lomefloxacin	0.12	3.53	0.04	2.14	0.07	1.28
Cinoxacin	0.07	3.78	0.04	1.94	0.04	1.05
	RSD% (1 µg/L)		RSD% (20 µg/L)		RSD% (250 µg/L)	
	R.T.	Area	R.T.	Area	R.T.	Area
Pefloxacin	0.12	5.30	0.09	2.61	0.07	1.73
Danofloxacin	0.11	2.63	0.02	2.42	0.05	1.34
	RSD% (0.5 µg/L)		RSD% (10 µg/L)		RSD% (125 µg/L)	
	R.T.	Area	R.T.	Area	R.T.	Area
Ciprofloxacin	0.13	3.61	0.08	2.18	0.13	1.68
Enrofloxacin	0.08	5.24	0.04	2.04	0.08	1.77
	RSD% (0.4 µg/L)		RSD% (8 µg/L)		RSD% (100 µg/L)	
	R.T.	Area	R.T.	Area	R.T.	Area
Sarafloxacin	0.08	4.39	0.03	2.27	0.07	0.87

LODs and LOQs

In order to evaluate the method's sensitivity, a multi-standard solution containing the 10 quinolones at concentrations as shown below was prepared and subjected to 7 replicate injections. The LODs and LOQs were calculated from the standard deviations of the area ratios of the 7 injections and determinations using the formulae LOD = 3.14×S and LOQ=4×LOD. The assay results were as shown in Table 6.

Table 6 LODs and LOQs of the 10 quinolones

No.	Compound	Concentration (μg/L)	RSD (%)	LOD (μg/L)	LOQ (μg/L)
1	Pipemedic acid	2	4.63	0.29	1.16
2	Enoxacin	2	2.85	0.18	0.72
3	Ofloxacin	2	1.63	0.10	0.40
4	Pefloxacin	1	4.48	0.14	0.56
5	Ciprofloxacin	0.5	4.40	0.07	0.28
6	Lomefloxacin	2	3.23	0.20	0.80
7	Danofloxacin	1	2.80	0.09	0.36
8	Enrofloxacin	0.5	2.80	0.05	0.20
9	Sarafloxacin	0.4	1.82	0.03	0.12
10	Cinoxacin	2	2.91	0.18	0.72

Spiked matrix test

A fish meat sample that contained none of the 10 quinolones was used as blank matrix for the spiked matrix test and spiked with standards at concentrations as shown in Table 7. The assay chromatograms of fish meat samples and spiked samples were as shown in Fig.4 and Fig.5, respectively. As can be inferred from the results in Table 7, the method was of high sensitivity and its LODs of quinolones in fish meat samples were well below the MRLs specified in GB/T 20366-2006 *Analysis of fourteen quinolones in food of animal origin by high performance liquid chromatograph tandem mass spectrometry*.

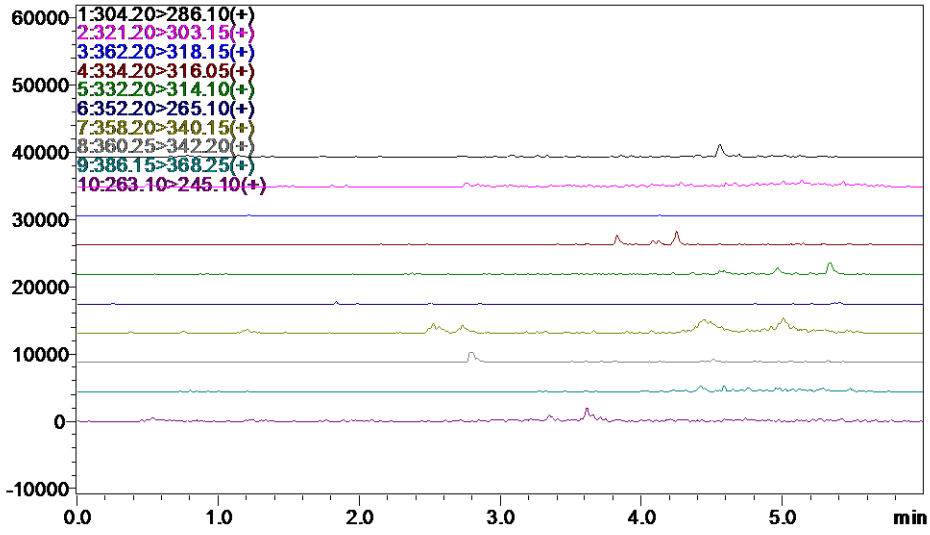


Fig. 4 MRM chromatograms of fish meat sample

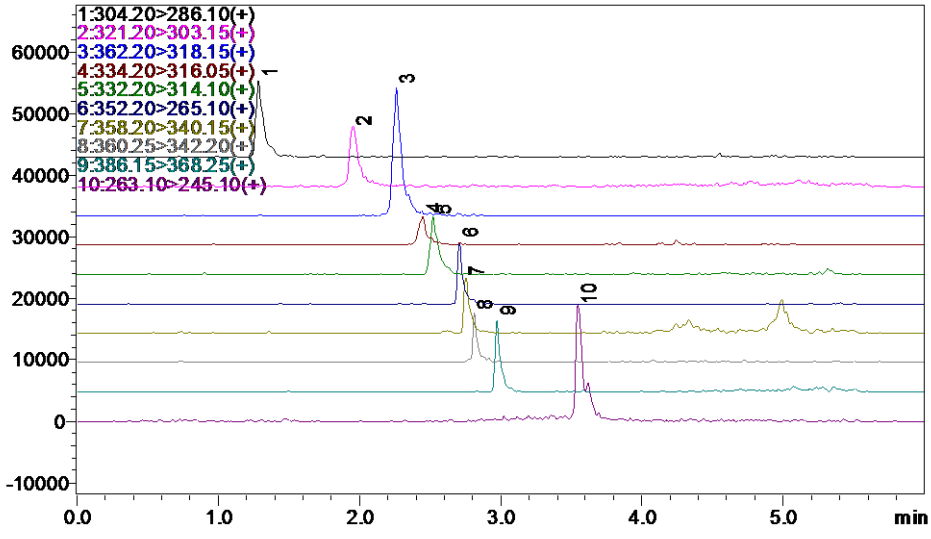


Fig. 5 MRM chromatograms of fish meat sample spiked with standard (at levels as shown in Table 7)

Assignment of peaks: 1. pipemedic acid; 2. enoxacin; 3. ofloxacin; 4. pefloxacin; 5. ciprofloxacin; 6. lomefloxacin; 7. danofloxacin; 8. enrofloxacin; 9. sarafloxacin; 10. cinoxacin

Table 7 Spike recoveries and S/N ratio

No.:	Compound	Spike level (μg/kg)	S/N	Measured Value (μg/kg)	Recovery (%)
1	Pipemedic acid	2	76	1.62	81.0
2	Enoxacin	2	46	1.36	68.0
3	Ofloxacin	2	154	1.76	88.0
4	Lomefloxacin	2	224	1.44	77.0
5	Cinoxacin	2	51	1.91	95.5
6	Pefloxacin	1	33	0.92	92.0
7	Danofloxacin	1	42	0.83	83.0
8	Ciprofloxacin	0.5	55	0.37	74.0
9	Enrofloxacin	0.5	41	0.38	76.0
10	Sarafloxacin	0.4	104	0.33	82.5

CONCLUSION

A method is proposed in this paper for the determination of 10 quinolones residues in fishery products using Shimadzu LC-30A ultra fast liquid chromatograph and LCMS-8040 triple quadrupole mass spectrometer. With the proposed method, 10 target analytes were separated and assayed within 6.0 minutes and the correlation coefficients of calibration curves of the 10 analytes were all in the range of 0.9989~0.9999. Precision test was performed on standard solutions of various concentrations. The %RSDs of retention time and peak area of 6 successive injections were below 0.50 % and 5.30 %, respectively, showing that the system had satisfactory precision. With the merits of ultrafast analysis speed, high sensitivity, and LODs well below the MRLs specified in GB/T 20366-2006 Method for the determination of quinolones in animal tissues - LC-MS/MS method, the proposed method is suitable for fast determination of quinolones residues in fishery products.

SHIMADZU
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V-31 Determination of Macrolide Antibiotic Residues in Aquatic Products using UFLC-Triple Quadrupole Mass Spectrometry

INTRODUCTION

In this paper, a method is described for the determination of 5 macrolide antibiotic residues in aquatic products using Shimadzu ultra fast liquid chromatograph and a triple quadrupole mass spectrometer. Samples were extracted, separated by ultra fast liquid chromatograph LC-30A, and then quantitatively assayed with triple quadrupole mass spectrometer LCMS-8040. 5 macrolide antibiotics were separated and detected rapidly within 3 minutes. The proposed method demonstrated satisfactory linearity for spiramycin in the range of 0.5~100 μg/L, kitasamycin and tylosin in the range of 0.2~50 μg/L, and erythromycin and tilmicosin in the range of 0.2~100 μg/L; the correlation coefficients of calibration curves were all greater than 0.9951. Precision tests were performed on mixed standard solutions of concentrations at 2 μg/L, 20 μg/L and 50 μg/L: the %RSDs of retention time and peak area of 6 consecutive injections fell in the ranges of 0.05~1.13% and 0.85~2.43%, respectively, showing that the method is of satisfactory precision. The LODs fell in the range of 0.016~0.11 μg/L, LOQs in the range of 0.063~0.38 μg/L, and spike recoveries in the range of 84.0~104.0%. It is suitable for the detection of macrolide antibiotics in aquatic products.

China's aquiculture has developed rapidly in recent years. With increased intensification of aquiculture, aquiculture diseases are becoming a tougher challenge. Various drugs are widely used in the production of aquatic products, and the problem of drug residue is becoming increasingly prominent. Macrolide antibiotics are a category of antibiotics that is widely used at large dosages and apt to enter environmental water. Most macrolide antibiotics tend to be present in water at trace levels which are hard to detect. A method was developed with reference to SN/T1777.2-2007

Determination of macrolide antibiotic residues in animal-derived food - Part 2: HPLC-MS/MS method for the fast determination of 5 macrolide antibiotics (spiramycin, tilmicosin, tylosin, kitasamycin and erythromycin) commonly seen in aquatic products using Shimadzu ultra fast liquid chromatograph LC-30A and a triple quadrupole mass spectrometer LCMS-8040. The proposed method was evaluated to detect the pollution caused by macrolide antibiotics in commercial fish meat products for the reference of relevant analysts.

EXPERIMENTAL

Apparatus

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and a triple quadrupole mass spectrometer LCMS-8040 was used in the experiment. The configuration included two LC-30AD pumps, a DGU-20A$_5$ online degasser, a SIL-30AC autosampler, a CTO-30A column oven, a CBM-20A communications bus module, a LCMS-8040 triple quadrupole mass spectrometer, and a LabSolutions Ver. 5.53 chromatography workstation.

Conditions of Analysis

LC Condition

Apparatus	:LC-30A system
Column	:Shimadzu Shim-pack XR-ODS III (2.0 mmI.D.×50 mmL., 1.6 μm)
Mobile phase	:A－0.1 % formic acid aqueous solution
	; B acetonitrile－
Flow rate	:0.4 mL/min
Injection volume	:20 μL
Column temperature	:40 °C
Elution mode	:Binary gradient with initial concentration of 15%B. See Table 1 for time program.

MS condition

Apparatus	:LCMS-8040
Ion source	:ESI, positive mode
Ionization voltage	:ESI (+), +4.5 kV
Nebulizing gas	:Nitrogen, 3.0 L/min
Drying gas	:Nitrogen, 15.0 L/min
Collision gas	:Argon
DL temperature	:250 °C
Heater block temperature	:400 °C
Scan mode	:MRM
Pause time	:30 ms
Dwell time	:3 ms
MRM parameters	:See Table 2

Table 1 Time program

Time (min)	Module	Command	Value
1.00	Pumps	B Conc.	60
1.01	Pumps	B Conc.	85
1.30	Pumps	B Conc.	90
1.40	Pumps	B Conc.	15
3.00	Controller	Stop	

Table 2 MRM Parameters

No.	Name	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
1	Spiramycin	843.60	174.15*	30	-41	-18
			101.10	-30	-48	-11
2	Kitasamycin	772.45	109.15*	-22	-45	-22
			174.15	-22	-34	-18

3	Erythromycin	734.50	158.10*	-40	-31	-17
			576.35	-40	-20	-28
4	Tilmicosin	869.65	174.10*	-24	-49	-18
			696.50	-24	-51	-25
5	Tylosin	916.60	174.10*	-20	-43	-18
			101.50	-20	-51	-11

* refers to quantitative ion.

Sample Preparation

Preparation of standard solutions: aliquot of standard substances of spiramycin, tilmicosin, tylosin, kitasamycin and erythromycin, accurately were weighed, and mixed standard stock solutions (1000 mg/L) were prepared with methanol as a solvent, and then diluted with mobile phase to obtain standard working solutions of concentrations at 0.2, 0.5, 1, 5, 10, 20, 50 and 100 μg/L.

Pretreatment method of samples: refer to SN/T1777.2-2007 *Determination of macrolide antibiotic residues in animal-derived food - Part 2: HPLC-MS/MS method* for details.

RESULTS AND DISCUSSION

Mass and MS/MS spectra of standard samples

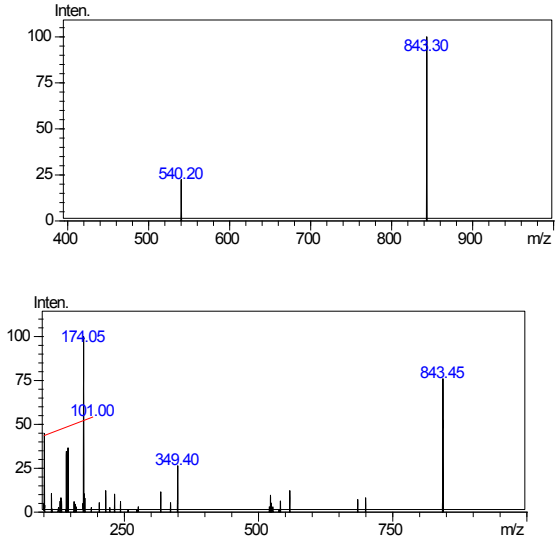


Fig. 1. Mass spectrum of spiramycin and MS/MS spectrum (CE -20V)

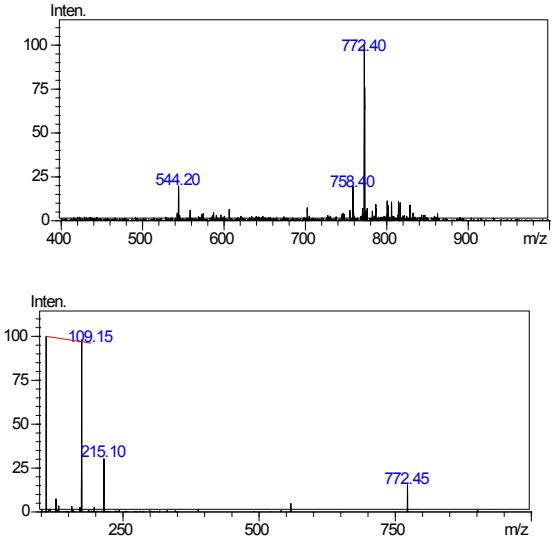


Fig. 2. Mass spectrum of kitasamycin and MS/MS spectrum (CE -32V)

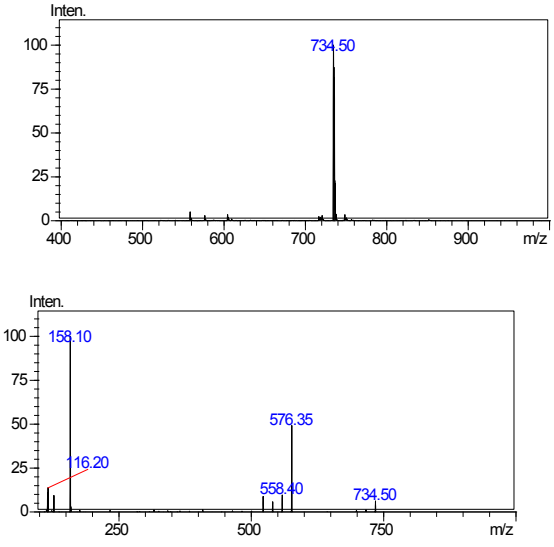


Fig. 3. Mass spectrum of erythromycin and MS/MS spectrum (CE -25V)

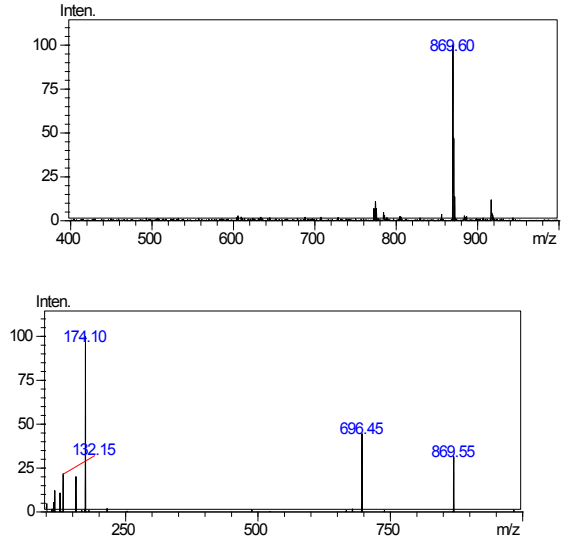


Fig. 4. Mass spectrum of tilmicosin and MS/MS spectrum (CE -45V)

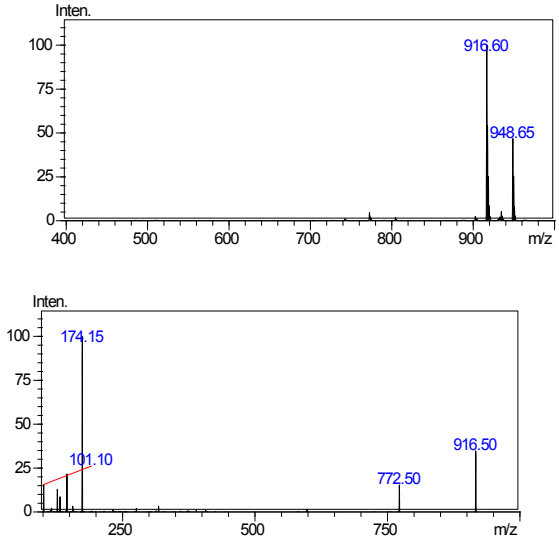


Fig. 5. Mass spectrum of tylosin and MS/MS spectrum (CE -35V)

MRM Chromatogram of Standard Samples

Fig. 6 shows MRM chromatograms of a mixed standard sample. 5 macrolide antibiotics were analyzed within 3 minutes.

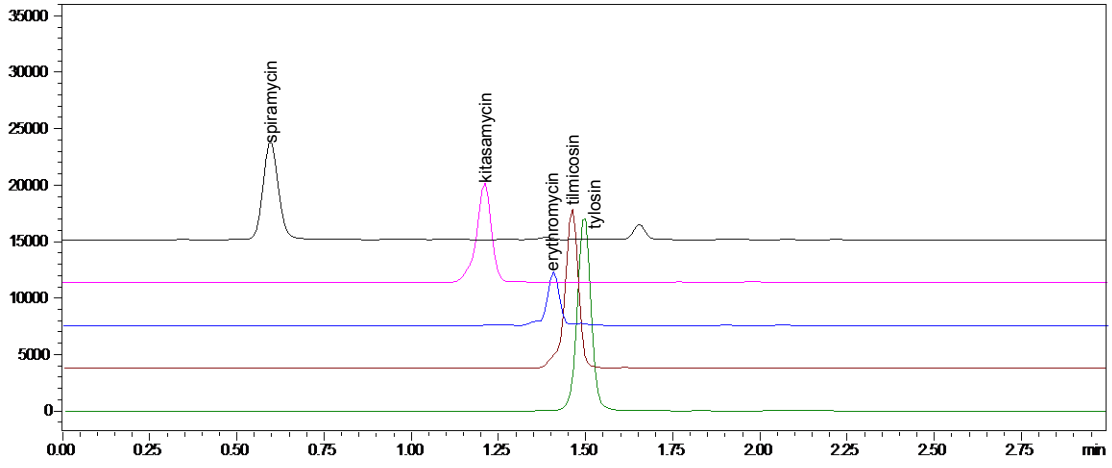


Fig. 6. MRM chromatogram of macrolide antibiotics standard samples (1 µg/L each)

Linearity

Mixed standard working solutions of concentrations at 0.2, 0.5, 1, 5, 10, 20, 50 and 100 µg/L were determined. Calibration curves were plotted as shown in Figs. 7-11 with the concentration as abscissa and the peak area as ordinate. The method demonstrated satisfactory linearity for spiramycin in the range of 0.5~100 µg/L, kitasamycin and tylosin in the range of 0.2~50 µg/L, and erythromycin and tilmicosin in the range of 0.2~100 µg/L. The plotted calibration curves were of good linearity. Their linear equations and correlation coefficients are shown in Table 3.

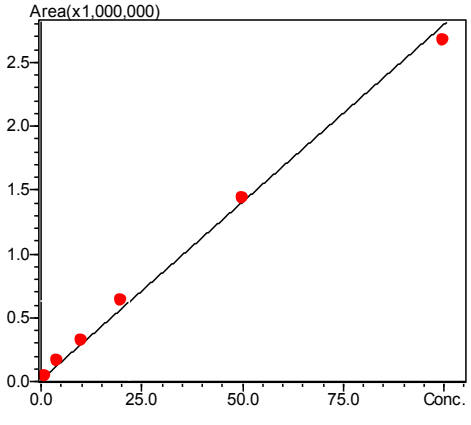


Fig. 7. Calibration curve of spiramycin

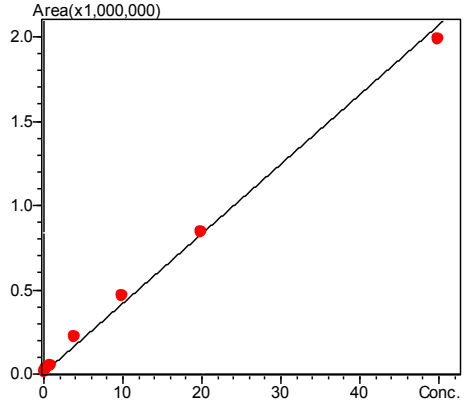


Fig. 8. Calibration curve of kitasamycin

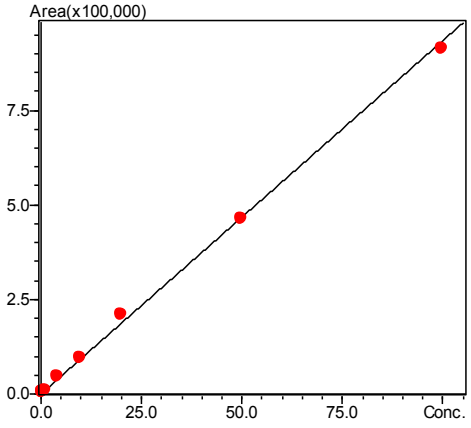


Fig. 9. Calibration curve of erythromycin

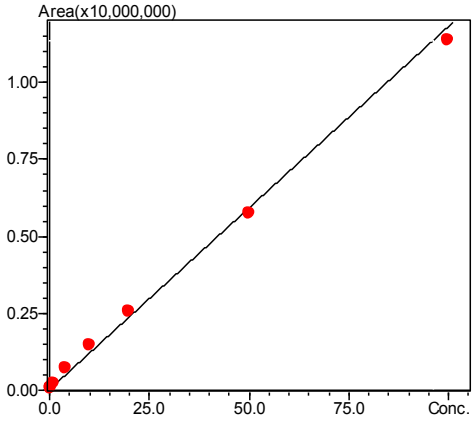


Fig. 10. Calibration curve of tilmicosin

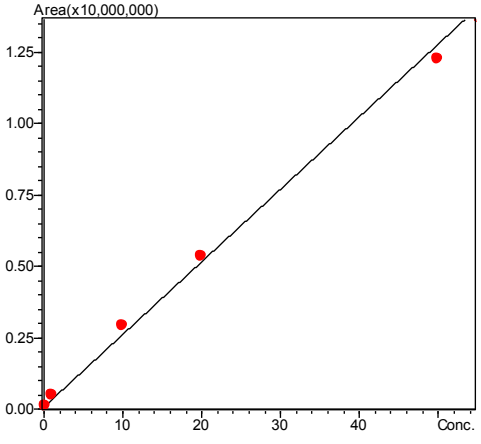


Fig. 11. Calibration curve of tylosin

Table 3. Parameters of the calibration curves of 5 macrolide antibiotics

No.	Name	Calibration Curve	Correlation Coefficient (r)	Linear Range
1	Spiramycin	Y = (28081.2)X + (6171.18)	0.9970	0.5-100
2	Kitasamycin	Y = (41302.3)X + (6801.93)	0.9969	0.2-50
3	Erythromycin	Y = (9338.55)X + (522.514)	0.9988	0.2-100
4	Tilmicosin	Y = (117967)X + (27799.9)	0.9951	0.2-100
5	Tylosin	Y = (254080)X + (75500.2)	0.9959	0.2-50

Precision test

Mixed standard solutions at concentrations of 2 µg/L, 20 µg/L and 50 µg/L were injected 6 consecutive times to assess precision of the method. The repeatability results of retention time and peak area are shown in Table 4. The %RSDs of retention time and peak area of standard solutions of 3 concentrations were 0.05~1.13% and 0.85~2.43%, respectively, showing that the method's precision was satisfactory.

Table 4 Repeatability - retention time and peak area (n=6)

Sample Name	RSD% (2 µg/L)		RSD% (20 µg/L)		RSD% (50 µg/L)	
	R.T	Area	R.T	R.T	Area	R.T
Spiramycin	1.13	2.01	0.67	1.37	0.42	1.10
Kitasamycin	0.13	1.35	0.11	0.85	0.19	0.88
Erythromycin	0.10	2.12	0.08	1.88	0.08	1.78
Tilmicosin	0.08	2.43	0.05	2.01	0.08	2.22
Tylosin	0.05	2.11	0.07	1.28	0.07	1.20

LOD

Standard samples of 0.5 µg/L were prepared and directly injected for analysis. The standard derivation (SD) of measurements was calculated. The limit of detection (LOD) and the lower limit of quantitation (LLOQ) were

calculated using these formulae: MDL= 3.14×S, LOQ=4×MDL. The determination results are shown in Table 5.

Table 5 LODs and LLOQs of macrolide antibiotics

No.	Name	Standard Deviation (SD)	LOD (μg/L)	LLOQ (μg/L)
1	Spiramycin	0.030	0.095	0.38
2	Kitasamycin	0.025	0.078	0.31
3	Erythromycin	0.035	0.11	0.44
4	Tilmicosin	0.0050	0.016	0.063
5	Tylosin	0.010	0.033	0.13

Spiked matrix test

Mixed standard solutions at concentration of 0.5 μg/kg were spiked into samples prepared according to the method as specified in 1.3. The MRM chromatograms of blank fish meat matrix and the MRM chromatograms of spiked matrix are shown in Fig. 12 and Fig. 13. It can be seen from the MRM chromatograms that the system responded well to all spiked matrix samples in terms of LOQ.

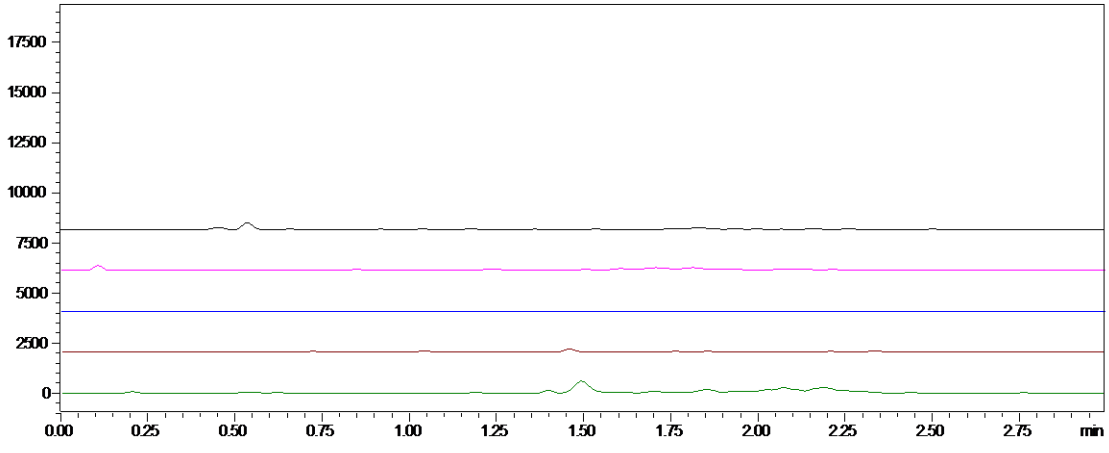


Fig. 12. MRM chromatograms of fish meat blank matrix sample

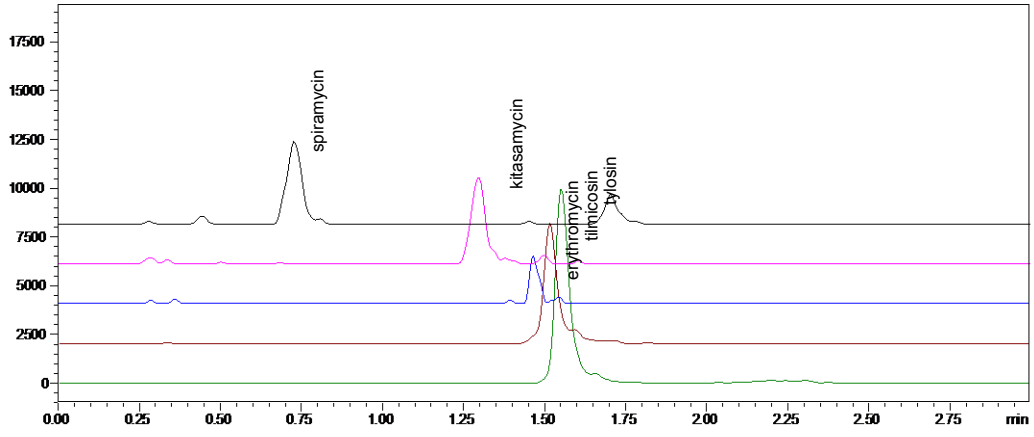


Fig. 13. MRM chromatograms of fish meat sample spiked with standards

Table 6.Spike recoveries of macrolide antibiotics

No.	Name	Tested concentration of Sample 1 (μg/kg)	Tested concentration of Sample 2 (μg/kg)	Average Recovery (%)
1	Spiramycin	0.42	0.47	89.0
2	Kitasamycin	0.41	0.50	91.0
3	Erythromycin	0.38	0.46	84.0
4	Tilmicosin	0.53	0.51	104.0
5	Tylosin	0.44	0.48	93.0

CONCLUSION

A method was developed for the determination of 5 macrolide antibiotics in fish meat using Shimadzu ultra fast liquid chromatograph LC-30A and a triple quadrupole mass spectrometer LCMS-8040. The method showed the merits of fast analysis speed, high sensitivity, and satisfactory precision. It demonstrated satisfactory linearity for determining spiramycin in the range of 0.5~100 μg/L, kitasamycin and tylosin in the range of 0.2~50 μg/L, and erythromycin and tilmicosin in the range of 0.2~100 μg/L. The correlation coefficients of calibration curves were greater than 0.995. The LODs for these antibiotics were 0.016~0.11 μg/L, LOQs were 0.063~0.38 μg/L, and spike recoveries were 84.0~104.0%. It is suitable for the detection of spiramycin, tilmicosin, tylosin, kitasamycin and erythromycin in aquatic products.

V-32 Determination of 13 β-receptor Agonists in Feed with UFLC-tandem Mass Spectrometry

INTRODUCTION

A method was developed for the determination of clenbuterol and other 12 β-receptor agonists in feed using a Shimadzu ultra-fast liquid chromatograph LC-30A and a triple quadrupole mass spectrometer LCMS-8040. In the method, samples were ground, extracted with hydrochloric acid and acidified methanol, and then loaded to a mixed mode cation exchange column for purification. The eluent obtained was dried with nitrogen flow at 45 °C. The residue was dissolved in mobile phase. Components were eluted with 0.2% formic acid-methanol for gradient elution and detected by ESI-MS/MS in MRM mode. Calibration curves were plotted for clenbuterol and other 12 β-receptor agonists using matrix spiked with standards. The curves displayed satisfactory linearity and their correlation coefficients (R^2) were better than 0.999. The method's recoveries ranged from 61.0% to 111.9% at the two spiked levels of 0.01 mg/kg and 0.05 mg/kg with %RSDs lower than 12% (n=8). The method showed a good applicability with an LOQ of 0.01 mg/kg that met the requirements of 0.05 mg/kg set in the Announcement No. 1063-6-2008 of the Ministry of Agriculture of China.

β-receptor agonists are a category of chemically synthesized phenylethanolamine derivatives including clenbuterol hydrochloride, ractopamine, salbutamol, cimaterol, bambuterol, etc. Generally speaking, feed added with appropriate amount of clenbuterol hydrochloride can increase feed conversion rate and animal growth speed and lean meat rate by more than 10%. Therefore, the substance is also referred to as lean meat powder. However, β-receptor agonists can cause serious damages to human body and are hereby banned globally. In China, β-receptor agonists were listed on the *Catalog of Drugs Prohibited from Use in Feed or Drinking Water for Animals* issued in 2002. The method specified in the Announcement No. 1063-6-2008 of the Ministry of Agriculture for the detection of lean meat powder in feed has an LOD of 0.01 mg/kg and an LOQ of 0.05 mg/kg. In this paper, a method was developed for detection of clenbuterol and other 12 β-receptor agonists in feed using Shimadzu ultra-fast liquid chromatography (UFLC) tandem mass spectrometry.

EXPERIMENTAL

Instruments

A combined system of a Shimadzu ultra-fast liquid chromatograph LC-30A and a triple quadrupole mass spectrometer LCMS-8040 was used in the experiment. The configuration included two LC-30AD pumps, a DGU-20A_5 online degasser, a SIL-30AC autosampler, a CTO-30A column oven, a CBM-20A communications bus module, a LCMS-8040 triple quadrupole mass spectrometer and a LabSolutions ver. 5.50 chromatography workstation.

Conditions of Analysis

LC condition

Column :Shim-pack XR-ODSIII 2.0 mm I.D.× 75 mm L., 1.6 µm
Mobile phase :A:0.2% formic acid aqueous solution
Mobile phase :B:methanol
Flow rate :0.4 mL/min
Column temperature :40 °C
Injection volume :1 µL

Time program:

Time(min)	Module	Command	Value
2.00	Pumps	B Conc.	16
5.00	Pumps	B Conc.	60
5.50	Pumps	B Conc.	95
6.50	Pumps	B Conc.	95
6.51	Pumps	B Conc.	3
9.00	Controller	Stop	

MS condition

Ionization mode :ESI-positive
Ionization voltage :+4.5 kV
Nebulizing gas :Nitrogen, 3.0 L/min
Drying gas :Nitrogen, 15 L/min
Collision gas :Argon
DL temperature :250 °C
Heater block temperature :400 °C
Acquisition mode :MRM
Pause time :15 ms
Dwell time :1 ms
Collision energy :See Table 1

Preparation of standard solutions and pretreatment of samples

Each standard solution of concentration at 1000 μg/mL was prepared with methanol for the 13 β-receptor agonists, and then diluted with methanol to get the mixed standard intermediate solutions of concentration at 1 μg/mL, which were then diluted with water to get mixed working solutions of concentrations of 1, 5, 10, 20 and 50 ng/mL.

The samples were pre-treated basically in accordance with the Announcement No. 1063-6-2008 of the Ministry of Agriculture *Determination of 13 β-receptor agonists in feeds - LC-tandem MS*. The difference between the proposed method and the standard method in sample pretreatment is that nitrogen blow drying was used in the proposed method in place of rotary evaporation.

Table 1MRM parameters

Name	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Terbutaline	226.00	152.15*	-23.0	-15.0	-29.0
		107.15	-23.0	-30.0	-18.0
Salbutamol	243.20	151.20*	-29.0	-17.0	-27.0
		225.25	-29.0	-9.0	-23.0
Zilpaterol	262.00	244.15*	-27.0	-12.0	-25.0
		185.15	-27.0	-23.0	-18.0
Cimaterol	219.80	202.20*	-23.0	-9.0	-20.0
		160.15	-23.0	-14.0	-30.0
Cimbuterol	234.00	160.15*	-25.0	-13.0	-30.0
		143.05	-25.0	-24.0	-26.0
Clenproperol	262.90	245.10*	-27.0	-9.0	-25.0
		132.15	-27.0	-24.0	-24.0
Ractopamine	302.00	284.25*	-30.0	-12.0	-19.0
		164.15	-30.0	-16.0	-30.0
Tulobuterol	227.70	154.10*	-24.0	-16.0	-28.0
		119.10	-24.0	-28.0	-21.0
Clenbuterol	276.90	203.10*	-29.0	-15.0	-20.0
		259.15	-29.0	-10.0	-17.0
Brombuterol	366.90	292.90*	-17.0	-19.0	-30.0
		349.00	-17.0	-13.0	-24.0

Bambuterol	368.10	294.20*	-17.0	-20.0	-30.0
		72.15	-17.0	-35.0	-27.0
Mabuterol	310.80	237.10*	-30.0	-16.0	-24.0
		293.15	-30.0	-11.0	-30.0
Clorprenaline	213.80	154.10*	-22.0	-15.0	-28.0
		196.15	-22.0	-10.0	-19.0

* refers to quantitative ion

RESULTS AND DISCUSSION

MRM chromatograms of mixed standard working solutions

MRM chromatograms of 20 ng/mL mixed standard working solutions are shown in Fig.1-Fig. 13. The peaks of the compositions were of symmetric shapes and satisfactory response.

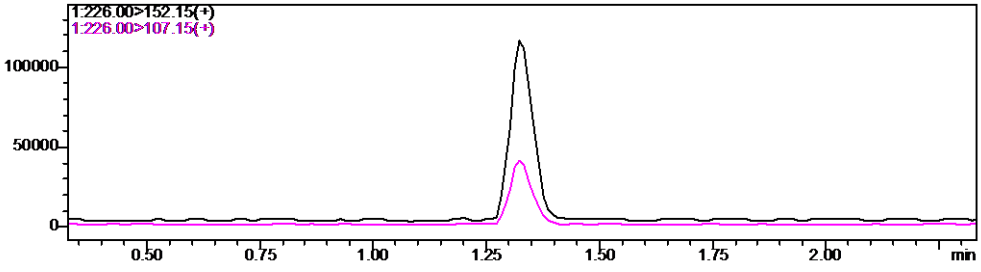


Fig. 1 MRM chromatograms of 20 ng/mL terbutaline

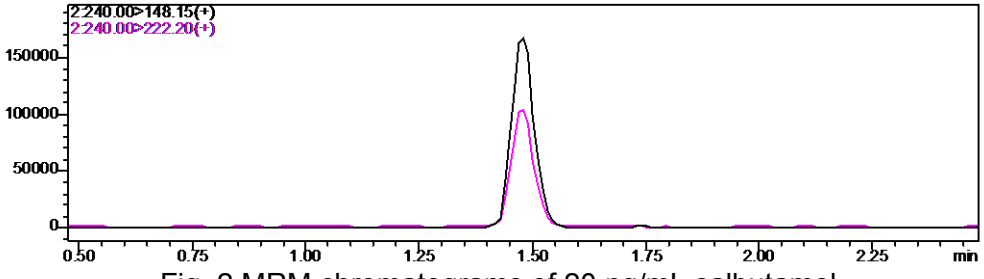


Fig. 2 MRM chromatograms of 20 ng/mL salbutamol

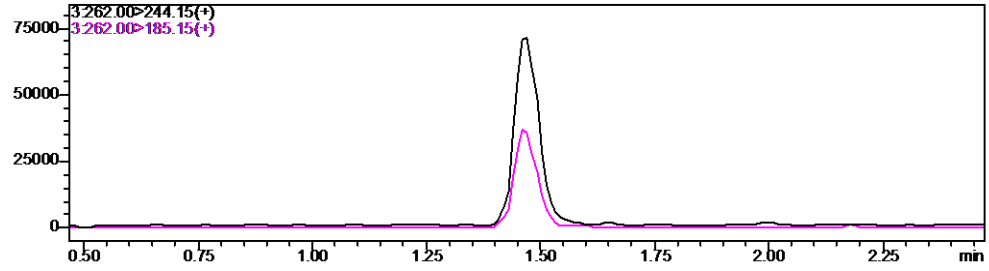


Fig. 3 MRM chromatograms of 20 ng/mL zilpaterol

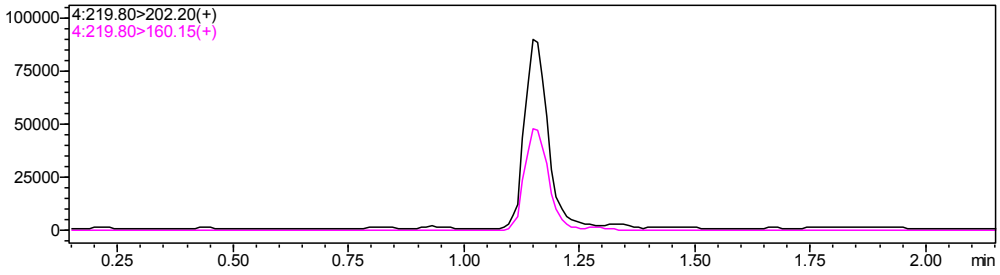


Fig. 4 MRM chromatograms of 20 ng/mL cimaterol

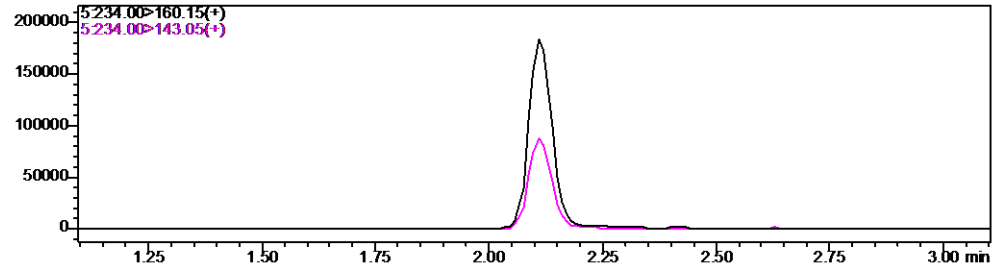


Fig. 5 MRM chromatograms of 20 ng/mL cimbuterol

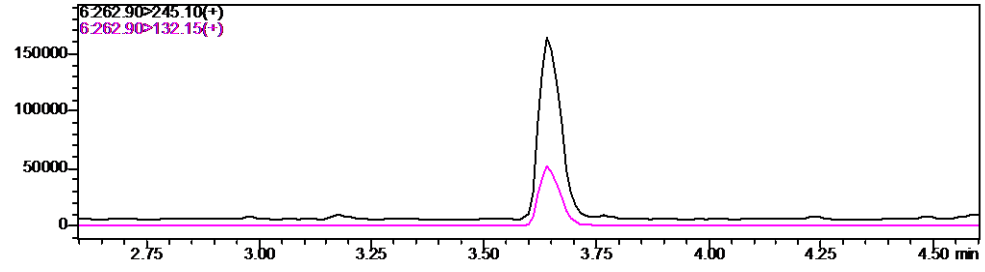


Fig. 6 MRM chromatograms of 20ng/mL clenproperol

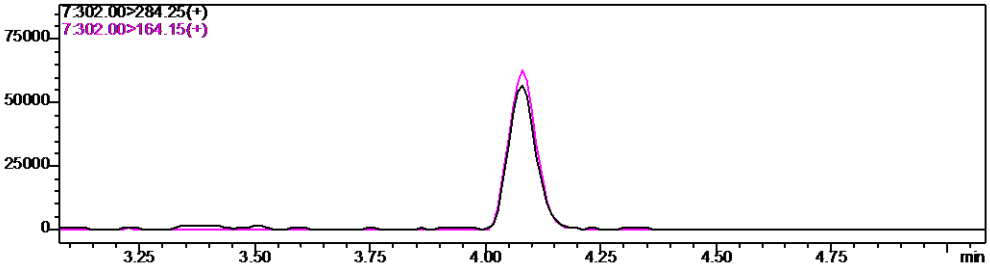


Fig. 7 MRM chromatograms of 20 ng/mL ractopamine

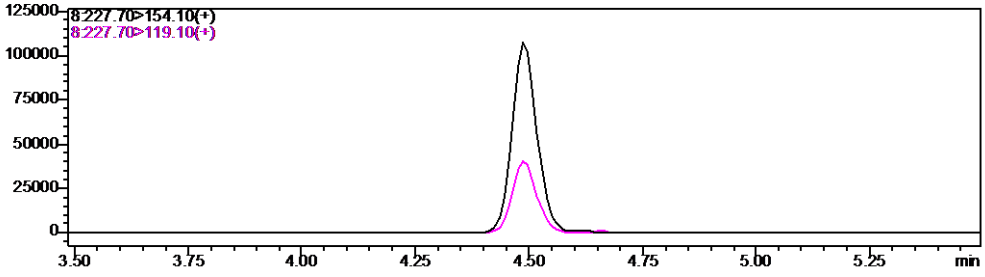


Fig. 8 MRM chromatograms of 20 ng/mL tulobuterol

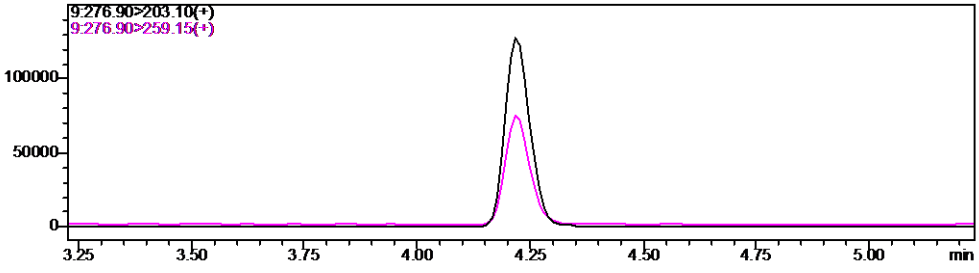


Fig. 9 MRM chromatograms of 20 ng/mL clenbuterol

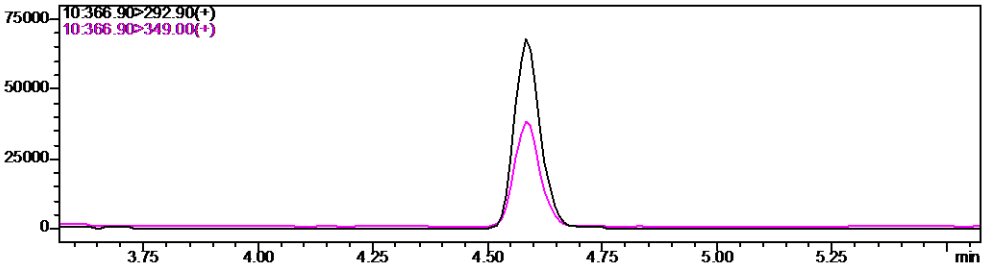


Fig. 10 MRM chromatograms of 20 ng/mL brombuterol

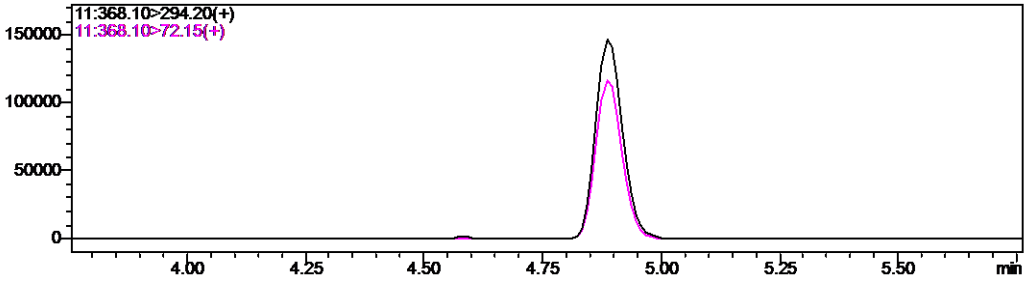


Fig. 11 MRM chromatograms of 20 ng/mL bambuterol

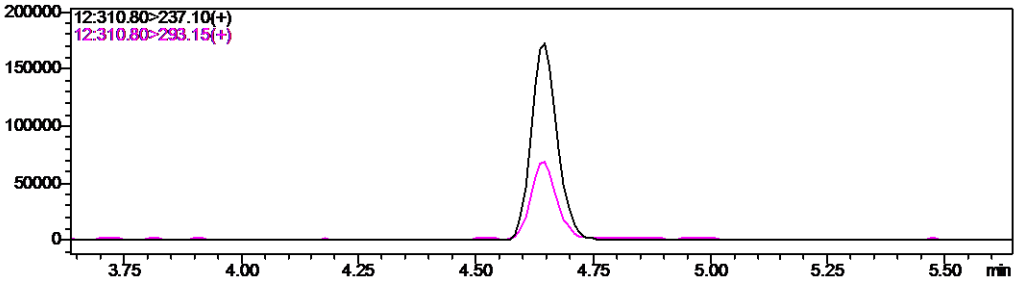


Fig. 12 MRM chromatograms of 20 ng/mL mabuterol

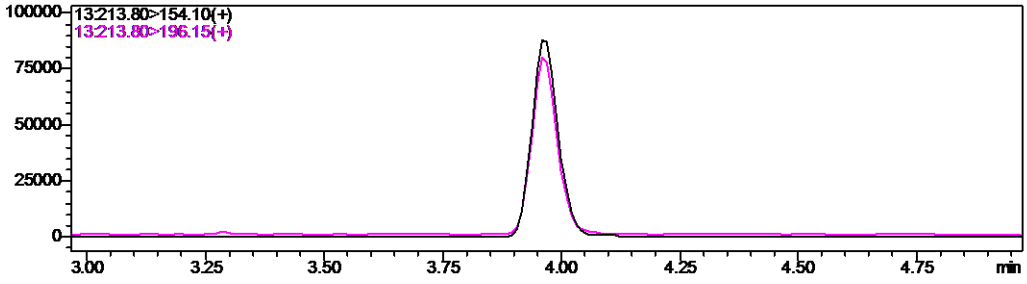


Fig. 13 MRM chromatograms of 20 ng/mL clorprenaline

Linearity

Working curves of matrix spiked with standards were plotted using blank matrix. Solutions of mixed matrix spiked with standards of concentrations of 1, 5, 10, 20 and 50 ng/mL were determined under the analysis conditions as specified in 1.2 and calibration curves were plotted using concentration as abscissa and peak area as ordinate. The plotted calibration curves were of satisfactory linearity. Relevant information is shown in Table 2.

