Application Note: 525

Streamlined Analysis of 400+ Pesticides in a Single Run Using the TSQ Quantum Access MAX Mass Spectrometer and TraceFinder Software

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Key Words

- TSQ Quantum Access MAX
- TraceFinder software
- T-SRM
- Pesticide analysis
- Food safety

Introduction

Growing concerns over food safety and the expanding world agricultural trade have lead to the enforcement of stricter pesticide regulations. In 2006, Japan introduced the Positive List System that established maximum residue levels (MRLs) for hundreds of agricultural chemicals in food, including approximately 400 pesticides, and set a uniform limit of 10 µg/kg (ppb) for chemicals for which MRLs have not been determined.[1] In 2008, the European Parliament implemented Regulation (EC) No. 396/2005, which harmonized all pesticide MRLs for European Union (EU) member states and set default limits of 10 µg/kg for all pesticide/commodity combinations for which no MRLs have been set.[2] A pesticide safety review of about 1,000 active substances on the market was mandated by EU Directive 91/414/EEC and, upon its completion in 2009, led to the approval of only about 250 substances and effectively set the permissible levels of over 700 de-listed pesticides to the default limit.[3] The EU and Japanese regulations are among the most stringent in the world and have fueled the need for faster and more sensitive analytical methods for cost-efficient, high-throughput screening and quantitation of multi-class pesticide residues.

Liquid chromatography-triple quadrupole tandem mass spectrometry (LC/MS/MS) enables highly selective, targeted, and sensitive quantitation and confirmation of hundreds of target pesticides in a single run. A multi-residue method was developed for screening and quantitation of 437 pesticides in one 45-minute run using Thermo Scientific TraceFinder software and a Thermo Scientific TSQ Series LC-MS/MS system. At least one, and often two or three, ion ratios were used to confirm each analyte. In addition, the use of the Quantitation-Enhanced Data-Dependent scan mode (QED-MS/MS) provided MS/MS mass spectra that was used for structural confirmation.

Goal

To analyze large numbers of pesticides in a single run on a triple quadrupole mass spectrometer using TraceFinder™ software with built-in workflows for streamlining method development and routine analysis.

Experimental Conditions

Sample Preparation

Pesticide standards were obtained from the U.S. Food and Drug Administration (FDA). The stock solution was prepared at a concentration of 3 mg/L. Calibration solutions, with concentrations of 0.1-250 µg/L (ppb), were prepared by serial dilution of the stock solution in 50:50 (v/v) acetonitrile/water.

Apple, orange, and asparagus matrices were prepared for analysis by using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, which is a sample preparation procedure used to extract pesticides from food.[4] The QuEChERS extracts were obtained from California Department of Food and Agriculture. For the QuEChERS extraction, 15 g of homogenized sample and 15 mL of acetonitrile were used. Then, 200 µL of final QuEChERS extract, 300 µL of acetonitrile, and 500 µL of water were transferred into an autosampler vial, spiked with 20 µL of the pesticides standard, and mixed well.

HPLC

Chromatographic analysis was performed using the Thermo Scientific Accela 1250 U-HPLC system. The autosampler was an HTC-PAL Autosampler (CTC Analytics, Zwingen, Switzerland). The chromatographic conditions were as follows:

Column:	Thermo Scientific Hypersil GOLD aQ column (100 x 2.1 mm, 1.9 µm particle size)		
Mobile Phase A:	Water with 0.1% formic acid and 4 mM ammonium formate		
Mobile Phase B:	Methanol with 0.1% formic acid and 4 mM ammonium formate		
Flow Rate:	300 µL/min		
Column Temperature:	40 °C		
Sample Injection Volume:	10 µL		
Gradient:	Gradient Time (min)	%A	%B
	0.00	98	2
	0.25	70	30
	35.00	0	100
	40.00	0	100
	40.01	98	2
	45.00	98	2

MS

All samples were analyzed on the Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer with a heated electrospray ionization (HESI) source. To maximize the performance of the mass spectrometer, time-specific SRM windows were employed at the retention times of the target compounds. In addition, Quantitation-Enhanced Data-Dependent scanning, which delivers SRM-triggered MS/MS data, was used for structural confirmation. Alternating positive and negative polarity switching was utilized in the method. The MS conditions were as follows:

Sheath Gas Flow Rate:	55 units
Aux Gas Flow Rate:	15 units
Spray Voltage:	3500 V
Capillary Temp:	280 °C
Heater Temp:	295 °C
Cycle Time:	0.2 s

Software

Method development, data acquisition, and data processing were performed with TraceFinder software. TraceFinder software streamlines method development, acquisition, and data review. It provides a comprehensive system incorporating processing methods, library searching capabilities, data review, reporting, and built-in methods for commonly found contaminants. The Compound Data Store (CDS) in TraceFinder software includes selective reaction monitoring (SRM) transitions and collision energies for several hundred pesticides, herbicides, personal care products, and pharmaceutical compounds (Figure 1).

In this experiment, the appropriate SRM transitions of the pesticides were chosen from the CDS and inserted into the method for detection. No compound optimization was necessary for compounds that were included in the Compound Data Store.

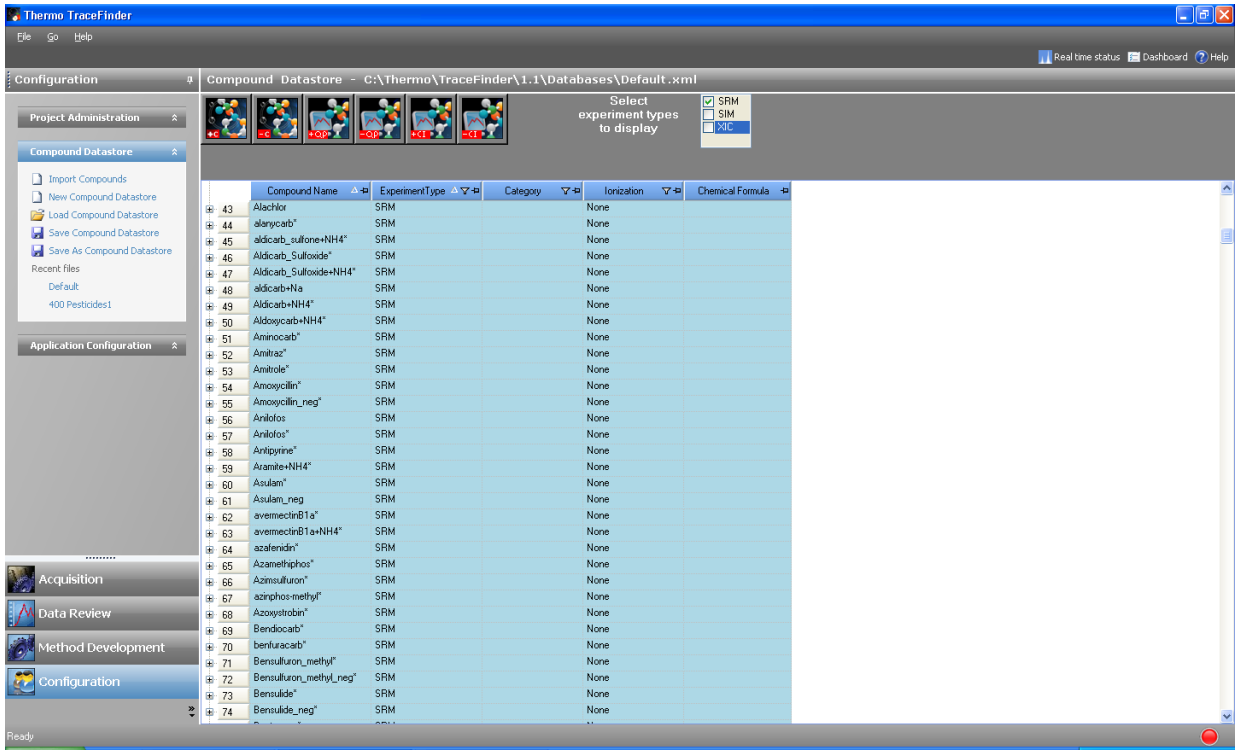


Figure 1. TraceFinder software Compound Data Store (CDS)

