Method: 52213

Determination of Pesticides in Grapes, Baby Food and Wheat Flour by Automated Online Sample Preparation LC-MS/MS

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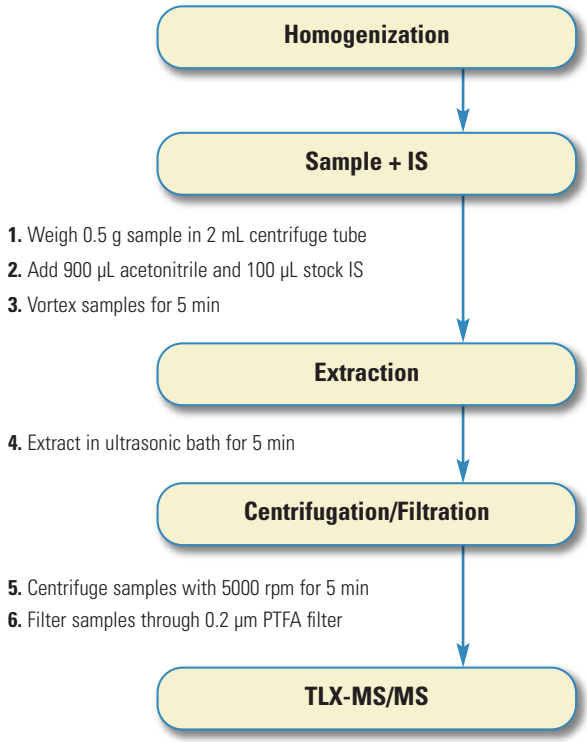
Key Words

- Transcend TLX
- TSQ Quantum Access MAX
- TurboFlow Technology
- Food Safety

1. Schematic of Method



2. Introduction

European Regulation 396/2005 sets maximum residue levels of pesticides in different products of plant and animal origin. These regulations present a significant analytical challenge with respect to the low limits of quantification which are required for some specified food matrices such as baby food. Many gas chromatography (GC) and high pressure liquid chromatography (HPLC) methods have been developed for multi-residue determination of pesticides and are in widespread use – employing a variety of sample preparation and cleanup techniques. In recent years the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method has become widely adopted for handling fruit and vegetables. However, QuEChERS requires many manual sample manipulation steps, making it labor-intensive when large numbers of samples have to be analyzed. It is therefore beneficial to consider options for automation of multi-residue methods, which can be cost-effective and can offer a high degree of reliability in recovery and repeatability. While the preliminary stages of homogenization and solvent extraction of food matrices inevitably require manual intervention, once a crude extract has been obtained, the procedure is fully automated thereafter. This automated procedure is included in the method, which utilizes turbulent flow chromatography with online liquid chromatography-mass spectrometry (LC-MS/MS).

Thermo Scientific Transcend TLX system coupled with the TSQ Quantum Access Max triple quadrupole mass spectrometer

Thermo SCIENTIFIC

3. Scope

This multi-residue pesticide method can be applied to fruits, cereals and composite baby foods at limits of detection (LODs) in the range of 0.8–10.3 µg/kg which are below respective EU maximum residue limits (MRLs). The method has been validated for 48 pesticides from different classes, but can be readily extended to a larger number of residues.

4. Principle

This method describes a novel sample preparation technique as a possible alternative to the QuEChERS method for high throughput pesticide analysis. Sample concentration, cleanup and analytical separation are carried out in a single run using an online coupled turbulent flow chromatography – reversed phase chromatography system (Thermo Scientific Transcend TLX system powered by Thermo Scientific TurboFlow technology). TurboFlow™ technology enables very effective separation of matrix and target compounds – resulting in relatively clean sample extracts. Macromolecules are removed from the sample extract with high efficiency, while target analytes are retained on the column based on different chemical interactions. After application of a wash step, the trapped compounds are transferred onto the analytical LC column and separated conventionally. The complete method involves internal standardization, solvent extraction of the homogenized food sample, centrifugation and injection into an automated cleanup system. Cleanup using TurboFlow technology has been optimized for maximum recovery of pesticide residues and minimal injection of co-extractives into the MS/MS. Identification of residues is based on ion-ratios using multiple reaction monitoring (MRM) of characteristic transition ions, and quantification using matrix-matched standards of one of the selected MRM ions.