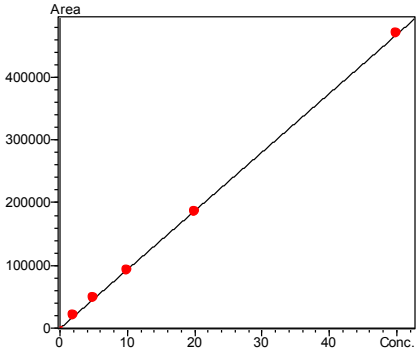


Fig. 14 Calibration curve of terbutaline

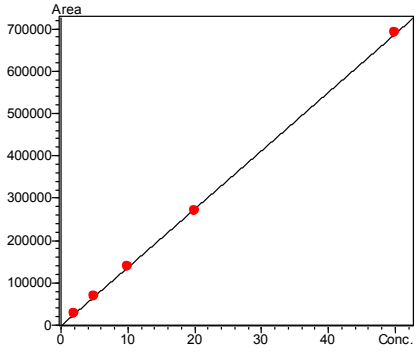


Fig. 15 Calibration curve of salbutamol

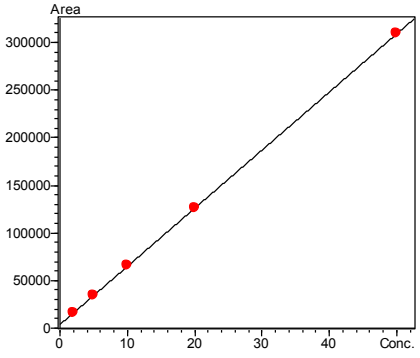


Fig. 16 Calibration curve of zilpaterol

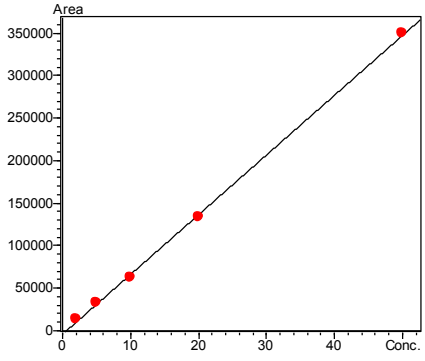


Fig. 17 Calibration curve of cimaterol

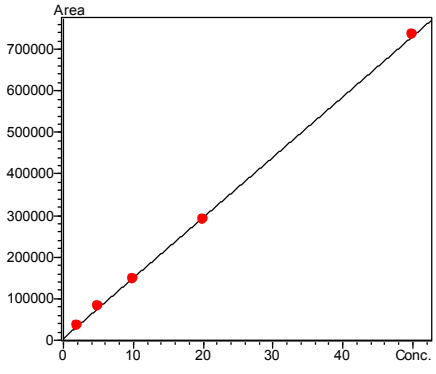


Fig. 18 Calibration curve of cimbuterol

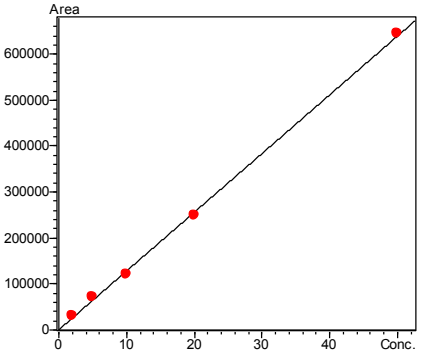


Fig. 19 Calibration curve of clenproperol

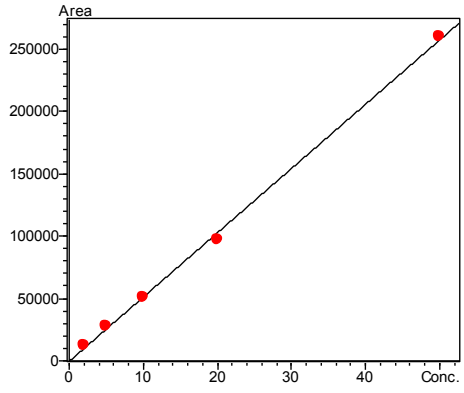


Fig. 20 Calibration curve of ractopamine

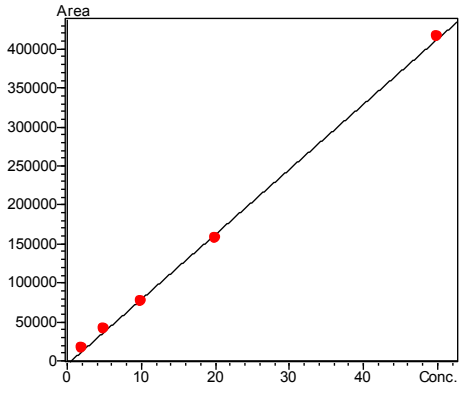


Fig. 21 Calibration curve of tulobuterol

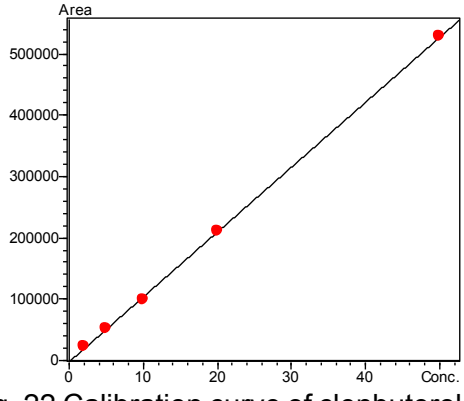


Fig. 22 Calibration curve of clenbuterol

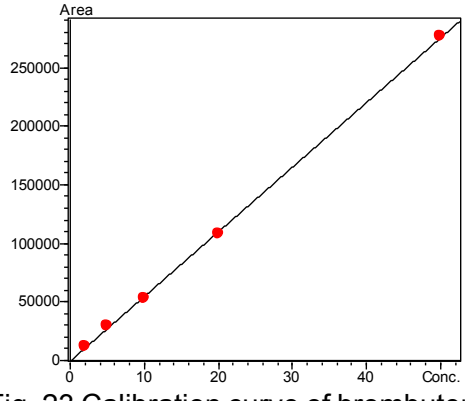


Fig. 23 Calibration curve of brombuterol

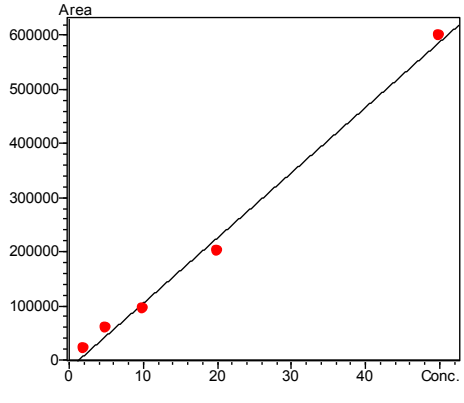


Fig. 24 Calibration curve of bambuterol

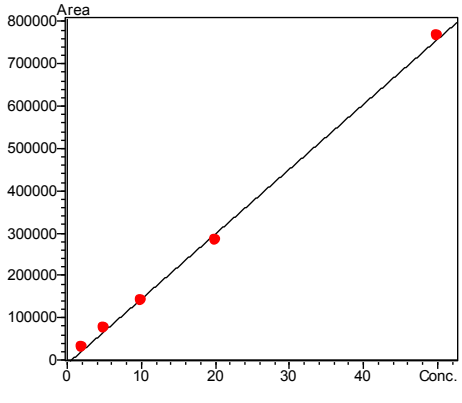


Fig. 25 Calibration curve of mabuterol

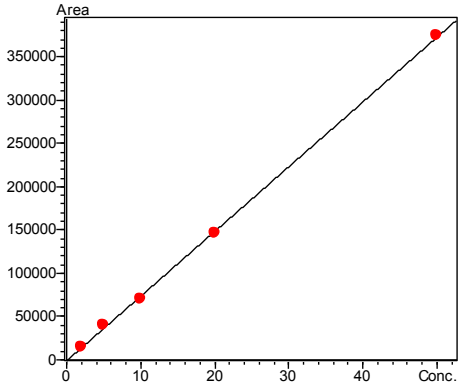


Fig. 26 Calibration curve of clorprenaline

Table 2 Information on the calibration curves of 13 β-receptor agonists

Name	Calibration Curve	Correlation Coefficient (R^2)
Terbutaline	Y = (8677.74)X + (8510.66)	0.9999
Salbutamol	Y = (15263.7)X + (14409.9)	0.9999
Zilpaterol	Y = (5729.03)X + (4590.08)	0.9999
Cimaterol	Y = (4402.70)X + (-3371.67)	0.9998
Cimbuterol	Y = (17946.0)X + (5670.37)	0.9999
Clenproperol	Y = (8800.96)X + (8520.94)	0.9999
Ractopamine	Y = (3496.88)X + (3771.73)	0.9999
Tulobuterol	Y = (6407.66)X + (7807.28)	0.9999
Clenbuterol	Y = (13103.5)X + (9273.56)	0.9999
Brombuterol	Y = (4607.60)X + (2947.62)	0.9999
Bambuterol	Y = (10498.4)X + (9675.91)	0.9999
Mabuterol	Y = (17391.0)X + (13189.9)	0.9999
Clorprenaline	Y = (9339.98)X + (8815.97)	0.9999

Repeatability test on standard samples

Mixed standard solutions of concentrations of 5 and 20 ng/mL were determined for 12 successive times and the %RSDs (n=12) of the obtained retention time and peak area were in the ranges of 0.06-0.73% and 1.41-6.93% respectively, indicating that the method had satisfactory precision. Results of the test are shown in Table 3.

Table 3 Repeatability data of standard solutions (n=12)

Name	5 ng/mL		20 ng/mL	
	%RSD RT	%RSD Area	%RSD RT	%RSD Area
Terbutaline	0.39	3.61	0.39	3.61
Salbutamol	0.37	2.28	0.37	2.28
Zilpaterol	0.36	6.20	0.36	6.20
Cimaterol	0.73	6.93	0.73	6.93
Cimbuterol	0.28	5.97	0.28	5.97
Clenproperol	0.15	4.87	0.15	4.87
Ractopamine	0.17	6.83	0.17	6.83
Tulobuterol	0.10	4.37	0.10	4.37
Clenbuterol	0.09	3.62	0.09	3.62
Brombuterol	0.11	4.66	0.11	4.66
Bambuterol	0.06	2.82	0.06	2.82
Mabuterol	0.09	3.05	0.09	3.05
Clorprenaline	0.16	3.15	0.16	3.15

Recovery and precision tests

The proposed method's recovery and precision were assessed with 8 replicate parallel tests of feed samples at concentrations of 0.01 mg/kg and 0.05 mg/kg respectively. Results of the tests are shown in Table 4.

Table 4 Recovery and precision of the pretreatment method (n=8)

Name	0.01 mg/kg		0.05 mg/kg	
	Recovery (%)	Precision (%)	Recovery (%)	Precision (%)
Terbutaline	61.8	7.74	61.0	1.23
Salbutamol	74.9	7.72	69.1	6.19
Zilpaterol	98.8	6.09	111.9	2.02
Cimaterol	90.2	11.95	90.0	3.56
Cimbuterol	80.6	10.48	89.9	2.14
Clenproperol	85.5	7.73	78.6	1.74
Ractopamine	83.8	3.66	89.8	3.40
Tulobuterol	87.4	7.33	99.0	3.07
Clenbuterol	86.8	4.92	77.8	2.04
Brombuterol	87.2	6.83	87.3	2.61
Bambuterol	82.5	8.72	97.2	1.37
Mabuterol	87.7	7.09	84.7	2.74
Clorprenaline	90.6	6.53	81.4	2.13

Sensitivity test

In order to assess the proposed method's sensitivity, 13 β-receptor agonists were spiked into blank samples of feed at the level of 0.01 mg/kg, the resulted chromatograms are shown in Fig. 27 and Fig. 28. Within the corresponding retention time, blank feed did not have any interference with target compounds. The method's LOD was 0.01 mg/kg for 13 β-receptor agonists.

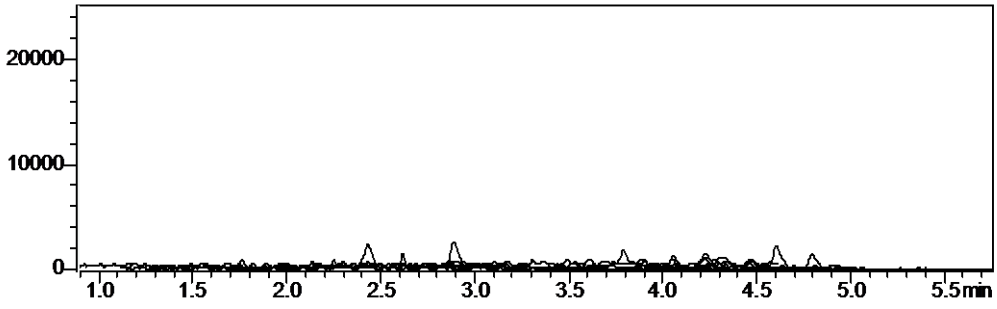


Fig. 27. MRM chromatograms of blank feed samples

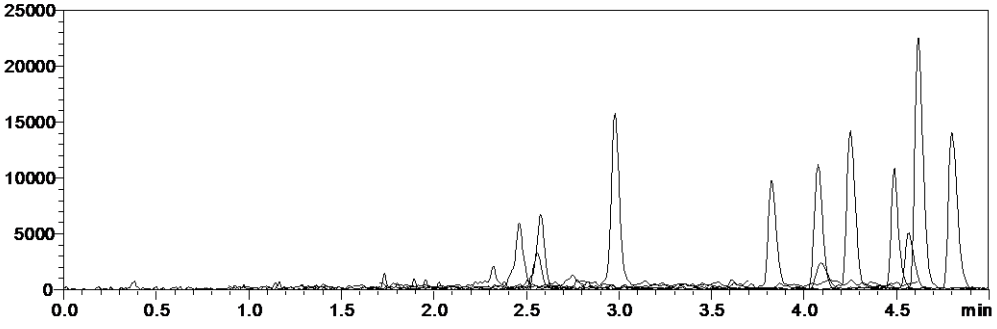


Fig. 28. MRM chromatograms of feed samples spiked with 0.01 mg/kg standards

CONCLUSION

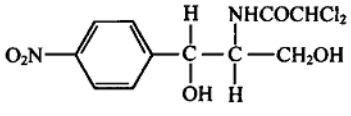
A method was developed for the determination of 13 β-receptor agonists in feed with a Shimadzu ultra-fast liquid chromatograph LC-30A and a triple quadrupole mass spectrometer LCMS-8040. The proposed method has fast analysis speed, high recovery, and satisfactory precision. The correlation coefficients of calibration curves were greater than 0.999. The method has an LOQ of 0.01mg/kg, meeting the requirements of 0.05mg/kg set in the Announcement No. 1063-6-2008 of the Ministry of Agriculture of China. It is concluded that the method with Shimadzu ultra-fast liquid chromatograph-tandem mass spectrometer can meet the requirements for the determination of β-receptor agonists in feed.

V-33 Determination of chloramphenicol in honey with online pretreatment LC system-mass spectrometery

INTRODUCTION

A method is proposed in this paper for determination of chloramphenicol in honey with online pretreatment LC system-mass spectrometer. The proposed method utilizes online pretreatment and concentration of samples by a specifically established valve switching system which significantly cut down users' time needed for sample pretreatment. The method was simple, convenient, highly sensitive, and capable of determining of chloramphenicol in honey with high reproducibility and an LOQ as low as 0.5 μg/kg.

Chloramphenicol (CAP, CAS:56-75-7), also called chloromycetin, is a broad-spectrum antibiotic which is commonly used for the treatment of bacterial infectious diseases in fishery and poultry husbandry production. Its chemical structural formula is as follows.



Chlorampenicol(CAP)

Because of the hematopoietic function inhibiting action of chloramphenicol, its application in animal-derived food is banned in many countries and a maximum residue limit (MRL) of zero is set for chloramphenicol in edible tissues of food animals. It is stipulated by the Ministry of Agriculture of China (in No. 227 announcement of the year 2002) that the afore-mentioned ban also applied in China and chloramphenicol was included in the *List of Food Additives That May Be Illegally Added into Food and Abused (the fifth batch)*. China is a major honey exporting country and chloramphenicol is a mandatory test item for imported/exported honey products. Therefore, it is absolutely necessary to develop simple, convenient, and sensitive detection methods for chloramphenicol.

In this paper, an online pretreatment LC system-mass spectrometer was used in conjunction with Shimadzu LCMS-8040 triple quadrupole mass spectrometric detector for fast and highly sensitive assay of trace amount of chloramphenicol at the same time significantly simplifying the pretreatment procedures for honey samples.

EXPERIMENTAL

Instrument

An LC-30A based online pretreatment system was used in the experiment in conjunction with LCMS-8040 triple quadrupole mass spectrometer. The flow circuit diagram of the system was shown below:

1) Sample introduction flow circuit: A sample introduction pump was used for introduction of samples into the pretreatment column, where the target analyte was retained while the matrix was carried away by the mobile phase into waste liquid bottle, thereby achieving the purpose of sample pretreatment.

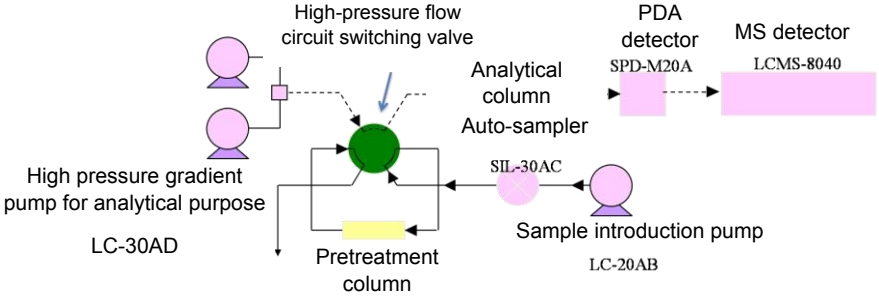


Fig.1 Flow circuit diagram of sample introduction

2) Sample analysis flow circuit: When sample flow path was switched to this circuit, analytical mobile phase would be transported by a high pressure gradient pump to the pretreatment column, where the mobile phase would elute the target analyte enriched in the pretreatment column out into the analytical column for separation and analysis with PDA and MS detectors.

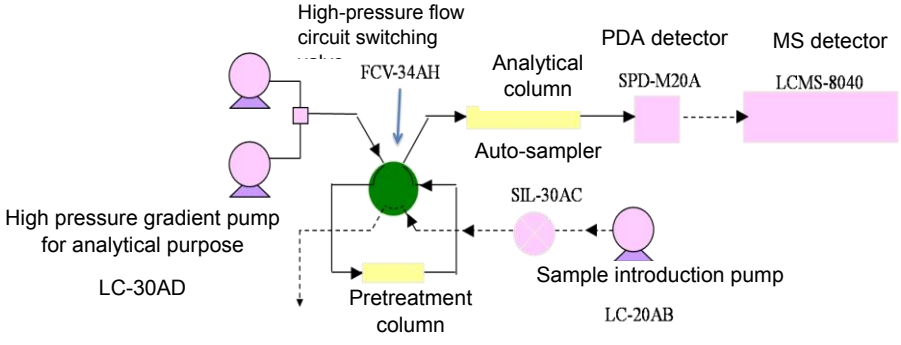


Fig.2 Flow circuit diagram of sample analysis

Conditions of Analysis

LC conditions

Loading conditions

Column	:MAYI-ODS (2.0 mm I.D.×10 mm L., 5 μm)
Introduction mobile phase	:A: aqueous solution; mobile phase B:acetonitrile
Flow rate	:2 mL/min
Injection volume	:5 μL

Conditions of Analysis

Column	:Shim-pack XR-ODS (2.0 mm I.D.×75 mm L., 2.2 μm)
Mobile phase	:A: aqueous solution; Mobile phase B:acetonitrile
Flow rate	:0.35 mL/min
Column temperature	:40°C
Elution mode	:Gradient elution with initial concentration of mobile phase B of 5%,

See Table 1 for the elution program.

Table 1 Time program

Time(min)	Module	Command	Value
1.00	Column Oven	CTO.RVL	1
1.00	Pumps	Pump B Conc.	5
1.00	Pumps	Pump C B.Conc	5
1.01	Pumps	Pump C B.Conc	90
2.50	Pumps	Pump B Conc.	95
3.00	Column Oven	CTO.RVL	0
3.00	Pumps	Pump B Conc.	95
3.00	Pumps	Pump C B.Conc	90
3.01	Pumps	Pump B Conc.	5
3.01	Pumps	Pump C B.Conc	5
5.00	Controller	Stop	

MS conditions

Ionization mode	:ESI(-)
Ionization voltage	:-3.5 kV
Nebulizing gas	:Nitrogen 2.5 L/min
Drying gas	:Nitrogen 15 L/min
Collision gas	:Argon
DL temperature	:250°C
Heater block temperature	:300°C
Acquisition mode	:Multiple Reaction Monitoring (MRM)
Dwell time	:100 ms
Pause time	:3 ms
MRM parameters	:See Table

Table 2 Optimized MRM parameters

Compound	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q1 Pre Bias (V)
Chloramphenicol	321.05	152.05*	12.0	18.0	29.0
		257.05	12.0	10.0	16.0
D5-chloramphenicol(IS)	326.00	262.15	23.0	11.0	16.0

Note: * refers to quantitative ion

Preparation of standard solutions

Preparation of standard working solutions: A 1.0 mg/mL standard stock solution was prepared using acetonitrile as solvent, then progressively diluted with water to get a series of working solutions of concentrations of 0.1, 0.5, 1, 2, 5, 10, 20, 50, and 100 ng/mL, respectively.

Sample pretreatment method

The proposed method made use of online pretreatment, therefore honey samples were simply diluted and filtered for direct analysis. The specific procedures were as follows: 5 g honey was accurately weighed (with a precision of 0.01 g) and added 50 mL water, subjected to a shaker for

mixing evenly followed by filtration with 0.22 μm micropore film before injection for assay.

RESULTS AND DISCUSSION

Optimization of loading time

The determination of sample loading time can have significant impact on the results when a sample pretreatment system is used. If the loading time is too short, the matrix may not be completely eluted; if the loading time is too long, the target analyte may suffer from wider peak span and lower recovery. In the light of this, sample loading time need to be determined early in the development of the method. In consideration of that the analyte chloramphenicol in honey, which contained a lot of carbohydrates, the mass spectrometer was not connected to the system during determination of loading time. A UV detector working at 200 nm was used instead for monitoring the elution of matrix. The sample introduction flow circuit was used at this time and the exit of the circuit was connected to the UV detector. The results showed that all carbonhydrates in samples were almost completely eluted within 1 min. Therefore, the sample loading time of the method was set to 1 min.

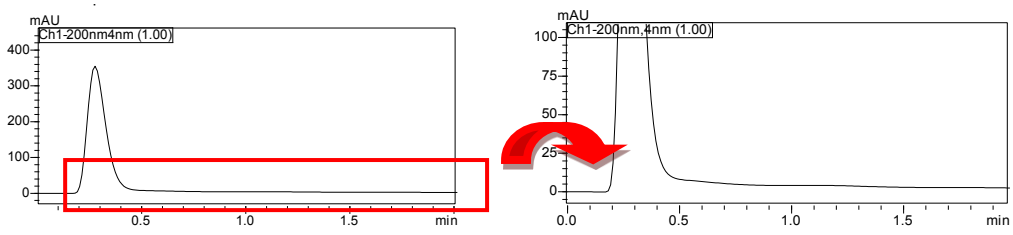


Fig.3 UV chromatogram of sample retention in the pretreatment column

Mass spectrum and MRM chromatogram

Mass spectrum of chloramphenicol was obtained by analyzing a 100 ng/mL standard solution in Q3 Scan mode. Chloramphenicol responded well to the method in negative ion mode, $[M-H]^-$=321.05. MRM chromatogram of a 10 ng/mL standard solution was shown in Fig.5.

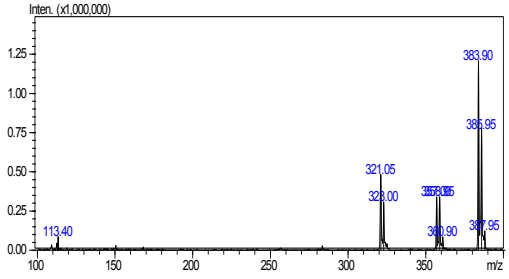


Fig.4 Scan chromatogram of a 100 ng/mL standard solution in Q3 Scan mode

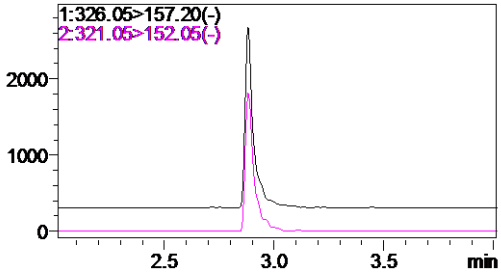


Fig.5 MRM chromatogram of a 10 ng/mL standard solution

Linear range

A series of standard working solutions of concentrations of 0.1, 0.5, 1, 2, 5, 10, 20, 50, and 100 ng/mL was subjected to quantitative analysis under the analytical conditions specified using internal standard. A calibration curve was plotted as shown in Fig 6 with concentration ratio as abscissa and peak area ratio as ordinate. The resulted calibration curve was of satisfactory linear relation and had a linear equation of Y=(0.183317)X + (-0.00508229) and a correlation coefficient of r=0.9997.

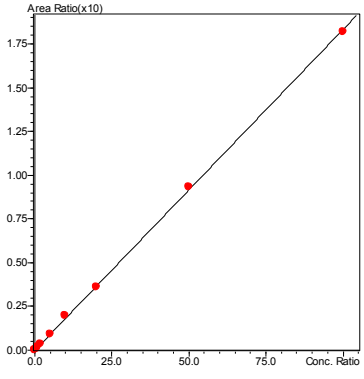


Fig.6 Calibration curve of chloramphenicol

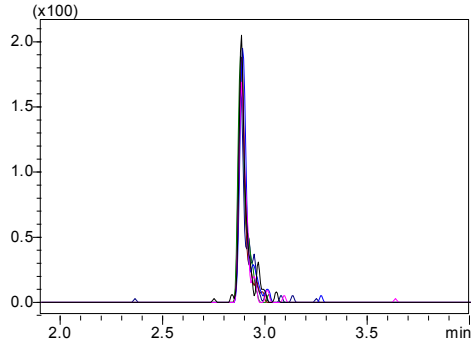


Fig.7 Overlapping chromatogram of 6 replicate injections of a 0.5 ng/mL standard solution

Precision test

The system's precision was assessed on 6 replicate injections of 0.5 ng/mL standard working solution. The resulted overlapping chromatogram is shown in Fig. 7. The %RSDs of retention time and peak area data were 0.13% and 3.12%, respectively, suggesting that the system had good precision.

Table 5 Repeatability - retention time and peak area (n=6)

No.	R.T.	Area
1	2.885	457
2	2.886	432
3	2.892	462
4	2.886	433
5	2.882	434
6	2.881	435
Average	2.885	442
RSD%	0.13	3.12

Sensitivity test

In order to assess the system's sensitivity, honey matrix samples spiked with standard at the spiked level of 5 μg/kg were analyzed and demonstrated good response to the method. The resulted chromatograms are shown in Fig.8. Since the pretreatment column in the system was provided with sample concentrating function, large volume samples could be loaded to the system. When 50 μL honey matrix sample, spiked with 0.5 μg/kg standard, was loaded, the system's S/N ratio was 36.65. The resulted chromatograms are shown in Fig. 9.

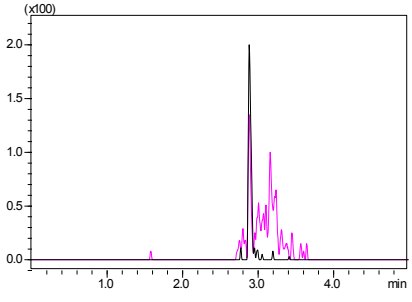


Fig.8 Chromatogram of a loading of 5 uL honey matrix spiked with 5 μg/kg standard

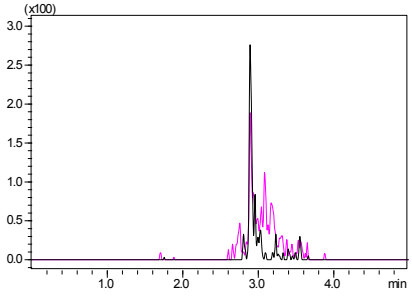


Fig.9 Chromatogram of a loading of 50 uL honey matrix spiked with 0.5 μg/kg standard

Recovery test

The method's recovery of 5 µg/kg chloramphenicol from spiked samples was carried out. The results show recovery of 83.0%.

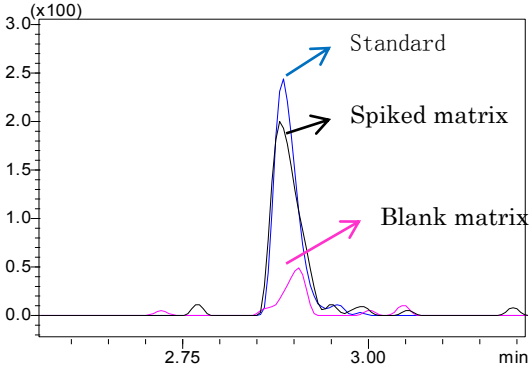


Fig.10 Overlapping chromatogram of blank matrix, spiked matrix and standard

CONCLUSION

A method was proposed in this paper for detection of chloramphenicol in honey with online pretreatment LC system-mass spectrometer. The method was capable of online pretreatment and concentrating honey samples and demonstrated good linearity for chloramphenicol in the concentration range of 0.1 ~100 ng/mL with a correlation coefficient of 0.9997. The method is suitable at level of 0.5 µg/kg chloramphenicol by loading large volume sample and making use of its online concentrating function. The 6 replicate injections of 0.5 ng/mL standard working solution shows %RSDs of retention time and peak area as 0.13% and 3.12%, respectively, showing that the system had good precision. The method achieved a recovery of 83.0% of 5 µg/kg samples.

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V-34 LCMS-8040

Quantitative analysis of multi-class antibiotic residues in milk using LC/MS/MS

INTRODUCTION

Antimicrobial drugs are widely used for treatment and prevention of diseases in dairy cattle. Residues of these drugs may, therefore, be present in milk and can be a health hazard to consumers. They may cause allergic reactions in sensitive persons and can increase risk of developing antibiotic resistant pathogenic bacteria. Hence, monitoring of antimicrobial residues in commercial milk is essential for ensuring the safety and adequacy of food.
Antibiotics belonging to different classes such as β-lactams, Sulfonamides, Macrolides etc. are generally administered to cattle to treat various bacterial infections. It is, therefore, desirable to develop a single analytical method for simultaneous determination of multi-class antibiotics. High specificity and sensitivity offered by LC/MS/MS technique makes it a preferred method of choice for multi-component analysis from complex matrices[1].
Multi-class antibiotic analysis using LC/MS/MS requires a system capable of acquiring data in both positive and negative polarities simultaneously due to different ionization tendencies of analytes. Multiple antibiotics belonging to the same class may fragment to give same product ions during MRM analysis. Hence, it is essential to have minimum crosstalk. LCMS-8040 with ultrafast polarity switching (15 msec), UFsweeper™ II technology (ensuring minimum cross talk), lowest dwell time and pause time (0.8 and 1 msec respectively) is, therefore, well suited for multi-class antibiotic analysis.

EXPERIMENTAL

Preparation of matrix matched standards :

2 mL of raw milk sample was mixed with 8 mL of acetonitrile using ultra sonicator for 5 mins. Mixture was centrifuged and 8 mL of the supernatant was collected. This supernatant was evaporated to dryness using low volume nitrogen evaporator. Dried residue was then reconstituted in 8 mL of water. The reconstituted solution was filtered through 0.22 µ syringe filter. This filtered solution was then used as a diluent to prepare antibiotics matrix matched mix standards at concentration levels of 0.5 ppb, 1 ppb, 2 ppb, 4 ppb, 5 ppb, 8 ppb, 10 ppb and 20 ppb.
Note: Matrix effect is a phenomenon seen in Electro Spray Ionization (ESI) LC/MS/MS analysis that impacts the data quality, especially when matrix is complex like food items. Milk is one such matrix that can exhibit matrix effect (either ion suppression or enhancement). A calibration curve based on matrix matched standards can demonstrate true sensitivity of the analyte in presence of matrix. Therefore, this approach was used to obtain more reliable and accurate data as compared to quantitation against neat (solvent) standards[2].

Analytical conditions:

Matrix matched antibiotics were analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera coupled with LCMS-8040 triple quadrupole system (Shimadzu Corporation, Japan). The details of analytical conditions are given below:

Nexera parameters	
Column	Shim-pack XR-ODS (50 mm L x 3 mm I.D.; 2.2 µ)
Mobile phase	A: water B: 0.1 % formic acid in acetonitrile
Flow rate	0.3 mL / min
Oven temp.	40 ˚C
Injection volume	15 µl

Gradient time program		
Time (min)	A conc. (%)	B conc. (%)
0.50	90	10
2.00	60	40
2.70	0	100
3.50	0	100
3.51	90	10
6.00	Stop	

LCMS-8040 parameters	
Interface	ESI
Polarity	positive and negative
Nebulizing gas flow	2 L / min
Drying gas flow	10 L / min
Desolvation line temp.	250 ˚C
Heat block temp.	350 ˚C

RESULTS AND DISCUSSION

LC/MS/MS method was developed for analysis of ten antibiotics belonging to different classes like β-lactams, Sulfonamides, Tetracyclines, Macrolides etc. in a single run. LOQ was determined for each antibiotic based on following criteria – **A**. % RSD for area < 16% (n=6), **B**. % accuracy between 80-120 % and **C**. Signal to noise ratio (S/N) > 10. Linearity and LOQ results for each antibiotic has been summarized in Table 1. Representative MRM chromatograms of blank matrix and matrix matched antibiotics at 1 ppb level are shown in Figures 1 and 2 respectively. MRM chromatograms of all antibiotics at LOQ levels and calibration graphs have been shown in Figure 3.

Table 1: Results of multi-class antibiotics analysis

Compound name	Antibiotic class	MRM transition	Retention time (min)	Calibration range (ppb)	Correlation coefficient (r^2)
Amoxicillin	β-lactam	366.10>134.20	1.17	1 - 20	0.9982
Trimethoprim	Dihydrofolate reductase inhibitor	290.50>261.00	2.09	0.5 - 20	0.9930
Ampicillin	β-lactam	350.10>106.10	2.20	1 - 20	0.9970
Oxytetracycline	Tetracycline	461.00>426.10	2.50	1 - 20	0.9993
Tetracycline	Tetracycline	445.00>154.10	2.55	1 - 20	0.9964
Sulfadiazine	Sulfonamide	250.60>156.00	2.58	0.5 - 20	0.9934
Chlortetracycline	Tetracycline	479.20>444.20	2.86	1 - 10	0.9990
Tylosin	Macrolide	916.20>174.20	3.14	1 - 20	0.9926
Sulfadoxine	Sulfonamide	310.80>156.00	3.35	0.5 - 20	0.9984
Chloramphenicol	Amphenicol	321.20>152.20	3.38	1 - 20	0.9973

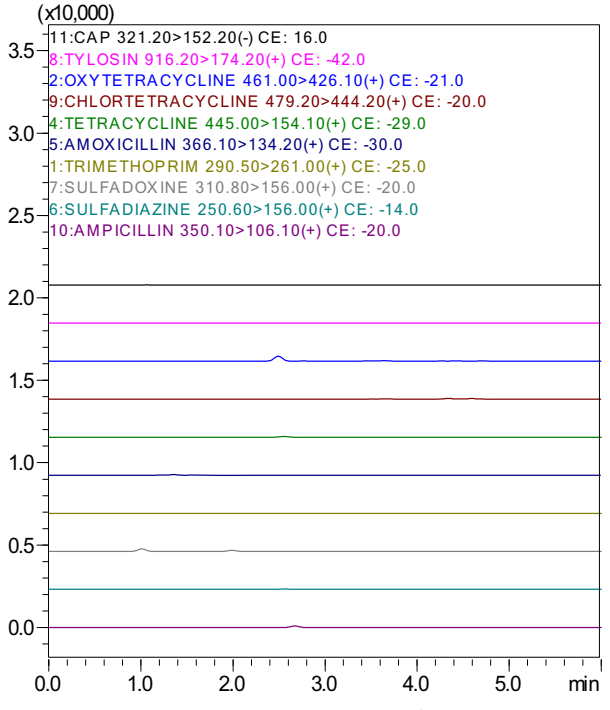

Figure 1: MRM chromatogram of blank matrix

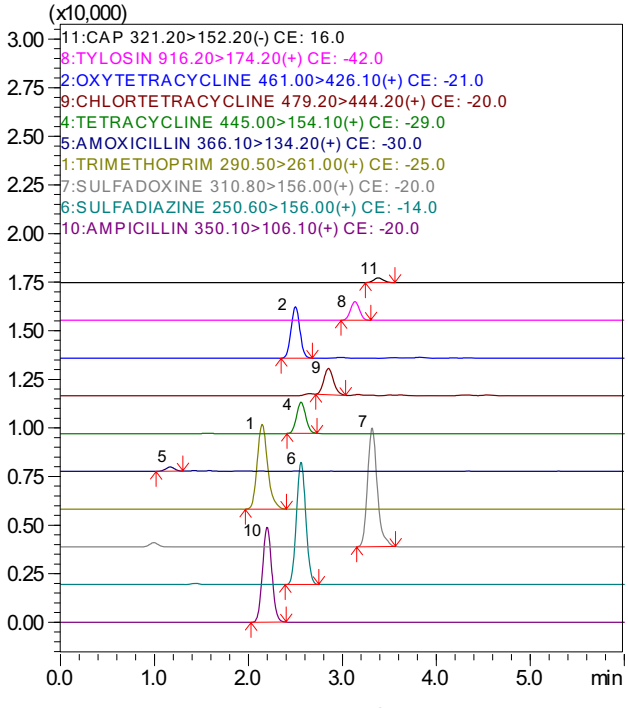

Figure 2: MRM chromatogram of antibiotics at 1 ppb level

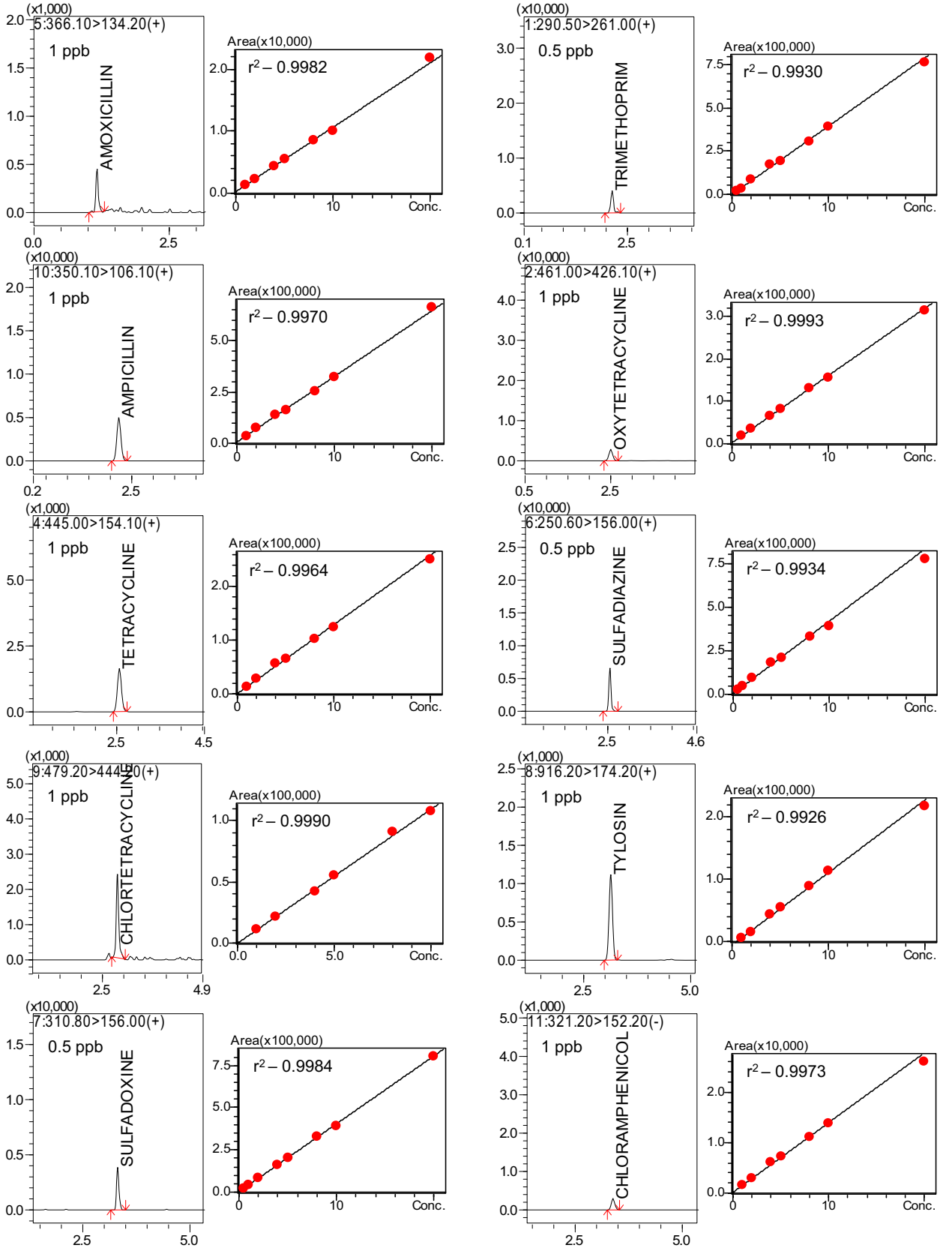

Figure 3: MRM chromatograms at LOQ levels and calibration graphs of antibiotics

CONCLUSION

- Quality checking of the milk sample becomes tedious when antibiotics belonging to different classes have to be analyzed. This often requires multiple LC/MS/MS methods. This task has been simplified here, by developing a single method for simultaneous analysis of ten antibiotics belonging to six different classes.
- Ultra-high sensitivity, ultra fast polarity switching (UFswitching), low pause time and dwell time along with UFsweeper™ II technology of LCMS-8040 system has enabled sensitive and selective multi-class antibiotic analysis from matrix like milk.

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LC-MS
Liquid Chromatograph Mass Spectrometer

V-35 Simultaneous Analysis of 36 Veterinary Drugs using Triple Quadrupole LC/MS/MS

The Japanese Ministry of Health, Labor, and Welfare (MHLW) has established an analytical standard for each veterinary drug found in food. Instrumentation measuring these standards must have sensitivity exceeding 1 ng / mL per analyte in order to detect trace levels of these drugs in food. Additionally, because the MHLW has established many compounds as residue standards, instrumentation used for these analyses must have the capability to make simultaneous measurements with sensitivity, specificity, and speed.

This report illustrates the simultaneous analysis of 36 veterinary drugs measured in 15 minutes using the Shimadzu LCMS-8050 Ultra Fast Triple Quadrupole Mass Spectrometer, featuring ultrafast polarity switching. The polarity switching speed of the LCMS-8050 is just 5 milliseconds.

Combination of the Nexera X2 LC and the LCMS-8050 provides incomparably fast run times without compromising the quality of the results.

■ HPLC conditions（Nexera X2）

Column	:	YMC-Triart C18 (Manufactured by YMC) (2.0 mmI.D. × 150 mmL.,1.9 μm)
Mobile phase A	:	0.1% formic acid in water
Mobile phase B	:	Acetonitrile
Time program	:	1% B. (0.00 min) →10% B. (0.10 min) →80% B. (11.00 min-12.00 min) →1% B. (12.01 -15.00min)
Flow rate	:	0.4 mL/min
Injection volume	:	5 μL
Column oven	:	40 ℃

■ MS conditions (LCMS-8050)

Ionization	:	ESI (Positive / Negative)
Nebulizing gas flow	:	2.0 L/min
Drying gas flow	:	10.0 L/min
Heating gas flow	:	10.0 L/min
Interface temperature	:	300 ℃
DL temperature	:	200 ℃
Heat block temperature	:	200 ℃
Interface voltage	:	+0.5, +1.5, +5.0kV (Positive) -1.0, -4.0kV (Negative)

＊ Interface voltage was optimized for each compound

Compound	Polarity	Precursor (*m/z*)	Product (*m/z*)	Area reproducibility (1 ng/mL)
Dexamethasone	+	393.30	337.30	10.3%
Clopidol	+	191.70	101.10	2.2%
Enrofloxacin	+	360.00	316.20	4.1%
Flubendazole	+	314.10	282.05	3.2%
Flumequine	+	262.10	244.05	0.9%
Mebendazole	+	296.10	264.00	2.1%
Nalidixic acid	+	233.10	215.05	1.5%
Orbifloxacin	+	396.00	352.20	6.8%
Oxolinic acid	+	261.90	160.00	4.2%
Sarafloxacin	+	386.00	299.10	9.3%
Tylosin	+	916.50	174.10	2.2%
Warfarin	+	309.05	163.00	0.9%
Ciprofloxacin	+	331.90	288.20	11.7%
Thiabendazole	+	202.10	175.00	2.5%
Levamisole	+	205.10	178.20	3.4%
Sulfachloropyridazine	+	285.10	156.10	1.2%
5-Hydroxythiabendazole	+	218.10	191.20	1.7%
Benzocaine	+	166.10	138.20	8.4%

Compound	Polarity	Precursor (*m/z*)	Product (*m/z*)	Area reproducibility (1 ng/mL)
Bromacil	+	261.00	205.00	0.6%
Diaveridine	+	261.15	123.10	0.9%
Famphur	+	326.00	93.10	0.9%
Josamycin	+	828.55	173.95	2.4%
Meloxicam	+	352.10	115.20	1.2%
Menbutone	+	259.25	241.10	2.8%
Oxibendazole	+	250.00	176.30	0.5%
Sulfaethoxypyridazine	+	295.15	156.05	1.6%
Valemuline	+	565.40	263.20	3.7%
Cafoperzone	+	646.40	143.30	9.4%
Difloxacin	+	400.10	356.20	2.2%
Methylprednisolone	+	375.20	161.20	3.6%
Nafcillin	+	415.10	199.10	1.3%
Ofloxacin	+	362.10	148.30	12.4%
Phenoxymethylpenicillin	-	349.10	208.30	1.1%
Thiamphenicol	-	353.80	185.10	11.3%
Florfenicol	-	356.10	185.30	5.4%
Clorsulon	-	377.90	342.20	2.2%

Table 1: MRM transition and area reproducibility at 1 ng / mL

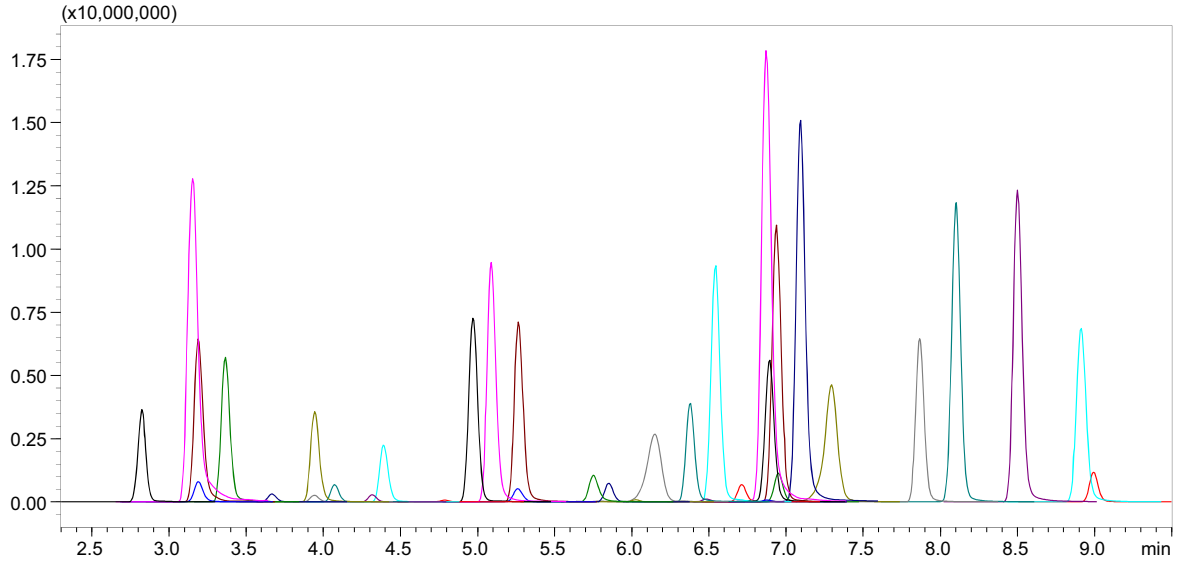


Fig. 1: Representative MRM chromatograms for 36 veterinary drugs at 0.5 μg / mL

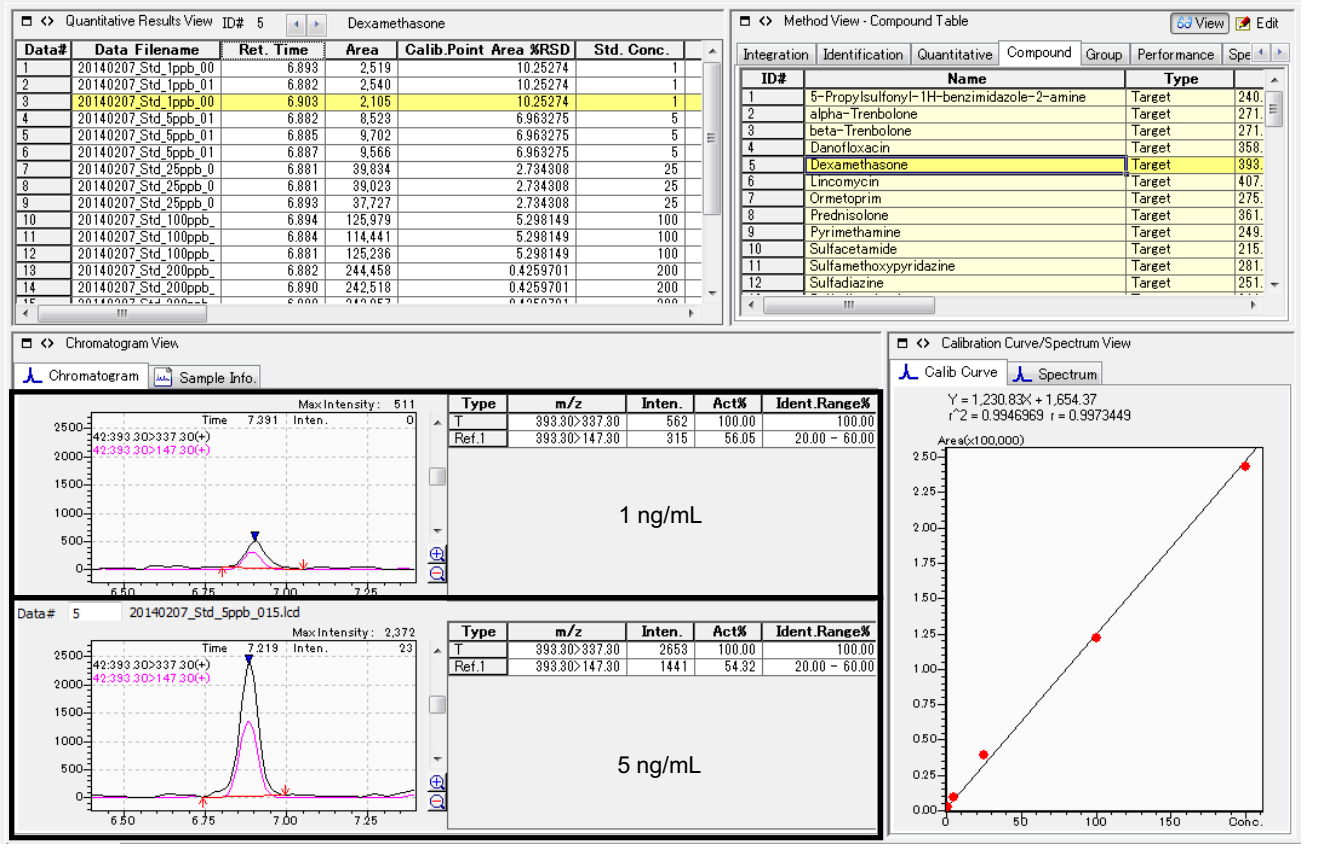


Fig. 2: Results of Dexamethasone

Area %RSDs of these 36 veterinary drugs are less than 20% at 1 ng / mL and all compounds show excellent linearity (R^2), greater than 0.99. Results above are from Dexamethasone.

V-36 Quantitative Analysis of Veterinary Drugs Using the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer

Foods in which chemical residues, like pesticides, feed additives, and veterinary drugs found in excess of maximum residue levels have been banned from sale in many countries around the world. Compounds that are subject to residue standards vary widely and the list is expected to grow. Because of this, there is a need for a highly sensitive and rapid analytical technique to analyze as many of these compounds as possible in a single run. This Application News introduces an example of the high-sensitivity analysis of 89 veterinary drugs in a crude extract of livestock and fishery products.