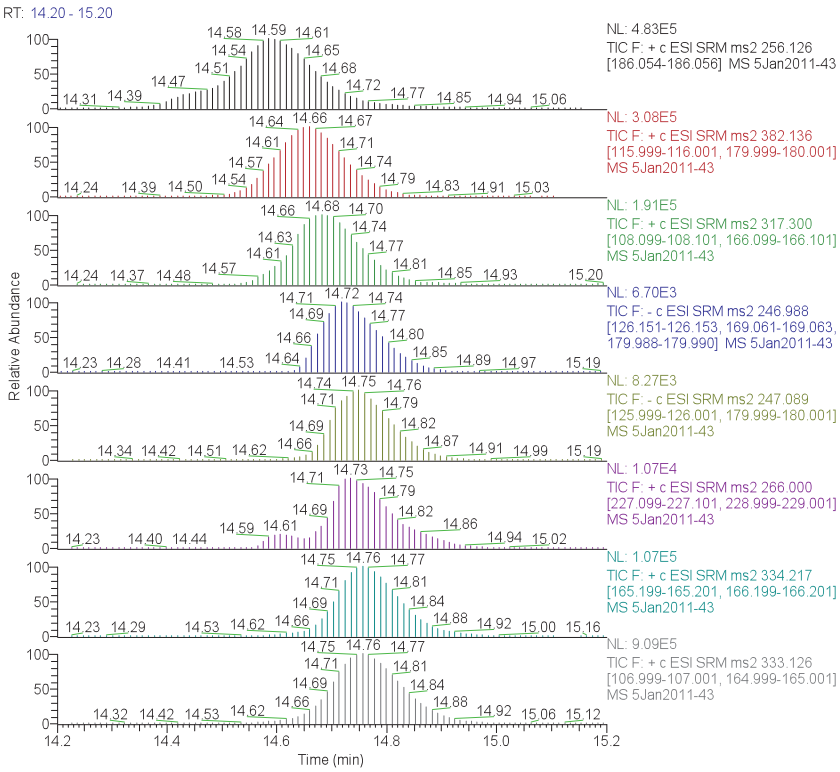


Figure 2. Eight extracted ion chromatograms showing number of scans with positive and negative switching

Results and Discussion

Multi-residue screening studies can generate very large SRM transition lists in a single experiment. T-SRM can be a useful tool to enhance qualitative and quantitative analyses. In a T-SRM experiment, using prior knowledge of the pesticide retention times, the method is set to look for specific transitions only during the expected retention time window. This increases the number of SRM transitions that can be monitored effectively per experiment. T-SRM also increases the scan time and duty cycle for monitoring individual compounds per experiment, providing more accurate and sensitive quantitation. In this screening, after retention times were determined by standard SRM run, a T-SRM method containing a total of 933 T-SRMs was constructed to analyze the compounds in one single mix. For most compounds, the time window was 60 s. Figure 2 shows that by using T-SRM, enough scans were obtained for closely and overlapping peaks with positive and negative polarity switching. T-SRM enabled the efficient detection of a large list of SRM transitions without compromising the scan time for each SRM.

A mixture of 437 pesticides representing a broad spectrum of chemical classes was separated and detected within 45 minutes (Table 1). For the concentration range studied (0.1-250 µg/L), limits of detection (LOD) were estimated from standard solutions. The LOD ranged from 0.1 to 50 µg/L, depending on the analytes.