5. Reagent List

		Part Number
5.1	Acetone, HPLC grade	A/0606/17
5.2	Acetonitrile, LC/MS grade	A/0638/17
5.3	Ammonium formate, for HPLC	A/5080/53
5.4	Methanol, Optima LC/MS grade	A456-212
5.5	Formic acid, extra pure for HPLC	F/1850/PB08
5.6	Isopropanol, HPLC grade	P/7507/17
5.7	Water, LC/MS grade	W/0112/17

6. Standard List

6.1 Pesticides: all standards from Sigma-Aldrich

Abamectin, ametryn, azinphos-me, azoxystrobin, bifenazate, carbaryl, carbendazim, carfentrazone-ethyl, chlormequate, clofentezin, cymoxanil, cypermethrin, dazomet, diazinon, dimethoate, dimethomorph A, dimethomorph B, ediphenfos, fenazaquin, fluazifop P, fluzilazol, hexithaizox, imazalil, imidacloprid, isoproturon, isoxaben, lactofen, malathion, metalaxyl, methomyl, metribuzin, myclobutanyl, omethoate, oxadyxil, oxamyl, pethoxamid, profenofos, promecarb, propoxur, pymetrozin, pyperonil-butoxide, pyrimethanyl, quinoxifen, spirodiclofen, tebuconazol, thiacloprid, triadimefon, trifloxistrobin.

6.2 Internal Standards

d_4-imidacloprid-, d_6-isoproturon, d_6-primicarb, d_{10}-parathion-ethyl (Sigma)

6.3 Quality Control Materials

FAPAS #963 (pasta matrix), FAPAS #966 (maize flour matrix), FAPAS #19110 (lettuce puree matrix)
(Note: FAPAS samples were selected primarily on content of target pesticides, however, matrices are different from the validated matrices with the exception of flour.)

7. Standards and Reagent Preparation

7.1 Concentration of mixed **pesticide working stock solution** (2 µg/mL and 1 µg/mL) in methanol. Prepare 2 µg/mL working stock standard solution by 10× dilution of intermediate stock standard solution in a 10 mL volumetric flask with methanol. Prepare 1 µg/mL working stock standard mix, by diluting intermediate stock standard solution by 20× in a 10 mL volumetric flask.

7.2 To prepare **individual stock standard solutions**, weigh 10 mg from each analyte into a 20 mL amber screw cap vial on the five digit analytical balance. Add 10 mL methanol from a calibrated pipette and note the weight of both analyte and solvent. If undissolved crystals are seen, put the vial in an ultrasonic bath until complete dissolution.

7.3 To prepare **intermediate stock standard solution**, pipette 200 µL from each individual stock standard into a 10 mL volumetric flask and fill up to the mark with methanol.

7.4 Concentration of **stock internal standard** (for sample spiking for internal standardization) is 100–100 ng/mL for d_4-imidacloprid and d_6-isoproturon, 10000 ng/mL for d_6-primicarb and 700 µg/mL d_{10}-parathion-ethyl in methanol. Prepare stock internal standard mixture by pipetting 7 mL of d_{10}-parathion-ethyl individual stock solution and 1 mL of intermediate stock internal standard mixture into a 10 mL volumetric flask and fill up to the mark with methanol.

7.5 To prepare **individual stock internal standard** solutions, weigh 10 mg of each analyte into a 20 mL amber screw cap vial on the five digit analytical balance. Add 10 mL methanol from a calibrated pipette and note the weight of both analyte and solvent.

7.6 To prepare **intermediate stock internal standard** mixture, pipette 1000 µL d_6-primicarb individual solution and 100–100 µL d_4-imidacloprid and d_6-isoproturon individual solutions into a 10 mL volumetric flask and fill to the mark with methanol.