Sample Preparation

The typical samples used in the analysis of veterinary drugs contain large amounts of lipids because they are commonly meat and fish samples. Sample preparation is extremely important to ensure excellent sensitivity and repeatability. To avoid the typical time-consuming and laborious solid phase extraction sample preparation procedure, the QuEChERS method, which is typically used for the preparation of vegetables, was selected to simplify sample preparation.
The QuEChERS method normally consists of two steps, the first is an acetonitrile extraction and the second a cleanup step, but this time only the acetonitrile extraction step was used.

* QuEChERS Extraction Salts kit: Restek Q-sep™ AOAC2007.01

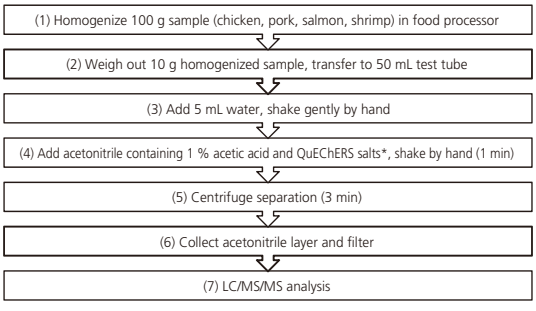
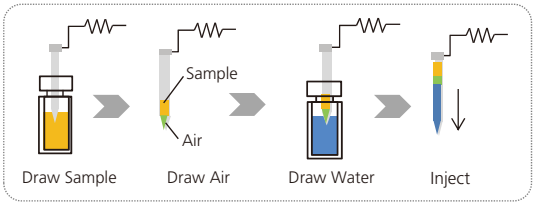


Fig. 1 Sample Preparation Procedure

Improved Peak Shape Using Sample / Water Co-Injection

When conducting reversed phase chromatography, the peaks of polar compounds may split or collapse depending on the relationship between the sample solvent and mobile phase. In cases where the sample solvent is rich in organic solvent, the elution strength must be lowered (by substitution or dilution) with the addition of water. As the pretreated sample solvent in this analysis consists of 100 % acetonitrile, injection in that state into the LC/MS will result in split peaks for some of the substances (Fig. 2 left).
To eliminate as much of the time and effort typically associated with sample preparation, the pretreatment features of the autosampler (SIL-30A) were utilized to conduct co-injection of sample and water, which resulted in improved peak shapes.



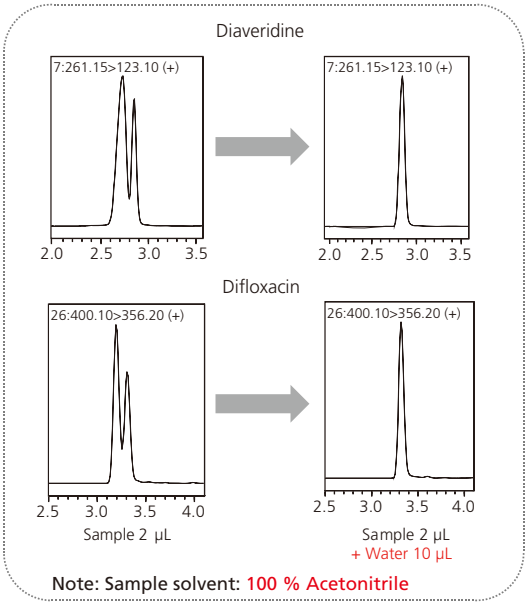


Fig. 2 Comparison of Peak Shape

MRM Analysis of Matrix Standards

Fig. 3 shows the MRM chromatogram of the matrix standard solution consisting of the sample solution with added standard solution (data obtained using pork extract solution). Table 1 shows the lower limits of quantitation for the standard solution without added matrix and with added matrix, respectively. In a crude extract obtained by acetonitrile extraction alone, sensitivity was comparable to that obtained for most of the compounds using only standard solution. Although there were several compounds for which the lower limit of quantitation was different in the standard solution than the matrix-added solution, rather than attributing this to matrix effects, it is thought to be caused by elevated background due to ions derived from contaminating components (Refer to Fig. 5).

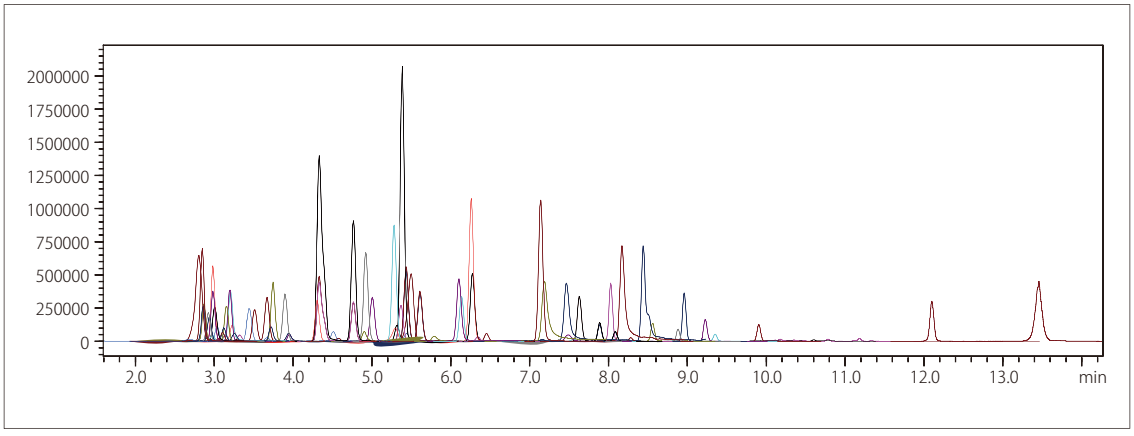


Fig. 3 MRM Chromatograms of 89 Veterinary Drugs (10 µg/L pork extract solution with added standard solution)

Table 1 LOQs of Veterinary Drugs in Neat Standards and Matrix Standards and Calibration Range of Veterinary Drugs in Matrix Standards

	Std. Solution	Matrix-Added Std. Solution	
	Min. Conc.	Min. Conc.	Max. Conc.
Gentamicin	0.5	1	50
Sulfanilamide	1	1	50
Levamisole	0.05	0.05	50
Lincomycin	0.01	0.01	10
5-Propylsulfonyl-1-benzimidazole-2-amine	0.05	0.05	10
Diaveridine	0.01	0.01	10
Trimethoprim	0.02	0.02	20
Marbofloxacin	0.01	0.01	50
Sulfisomidine	0.02	0.02	20
Norfloxacin	0.5	0.5	50
Ormetoprim	0.02	0.02	10
Thiabendazole	0.01	0.01	10
Ciprofloxacin	0.05	0.5	10
Neospiramycin I	0.01	0.05	10
Danofloxacin	0.1	0.1	10
Enrofloxacin	0.05	0.1	50
Oxytetracycline	0.01	0.1	50
Xylazine	0.01	0.01	10
Orbifloxacin	0.05	0.05	50
Sulfacetamide	1	1	50
Clenbuterol	0.01	0.01	10
Tetracycline	0.05	0.01	50
Spiramycin I	0.01	0.01	50
Sarafloxacin	0.5	0.5	50
Difloxacin	0.05	0.1	50
Sulfadiazine	0.02	0.1	20
Sulfathiazole	0.02	0.1	20
Sulfapyridine	0.02	0.1	20
Carbadox	0.05	0.05	10
Pyrimethamine	0.02	0.02	20
Sulfamerazine	0.02	0.02	20
Chlortetracycline	0.1	0.1	50
Tilmicosin	0.1	0.1	50
Thiamphenicol	1	1	50
Sulfadimidine	0.02	0.02	20
Sulfametoxydiazine	0.01	0.02	10
Sulfamethoxypyridazine	0.02	0.02	20
Sulfisozole	0.01	0.01	50
Trichlorfon (DEP)	0.05	0.05	50
Sulfamonomethoxine	0.02	0.02	20
Furazolidone	1	1	50
Difurazone	0.05	0.05	50
Erythromycin A	0.01	0.01	50
Cefazolin	0.5	0.5	50

	Std. Solution	Matrix-Added Std. Solution	
	Min. Conc.	Min. Conc.	Max. Conc.
Sulfachloropyridazine	0.02	0.02	20
Sulfadimethoxine	0.02	0.02	10
Tylosin	0.05	0.05	50
Sulfamethoxazole	0.02	0.1	10
Sulfaethoxypyridazine	0.02	0.02	10
Tiamulin	0.01	0.01	50
Florfenicol	0.5	10	50
2Acetylamino 5nitrothiazole	0.05	0.05	50
Sulfatroxazole	0.01	0.01	5
Leucomycin	0.01	0.01	50
Sulfisoxazole	0.01	0.05	50
Oxolinic acid	0.01	0.1	50
Chloramphenicol	0.5	1	50
Clorsulon	0.5	1	50
Sulfabenzamide	0.01	0.01	10
Ethopabate	0.01	0.01	10
Sulfadoxine	0.02	0.02	20
Sulfaquinoxaline	0.02	0.02	10
Prednisolone	0.1	0.05	20
Ofloxacin	0.5	0.5	50
Flubendazole	0.01	0.01	50
Methylprednisolone	0.5	0.5	50
Nalidixic acid	0.01	0.01	50
Dexamethasone	0.5	0.5	50
Flumequine	0.01	0.01	50
Benzylpenicillin	0.5	0.5	50
Sulfanitran	0.2	0.2	50
Sulfabromomethazine	0.01	0.01	50
betaTrenbolone	0.02	0.1	50
Emamectin B1a	0.01	0.01	50
alphaTrenbolone	0.02	0.1	50
Piromidic acid	0.01	0.05	50
Zeranol	1	0.1	50
Ketoprofen	0.01	0.05	50
Testosterone	0.01	0.05	10
Famphur	0.05	0.05	50
Fenobucarb (BPMC)	0.01	0.01	50
Clostebol	0.05	0.05	50
Dichlofenac	0.01	0.01	50
Melengestrol Acetate	0.05	0.05	50
Temephos (Abate)	0.01	0.5	50
Allethrin	0.1	1	50
Closantel	0.01	0.01	10
Monensin	0.01	0.01	10

(Unit: µg/L)

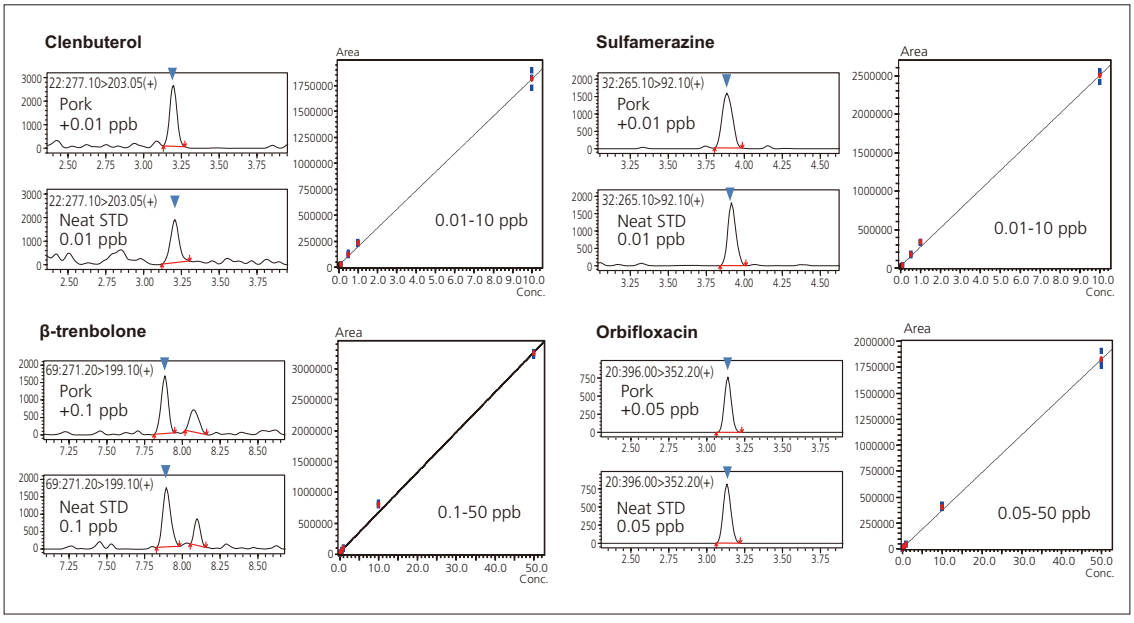


Fig. 4 MRM Chromatograms in the Vicinity of the LOQ and Calibration Curves of Typical Compounds

Recoveries of Veterinary Drugs in Crude Extracts from Livestock and Fishery Products (Matrix Effect Verification)

We examined whether or not the matrix affected measurement of actual samples. This time, four types of food product samples were used, including shrimp, chicken meat, pork, and salmon. Standard solution was added to the acetonitrile extraction solution of each of these to obtain a final concentration of 10 µg/L, after which the rates of recovery were determined. The results indicated that 90 % of the compounds were recovered at rates of 70 to 120 % and measurement was accomplished without any adverse matrix effects even though the crude extract solution was subjected only to acetonitrile extraction.

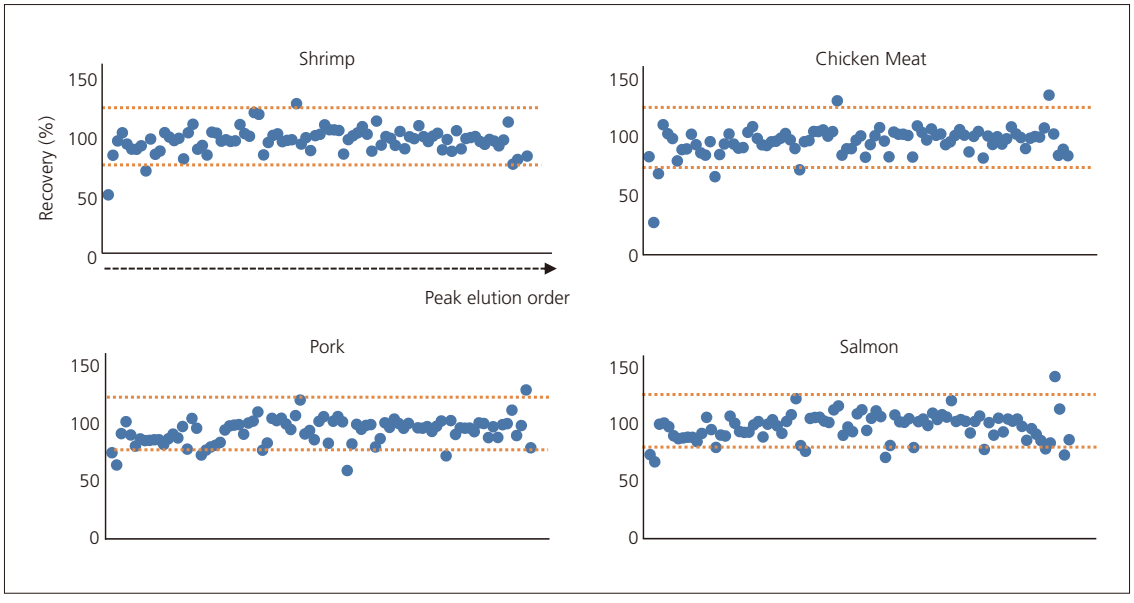


Fig. 5 Recoveries of Veterinary Drugs in Each of the Matrices

Acetonitrile Extraction Efficiency Using QuEChERS Method

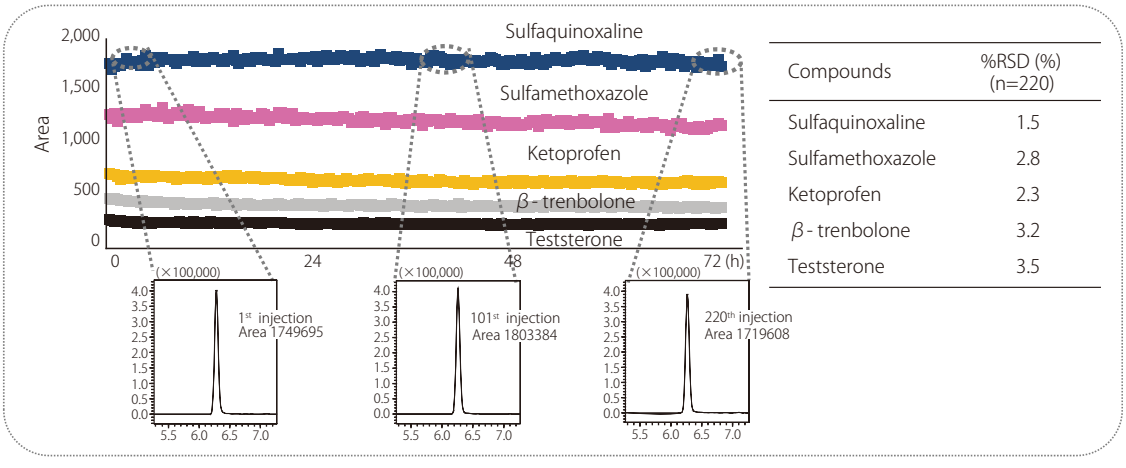
To check the efficiency of acetonitrile extraction by the QuEChERS method, standard solution was added at stage (2) of Fig. 1 to obtain a concentration of 10 µg/L, and the recoveries were determined. Good recoveries of approximately 80 % were obtained in cases both with and without the addition of matrix. However, relatively poor recoveries were seen for highly polar compounds such as tetracycline and quinolone. For these compounds, it is necessary to examine the use of a separate extraction solvent and extraction reagent.

Table 2 Recoveries (Pre-Spike)

Recovery	Without Matrix	With Matrix (Pork)	Compounds with Poor Recovery
< 50 %	17 (19 %)	13 (15 %)	Tetracyclines Quinolones
50 % - 70 %	1 (1 %)	8 (9 %)	
> 70 %	71 (80 %)	68 (76 %)	

Robustness

We checked the long-term stability of the instrument using a solution of pork crude extract (spiked with 10 µg/L standard solution). Even after continuous measurement of an extremely complex matrix over a period of 3 days, we were able to obtain stable data.



Compounds	%RSD (%) (n=220)
Sulfaquinoxaline	1.5
Sulfamethoxazole	2.8
Ketoprofen	2.3
β- trenbolone	3.2
Teststerone	3.5

Fig. 6 Area Plot and %RSD of Typical Compounds with Continuous Analysis

Table 3 Analytical Conditions

Column	: Shim-pack XR-ODS Ⅱ (75 mm × 2.0 mm I.D., 2.2 µm)
Mobile Phase A	: 0.1 % Formic Acid - Water
Mobile Phase B	: Acetonitrile
Time Program	: 1 %B (0 min) → 15 %B (1 min) → 40 %B (6 min) → 100 %B (10-13 min) → 1 %B (13.01-16 min)
Flowrate	: 0.2 mL/min.
Injection Volume	: 2 µL (2 µL sample solution + 10 µL water)
Oven Temperature	: 40 °C
Ionization Mode	: ESI (Positive / Negative)
Probe Voltage	: +2.0 kV / -1.0 kV
Neburizing Gas Flow	: 3.0 L/min.
Drying Gas Flow	: 10.0 L/min.
Heating Gas Flow	: 10.0 L/min.
Interface Temperature	: 400 °C
DL Temperature	: 200 °C
Block Heater Temperature	: 400 °C

4. OTHERS

PREFACE

Along with chemical, biological and vet drugs hazard, physical contamination is also a growing cause of health concern. The physical contamination mainly involves toxic elements present in food, coming from additives or environment, needs to be controlled due to their toxic effects on human health. Most of the countries including US EPA, US FDA, EU Regulations, have set MRLs for heavy metals.

In addition to above contaminants, food composition may have natural or artificial chemicals which, depending on their concentration, may have adverse effect on health. These components need to be identified and quantified prior to human consumption. For example, higher trans fatty acids may raise your bad (LDL) cholesterol levels and lower your good (HDL) cholesterol levels. Eating *trans* fats increases your risk of developing heart disease and stroke. It's also associated with a higher risk of developing type 2 diabetes. Genetically modified food may cause side effects like inflammation, kidney and liver malfunction, and reduced fertility.

The packaging of food plays an important role during the transportation and storage, which may cause contamination in food leading to health hazard. So EU regulations have set monitoring methods for leachable and extractable from food packaging materials.

Shimadzu Corporation provides solution to all above areas of food safety monitoring through development of advance analytical instrument technologies like ICPE, AAS with GF, FTIR, NIR, GC-ECD, MultiNA, EDXRF, UV-Vis, etc. The following chapter demonstrates some of the applications developed using these technologies for ensuring safe food.

O-1 ANALYSIS OF 3-MCPD FATTY ACID DIESTERS IN PALM OIL USING A TRIPLE QUADRUPOLE LC/MS/MS [LCMS-8030]

INTRODUCTION

3-MCPD (3-monochloropropane-1,2-diol) is a byproduct that is formed in the production of condiments such as soy sauce when hydrochloric acid is used to accelerate the hydrolysis of vegetable proteins such as defatted soybean and wheat gluten. According to the risk assessment of 3-MCPD by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 3-MCPD is not considered to be genotoxic or carcinogenic. However, animal tests have indicated that it adversely affects the kidneys if ingested in large quantities over a long period of time. In Japan, it has been confirmed that there is no 3-MCPD present in *honjozo* (authentically-brewed) soy sauce produced by a traditional method, which accounts for 85 % of the soy sauce produced in Japan. The general dietary intake of 3-MCPD that can be ingested without causing problems is not regulated in Japan. However, measures have been implemented to improve upon production methods and limit the inclusion of 3-MCPD. Recently, the presence of 3-MCPD fatty acid esters have been reported in many foods, containing refined edible oils. The toxicity of 3-MCPD fatty acid esters has not yet been clarified, therefore the analysis of 3-MCPD fatty acid ester is very important. The application of GC/MS following derivatization with phenylboronic acid (DGF Standard methods 2009, Section C- Fats) has traditionally been used for analysis of 3-MCPD fatty acid esters, yet direct analysis by LC/MS/MS without derivatization is gaining attention as an attractive alternative method. Significant amounts of 3-MCPD fatty acid esters are present in numerous natural vegetable oils, and their concentration is particularly high in palm oil. Here, we introduce the quantitative analysis of 3-MCPD fatty acid esters in palm oil using LC/MS/MS.

EXPERIMENTAL

Instrument parameters

LC conditions

Column :Shim-pack XR-ODS II (75 mm L x 2 mm I.D.; 2.2 µm)

Mobile phase :A:3 mM/L ammonium acetate in methanol :acetonitrile (9:1 v/v)
B:3 mM/L ammonium acetate in methanol:acetonitrile :acetone (1:1:8 v/v)

Flow rate : 0.4 mL/min

Oven temperature : 40 °C

Injection volume : 2 µL

Gradient program :

Time (min)	Pump B conc.
0.01	0
2.50	0
7.50	65
7.51	100
10.00	100
10.01	0
15.00	0

MS conditions

Ionisation : Electrospray solution (ESI); positive (+4.5 kV)

Mode : MRM

MRM transitions : 3-MCPD-Dipalmitoyl Ester 604.40 > 331.10
3-MCPD-Dioleoyl Ester 656.50 > 357.20

Nebulising gas flow : 1.5 L/min

Drying gas flow :20 L/min

DL temperature :300 °C

Heat block temperature :400 °C

DL/Q-array voltage :use tuning file

Sample Preparation

169.9 mg of palm oil was weighed out, dissolved in 1 mL of hexane and diluted 100 times with acetone (588.6 times dilution), and then analyzed.

RESULTS AND DISCUSSION

Synthetic 3-MCPD-dipalmitoyl ester and 3-MCPD-dioleoyl ester were used as standard samples. Electrospray ionization (ESI) was used as the ionization method and the 3-MCPD-di-fatty acid esters were detected as NH4 + adduct ions due to the addition of ammonium acetate in the mobile phase. The MS/MS spectra obtained using the adduct ions as the precursor are shown in Figure 1. Figure 1 shows MS/MS spectra at varying collision energies (CE) from the top, middle, and bottom spectra generated by 10, 30, and 40 V, respectively. As each one of the fatty acids is desorbed, it is detected as a product ion. Figure 2 shows the MRM chromatograms of the standard samples (1 µg/L).

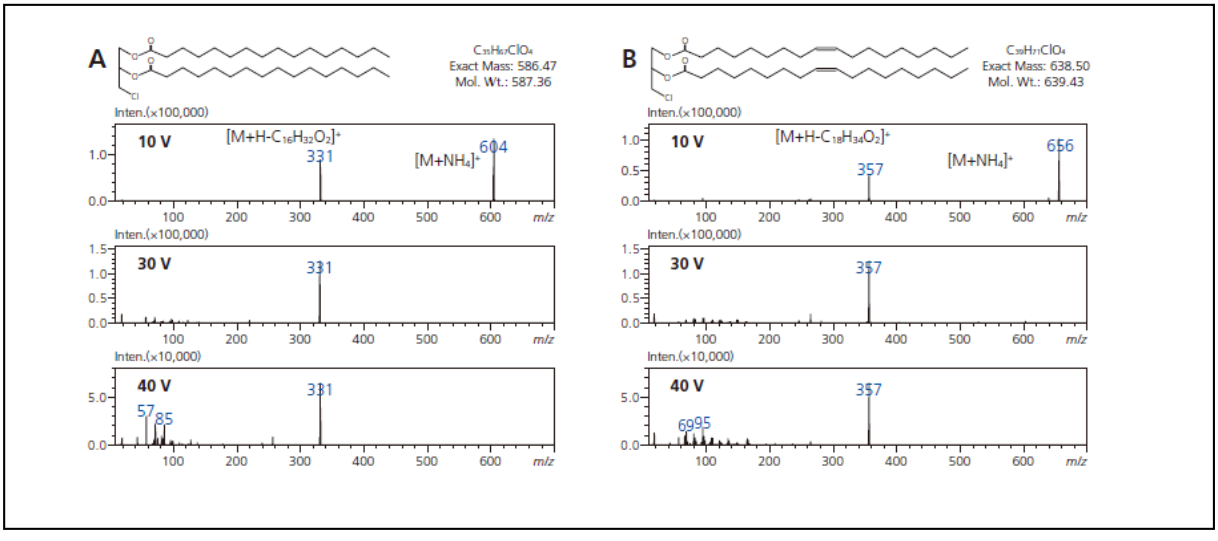


Figure 1. MS/MS Spectra of the Synthetic Samples (A: 3-MCPD-Dipalmitoyl Ester, B: 3-MCPD-Dioleoyl Ester)

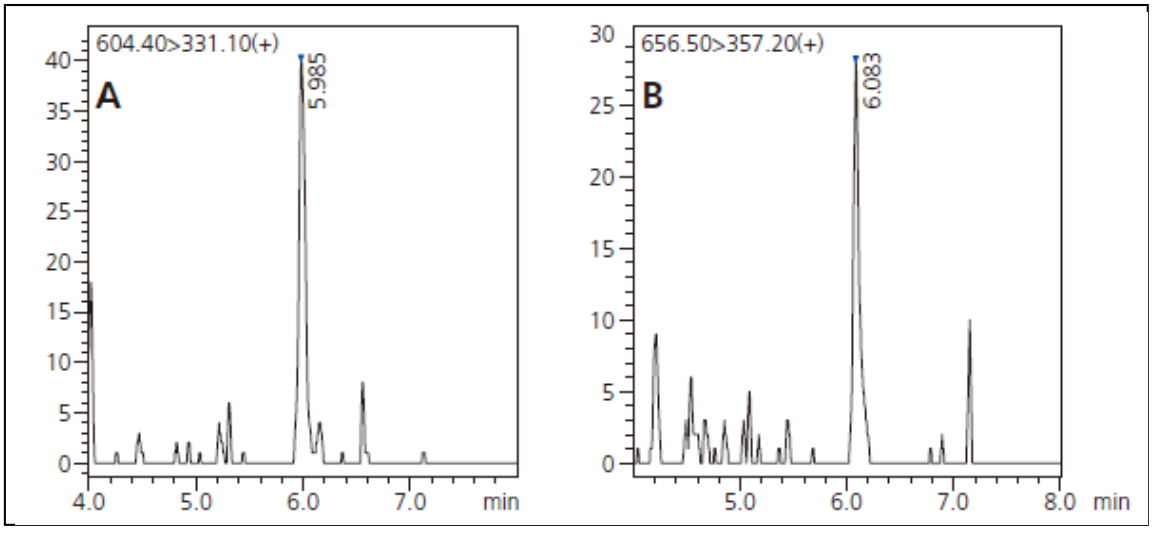


Figure 2. MRM Chromatograms of the Synthetic Samples (1 µg/L, A: 3-MCPD-Dipalmitoyl Ester, B: 3-MCPD-Dioleoyl Ester)

Next, the calibration curves for 3-MCPD-dipalmitoyl ester and 3-MCPD-dioleoyl ester are shown in Figure 3A and Figure 3B, respectively. Excellent linearity was obtained over a wide range from 1–1000 µg/L, with correlation coefficient (R^2) values greater than 0.999. The repeatability using 6 repeat measurements of 3-MCPD-dipalmitoyl ester and 3-MCPD-dioleoyl ester was 15.47 and 19.64 area % RSD, respectively, at 1 µg/L, and 6.54 and 9.32, respectively, at 10 µg/L.

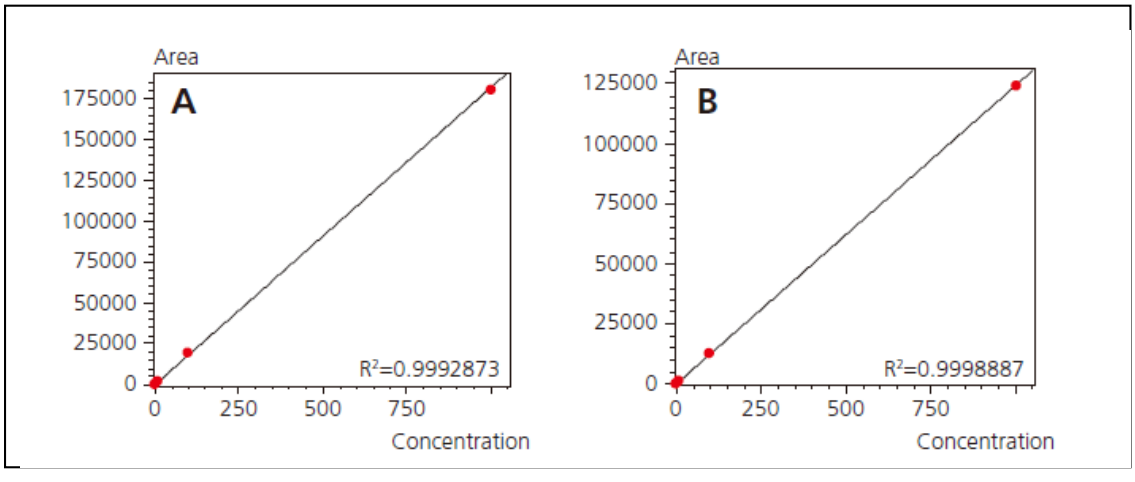


Figure 3. Calibration Curves (1–1000 µg/L, n = 6)

3-MCPD-dipalmitoyl ester and 3-MCPD-dioleoyl ester were detected in this diluted solution at approximately 10 µg/L (Figure 4A and B).This corresponds to a concentration in palm oil of about 6 mg/L of 3-MCPD-dipalmitoylester and 3-MCPD-dioleoylester, respectively.

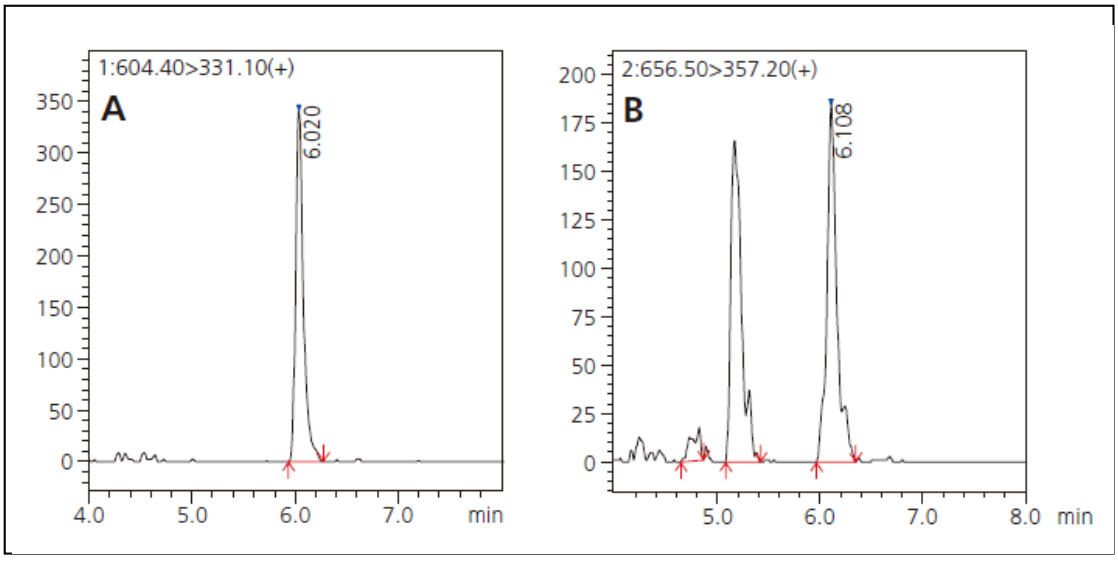


Figure 4. MRM Chromatograms of 3-MCPD Fatty Acid Diesters in Palm Oil (A: 3-MCPD-Dipalmitoyl Ester, B: 3-MCPD-Dioleoyl Ester)

CONCLUSION

Thus, it is possible to use a liquid chromatograpgy/triple quadrupole mass spectrometer for detection of 3-MCPD fatty acid esters from palm oil using a simple pretreatment procedure that is limited to sample dilution.

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O-2 RAPID ANALYSIS OF TRIGLYCERIDES AND FATTY ACIDS IN FOOD OILS USING DART-MS WITH HIGH-SPEED POLARITY SWITCHING

INTRODUCTION

Conventionally, triglycerides have been commonly analysed by using liquid chromatograph or gas chromatograph. Some of the problems with those methods are cumbersome sample preparation, lengthy analysis time and memory effect. Previous reports on DART-MS analysis of triglycerides and fatty acids have involved separate acquisitions of positive spectra and negative spectra, but the ultra-fast polarity switching feature has been successfully applied to carry out high-throughput parallel analyses of fatty acid compositions in triglycerides and free fatty acids using Direct Analysis in Real Time (DART) mass spectrometry.

EXPERIMENTAL

Commercially available edible oils such as olive oil and Chinese chili oil were obtained from a local grocery store. Small amount of the samples were picked up and held in the DART ionization gas stream using glass capillaries. DART-OS ion source (IonSense, Inc., MA, USA) was coupled with triple quadrupole mass spectrometer LCMS-8030/8040 from Shimadzu Corporation, Kyoto, Japan, which is capable of ultra-fast polarity switching (refer Figure 1). Scan range was set between *m/z* 50 -1200 for both positive and negative mode, with which the performance of 2 scans/sec was achieved.

Instrument parameters

MS conditions

DART	:DART OS (IonSense, Inc., USA)
MS	:LCMS-8030 (Shimadzu Corporation, Japan)
Ionization	:Electrospray ionization,
Positive/Negative	
Ultra Fast Polarity Switching	: 15 msec
Heater Temperature	:300 °C – 500 °C

Scan type :Q3 scan, *m/z* 50 - 1200
Ultra Fast Scanning :Up to 15,000 u/sec

Figure 1. DART OS ion source coupled with LCMS-8040 triple quadrupole mass spectrometer

RESULTS AND DISCUSSION

Method development for oils

The samples were analyzed using three different DART gas temperature settings of 300 °C, 400 °C and 500 °C (refer Figure 2). Figure 3 shows typical mass spectra that were analyzed from Chinese chili oil. From the Chinese chili oil, capsaicin (*m/z* 306, positive ion) was detected. The best temperature setting for this compound was 300 °C among the three temperature settings that were examined. As the heater temperature was raised to 400 °C and 500 °C the positive ion signals of triglycerides around *m/z* 900 became more intense. Diglycerides were detected around *m/z* 600. Triglycerides were believed to be primarily ammonia adduct ions and diglycerides dehydrated ions. From the negative ion spectra, signals for linoleic acid (*m/z* 279) and oleic acid (*m/z* 281) were found throughout the temperature range used in this experiment (refer Figure 4). It was decided that the optimum DART heater temperature setting was 500 °C for the

purpose of pattern analysis of triglycerides and fatty acids, and hence was kept 500 °C throughout the rest of the experiment.

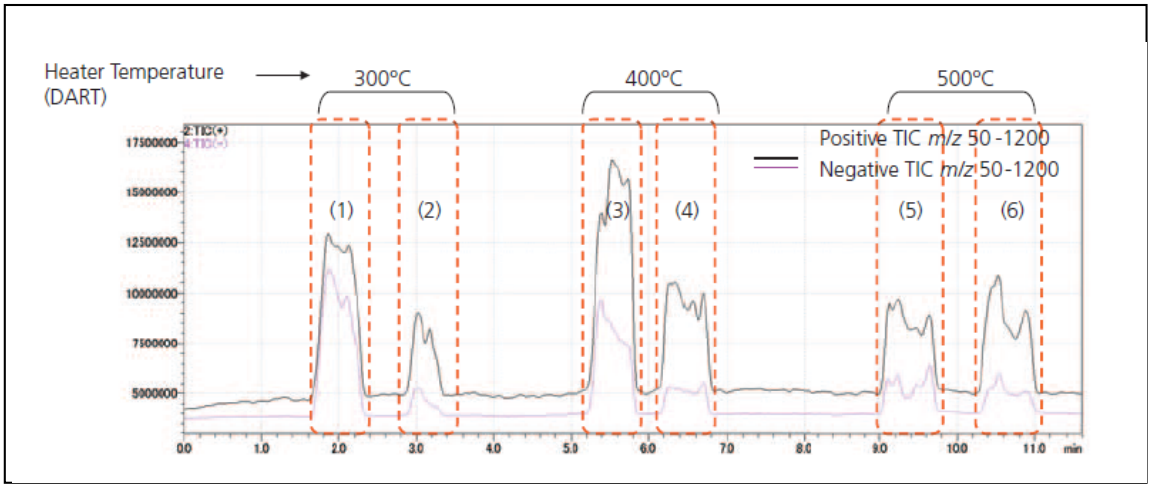


Figure 2. Total ion current chromatograms for DART analysis of oils

(1) Chinese chili oil; heater temperature 300 °C
(2) Salad oil; heater temperature 300 °C
(3) Chinese chili oil; heater temperature 400 °C
(4) Salad oil; heater temperature 400 °C
(5) Chinese chili oil; heater temperature 500 °C
(6) Salad oil; heater temperature 500 °C

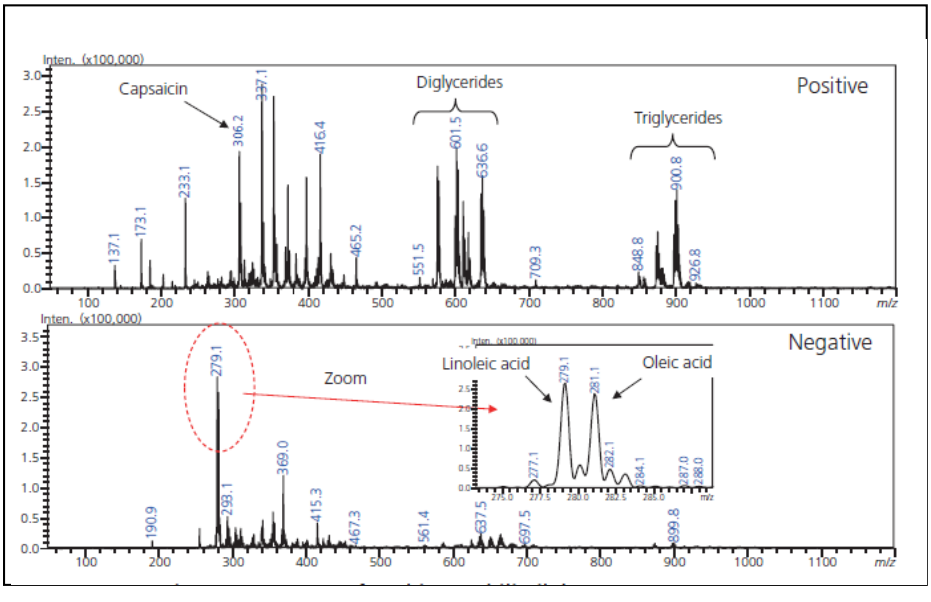


Figure 3. Mass spectra for Chinese chili oil; heater temperature 400 °C

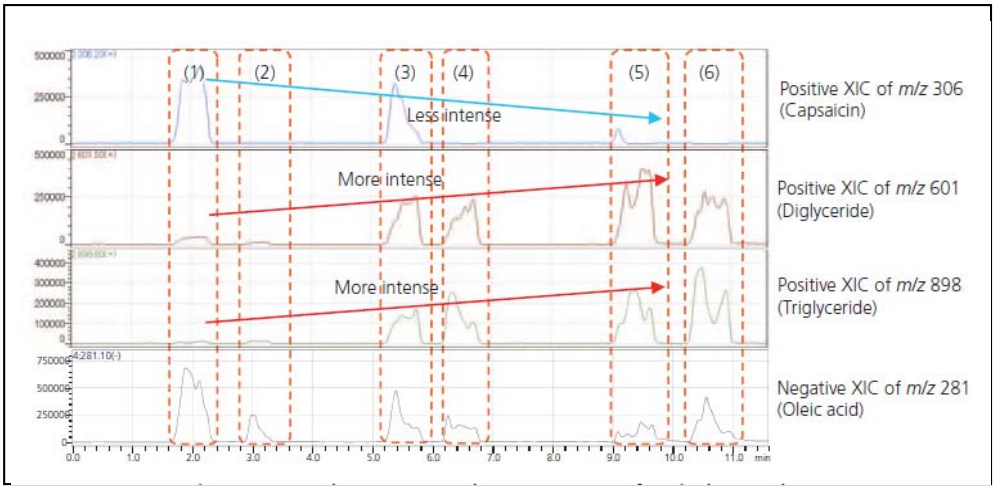


Figure 4. Extracted Ion Current Chromatograms of typical *m/z* values

Chinese chili oil

Figure 5 shows mass spectra of Chinese chili oil at heater temperature 500 °C. Taking a closer look at triglycerides of Chinese chili oil, it was found that there were signals of triglycerides comprising of (1) oleic acid molecules only, (2) two oleic acid and a linoleic acid molecules, (3) an oleic acid and two linoleic acid molecules and (4) linoleic acid molecules only, in similar intensity, which correlates with the balance of fatty acid signals of oleic acid and linoleic acid in the negative ion spectra. It was also possible to determine the presence of palmitic acid components by comparing the triglyceride ion clusters that appear in lower *m/z* range in the positive spectra and palmitic acid signal in the negative spectra.

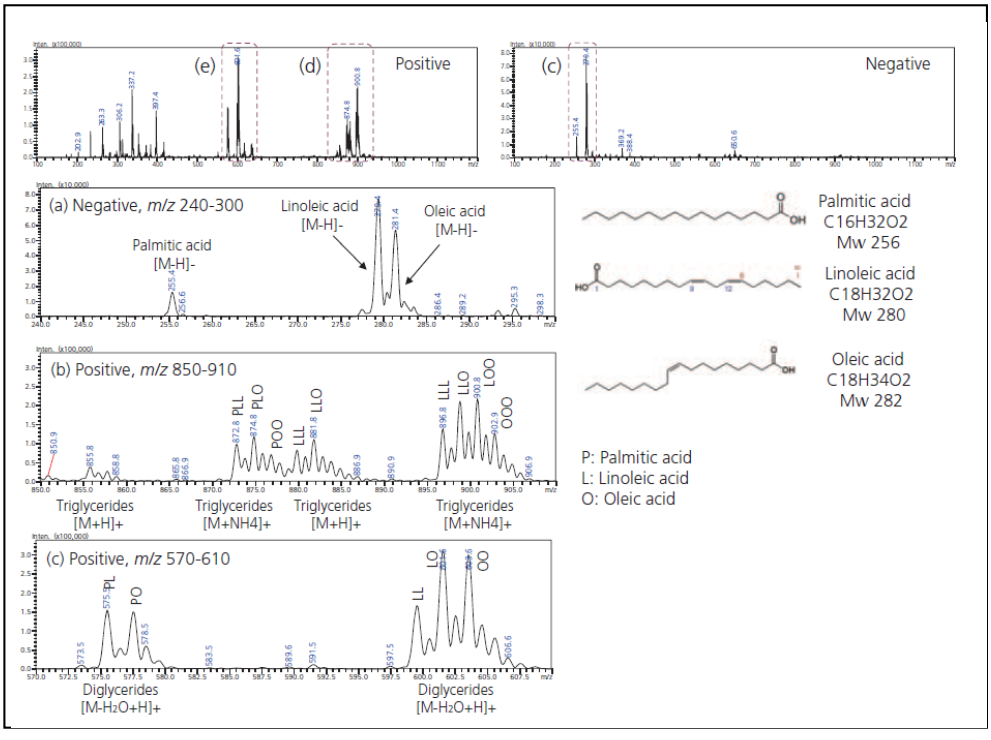
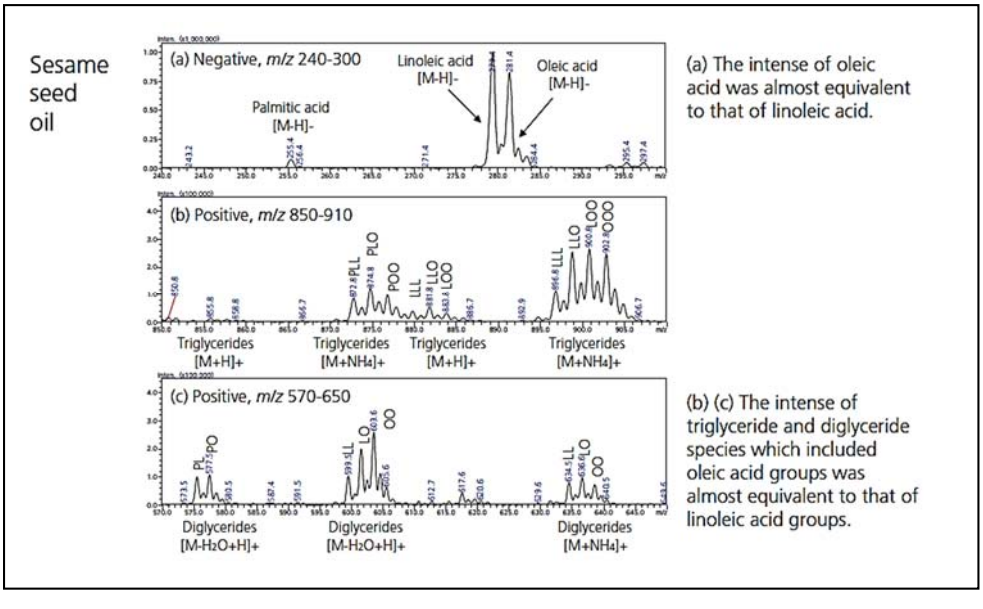


Figure 5. Mass spectra for Chinese chili oil; heater temperature 500 °C

Olive oil and sesame seed oil

DART-MS analyses were carried out for olive oil, sesame seed oil in the same fashion, and good correlations between triglyceride compositions in the positive ion spectra and fatty acid abundance ratio in the negative ion spectra were readily observed in one DART-MS run.



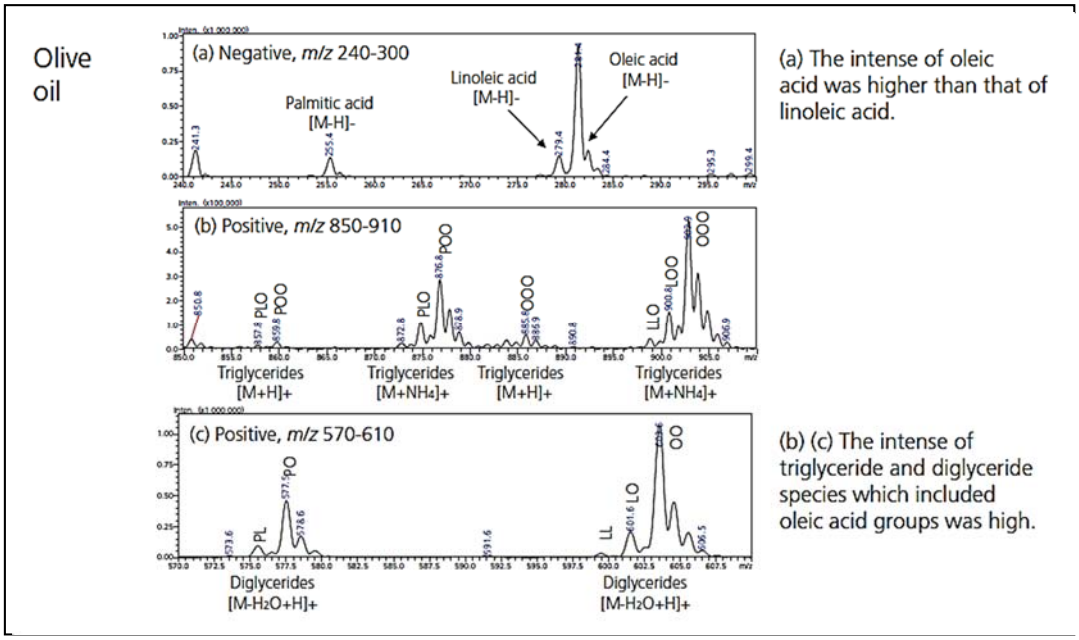


Figure 6. Mass spectra for olive oil and sesame seed oil

CONCLUSION

Ultra-fast polarity switching was useful for high-throughput parallel pattern analyses of fatty acid compositions in triglycerides and free fatty acids using DART mass spectrometer. Good correlations were seen between triglyceride and diglyceride compositions in the positive ion spectra and fatty acid abundance ratio in the negative ion spectra.

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O-3 RAPID, HIGH THROUGHPUT QUANTITATION OF THUJONE IN ABSINTHE BY UHPLC/MS/MS

INTRODUCTION

Absinthe is an anise-flavored, distilled alcoholic beverage that was vilified and banned in many countries before the World War I. It was targeted because the beverage was alleged to have hallucinogenic effects; with the compound thujone (refer Figure 1) thought to be the culprit. By the 1990's, the advent of new food safety laws and a renewed interest in absinthe led to a lifting of the ban in many countries, but in some areas legal limits on thujone levels remain. In order to comply with food and beverage safety laws, a rapid and accurate measurement of thujone in absinthe is required. To accomplish this, a UHPLC/MS/MS method meeting these requirements of selectivity, sensitivity, and speed was developed.

EXPERIMENTAL

A Shimadzu Nexera UHPLC with an LCMS-8040 triple quadrupole mass spectrometer was utilized for this analysis. An authentic standard of α,β-thujone was obtained from PhytoLab (Vestenbergsgreuth, Germany). Fisher LC/MS Grade water and methanol were used for chromatography. Sigma-Aldrich absolute ethanol and Fluka formic acid were also used. Three brands of absinthe (Grüne Fee, La Sorcière and Grande Absente) were purchased for testing and Smirnoff triple distilled vodka was used as a control.