Table 1. LC-MS/MS data for 437 pesticide standards

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Acephate	184.08	143.05	10	95.20	25			2.11	+
Acetamiprid	223.10	126.10	22	90.20	36			3.87	+
Acibenzolar-S-methyl	211.09	136.00	32	140.00	24			13.17	+
Acifluorfen	360.00	316.00	10					15.26	-
Acrinathrin+NH_4	559.00	208.00	16	181.00	33	317.00	12	27.78	+
Akton	374.80	304.90	20	97.10	40			22.05	+
Alachlor	270.10	162.00	19					15.86	+
Aldicarb sulfone+NH_4	240.12	86.20	22	148.05	12			2.38	+
Aldicarb sulfoxide	207.00	132.00	10	89.00	16			2.3	+
Aldicarb sulfoxide +NH_4	224.20	89.00	19	131.70	15			2.3	+
Aldicarb+NH_4	208.10	116.10	10	89.20	17			4.97	+
Allethrin	303.16	135.05	13	123.11	18	91.16	33	23.14	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Allidochlor	174.09	98.23	12	41.44	23	39.44	45	5.24	+
Ametryn	228.20	185.90	19	96.00	26			8.94	+
Amicarbazone	242.18	143.10	12	85.20	32			6.21	+
Aminocarb	209.12	137.10	25	152.10	15			2.2	+
Amitraz	294.08	122.19	33					2.77	+
Ancymidol	257.11	135.05	26	81.21	26	77.20	45	7.44	+
Anilofos	368.00	199.00	16	171.00	23	125.00	34	18.96	+
Aramite+NH_4	352.00	191.00	12	255.00	10			23.2	+
Aspon	378.90	210.90	21	115.10	33			25.28	+
Asulam	231.00	156.00	12	92.00	25			2.29	+
Atrazine	216.00	174.00	16					9.32	+
Avermectin B1a +NH_4	890.45	305.28	22	307.00	29	567.41	11	27.65	+
Avermectin B1a+Na	895.39	751.50	45	183.08	50			27.65	+
Avermectin B1b +NH_4	876.45	291.00	30	553.40	15	145.00	35	26.77	+
Azaconazole	300.00	158.93	27	230.92	17	122.99	51	11.07	+
Azafenidrin	338.11	264.03	30	302.10	17	298.98	20	10.94	+
Azamethiphos	324.98	182.91	17	112.04	36	138.96	23	6.41	+
Azinphos-ethyl	345.96	132.10	16	160.10	7			16.14	+
Azinphos-methyl	317.93	260.98	8	125.03	19			11.9	+
Azoxystrobin	404.12	372.14	14	329.11	32			13.86	+
Benalaxyl	326.18	148.00	22	208.00	15			18.7	+
Bendiocarb	224.16	167.06	10	109.10	20			6.94	+
Benodanil	324.01	241.98	25	261.96	18	132.03	19	14.84	+
Benoxacor	260.03	148.69	17	133.98	13			11.31	+
Bensulide	398.00	314.00	12	158.00	25	218.00	18	18.34	+
Bentazone	239.07	132.00	28	197.00	22			6.51	-
Benthiavalicarb	382.14	180.00	33	116.00	23			14.65	+
Benzoximate	364.35	199.20	11	105.20	33			20.06	+
Bifenazate	301.23	170.00	20	152.00	40			16.02	+
Bifenox	342.00	310.00	15					18.99	+
Bifenthrin+NH_4	440.00	181.00	14	166.00	42			29.35	+
Bispyribac-sodium	453.14	296.96	19					13.92	+
Bitertanol	338.08	269.00	10	99.00	16			20.15	+
Boscalid	343.24	307.00	19	271.00	34			14.21	+
Brodifacoum	522.88	335.00	23	178.20	35			28.91	+
Bromadiolone	525.07	249.96	37	263.27	40	218.93	50	23.67	-
Bromoxynil	276.07	81.00	36	79.00	36			8.86	+
Bromuconazole1	377.92	158.92	28	160.88	28	123.02	35	15.16	+
Bromuconazole2	377.92	158.92	28	160.88	28	123.02	35	17.83	+
Bufencarb	222.11	95.20	34	77.20	43			17.19	+
Bupirimate	317.30	166.10	25	108.10	27			14.68	+
Buprofezin	306.21	201.00	12	116.00	18			20.78	+
Butachlor	312.20	238.00	11					22.92	+
Butafenacil+NH_4	492.31	331.00	26	180.00	46			16.26	+
Butocarboxin	208.10	109.20	15	91.40	39			13.82	+
Butoxycarboxin	223.11	106.10	10	86.20	20			2.35	+
Butoxycarboxin+NH_4	240.11	86.20	18	106.10	25			2.36	+
Butralin	296.14	240.03	14	222.03	22	208.00	28	24.95	+
Butylate	218.20	156.00	11					21.14	+
Cadusafos	270.97	158.90	16	97.00	36			20.21	+
Carbaryl	202.08	145.00	12	127.00	30			8.13	+
Carbendazim	192.10	160.06	20	132.10	33			2.75	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Carbetamide	237.12	192.05	10	118.10	15			5.83	+
Carbofuran	222.14	165.06	14	123.10	25			6.91	+
Carbofuran-3-hydroxy	238.08	220.08	9	181.08	11			3.64	+
Carboxin	235.95	142.97	17	86.98	24			7.6	+
Carfentrazone-ethyl	412.19	384.00	15	366.20	19			17.94	+
Carpropamid	334.00	139.00	22	196.00	14	103.00	38	18.84	+
Chlorantraniliprole	482.13	450.89	21	283.81	19			11.79	+
Chlorbromuron	292.91	203.88	20	181.95	19	124.94	33	13.73	+
Chlordimeform	197.02	117.20	29	89.00	50			3.15	+
Chlorfenvinphos	358.81	155.20	14	99.10	33			19.16	+
Chlorfluazuron	541.90	385.00	25					26.79	+
Chlorfluazuron	539.70	383.00	20					26.79	+
Chloroxuron	291.11	72.20	20	46.20	19			15.97	+
Chlorpropham	214.00	172.00	12	154.00	19			9.96	+
Chlorpyrifos	350.00	198.00	18	97.00	35			23.81	+
Chlortoluron	213.08	140.00	22	168.00	20			9.18	+
Clethodim	360.19	164.00	20	268.00	14			21.38	+
Clofentezine	303.07	138.00	18	102.00	36			20.42	+
Clothianidin	250.12	169.06	14	132.10	18			3.38	+
Coumaphos	363.02	226.90	25					19.38	+
Coumaphos oxon	347.02	290.92	18	210.92	28	318.93	14	12.72	+
Crotoxyphos	332.07	126.99	23	99.04	27			14.36	+
Dumyluron	303.00	185.00	14	125.00	34	119.00	22	15.44	+
Cyanazine	241.10	214.00	17					6.18	+
Cyazofamid	325.22	108.00	15	261.00	10			17.23	+
Dycloate	216.00	154.00	12	134.00	14	83.00	18	19.81	+
Cyclohexamide	299.18	264.16	14	246.12	19	159.16	30	5.5	+
Cycluron	199.11	89.10	16	72.20	24			10.42	+
Cyflufenamid	413.00	295.00	16	241.00	25	203.00	42	20.34	+
Cyfluthrin	434.10	191.00	17					26.68	+
Cyhalothrin+NH_4	467.00	225.00	18	450.00	10			26.79	+
Cymoxanil	199.06	128.10	10	111.10	20			4.07	+
Cyphenothrin	393.08	315.89	23	376.00	10			20.84	+
Cyproconazole	292.13	125.00	32					15.58	+
Cyromazine	167.09	85.17	19	68.23	28	81.21	26	1.97	+
Daimuron	269.00	151.00	14	91.00	45	119.00	25	14.55	+
DEF	315.02	169.00	17	259.09	13			26.36	+
Deltamethrin	506.10	281.00	11					26.9	+
Demeton S-methyl	231.01	89.16	10	61.26	32			7.06	+
Demeton-O	259.00	89.10	11	61.21	29			11.72	+
Demeton-S	259.00	89.25	12	61.20	47			11.72	+
Desmedipham+NH_4	318.16	182.00	15	136.00	28			11.72	+
Desmetyrn	214.11	172.07	18	82.21	30	57.34	33	6.63	+
Di-allate	269.99	86.15	17	109.04	30	143.03	20	20.67	+
Diamidafos (Nellite)	201.10	107.20	28					3.96	+
Diazinon	305.03	169.10	25	153.13	23			18.51	+
Diazinon Oxon	289.00	233.00	20					16.12	+
Dichlorfenthion	314.98	258.82	16					26.36	+
Dichlormid	208.04	81.26	13	98.18	13	41.47	20	6.85	+
Dichlorvos	221.00	109.00	18	145.00	14	127.00	10	6.72	+
Dichlorvos+NH_4	238.00	109.00	24	221.00	18	127.00	24	6.72	+
Diclobutrazol	328.14	159.00	35	70.20	25			16.24	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Dicrotophos	238.10	193.10	10	112.10	14			3.04	+
Diethofencarb	268.21	226.00	13	180.10	18			12.43	+
Difenacoum	445.13	179.00	30	256.97	21	177.83	59	26.63	+
Difenoconazole	406.17	251.00	25	111.00	55			21.12	+
Difenoxuron	287.09	123.04	22	72.22	26	95.15	30	11.19	+
Dimepiperate	264.12	146.08	11	119.13	17	91.15	35	20.36	+
Dimethametryn	256.13	186.05	22					14.59	+
Dimethenamid	276.00	243.97	14	168.02	23	111.15	33	12.76	+
Dimethoate	230.11	199.10	12	125.10	23			3.68	+
Dimethomorph	388.14	301.00	22	165.00	34			15.25	+
Dimethylvinphos1	331.00	127.04	13	99.06	26			14.47	+
Dimethylvinphos2	331.00	127.04	13	99.06	26			15.55	+
Dimetilan	241.10	72.20	21					4	+
Dimoxystrobin	327.13	205.00	12	116.00	25			17.73	+
Diniconazole	326.17	148.20	27	70.20	35			18.7	+
Dinotefuran	203.02	129.00	10	114.00	15			2.31	+
Dioxacarb	224.08	167.06	10	123.10	18			6.94	+
Dioxathion	473.99	271.09	10	153.04	28			22.8	+
Diphenamid	240.12	134.13	21	167.09	24	165.09	48	11.18	+
Diphenylamine	170.09	114.09	17	100.13	22	69.21	26	7.91	+
Dipropetryn	256.15	214.06	19	144.06	29	172.03	21	14.46	+
Disulfoton	274.94	89.27	5	61.28	34			19.59	+
Ditalimfos	300.10	145.30	22	144.20	21			14.47	+
Dithiopyr	402.10	354.00	20	272.30	32			21.84	+