8. Apparatus

		Part Number
8.1	Fisher precision balance	XP-1500FR
8.2	Sartorius analytical balance	ME235S
8.3	Thermo Scientific Barnstead EASYpure II water	3125753
8.4	Ultrasonic bath Elmasonic S40H	1002006
8.5	ULTRA-TURRAX® – G25 dispergation tool	1713300
8.6	ULTRA-TURRAX	3565000
8.7	Vortex shaker	3205025
8.8	Vortex universal cap	3205029
8.9	Accu-Jet pipettor	3140246
8.10	Thermo Scientific Heraeus Fresco 17 micro centrifuge	3208590
8.11	Transcend™ TLX-1 system	
8.12	Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer	

9. Consumables

		Part Number
9.1	LC vials	24014019
9.2	Pipette Finnpipette 100–1000 µL	3214535
9.3	Pipette Finnpipette 10–100 µL	3166472
9.4	Pipette Finnpipette 500–5000 µL	3166473
9.5	Pipette holder	3651211
9.6	Pipette tips 0.5–250 µL, 500/box	3270399
9.7	Pipette tips 1–5 mL, 75/box	3270420
9.8	Pipette tips 100–1000 µL, 200/box	3270410
9.9	Spatula, 18/10 steel	3458179
9.10	Spatula, nylon	3047217
9.11	Tube holder	3204844
9.12	Wash bottle, PTFE	3149330
9.13	2 mL vial rack	12211001
9.14	0.2 µm PTFA syringe filter	F2513-4
9.15	1 mL disposable plastic syringe	S7510-1
9.16	1.7 mL centrifuge plastic tube	3150968
9.17	TurboFlow Cyclone MCX-2 (50 × 0.5 mm) column	CH-953457
9.18	Thermo Scientific Hypersil GOLD 150 × 4.6 mm, 5 µm column	25005-154630
9.19	UNIGUARD holder	850-00
9.20	Hypersil GOLD™ 10 × 4 mm, 5 µm guard column	25005-014001

10. Glassware

		Part Number
10.1	Volumetric flask, 10 mL	FB50143
10.2	Volumetric flask, 25 mL	FB50147
10.3	1 mL glass pipette	FB50211
10.4	1 L bottle	9653650
10.5	500 mL bottle	9653640

11. Procedure

11.1 Sample Preparation

Solid Samples

Extract solid samples prior to injection into the Transcend system coupled to the TSQ Quantum Access MAX™ mass spectrometer. If samples are table grapes, these are treated as semisolid samples and need to be homogenized prior to extraction. Baby food and flour samples are treated as fine and homogenous solid matrices, so intensive manual mixing with a spatula is satisfactory.

11.2 Homogenization of Semisolid Samples

~~11.1.2~~ Select approximately 10–15 individual grapes randomly from the bunch and put into an appropriate size (depending on grape type and size ~100 mL) beaker and label it.

11.2.2 Attach the G25 dispergation tool to the ULTRA-TURRAX homogenizer

11.2.3 Start homogenization at middle rotation speed (speed level 2-3) and continue it to form a smooth puree

11.3 Extraction

11.3.1 Weigh 0.5 g sample into a 1.7 ml centrifuge tube

11.3.2 Add 900 µL acetonitrile stock IS

11.3.3 Vortex the sample for 5 min (to wet all the solid samples throughout)

11.3.4 Put the well-mixed samples into the Ultrasonic bath for 5 min.

11.3.5 Centrifuge in the micro centrifuge at 5000 rpm for 5 min.

11.3.6 Remove supernatant and filter it through 0.2 µm PTFE syringe filter directly into the LC vial

12. Analysis

Sample concentration, cleanup and analytical separation are carried out in a single run using an automated online sample preparation system, which includes the Transcend system and Thermo Scientific Aria operating software. TurboFlow technology with the Transcend system enables very effective separation of matrix and target compounds due to its special size exclusion and reversed phase chemistry. Macromolecules are removed from the sample extract with high efficiency, while target analytes are retained on the column based on different chemical interactions. After application of a wash step, the trapped compounds are transferred onto the analytical LC column and separated conventionally. Consequently the method was optimized for both TurboFlow technology and analytical chromatography.