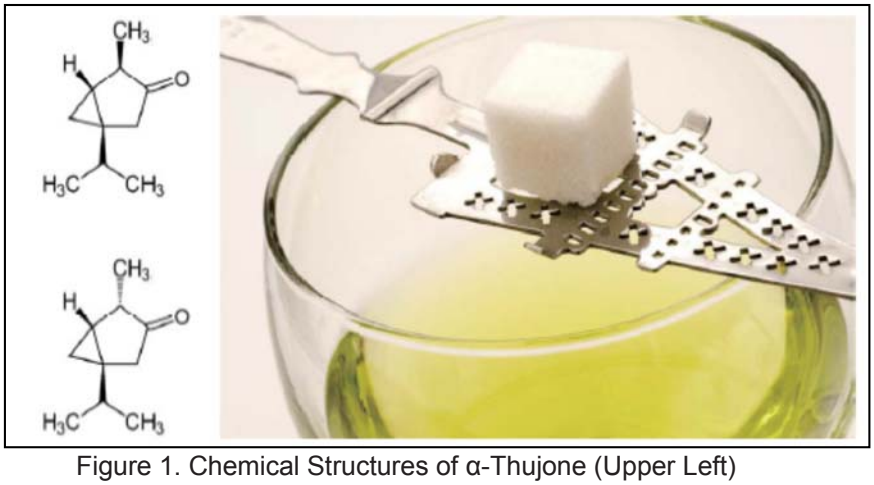


Figure 1. Chemical Structures of α-Thujone (Upper Left)
and β-Thujone (Lower Left) as well as an example of a typical absinthe cocktail

Sample Preparation

A standard curve was generated by spiking α,β-thujone into vodka to a known final concentration. Absinthe was diluted 20 times using 40 % ethanol in water, or spiked with 5000 ng/mL α,β-thujone and then diluted 20 times with 40 % ethanol in water.

Instrument parameters

System congifuration

HPLC :Shimadzu Nexera UHPLC system

MS :Shimadzu LCMS-8040 Triple Quadrupole Mass Spectrometer

LC conditions

Column :Thermo Scientific Hypersil GOLD (100 mm L x 2.1 mm ID; 1.9 µm)

Mobile Phase ::A - LC/MS grade water with 0.1% formic acid
B - LC/MS grade methanol (all mobile phases were purchased from Fisher Scientific)

Flow Rate :0.3 mL/min

Column Temperature :40 °C

Injection Volume :10 µL

Gradient Program :

Time (min)	Pump B conc.
0.01	40
0.50	40
5.00	95
6.00	95
6.01	40
10.00	40

MS conditions

Ionization : Electrospray Ionisation; positive

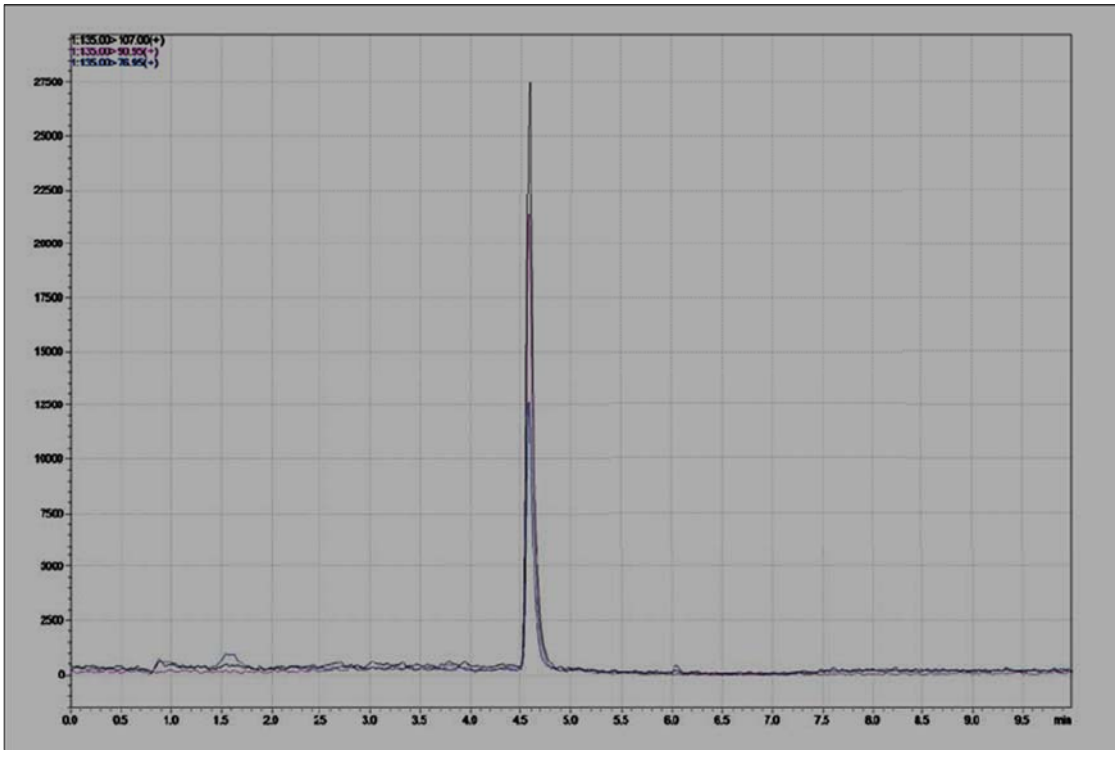
Mode : Multiple Reaction Monitoring

MRM Transitions : Quantifier (135 > 107);
Reference Ions (135.00 > 90.95; 135.00 > 76.95)

RESULTS AND DISCUSSION

Analysis was conducted on three separate days. Commercially available absinthes were diluted 20-fold with 40 % ethanol and the concentration of β-thujone was calculated from a calibration curve of 5-10,000 ng/mL. The level determined for both unspiked and spiked absinthe agreed within 10 % over the three runs. The calculated concentrations of the spiked absinthe (5 µg/mL spiked) also found within 10 % of the expected concentration. The measured carryover from ULOQ to a blank was less than 10 % of the LLOQ.

Due to the presence of isobars in the commercially available absinthes, two reference ions were chosen to ensure proper identification in the sample matrix (refer Figure 2).



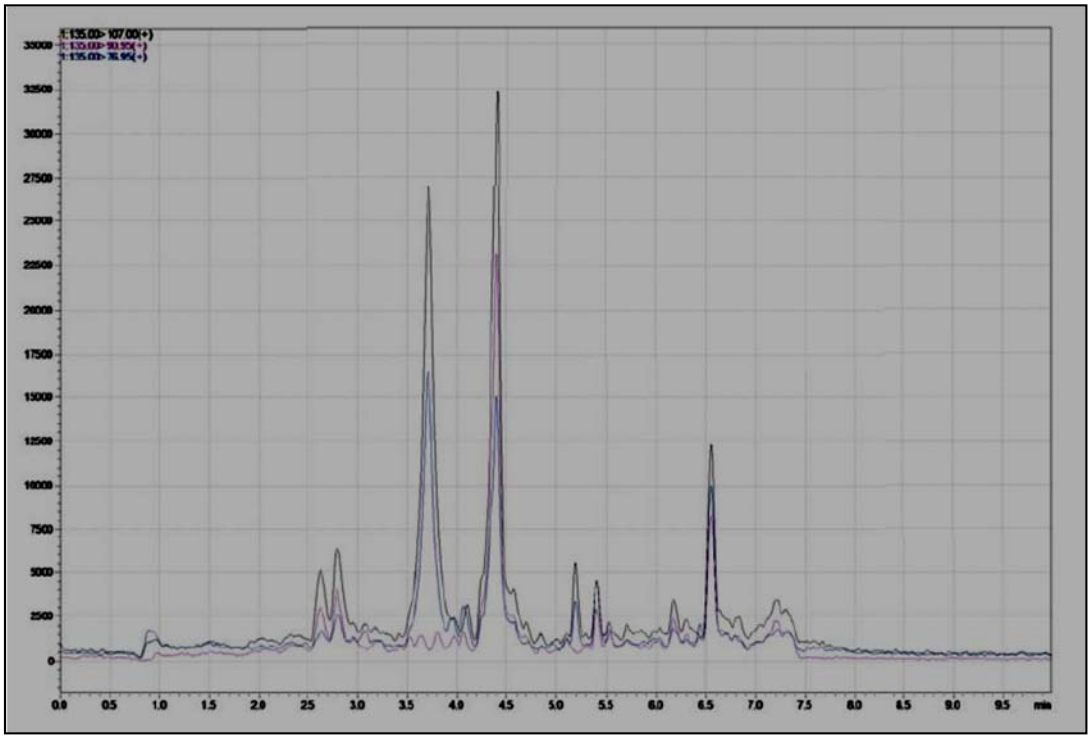


Figure 2. Typical chromatograms for a standard (top) and an absinthe sample (bottom), illustrating why 2 reference ions are monitored.

The absinthe samples analysed and their corresponding thujone contents (refer Table 1) were calculated on calibration range of 5 – 10,000 ng/mL (refer Figure 3).

Table 1. Average calculated concentration of α,β-thujone in three commercially available absinthe brands analyzed on three separate days

Absinthe Brand	Average Calculated Concentration α,β-thujone (µg/mL)	% CV
Grüne Fee-unspiked	2.38	2.4%
Grüne Fee-5 µg/mL spike	6.96	5.1%
La Socière-unspiked	3.99	2.6%
La Socière-5 µg/mL spike	8.82	3.6%
Grande Absente-unspiked	0.558	8.5%
Grande Absente-5 µg/mL spike	5.20	6.3%

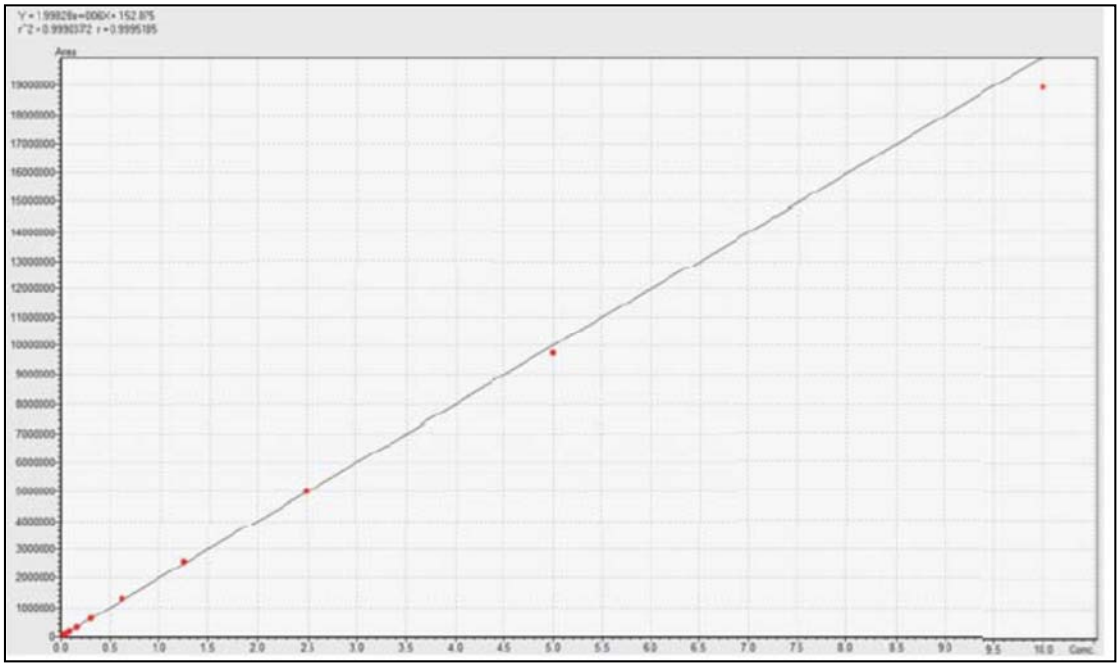


Figure 3. Calibration Curve for α,β-thujone spiked into vodka at concentrations ranging from 5-10,000 ng/mL

CONCLUSION

A rapid dilute-and-shoot method enabling the analysis of α,β-thujone in commercially available absinthes has been developed. This method could be utilized to ensure that absinthes sold in the US conform with the FDA guideline of containing less than 10 ppm thujone.

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O-4 ANALYSIS OF PHTHALATE ESTERS IN ENVIRONMENTAL WATER SAMPLES BY ONLINE-SPE-LC COUPLED WITH HIGH-SPEED TRIPLE QUADRUPLE MASS SPECTROMETER

INTRODUCTION

Phthalate esters are produced in large quantities throughout the world and used as primary plasticizers. These compounds, however, are of environmental concern due to their suspected endocrine disrupting potential. Furthermore, phthalate di-esters can be bio-transformed into mono-esters. Subsequently, these compounds may be transported from wastewater to environmental water primarily due to insufficient wastewater treatment. Since both phthalate di- and mono-esters are likely to exist as a mixture in the environment, it is important to develop a simultaneous quantification method for both forms. In this study we developed a rapid online-SPE-LC system coupled with a high-speed triple quadrupole mass spectrometer for the simultaneous determination of phthalate di- and mono-esters to trace levels.

EXPERIMENTAL

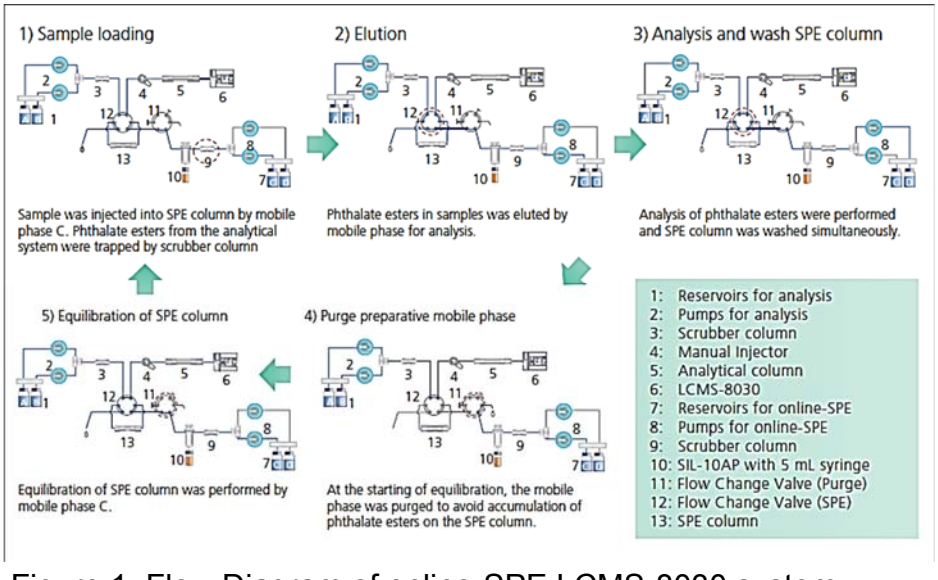


Figure 1. Flow Diagram of online-SPE LCMS-8030 system.

The analysis was carried out on Shimadzu Nexera UHPLC system coupled with LCMS-8030 triple quadrupole mass spectrometer. The sample pretreatment with online SPE has been depicted in Figure 1.

Instrument parameters

Online-SPE conditions

Scrubber column : Shim-pack XR-ODS (50 mm L x 4.6 mm I.D.; 2.2 µm)
Preparative column : EVN-MASK (purchased from Chemco Inc.)
(10 mm L x 2 mm I.D., 8 µm)
Mobile phase : A -LC/MS grade water with 0.1% formic acid
B –Acetonitrile
Flow rate : 2 mL/min
Injection volume : 1000 µL

LC conditions

Scrubber column : Shim-pack XR-ODSII (50 mm L x 2 mm I.D.; 2.2 µm)
Analytical Column : Shim-pack XR-ODSII (75 mm L x 2 mm I.D.; 2.2 µm)
Mobile Phase : A - LC/MS grade water with 0.1% formic acid
B - LC/MS grade acetonitrile
Flow Rate : 0.25 mL/min
Column Temperature: 40 °C
Gradient Program :

Time (min)	Pump B conc.
0.01	30
2.50	30
10.00	98
12.50	98
12.10	30
15.00	30

MS Conditions

Ionisation : Electrospray Ionisation (ESI);
Positive for di-esters;negative for mono-esters
Polarity switching : 15 msec
DL temperature : 250 °C
Heat block temperature : 400 °C
Nebulising gas : 2 L/min (nitrogen)
Drying gas : 10 L/min (nitrogen)

MRM transitions :

Compound name	Abbreviation	+/-	Q1 m/z	Q1 m/z	Q3 m/z	Dwell Time
Monomethylphthalate	MMP-N	-	179	11	77.3	200
Monomethylphthalate	MMP-IS	-	183	11	79.3	200
monoethylphthalate	MEP-N	-	193	19	77.3	50
monoethylphthalate	MEP-IS	-	197	19	79.3	50
monoethylhydroxyhexylphthalate	MEHHP-N	-	293	18	121.2	30
monoethylhydroxyhexylphthalate	MEHHP-IS	-	297	18	124.2	30
monobutylphthalate	MnBP-N	-	221	20	77.3	30
monobutylphthalate	MnBP-IS	-	225	20	79.3	30
monobenzylphthalate	MBzP-N	-	255	23	77.3	30
monobenzylphthalate	MBzP-IS	-	259	23	79.3	30
monoethylhexylphthalate	MEHP-N	-	277	14	134.3	30
monoethylhexylphthalate	MEHP-IS	-	281	14	137.3	30

Compound name	Abbreviation	+/-	Q1 m/z	Q1 m/z	Q3 m/z	Dwell Time
monooctylphthalate	MOP-N	-	277	26	77.3	30
monooctylphthalate	MOP-IS	-	281	26	79.3	30
monononylphthalate	MNP-N	-	291	19	141.3	30
monononylphthalate	MNP-IS	-	295	19	141.3	30
dimethylphthalate	DMP-N	+	195	-11	163.1	3
diethylphthalate	DEP-N	+	223.1	-20	149.05	3
dibutylphthalate	DBP-N	+	279.2	-17	149.05	3
dioctylphthalate	DOP-N	+	391.3	-23	149.05	50
diethylhexylphthalate	DEHP-N	+	391.3	-23	149.05	50
benzylbutylphthalate	BzBP-N	+	313.2	-25	91.1	3

Development of analytical system

High speed simultaneous analysis of phthalate esters was achieved by using high speed polarity switching (10 msec) technology. Background contaminations were minimized with a scrubber column inserted after a mixing chamber. Background peaks were, however, still detected with the scrubber column in place which suggested that auto-sampler and valves can also be a source of contamination for DBP, DEHP, DOP.

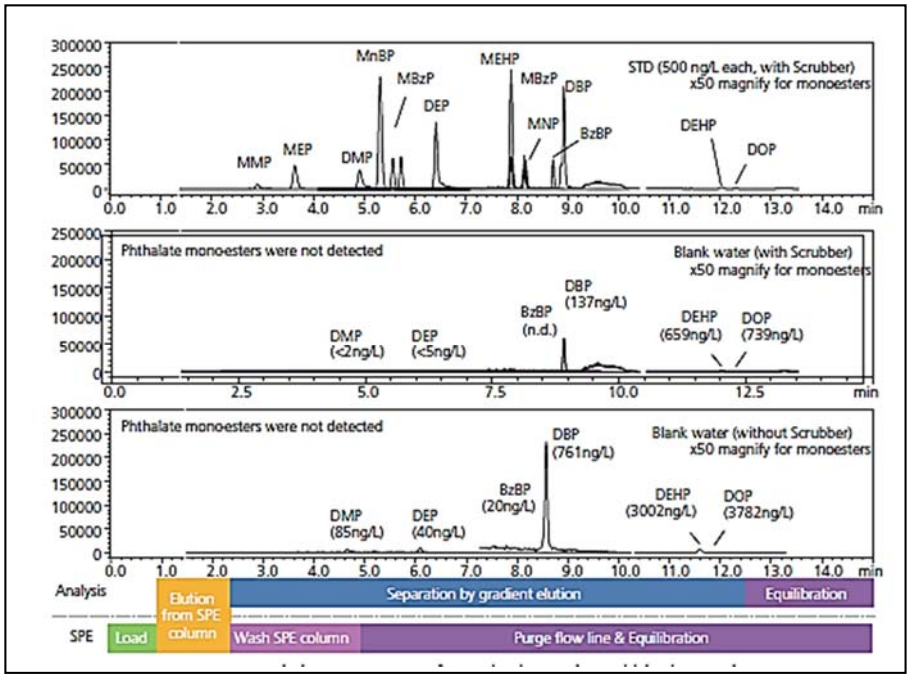


Figure 2. Typical chromatogram of standard sample and blank samples

Method performance characteristics in river water

Compound	RT (min)	Recovery (%) at 500 ng/L*	Linear range (ng/L)	R^2	RSD (%) at LOQ
MMP	2.85	69.5 ± 11.6	100-10000	0.9997	9.3
MEP	3.65	103.6 ± 14.6	20-10000	0.9995	14.2
MEHHP	5.32	72.8 ± 0.1	10-10000	0.9996	13.0
MnBP	5.57	133.9 ± 16.1	10-10000	0.9991	11.1
MBzP	5.73	89.2 ± 3.5	5-10000	0.9974	12.7
MEHP	7.87	68.3 ± 12.7	5-10000	0.9994	8.5
MOP	8.12	53.3 ± 2	5-10000	0.9978	14.2
MNP	8.14	55.6 ± 12.6	5-10000	0.9987	17.8
DMP	4.95	97.4 ± 5.1	2-10000	0.9997	17.4
DEP	6.42	100.8 ± 17.8	5-10000	0.9991	8.4
DBP	8.92	101.1 ± 15.5	50-10000	0.9998	4.2
DEHP	12.04	105.3 ± 19.6	20-10000	0.9956	8.4
DOP	12.30	105.4 ± 4.7	20-10000	0.9966	7.2
BzBP	8.71	115.5 ± 9.8	5-10000	0.9994	19.7

$$*\text{Recovery}(\%) = \frac{\text{peak area of each components loaded by online - SPE (500 ng/L x 1 mL)}}{\text{peak area of each components loaded by manual injector (100 µg/L x 5 µL)}}$$

CONCLUSION

A novel simultaneous analysis method of phthalate di- and mono-esters combined with online-SPE was developed.

The cycle time including online-SPE and column separation was 15 minutes.

Background contamination from online-SPE and LCMS system were successfully minimized.

O-5 DETERMINATION OF 20 PHTHALIC ACID ESTERS IN ALCOHOLIC DRINKS BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

INTRODUCTION

Phthalic acid esters (PAEs) are a group of commercial chemicals known to cause birth defects or reproductive harm. They are widely used to make plastics more malleable and help lotions penetrate skin. A number of phthalate esters are PAEs can migrate from plastic materials to the environment. They are often found in water, soil, air, food products and the human body. As well known, alcoholic drinks have always been popular around the world. In alcoholic drinks production, plastic containers are typically used in the storage and transportation process, which could make some phthalate esters leak easily from PVC tubes or vessels as well as plastic caps. The aim of this study is to determine the level of phthalate esters migration in alcoholic drinks by fast liquid chromatography-electrospray tandem mass spectrometry. This method is simple and rapid with acceptable sensitivity to meet the requirements for the analysis of PAEs in alcoholic drinks.

EXPERIMENTAL

Sample preparation

Accurately weigh 5.0 g of alcoholic drinks into a glass tube. After centrifugation for 20 min at 6000 rpm, the supernatant was analyzed by LC/MS/MS.

Standard PAEs

Dimethyl phthalate (DMP), diethyl phthalate (DEP), bis(2-ethoxyethyl) phthalate (DEEP), bis(2-methoxyethyl) phthalate (DMEP), diallyl (o-)phthalate (DAP), dipropyl phthalate (DPRP), diisopropyl phthalate (DiPRP), diphenyl phthalate (DIPP), benzyl butyl phthalate (BBP), diisobutyl phthalate (DIBP), bis(2-butoxyethyl) phthalate (DBEP), dibutyl phthalate (DBP), dipentyl phthalate (DPP), diisoamyl phthalate (DiAP), bis(4-methyl-2-pentyl) phthalate (BMPP), bis(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DNOP), dicyclohexyl phthalate (DCHP),

di-n-hexyl phthalate (DHXP), and diheptyl phthalate (DHP). All of the standard PAEs were purchased from ANPEL Scientific Instrument Co., Ltd.

Instrument parameters

System configuration

HPLC : Nexera (UHPLC)
Degassing unit : DGU-20A$_5$
Pumping uint : LC-30AD
Autosampler : SIL-30AC
Column oven : CTO-30A
MS : LCMS-8040

LC conditions

Guard colulmn : Inertsil ODS-4 (50 mm L × 3.0 mm I.D., 2.0 µm)
Column : Shim-pack XR-ODSIII column (150 mm L × 2.0 mm I.D., 2.2 µm)
Mobile phase : A - 5 mmol/L ammonium acetate in water
B - Methanol
Flow rate : 0.4 mL/min
Oven temperature : 45 °C
Injection volume : 10 µL
Gradient program :

Time (min)	Pump B conc.
0.01	45
6.50	90
7.00	100
9.90	100
10.00	45

MS conditions

Ionisation : Electrospray solution (ESI); positive
Mode : MRM
Nebulising gasflow : 3 L/min
Drying gas flow : 15 L/min
DL temperature : 250 °C
Heat block temperature : 450 °C
Dwell time : 15 msec
Pause time : 3 msec

MRM transitions :

Compound	Precursor ion (*m/z*)	Production (*m/z*)	Q1 pre bias (V)	CE (V)	Q3 pre bias (V)
DMP	194.7	163.0*	-23	-11	-17
		77.1	-23	-31	-14
DEMP	283.1	207.0*	-21	-6	-22
		149	-21	-28	-29
DEP	223.1	149.0*	-30	-20	-30
		177.1	-30	-10	-18
DEEP	311.1	73.1*	-23	-13	-30
		221.1	-23	-6	-24
DAP	247.1	189.1*	-28	-11	-20
		149.1	-28	-16	-27
DPRP	251.1	149.0*	-30	-20	-30
		191.1	-30	-9	-20
DiPRP	251.1	149.0*	-30	-20	-30
		191.1	-30	-9	-20
DIPP	319.1	225.0*	-23	-9	-24
		77.1	-23	-36	-30
BBP	313.1	91.1*	-30	-20	-17
		149	-30	-12	-16
DIBP	279.1	149.0*	-30	-20	-30
		205.1	-30	-8	-22
DBP	279.1	149.0*	-30	-20	-30
		205.1	-30	-8	-22
DBEP	367.1	101.1*	-27	-12	-19
		249.1	-27	-7	-27
DPP	307.1	149.1*	-30	-20	-30
		219.1	-30	-7	-24
DiAP	307.1	149.1*	-30	-20	-30
		219.1	-30	-7	-24
DCHP	331.1	149.1*	-30	-25	-28
		167.1	-30	-13	-18
BMPP	335.1	149.1*	-30	-25	-27
		167	-30	-11	-18
DHXP	335.2	149.1*	-30	-20	-30
		233.2	-30	-8	-25
DHP	363.2	149.1*	-30	-20	-30
		247.2	-30	-8	-27
DEHP	391.3	149.1*	-30	-25	-30
		167.1	-30	-13	-17
DNOP	391.4	149.0*	-30	-20	-30
		261.1	-30	-8	-29

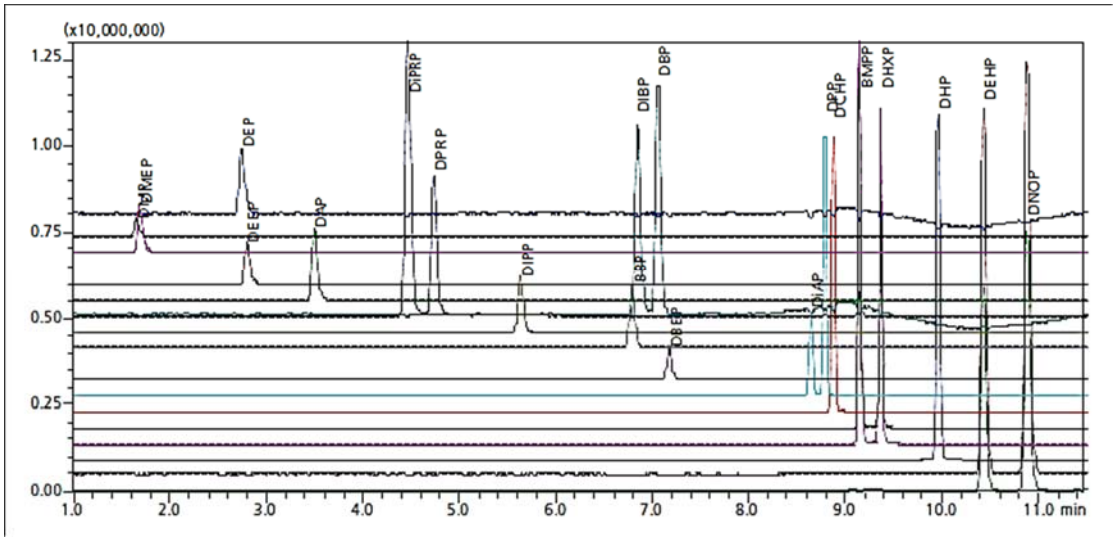


Figure 1. MRM chromatograms of 20 phthalic acid esters (200 µg/L)

20 phthalic acid esters were separated in 11 min. The MRM chromatograms in positive mode are shown in Figure 1. A linear relationship was found between peak area and different concentrations of 20 phthalate esters within 5, 10, 20, 50 and 200 µg/L. Correlation coefficients (R^2) more than 0.999, the limits of detection (LODs) and the limits of quantitation (LOQs) were obtained as shown in Table 1.

Table 1. The calibration curve, LOD and LOQ of 20 PAEs

Compound	Calibration curve	r	LOD (µg/L)	LOQ (µg/L)
DMP	Y = 12673.3X + 2300.05	0.9998	0.62	1.85
DMEP	Y = 26744.3X - 12057.1	0.9999	0.23	0.70
DEP	Y = 41199.3X + 60434.8	0.9998	1.67	5.00
DEEP	Y = 24761.6X - 25617.3	0.9999	0.18	0.56
DAP	Y = 43997.2X - 53600.0	0.9999	0.12	0.36
DiPRP	Y = 166476X - 70183.0	0.9999	0.05	0.15
DPRP	Y = 82151.0X - 14189.4	0.9999	0.09	0.26
DIPP	Y = 28688.5X - 89879.0	0.9990	0.16	0.47
BBP	Y = 36060.8X + 13664.7	0.9999	0.10	0.29
DIBP	Y = 105063X + 1597740	0.9999	1.63	4.94
DBP	Y = 131073X + 373016	0.9999	0.83	2.50
DBEP	Y = 16453.9X - 2429.68	0.9999	0.13	0.38
DiAP	Y = 39687.3X + 274520	0.9989	0.03	0.10
DPP	Y = 115069X + 108679	0.9999	0.02	0.06
DCHP	Y = 114309X + 233683	0.9999	0.02	0.05
BMPP	Y = 143127X + 451840	0.9996	0.02	0.05
DHXP	Y = 129006X + 132748	0.9999	0.02	0.06
DHP	Y = 128802X - 125656	0.9999	0.03	0.10
DEHP	Y = 164622X - 14942.3	0.9998	0.47	1.42
DNOP	Y = 120832X - 232093	0.9998	0.46	1.40

In this study, the repeatability of 20 phthalic acid esters at different concentrations (20, 50 and 100 µg/L) was investigated. The % RSDs of retention time were better than 0.26 % and % RSDs of peak area were less than 4.79 %, as shown in Table 2. The mixed standard solution was spiked into the blank alcoholic drink at the levels of 50 µg/kg and 100 µg/kg to evaluate the recovery of this method. A good recovery of 78 % to 127 % was obtained for each of the compound. The results are shown in Table 3. A 50 % alcohol distilled liquor made by Hunan-based liquor producer in China contained a maximum of 1.04 mg of DBP per kg. Three samples, including Chinese liquor, wine and whisky from the local market were chosen for analysis. The results showed that 154.1 µg/kg DBP and 18.7 µg/kg DIBP were detected in one of the Chinese liquor, and no detection was seen in other samples.

Table 2. Repeatability of peak area and RT at different concentrations (n=6)

Compound	%RSD (20 µg/L)		%RSD (50 µg/L)		%RSD (100 µg/L)	
	Area	R.T.	Area	R.T.	Area	R.T.
DMP	3.23	0.08	3.14	0.21	1.76	0.24
DMEP	0.73	0.08	0.71	0.19	0.74	0.20
DEP	4.79	0.17	3.90	0.18	2.76	0.24
DEEP	1.04	0.18	1.18	0.19	1.22	0.26
DAP	0.85	0.18	1.25	0.18	0.81	0.26
DiPRP	0.93	0.18	0.49	0.14	1.17	0.24
DPRP	0.61	0.17	0.87	0.13	0.91	0.22
DIPP	0.84	0.16	1.00	0.10	1.27	0.19
BBP	1.29	0.13	0.36	0.07	1.19	0.15
DIBP	3.55	0.12	3.34	0.07	1.82	0.15
DBP	4.79	0.11	1.50	0.07	1.40	0.14
DBEP	1.33	0.12	0.66	0.06	0.41	0.14
DiAP	1.56	0.05	1.44	0.02	1.10	0.05
DPP	1.30	0.09	0.26	0.09	1.74	0.07
DCHP	1.51	0.04	0.75	0.02	0.56	0.05
BMPP	1.35	0.04	0.83	0.02	1.23	0.03
DHXP	1.24	0.04	0.66	0.02	1.57	0.02
DHP	1.86	0.04	0.99	0.03	1.45	0.02
DEHP	0.59	0.06	0.42	0.04	1.65	0.04
DNOP	1.76	0.08	0.85	0.06	0.98	0.06

Table 3. Recovery of 20 PAEs in an alcoholic drink

No.	Compound	50 µg/kg		100 µg/kg	
		Measured value (µg/kg)	Recovery (%)	Measured value (µg/kg)	Recovery (%)
1	DMP	42	91	92	100
2	DMEP	48	104	101	110
3	DEP	45	97	101	110
4	DEEP	49	107	102	111
5	DAP	49	107	102	111
6	DiPRP	48	104	101	110
7	DPRP	48	105	105	114
8	DIPP	54	118	117	127
9	BBP	47	102	98	106
10	DIBP	45	99	98	106
11	DBP	48	104	101	110
12	DBEP	48	104	102	111
13	DiAP	36	78	77	84
14	DPP	46	99	91	99
15	DCHP	43	92	85	93
16	BMPP	45	99	91	99
17	DHXP	46	101	91	99
18	DHP	46	99	89	97
19	DEHP	46	100	97	105
20	DNOP	45	99	96	105

CONCLUSION

A UHPLC/MS/MS method has been developed for determination of 20 PAEs in alcoholic drinks. All of them were separated in 11 minutes, and analyzed in positive ESI mode. The calibration curves of 20 PAEs were constructed over a concentration range of 50-200 µg/L with correlation coefficients (r) more than 0.999. Good repeatability on both retention times and peak areas were obtained. The limits of detection (LODs) and the limits of quantitation (LOQs) for 20 PAEs were better than 2 µg/L, 5 µg/L respectively. The method was established for fast, reliable quantitative determination of 20 PAEs in alcoholic drinks.

O-6 SIMULTANEOUS ANALYSIS OF ALKYLPHENOL ETHOXYLATES USING ULTRA-HIGH SPEED LC/MS/MS

INTRODUCTION

Alkylphenol ethoxylates (APEs), kinds of non-ion surfactants, are used in the manufacture of textiles as detergent and dispersant in industry. The quantity of production of nonylphenol ethoxylates (NPEs), in which the carbon number of an alkyl group is 9 (refer Figure 1), has most as about 80 % in all APEs, and most of remaining is octylphenol ethoxylates (OPEs), the carbon number 8 (refer Figure 1). It is known that APEs can break down to alkyl phenol form by biodegradation. On the other hand, it has been becoming an environmental problem that the surfactant contained in wash drainage pollutes water so it is desired to measure these surfactants with sufficient accuracy. Here, we developed the simultaneous analysis method using LC/MS/MS of typical APEs.

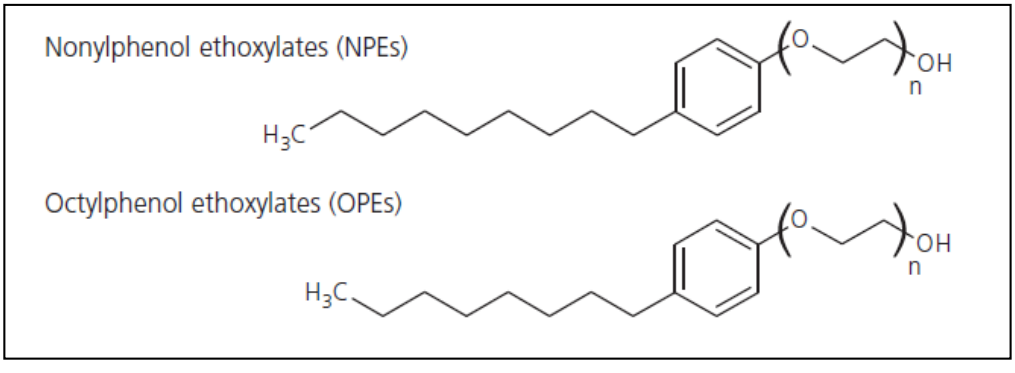


Figure 1. Structure of NPEs and OPEs

EXPERIMENTAL

The commercial reagents of APEs and the textile products were used for this experiment. Standards of APEs were diluted with methanol to suitable concentration and then loaded to LC/MS/MS. As an LC/MS/MS system, Nexera X2 UHPLC system was connected to LCMS-8030 triple quadrupole mass spectrometer. Separation occurred on a ODS column, where temperature was maintained at 40°C. Samples were eluted at flow rate 300 µL/min with a binary gradient system; the mobile phase consisted of (A) 10 mM ammonium acetate buffer and (B) acetonitrile. LC/MS/MS with

electrospray ionization (ESI) source was operated in multiple precursor ion scanning modes with ultra-high scanning speed in screening analysis for APEs and multiple-reaction-monitoring (MRM) mode for quantitative analysis.

Instrument parameters

System configuration

HPLC : UHPLC Nexera X2 system

MS : LCMS-8030 Triple quadrupole mass spectrometer

LC conditions

Column : Zorbax Eclipse XDB-C18 (150 mm L × 2.1 mm I.D.,5 µm) or Cadenza CD-C18 (150 mm L × 2 mm I.D., 3 µm)

Mobile phase : A - 10 mM ammonium acetate in water
B - Acetonitrile

Flow rate : 0.3 mL/min

Column temperature: 40 °C

Injection volume : 5 µL

Gradient program :

Time (min)	Pump B conc.
0.01	60
1.00	60
5.00	98
10.00	98
10.01	60
15.00	60

MS conditions

Ionization : Electrospray (ESI); positive (+4.5 kV)

Mode : MRM

MRM parameters : as tabulated below

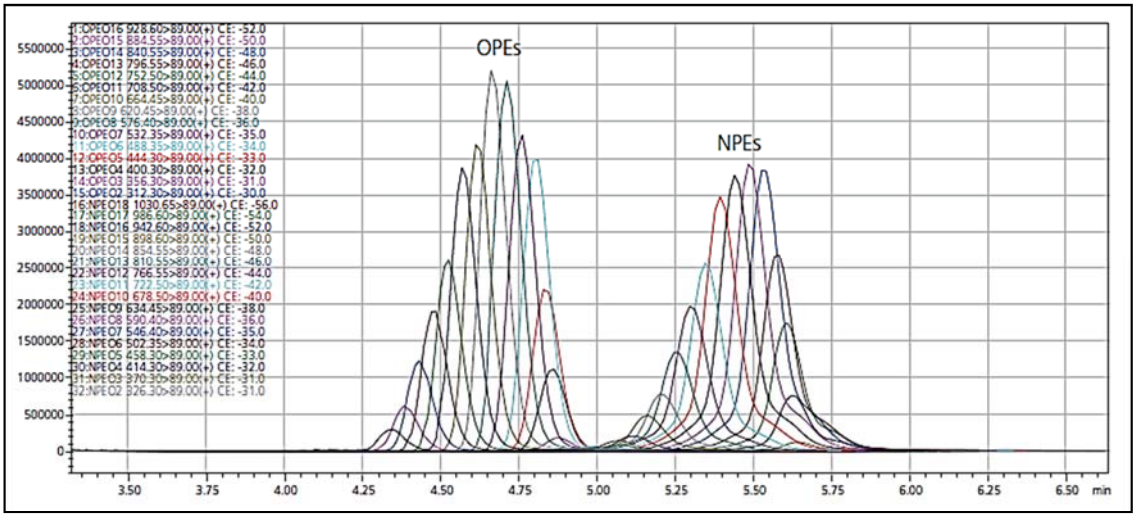
Compound	Q1 (u)	Q3 (u)	CE (V)	Compound	Q1 (u)	Q3 (u)	CE (V)
OPE 16	928.6	89.0	52	NPE 18	1030.7	89.0	56
OPE 15	884.6	89.0	50	NPE 17	986.6	89.0	54
OPE 14	840.6	89.0	48	NPE 16	942.6	89.0	52
OPE 13	796.6	89.0	46	NPE 15	898.6	89.0	50
OPE 12	752.5	89.0	44	NPE 14	854.6	89.0	48
OPE 11	708.5	89.0	42	NPE 13	810.6	89.0	46
OPE 10	664.5	89.0	40	NPE 12	766.6	89.0	44
OPE 9	620.5	89.0	38	NPE 11	722.5	89.0	42
OPE 8	576.4	89.0	36	NPE 10	678.5	89.0	40
OPE 7	532.4	89.0	35	NPE 9	634.5	89.0	38
OPE 6	488.4	89.0	34	NPE 8	590.4	89.0	36
OPE 5	444.3	89.0	33	NPE 7	546.4	89.0	35
OPE 4	400.3	89.0	32	NPE 6	502.4	89.0	34
OPE 3	356.3	89.0	31	NPE 5	458.3	89.0	33
				NPE 4	414.3	89.0	32
				NPE 3	370.3	89.0	31

RESULTS AND DISCUSSION

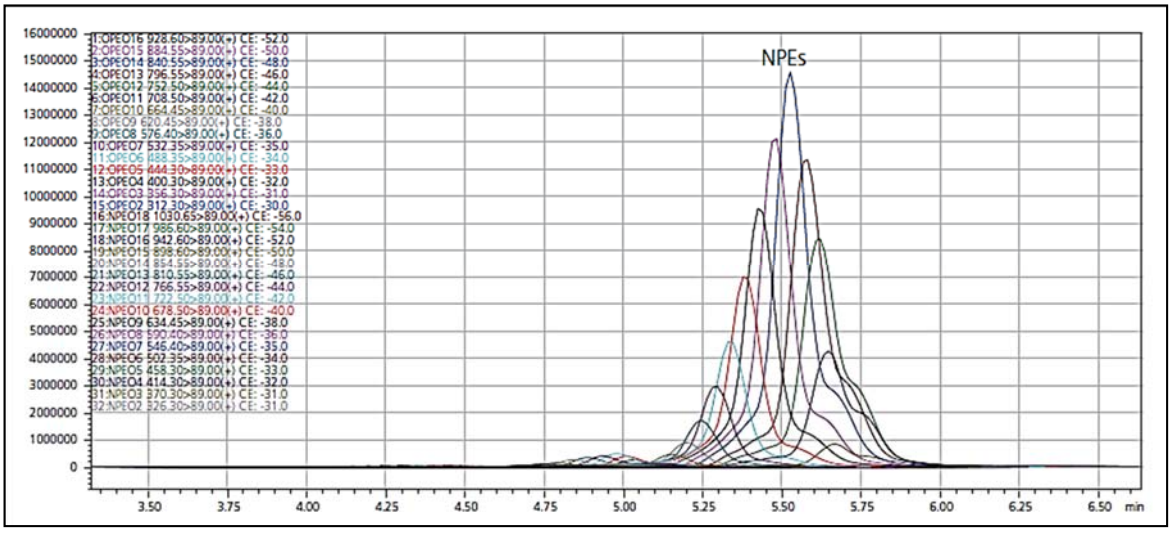
Quantitative method development for APEs

NPEs and OPEs as standard APEs reagents were analyzed in terms of being widely used. Flow injection analysis (FIA) with MS scanning mode was carried out for determination of the ionization polarity of compounds and subsequently FIA for MRM transition optimization were performed. As a result of that, all NPEs and OPEs were detected with their precursor ion, [M+H]+ in positive polarity and same fragment ion of *m/z* 89 was similarly obtained as optimal MRM transition for NPEs which number of added ethylene oxide (EO) is > 2, same fragment of *m/z* 89 for OPEs, EO > 2. OPEs were eluted first in the order as OPE 16, 15, 14,...,3 and then NPEs were eluted in the order as NPE 18, 17, 16,...,3. The dilution series of these compound standards were analyzed and all compounds were detected with excellent sensitivity and good linearity. In addition, the quantitative analysis of the textile products was carried out and some APEs were detected. It turned out that this method is effective in real samples. Typical chromatograms of APEs; standards and real sample are shown in Figure 2.

(a) Chromatograms of APEs standards



(b) chromatograms of a textile product



Screening method development for APEs

Furthermore, in order to conduct screening analysis of how many carbon numbers of an alkyl group in APEs, the method which performed the high-speed precursor ion scans (scan rate > 3000 u/sec) of *m/z* 89 for OPEs and NPEs, with multiple collision energy settings, was used (Figure 3). It is known that a precursor ion scan detected with high scanning speed using triple quadrupole mass spectrometer can show mass shift for higher *m/z;* however, in this analysis, the mass shift was not seen because of high-speed-correspondence in mass spectrometer. The chromatograms and mass spectra of APEs have been shown in Figure 4.

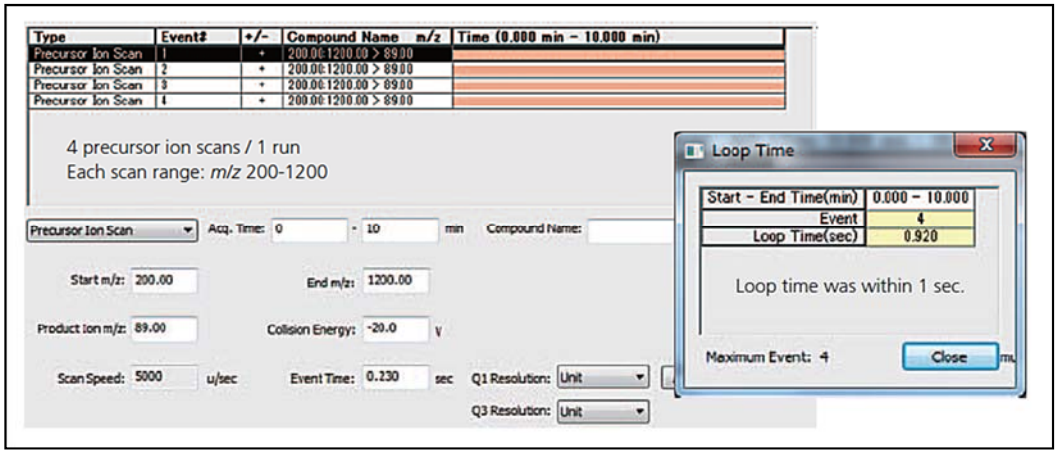


Figure 3. Screening method for APEs; 4 precursor ion scans; CE = 20, 30, 40, 50 V

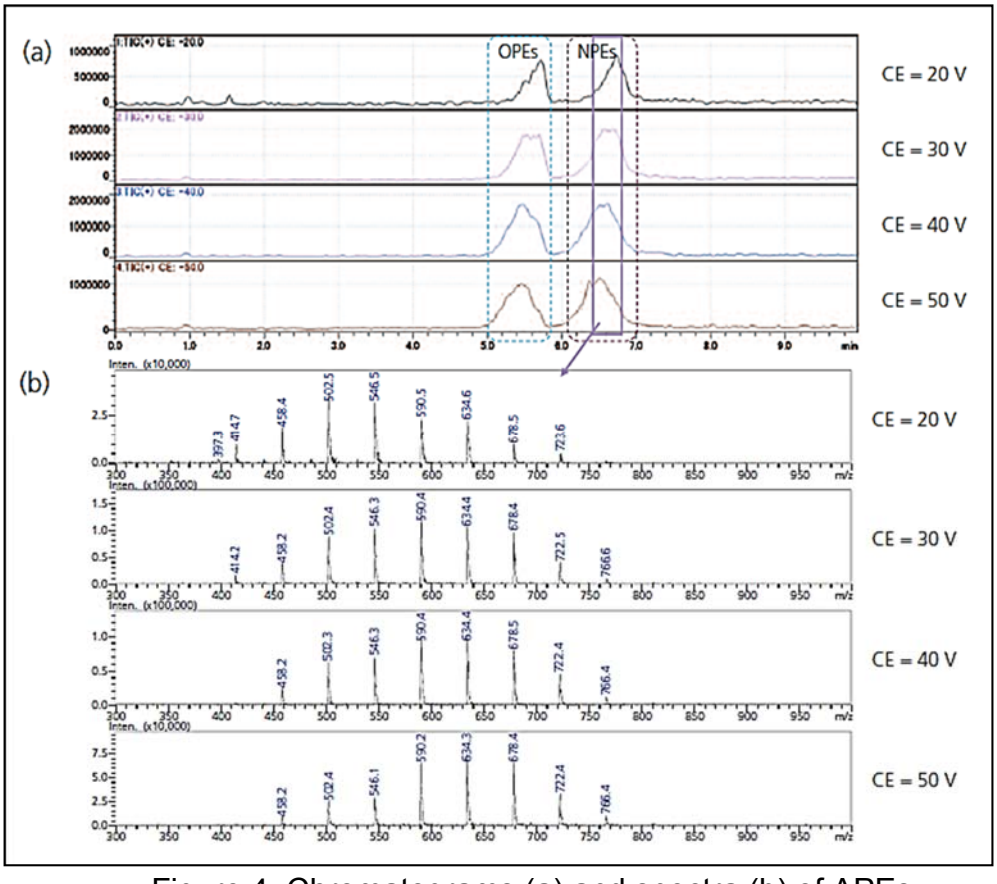


Figure 4. Chromatograms (a) and spectra (b) of APEs

CONCLUSION

Simultaneous analysis method of APEs using ultra-high speed scanning technique of LC/MS/MS was developed. MRM transitions for all APEs had common fragment ion (*m/z* 89). The LC/MS/MS method consisting of multiple precursor ion scan (for fragment ion *m/z* 89) with high scan speed was useful for screening of APEs.

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Excellence in Science

O-7 SIMULTANEOUS ANALYSIS OF CATIONIC, ANIONIC AND NEUTRAL SURFACTANTS FROM DIFFERENT MATRICES USING LC/MS/MS

INTRODUCTION

Surfactants

The term surfactant (surface active agent) designates a substance which exhibits some superficial or interfacial activity. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents and dispersants. In 2008, the worldwide production of synthetic surfactants was estimated to be as high as 13 million metric tons. Most of these surfactants after use, are sent to sewage treatment plants (STP). Here, (bio)degradation processes and/or adsorption on sludge particles remove these chemicals from waste waters to a greater or lesser extent, depending on the particular nature of the surfactant molecule. After treatment, the STP effluents are discharged into the environment. Some surfactants are known to be toxic to animals, ecosystems, humans and can increase the diffusion of other environmental contaminants. Some of these surfactants are also potential carcinogens. Despite this, they are routinely deposited in numerous ways on land and in water systems, whether as part of an intended process or as industrial and household waste and therefore, it becomes essential to monitor their levels in environmental effluents.

Basic structure and classification

Surfactants are usually organic compounds that are amphiphilic, meaning they contain both hydrophobic groups (their tails) and hydrophilic groups (their heads). Therefore, a surfactant molecule contains both, water insoluble (or oil soluble), component and a water soluble component. The hydrophobe is usually C8 to C18 hydrocarbon, and can be aliphatic, aromatic or a mixture of both. The hydrophilic groups give the primary classification to surfactants, making them anionic, cationic and nonionic in nature. Anionic surfactants dissociate in water to form an amphiphilic anion and a cation, which is in general an alkaline metal (Na+, K+) or a quaternary ammonium ion. Nonionic surfactants do not ionize in aqueous solution, because their hydrophilic group is of a non-dissociable type such as alcohol, phenol, ether, ester or amide. A large proportion of these nonionic surfactants are made hydrophilic by the presence of a polyethylene glycol chain, obtained by the poly-condensation of ethylene oxide. They are called poly-ethoxylated nonionic. Cationic surfactants are

dissociated in water into an amphiphilic cation and an anion, most often of the halogen type. A very large proportion of this class corresponds to nitrogen compounds such as fatty amine salts and quaternary ammoniums, with one or several long chain of the alkyl type, often coming from natural fatty acids. When a single surfactant molecule exhibits both anionic and cationic dissociations it is called amphoteric or zwitterion. In this paper, we have presented data for four surfactants namely Perfluoro-octanoic Acid (PFOA), Sodium Dodecyl Sulfate (SDS) both of which are anionic surfactants, Cetrimide which is cationic surfactant and Octylphenol Ethoxylates (OPEO) which is nonionic.