Diuron	233.11	72.00	20	46.30	35			8.81	+
DNOC	199.14	117.10	28	89.00	53			3.15	+
Dodemorph	282.23	116.16	20	98.22	25	69.29	31	11.66	+
Doramectin	916.40	331.40	35	593.50	25			28.79	+
Edifenphos	310.98	283.00	12	109.11	35			18.62	+
Emamectin	886.70	158.00	33	302.00	20			24.99	+
Emamectin B1b	872.40	158.20	33	302.30	20			24.02	+
Epoxiconazole	330.20	121.00	21	123.00	20			16.84	+
Eprinomectin B1a	936.53	490.22	52	352.13	57			27.15	+
EPTC	190.07	128.20	13	86.20	14			16.67	+
Esprocarb	266.20	91.00	24	71.10	17			22.34	+
Etaconazole	328.19	159.00	32	123.00	58			16.62	+
Ethaboxam	321.00	183.10	24	200.10	28			8.89	+
Ethalfluralin	334.22	166.20	21	165.20	20			14.76	+
Ethidimuron	265.09	208.20	16	114.20	20			3.32	+
Ethiofencarb	226.09	107.00	16					13.16	+
Ethiolate	162.10	132.16	23	147.16	15	117.14	30	22.92	+
Ethion	384.92	142.97	29	97.09	49			23.56	+
Ethion monoxon	368.85	199.20	13	142.90	27			17.7	+
Ethiprole	397.12	351.00	20	255.00	34			14.03	+
Ethirimol	210.20	140.10	23	98.10	28			4.82	+
Ethofumesate	286.96	258.90	11	120.90	20			12.86	+
Ethoprophos	243.07	97.10	30	131.10	40			15.93	+
Ethoxyquin	218.00	174.00	34	160.00	34			8.81	+
Etobenzanid	340.13	179.10	20	121.00	33			19.13	+
Etofenprox	394.15	177.07	14	107.11	38	135.03	28	28.5	+
Etoxazole	360.21	177.10	22					19.06	+
Etrimfos	293.10	265.00	17					17.81	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Famoxadone+NH_4	392.11	331.22	8	238.03	18			20.08	+
Famphur	325.96	217.03	21	280.98	13			10.36	+
Famphur oxon	327.14	201.00	26	265.00	19	186.01	35	4.91	+
Fenamidone	312.20	236.20	16	264.20	12			13.57	+
Fenamiphos	304.03	217.01	24	234.03	8			17.47	+
Fenamiphos sulfone	336.09	279.87	17	199.98	28			16.95	+
Fenarimol	331.12	268.00	23	81.00	34			16.32	+
Fenazaquin	307.20	57.20	23	160.90	18			20.77	+
Fenbuconazole	337.04	125.14	35	70.41	27			17.8	+
Fenhexamid	302.09	97.00	26	55.00	36			15.84	+
Fenitrothion	277.95	245.95	17	125.10	21			12.76	+
Fenoxycarb	302.17	116.00	13	88.00	20			18.07	+
Fenpiclonil	254.07	172.01	17					7	+
Fenpropathrin	350.20	97.00	34	125.00	16			23.82	+
Fenpropathrin+NH_4	367.20	125.00	18	97.00	34			25.65	+
Fenpropimorph	304.40	147.10	31	130.10	26			13.16	+
Fenpyroximate	422.21	366.00	15	214.00	34			25.9	+
Fensulfothion	309.18	251.00	21	163.00	18			14.17	+
Fenthion	278.95	247.01	13	169.06	20			12.76	+
Fenthion sulfone	328.09	311.04	9	109.12	37			9.11	+
Fenthion sulfoxide	294.90	108.90	32	114.90	27			8.39	+
Fenuron	165.03	72.10	17	46.30	18			3.53	+
Flonicamid	230.12	174.10	18					13.18	+
Florasulam	360.00	129.00	26	192.00	18			4.98	+
Florasulam+NH_4	377.00	129.00	30					4.98	+
Fluazinam	463.19	416.00	20	398.00	17			23.95	-
Flubendiamide	681.00	253.94	29	273.93	19	271.89	19	19.03	+
Flucarbazone	397.13	129.90	21	115.00	48			5.01	+
Fludioxinil	266.00	229.00	17	227.10	10			14.74	+
Fludioxonil	246.99	179.99	34	169.06	32	126.15	34	14.74	-
Flufenacet	364.23	194.00	12	152.00	20			16.23	+
Flufenoxuron	487.16	304.00	20	156.00	16			25.95	-
Flumetsulam	326.00	109.00	53					3.46	+
Flumioxazin	355.06	170.81	24	212.82	17	142.87	29	20.84	+
Fluometuron	233.08	72.10	18	46.30	17			8.81	+
Fluopicolide	383.01	172.94	23	144.95	47	365.01	17	14.44	+
Fluorochloridone	329.11	302.04	12	188.98	20			17.61	+
Fluoxastrobin	459.20	427.10	18	188.00	37			16.67	+
Fluquinconazole	376.17	349.20	21	307.00	20			15.8	+
Flusiazole	316.18	247.10	19	165.00	34			18.02	+
Flutolanil	324.21	242.00	26	262.00	18			14.84	+
Flutriafol	302.16	70.10	19	123.00	33			10.18	+
Fluvalinate	503.00	181.00	34	208.00	12			28.24	+
Fonophos	246.98	109.10	23	137.10	12			18.44	+
Forchlorfenuron	248.14	129.00	18	93.00	26			10.77	+
Formetanate	222.10	165.00	30					10.01	+
Fosthiazate	284.00	228.00	12	104.00	23			8.77	+
Fuberidazole	185.05	157.05	23	156.03	29	130.18	23	3.41	+
Furalaxyl	302.11	242.10	17	95.00	35			13.23	+
Furathiocarb	383.19	195.00	20	252.00	14			22.38	+
Griseofulvin	353.10	215.00	19	285.06	18	165.03	19	10.97	+
Halofenozide	329.10	121.14	22	77.33	37	155.15	29	13.57	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Haloxyfop-methyl	376.03	315.96	17	287.98	25	91.13	31	20.84	+
Hexaconazole	314.14	70.20	20	159.00	20			19.39	+
Hexaflumuron	458.92	439.00	12	175.00	39			22.79	-
Hexazinone	253.09	171.05	17	85.19	29	71.27	30	7	+
Hexythiazax	353.24	228.20	18	168.10	25			24.03	+
Hydramethylnon	495.27	323.00	35	150.90	55			23.22	+
Imazalil	297.18	159.00	24	201.00	18			10.18	+
Imazamox	306.09	261.10	23	193.10	27			4.05	+
Imazapyr	262.06	216.98	19	201.97	27			9.64	+
Imazaquin	312.00	267.00	22	199.00	30	252.00	27	7.29	+
Imibenconazole	411.00	125.00	36	171.00	21			23.76	+
Imidacloprid	256.12	209.10	18	175.10	20			3.29	+
Inabenifide	339.26	80.20	38	78.90	55			13.09	+
Indanofan	341.00	187.00	14	175.00	17			16.23	+
Indoxacarb	528.30	203.00	40	293.00	15			21.9	+
Ipconazole	334.13	70.20	22	125.00	42			21.54	+
Iprobenfos	289.02	204.96	11	91.23	24			17.82	+
Iprovalicarb	321.16	119.00	20	203.00	10			15.55	+
Isocarbamid	186.08	145.05	22					21.54	+
Isocarbophos	307.12	230.93	17	171.12	22			10.71	+
Isofenfos	346.04	216.94	23	244.99	12			19.79	+
Isofenfos O-analog	330.15	121.10	43					16.84	+
Isoprocarb	194.09	95.00	16	137.00	11			9.5	+
Isopropalin	310.15	225.94	20	222.07	20	210.01	19	26.19	+
Isoprothiolane	291.00	189.00	22	231.00	12			14.5	+
Isoproturon	207.10	72.00	19	165.15	14			10.09	+
Isoxaben	333.13	165.00	20	107.00	61			14.76	+
Isoxaflutole	360.25	220.00	42					19.04	+
Isoxathion	314.00	286.00	10	105.00	18	258.00	12	20.09	+
Isozophos	314.03	162.01	16	97.03	34	120.02	28	15.81	+
Ivermectin B1a +NH_4	892.50	307.00	28	569.00	17			29.92	+
Kresoxim-methyl	314.07	267.14	8	222.13	15			17.77	+
Lactofen+NH_4	479.00	344.00	15	223.00	36			23.62	+
Linuron	249.10	182.00	18	160.00	17			12.87	+
Loxynil	369.86	242.95	28		28			11.26	-
Lufenuron	509.21	326.00	18	175.00	37			24.97	-
Malathion	330.97	126.99	13	99.02	25	124.98	32	14.48	+
Mandipropamid	412.10	327.90	15	355.90	11			15.16	+
Matoxuron	229.02	72.22	25	156.03	24			5.25	+
Mefenacet	299.17	148.00	14	120.10	31			15.4	+
Mefluidide	328.09	311.04	14	135.12	30	121.10	41	7.58	+
Mepanipyrim	224.14	106.00	27	77.00	40			15.48	+
Mephospholan	270.03	139.98	25	196.02	14	167.96	17	6.7	+
Mepronil	270.15	228.00	16	119.00	21			14.37	+
Mesotrione	340.16	227.95	16					4.72	+
Metaflumizone	505.15	302.04	22	285.10	52	117.15	34	24.67	-
Metalaxyl	280.11	220.10	16	192.10	16			10.36	+
Metazachlor	278.02	134.07	24	105.11	41			9.96	+
Metconazole	320.20	70.10	22	124.90	41			19.62	+
Methabenzhiazuron	222.13	165.00	17					6.91	+
Methacrifos	258.05	209.01	12	125.04	25	79.21	32	11.44	+
Methamidophos	142.00	94.00	20	125.00	10			1.95	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Methidathion	302.90	85.20	23	144.92	5			10.92	+
Methiocarb	226.09	169.00	10					8.28	+
Methomyl	163.05	106.10	10	88.10	10			2.63	+
Methoprotryne	272.20	240.00	10	198.00	15			9.55	+
Metobromuron	259.10	170.00	20	148.00	25			9.34	+
Metolachlor	284.14	252.10	17	148.20	24			16.14	+
Metominostrobin	285.08	193.96	17	166.02	28	139.95	41	11.15	+
Metosulam	418.00	174.88	27	139.96	45	189.68	24	8.21	+
Metrafenone	409.03	209.10	16	227.10	20			20.13	+
Metribuzin	215.09	187.07	17	130.97	17			6.23	+
Mevinphos1	225.09	127.10	15	192.80	8			3.63	+
Mevinphos2	225.09	127.10	15	192.80	8			4.57	+