		TurboFlow Technology								LC					
Step	**Duration [s]**	**Flow**	**Grad**	**A%**	**B%**	**C%**	**D%**	**Tee**	**Loop**	**Flow**	**Grad**	**A%**	**B%**	**C%**	**D%**
1	60	1.50	step		100			–	out	0.50	step		100		
2	60	1.50	step		95		5	–	out	0.50	step		100		
3	80	0.16	step		100			Tee	in	1.44	step		100		
4	60	1.00	step			100		–	in	1.60	ramp		55		45
5	60	1.00	step	10			90	–	in	1.60	ramp		40		60
6	220	0.20	step		100			–	out	1.60	ramp				100
7	60	0.20	step		100			–	out	1.60	step				100
8	180	0.20	step		100			–	out	1.00	step		100		
		Mobile phases for the TurboFlow method: A: water pH=3 B: water C: 40% acetonitrile 40% isopropanol and 20% acetone D: 5 mM ammonium-formiate in methanol + 0.1% formic acid								Solvent channels for LC: A: acetonitrile B: 5 mM ammonium-formiate in water + 0.1% formic acid C: water D: 5 mM ammonium-formiate in methanol + 0.1% formic acid *Note: LC channel C can be used for column wash purposes*					

Table 1: Gradient program table for Aria™ control software

12.1 LC Conditions for Transcend TLX System

Operation was carried out in focus mode setup (Figure 1) with 1:1 splitting before the TSQ Quantum Access MAX mass spectrometer entrance using a divert valve connection. The TurboFlow Cyclone MCX-2 column was installed as the TurboFlow column (9.17). The Hypersil GOLD column equipped with a guard column was used as the analytical LC column (9.18–9.20). Installed loop volume was 200 µL.

Sample load (Step 1) was applied with 1.5 mL/min flow rate, whereby matrix components were eluted in the waste, and target pesticides were trapped on the TurboFlow column. After washing the TurboFlow column with 5% organic/aqueous mixture (Step 2), the trapped pesticides were eluted and transferred (Step 3) after 2 min from the TurboFlow column to the analytical LC column. Simultaneous dilution of the eluate occurs enabling pre-concentration of pesticides at the beginning of the analytical column. The analytical LC column was equilibrated and conditioned during loading and washing steps. After transfer of the pesticides, the analytical separation started with gradient elution (Steps 4–7), while the TurboFlow column was washed and conditioned, and the loop was filled with the eluent. After the gradient run, the Hypersil GOLD column was washed in acetonitrile and conditioned for the next run. The total run time of the method with automated online sample preparation and analytical separation was 13 min. Table 1 gives details of the method program. In order to minimize sample carry-over and cross-contamination, the injection needle as well as the injection valve was washed 4 times with both cleaning solvents.

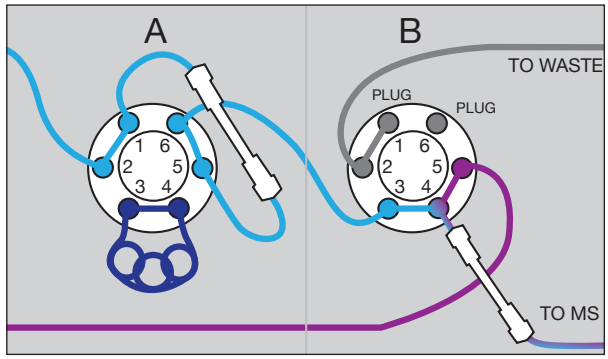


Figure 1: Focus mode system set up and method setting in Aria control software on the Transcend TLX system

12.1.1 Injector settings

Injector: Transcend TLX autosampler with 100 µL injection syringe volume

Sample holder temperature: 10 °C

Cleaning solvents: Solvent channel 1–80%MeOH/acetone
Solvent channel 2–50%MeOH/H_2O