EXPERIMENTAL

Instrument parameters

HPLC : UHPLC Nexera X2 system
MS : LCMS-8040 Triple quadrupole mass spectrometer

LC conditions
Column : Shim-pack XR-ODS II (100 mm L × 3 mm I.D., 2.2 µm)
Mobile phase : A - 20 mM ammonium acetate in water B - Methanol
Flow rate : 0.45 mL/min
Column temperature: 55 °C
Injection volume : 5 µL
Gradient program :

Time (min)	Pump B conc.
0.01	75
4.00	100
5.00	75
7.00	75

MS conditions
Ionization : Electrospray (ESI); positive & negative
Mode : MRM
DL temperature : 250 °C
Heat block temperature : 400 °C
Nebulising gas : 3 L/min
Drying gas : 15 L/min
MRM parameters : as tabulated below

Surfactant	MRM transition	Polarity
PFOA	413 > 369	Negative
SDS	265 > 97	Negative
Cetrimide	256 > 60	Positive
OPEO	664 > 89	Positive

Standard preparation

A mixture of surfactants standards namely Cetrimide, Perfluorooctanoic Acid (PFOA), Sodium Dodecyl Sulfate (SDS) and Octylphenol Ethoxylates (OPEO) were prepared in methanol for calibration points ranging from 10 ppb to 1000 ppb.

Sample preparation

Tap water and sea water samples were collected from Marol area in Andheri and Juhu in Vile Parle respectively in Mumbai, Maharashtra, India. The tap water sample was spiked with the standard surfactant mixture to obtain a resultant concentration of 100 ppb. This sample was filtered through a 0.2 µm membrane filter and analyzed by LC/MS/MS. Similar treatment was given to sea water sample.

RESULTS AND DISCUSSION

The MRM transitions selected for surfactants are given in Table 1 which are based on the probable fragmentation pathway shown in Figure 2. No peak was seen in diluent (methanol) injection at the retention times of the surfactants for selected MRM transitions which confirms the absence of any interference from diluent (shown in Figure 3). Representative MRM chromatogram of 10 ppb standard surfactant mixture is shown in Figure 4. Linearity studies were carried out using external standard calibration method and the results of the same are shown in Table 2. For each concentration level % RSD was found to be within the acceptance criteria. The analytical methodology was tested on water samples from various sources. This exercise was aimed at screening surfactants from different water sources and recoveries were studied from spiked samples. Tap water and sea water were individually spiked with mix surfactant standards to get a final concentration of 100 ppb and subjected to LC/MS/MS. Recovery percentages for Cetrimide, SDS, OPEO and PFOA were found to be ranging between 50-120 % (shown in Table 3). The lower recoveries can be improved by applying extraction procedures to the samples. Blank and spiked sample chromatograms are shown in Figures 5-8.

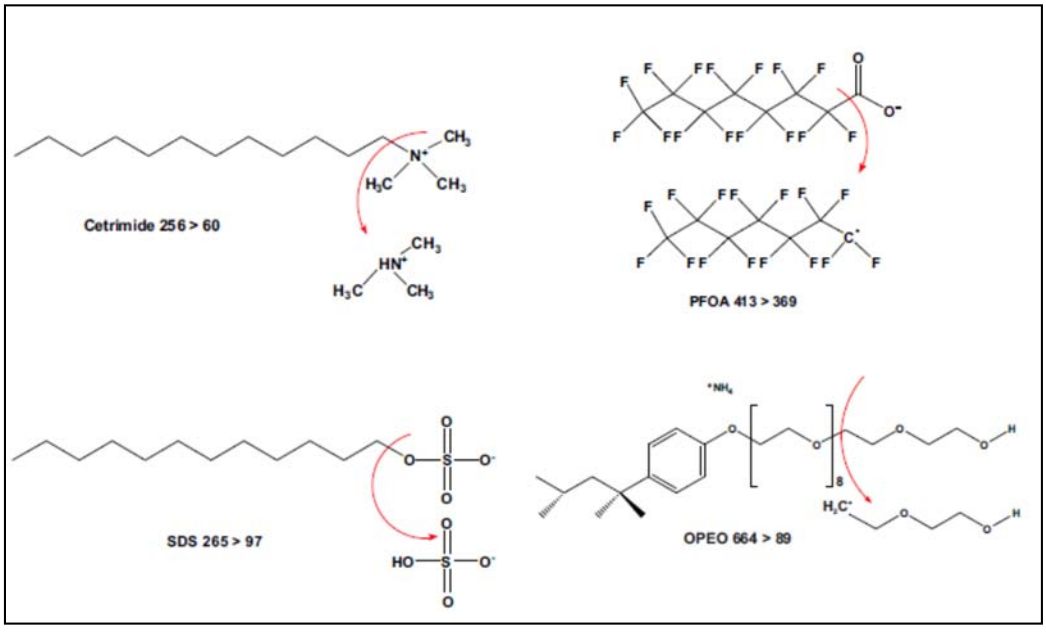

Figure 2. Probable fragmentation pathway of selected surfactants

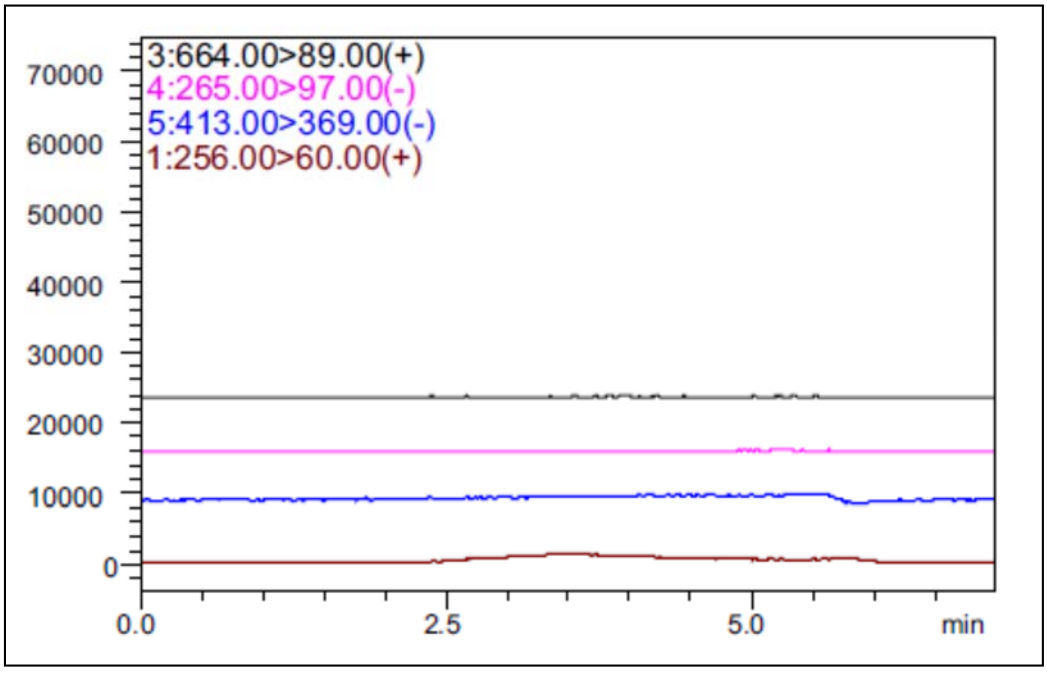

Figure 3. MRM chromatogram of diluents (methanol)

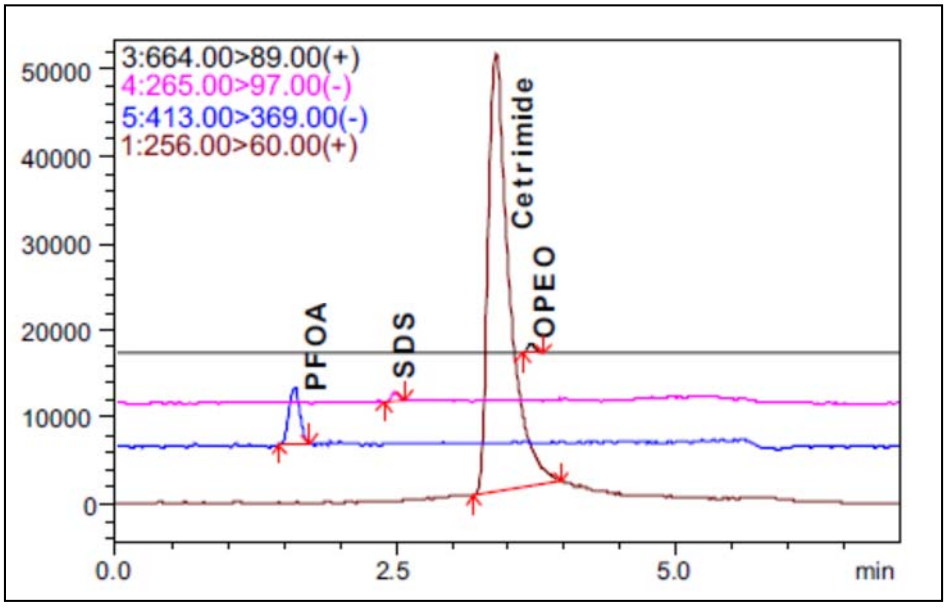

Figure 4. MRM chromatogram of 10ppb of standard surfactants mixture in methanol

Table 2. Calculated values of % RSD for retention time and area for at 100 ppb concentration

ID	Compound Name	% RSD (n=6)		Linearity	LOD (ppb)	LOQ (ppb)
		RT (min)	Area	(10 - 1000ppb)		
1	PFOA	0.21	3.41	0.9995	0.55	1.66
2	SDS	0.27	12.68	0.9998	1.63	4.95
3	Cetrimide	0.06	1.70	0.9999	0.04	0.12
4	OPEO	0.19	10.43	0.9999	0.30	0.90

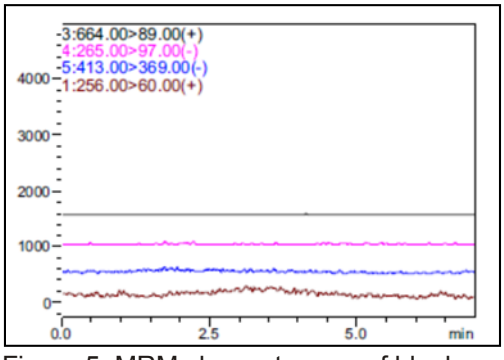

Figure 5. MRM chromatogram of blank tap water

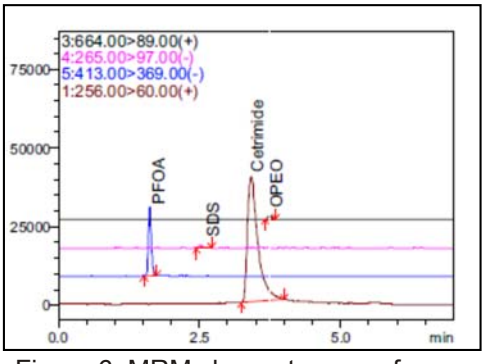

Figure 6. MRM chromatogram of spiked tap water

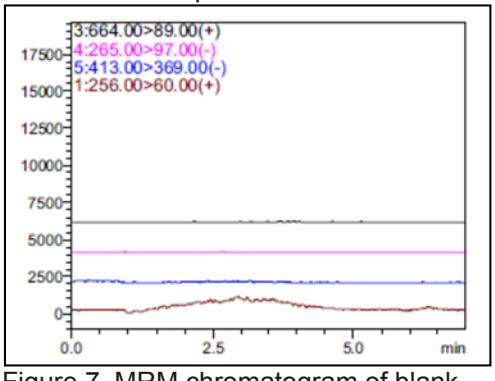

Figure 7. MRM chromatogram of blank sea water sample

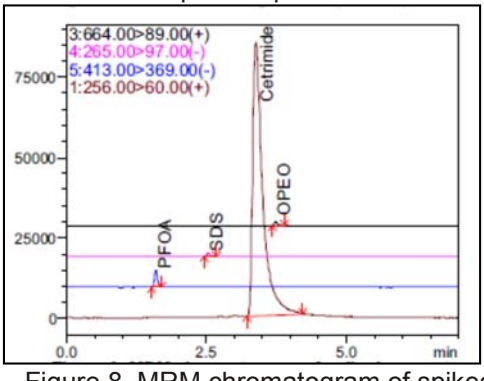

Figure 8. MRM chromatogram of spiked sea water sample

Results of the recovery studies

Surfactants	%Recovery	
	Sea water sample	Tap water sample
PFOA	77	124
SDS	86	114
Cetrimide	102	54
OPEO	73	71

CONCLUSION

The ultrafast polarity switching of 15msec exhibited by LCMS-8030 system along with its compatibility with UHPLC Nexera enabled simultaneous analysis of surfactants with different ionizing tendencies within short analysis time.

The analytical method discussed here can be extrapolated to real environmental samples for screening surfactant levels. This method can also be extended to monitor surfactant levels in consumer products.
Sensitivity of Nexera coupled with LCMS-8030 has facilitated quantitation of surfactants over the concentration range of 10 ppb to 1000 ppb with R2 values greater than 0.9995. Repeatability studies have shown that %RSD for area and retention times are within criteria.

O-8 CHARACTERIZATION OF FLAVONOIDS AND PHYTOESTROGENS IN AN EXTRACT OF PUERARIA MIRIFICA BY UHPLC/MS/MS

INTRODUCTION

Pueraria mirifica [Leguminosae] is a plant species native to Thailand whose tuberous roots (refer Figure 1) are used medicinally for their anti-ageing properties. *P. mirifica* is a source of several phytoestrogens and flavonoids such as deoxymiroestrol, puerarin, genistein, and many others. Renewed interest in the herb as well as higher standards of identity and purity demand accurate and precise methods for detecting the various compounds in P. mirifica extracts both qualitatively and quantitatively. UHPLC/MS/MS is an effective tool to characterize such extracts and accurately measure its key components. A selective and sensitive UHPLC/MS/MS method, meeting these requirements was developed.

Figure 1. Roots of *Pueraria Mirifica*

EXPERIMENTAL

Instrument parameters

HPLC : UHPLC Nexera system
MS : LCMS-8040 Triple quadrupole mass spectrometer

LC conditions
Column : Shim-pack XRODS III (50 mm L × 3 mm I.D., 2.2 µm)
Mobile phase : A - 0.1 % formic acid in water B - Acetonitrile
Flow rate : 0.5 mL/min
Column temperature: 50 °C
Injection volume : 5 µL

MS conditions
Ionization : ESI / APCI / DUIS (Dual Ion Source); positive & negative
Mode : MRM
DL temperature : 300 °C
Heat block temperature : 200 °C
Nebulising gas : 3 L/min
Drying gas : 15 L/min

RESULTS AND DISCUSSION

Table 1 shows calculated results for quantitative analysis of selected flavonoids in *Pueraria Mirifica.*

Table 1. Quantitative analysis of flavanoids in *Pueraria Mirifica*

	Ret. Time	Channel	Peak #	Area	Height	Conc (ng/mL)	
Puerarin	4.084	Ch1254nm	17	228007	90540	8250	*
Daidzin	4.427	Ch1254nm	19	55601	26514	2012	**
Genistin	4.847	Ch1254nm	23	16461	8938	335.6	***
Daidzein	5.526	Ch1254nm	28	23838	12704	486	*
Genistein	6.071	Ch1254nm	31	9341	4960	190	***
Deoxymiroestrol (total)	6.463	Ch1254nm	34	11470	4752	234	***

* Calculated by LCMS with external standards
** Calculated by puerarin relative UV response
*** Calculated by dadzein relative UV response

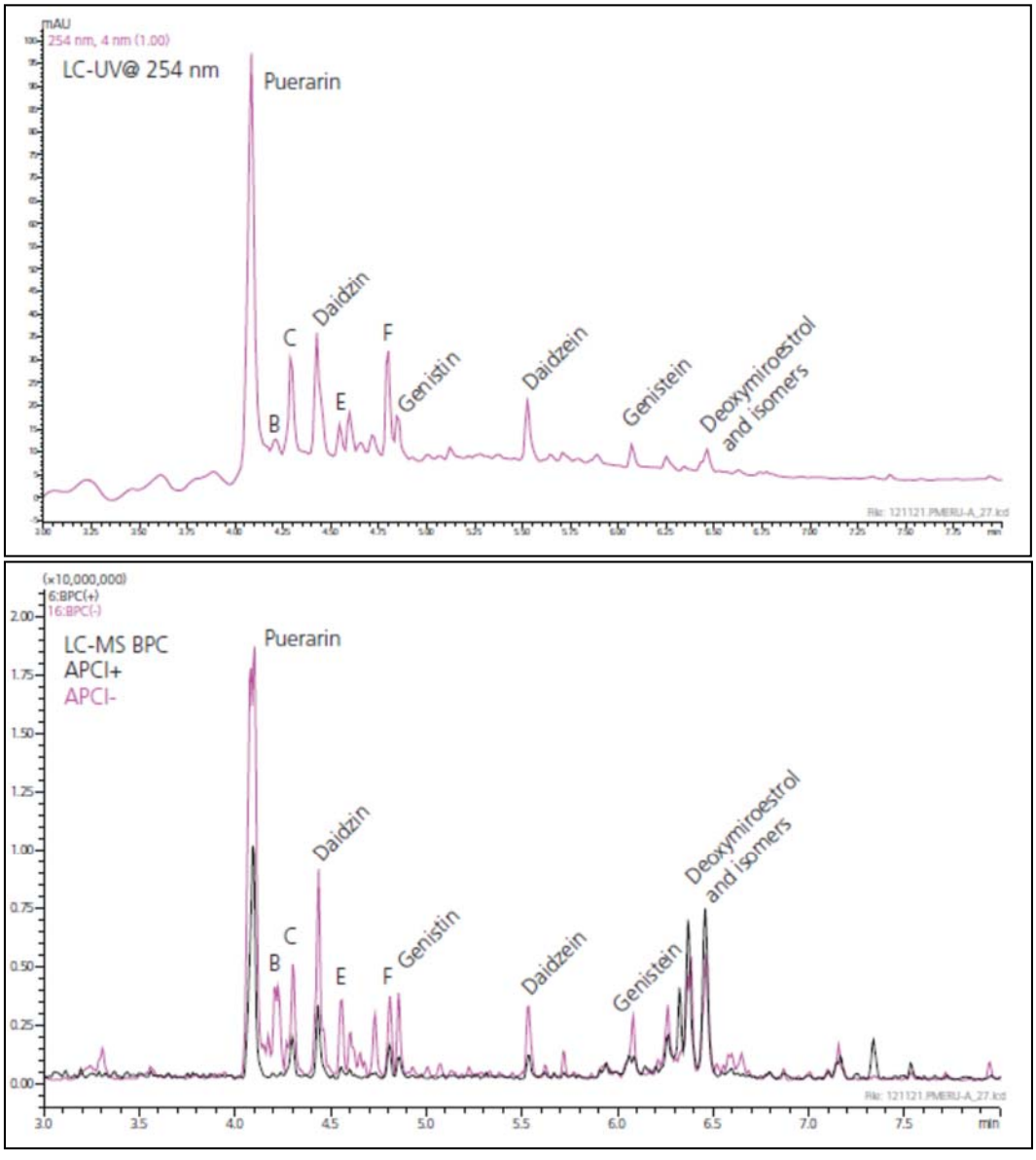

Figure 2. LC-UV chromatogram (top) and LC-MS base peak chromatogram (bottom) of an injection of the *P. mirifica* extract

MS measurement was carried out in several modes, including full scan, selected ion monitoring, multiple reaction monitoring, and product ion scan modes. Tandem mass spectra were compared with authentic standards or published spectra to propose identifications for each compound. In particular, the tandem mass spectra of O-linked and C-linked flavonoid

glycosides were examined and could be distinguished based on the fragmentation patterns of the glycoside ring. In addition, several isomers of

deoxymiroestrol were detected and identified based upon their similar fragmentation patterns.

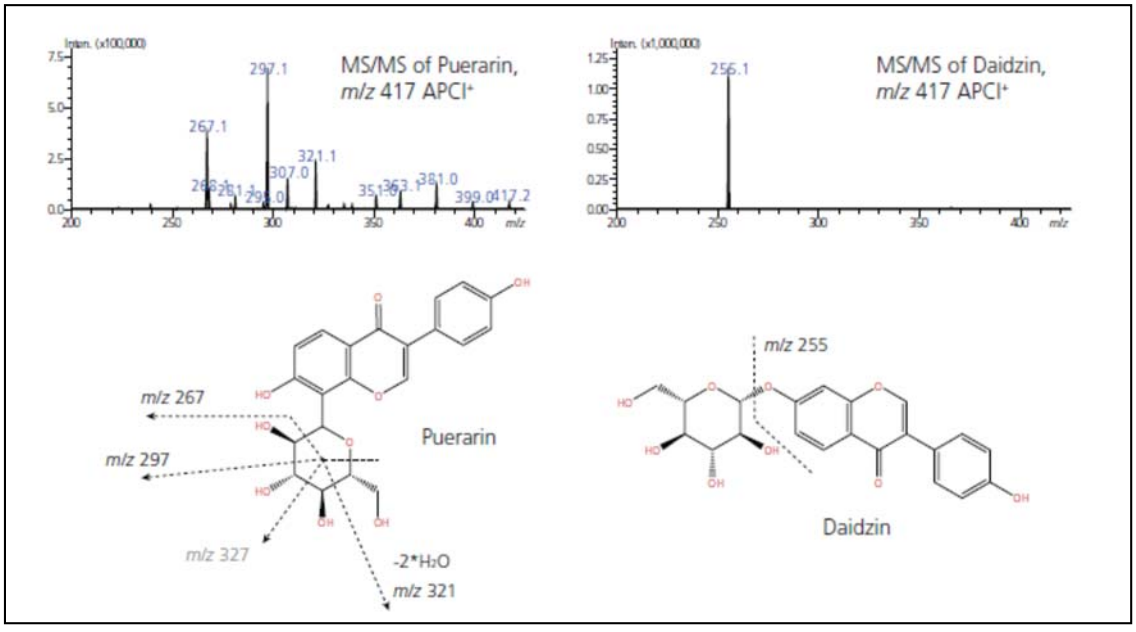

Figure 3. Tandem mass spectra of puerarin and the peak identified as daidzin in positive mode APCI (Top left and right, respectively).

Structure assignment for fragments of puerarin (bottom left) and daidzin (bottom right). The C-linked glycoside puerarin fragments differently than the O-linked glycoside daidzin.

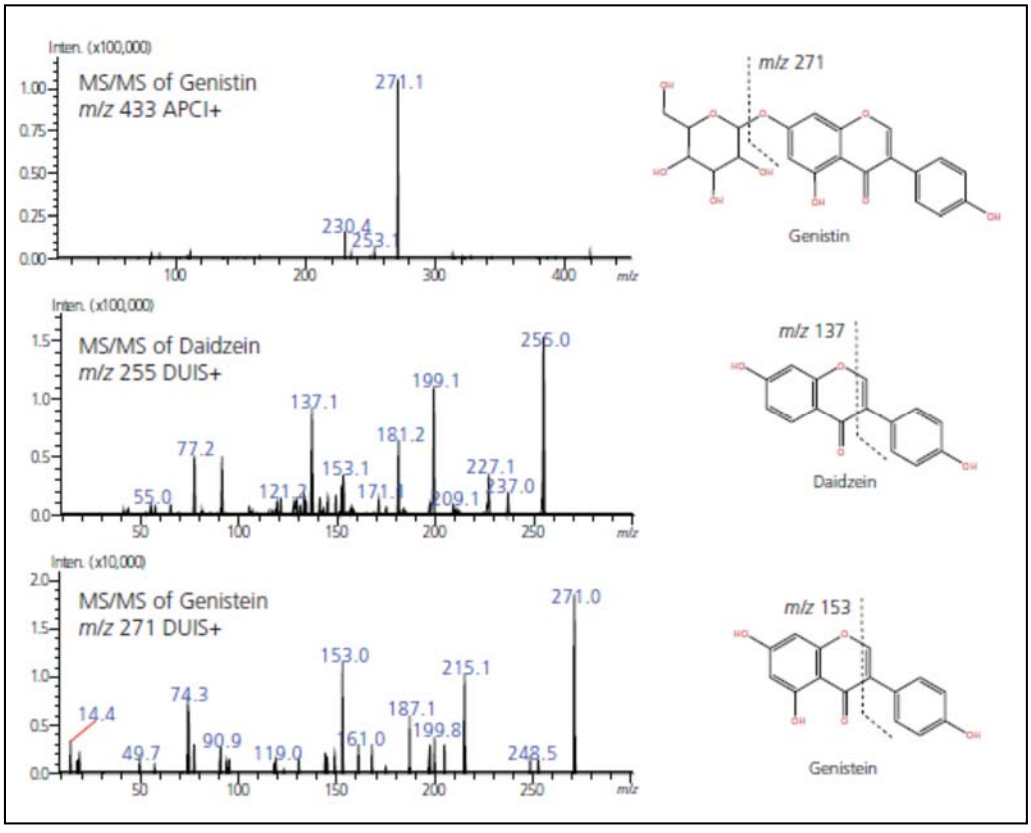

Fig. 4 Tandem mass spectrum and structure assignment of genistin, daidzein, and genistein.

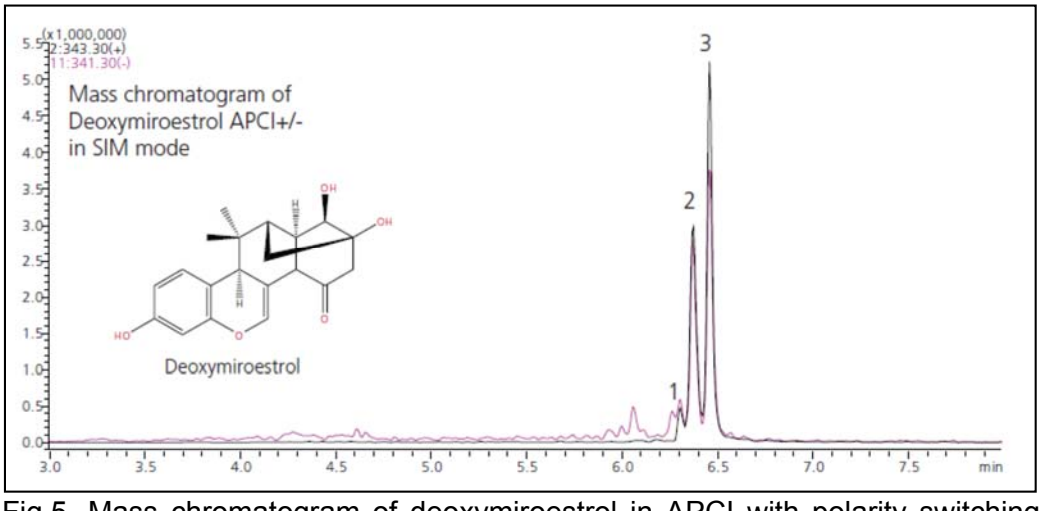

Fig.5. Mass chromatogram of deoxymiroestrol in APCI with polarity switching. Several isomers of deoxymiroestrol, labeled 1–3, can be clearly distinguished

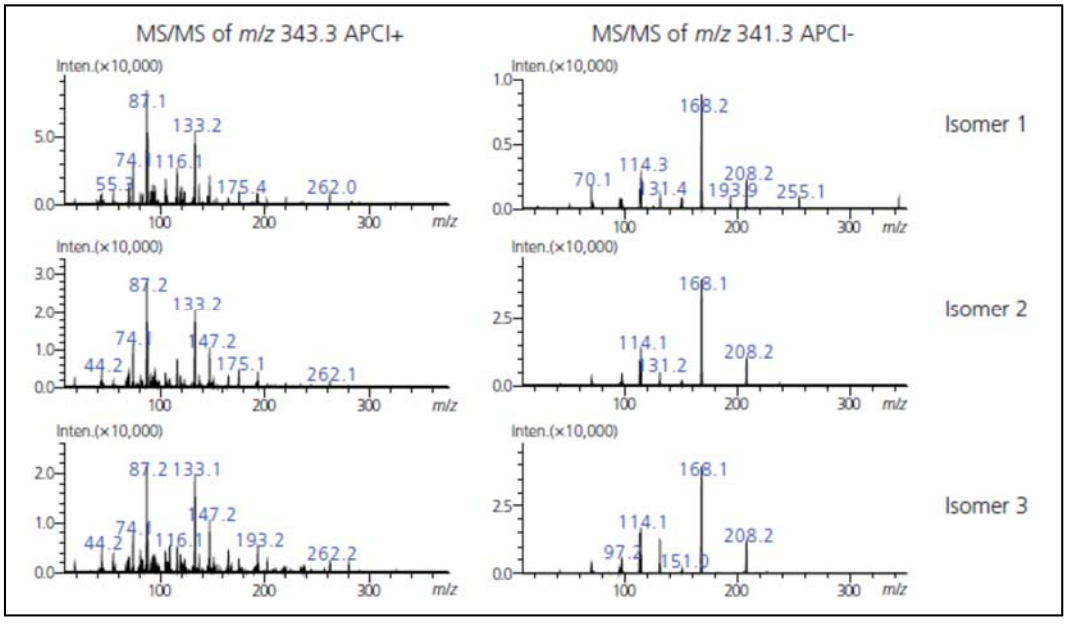

Fig. 6. Tandem mass spectra of three isomers of deoxymiroestrol. The left column are APCI+ spectra and the right column are APCI- spectra. Each row corresponds to one of the isomers of deoxymiroestrol.

CONCLUSION

UHPLC/MS/MS was used to characterize an extract of *P. mirifica*, simultaneously revealing several O- and C-linked glycosides as well as several newly detected isomers of deoxymiroestrol. The fast scan speed and rapid polarity switching of the LCMS-8040 triple quadrupole mass spectrometer allowed a full range of data to be acquired for each run with ESI and APCI ionization (DUIS mode), enabling comprehensive characterization of the extract.

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O-9 ANALYSIS OF MICROCYSTINS IN DRINKING WATER BY UHPLC/MS/MS

INTRODUCTION

Microcystins (MCs) are natural toxins produced by certain general cyanobacteria, which has a strong carcinogenic effect. MCs are seriously harmful to the residents, because they couldn't be degraded and removed. Therefore, the quantity of MCs in drinking-water is regulated by the WHO. This note employed a ultra-high performance liquid chromatography/electrospray ionization tandem mass spectrometry (UHPLC/MS/MS) method to determinate 10 MCs in drinking-water. The water samples were prepared without any pretreatment before determination. The method is simple, rapid and highly sensitive, which can meet the requirements for the analysis of MCs in drinking water.

EXPERIMENTAL

Sample preparation

Drinking water samples were directly filtered and MCs were determined using an UHPLC/MS/MS instrument.

Standards

Ten MCs (MC-RR, Dimethyl-RR, LR, LY, LW, LA, YR, WR, CF and NOD) were dissolved in water. MC standards were purchased from Enzo Life Sciences.

Instrument parameters

System configuration

HPLC	: Nexera UHPLC system
Pumping unit	: LC-30AD
Column oven	: CTO-30A
Degassing unit	: DGU-20A$_5$
Autosampler	: SIL-30AC
MS	: LCMS-8040

LC conditions

Column : Shim-pack XRODS III (50 mm L x 2.0 mm I.D., 1.6 µm)

Mobile phase : A – 0.1 % formic acid in water
B – 0.1 % formic acid in acetonitrile

Flow rate : 0.4 mL/min

Injection volume : 20 µL

Gradient program :

Time (min)	Pump B conc.
0.01	30
1.50	80
4.00	80
4.10	30
5.50	30

MS conditions

Interface : Electrospray ionization (ESI); positive

Mode : MRM

DL temperature : 250 ºC

Heat block temperature : 400 ºC

Nebulising gas : 3 L/min

Drying gas : 15 L/min

Dwell time : 50 msec

Pause time : 3 msec

MRM parameters : as tabulated below

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Q1 pre bias (V)	CE (V)	Q3 pre bias (V)
DE-RR	512.8	135.20*	-26.0	-34.0	-24.0
		103.05	-26.0	-55.0	-18.0
RR	519.8	135.20*	-26.0	-36.0	-23.0
		105.00	-26.0	-49.0	-19.0
NOD	825.35	135.20*	-32.0	-60.0	-25.0
		227.25	-32.0	-55.0	-24.0
YR	1045.45	135.20*	-32.0	-60.0	-25.0
		227.25	-32.0	-55.0	-24.0
LR	995.40	135.20*	-38.0	-65.0	-25.0
		213.10	-38.0	-64.0	-21.0
WR	1068.45	135.20*	-42.0	-65.0	-24.0
		213.10	-42.0	-64.0	-21.0
LA	910.35	135.20*	-34.0	-60.0	-24.0
		213.10	-34.0	-52.0	-22.0
LY	1002.40	135.20*	-38.0	-59.0	-24.0
		446.15	-38.0	-39.0	-30.0
LW	1025.40	135.20*	-40.0	-62.0	-25.0
		213.10	-40.0	-59.0	-22.0
LF	986.40	135.20*	-38.0	-60.0	-24.0
		375.20	-38.0	-40.0	-26.0

*: quantitative transition

RESULTS AND DISCUSSION

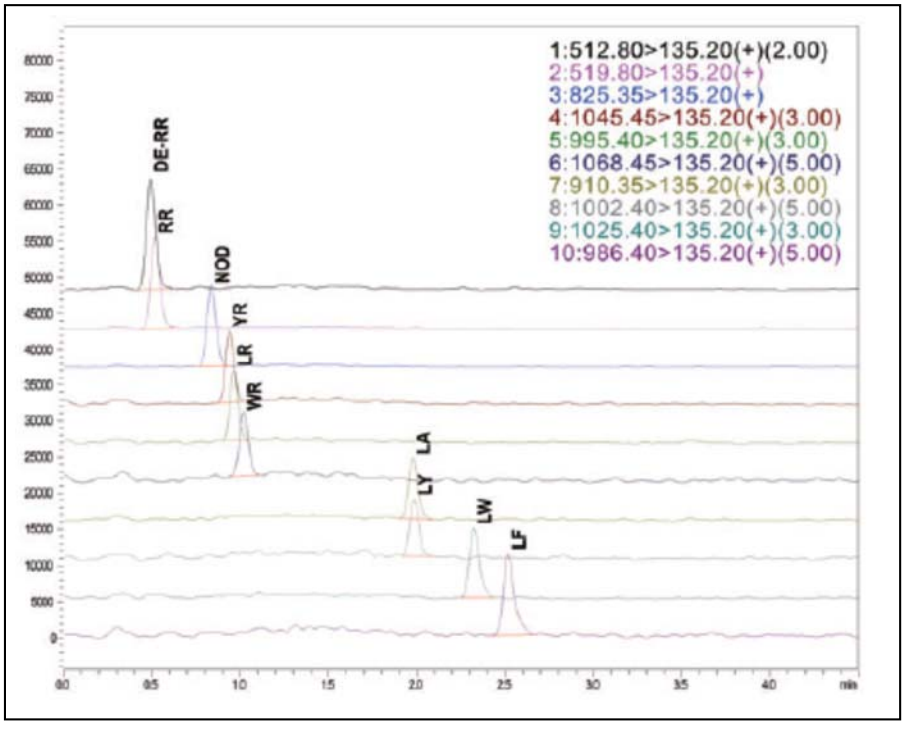

Figure 1. MRM chromatograms of 10 MCs (1 µg/L)

Ten microcystins were quickly seperated and analyzed in 5.5 min. The MRM chromatograms of 10 MCs in positive ion mode are shown in Figure 1. A linear relationship was found between peak area and different concentrations of 10 MCs within 0.02-50 µg/L. The calibration curves of 10 MCs were constructed with correlation coefficients (r^2) more than 0.999 as shown in Table 1.

Table 1. The calibration curve of 10 MCs

Compound	Calibration curve	r	Linear range (µg/L)
DE-RR	Y = 23620.7X - 713.367	0.9999	0.02-50
RR	Y = 7876.04X - 306.677	0.9999	0.02-50
NOD	Y = 2554.31X + 139.085	0.9999	0.1-50
LR	Y = 1130.75X + 0.304781	0.9999	0.1-50
YR	Y = 1045.52X - 179.194	0.9999	0.5-50
WR	Y = 583.910X - 77.2702	0.9999	0.5-50
LY	Y = 1555.45X - 106.796	0.9999	0.1-50
LA	Y = 1688.33X + 70.7246	0.9999	0.1-50
LW	Y = 2787.07X - 231.222	0.9999	0.1-50
LF	Y = 1767.59X + 165.616	0.9999	0.5-50

In this study, the repeatability of 10 MCs in different concentrations (1 and 10 µg/L) was investigated. The % RSD of retention time were from 0.077 to 0.369 and % RSD of peak area were from 0.843 to 9.672 (refer Table 2).

Table 2 Repeatability of 10 MCs in different concentrations (n=6)

Compound	%RSD (1 µg/L)		%RSD (10 µg/L)	
	Area	R.T.	Area	R.T.
DE-RR	1.599	0.369	0.843	0.183
RR	3.965	0.307	1.627	0.215
NOD	4.735	0.134	1.387	0.120
YR	4.882	0.262	4.548	0.147
LR	4.811	0.132	4.315	0.162
WR	9.672	0.196	4.947	0.156
LA	4.310	0.098	2.911	0.091
LY	2.810	0.145	1.055	0.125
LW	4.586	0.077	2.764	0.091
LF	4.417	0.245	4.987	0.079

The mixed standard sample was spiked into the blank water at levels of 0.02 µg/L or 0.1 µg/L to show the method LOQ. All the analyses were performed using above analytical conditions. The chromatograms of 10 MCs are shown in Figure 2. The results showed that there was no detection of MC'S in tap water samples.

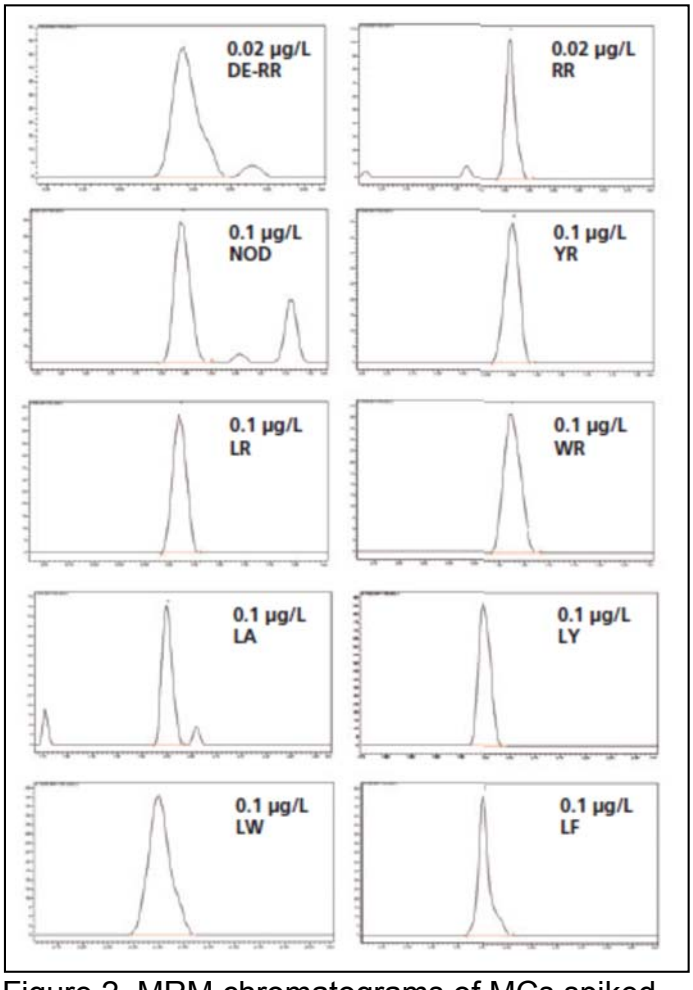

Figure 2. MRM chromatograms of MCs spiked in tap water at 0.02 µg/L or 0.1 µg/L

CONCLUSION

A UHPLC/MS/MS method has been developed for 10 MCs in water. All of the target compounds were separated in 5.5 minutes, and analyzed in ESI positive mode. The calibration curves of 10 MCs were constructed over a concentration range of 0.02-50 µg/L with correlation coefficients (r^2) more than 0.999. Good repeatability on both retention time and peak area was obtained. The limits of quantitation (LOQs) for 10 MCs were within 0.02-0.5 µg/L. A reliable method was established for fast quantitative determination of 10 MC's in water.

O-10 Determination of Cadmium Content in Rice with GFASS of Direct Suspension Sampling

INTRODUCTION

"Cadmium rice" has aroused widespread concern in the whole society after media exposure. Rice is one of the most common staple food sources in people's lives, so it is of great significant to determine the cadmium content in rice accurately and quickly.

In conventional detection needs, the rice samples should be digested before detection. Common digestion methods include wet digestion, dry digestion and microwave digestion. Even in the rapidest microwave digestion method, it will take no less than 3h in the whole digestion and evaporation process.

In this paper, a method was proposed for accurate determination of cadmium content in rice with GFASS of direct suspension sampling after the rice powder was suspended in agar solution. This method does not require digestion of samples, effectively saving sample pretreatment time and achieving the purpose of rapid and accurate analysis.

The comparision of different pretreatment methods is shown in following table.

Pretreatment method	Time consumption of digestion	Consumption of acids and other reagent
Suspension	Do not need digestion	A little agar solution and 1% HNO_3
Microwave digestion	30 min for digestion and 60 min for catching acid	5~10 mL nitric acid
Wet digestion	More than 1h for digestion and about 60 min for catching acid	Not less than 10 mL nitric acid
Dry digestion	About 6~8h	About 5 mL nitric acid

EXPERIMENTAL

Instrument : SHIMADZU AA-6300C

Sample
Rice standard: GBW10010 (Provided from China National Standardard Materials Center)

Pretreatment

Preparation of agar solution
0.12 g agar powder was accurately weighed, transferred to a 200 mL beaker, 100 mL water was added, heated on an electric hot plate to transparent solution, then supplemented to 100 mL, statically cooled down to room temperature and subject to surface film removal for standby application.

Preparation of suspension
0.25 g standard rice sample was accurately weighed, transferred to a 25 mL volumetric flask, brought to metered volume with 1.2 g/L agar solution, subject to shaking and vortex and then diluted 2.5 times with 1% nitric acid solution for determination. A blank group was prepared with the same method without standard rice sample. The details are shown in following pictures.

Agar Weighing agar Heating

Rice powder Adding agar solution Rice suspension

1%HNO_3 and rice suspension Diluting suspension Samples

Instrument Conditions and Parameters

The experiments were conducted with GFASS of direct suspension sampling. The apparatus optical parameters and graphite furnace temperature programming were shown in Table 2and Table 3.

Table 2. Optical Parameters

ELEMENT	CD
WAVELENGTH(NM)	228.8
LIGHTING MODE	BGC-D2
SLIT WIDTH(NM)	0.2
CURRENT(MA)	8

Table 3. Graphite Furnace Temperature Programming

	TEMPERATURE (ºC)	TIME (S)	HEATING METHOD	GAS FLOW
1	90	10	RAMP	0.10
2	90	10	STEP	1.00
3	150	5	RAMP	1.00
4	250	10	RAMP	0.10
5	500	10	RAMP	1.00
6	500	10	STEP	1.00
7	500	3	STEP	0.00
8	2200	2	STEP	0.00
9	2400	2	STEP	1.00

RESULTS AND DISCUSSION

Standard solutions at concentrations of 0.0, 0.4, 0.8 and 1.2 μg/L were prepared. The standard curve was plotted with absorbance as ordinate and concentration as abscissa, as shown in Figure 1, in which, the injection volume was 10 μL and 5 μL palladium nitrate at the concentration of 100 mg/L was added as the matrix modifier. Within the standard curve concentration range, there was a good linear relation between absorbance of Cd and concentration, with correlation coefficient r=0. 9995.

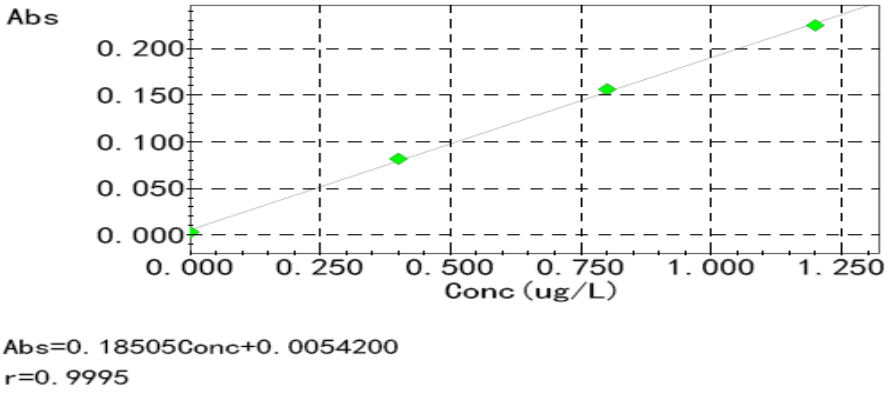

Fig. 1 Calibration curve of Cd

Cd content in the standard samples of rice was calculated with standard addition method and RSDs of replicate rejections were investigated. The specific results were shown in Table 4.

Table 4 Determination Results

Element	Detection content (ng/mL)	Sample weight (g)	Constant volume (mL)	Dilution ratio	Actual sample conc. (ng/g)	Reference conc. (ng/g)	RSD (%)
Cd	0.3369	0.2560	25	2.5	82.25	87±5	3.04

Note

Agar powder was insoluble in water at room temperature and should be heated and dissolved;

Agar solution concentration greatly affected the stability of the suspension system. The suspension system formed was easy to stratify at low concentration;

The temperature was kept at 90~95 ºC for 10~20 s at the desolvation stage to prevent sample bumping

CONCLUSION

GBW10010 rice samples were suspended in 1.2 g/L agar solution, and the cadmium content in rice was detected with GFASS of direct suspension sampling. This method saved a lot of pretreatment time and could effectively improve the analysis speed compared with sample digestion method before analysis

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O-11 Determination of Se in tea leaves by HVG-ICP-AES

INTRODUCTION

A method for determination of Se using ICP-AES and HVG-1 is established. HVG-1(Hydride Vapor Generator) is a gas-generator accessory for ICP-AES, which can increase the sensitivity of ICP-AES for about 1-2 orders of magnitude. Usually the gas generated from the HVG-1 is brought into the ICP with Argon and the waste is discharged with a peristaltic pump. As for the injection speed can be controlled more convenient, the sensitivity is better with fast injection speed. Under the optimum conditions, the limit of detection of Se in tea leaves base is 0.005 μg /L. The recoveries for Se determination range from 94% to 98%. With high accuracy and good precision, this method is suitable for trace determination of Se.

EXPERIMENTAL

Instrument : ICPE-9000 coupled with HVG-1 (Shimadzu)

Sample : A green tea sample and a black tea sample

Sample Pretreatment:

0.5 g samples were weighed accurately, and transferred into 50 mL tanks of PTFE, and 5 mL HNO_3 was added, and then heated in microwave digestion system at 180 ºC ,15 min for digestion. After the process, till the tanks cooling to room temperature, the samples were transferred to glass beakers respectively. 1.0 mL $HCLO_4$ was added, and then heated the beakers on a hot plate at 190 ºC until white smoke emerged.After cooling, sample solutions were transferred to 25 mL flasks with 5% HNO_3, and then set up to the scale using 5% HNO_3 respectively and use these as the analytical samples.

Calibration Curve Sample:

Prepare a standard solution (1000 mg/L) for use in atomic absorption analysis, and appropriately dilute with ultra pure water. Each standard solution made in 5% HNO_3 base solutions, similar to sample solutions.

Analysis

Using the ICPE-9000 coupled with HVG-1, we conducted quantitation of the analytical samples of black tea and green tea leaves.

Table 1. Analytical Conditions

INSTRUMENT	ICPE-9000
RADIO FREQUENCY POWER	1.2(KW)
PLASMA GAS	10(L/MIN)
AUXILIARY GAS	0.6(L/MIN)
CARRIER GAS	0.7(L/MIN)
SAMPLE INTRODUCTION	HVG GAS INJECTION
SAMPLE ASPIRATION	1.0(L/MIN)
MISTING CHAMBER	CYCLONE CHAMBER
PLASMA TORCH	MINI TORCH
VIEW DIRECTION	AXIAL

RESULTS AND DISCUSSION

The measurement results and the recoveries of green tea and black tea leaves are shown in Table2 and Table3. It shows that measurement results and the recoveries are all fine.

Selenium in tea leaves was determinate by ICP-AES and Hydride Vapor Generator coupling system. The tea leaves contained large amount of salt normally, such as phosphate and sulfate and so on, usually the complex matrix of samples can produce negative influence to measuring. But the hydride generation method can effectively avoid the interference caused by complex matrices, due to its gas injection essence.

Fig. 1 shows the ICP-HVG system. Fig. 2 shows the spectral profiles, and Fig. 3 shows the calibration curves.

The ICPE-9000 used for measurement is equipped with an echelle spectrometer and CCD detector, making it possible to conduct simultaneous analysis of all elements at all wavelengths, thereby permitting high throughput measurement even for multiple analysis. Further, compared to the conventional torch, the mini torch offers lower consumption of argon gas, thereby reducing running costs. In ICP, the optimal wavelength varies depending on the elements and concentrations in the sample matrix, but with the ICPE-9000, the optimal wavelength is automatically selected for each sample.