Mexacarbate	223.15	151.00	26	166.00	16			3.07	+
Milbemycin A3	511.40	493.20	10	475.20	10			26.77	+
Milbemycin A4+NH_4	560.40	525.20	10	507.20	12			27.95	+
Milbemycin A4-H_2O	525.40	507.20	10	489.20	10			27.96	+
Molinate	188.06	126.20	16	83.10	20			13.75	+
Monocrotophos	224.08	127.05	28	193.10	19			2.83	+
Monolinuron	215.08	126.00	17	99.00	36			8.31	+
Moxidectin	640.20	528.50	15	498.50	20			29.19	+
Myclobutanil	289.13	125.00	31	70.20	19			15.58	+
Naled	396.12	324.13	20	308.15	22			16.22	+
Naphthol	145.11	115.10	18	102.12	22			18.1	+
Napropamide	272.14	171.07	20	129.15	16	114.17	22	16.4	+
Naptalam sodium	331.14	105.16	18	139.04	19			13.57	+
Neburon	275.10	57.20	35	88.00	30			17.82	+
Nitenpyram	271.22	225.00	12	237.00	20			2.53	+
Nitralin	346.12	303.98	15	241.87	17	196.00	36	17.44	+
Nitrothal-isopropyl	313.03	148.95	15	91.14	41			15.23	+
Norflurazon	304.07	284.00	25	88.00	39			11.01	+
Novaluron	493.26	158.00	18	141.00	42			23.17	+
Novaluron	491.23	471.00	15	305.00	19			23.18	-
Noviflumuron	527.00	344.00	15	193.00	35			25.7	+
Nuarimol	315.11	251.90	26	81.00	36			13.33	+
Octhilinone	214.14	102.12	16	57.36	17			16.78	+
Ofurace	299.09	254.05	17	236.04	21	160.09	28	7.25	+
Omethoate	214.07	183.00	13	155.00	18			2.23	+
Orbencarb	258.06	125.05	28	100.15	13	89.13	43	19.38	+
Oryzalin	345.00	281.00	19	147.00	30	78.00	38	16.82	-
Oxadiazon	362.06	302.93	18	219.69	25	184.89	35	23.09	+
Oxadixyl	279.00	219.00	15	132.00	25			5.92	+
Oxamyl+NH_4	237.10	72.08	15	90.09	10			2.44	+
Paclobutrazole	294.10	70.00	20	125.00	35			14.26	+
Parathion	292.00	236.00	15	97.00	30			17.68	+
Parathion-methyl	263.94	232.07	18	109.13	20	124.90	25	12.11	+
Penconazole	284.12	159.00	35	70.10	17			18.43	+
Pencycuron	329.00	125.00	30	218.00	16			20.49	+
Pendimethalin	282.09	212.00	11	194.11	18	119.07	25	24.1	+
Penoxsulam	484.06	195.20	29	194.70	36			9.33	+
Permethrin+NH_4	408.00	183.00	22	355.00	10			28.45	+
Phenmediphame	301.17	136.00	22	168.00	10			12.23	+
Phenothrin	368.20	183.00	24	237.04	12	165.03	42	28.24	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Phenthoate	320.93	247.02	11	79.26	46			18.01	+
Phorate	260.97	75.08	14	142.94	19			19.08	+
Phorate oxon sulfone	276.98	142.92	22	97.00	36	152.97	16	9.2	+
Phorate sulfone	276.05	94.15	35	173.97	21			9.85	+
Phosalone	368.00	182.00	17					20	+
Phosmet	317.91	160.05	15	133.15	39			12.18	+
Phosphamiden	317.08	300.01	10	127.04	25	226.93	19	6.07	+
Phoxim	299.00	129.00	10	77.00	20			19.81	+
Phropham	180.00	138.00	10	120.00	15	92.00	26	9.11	+
Picloram	241.00	195.00	24					2.67	+
Picoxystrobin	368.20	145.00	23	205.10	11			18.1	+
Pinoxaden	401.19	317.00	23	57.10	34			20.09	+
Piperonyl butoxide	356.19	177.00	13	119.00	33			22.74	+
Piperophos	354.09	170.85	22	212.83	16	142.90	32	20.84	+
Pirimicarb	239.09	182.00	16	72.00	21			4.59	+
Pirimiphos ethyl	334.07	198.11	24	182.14	26			21.8	+
Pirimiphos-methyl	306.01	164.12	24	108.18	34			18.17	+
Pretilachlor	312.20	252.00	17					22.53	+
Prochloraz	376.21	308.00	14	266.00	18			18.99	+
Profenophos	372.90	302.80	19	143.86	36	127.97	40	22.05	+
Prohexadione	211.07	167.19	17	123.24	17	111.18	23	4.83	+
Promecarb	208.09	151.00	10	109.00	17			13.82	+
Prometon	226.21	184.00	21	141.90	24			7.65	+
Prometryn	242.21	157.90	24	199.90	20			11.65	+
Propachlor	212.06	169.99	15	94.13	25	77.18	41	9.95	+
Propamocarb	189.05	102.10	19	144.05	14			2.32	+
Propanil	215.99	160.02	21					12.9	+
Propargite	368.18	231.00	11	174.90	18			24.9	+
Propazine	230.00	124.00	17					15.09	+
Propetamphos	282.04	138.08	18	156.00	10			15.22	+
Propiconazole	342.20	159.00	29	69.20	21			18.91	+
Propoxur	210.07	111.10	16	168.06	10			6.62	+
Prothioconazole	341.98	306.00	16	100.00	30			19.09	+
Prothoate	286.04	97.02	35					10.73	+
Pymetrozine	218.00	105.00	25	79.00	30			2.18	+
Pyracarbolid	218.20	124.90	21	96.90	31			7.03	+
Pyraclofos	361.10	257.00	23					20	+
Pyraclostrobin	388.22	194.00	14	163.00	26			20.01	+
Pyraflufen-ethyl	413.10	339.00	19					19.46	+
Pyrasulfotole	361.06	159.08	46	64.35	61	79.25	18	24.99	+
Pyrazophos	374.04	222.10	22	194.04	36			19.73	+
Pyridaben	365.20	309.10	13	147.00	23			26.81	+
Pyridalyl	489.95	109.00	29	163.90	38			30.53	+
Pyridaphenthion	340.94	189.09	23	205.04	22			15.62	+
Pyridate	379.20	207.00	19					28.28	+
Pyrifenox	294.97	93.12	26	92.07	52	67.19	50	12.88	+
Pyrimethanil	200.07	107.00	26	82.00	30			9.74	+
Pyriproxyfen	322.22	96.00	16	185.30	27			23.49	+
Pyroquilon	174.10	132.13	23	117.15	31	130.13	38	6.77	+
Pyrosulam	434.95	195.20	28	194.10	39			7.42	+
Quinalphos	299.05	163.01	23	147.06	24	38.00		17.63	+
Quinoxyfen	307.88	196.80	33	161.90	47			23.92	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Resmethrin	356.16	171.01	15	143.01	26	128.03	43	27.36	+
Rotenone	395.30	213.20	23	192.10	26			17.69	+
Salflufenacil	518.19	348.94	30	459.00	16			12.36	+
Schradan	287.12	242.02	14	135.08	26	92.15	40	4.25	+
Secbumeton	226.21	169.90	19	99.90	33			7.91	+
Sethoxydim	328.00	178.00	20					7.58	+
Siduron	233.12	137.00	20	94.00	38			12.55	+
Simazine	202.10	132.00	20	104.00	27			6.7	+
Simetryne	214.10	124.00	20	96.00	26			6.56	+
Spinetoram1	748.32	141.92	30	98.03	37			22.65	+
Spinetoram2	760.2	141.88	31					24.11	+
Spinosyn A	732.50	142.00	35	98.00	47			21.19	+
Spinosyn D	746.50	142.00	34	98.00	47			22.6	+
Spirodiclofen	411.00	313.10	15	213.10	25			25.6	+
Spiromefesin	371.30	273.30	15	255.30	25			24.73	+
Spirotetramat	374.20	330.20	17	302.20	19			16.21	+
Spiroxamine	298.22	144.00	21	100.00	35			14.74	+
Sulfentrazone	404.00	387.00	10	307.00	15			7.9	+
Sulfotep-ethyl	323.19	219.00	16	247.10	15			24.39	+
Sulfuramid	525.99	219.02	26	168.94	27	269.07	23	25.97	-
Sulprofos	322.93	218.95	17	246.95	12			24.39	+
Tebuconazole	308.22	70.20	21	125.00	34			18.57	+
Tebufenozide	353.12	133.00	19	297.00	10			17.95	+
Tebufenpyrad	334.21	145.20	28	117.00	36			22.77	+
Tebupirimfos	319.10	210.20	22					14.7	+
Tebuthiuron	229.16	172.06	18	116.10	28			7.29	+
Teflubenzuron	379.16	339.00	13	196.00	22			23.82	-
Tefluthrin	419.03	174.85	27	140.72	47			8.21	+
Temephos	466.95	419.13	20	405.08	14			24.23	+
Tepraloxydim	340.00	220.00	34	248.00	18			8.38	-
Terbufos	288.97	103.10	12	57.50	21			22.23	+
Terbufos sulfone	338.08	171.00	16	115.01	31	97.06	42	12.39	+
Terbumeton	226.22	169.90	20	113.90	25			7.66	+
Terbutryn	242.22	185.90	20	91.00	28			12.03	+
Tetrachlorvinphos-a	365.00	204.00	40	127.00	16			17.79	+
Tetrachlorvinphos-a+NH_4	382.00	127.00	20					17.79	+
Tetrachlorvinphos-b	366.87	127.03	16	205.96	37	240.74	23	17.79	+
Tetrachlorvinphos-b+NH_4	383.88	126.95	19	205.81	49	240.88	24	17.79	+
Tetraconazole	372.19	159.00	39	70.00	24			17.13	+
Tetramethrin	332.10	127.04	28	174.03	19	226.92	18	14.29	+
Thiabendazole	202.04	175.05	28	131.05	35			3.2	+
Thiacloprid	253.13	126.10	22	90.20	37			4.68	+
Thiamethoxam	292.15	211.10	14	132.05	24			2.76	+
Thiazopyr	397.05	377.04	22	335.00	26			18.67	+
Thidiazuron	221.13	102.10	16	94.20	14			7.13	+
Thiobencarb	258.07	125.00	18	100.20	15			19.38	+
Thiofanox+NH_4	236.09	57.20	16	76.10	12			8.52	+
Thiometon+Na	268.88	89.10	25	61.10	36			14.52	+
Thiophanate-methyl	343.21	151.06	24	311.20	12			6.78	+
Tolclofos-methyl	301.00	175.00	22					6.16	+
Tolfenpyrad	384.08	196.95	29	181.69	30			23.59	+
Tralkoxydim	330.00	284.00	13	138.00	22			16.13	+