Injector settings:

- Pre Clean with solvent 1 [steps]: 2
- Pre Clean with solvent 2 [steps]: 2
- Pre Clean with sample [steps]: 1
- Filling speed [µL/s]: 50
- Filling strokes [steps]: 2
- Injection port: LC Vlv1 (TurboFlow method channel)
- Pre inject delay [ms]: 500
- Post inject delay [ms]: 500
- Post clean with solvent 1 [steps]: 4
- Post clean with solvent 2 [steps]: 4
- Valve clean with solvent 1 [steps]: 4
- Valve clean with solvent 2 [steps]: 4
- Injection volume: 10 µL

12.1.2 Mass Spec conditions

Mass spectrometric detection was carried out by TSQ Quantum Access MAX triple stage quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) in selected reaction monitoring (SRM) mode. All SRM traces were individually tuned for each target pesticide. MS programming was set in Thermo Scientific Xcalibur software in Eazy set up mode.

Settings were:

- Scan type: SRM (details in table below)
- Cycle time [s]: 0.3
- Peak width: 0.7 Da FWHM
- Collision gas pressure [mTorr]: 1.0
- Capillary Temperature [°C]: 290
- Vaporizer Temperature [°C]: 290
- Sheath gas pressure [arb]: 40
- Aux gas pressure [arb]: 10
- Ion sweep pressure [arb]: 0
- Spray voltage [V]: 3200
- Polarity: positive for all compounds
- Trigger: 1.00e5

12.2 Calculation of Results

Calibration by the internal standardization is applied for the determination of pesticides. This quantification method requires determination of response factors R_f defined by the equation below. Calculation of final results is performed using the following equations.

Calculation of the response factor:

$$R_f = \frac{A_{St} \times c_{[IS]}}{A_{[IS]} \times c_{St}}$$

$\mathbf{R_f}$ – the response factor

$\mathbf{A_{St}}$ – the area of the pesticide peak in the calibration standard

$\mathbf{A_{[IS]}}$ – the area of the internal standard peak of the calibration standard

$\mathbf{c_{St}}$ – pesticide concentration of the calibration standard solution

$\mathbf{c_{[IS]}}$ – the internal standard concentration of the calibration standard solution

Calculations for each sample of the absolute amount of pesticide that was extracted from the sample:

$$X_{analyte} = \frac{A_{analyte} \times X_{[IS]}}{A_{[IS]} \times R_f}$$

$\mathbf{X_{analyte}}$ – the absolute amount of pesticide that was extracted from the sample

$\mathbf{A_{analyte}}$ – the area of pesticide peak in the sample

$\mathbf{A_{[IS]}}$ – the area of the internal standard peak in the sample

$\mathbf{X_{[IS]}}$ – the absolute amount of internal standard added to the sample

The concentration of pesticide in the sample [ng/g]:

$$c = \frac{X_{analyte}}{m}$$

m – the weight of sample [g]

$\mathbf{X_{analyte}}$ – absolute analyte amount [ng]

13. Method Performance Characteristics

In-house validation of the method was carried out on all matrices and target pesticides. International Union of Pure and Applied Chemistry (IUPAC)/Association of Official Analytical Chemists (AOAC) guideline for single laboratory validation[1,2] was used as a template and it was also demonstrated that method performance characteristics fulfilled the legislative criteria set for pesticide residue methods.[3]

13.1 Selectivity

Method (SRM) selectivity was confirmed based on the presence of specific ion transitions at the corresponding retention time (Table 2), as well as the observed ion ratio values corresponding to those of the standards. Acceptance criteria for retention time and ion ratios were set according to Reference 1.

13.2 Linearity, Response Factor

The linearity of calibration curves was assessed over the range from 10–500 ng/g. In all cases, the correlation coefficients of linear functions were better than 0.985. The calibration curves were created at five levels (matrix-matched) and injected in duplicate. R_f values for internal standardization were determined from the calibration curves for each matrix, and internal standards by calculating cumulative average response factors over the whole calibration range.