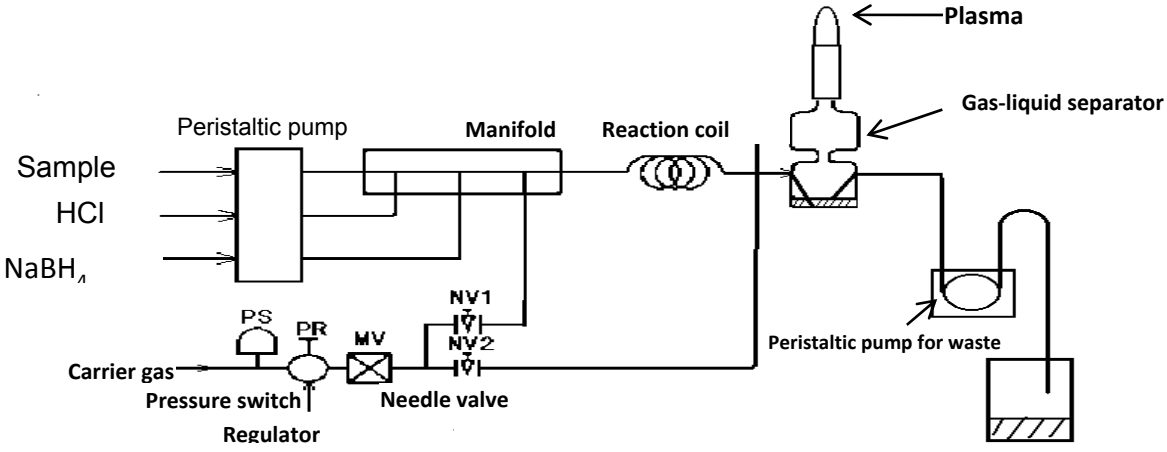

Figure 1. The ICP-HVG System

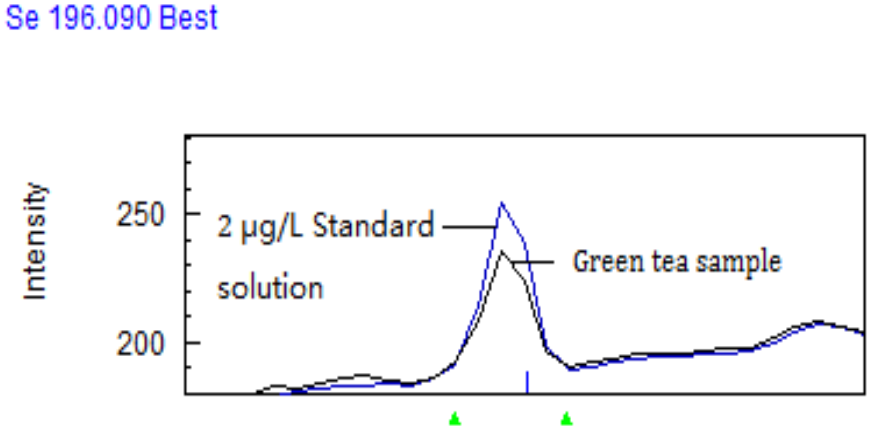

Figure 2. Spectral Profiles

Table 2. Quantitative Results of tea leaves samples

ELEMENT	BLACK TEA(MG/KG)		GREENTEA(MG/KG)	
	MEASURED	TOTAL IN SAMPLE	MEASURED	TOTAL IN SAMPLE
SE	0.97	51.68	1.27	62.93

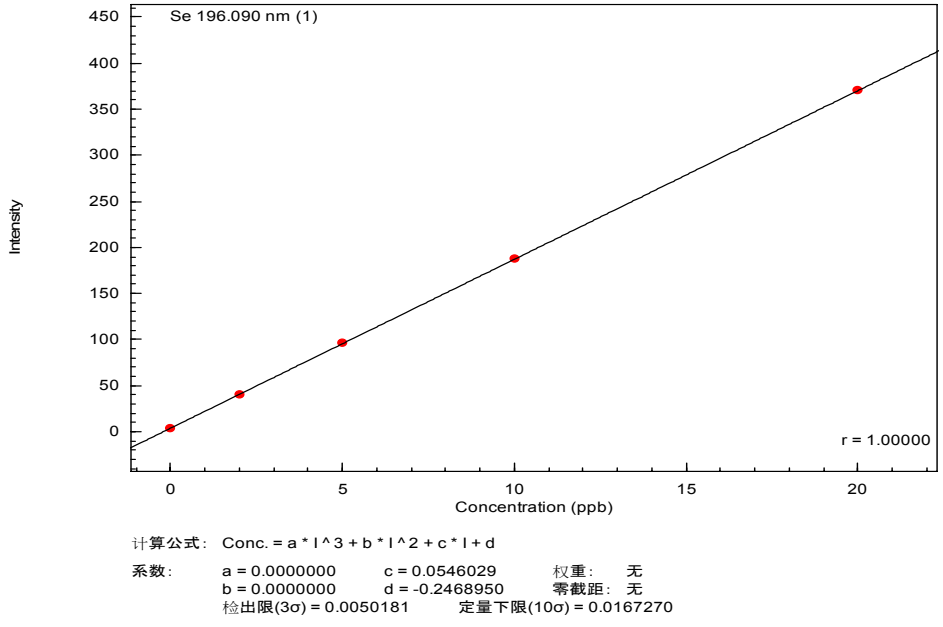

Figure 3. Calibration Curves

Table 3.Spiked samples and recovery results

Sample	Before Spiked(μg /L)	Added amount (μg /L)	After Spiked (μg /L)	Recovery (%)
Black Tea	0.97	4.0	4.92	98.7
Green Tea	1.27	4.0	5.06	94.7

CONCLUSION

This application introduces the analysis the element of Se in tea leaves using the ICPE-9000 coupled with HVG-1. The ICPE-9000 used for measurement is equipped with an Echelle spectrometer and CCD detector, making it possible to conduct simultaneous analysis of all elements at all wavelengths, thereby permitting high throughput measurement even for multiple analytes. The hydride generation method can effectively avoid the interference caused by complex matrices, due to its gas injection essence. The results show that the recoveries for Se determination range from 94% to 98%. With high accuracy and good precision, this method is suitable for trace determination of Se.

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O-12 Measurement of Arsenic and Mercury Levels in Concentrated Juice

INTRODUCTION

This article used the Imported and Exported Fruit Juice (Puree) Test Regulations (SN/T 2803-2011), analysis method for Arsenic, Cadmium, Lead, Selenium, and Zinc in Food Products (AOAC 986.15), and Measurement of Total Mercury and Organic Mercury in Food Products (GB/T 5009.17-2003) as reference. After the samples were subjected to wet treatment, the arsenic and mercury levels in concentrated juice were determined by Hydride Generation Atomic Absorption Spectrometry (HGAAS). The experimental results show that the recovery rate of arsenic was 100.9 % and its linear correlation coefficient 0.9995, and the recovery rate of mercury was 90.6 % and its linear correlation coefficient 0.9991. This method is easy to perform, is highly accurate, and meets the requirements for quick measurement.

Arsenic and mercury are heavy metal pollutants that exhibit high biotoxicity in the environment, and are, under normal conditions, present in animals and plants at trace levels. Trace amounts of arsenic and mercury are, therefore, present in our food products. With the development of industry and agriculture, extensively used pesticides, herbicides, and insecticides have become potential hazards. China is a major exporting country of concentrated juice, which is primarily exported to the U.S. and Europe. Frequent food safety accidents in recent years have caused economic and reputation losses to China's export trade. The Hygiene Standard for Fruit and Vegetable Juice (GB 19297-2003) of China requires that total arsenic level shall not exceed 0.2 mg/L, and the European Fruit Juice Association requires that arsenic level shall not exceed 0.1 mg/kg and mercury level not exceed 0.01 mg/kg in apple juice.

In this article, arsenic and mercury levels in concentrated juice were determined using the methods specified in Arsenic, Cadmium, Lead, Selenium, and Zinc in Food Products (AOAC 986.15), and Measurement of Total Mercury and Organic Mercury in Food Products (GB/T 5009.17-2003).

EXPERIMENTAL

Instruments

AA-7000 (Shimadzu)

HVG-1 hydride vapor generator

Reagents

- Deionized water
- Experimental vessels soaked in a nitric acid solution for 24 hours
- 1000 μg/mL arsenic and mercury standard stock solutions
- 100 ng/mL arsenic and mercury standard solutions

Instrumental Parameters

See Table 1 for the analysis parameters.

Table 1 Experimental Conditions

Element	Wavelength (nm)	Lamp Current (mA)	Analysis Method	Mode of Background Deduction
As	193.7	12	HGAAS	BGC-D_2
Hg	253.7	10	HGAAS	BGC-D2

Element	Flame Type	Acetylene Flowrate (L/min)	Air Flowrate (L/min)
As	Air-acetylene	2.0	15.0
Hg	No flame	–	–

Sample Pretreatment

After shaking up and treating the sample, 1.0 g of the sample was weighed out and transferred into a 100 mL glass beaker. Then 15 mL of concentrated nitric acid and 3 mL of perchloric acid were added, and the solution was heated slowly on a plate heater while ensuring that the liquid did not boil away and its temperature did not exceed 160 °C. During the heating process, 10 mL of concentrated nitric acid was added. When the volume of the sample solution in the beaker evaporated down to approximately 3 mL, the beaker was removed and allowed to cool down to room temperature. The sample was then treated, transferred to a 10 mL volumetric flask, brought up to the mark using a nitric acid solution (1%)

and shaken, after which it was ready for measuring. The same method was used for the preparation of the blank solution.

To ensure matrix matching, perchloric acid with a concentration of 30 % was used in the series of standard samples.

RESULTS AND DISCUSSION

Linear Equation and Detection Limits

The rotating speed of the hydride vapor generator and the pressure of the sample injection tube were adjusted, and the sample was introduced after the flowrate had stabilized.

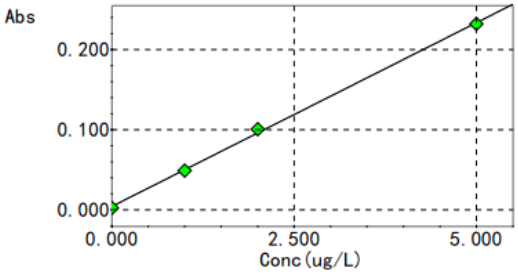

Fig. 1 Linear Equation for Arsenic Measurement

The linear equation of the calibration curve in Fig. 1 shows that the concentration of arsenic was 0.0042214 μg/L (Abs = 0.045864) and the correlation coefficient R^2 was 0.9995. The blank solution was measured 10 times according to the experimental method, and the detection limit was calculated by dividing 3 times the standard deviation by the slope of the curve. The detection limit of arsenic was 0.11 μg/L.

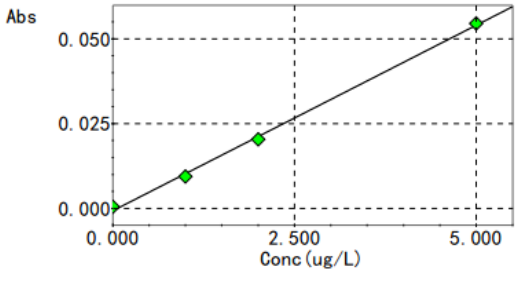

Fig. 2 Linear Equation for Mercury Measurement

The linear equation of the calibration curve in Fig. 2 shows that the concentration of mercury was 0.00067143 μg/L (Abs = 0.010936) and the correlation coefficient R^2 was 0.9991. The blank solution was measured 10 times according to the experimental method, and the detection limit was calculated by dividing 3 times the standard deviation by the slope of the curve. The detection limit of mercury was 0.08 μg/L.

Results of Sample Measurement

Table 2 Juice Analysis Results

Item	As		Hg	
	As-1	As-2	Hg-1	Hg-2
Measured value (μg/L)	0.798	0.757	0.208	0.193
Sample weight (g)	0.9884	0.9948	0.9991	0.9979
Metered volume (mL)	10	10	10	10
Dilution factor	5	5	1	1
Sample level (mg/kg)	0.040	0.038	0.0021	0.0019

Table 3 Recovery Test

Sample	Before Addition (μg/L)	Amount Added (μg/L)	After Addition (μg/L)	Recovery Rate (%)
As-2	0.757	1.00	1.772	100.9
Hg-1	0.208	1.00	1.094	90.6

CONCLUSION

In this article, the arsenic and mercury levels in concentrated juice were determined by HGAAS. This method is easy to perform, is highly accurate and precise, and can fully meet the requirements for measuring arsenic and mercury in concentrated juices.

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O-13 Determination of Rare Earth Elements in Tea by ICP-AES with Ultrasonic Aerosol Generator

INTRODUCTION

With the food quality and security being paid more attention, food quality is also being managed more and more strictly. Chinese National Standard for Contaminants Limitation in Food (GB 2762-2012) was released in 2012. The standard set the maximum allowable amounts of total amount of oxides of rare earth elements in tea leaves (<2.0 mg/kg). A method for determination of rare earth elements using ICP-AES with UAG-1 is established. UAG-1(Ultrasonic Aerosol Generator) is an accessory for ICP-AES, which can increase the sensitivity of ICP-AES for more than 3 times. With this method, tea leaves samples were digested with HNO_3-$HClO_4$ mixed system by wet digestion. Standard addition method was applied. Accuracy, precision, limits of detection, recovery of the method for rare earth elements were evaluated.

EXPERIMENTAL

Standard reference material (GBW10016) was purchased from National Institute of Metrology. The system is shown in Fig 1.

Fig. 1 ICPE-9000 and UAG-1

Sample pretreatment

A standard reference material (GBW10016), a green tea sample and a Pu'er tea sample were measured.1.0 g samples were weighed accurately, and transferred into 100 mL backers, and 10 mL HNO_3 and 2 mL $HClO_4$ were added, and then heated on a hot plate at 190 ºC for digestion. The samples were evaporated to nearly dry. After cooling, sample solutions were transferred to 25 mL flasks with 5% HNO_3, and then set up to the scale using 5% HNO_3.

Table 1 Analytical conditions

Instrument	**ICPE-9000 with UAG-1**
Radio Frequency Power	**1.0 kW**
Plasma Gas	**14 L/min**
Auxiliary Gas	**1.2 L/min**
Carrier Gas	**0.6 L/min**
Sample Introduction	**Ultrasonic Aerosol Generator**
Misting Chamber	**Cyclone Chamber**
Plasma Torch	**Standard Torch**
View Direction	**Axial**

RESULTS AND DISCUSSION

Optimum wavelengths and detection limit

Table 2 Wavelengths and detection limit

Element	Wavelength (nm)	Detection Limit (mg/L)
Ce	413.380	0.0022
Dy	387.212	0.0028
Er	349.910	0.0018
Eu	381.967	0.00034
Gd	342.247	0.0019
Ho	345.600	0.00040
La	408.672	0.0014
Lu	350.739	0.00091
Nd	406.109	0.0035
Pr	440.884	0.0043
Sm	460.949	0.0016
Tb	350.917	0.0011
Tm	346.220	0.00079
Y	360.073	0.00053
Yb	328.937	0.00011

Measurement results

The results of the standard material GBW10016 (tea leaves) are shown in Table 3, quantitation results of Pu'er tea and green tea samples are shown in Table 4. Results of spike test of green tea sample are shown in Table 5.

Table 3 Quantitation results of GBW10016

Element	GBW10016 Certified Value	GBW10016 Quantitation Results	Unit	RSD (%)
Ce	0.39±0.05	0.39	μg/g	6.51
La	0.25±0.02	0.248	μg/g	1.95
Nd	0.15±0.02	0.15	μg/g	6.41
Y	0.23±0.03	0.208	μg/g	0.52
Yb	0.018±0.004	0.02	μg/g	2.88

Table 4 Quantitation Results of tea leaves samples

ELEMENT	PU'ER TEA (MG/G)	RSD (%)	GREEN TEA (MG/G)	RSD (%)
CE	1.865	2.66	N.D	-
DY	N.D	-	N.D	-
ER	0.1475	3.03	N.D	-
EU	0.035	3.24	N.D	-
GD	N.D	-	N.D	-
HO	N.D	-	N.D	-
LA	1.24	1.39	N.D	-
LU	N.D	-	N.D	-
ND	0.973	1.41	N.D	-
PR	N.D	-	N.D	-
SM	N.D	-	N.D	-
TB	N.D	-	N.D	-
TM	N.D	-	N.D	-
Y	1.45	0.51	0.04	3.33
YB	0.173	0.90	N.D	-

Table 5 Spiked and Recovery Results

ELEMENT	BEFORE SPIKED (MG/L)	ADDED AMOUNT (MG/L)	AFTER SPIKED (MG/L)	RECOVERY (%)
CE	0.0032	0.02	0.0219	93.5
DY	-0.0004		0.0181	92.5
ER	-0.0006		0.0183	94.5
EU	-0.0001		0.0182	91.5
GD	0.0011		0.0194	91.5
HO	-0.0002		0.0182	92.0
LA	0.0015		0.0207	96.0
LU	-0.0007		0.018	93.5
ND	0.0024		0.0212	94.0
PR	-0.003		0.0182	106.0
SM	-0.0004		0.0185	94.5
TB	-0.0003		0.0184	93.5
TM	0.0003		0.0185	91.0
Y	0.0016		0.0198	91.0
YB	0.0001		0.0182	90.5

CONCLUSION

Rare earth elements in tea leaves samples including standard sample (GBW10016) were determinate by ICP-AES with ultrasonic aerosol generator system. Base on the qualitative results of samples with the ICPSolution software, the tea leaves contained large amount of salt normally, such as phosphate and sulfate and so on. The complex matrix of samples can produce negative influence to measuring. Therefore, standard addition method was used instead of calibration curve method, in order to eliminate matrix interference. The results showed that the quantitation results of rare earth elements in GBW10016 matched the certified values well. The recovery results for rare earth elements ranged from 90.5% to 106.0%. The detection limits of rare earth elements in tea leaves was less than 5 ng/mL, which are superior to ICP-AES. This method is suitable for trace-determination of rare earth elements in tea leaves.

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IRTracer-100, LabSolutions IR, Chemometrics

O-14 Quantitative Determination of Protein, Total Fat, and Carbohydrate Contents in Milk by FT-NIR Spectroscopy Method

INTRODUCTION

The traditional methods of analyzing main constituents in milk, for example the Kjeldahl method for protein, Röse-Gottlieb or Mojonnier methods for fat, and polarimetry method for lactose, are time consuming and expensive. The near infrared spectroscopy (NIR) method provides a simultaneous quantitation of a number of milk constituents like proteins, fats and carbohydrates. It is a non-destructive and rapid measurement. Sample dilution is not necessary and even comparatively thick samples can be measured. The NIR spectrum consists of combination bands and overtone vibrations of the fundamental middle infrared bands, and mainly –CH, –OH, and –NH bonding vibrations are observed in the NIR region. However, unlike fundamental middle-IR bands, NIR bands are generally weak intensity, broad and overlapping. Because of the high similarity in NIR spectra, chemometrics data analysis such as principle component regression (PCR), multi-linear regression (MLR) or partial least squares (PLS) regression is required to correlate spectral data with the reference values of measuring components. Here, we introduce a method for quantitative determination of protein, total fat and carbohydrate contents in milk using NIR spectrometry and PLS quantitative calibration method.

EXPERIMENTAL

Twelve commercially available cow milks were measured over the range of 3850 cm^{-1} to 10000 cm^{-1} by FT-NIR transmission method with a pathlength of 1 mm. The measurement conditions used are shown in Table 1. Each sample was measured three times and out of the twelve milk samples, ten were used as references to establish a PLS calibration curve using LabSolutions IR workstation with Chemometrics function. While, Milk 07 and Milk 08 were used as samples for quantitative determination of protein, total fat and carbohydrate.

Table 1. Instrument and Analytical Conditions

Instruments	: IRTracer-100, Near-Infrared Kit
Resolution	: 8 cm^{-1}
Accumulation	: 100
Apodization	: Happ-Genzel
Detector	: InGaAs

Table 2: Labelled nutritional contents of 12 milk samples

Sample	g / 100 mL		
	Protein	Total Fat	Carbohydrate
Milk 01	3.2	3.8	4.8
Milk 02	3.7	1.3	5.7
Milk 03	3.8	0.1	4.9
Milk 04	4	3.7	3.9
Milk 05	4	4	5
Milk 06	3.7	1.5	5
Milk 07	3.5	3.6	5
Milk 08	5	1	5.3
Milk 09	5	1	5.5
Milk 10	3.3	4.1	5
Milk 11	3.2	1.2	7
Milk 12	3.2	3.4	4.9

RESULTS AND DISCUSSION

Figure 1 shows the overlapped NIR spectra of Milk 01 to Milk 12 from 5300 cm^{-1} to 10000 cm^{-1}.

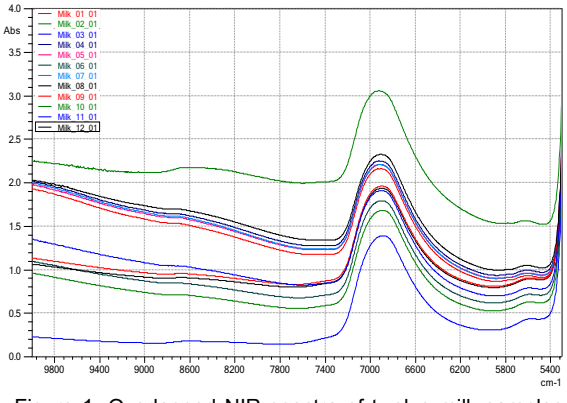

Figure 1: Overlapped NIR spectra of twelve milk samples, Milk 01 to Milk 12.

Second derivative spectra were actually used in the PLS data analysis for better resolution of overlapping and shoulder peaks, as well as removal of baseline fluctuation. Good correlation coefficients of greater than 0.95 were obtained for the PLS calibration modeling with low Mean Squared Error of Prediction (MSEP) and Standard Error of Prediction (SEP) as shown in Table 3.

Table 4 shows the quantitation results of Milk 07 and Milk 08 by the PLS method. From the repeated measurements, the percentage variation from mean is less than 10%. The measured values were very closed to the labelled values. In general, for greater accuracy more calibration samples will be required for establishment of PLS calibration.

Table 3. PLS calibration parameters of protein, total fat and carbohydrate in milk using ten reference samples

Calibration Table			
Algorithm	PLS II		
Number of components	3		
Number of references	30 (three measurements per sample)		
Range [cm^{-1}]	5300 – 10000		
Pre-process	MSC (5500 - 9000) Derivative, Order=2, Points=23		
Scale	Autoscale		
Component	Protein	Total Fat	Carbohy-drate
Number of factors	5	5	5
Correlation coefficient	0.9556	0.9968	0.9743
MSEP	0.0839	0.0062	0.0491
SEP	0.2897	0.0786	0.2216

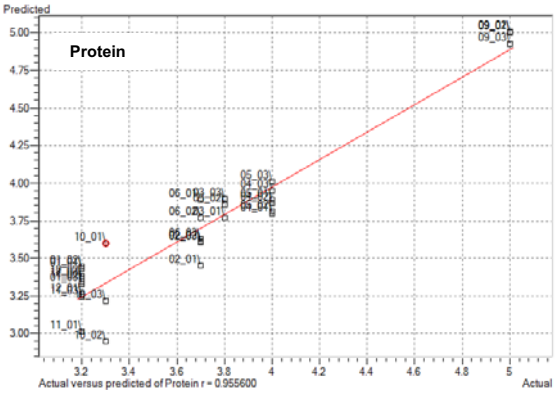

Figure 2. PLS calibration for protein predicted versus actual values (labelled).

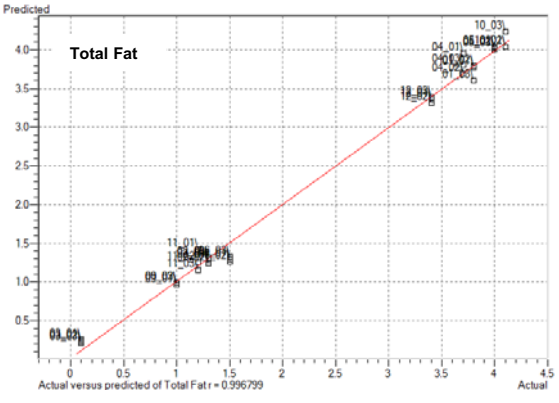

Figure 3. PLS calibration for total fat predicted versus actual values (labelled)

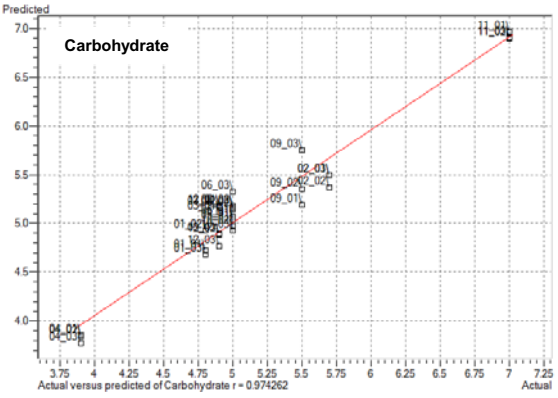

Figure 4. PLS calibration for carbohydrate predicted versus actual values (labelled).

Table 4. Quantitation results of protein, total fat and carbohydrate in samples Milk 07 and Milk 08

Sample	Predicted (g/100 mL)				Labelled (g/100 mL)
	Milk 07-1	**Milk 07-2**	**Milk 07-3**	**Mean**	
Protein	3.49	3.65	3.25	3.46	3.5
Total Fat	3.76	3.74	3.97	3.82	3.6
Carbohy-drate	5.51	4.81	4.81	5.04	5
Sample	**Milk 08-1**	**Milk 08-2**	**Milk 08-3**	**Mean**	**Labelled**
Protein	4.82	4.84	4.82	4.83	5
Total Fat	0.88	0.86	0.91	0.88	1
Carbohy-drate	5.46	5.21	5.32	5.33	5.3

CONCLUSION

The FT-NIR with PLS data analysis offers an alternative quantitation method for protein, total fat and carbohydrate contents in milk without the need for sample pre-treatment. Furthermore, the method is cost effective and faster than the traditional methods.

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ICPE-9000, AA-7000F and HVG-1

O-15 Analysis of Arsenic in Canned Fish with Hydride Vapour Generation Method

INTRODUCTION

The quantitative analysis of arsenic in food can be carried out using HVG with flame atomic absorption spectrophotometry (AAS)[1] and inductively coupled plasma-atomic emission spectrometry (ICP-AES) methods. Inorganic arsenic exists in trivalent and pentavalent forms, and in seafood, arsenic is mainly found in organic form[2]. In order to decompose this organic arsenic and any other co-existing organic substances, nitric acid is added to the sample followed by thermal decomposition. As a result of this process, arsenic is changed to pentavalent form. However, because arsenic reacts to form hydrides in trivalent form, the sample must be pre-reduced by adding potassium iodide in order to ensure all the arsenic is in trivalent form. On the other hand, reduction will not take place properly if there is any nitric acid left and so after thermal decomposition, the sample is heated until almost dry to evaporate all the nitric acid. This data sheet demonstrates the analysis of arsenic in canned fish using both AAS-HVG and (ICP-AES)-HVG methods.

EXPERIMENTAL

The 1000ppm Arsenic (III) standard solution and sodium borohydride were from Sigma-Aldrich (Fluka), USA whereas potassium iodide (KI) was from JT Baker, USA. Hydrochloric acid (HCl) and sodium hydroxide were purchased from Merck, Germany. Ultra pure water was produced by reverse osmosis, electrodeionisation, UV and finally filtered by a 0.22 mm filter to produce water with resistivity of 18 MΩ using the Milli-Q system from Millipore, USA. The Arsenic hollow cathode lamp was purchased from Hamamatsu, Japan.

The sample used in this analysis was Canned Fish Certified Reference Material (CRM), T07121QC, from the Food and Environment Research Agency (FAPAS), USA. It contains 2550 μg/kg arsenic and the satisfactory range is 1842-3259 μg/kg Arsenic. The Canned Fish sample, 0.25g was prepared using nitric acid-microwave system, followed by mild boiling to remove nitric acid and then topped up to 25ml with 1M HCl. There were two sets of preparation. Finally, the sample was diluted 10 times and added with KI to a final concentration of 1% before analysis.

The sodium borohydride and HCl reagents used in HVG were prepared as in Shimadzu HVG-1 Instruction Manual[3].

❑ Analytical Conditions

The flame AAS and ICP-AES analysis conditions were shown in Tables 1 and 2 respectively.

Table 1: AA-7000F Analytical Conditions

Wavelength	193.7nm
Lamp current	12mA
Slit width	0.7nm
Background correction	Deuterium lamp
HVG quartz cell heating	Air-Acetylene flame

Table 2: ICPE-9000 Analytical Conditions

Radio Frequency Power	1.20	(kW)
Plasma Gas Flow Rate	10.0	(L/min)
Auxiliary Gas Flow Rate	0.60	(L/min)
Nebulizer Gas Flow Rate	0.80	(L/min)
Plasma Torch	Mini-torch	
Observation	Axial	

RESULTS AND DISCUSSION

The Arsenic calibration curves and profiles by AAS-HVG were shown in Figures 1 and 3 whereas Figures 2 and 4 are for ICP-HVG. The results were summarized in Table 3. Both the AAS-HVG and ICP-HVG results were in the satisfactory range.

Table 3: Results of AAS-HVG and ICP-HVG

	AAS-HVG	ICP-HVG	CRM (T07121QC)
Measured Concentration	2872 µg/kg	2709 µg/kg	1842-3259 µg/kg
	2966 µg/kg	2712 µg/kg	
Instrument Detection Limit	0.1 ppb(4) (3 σ blank)	0.1 ppb(2) (3 σ blank)	

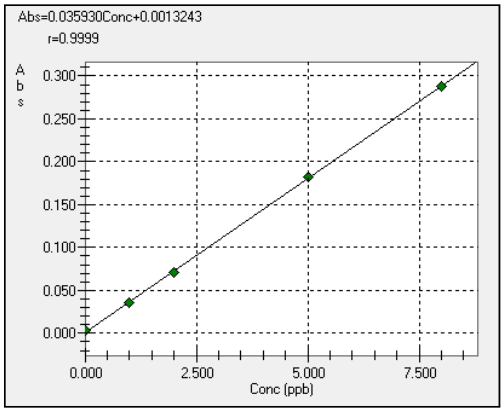

Figure 1: Arsenic calibration curve with AAS-HVG

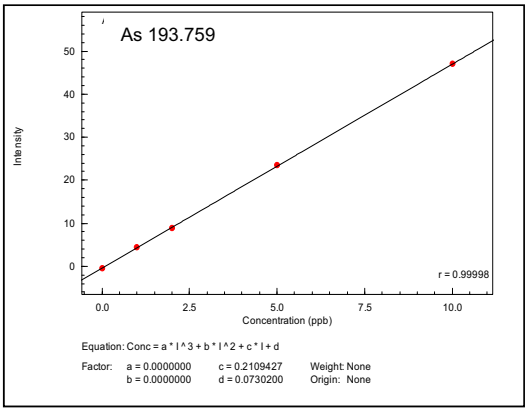

Figure 2: Arsenic calibration curve with ICP-HVG

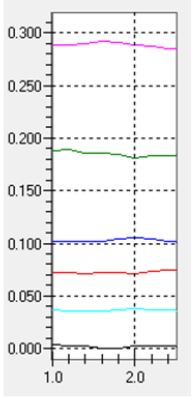

Figure 3: AAS-HVG Arsenic profiles

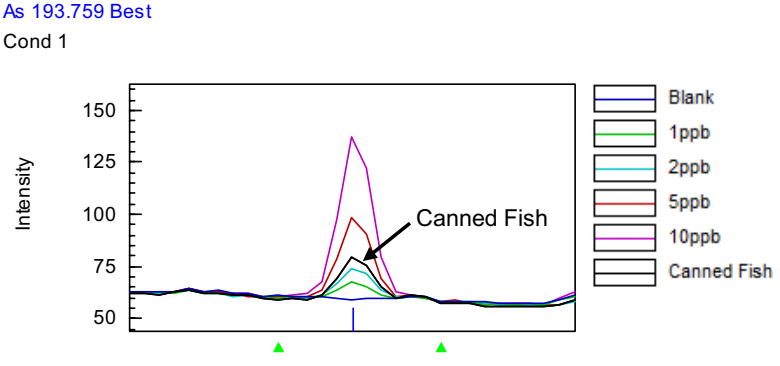

Figure 4: ICP-HVG Arsenic Profiles

CONCLUSION

Arsenic can be analysed using both AAS-HVG and ICP-HVG methods. Arsenic reacts with nascent hydrogen to generate gaseous hydride. Introducing the hydride into the flame atomizer or into the plasma enables high sensitivity analysis. It is shown that with microwave digestion system, removal of nitric acid and pre-reduction with potassium iodide, both AAS-HVG and ICP-HVG can determine arsenic in canned fish at ppb level.

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ICP-AES

Inductively Coupled Plasma Atomic Emission Spectrometry

O-16 Analysis of Fish Sample with ICP-AES for Trace Element Contamination

INTRODUCTION

Fish is one of the most important food resource and is widely consumed in many parts of the world because of its high protein content, low saturated fats and omega-3 fatty acid. It is also rich in calcium and phosphorus. However due to industrial pollution, many fish have trace levels of contaminants such as arsenic and lead which are absorbed by surrounding waters and from foods they eat. Hence, toxic metal accumulation in fish due to toxic effluents can have an adverse effect on the health of human beings. The itai-itai disease was the documented case of mass cadmium poisoning in Toyama Prefecture, Japan, starting around 1912.

This application data sheet demonstrate the ability of ICPE-9000 simultaneous ICP atomic emission spectrometer in quantitative analysis of trace elements in fish.

EXPERIMENTAL

Fish Protein Certified Reference Material for Trace Metals (DORM-4) was used as fish sample. The sample was weighed out to 0.5g into a digestion vessel. 5.0mL concentrated nitric acid, 2.0mL of hydrogen peroxide and 1.0mL of water were added. The sample was digested using microwave-assisted digestion system and the digestion procedure was based on AOAC 999.10 [1]. After the digestion process, deionized water was added to the digested sample to make up to a final total volume of 20.0mL. A duplicate sample was prepared to check the reproducibility of the method.

The calibration standards were prepared from 100ppm ICP multi-element standards and 1000ppm AAS standards from Merck, Germany, and were acid matched to the digested samples. Table 1 shows the target elements, wavelengths selected and the calibration curve standards concentration prepared for each element.

Table 1. Target elements, wavelengths and calibration curve standard concentrations prepared

Element	Wavelength (nm)	Standard (ppm)			
		1	2	3	4
As	189.042	0	0.01	0.05	0.2
Cd	214.438	0	0.01	0.05	0.2
Cr	205.552	0	0.01	0.05	0.2
Cu	324.754	0	0.5	1	2
Fe	238.204	0	5	10	20
Ni	231.604	0	0.01	0.05	0.2
Pb	220.353	0	0.01	0.05	0.2
Se	196.090	0	0.01	0.05	0.2
Zn	206.200	0	0.5	1	2

Table 2. ICP-AES Instrument and analytical conditions

Instrument	:	ICPE-9000
Radio Frequency Power	:	1.20 (kW)
Plasma Gas Flow Rate	:	10.0 (L/min)
Auxiliary Gas Flow Rate	:	0.60 (L/min)
Nebulizer Gas Flow Rate	:	0.70 (L/min)
Sample Introduction	:	Coaxial Nebulizer
Spray Chamber	:	Cyclone Chamber
Plasma Torch	:	Mini-torch
Observation	:	Axial

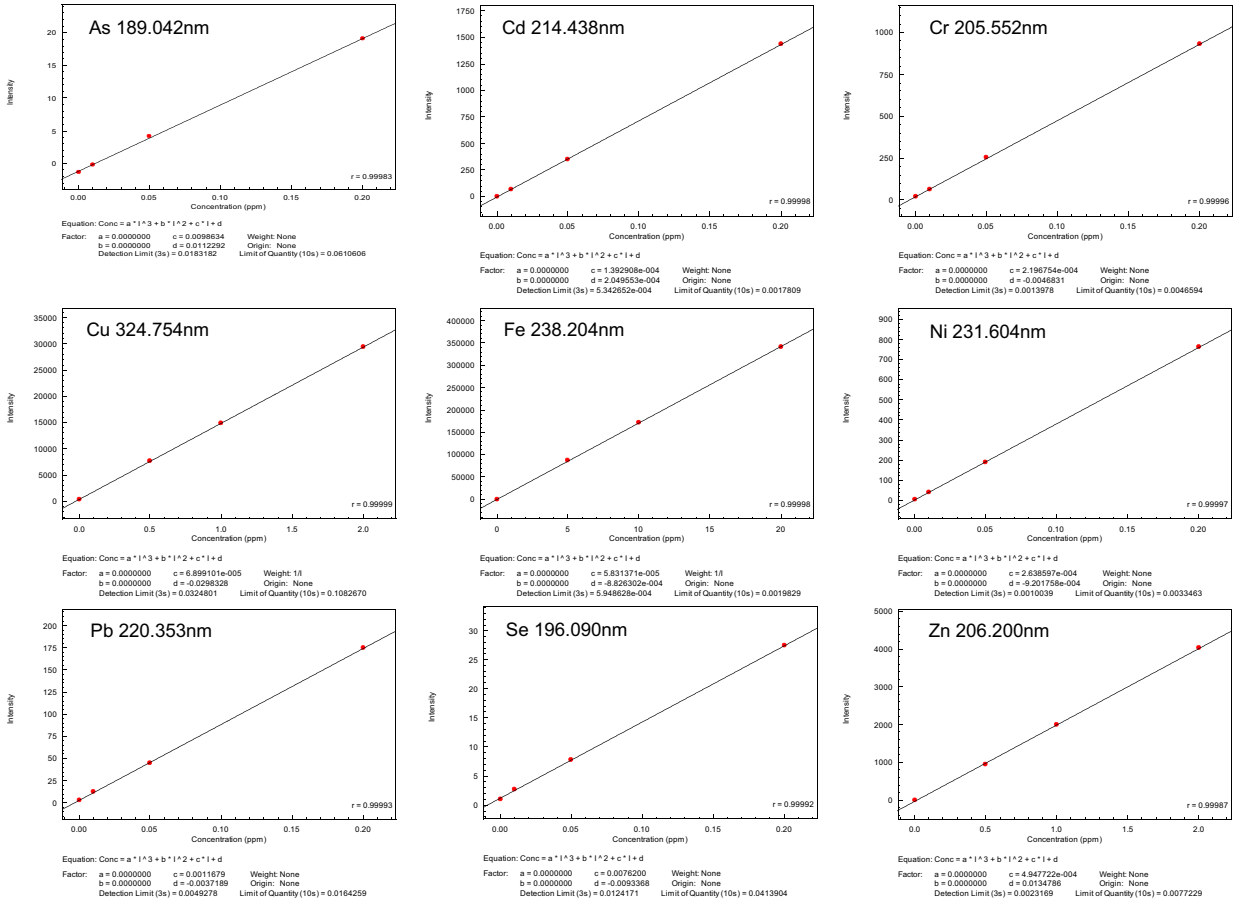

Figure 1. Quantitation calibration curves of nine elements

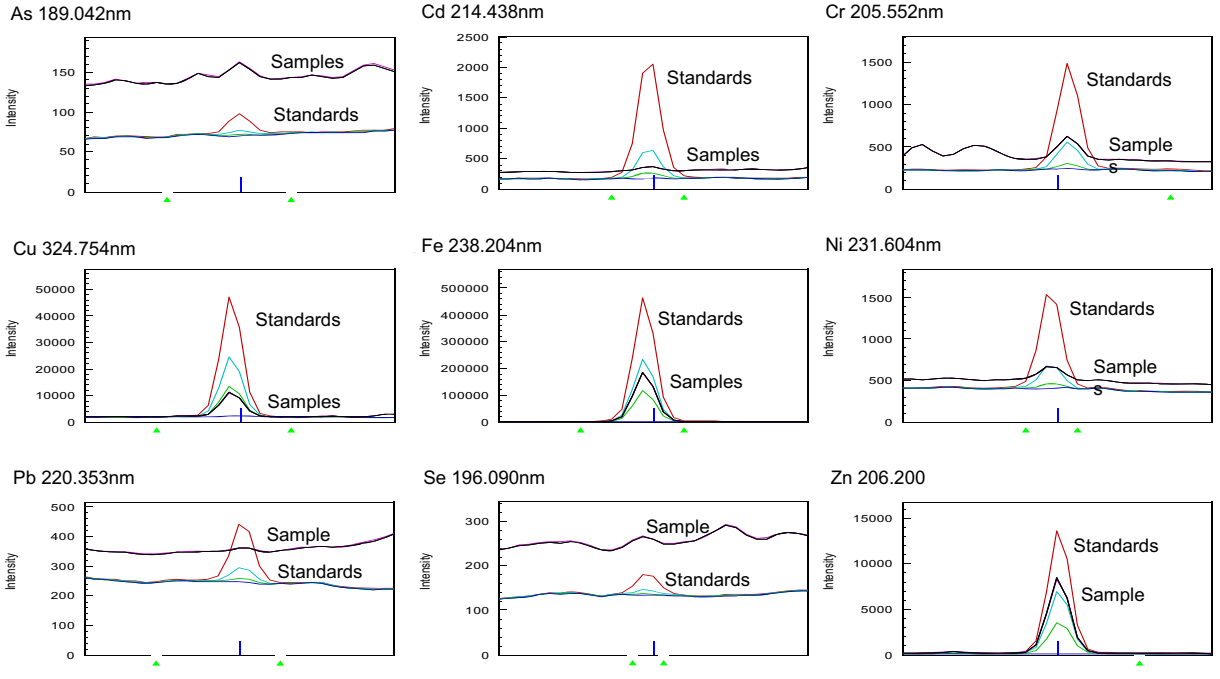

Figure 2. Peak Profiles of nine elements

Table 3. Quantitation results of Fish CRM DORM-4

Element	Fish CRM DORM-4		
	Measured Value (mg/kg)	(Duplicate) Measured Value (mg/kg)	Certified Value (mg/kg)
As	7.07	6.83	6.80 ± 0.64
Cd	0.312	0.312	0.306 ± 0.015
Cr	1.74	1.74	1.87 ± 0.16
Cu	15.5	15.6	15.9 ± 0.9
Fe	317	318	341 ± 27
Ni	1.17	1.18	1.36 ± 0.22
Pb	0.392	0.439	0.416 ± 0.053
Se	3.36	3.46	3.56 ± 0.34
Zn	50.3	51.9	52.2 ± 3.2

Table 4. Quantitation results and percentage recovery of CRM-TMF

Element	CRM-TMF		
	Measured Value (mg/kg)	Certified Value (mg/kg)	Percentage Recovery (%)
As	103	100	103
Cd	5.32	5	106
Cr	21.2	20	106
Cu	46.6	50	93
Fe	101	100	101
Ni	19	20	95
Pb	9.69	10	97
Zn	989	1000	99

RESULTS AND DISCUSSION

The calibration curves are displayed in Figure 1 and the peak profiles of standards and samples are displayed in Figure 2.

The quantitation results obtained matched the certified values of Fish Protein CRM DORM-4 and are shown in Table 3. Certified Reference Material-Trace Metals in Fish Solution, CRM-TMF, was diluted 1000 times and used to check the calibration curves. The quantitation results and percentage recovery are shown in Table 4.

CONCLUSION

Shimadzu ICPE-9000 can provide a rapid method to analyze trace elements in fish simultaneously. The results show excellent correlation with the certified reference material.

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AA-7000, GFA-7000 and ASC-7000

O-17 Analysis of Phosphorus in Waste Water and Food Using Electrothermal Atomic Absorption Spectrophotometry (ETAAS)

INTRODUCTION

Phosphorus plays an important role to ensure proper functioning of the human body. However, high levels of Phosphorus in bodies of water can lead to impairment of drinking water. This results in harmful algae blooms, which reduces spawning grounds and nursery habitats, kills fish, and forms oxygen-starved hypoxic or "dead" zones [1]. The quantitative analysis of Phosphorus can be carried out using ETAAS method [2]. This application news demonstrates the analysis of Phosphorus in food and waste water Certified Reference Materials (CRM) using Shimadzu AA-7000 AAS with GFA-7000 graphite furnace controller and the ASC-7000 autosampler.

EXPERIMENTAL

The 10,000 ppm Phosphorus standard solution and Lanthanum Nitrate Hexahydrate were from Merck, Germany. Type E-1 [3] ultra pure water with resistivity of 18MΩ was used. The Phosphorus hollow cathode lamp was purchased from Heraeus Noblelight, Germany. The AAS analysis conditions were shown in Tables 1 to 3.

Table 1: AAS conditions

Wavelength	213.6 nm
Lamp current	10 mA
Slit width	0.7 nm
Background correction	Deuterium
Matrix modifier	0.1% La

Table 2: ETAAS heating programme

Step	Temperature (°C)	Time (s)	Heat Mode	Sensitivity	Ar Flow (L/min)
1	60	10	Ramp	Regular	0.1
2	120	30	Ramp	Regular	0.1
3	250	10	Ramp	Regular	0.1
4	1200	10	Ramp	Regular	1.0
5	1200	10	Step	Regular	1.0
6	1200	3	Step	High	0.0
7	2800	3	Step	High	0.0
8	2800	2	Step	Regular	1.0

Pyrocoated graphite tube
Sampling at step 7

Table 3: Preparation of standards by autosampler

Standard	Diluent	0.1% La	4 ppm Phosphorus	Total Volume
0.0 ppm	20 µl	5 µl	0 µl	25 µl
1.0 ppm	15 µl	5 µl	5 µl	25 µl
2.0 ppm	10 µl	5 µl	10 µl	25 µl
3.0 ppm	5 µl	5 µl	15 µl	25 µl
4.0 ppm	0 µl	5 µl	20 µl	5 µl

The samples used in this analysis were:

a) Certified Reference Material (CRM) – "Trace Metals in Fish" from High Purity Standards, USA. It contains 100 ppm Phosphorus and was diluted 50 times prior to analysis.
b) CRM Waste Water catalogue no 739 "Simple Nutrients" from ERA, USA. It contained 4.24 ppm Phosphorus.
c) CRM Waste Water catalogue no 741 "Complex Nutrients" from ERA, USA which contained 5.73 ppm Phosphorus.

Both "Simple Nutrients" and "Complex Nutrients" samples were diluted 2 times using the autosampler as shown in Table 4 below.

Table 4: Preparation of samples by autosampler

Sample	Diluent	0.1% La	Total Volume
10 µl	10 µl	5 µl	25 µl

RESULTS AND DISCUSSION

The Phosphorus calibration curve and ETAAS peak profiles were shown in Figure 1 and Figure 2 respectively. Figure 3 showed the ETAAS peak profiles of samples. The accuracy of the analysis was satisfactory as shown in Table 5. Both the 1% absorption and instrument detection limit (a concentration that gives absorbance equal to 3 times the standard deviation of blank [4] was 0.1 ppm.

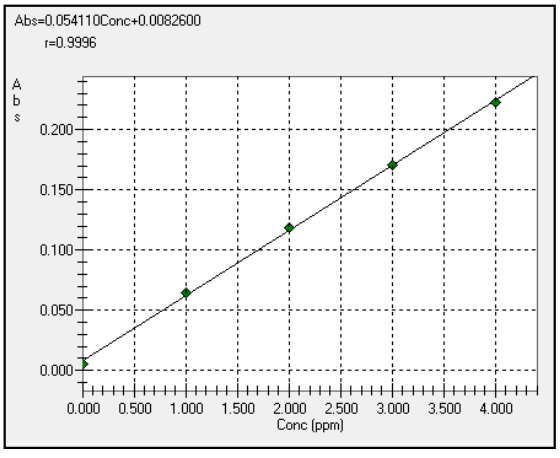
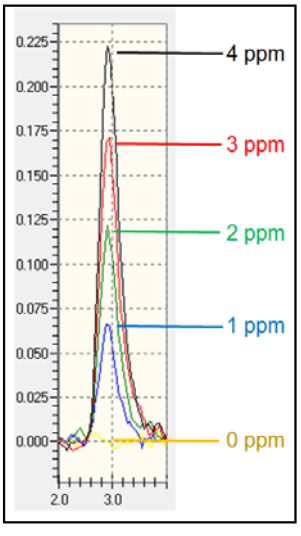


Figure 1: Phosphorus calibration curve



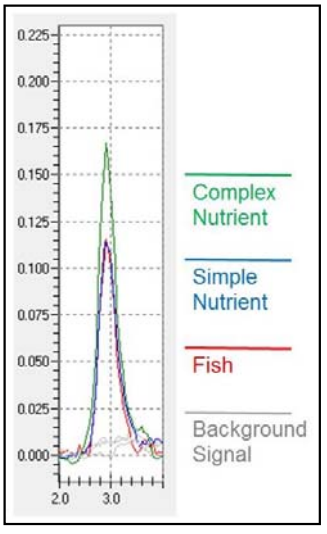


Figure 3: ETAAS peak profiles for CRM samples

Table 5: Phosphorus results for CRM samples

Sample	Certified Concentration	Measured Concentration	Percentage Accuracy
Trace Metals in Fish	100.00 ppm	98.17 ppm	98.2%
Simple Nutrient	4.24 ppm	4.00 ppm	94.3%
Complex Nutrient	5.73 ppm	6.03 ppm	100.5%

CONCLUSION

The quantitative analysis of food and waste water samples was carried out accurately using AA-7000 with GFA-7000 and ASC-7000.

O-18 HEADSPACE ANALYSIS OF TRIHALOMETHANES (THM) IN DRINKING WATER USING SHIMADZU GC-2010PLUS WITH ECD

INTRODUCTION

Generally chlorine and other commercial chlorinated disinfectants are used to control microbial contamination in water. As a result of this, free chlorine reacts with naturally occurring organic and inorganic matter (such as algae) to form toxic Chlorinated impurities. Trihalomethanes (THM) are among such major toxic impurities formed in water.

Some studies have suggested a small increase in the risk of bladder and colorectal cancers due to THM. Other investigations have found that chlorination by-products may be linked to heart, lung, kidney, liver, and central nervous system damage.

Different halogenated byproducts present in chlorinated water are given below.

TABLE I - By-products present in drinking water treated with chlorine

Trihalometanes	**Haloacetic acids**
chloroform	monochloroacetic acid
bromodichloromethane	dichloroacetic acid
dibromochloromethane	trichloroacetic acid
bromoform	monobromoacetic acid
	dibromoacetic acid
	bromochloroacetic acid
Cyanogenic halides	**Haloacetonitriles**
cyanogenic chloride	dichloroacetonitrile
cyanogenic bromide	trichloroacetonitrile
Halopicrines	dibromoacetonitrile
chloropicrin	tribromoaetonitrile
bromopicrin	bromochloroacetonitrile
Chloral hydrates	**Haloacetones, Haloaldehydes, Halophenols**
MX[3-chloro-4-(dichlorometil)-5-hydroxi-*H*)-furanone]	

THM are much more prevalent in public water supplies because most of the water treatment plants use chlorination as a disinfection technology. However, though THM are more common in public water systems, they are a threat to any water supply that uses chlorine, including private water wells.

In this application news, presence of four major Trihalomethanes (THM), Chloroform (CF), Bromoform (BF), Bromodichloromethane (BDCM) and Dibromochloromethane (DBCM) in water, collected from two different tanks from two different locations, are studied using Shimadzu's Gas Chromatograph GC-2010Plus and Electron Capture Detector (ECD) along with Headspace Sampler (HS) Versa.

EXPERIMENTAL

Analysis of Standard and Sample Solution

A stock solution of THM standards was procured from Supelco (P/N- 30036) for experiment. A working standard solutions are prepared in Methanol with different concentrations from above stock solution. Final working solutions for the linearity study are prepared in organic free water. Calibration curves are plotted for all the four THM.

Water samples are collected from Central and Western region of Mumbai, for this study. These samples are collected from the tanks after and before water wash at the interval of one month. To collect water sample, taps are kept open with water running till temperature of water is observed constant. The water sample is collected in 50 ml glass bottles with no air bubble present insid . The bottles are filled up to the top and sealed without allowing any free headspace, to avoid losses of THM. The temperature of samples was maintained at 4^0C till further analysis. For the analysis 1mL of sample water is taken in 22 ml HS vials which contained previously weighed 1g of Sodium Sulphate (Na_2SO_4).

Analytical Parameters for THM Analysis

Shimadzu GC-2010 ECD			
Oven	°C/min	Next °C	Hold Min
Initial	----	40	0
Ramp rate	15	250	6
Injector port Temp.	250 °C		
Injection Type	Split		
Gas Type	Helium		
Column	Rtx-624		
Length	60m		
Diameter	0.32mm		
Film thickness	1.8µm		
Detector	ECD		
Detector Temp.	300 °C		
Current	0.2nA		

Headspace Versa	
Platen/Sample Temp.	80 °C
Valve Oven Temp.	90 °C
Transfer Line Temp.	100 °C
Sample Equilabration Time	15mins
Mixing	ON
Pressurize	10.0 psig
Loop Fill Pressure	5.0 psig

RESULTS AND DISCUSSION

Linearity study is performed using analytical conditions as given above. Calibration curve is plotted for all four THM in the range from 10.0ug/L to 500 ug/L. Figure1 shows the representative chromatographic separation of four THM at 10 ug/L level.

Calibration curves for all four THM are shown in Figure 2.

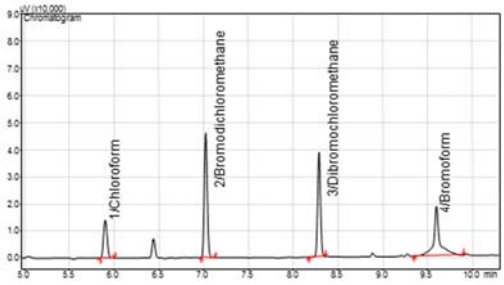


Figure 1: Chromatogram of Standard THMs at 10.0ug/L

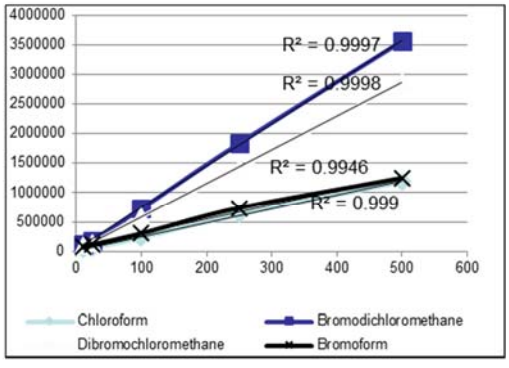


Figure 2: Linearity Study of THM

Table 1: Detection levels of THM

Name	LOD(ug/L)
Chloroform (CF)	0.145
Bromodichloromethane (BDCM)	0.043
Dibromochloromethane (DBCM)	0.051
Bromoform (BF)	0.125

Table 1 shows detection limits (LOD) for four THM standards. The Figure 3 shows the overlay chromatograms of underground water tank from Western Mumbai region, before and after wash. Similarly Figure 4 shows the overlay chromatograms of underground water tank from Central Mumbai region, before and after wash.