Table 1. LC-MS/MS data for 437 pesticide standards (continued)

Compound	Precursor Ion	Quantitation Ion	CE	Confirming Ion 1	CE	Confirming Ion 2	CE	RT (min)	Polarity
Tralomethrin+NH_4	682.80	440.60	18	665.80	10	412.60	22	27.59	+
Triadimefon	294.17	197.10	16	225.10	16			14.86	+
Triadimenol	296.10	70.00	15					14.26	+
Triazophos	313.99	162.10	21	119.17	36			15.82	+
Trichlamide	340.00	121.00	22					19.14	+
Trichlorfon	256.90	127.00	19	109.10	19			4.57	+
Tricyclazole	190.07	163.06	24	136.10	30			5.33	+
Tridemorph	298.00	130.00	28	98.00	32			19.42	+
Trifloxystrobin	409.30	186.00	21	206.10	16			21.54	+
Triflumizole	346.16	278.10	12	73.00	18			21.4	+
Triflumuron	359.10	156.20	17	139.00	31			20.24	+
Triforine-a	434.90	390.00	12					12.45	+
Triforine-b	432.90	388.00	12					12.46	+
Triforine-c	436.90	392.00	12					12.45	+
Trinexapac-ethyl	253.11	207.02	11	69.27	20	165.02	17	10.28	+
Triconazole	318.12	70.00	25	125.00	30			16.16	+
Uniconazole	292.13	70.20	25	125.00	32			17.32	+
Vamidothion	288.07	146.05	14	118.10	27			3.6	+
Vernolate	204.15	128.21	11	86.22	13	43.47	19	19.47	+
Warfarin	307.03	160.94	20					26.95	+
Zoxamide	336.22	187.00	23	159.00	38			18.7	+

Excellent linearity in detector response was observed over the calibration range. The correlation coefficients of 319 analytes were greater than 0.99, and those of 52 analytes were greater than 0.98. The total ion chromatogram is shown in Figure 3.

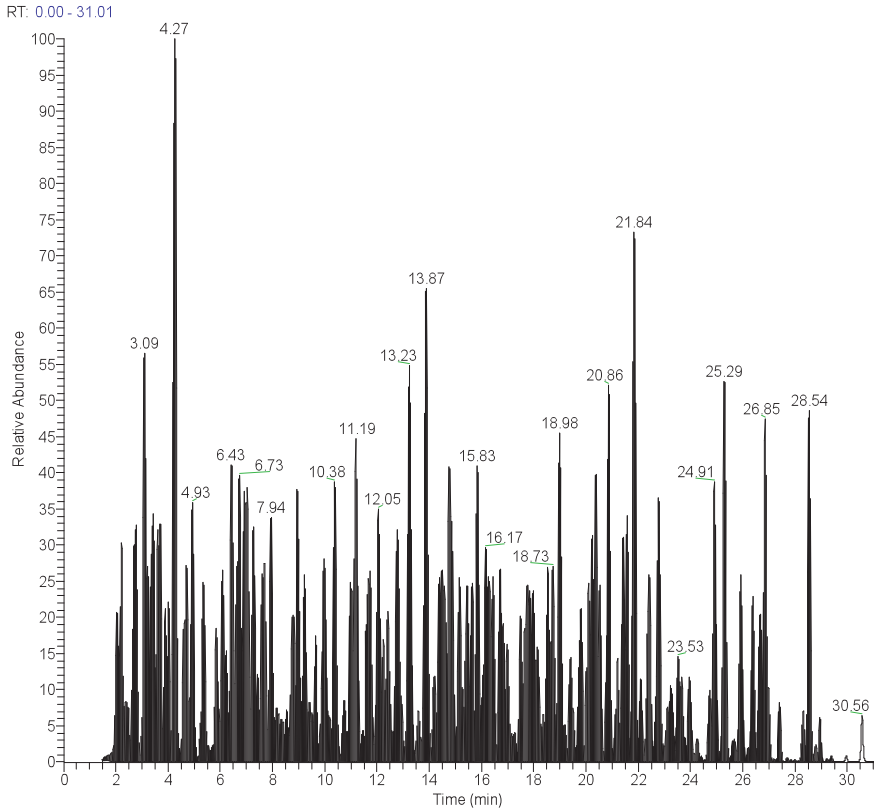
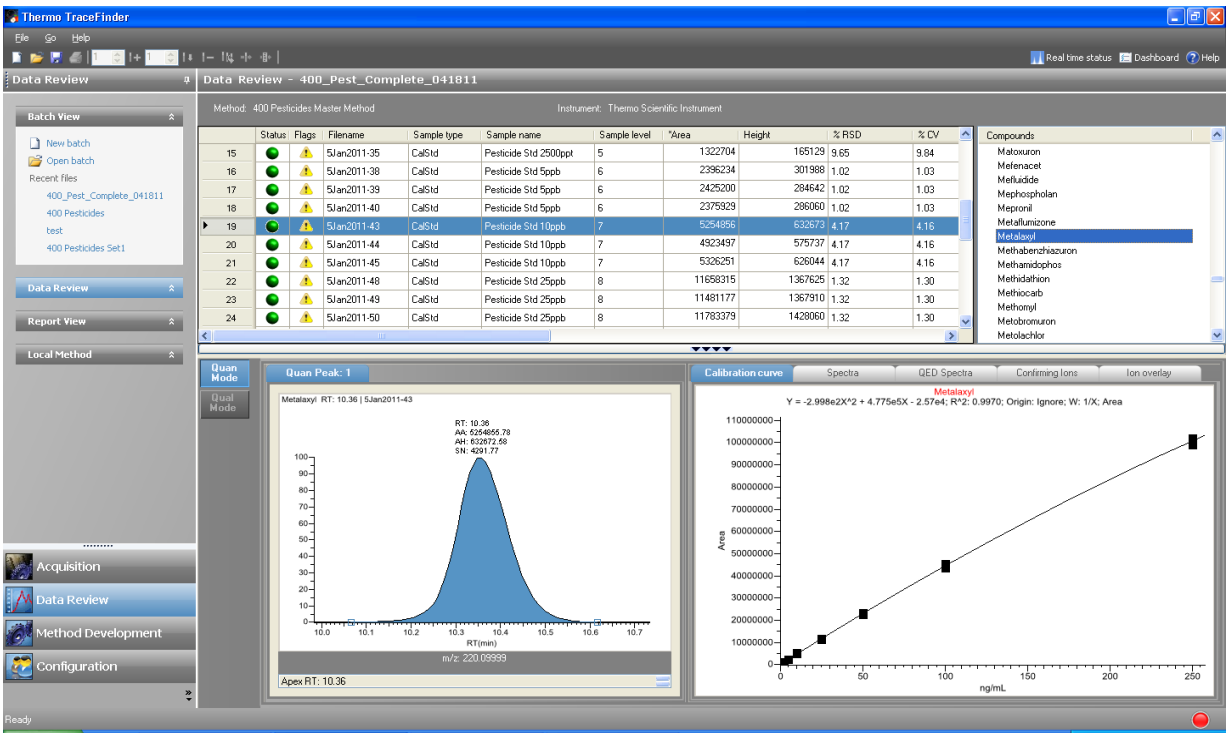


Figure 3. Chromatogram of 437 pesticides (10 µg/L standard solution)

The analysis of the pesticides was reviewed in the Data Review section of TraceFinder software (Figure 4). In this section, calibration curves, ion ratios, peak integration, and MS spectra can be monitored, and samples that meet user-set criteria can be flagged. In addition, user adjustments, such as peak re-integration, are permitted. The effects of the changes on the results are instantly updated in the results grid and standard reports. The extracted ion chromatogram and solvent standard calibration curve for two example pesticides, metalaxyl and pyridaben, are shown in Figure 4. Three replicates of each calibration standard were injected at each level.