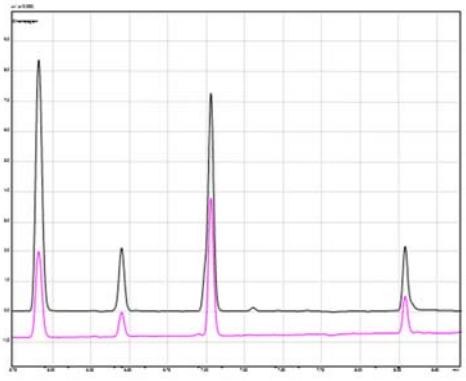


Figure 3: Comparison of water samples collected from Western region of Mumbai

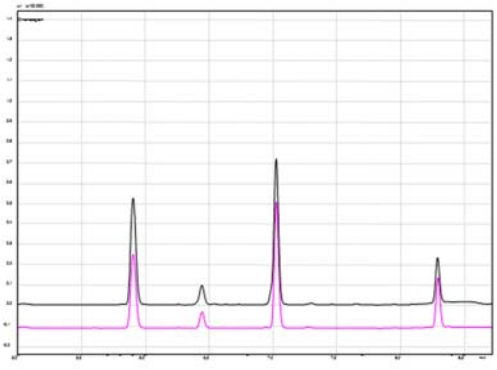


Figure 4: Comparison of water samples collected from Central region of Mumbai

Table 2: Summary of THM observed in water samples collected from Central and Western Region in Mumbai.

THM	Central		Western	
	Before ppb	After One Month ppb	Before ppb	After One Month ppb
CF	41.74	62.16	34.31	98.31
BDCM	19.83	25.54	15.16	26.02
DBCM	9.00	8.42	4.84	8.67
BF	18.42	25.59	20.34	25.52

From the above experiment, it is observed that initial water sample collected before the tank wash shows lower concentrations of THM in both Central and Western region of Mumbai. While as the sample collected after one month of washing show higher levels of THM. This may be due to presence of fresh water, which is source for micro organisms to act on levels of THM. Table 2 shows the summary of quantitative results obtained after HS-GC analysis.

CONCLUSION:

A simple and sensitive method for quantitative study of THM was developed on Shimadzu's Gas Chromatograph GC-2010Plus with ECD coupled with headspace sampler Versa. The presented method shows the very good linearity of THM standards at sub ppb levels. This application demonstrates the quantitative study of THM in public water resources.

O-19 PROFILING OF TRIACYLGLYCERIDES PRESENT IN EDIBLE OILS CONSUMED IN INDIA USING LC/MS/MS

INTRODUCTION

A triacylglyceride (TAG) is an ester derived from glycerol and three Fatty Acids (FA). TAGs are found in both plant oils and animal fats. Different varieties of oils are used for culinary purposes across the globe. Hence, their characterization is important for nutritional reasons. However, separation and identification of components of the complex mixtures of TAG that constitute fats and oils is a challenging.

Characterization of the TAGs present in edible oils requires acquisition of precursor ion m/z by full scan MS mode. Full scan MS spectra are generally complicated because of presence of varieties of TAGs. Furthermore, it is necessary to fragment all these precursor ions so as to understand fatty acid composition of TAGs. Selecting these precursor ions manually and creating a method to fragment them is also a tedious task.

This task is simplified by Synchronized Survey Scan (SSS) available in LCMS-8040 with LabSolutions software. Here, product ion spectra of all the major precursor ions are obtained as a dependent event of full scan MS in a single analysis, attributed to ultrafast scanning speed of 15000 u/sec of LCMS-8040.

EXPERIMENTAL

Sample Preparation:

10 µL of oil sample was diluted in 20 mL of 2-propanol (IPA) and was analyzed by Ultra High Performance Liquid Chromatography (UHPLC) Nexera system coupled with LCMS-8040 triple quadrupole system (Shimadzu Corporation, Japan).

LC/MS/MS Analytical Conditions:

Oil samples were analyzed using LC/MS/MS triple quadrupole system. The Dual Ion Source (DUIS) consists of an integrated probe for analysis of both ESI and APCI techniques concurrently and continuously, without relying on switching between modes. The analysis was performed using Shimadzu's Synchronized Survey Scan (SSS) function (shown in Figure 1). With this automatic MS/MS function, the original MS measurement is used as a trigger, enabling the product ion mass spectra (MS^2 spectra) to be acquired. In this way, by measuring the molecular ion spectra (Q3 scan MS spectra), and then using this as the trigger for measuring the product ion mass spectra (MS^2 spectra), both MS and MS^2 spectra can be acquired simultaneously for a single peak. Neutral loss (NL) scan was also

used so as to get additional confirmation of fatty acid composition of TAGs. The details of analytical conditions are given below.

Column	Inertsil C8 (50 mm L x 4.6 mm I.D. x 3 µm)
Mobile phase	10 mM ammonium acetate in methanol
Flow rate	0.7 mL/min
Oven temperature	50 °C
Injection volume	5 µL
MS interface	DUIS
Nitrogen gas flow	Nebulizing gas 1.5 L/min; Drying gas 15 L/min
MS temperature	Desolvation line 250 ^{0}C; Heat block 400 ^{0}C
MS analysis mode	1. Q3 scan (m/z range 300-1000) 2. SSS (m/z range 200-1000) 3. Neutral loss scan

Type	Event#	+/-	Compound Name m/z
Q3 Scan	1	+	300.00:1000.00
I- Product Ion Scan	2	+	100.00 > 200.00:1000.00

Figure 1: MS method using SSS function

RESULTS AND DISCUSSION

Edible oils from palm, sesame, *mahua (Madhuca longifolia),* sunflower etc. were analyzed using above mentioned method. Workflow is explained here using the data from the analysis of *mahua* oil obtained from *Madhuca longifolia* seeds. This oil is used by some tribes in India for cooking purpose. Other edible oils were analyzed in similar manner.

FA composition of *mahua* oil is given in Table 1. Q3 full scan chromatogram of *mahua* oil is shown in Figure 2. Each TAG shows predominantly an $(M+NH_4)^+$ adduct ion (shown in Figure 3) due to the addition of ammonium acetate in the mobile phase. The true mono isotopic Molecular Weight (M.W.) of a TAG is approximately 0.8 Da greater than the number obtained by adding the integer atomic weights of the elements, owing to the mass defect of hydrogen (actual atomic weight 1.00794) and the large number of hydrogen in the molecule.

Table 1: Fatty acid composition of *mahua* oil[1]

Fatty Acid Name	Lipid Number	Abbreviation Used	Molecular Weight	Neutral Loss monitored (FA M.W. + NH_3)	% fatty acid composition of *mahua* oil
Oleic	C18:1	O	282.00	299.00	36.7 ± 0.27
Palmitic	C16:0	P	256.00	273.00	21.3 ± 1.01
Stearic	C18:0	S	284.00	301.00	24.3 ± 0.30
Linoleic	C18:2	L	280.00	297.00	15.2 ± 0.64
Arachidic	C20:0	A	312.50	329.50	1.3 ± 0.15
Myristic	C14:0	M	228.00	245.00	-
Euric	C22:1	E	338.00	355.00	-
Behenic	C22:0	B	340.50	357.50	-
Lignoceric	C24:0	Ln	368.60	385.60	-

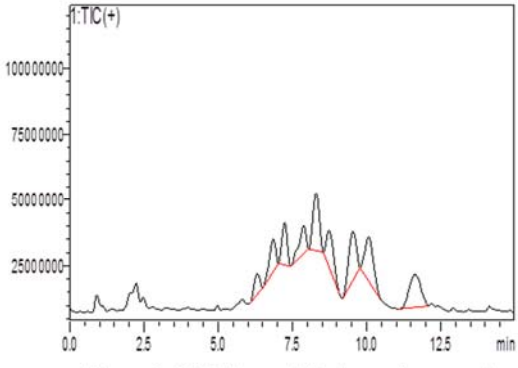


Figure 2: Q3 full scan MS chromatogram of *mahua* oil

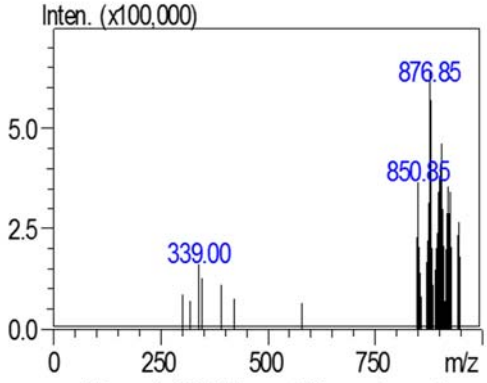


Figure 3: Q3 full scan MS spectrum of *mahua* oil

Additional information can be obtained from extracted ion chromatograms. Overlay of extracted chromatograms for m/z 880.80, 878.90, 876.80, 874.70 and 872.80 is shown in Figure 4. These m/z values correspond to triglycerides made

up of combination of fatty acids with 0, 1, 2, 3 and 4 double bonds respectively. Such comparison indicates the degree of unsaturation of edible oil. The dominant ion is m/z 876.80 and the product ion spectrum of m/z of 876.80 indicates the probable composition as presence of two oleic acid or one linoleic acid (Refer to Table 1).

Product ion spectra of all major precursor ions are obtained using Synchronized Survey Scan. Representative product ion spectrum of m/z 872.9 is shown in Figure 5. Product ion m/z 855.90 is formed due to the loss of ammonium adduct where as m/z 575.65 and 599.30 are formed due to combined loss of one of the fatty acids and ammonia from the TAG species.

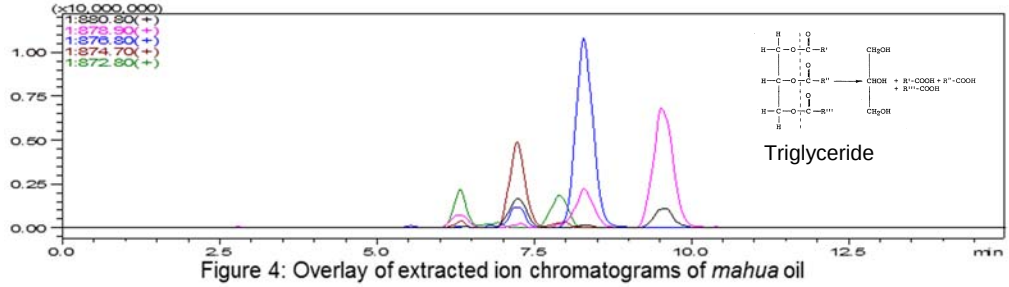


Figure 4: Overlay of extracted ion chromatograms of *mahua* oil

Interpretation of product ion spectra are generally straightforward. First, the identity of the FA lost to give each Diacyl product ion is determined. This is done by subtracting the mass of the Diacyl product ion from the mass of the TAG precursor ion and subtracting further 17 for ammonia to get the M.W. of the FA lost in the fragmentation process of the sn (stereospecific number) position of FA. Then relative abundance of Diacyl product ions are compared so as to assign positions of fatty acids on the glycerol. Assignment is based on the observation that the loss of the sn-1 FA is generally preferred to sn-2 FA.[2] Results obtained from Q3 full scan MS and SSS as well as their interpretation are given in Table 2.

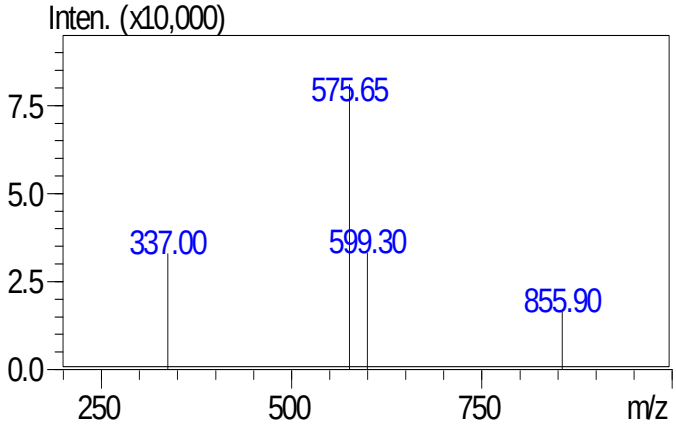


Figure 5: Representative product ion spectrum of m/z 872.90 obtained using SSS

Table 2: Results of Q3 scan and SSS analysis of *mahua* oil

Retention time (min)	TAG adduct ion (m/z)	Diacyl product ion (m/z)	M.W. of FA lost*	Name of FA lost from TAG species	Abundance of Diacyl product	Probable FA composition of TAG**
6.31	872.90	575.65	280.45	Linoleic	93138	LLP
		599.30	256.40	Palmitic	78383	
6.85	848.80	575.55	256.25	Palmitic	235777	PLP
		551.55	280.25	Linoleic	124438	
7.22	874.70	601.60	256.10	Palmitic	251762	OLP
		575.50	282.20	Oleic	193787	
		577.40	280.30	Linoleic	138732	
7.65	900.90	601.60	282.30	Oleic	99017	OLO
		603.70	280.20	Linoleic	70447	
7.84	850.70	577.65	256.05	Palmitic	1110286	POP
		551.40	282.30	Oleic	303603	
8.31	876.80	577.55	282.25	Oleic	387032	PLS/POO
		603.60	256.20	Palmitic	330750	
		579.55	280.25	Linoleic	110746	
		575.50	284.30	Stearic	107569	
8.77	902.90	603.55	282.35	Oleic	234842	OSL
		605.55	280.35	Linoleic	79227	
		601.55	284.35	Stearic	58450	
9.52	878.90	577.50	284.40	Stearic	215388	SOP
		605.45	256.45	Palmitic	128274	
		579.50	282.40	Oleic	71130	
10.12	904.90	605.55	282.35	Oleic	233175	SLS/SOO
		603.55	284.35	Stearic	185741	
		607.85	280.05	Linoleic	27498	
10.54	930.80	633.60	280.20	Linoleic	49143	OLA***
11.61	906.90	605.65	284.25	Stearic	114606	SOS/POA
		607.60	282.30	Oleic	80971	
		577.45	312.45	Arachidic	13423	
		633.60	256.30	Palmitic	7022	

The $[M+NH_4]^+$ ions of TAGs undergo the characteristic loss of a single neutral species RCOOH + NH_3, for each fatty acyl group present and so neutral loss scan mode can be used for analysis of ammoniated adducts of TAGs.[3] Several neutral loss scans as mentioned in Table 2 were used to determine a complete fatty acyl profile of the TAGs present in edible oils. Results obtained from neutral loss scan and their interpretation is given in Table 3.

Table 3: Results of neutral loss scan analysis of *mahua* oil

Retention time (min)	Fatty acid neutral loss monitored	Observed precursor ion (m/z)	Abundance	Probable TAG composition
6.31	Linoleic	872.90	High	LLP
	Palmitic		medium	
6.85	Palmitic	848.80	high	PLP
	Linoleic		medium	
7.22	Oleic	874.70	high	OLP
	Palmitic		medium	
	Linoleic		low	
7.65	Oleic	900.90	high	OLO
	Linoleic		medium	
7.84	Palmitic	850.70	high	POP
	Oleic		medium	
7.90	Palmitic	852.80	high	PSP
	Stearic		medium	
8.31	Palmitic	876.80	high	PLS/POO
	Oleic		medium	
	Stearic		low	
	Linoleic		low	
8.77	Oleic	902.90	high	OSL
	Linoleic		medium	
	Stearic		low	
9.52	Stearic	878.90	high	SOP
	Palmitic		medium	
	Oleic		low	
9.57	Stearic	880.80	high	SPS
	Palmitic		medium	
10.12	Oleic	904.90	high	SLS/SOO
	Stearic		medium	
	Linoleic		low	
10.54	Oleic	930.80	high	OLA
	Arachidic		medium	
	Linoleic		low	
11.61	Stearic	906.80	high	SOS
	Oleic		medium	

CONCLUSION

1. Profiling of TAGs present in *mahua* oil is done for the first time using LC/MS/MS triple quadrupole system.
2. Synchronized Survey Scan enables acquisition of MS and MS^2 spectra simultaneously for given peak and hence simplifies the task of profiling of TAGs from edible oils.
3. Ultra high scanning speed of 15000 u/sec allows the acquisition of Q3 full scan, SSS and NL in a single run and hence large amount of information can be obtained to determine fatty acid composition of TAGs present in edible oil.

SHIMADZU
Excellence in Science

O-20 DETERMINATION OF OIL AND GREASE IN WATER USING IRAFFINITY-1

INTRODUCTION

The concentration of dispersed oil and grease (O&G) in water is an important parameter for water quality and safety. This paper presents analysis of O&G in water and waste water using FTIR with respect to IS 3025 (Part 39) with Shimadzu IRAffinity-1. The above method is based on Hexane as an extraction solvent, which as a hydrocarbon containing solvent interfere with the FTIR analysis in determination of O&G.

Hence, this method is developed using carbon tetrachloride as an extraction solvent. Carbon tetrachloride is proved to be a suitable alternative to Hexane and the method has shown sufficient sensitivity for monitoring O& G in water as specified by IS 3025 (Part 39).

EXPERIMENTAL

Standard samples were analyzed using Shimadzu's IRAffinity-1 with deuterated L-alanine triglycine sulfate (DLATGS) detector. It has many other features like built in auto dryer, Flexible Joint Support (FJS) system, etc. The software used to acquire spectra was IR solution Ver. 1.40. Spectra were acquired over the range of 3200 – 2700 cm-1 at 4 cm-1 with 45 scans against carbon tetrachloride as a pure solvent. The peak maximum was chosen at 2926 cm-1 for calibration. Linear Base–line correction was applied to all spectra before plotting standard calibration curve. The reagents and standards were prepared as per IS 3025 (Part 39).

Apparatus:

1. Separating funnel – 1 liter capacity Teflon stopcock.
2. Quartz cell with cell holder.
3. Filter paper – Whatman No. 40 or Equivalent.

Reagents:

All reagents used were of spectral grade.

Hydrochloric acid – 1:1 (HCl).

Carbon tetrachloride (CCl4).

Anhydrous Sodium Sulphate. (Na2SO4) Reference oil (used for calibration): mixture of 25% benzene, 37.5% iso-octane and 37.5% hexadecane.

Standard Preparation:

For calibration, reference oil was prepared as per above mentioned proportion and stored in sealed container to avoid evaporation. Stock solution was prepared by weighing 1 mg of reference oil in a 100 ml volumetric flask and diluting it up to mark by CCl4. From the stock solution series of working standards were prepared by using a volumetric technique from 1 – 40 ppm.

Sample preparation:

1) Acidify 1 liter of sample by using HCl to pH=2.0.
2) Extract above sample with 30 ml of CCl4 three times.
3) Filter above extract over 10 g of anhydrous sodium sulfate and dilute the extract to 100 ml with solvent.
4) Measure the absorbance of the solution at the frequency 2926 cm-1.

Calibration – Linearity:

Over the calibration range excellent linearity was observed with correlation coefficient of (R^2) 0.9998 (See fig.1).

RESULTS AND DISCUSSION

Figure 1 shows overlay FTIR spectra of all standard samples from 3200 cm-1 to 2700 cm-1.

On the basis of calibration curve obtained, 5.0 ppm sample was analyzed and the good repeatability (SD=0.029 and RSD as 0.59) as shown below in table 1.

Table 1: Absorbance for 5.0 ppm

Sample No.	Values obtained (ppm)
1	4.925
2	4.937
3	4.936
4	4.948
5	4.986
6	4.897
Standard Deviation	0.029
Relative Standard Deviation	0.59

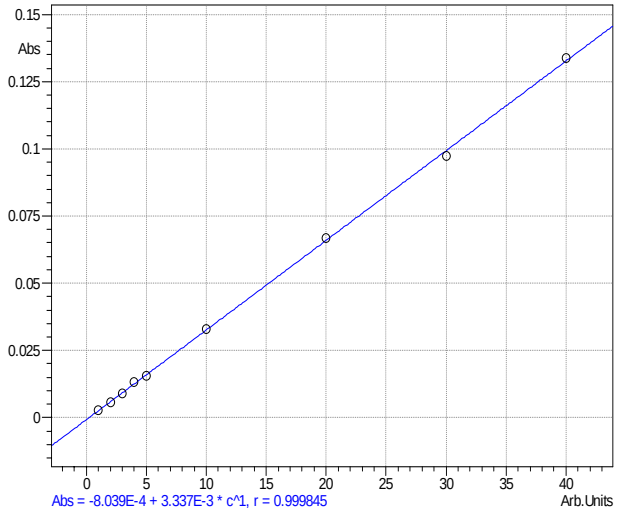


Figure 2: Calibration Curve for Mineral oil Standard:

This application note demonstrates O&G in water can be easily analyzed by IRAffinity-1. Excellent calibration curve is achieved with good linearity (Correlation coefficient = 0.9998, see fig. 2).

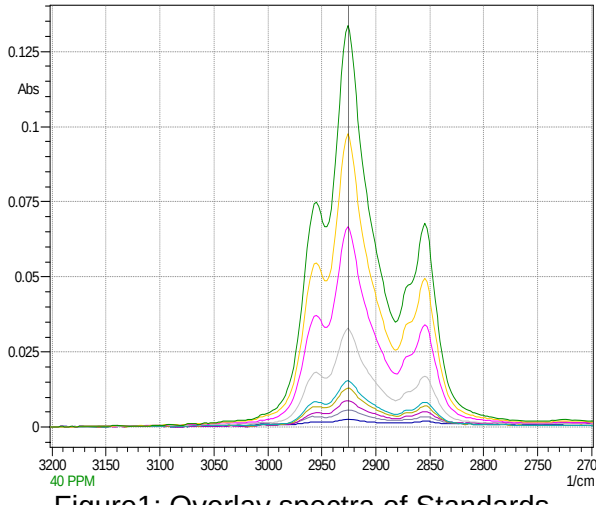


Figure1: Overlay spectra of Standards

CONCLUSION

Standard Method IS 3025 (Part 39) works well with CCl4 as an extraction solvent. As per method working range is specified 4ppm to 40ppm but by using the above method we can easily determine concentration from 1ppm to 40ppm.

Spectrophotometric Analysis

O-21 Flame AA Analysis of Cadmium (Cd) and Lead (Pb) in Simulated Seawater and Polished Rice Using Chelating Polymer Solid Phase Extraction

INTRODUCTION

The testing methods for industrial wastewater (JIS K 0102) as amended in 2013, newly adopt the use of solid phase extraction with a chelating resin for sample pretreatment of Cu, Zn, Pb, Cd, Fe, Ni, and Co. Using this process permits separation and concentration of the target elements from such interfering components as sodium, potassium, magnesium and calcium, etc. Solvent extraction using a chelating agent has conventionally been employed for the same purpose, but solid phase extraction using a chelating resin offers such benefits as reduced solvent consumption and pollution, and simplified processing.
Here, using the AA-7000, we introduce examples of analysis by the flame method of Cd and Pb in simulated seawater and a solution of decomposed polished rice using commercially available chelating resin cartridges for solid phase extraction.

EXPERIMENTAL

For the simulated seawater, the sample stock solution was used as is. For the polished rice solution, 2 g of polished rice was weighed out in a beaker, nitric acid and perchloric acid were added, and decomposition was conducted using a hot plate. After cooling, the solution was filtered, and 100 mL of the filtrate was used as the sample stock solution.
The pH of the sample stock solution was then adjusted to about pH 5.6, after which the solution was passed through conditioned chelating cartridge, thereby trapping the measurement elements in the chelating cartridge. As the chelating resin in this case had a pH of about 5.6, elements such as Cd and Pb were effectively retained in the cartridge, while elements such as Na, K, Mg and Cd were not retained. After washing the chelating cartridge in distilled water, the captured elements were eluted from the cartridge using 5 mL of 3 mol/L nitric acid solution. The eluate was then adjusted to a volume of 10 mL, and this served as the measurement solution. Fig. 1 and 2 show the chelate cartridge conditioning and sample solution extraction process, respectively. As the pH dependence of the retained element and the type and concentration of the reagent used vary with the chelate cartridge that is used, it is necessary to refer to the manufacturer's instruction manual.
For validation of the extraction process, a spiked recovery test solution was prepared prior to conducting the extraction by adding standard solution to the sample solution, and then conducting the same processing as for the actual sample solution. The spiked concentrations in the simulated seawater consisted of 0.01 mg/L of cadmium and 0.1 mg/L of lead. For the rice, the prepared concentrations based on solid sample conversion were 0.2 mg/kg of cadmium and 2 mg/kg of lead.

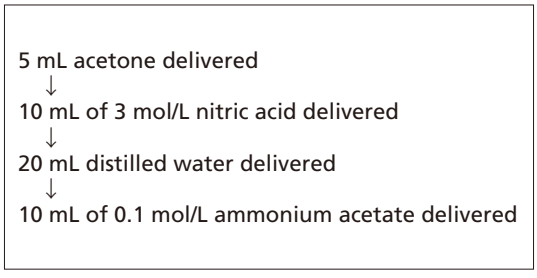


Fig. 1 Conditioning of Chelating Cartridge

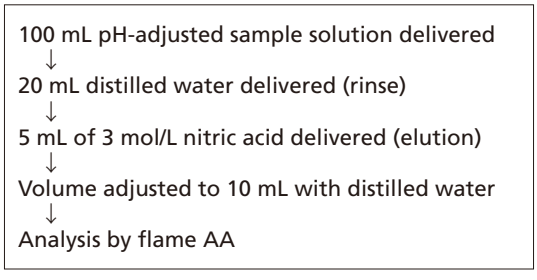


Fig. 2 Chemical Separation of Sample Solution with Chelating Cartridge

Analytical Method and Conditions

Measurement was conducting using the calibration curve method. The standard solutions were prepared by diluting commercially available standard solutions for atomic absorption measurement. The main measurement parameters of the instrument are shown in Table 1.

Table 1 Instrument Measurement Parameters

	Cd	Pb
Measurement wavelength	228.8 nm	283.3 nm
Slit width	0.7 nm	
Ignition mode	BGC-D2	
Flame type	Air-C_2H_2	

RESULTS AND DISCUSSION

Fig. 3 and 4 show the calibration curves for Cd and Pb, respectively. The standard solutions were spiked with nitric acid to match the concentrations of the measurement samples.

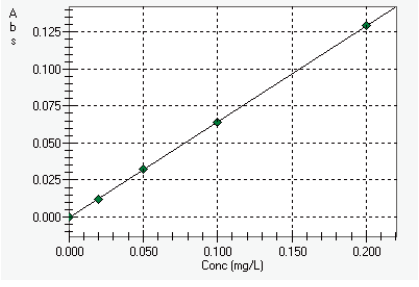


Fig. 3 Calibration Curve for Cd

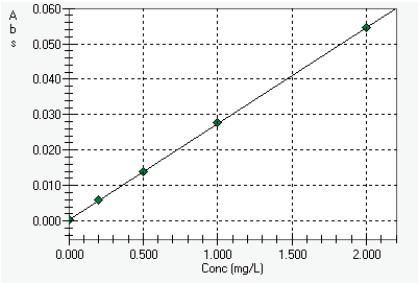


Fig. 4 Calibration Curve for Pb

(1) Measurement Results for Simulated Seawater

Table 2 shows the concentrations of Na and Mg in the original seawater and post-extraction seawater. The concentrations of both Na and Mg in the extracted seawater are less than 1/1000 those in the original simulated seawater, indicating efficient removal of interfering components.

Table 3 shows the measurement results for Cd and Pb. Concentration of the sample permitted measurement by the flame method even at low sample concentrations. Excellent spike and recovery results at greater than 95 % were also obtained. Fig. 5 shows the absorbance following integration.

Table 2 Na and Mg Concentrations in Original Seawater and Extracted Seawater

Element	In Original Seawater	In Extracted Seawater
Na	10000 mg/L	2 mg/L
Mg	1300 mg/L	0.6 mg/L

Table 3 Measurement Results for Cd and Pb in Seawater

Element	Cd	Pb
Simulated Seawater	0.0017 mg/L	<0.02 mg/L
Spike Recovery	98 %	95 %
Quantitation lower limit(*)	0.0007 mg/L	0.02 mg/L

(*) The lower limit of quantitation refers to the concentration in seawater corresponding to an absorbance of 0.004 when measurement is conducted using 10 mL after conducting chelating solid extraction of 100 mL of seawater.

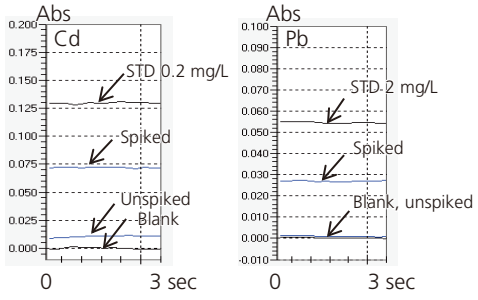


Fig. 5 Signal of Cd and Pb in Seawater and Standard

(2) Measurement Results for Polished Rice

Table 4 shows the sample measurement results in which excellent spike and recovery results were obtained. In the case of cadmium, quantitation was possible at less than one-tenth of 0.4 mg/kg, the national standard for edible rice in Japan.

Table 4 Measurements Results for Cd and Pb in Polished Rice

Element	Cd	Pb
Polished rice	0.03 mg/kg	<0.4 mg/kg
Spike Recovery	95 %	98 %
Quantitation lower limit(*)	0.03 mg/kg	0.8 mg/kg

(*) The lower limit of quantitation refers to the concentration in polished rice corresponding to an absorbance of 0.004 when measurement is conducted after decomposition of 2 g of polished rice and subsequent solid extraction using 10 mL of chelating resin.

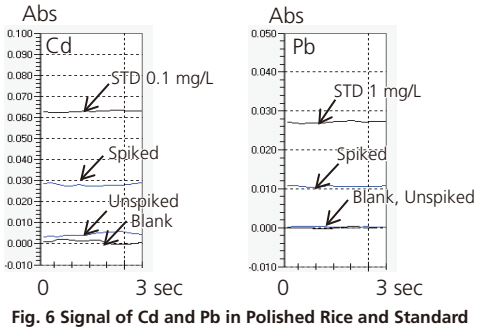


Fig. 6 Signal of Cd and Pb in Polished Rice and Standard

Application of solid phase extraction using chelating polymer for sample pretreatment permits the concentration of measurement analytes and the elimination of interfering substances using a simple operation, and can achieve the same high-sensitivity measurement results that are possible using solvent extraction pretreatment. Furthermore, since the obtained process solution consists of a nitric acid solution, it can be used not only for flame atomic absorption, but for graphite furnace atomic absorption, as well as ICP emission analysis and ICP mass spectrometric analysis.

The AA-7000 series includes models that support not only flame measurement and graphite furnace measurement, but automatic switching between the two modes as well, to accommodate a wide variety of analysis requirements.

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Inductively Coupled Plasma Atomic Emission Spectrometry

O-22 Analysis of Harmful Elements in Herbal Medicines by ICPE-9800 Series

INTRODUCTION

Herbal medicines, products consisting of animals and plants, fungi, and minerals with naturally occurring efficacy, are used without purification as drugs (over-the-counter drugs), foods, functional foods, and dietary supplements. Safety standards for harmful elements used in pharmaceuticals are provided for in each country. Table 1 shows examples of regulations regarding the permissible levels of harmful elements in herbal medicines, including the levels suggested by WHO[1] and the import/export reference values of China[2].

Here, using the Shimadzu ICPE-9800 series multi-type ICP atomic emission spectrometer, we conducted an analysis of an herbal medicine. The ICPE-9800 series, with its mini-torch plasma and spectrometer which permits all element/all wavelength simultaneous analysis, provides high sensitivity, high precision, and high throughput at low cost.

Table 1 Harmful Element Regulation of Herbal Medicines (mg/kg)

Element	As	Cd	Cu	Hg	Pb
WHO recomended value		0.3			10
China Import/Export Herbal Medicine Reference Value	2	0.3	20	0.2	5

Sample

Herbal medicines distributed in Japan.

EXPERIMENTAL

Low boiling point-elements such as arsenic (As), mercury (Hg), etc. are subject to loss due to volatilization during operations such as heating and acid addition. Therefore, a pretreatment process is required that will keep element loss to a minimum, while at the same time be efficient. Here, we conducted sample digestion using a microwave sample preparation system.

To 0.5 g of dry sample, 7.5 mL of concentrated nitric acid and 0.5 mL of concentrated hydrochloric acid were added, and digestion was conducted using a microwave sample preparation system. After digestion, distilled water was added to the process liquid to bring the total volume to 25 mL, and this was used as the analytical sample. Separately, after preparing another sample in the same manner, a standard solution containing the target elements was added, and this served as the spike-and-recovery test solution.

Instrument and Analytical Conditions

For measurement, the Shimadzu ICPE-9800 series multi-type ICP emission spectrometer was used. The analytical conditions used are shown in Table 2. The adoption of an echelle spectrometer and a CCD detector permits simultaneous analysis of all the elements at all the wavelengths, in addition to high-throughput measurement even with many target elements and samples. Also, the mini-torch plasma, Eco mode, and vacuum-housed spectrometer serve to greatly reduce running costs due to gas consumption.

Herbal medicines often contain large amounts of coexisting elements, including calcium (Ca), potassium (K), and magnesium (Mg). Typically, when a sample contains many coexisting elements, some level of error may affect the analysis value due to ionization interference. However, the ICPE-9800 series mini-torch produces a high-temperature plasma which in addition to providing high sensitivity, also suppresses the effect of ionization interference.

Table 2 Analytical Conditions

Instrument	: ICPE-9800 series
Radio Frequency Power	: 1.2 kW
Plasma Gas Flowrate	: 10 L/min
Auxiliary Gas Flowrate	: 0.6 L/min
Carrier Gas Flowrate	: 0.7 L/min
Sample Introduction	: Nebulizer 10
Misting Chamber	: Cyclone chamber
Plasma Torch	: Mini Torch
Observation	: Axial (AX)
Measurement Time	: 2.5 min/sample (Including rinse time)

Analysis

Quantitative analysis of As, Cd, Cr, Cu, Hg, Pb, and Sn was conducted using the calibration curve method.

RESULTS AND DISCUSSION

Table 3 shows the semi-quantitative results for the principal components using qualitative analysis. Semi-quantitative results are calculated automatically by the database built into the software. With the ICPE-9800 series, qualitative data for all elements are acquired and saved at the same time that quantitative analysis is conducted. This feature, even after quantitative analysis, makes it possible to identify the principal elements and their concentrations, making it possible to consider the impact on the analyte elements. Table 4 shows the analytical results, and Table 5 shows the results of spike-and-recovery test. Fig. 1 shows the spectral profiles. The detection limit is below the WHO recommended value as well as the China import and export reference values, indicating sufficient sensitivity. Further, as for the spike-and-recovery rate, good results were shown for all elements, and it is clear that accurate quantitation is possible without adverse influence from coexisting elements such as Ca and K present at high concentrations.

CONCLUSION

Use of the ICPE-9800 series offers high sensitivity, as well as accurate and low-cost measurement of harmful elements in herbal medicines.

Table 3 Semi-Quantitative Results for Herbal Medicines by Qualitative Analysis (wt%)

	Ca	K	Mg	S	Al	P	Si	Fe	Mn	Ba	Sr	Na
Horny goat weed	3.7	1.1	0.35	0.45	0.18	0.31	0.16	0.12	0.060	0.021	0.021	0.008
Fang feng (Saposhnikoviae Radix)	1.6	0.65	0.38	0.35	0.25	0.25	0.11	0.09	0.006	0.013	0.025	0.070

Table 4 Analytical Results for Herbal Medicines (µg/g)

Element / Sample Name	As	Cd	Cr	Cu	Hg	Pb	Sn
1. Cardamom	<	0.06	<	5.4	<	0.3	<
2. Cinnamon	<	<	0.4	6.6	<	0.6	<
3. Horny goat weed	0.4	0.13	2.8	4.5	<	1.5	<
4. Carrot	<	0.03	0.04	5.0	<	<	<
5. Rehmanniae Radix	<	<	0.4	3.8	<	<	<
6. Paeoniae radix	<	<	0.3	4.2	<	<	<
7. Fang feng (Saposhnikoviae Radix)	<	<	0.5	6.6	<	<	<
8. Turmeric (Curcumae Radix)	<	0.04	0.1	2.1	<	4.3	<
Detection Limit	0.2	0.007	0.02	0.04	0.07	0.1	0.04
WHO Recommended Value		0.3				10	
China Import/Export Herbal Medicine Reference Value	2	0.3		20	0.2	5	

Detection limit: 3 times the concentration standard deviation obtained from 10 repeated measurements of the calibration curve blank × Dilution factor (50)

<: Below the limit of detection

Table 5 Recovery Rate in Spike/Recovery Test (%)

3. Horny goat weed	99	98	99	101	101	96	98
7. Fang feng (Saposhnikoviae Radix)	100	97	99	102	102	98	100

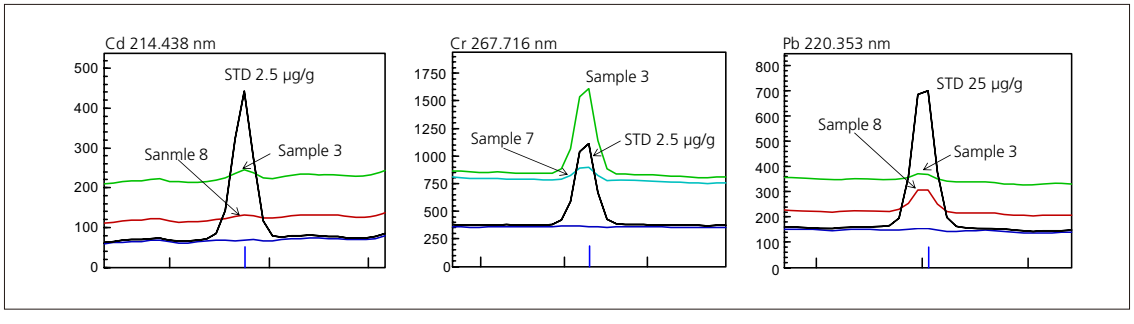

Fig. 1 Spectral Profiles of Cd, Cr, and Pb in Herbal Substances
*** The concentrations in the figures refer to the concentrations in the samples (solid)**

O-23 Analysis of Sugars and Sugar Alcohols in Energy Drink by Prominence-i with Differential Refractive Index Detector

Sugars and sugar alcohols display almost no ultraviolet absorption, and are therefore typically detected using a differential refractive index detector or evaporative light scattering detector. By using a ligand exchange column for sugar analysis, it is possible to distinguish among the different isomers based on the position of the hydroxyl group in the chair conformation of glucose and fructose for example. In other words, the hydroxyl group of the sugar and the metal ion of the stationary phase form a complex, making it possible to achieve separation due to the difference in the strength of the complex formation. Also, maintaining a column temperature of 80 °C suppresses sugar anomer separation and peak dispersion, thereby achieving good separation of adjacent peaks.
The new Prominence-i integrated high-performance liquid chromatograph can be connected to the RID-20A differential refractive index detector. The column oven, which can accommodate a 30 cm column and maintain temperature control up to 85 °C, therefore supports applications that require a long column.
In Application News No. 467, we introduced an example of analysis of sugars in juice, in which the Prominence-i was connected to a differential refractive index detector. Here, we introduce an example of simultaneous analysis of sugars and sugar alcohols in an energy drink using the Prominence-i and RID-20A.

Analysis of a Standard Mixture of Six Sugars

Sorbitol, xylitol, mannitol and erythritol are a type of sugar alcohol that because of their relative sweetness, are used as sweeteners. When conducting simultaneous analysis of sugars and sugar alcohols, a hydrophilic compound analytical column, such as the SPR-Ca or SPR-Pb, is suitable along with the use of a combination of the size exclusion and ligand exchange modes of analysis. Fig. 1 shows the results of analysis of a standard solution of six sugar alcohol substances (10 g/L each of maltose, glucose, fructose, erythritol, mannitol and sorbitol) using the SPR-Ca column with a 10 µL injection. The analytical conditions are shown in Table 1.
Fig. 2 shows the results of analysis of a standard solution of six sugar substances including sugar alcohols (10 g/L each of maltose, glucose, fructose, mannitol, xylitol, sorbitol) using a 10 µL injection, and Table 2 shows the analytical conditions that were used. The SPR-Pb was used as the analytical column.

Table 1 Analytical Conditions

Column	: Shim-pack SPR-Ca (250 mm L × 7.8 mm I.D., 8 µm)
Mobile Phase	: Water
Flowrate	: 0.6 mL/min
Column Temp.	: 80 °C
Injection Volume	: 10 µL
Detection	: RID-20A Polarity +, Cell temp. 40 °C, Response 1.5 sec

Table 2 Analytical Conditions

Column	: Shim-pack SPR-Pb (250 mm L × 7.8 mm I.D., 8 µm)
Mobile Phase	: Water
Flowrate	: 0.6 mL/min
Column Temp.	: 80 °C
Injection Volume	: 10 µL
Detection	: RID-20A Polarity +, Cell temp. 40 °C, Response 1.5 sec

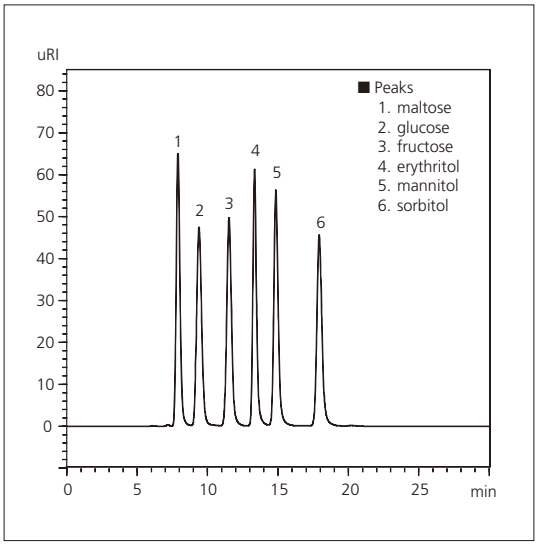

Fig. 1 Chromatogram of a Standard Mixture of Six Sugars (10 g/L each, 10 µL Injected)

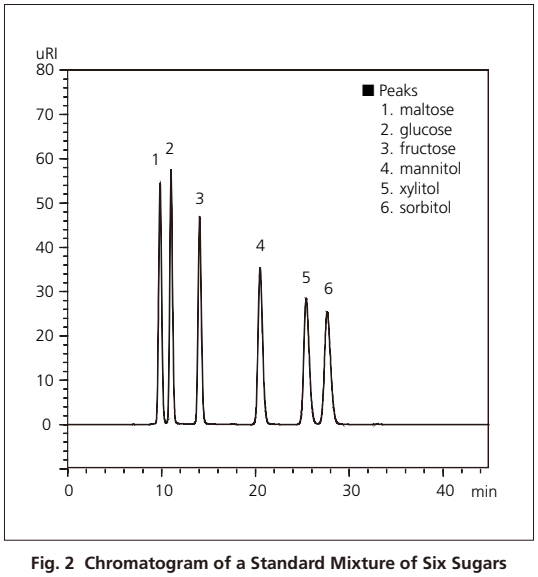

Fig. 2 Chromatogram of a Standard Mixture of Six Sugars (10 g/L each, 10 µL Injected)

Linearity

Fig. 3 shows the calibration curves generated using the analytical conditions of Table 2. When generating the curves for the six components over a concentration range of 0.2 to 10 g/L (using the average of three area values, respectively), excellent linearity with a coefficient of determination greater than R^2=0.9999 was obtained for each component.

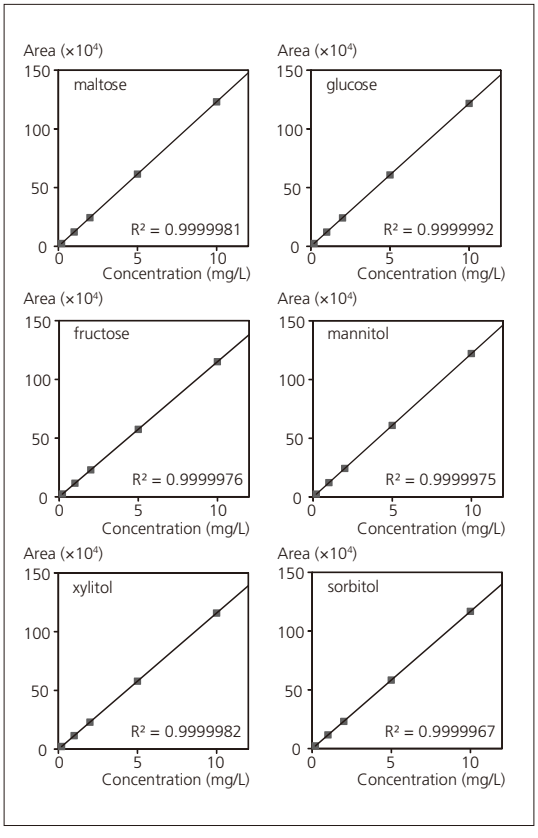

Fig. 3 Calibration Curves of a Standard Mixture of Six Sugars (0.2 – 10 g/L, 10 µL Injected)

Analysis of Energy Drink

Figs. 4 and 5 show the chromatograms obtained from measurement of energy drinks A and B, respectively. Energy drink A was diluted 10:1 with water, and energy B, 20:1 with water, and after each was filtered through a 0.2 µm membrane filter, 10 µL of each sample was injected. The analytical conditions were the same as those of Table 2.

Xylitol and sorbitol were detected in energy drink A, and glucose and fructose were detected in energy drink B. Table 3 shows the quantities of each of these sugars in the respective energy drinks.

Table 3 Content of Respective Sugars in Energy Drinks

	Content (g/L)	
	Energy Drink A	Energy Drink B
Glucose	ND	59
Fructose	ND	101
Xylitol	25	ND
Sorbitol	14	ND

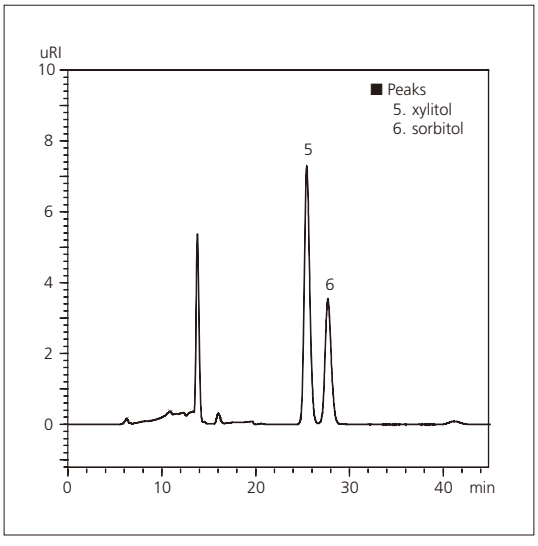

Fig. 4 Chromatogram of Energy Drink A (10 µL Injected)

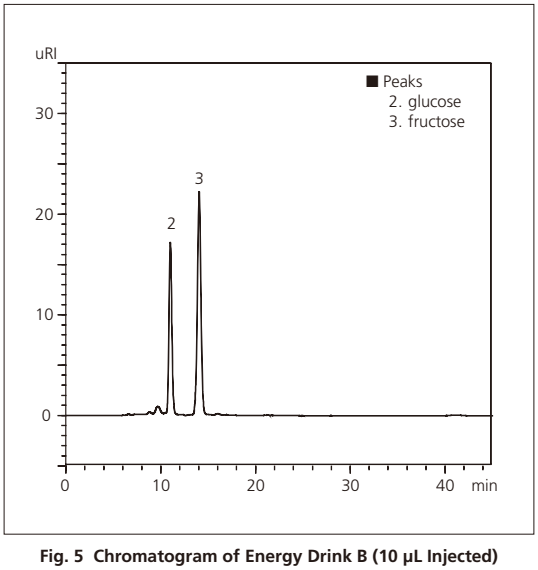

Fig. 5 Chromatogram of Energy Drink B (10 µL Injected)

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LC-MS
Liquid Chromatograph Mass Spectrometer

O-24 High Speed Analysis of Haloacetic Acids in Tap Water Using Triple Quadrupole LC-MS/MS

Haloacetic acids (HAAs), by-products of water disinfection, are formed from naturally-occurring organic and inorganic materials in water which react with the disinfectants chlorine and chloramine. Certain haloacetic acids have been shown to cause adverse reproductive or developmental effects in laboratory animals. Three HAAs regulated by numerous government bodies such as the US EPA include chloroacetic acid (CAA), dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA). A Liquid Chromatography Mass Spectrometry (LC-MS/MS) method for measuring HAAs capable of direct injection of water samples has been developed to replace previously used methods requiring tert-butyl-methyl ether liquid extraction and diazomethane derivitization prior to GC analysis, thus reducing the effort required for sample preparation. Reduced sample preparation times combined with rapid UHPLC chromatography increase the productivity of water control laboratories. This data sheet illustrates results from a high speed method acquired using a LCMS-8050 triple quadrupole mass spectrometer coupled with a Nexera X2 UHPLC.

■ Comparison of Sensitivity and Reproducibility between Standard and High Speed Methods

In the high speed method, CAA, DCAA, and TCAA eluted at 3.1, 3.4, and 5.2 minutes, shortening the run time by 25 minutes relative to the standard method (Figure 1). Figure 2 illustrates each HAA MRM chromatogram and area reproducibility at 0.001 mg/L. Each HAA demonstrates excellent reproducibility and sensitivity at this concentration.