(A) Metalaxyl



(B) Pyridaben

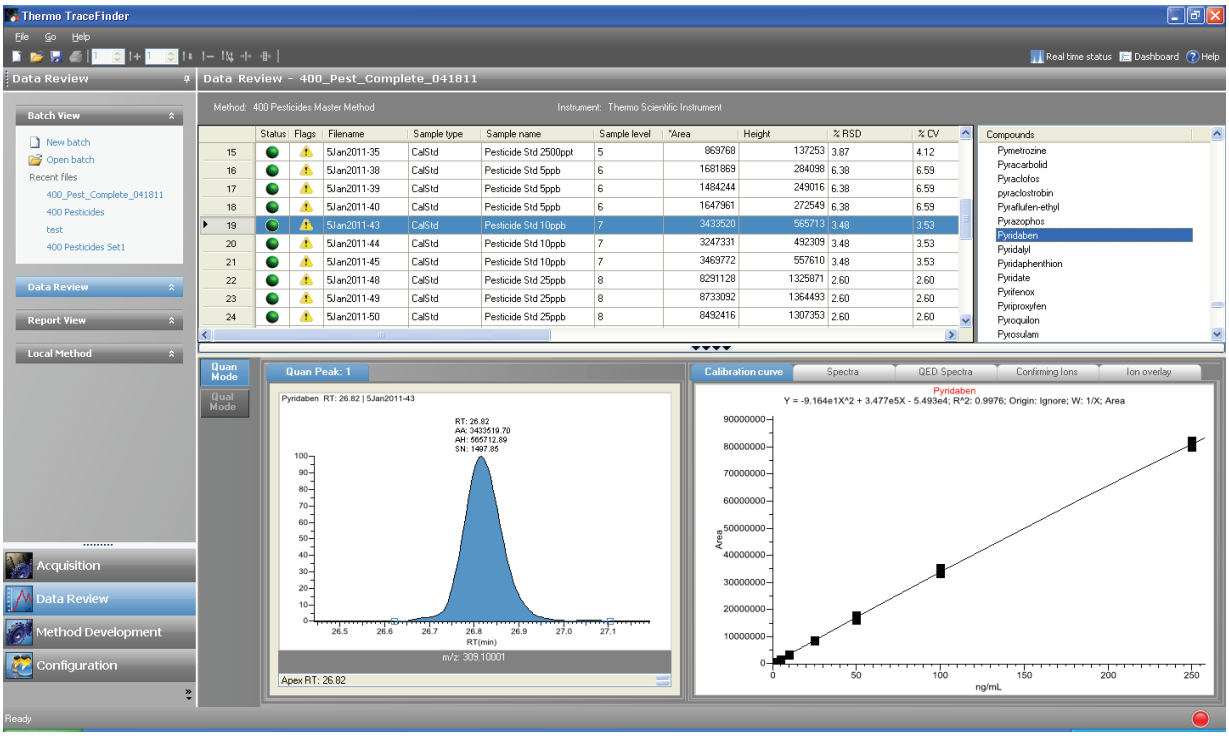


Figure 4. TraceFinder software view of extracted ion chromatogram and solvent standard calibration curve [metalaxyl (A) and pyridaben (B), 10 µg/L, 3 replicates, quadratic curve fit]

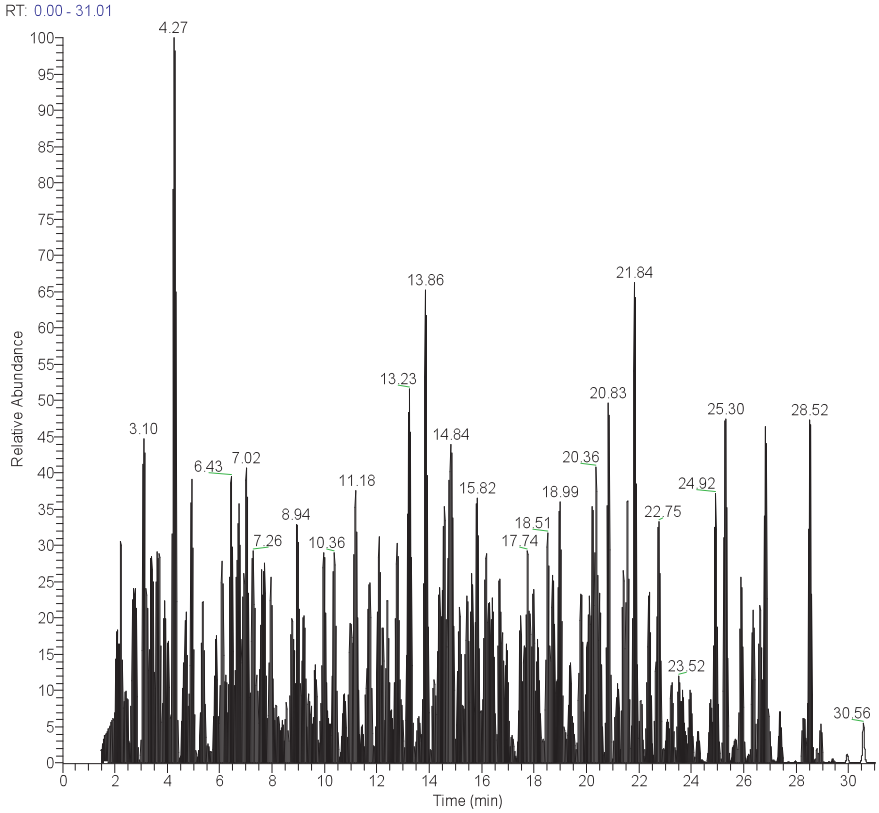
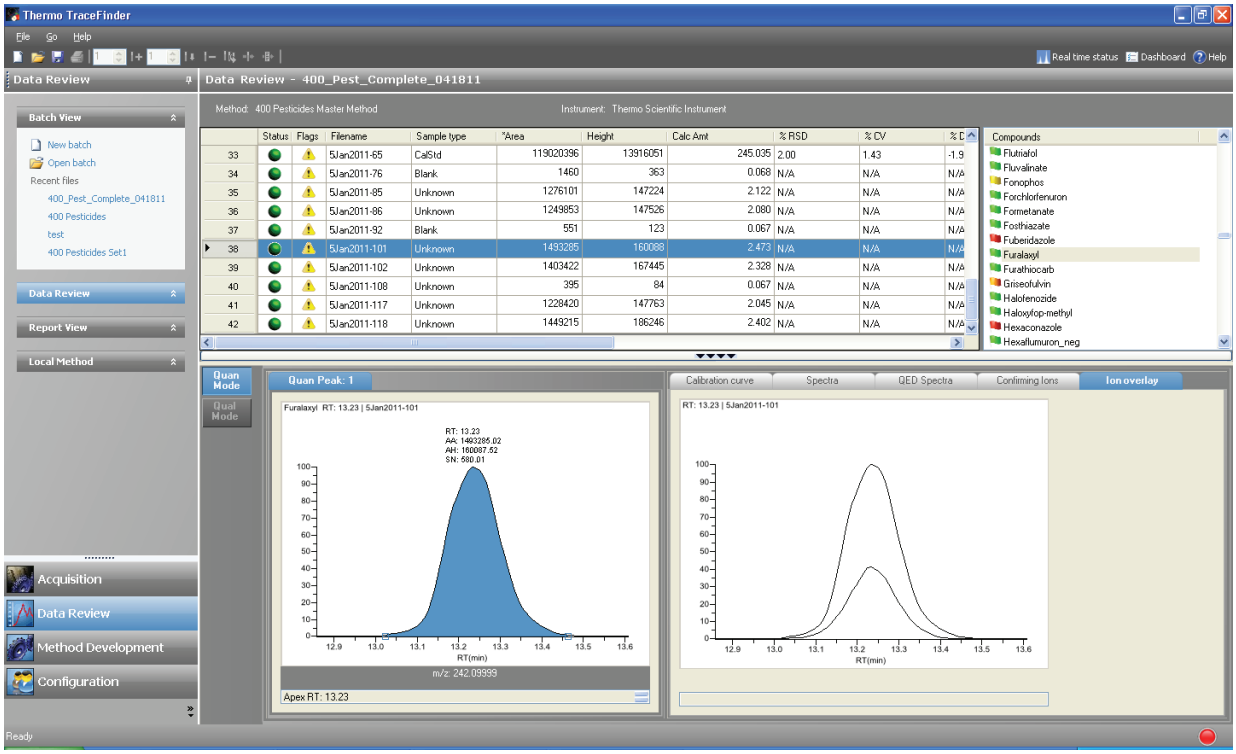


Figure 5. Chromatogram of 437 pesticides in orange extract at 2 µg/kg



Green flag – Compound found, above limit of reporting (LOR), all criteria passed.
Orange flag – Compound close to limit of detection (LOD) or LOR. User may want to double check results.
Yellow flag – Compound not found.
Red flag – Error, such as ion ratio, linearity, carryover, etc.

Figure 6. TraceFinder software view of quantitation peak and ion overlay of confirmation by secondary SRM (furalaxyl in orange extract 2 µg/kg)

To evaluate the applicability of this technique to complex food samples, the pesticide mixture was spiked into apple, orange, and asparagus matrices and analyzed. Figure 5 shows the chromatogram of 437 pesticides at 2 µg/kg in the orange matrix. The majority of the pesticides were detected at 2 µg/kg. The confirmation of target analytes was achieved by the second or third SRM. In Figure 6, the quantification ion and two qualification ions for furalaxyl are displayed in the Data Review section of TraceFinder software. The acceptance criteria percentage can be set for the ion ratio confirmation. If the ion ratio fails, the Confirmation Ion box is flagged in red by the software.

QED-MS/MS experiments were also applied to pesticide analysis in orange, asparagus, and apple extract to confirm the existence of compounds while they were being quantified. A full-scan MS/MS mass spectrum was obtained by data dependent scanning for confirmatory analysis during the SRM experiment. After a particular SRM transition reached the specified intensity threshold, the instrument automatically triggered the QED-MS/MS scan using the Reverse Energy Ramp (RER) scan function. The collision energy was linearly ramped from a high to a low value while Q3 was scanned from low *m/z* to high *m/z*. A highly sensitive, fragment-rich spectrum that was used to positively confirm the existence of a compound was collected. An example of a QED-MS/MS full scan spectrum is shown in Figure 7 for the compound fenamiphos. This QED-MS/MS scan function fragmented the precursor ion *m/z* 304 for fenamiphos over a reversed energy ramp of 10 to 50 eV.