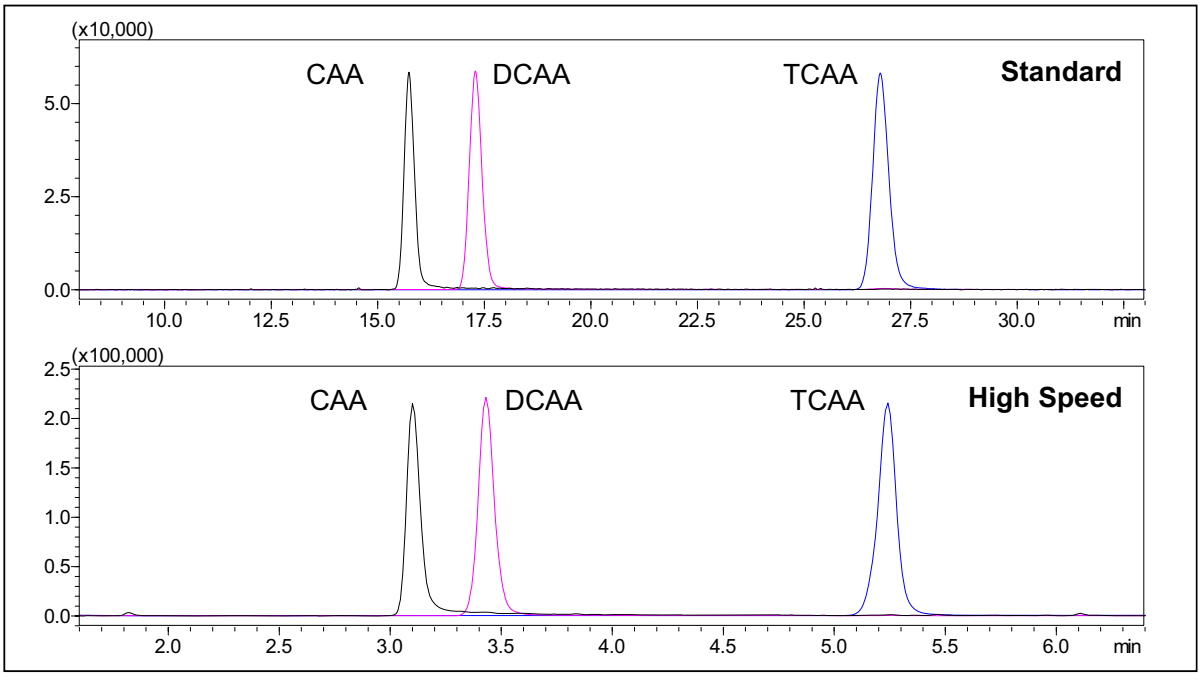

Fig. 1 MRM Chromatograms of Haloacetic Acids
(Top: Standard Analytical Method, Bottom: High Speed Analytical Method)

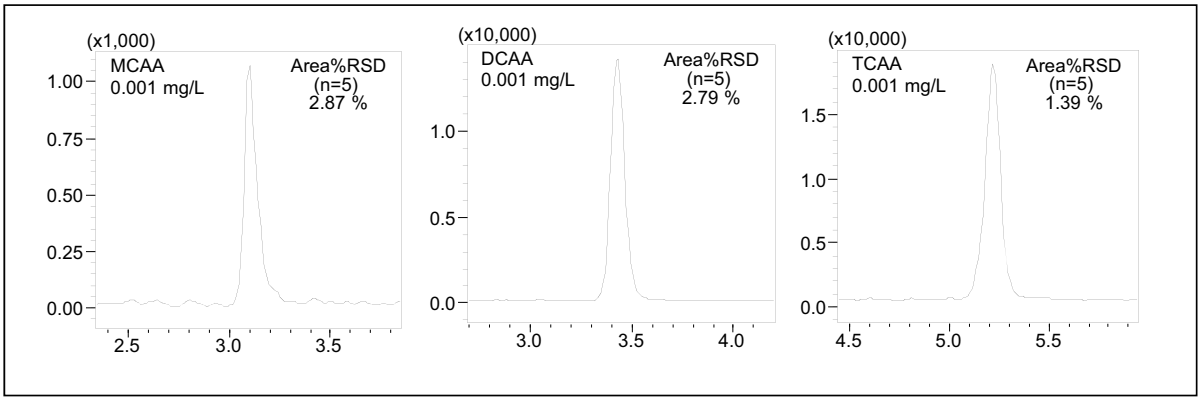

Fig. 2 MRM Chromatograms of CAA, DCAA and TCAA in neat solution at 0.001 mg/L. Reproducibility at 0.001 mg/L, n=5.

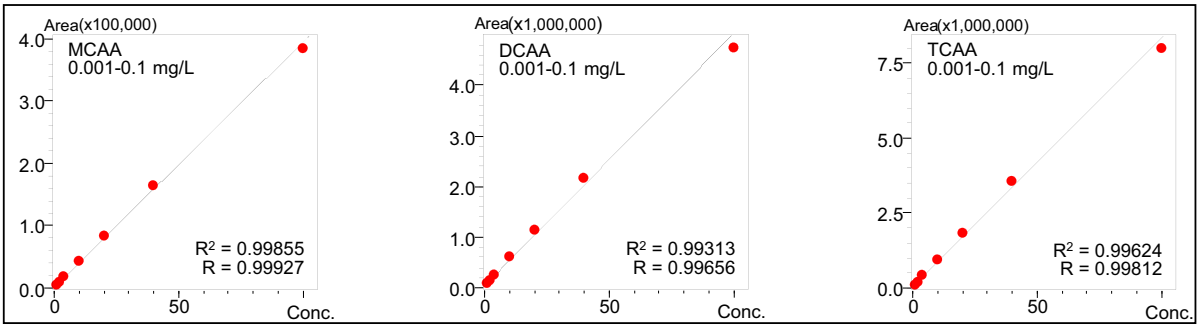

Fig. 3 Linearity of Peak Area of CAA, DCAA and TCAA

Table 1 Analytical Conditions

Column	: CAPCELL PAK MGIII (150 mm X 3 mm, 3 µm)
Mobile Phases	: A 0.2 % Formic acid-water
	: B 0.2 % Formic acid-methanol
Flow Rate	: 0.5 mL/min
Column Temperature	: 50 ℃
Injection Volume	: 25 µL
Probe Voltage	: -3.5 kV (ESI-negative mode)
DL Temperature	: 150 ℃
Block Heater Temperature	: 100 ℃
Interface Temperature	: 100 ℃
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 5 L/min
Heating Gas Flow	: 15 L/min
MRM Transition	: CAA; *m/z* 93.00>35.00, DCAA; *m/z* 126.90>82.90, TCAA; *m/z* 161.10>116.90

■ Recovery Test on Tap Water

A recovery test on tap water from four locations was conducted using this high speed method. Figure 4 demonstrates the quality of chromatograms produced when these three HAAs were spiked at 0.001 mg/L into each of the four tap water samples with no further sample preparation. Regardless of tap water location (Figure 5), excellent recoveries ranging from 90 to 110% were obtained for each sample. (Table 2)

Table 2 Quantitative Results and Recovery Tests of Tap Water

	Sample 1		Sample 2		Sample 3		Sample 4	
	Tap water conc. (mg/L)	Recovery (%)	Tap water conc. (mg/L)	Recovery (%)	Tap water conc. (mg/L)	Recovery (%)	Tap water conc. (mg/L)	Recovery (%)
CAA	Tr.	102.6	0.00076	103.6	0.00069	94.9	0.00034	100.4
DCAA	Tr.	108.3	0.01151	101.7	0.00742	102.9	0.00635	92.3
TCAA	Tr.	107.1	0.00861	107.2	0.00622	104.5	0.00452	102.9

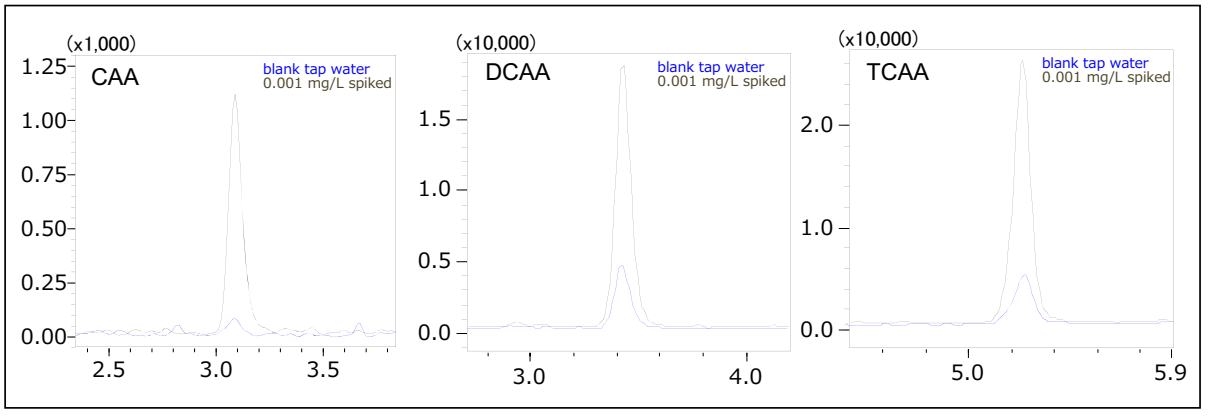

Fig. 4 MRM Chromatograms of Blank Tap Water (Blue) and CAA, DCAA and TCAA Spiked on Blank Tap Water (Sample 1: 0.001 mg/L each)

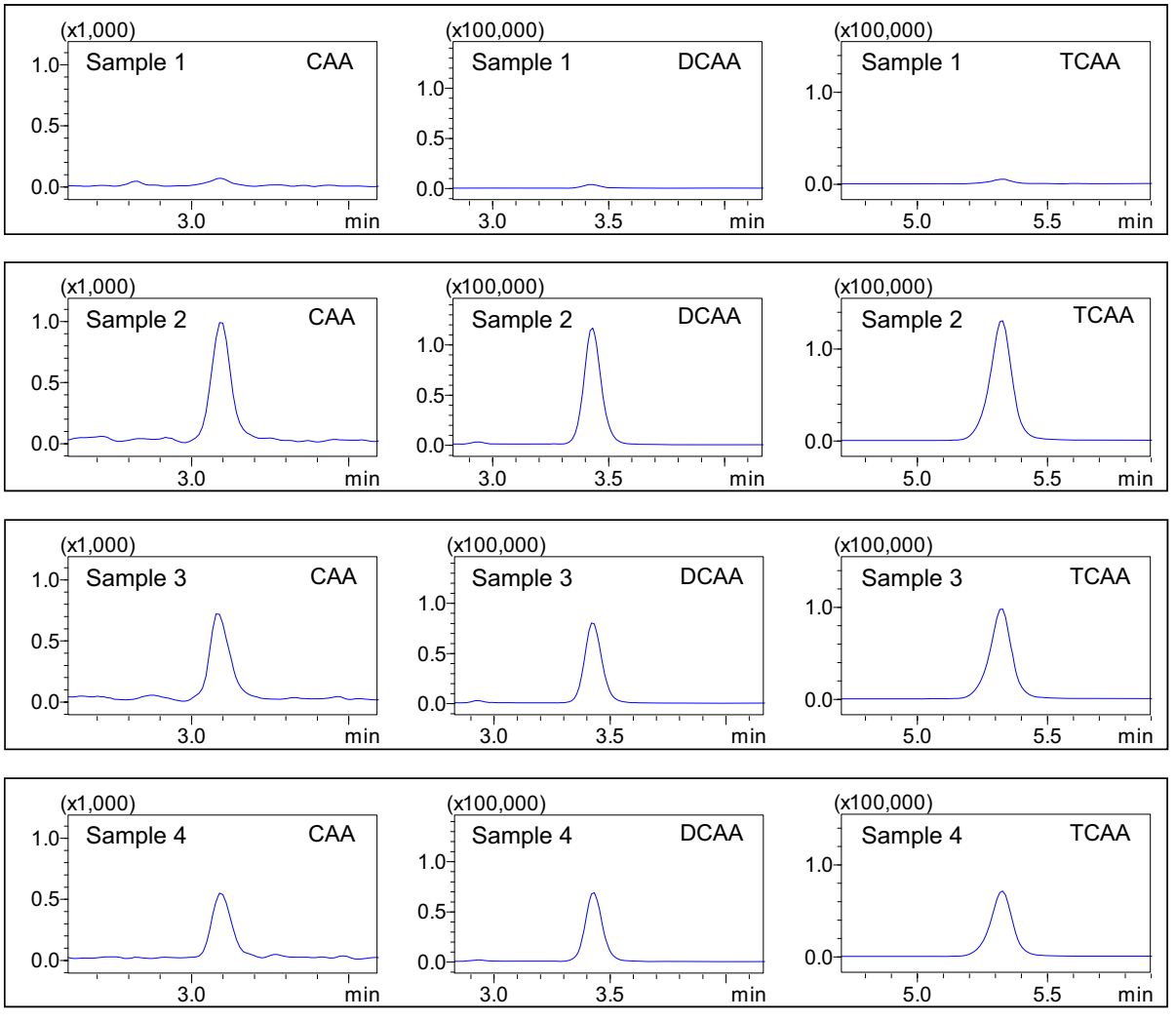

Fig. 5 MRM Chromatograms of Tap Water (Sample 1 to 4)

■ Intuitive Data Processing with LabSolutions Quant Browser

In a busy water control laboratory, it is important to not only increase the speed of measurement but also the throughput of data processing. Quant Browser provides an intuitive, quantitative data processing environment allowing multi-chromatogram visualization of different data files synchronized to analyze a compound of interest.

When measuring analytes from any matrix, there is a possibility of interferences, therefore, the results can be easily reviewed and confirmed by comparing the sample and standard data within a single chromatogram panel.

Figure 6 provides a Quant Browser screen capture displaying a CAA chromatogram from tap water (upper) and from standard solution (lower). With only a glance, it is clear there is no interference in the tap water chromatogram.

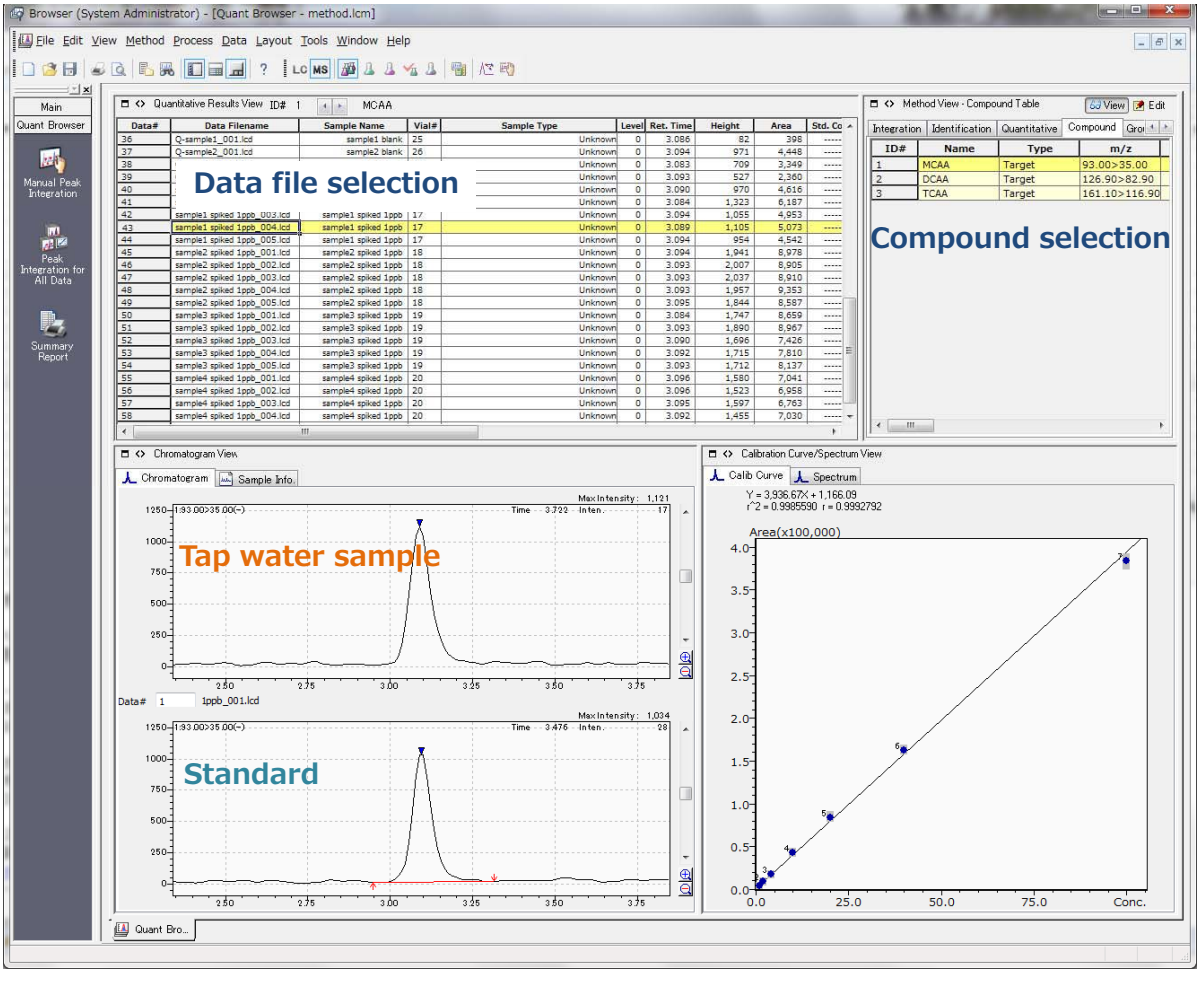

Fig. 6 Multiple Quantitative Data Processing with Quant Browser in LabSolutions

Liquid Chromatography Mass Spectrometry

O-25 Analysis of Phenols in Drinking Water Using Triple Quadrupole LC/MS/MS (LCMS-8040)

Phenols can be formed as wastewater purification and disinfectant by-products, and Japan's Ministry of Health, Labour and Welfare have designated six phenols, including phenol, 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, and 2,4,6-trichlorophenol as subject to water quality standards requirements. The method designated (by the ministry notification) for analysis of these six phenol components is solid-phase extraction – derivatization – GC/MS.
Here, we introduce an example of phenol analysis by UHPLC/MS/MS. Unlike the use of GC/MS for this analysis, LC/MS/MS does not require derivatization, and therefore simplifies the analysis process[1), 2)].

UHPLC/MS/MS Analysis

Sample pretreatment was conducted using the same solid phase extraction procedure as that designated in the notification (solid-phase extraction – derivatization – GC/MS) (Fig. 2). For the solid phase column, an N-containing poly (styrene-divinylbenzene-methacrylic acid) copolymer was used.
Fig. 1 shows the results obtained from measurement of a standard solution containing 0.4 µg/L of each of the six analytical target substances. Since the test water sample concentration is increased 50-fold using solid phase extraction, the equivalent concentration in the test water becomes 0.008 µg/L. Table 1 shows the linearity of the calibration curves over a concentration range equivalent to 0.008 to 1 µg/L in the test water sample, and the repeatability using a concentration of 0.008 µg/L. Excellent linearity and repeatability were obtained with respect to all of the components.

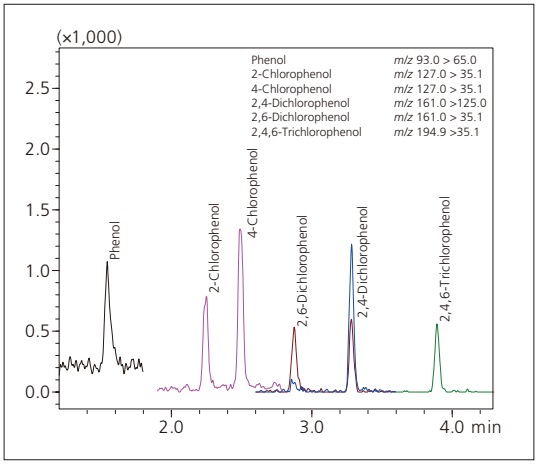

Fig. 1 Mass Chromatograms (MRM) of Phenols

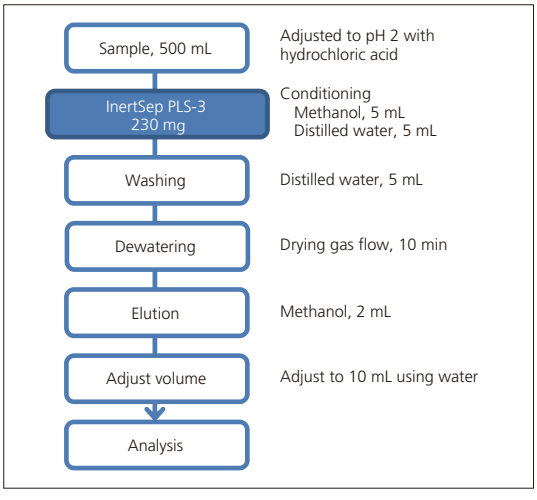

Fig. 2 Pretreatment Flow

Table 1 Calibration Curves and Repeatability

	Injection Sample Concentration (µg/L)	Test Water Sample Concentration (µg/L)	Coefficient of Determination R^2	Area Repeatability %RSD (Calibration point minimum concentration)
Phenol	0.4 – 50	0.008 – 1	0.99938	7.4
2-Chlorophenol	0.4 – 50	0.008 – 1	0.99967	4.5
4-Chlorophenol	0.4 – 50	0.008 – 1	0.99960	5.0
2,4-Dichlorophenol	0.4 – 50	0.008 – 1	0.99966	3.9
2,6-Dichlorophenol	0.4 – 50	0.008 – 1	0.99960	7.0
2,4,6-Trichlorophenol	0.4 – 50	0.008 – 1	0.99960	7.8

Spike and Recovery Test for Drinking Water

Using this analytical method, we conducted spike and recovery testing of the phenols in tap water. Fig. 3 shows mass chromatograms (MRM) of a blank tap water sample subjected to pretreatment, and a test water sample spiked with six different phenol compounds, each at a concentration equivalent to 0.08 µg/L in the test sample. These spike concentrations were approximately equivalent to 1/10 the reference values of the phenols (in terms of the amount of phenol, 0.005 mg/L or less). Regarding the tap water samples analyzed here, there was no indication of significant interference due to contaminating components (Fig. 3). In addition, good recoveries were obtained, ranging between and 90 to 110 % (Table 2).

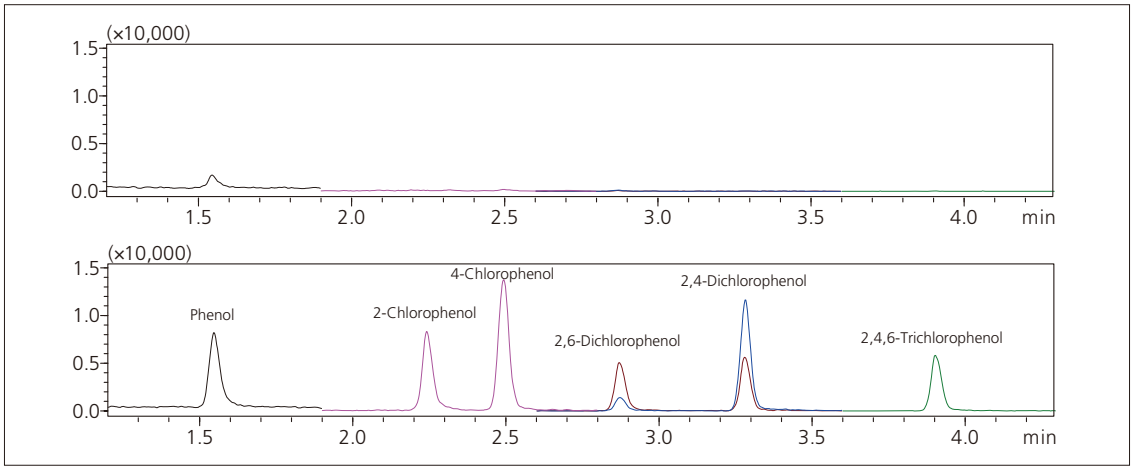

Fig. 3 Mass Chromatograms (MRM) of Drinking Water (Upper: Blank, Lower: 0.08 µg/L spiked)

Table 2 Results of Spike and Recovery Test (n=5)

	Recovery % (Corresponding to 0.08 µg/L)	Recovery % (Corresponding to 0.4 µg/L)
Phenol	103.7	99.6
2-Chlorophenol	104.8	100.1
4-Chlorophenol	104.1	100.2
2,4-Dichlorophenol	104.6	100.4
2,6-Dichlorophenol	102.0	100.3
2,4,6-Trichlorophenol	105.6	99.3

Table 3 Analytical Conditions

Column	: InertSustain C18 HP (100 mm L. × 2.1 mm I.D., 3 µm)
Mobile Phases	: A) Water
	: B) Methanol
Flowrate	: 0.5 mL/min
Time Program	: B conc. 40 % (0 min) – 95 % (4.8 – 5.4 min) – 40 % (5.41 – 7.5 min)
Column Temperature	: 40 °C
Injection Volume	: 50 µL
Probe Voltage	: -3.5 kV (APCI-negative mode)
DL Temperature	: 200 °C
Block Heater Temperature	: 200 °C
Interface Temperature	: 350 °C
Nebulizing Gas Flow	: 3 L/min (Air)
Drying Gas Flow	: 5 L/min (N_2)
MRM Transition	: Phenol: *m/z* 93.0 > 65.0, 2-Chlorophenol: *m/z* 127.0 > 35.1, 4-Chlorophenol: *m/z* 127.0 > 35.1, 2,4-Dichlorophenol: *m/z* 161.0 > 125.0, 2,6-Dichlorophenol: *m/z* 161.0 > 35.1, 2,4,6-Trichlorophenol: *m/z* 194.9 > 35.1

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GC-MS
Gas Chromatograph Mass Spectrometer

O-26 Analysis of Fatty Acids Using PCI-GC-MS/MS

INTRODUCTION

While some fatty acids, such as the n-3 fatty acids EPA and DHA, are beneficial to human health because they lower the amount of blood-borne neutral fat, too much intake of saturated fatty acids raises the risk of some diseases. For this reason, there is a need for the batch analysis of these fatty acids in the life sciences and food engineering sectors. Despite requiring methylation, GC-MS has gained attention because of its suitability for multicomponent batch analyses.

In fatty acid analyses utilizing GC-MS, the EI (electron ionization) method is used for ionization. With the EI method, there are many types of fragment ions, making it easy to select an *m*/*z* to enable separation by mass from impurities. However, because of the large number of fragment ions, the sensitivity of the individual ions is reduced, making it difficult to detect trace quantities of fatty acids. In contrast, with the PCI (positive chemical ionization) method, protonated molecular ions can be detected, from which molecular weight data can be obtained. Since there is only a small number of fragment ion types, the sensitivity is increased. This means, however, that the ion types that can be selected for monitoring are limited and there may not be any ions that can be separated by mass from impurities.

This application data sheet introduces the results of an investigation of sensitivity based on the EI-SIM, PCI-SIM, EI-MRM, and PCI-MRM methods. In addition, in Application Data Sheet No. 86, we introduce the results of an investigation of separation from impurities in the analysis of fatty acids in foods.

EXPERIMENTAL

The Supelco® 37 Component FAME Mix (P/N: 47885-U, SIGMA-ALDRICH) was utilized as the standard sample. The standard sample was diluted in stages with dichloromethane, and used for sensitivity evaluation. Table 1 shows the analysis conditions. Analysis methods included in the GC/MS Metabolite Database Ver. 2 were used for the monitoring *m*/*z* and for the EI-SIM, PCI-SIM, EI-MRM, and PCI-MRM methods.

Table 1: Analysis Conditions

GC-MS:	GCMS-TQ8030
Column:	SP-2560 (Length 100 m; 0.20 mm I.D.; df = 0.25 μm)
Glass insert:	Splitless insert with wool (P/N: 221-48876-03)
[GC]	
Sample injection unit temp.:	250 °C
Column oven temp.:	40 °C (2 min) → (4 °C /min) → 240 °C (15 min)
Injection mode:	Split
Split ratio:	10
Carrier gas control:	Linear velocity (20.0 cm/sec)
Injection volume:	1 μL

[MS]	
Interface temp.:	250 °C
Ion source temp.:	200 °C
Measurement mode:	
GC-MS:	SIM
GC-MS/MS:	MRM
Ionization method:	EI and PCI methods
PCI reagent gas:	Isobutane
PCI reagent gas pressure:	70 kPa

RESULTS AND DISCUSSION

Fig. 1 shows mass chromatograms for 100 pg methyl cis-10-heptadecenoate (Z, 17:1n-7) obtained in the analysis modes. Sensitivity was evaluated by calculating the lower limit of quantitation using a t-test on the area reproducibility results for which the %RSD was 20 % or less with the analysis repeated 8 times. The lower limits of quantitation in each analysis mode are shown in Table 2. Table 3 shows the efficacy of each analysis mode for saturated and unsaturated fatty acids, with analysis methods having a sensitivity difference within twice the lower limit of quantitation for the analysis method offering the highest sensitivity taken as advantageous. For unsaturated fatty acids, the PCI method was superior to the EI method.

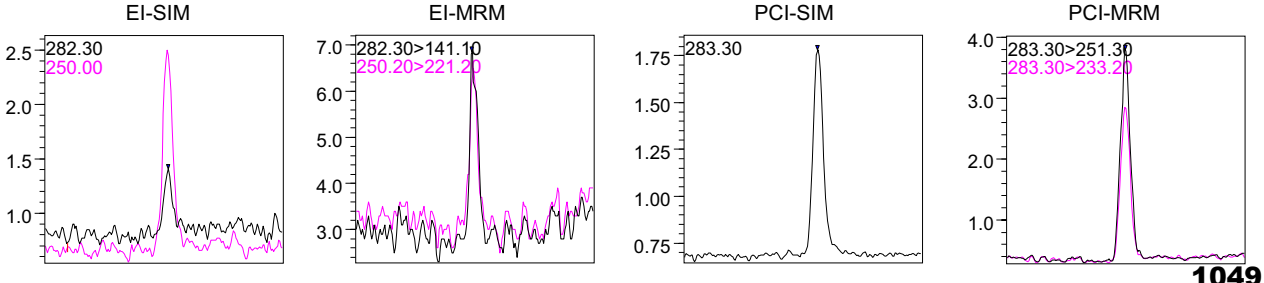

Fig. 1: Mass Chromatograms for Methyl Cis-10-Heptadecenoate (Z, 17:1n-7) Measured in Individual Analysis Modes

Table 2: Lower Limits of Quantitation in Each Mode for 37 Fatty Acid Methyl Esters

		EI SIM LOQ (pg)	EI MRM LOQ (pg)	PCI SIM LOQ (pg)	PCI MRM LOQ (pg)
1	Methyl butanoate;4:0	26.0	2.8	8.3	1.9
2	Methyl caproate;6:0	39.2	3.5	5.9	3.6
3	Methyl caprylate;8:0	53.9	10.5	4.8	4.8
4	Methyl caprate;10:0	36.8	32.9	7.2	8.8
5	Methyl undecanoate;11:0	44.0	22.0	30.1	20.6
6	Methyl laurate;12:0	74.4	56.5	6.6	10.9
7	Methyl tridecanoate;13:0	42.2	48.7	5.2	13.2
8	Methyl myristate;14:0	69.7	5.6	5.5	10.1
9	Methyl myristoleate;(Z)14:1n-5	134.0	178.3	4.7	2.7
10	Methyl pentadecanoate;15:0	32.1	29.3	4.8	36.6
11	Methyl cis-10-pentadecenoate;(Z)15:1n-5	33.7	225.3	4.0	4.6
12	Methyl palmitate;16:0	74.5	12.6	7.3	15.2
13	Methyl palmitoleate;(Z)16:1n-7	249.5	36.0	19.0	16.2
14	Methyl margarate;17:0	22.0	5.6	20.0	29.2
15	Methyl cis-10-heptadecenoate;(Z)17:1n-7	245.9	215.7	22.1	14.5
16	Methyl stearate;18:0	11.6	10.2	8.9	35.3
17	Methyl elaidate;(E)18:1n-9	173.9	180.7	5.5	19.8
18	Methyl oleate;(Z)18:1n-9	58.1	353.2	6.4	9.8
19	Methyl linolelaidate;(E)18:2n-6	52.0	253.9	28.8	23.2
20	Methyl linoleate;(Z)18:2n-6	160.7	297.9	23.2	16.5
21	Methyl arachisate;20:0	11.6	9.1	11.5	58.5
22	Methyl ganma-linolenate;(Z)18:3n-6	349.8	167.8	17.6	81.2
23	Methyl cis-11-icosenoate;(Z)20:1n-9	145.1	45.1	22.0	36.0
24	Methyl linolenate;(Z)18:3n-3	213.1	414.6	23.9	135.7
25	Methyl heneicosanoate;21:0	41.0	33.7	25.5	108.5
26	Methyl cis-11,14-Icosadienoate;(Z)20:2n-6	238.4	282.1	13.4	36.5
27	Methyl behenate;22:0	7.0	29.6	23.3	279.0
28	Methyl eicosa-8,11,14-trienoate;20:3n-6	140.5	405.2	31.7	220.3
29	Methyl erucate;22:1n-9	143.3	387.8	31.2	96.1
30	Methyl cis-11,14,17-Icosatrienoate;(Z)20:3n-3	446.0	-	24.3	284.5
31	Methyl tricosanoate;23:0	24.8	54.2	19.3	357.2
32	Methyl arachidonate;(Z)20:4n-6	292.2	181.2	45.5	151.7
33	Methyl cis-13,16-Docosadienate;(Z)22:2n-6	283.2	335.3	315.7	128.1
34	Methyl lignocerate;24:0	10.3	52.6	41.8	503.8
35	Methyl cis-5,8,11,14,17-Eicosapentaenoate;(Z)20:5n-3	437.4	286.5	54.9	184.9
36	Methyl nervonate;(Z)24:1n-9	230.7	445.2	56.5	99.4
37	Methyl cis-4,7,10,13,16,19-Docosahexaenoate;(Z)22:6n-3	281.7	161.5	304.2	255.7

Notes:
- Compound names in blue indicate unsaturated fatty acids.
- [ ] cells indicate that the LOQ is within twice the value for the analysis mode with the highest sensitivity.

Table 3: Sensitivity Predominance for Each Analysis Mode

Measurement Mode	Total Fatty Acids	Saturated Fatty Acids	Unsaturated Fatty Acids
EI SIM	7	6	1
EI MRM	10	9	1
PCI SIM	32	13	19
PCI MRM	18	7	11

Notes:
- The analysis methods having a sensitivity difference within twice the lower limit of quantitation for the analysis method offering the highest sensitivity are taken as advantageous.

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GC-MS
Gas Chromatograph Mass Spectrometer

O-27 Analysis of Fatty Acids in Food Using PCI-GC-MS/MS

INTRODUCTION

While some fatty acids, such as the n-3 fatty acids EPA and DHA, are beneficial to human health because they lower the amount of blood-borne neutral fat, too much intake of saturated fatty acids raises the risk of some diseases. For this reason, there is a need for the batch analysis of these fatty acids in the life sciences and food engineering sectors. Despite requiring methylation, GC-MS has gained attention because of its suitability for multicomponent batch analyses.

In fatty acid analyses utilizing GC-MS, the EI (electron ionization) method is used for ionization. With the EI method, there are many types of fragment ions, making it easy to select an *m*/*z* to enable separation by mass from impurities. However, because of the large number of fragment ions, the sensitivity of the individual ions is reduced, making it difficult to detect trace quantities of fatty acids. In contrast, with the PCI (positive chemical ionization) method, protonated molecular ions can be detected, from which molecular weight data can be obtained. Since there is only a small number of fragment ion types, the sensitivity is increased. This means, however, that the ion types that can be selected for monitoring are limited and there may not be any ions that can be separated by mass from impurities.

This application data sheet introduces the results of an investigation of separation from impurities based on the EI-SIM, PCI-SIM, EI-MRM, and PCI-MRM methods. In addition, in Application Data Sheet No. 85, we introduce the results of an investigation of sensitivity in the analysis of fatty acids in foods.

EXPERIMENTAL

Saury (fish) was used to investigate the separation from impurities in each analysis mode. The fatty acid methylation kit (P/N: 06482) sold by Nacalai Tesque was utilized for the pretreatment. The pretreatment method is shown in Fig. 1.

The edible flesh from the saury was collected and pulverized with a mill, after which 200 mg was measured out. After adding 2 mL of the extraction liquid and agitating, the mixture was centrifuged, and 500 μL of extracted liquid was obtained. The extracted liquid was dried under a nitrogen flow, and 500 μL each of reagents A and B were added. After leaving the mixture to stand for 1 hour at 37 °C, 500 μL of reagent C was added, and it was left to stand at 37 °C for a further 20 minutes. Afterward, 2 mL of the extraction liquid was added, and after centrifuging, the organic phase was collected. Deionized water was used to clean 1 mL of the organic phase, resulting in the test solution.

Refer to Application Data Sheet No. 85 for the analysis conditions for the EI-SIM, PCI-SIM, EI-MRM, and PCI-MRM methods.

Analysis methods included in the GC/MS Metabolite Database Ver. 2 were used for the analysis conditions and monitoring *m*/*z*.

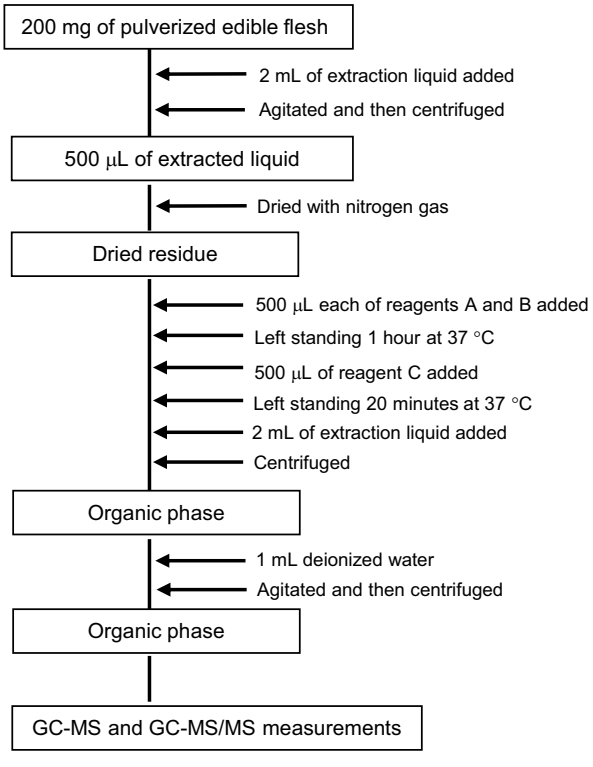
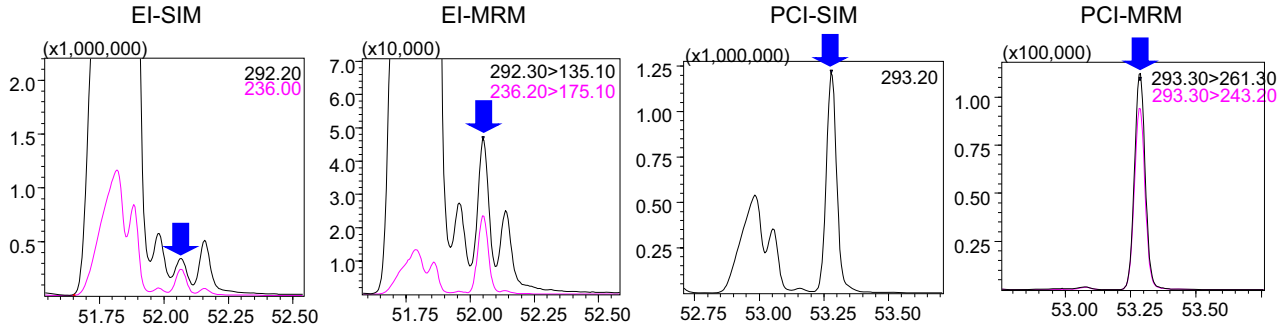


Fig. 1: Pretreatment of the Saury

RESULTS AND DISCUSSION

The sample extracted from the saury was measured in each analysis mode, and the separation from impurities investigated. Most of the fatty acid methyl esters could be separated from the impurities regardless of the analysis mode. However, a portion of the fatty acids was hard to completely separate from the impurities, both with EI-SIM and EI-MRM. Fig. 2 shows examples of measuring fatty acid methyl esters for which the degree of separation from impurities varied significantly depending on the analysis mode. Methyl linolenate;(Z)18:3n-3 and methyl cis-11,14,17-Icosatrienoate;(Z)20:3n-3 were hard to separate from the impurities, both with EI-SIM and EI-MRM. Some degree of separation was possible with PCI-SIM, but there was only one kind of monitoring *m*/*z*, so problems with peak identification could be expected. In contrast, with PCI-MRM, mass separation excluded impurities eluted nearby, making peak identification easy.

Methyl linolenate;(Z)18:3n-3



Methyl cis-11,14,17-Icosatrienoate;(Z)20:3n-3

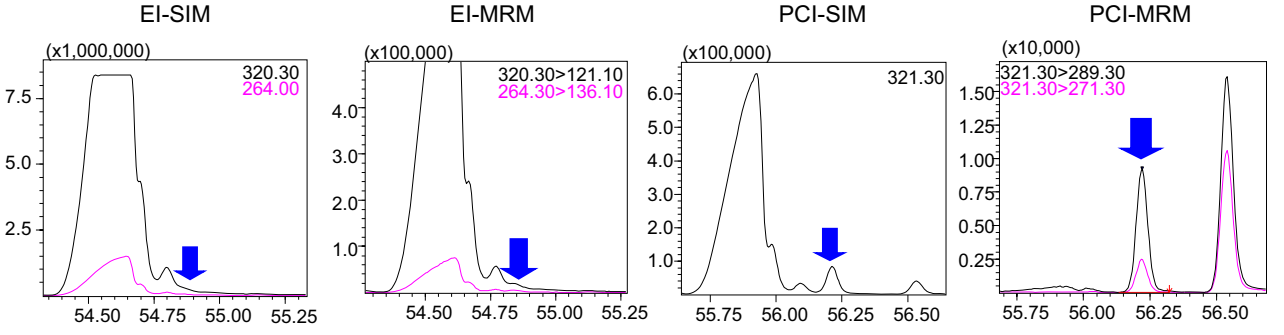


Fig. 2: Mass Chromatograms for Methyl linolenate;(Z)18:3n-3 and Methyl cis-11,14,17-Icosatrienoate;(Z)20:3n-3 Contained in an Extract of Saury Measured in Individual Analysis Modes

CONCLUSION

We analyzed fatty acids in foods to investigate mass separation from impurities and sensitivity for the EI-SIM, EI-MRM, PCI-SIM, and PCI-MRM analysis modes. The results revealed that, as shown in Application Data Sheet No. 85, the PCI method is the most sensitive, and for unsaturated fatty acids in particular, provides more sensitive detection than the EI method. Also, PCI-MRM was found to be the most ideal for mass separation from impurities, making peak identification easy.
It is thus evident that the PCI-MRM method is effective for multicomponent batch analyses of fatty acids.

5. TIPS

Few technical tips:

Selecting a Mass spectrometer-

Triple quadrupole mass spectrometer is one of the most powerful analytical tools when it comes to specifically quantitating analytes in presence of complex matrices. Following are the list of few points one may look into when selecting a triple quadrupole mass spectrometer for Food safety analysis.

1) This specificity of a triple quadrupole mass spectrometer is attributed to the feature of MRM. Hence, fast switching of MRM channels during the course of an analysis is essential to obtain reliable and reproducible data for large number of analytes. This capability can be understood by the dwell time and pause time ranges of a system.
2) In addition, due to presence of common fragment ions resulting from different target analyte molecules belonging to the same group of compounds, it is essential that the triple quadrupole system is capable of fast elimination of residual daughter fragments to prevent crosstalk.
3) With such a huge number of targeted molecules, optimization of instrument conditions (eg voltages, MRM transitions and so on) is preferred to be automatic rather manual.
4) In a multi-analyte analysis, due to need of high throughput, it is preferred to analyse both positive and negative analytes in a single run. The polarity switching time of a system can give a glimpse into whether a system needs separate runs to detect positive and negative analyte molecules.
5) Since food matrix is complex, system maintenance is an essential part of analysis. It is, therefore, prefrerred to have a system that gives minimum downtime and requires minimum skill for cleaning and maintaining the system. For example, changing of probe of cleaning of capillary and cone etc. must be easily possible at user level without much expertise required.

Other tips:

Since prevention is better than cure, it is always helpful to take precautionary measures during analysis to increase the system's productive hours and reduce downtime.

1) Use of guard column is always advisable to prevent frequent contamination of the anlaytical column as well as the analytical system.
2) Use of semi-micro column (~2 mm I.D.) are preferred over conventional columns (~ 4.6 mm I.D.) since it gives higher sensitivity w.r.t better S/N ratio.
3) Flow control valves to swtich the entry of LC eluate between waste and the mass spectrometer helps to send only the peaks of interest into the mass spectrometric system and channel out contaminants to waste. This is very useful to prevent frequent contamination of mass spectrometric system thereby aiding higher throughput.
4) Always filter samples and buffers through 0.22 µ filters before analysis. The chemistry of filters may need to be considered based of sample nature. [Refer annexure 1 for more details]
5) Mobile phase and sample solvents must be selected to aid ionization and care must be taken to prevent ion suppression. [refer annexure 2 for mobile phase selection guide].
6) Purge auto-sampler at least once in a day. R0 solution should be ideally same as mobile phase composition without buffers/ any additives.
7) Purge pumps before starting any analysis and also when mobile phase is changed or any air bubbles in the flow line.
8) Use only LCMS grade solvent for the analysis.
9) Avoid using too high or too low flow rates. Optimum flow rates for ESI analyses are 0.2 - 0.5 mL/min. For APCI, it can be between 0.6 - 1 mL/min. Lower flow rate is favored for reduced background level and contamination of MS. Use splitter whenever required.

10) During MRM, use of qualifier ions alongwith quantifiers is suggested. This increases specificity of the target analyte by distinguishing it from interfering matrix components.
11) Use of internal standard alongwith the target analytes will give a more reliable data by controlling false positive and false negative results to a very great extent.
12) When adduct ions are encountered, one may decide to select these ions for MRM if they are stable adduct forming as a result of mobile phase additives. However, if these adducts are undesirable, then, one may need to adjust temperatures, gas flow rates or voltages so as to break these adducts.
13) For multi-target analytes, Interface parameters such temperature, gas flow rates and voltages are set generally as default values. However, if some components show decreased sensitivity, one may need to adjust these parameters such that these signals for these components increase without much compromise on the other analytes.
14) Time based staggering of MRMs may be done for better data quality when number of target analytes are very high. In this case one has to careful on the selection of column and its availability since these staggered MRM transitions are retention time based.
15) Dwell times are to be adjusted so as to obtain reproducible and reliable data quality. Adjust dwell times such that the loop times are not too high [preferably <0.5sec].
16) Wipe the heater block and sampling cone (curtain plate), heater flange, inside wall of ionization unit with water/methanol in case of soiling. Use lint free tissue paper (e.g. Kim Wipe) for the same.
17) In case of soiling or high background noise after continuous analysis, baking of the system can be performed. For this purpose, ACN/methanol can be passed to MS at flow rate of 0.3-0.6 mL/min. Temperature of MS (Interface, heat block and desolvation line) can be set to maximum. This step has to be performed only for 1-2 hrs. Do not use column when performing this step.(Note: In case of APCI,

use only methanol). Refer respective instruction manuals for detailed steps on maintenance.

18) Always keep minimum consumable spares in stock. Also, ensure that the system regularly undergoes preventine maintenance to prevent unnecessary downtime.

ANNEXURE 1

Membrane Selection Guide

Syringe Filter Membrane Selection Guide

When filtering samples, it is important to use the correct membrane for your application. This guide should help you determine which membrane is most suitable for your requirements.

Cellulose Acetate (CA)

Cellulose acetate is a very low protein binding membrane that is ideal for aqueous based samples and biological samples. It is a lower protein binder than either polyethersulphone or PVDF membranes, but has a lower chemical resistance than regenerated cellulose. CA is a strong membrane mechanically and hydrophilic.

Membrane: HPLC Certified Cellulose Acetate
Protein Binding: <24 µg/cm2
Use with: Aqueous samples
Don't use with: Organic solvents
Sterilisation: Syringe filters can be sterilised by autoclave at 125° for 15 minutes.
Applications: Biological samples, tissue culture media filtration, preparation of aqueous samples for HPLC

Glass Microfibre (GMF)

Glass Microfibre filters should be used as a pre-filter for samples with a high particulate content. GMF membranes are available with a higher pore size than other membranes and is tolerant to most solvents.

Membrane: Binder-free Glass Microfiber

Use with: Heavily contaminated samples
Don't use with: Benzyl alcohol
Sterilisation: Syringe filters can be sterilised by autoclave at 125° for 15 minutes.
Applications: Dissolution testing, filtration of high particulate samples, air filtration, recovery of DNA from biological samples

Nylon

Nylon membranes are extremely low in extractables and mechanically very strong. They possess good thermal stability up to 50°C and are a good all-round filter for HPLC samples. Naturally hydrophilic membrane that provides broad compatibiity with aqueous and organic samples.

Membrane: HPLC Certified Nylon.
Use with: Bases, Most HPLC solvents, Alcohols, Aromatic Hydrocarbons, THF.
Don't use with: Acids, Aggressive Halogenated hydrocarbons, protein samples (Nylon is a high binder)
Sterilisation: Syringe filters can be sterilised by autoclave at 125° for 15 minutes.
Applications: General Laboratory filtration, most HPLC samples

Polyethersulphone (PES)

Polyethersulphone is a hydrophilic membrane that has very low protein binding and high flow characteristics. It is also certified for ion chromatography. Polyethersulphone is more heat resistant than most membranes, and can be used to 100°C.

Membrane: ICP Certified PES (Polyethersulfone)
Use with: Strong bases, alcohols, proteins, peptides
Don't use with: acids, ketones, esters, halogenated or aromatic hydrocarbons.

Sterilisation: Syringe filters can be sterilised by autoclave at 125° for 15 minutes.
Applications:Ion chromatography, tissue culture filtration, filtration of proteins and nucleic acids, high-temperature liquids.

Polypropylene (PP)

Hydrophilic polypropylene membranes are chemically resistant and suitable for a wide variety of organic and aqueous based samples. They are low protein binders and can be used with strong acids and bases without prewetting.

Membrane: Hydrophilic Polypropylene
Use with: Acids/Bases, general HPLC analysis
Limited resistance with: MeCl and Chloroform.
Sterilisation: Syringe filters can be sterilised by autoclave at 125° for 15 minutes.
Applications: Filtration of biological samples, aggressive organic solvents.

PTFE

Polytetrafluoroethylene (PTFE) membranes are chemically resistant to nearlly all solvents, acids and bases. The membrane has low extractables and good thermal stability. PTFE is hydrophobic and requires prewetting prior to use with aqueous solvents.

Membrane: HPLC certified PTFE, with polypropylene support

Use with: Aggressive solvents, strong acids, alcohols, bases, aromatics
Don't use with: Aqueous samples without prewetting (causes high backpressure)
Sterilisation: Syringe filters can be sterilised by autoclave at 125° for 15 minutes.
Applications: Filtration of aggressive organic or highly basic solutions, transducer protectors.

PVDF

Polyvinylidene difluoride is a hydrophilic membrane that is suitable for most general biological sample filtration. It has a broad chemical compatibility and is a low protein binder and so a good choice where high protein recovery is required.

Membrane: HPLC-certified PVDF
Use with: Alcohols, weak acids, proteins, peptides and other biomolecules
Don't use with: Some strong acids, bases, esters, ethers or ketones.
Sterilisation: Syringe filters can be sterilised by autoclave at 125° for 15 minutes.
Applications: General biological filtration, filtration of samples for high protein recovery.

Regenerated Cellulose (RC)

Regenerated cellulose is a hydrophilic solvent resistant membrane. It is a very low protein binder and is ideal for low nonspecific binding applications; tissue culture media filtration and general biological sample filtration

Membrane: HPLC Certified Regenerated Cellulose
Use with: Proteins, Peptides and other biomolecules
Don't use with: Strong acids, Chloroform, THF.
Sterilisation: Syringe filters can be sterilised by autoclave at 125° for 15 minutes.
Applications: Low nonspecific binding applications, Tissue culture media filtration and general biological sample filtration.

Annexure 2

Mobile phase for LCMS

- For ESI interface in LCMSMS, acetonitrile/water and methanol/water are solvents of choice to start with.
- For APCI interface, methanol/water is recommended; acetonitrile/ water may cause coking on the corona needle in negative mode.
- The ratio of solvents may not be so critical for ionisation, but higher water content may cause lower ionization efficiency. However, ratio plays an important role in peak elution.
- Gradient elution with changing aqueous phase between 0 and 100% can be used.
- Suitable solvents for LCMS samples include water, acetonitrile, methanol, ethanol, n-propyl alcohol, Isopropanol, t- Butyl alcohol, acetone, pyridine, THF, aniline, chloroform etc
- Non-suitable solvents include Benzene, Toluene, Hexane, Cyclohexane etc.

Buffers and pH control for LCMS:

- Non-volatile buffer like phosphate is not recommended. Use volatile buffer to replace phosphate buffer, for eg: ammonium acetate/formate, ammonia, trifluoroacetic acid, etc
- Using a lower buffer concentration eg: 1 – 50 mM is desirable to prevent ion suppression.

Generally, pH mobile phase or solvent used governs the type of ions formed, for eg: acidic pH aids positive ion formation whereas basic pH causes negative ion formation. The following guide may aid buffer selection for pH control:

- pH 1.8 - 2.5 : TFA, conc. < 0.1%
- pH 2.5 - 4 : Formic Acid, conc. ~ 0.1%

- pH 4 - 5 : Acetic Acid, conc. 0.1~5%
- pH 7 : ammonium acetate, ammonium formate
- pH > 7 : ammonia aqueous solution

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