TraceFinder software includes a large number of report templates. Reports can be created in PDF format, printed directly to the printer, or saved in XML format, which is useful for LIMS systems. Figure 8 shows the onscreen preview function of a report generated by TraceFinder software. The chromatogram shown is an apple sample spiked with 437 pesticides at 2 µg/kg. The top of the page contains a sample summary, and the quantitated results follow beneath the chromatogram. TraceFinder software can generate results for the entire batch with the click of a button, or the user can choose to view reports individually and print only those of interest.

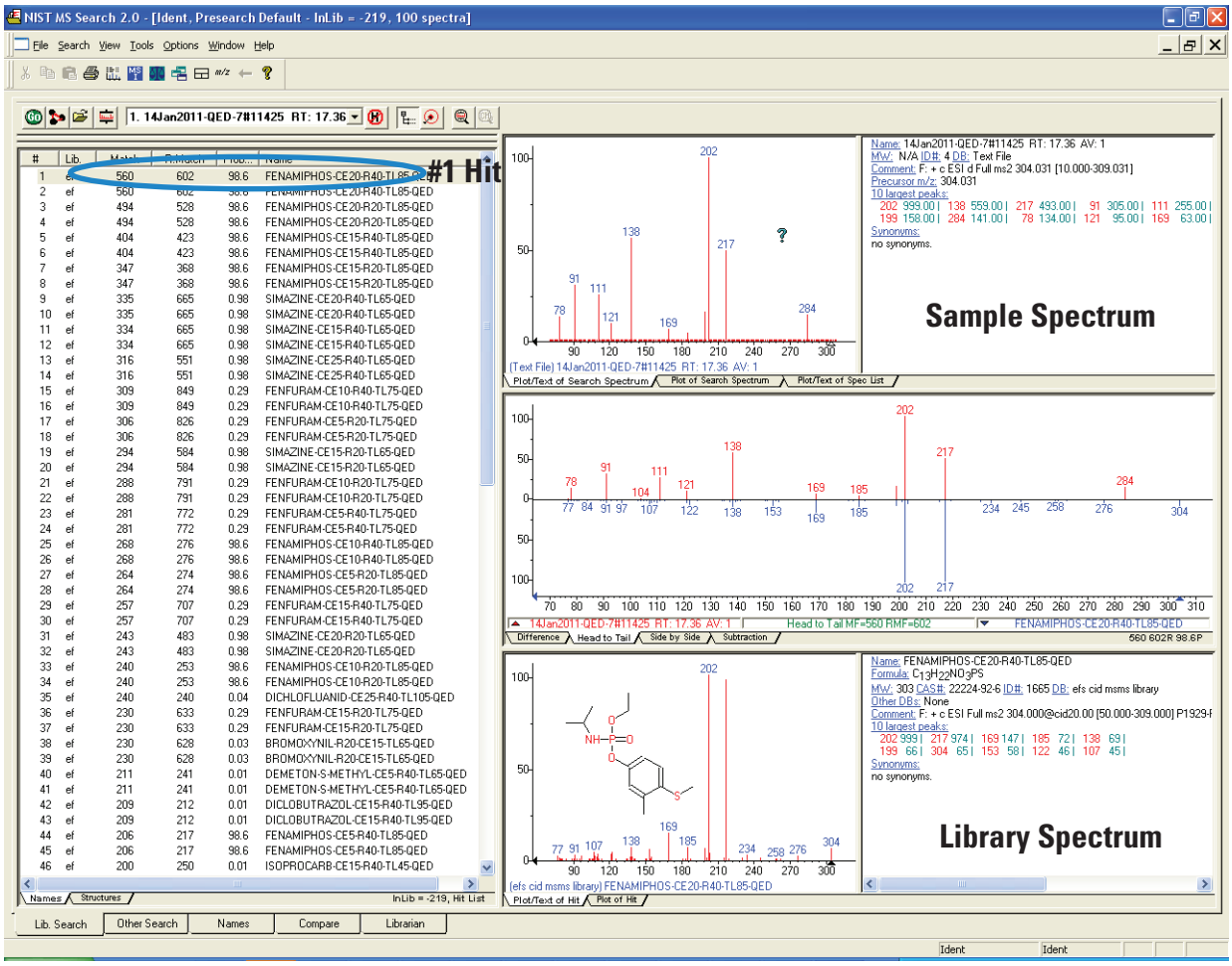


Figure 7. QED spectrum of fenamiphos at 2 µg/kg in asparagus. Searching against the standard library available on the TSQ Quantum Access MAX™ instrument platform yields a positive confirmation.

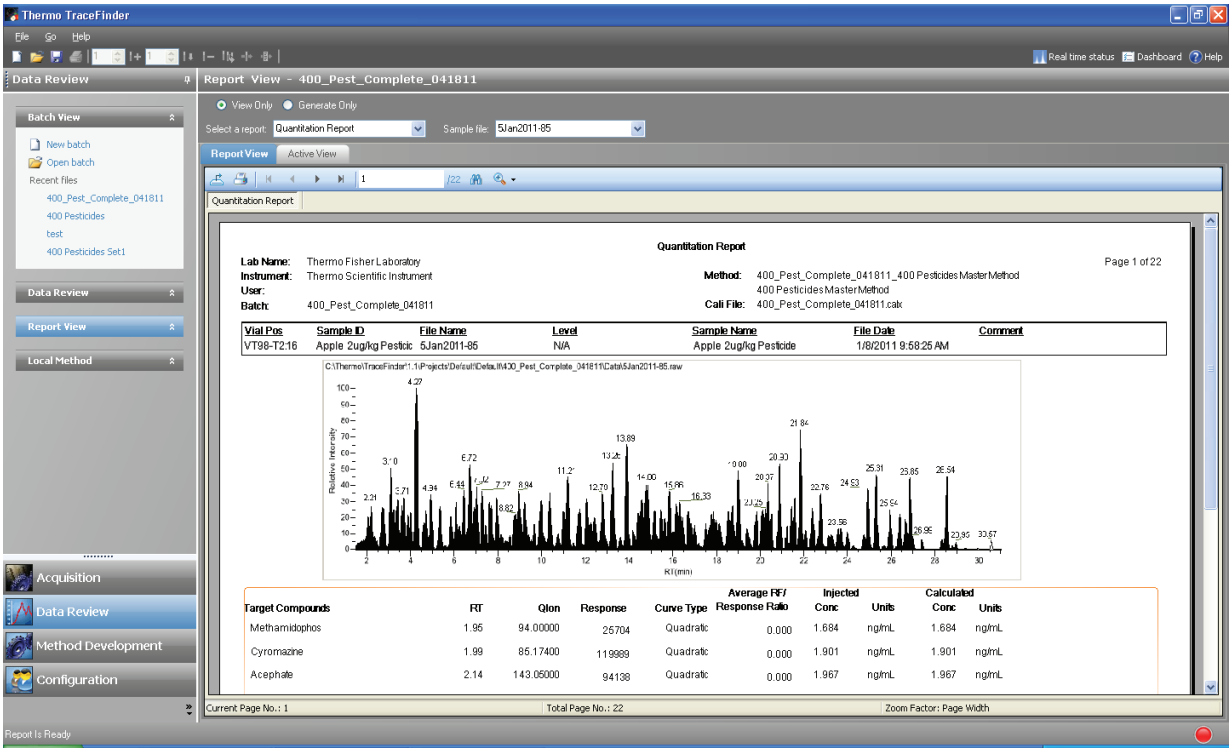


Figure 8. Report View section of TraceFinder software showing quantitative results

Conclusion

A multi-residue method was developed for the screening and determination of 437 pesticides in 45 minutes in a single run on a triple quadrupole mass spectrometer. Data analysis was streamlined by using TraceFinder software, which is ideally suited for quantitation of large amounts of data. For this large-scale multi-pesticide residue study, a timed SRM experiment provided accurate and sensitive analysis, without compromising the dwell time (and duty cycle) for detecting each compound per experiment. Quantitation-Enhanced Data-Dependent scanning provided confirmatory data following quantitative analysis. The majority of the pesticides were detected in the spiked matrices at concentrations lower than the MRLs established by EU and Japan.

Acknowledgment

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References

1. http://www.mhlw.go.jp/english/topics/foodsafety/positivelist060228/introduction.html
2. http://europa.eu/legislation_summaries/food_safety/plant_health_checks/l21289_en.htm
3. http://ec.europa.eu/food/plant/protection/evaluation/rev_prog_exist_pest_en.htm
4. Steven J. Lehotay, Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERs) Approach for Determining Pesticide Residues. *Methods in Biotechnology*, **2006**, *19*, 239-261.

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