13.3 Accuracy

Method accuracy and precision was assessed by recovery studies using blank matrices spiked at three concentration levels injected in six individually prepared replicates. Samples were spiked at 10, 100 and 250 ng/g concentration levels. Found concentrations, recovery and relative standard deviation (%RSD) were calculated (Table 3). Recovery values are deemed acceptable if between 70–125%. Additional accuracy was established for selected target analytes by analysing FAPAS #963, 966 and 19110 proficiency test materials. All measured concentrations of the relevant compounds (diazinon, tebuconazole, trifloxistrobin, malathion, azoxystrobin and dimethomorph) were within the acceptable satisfactory ranges.

13.4 Precision

Method within-day and between-day precision values were determined for each matrix at middle spiking level (100 ng/g) each in 6 replicates and expressed as %RSD over 3 days with individually prepared samples. Mean within-day precision values were determined as average of the 3 individual days' mean precision, while between-day precision was expressed as mean of the overall precision data. Measured values are shown in Table 4.

13.5 Limits of Detection (LODs) and Quantification (LOQs)

LODs and LOQs were estimated following the IUPAC approach which consisted of analyzing the blank sample to establish noise levels and then testing experimentally estimated LODs and LOQs for signal/noise, 3 and 10 respectively. The method LOD values are listed in Table 5. The expectation of the method was to meet MRL values at least at LOD level. The lowest MRL values were defined for baby food matrices (10 ng/g), which were achieved in all cases.

13.6 Robustness

A robustness study was performed by varying parameters like extraction time, centrifugation speed, time by 20%, shaker (horizontal shaker, vortex) and extraction mode (ultrasonic bath, vortex shaking). Results were compared to the original method and significant differences were sought based on ANOVA analysis. None of the parameters which were varied led to significant differences in measured values, consequently indicating that the method was robust.

14. Conclusion

The method described here enables convenient, fast and cost-effective automated determination of selected pesticides, from polar to non-polar compound chemistry, in different matrix types. Based on the short total run time and Transcend system with TurboFlow technology, 100 samples per day can be analyzed under controlled sample preparation conditions. Method performance characteristics were established by in-house validation for baby food, grapes and wheat flour matrices. The method performance indicates it is suitable for routine use for regulatory purposes and can be readily extended to a larger and wider range of pesticide residues.

15. References

1. http://www.aoac.org/Official_Methods/slv_guidelines.pdf
2. http://www.scribd.com/doc/4922271/Harmonized-Guidelines-for-Single-Laboratory-Validation-of-Methods-Of
3. http://ec.europa.eu/sanco_pesticides/public/index.cfm

16. Annex

16.1 Tables and Chromatograms

Analyte	Precursor Ion	Product Ion (CE)	Product Ion2 (CE)	Retention Time [min]
Abamectin	890.2	305.1 (25)	567.4 (12)	10.1
Ametryn	228.1	96.1 (25)	116.1 (26)	7.76
Azinphos methyl	339.8	132.1 (19)	160.2 (12)	7.87
Azoxystrobin	404.1	344.1 (25)	372.1 (14)	7.99
Bifenazate	301.1	198.1 (7)	170.1 (19)	8.36
Carbaryl	219.1	202.1 (5)	127.1 (32)	7.31
Carbendazim	191.8	160.1 (18)	132.1 (29)	5.96
Carfentrazone-ethyl	429.1	412.2 (12)	384.2 (18)	8.71
Chlormequate	122.1	58.5 (31)	63.3 (21)	4.06
Clofentezin	304.7	138.1 (26)	102.1 (38)	9.07
Cymoxanil	199.3	83.9 (20)	111.1 (20)	6.71
Cypermethrin	433.1	416.3 (5)	191.2 (15)	8.72
Dazomet	163.1	120.1 (11)	90.2 (9)	5.83
Diazinon	304.9	169.1 (21)	153.1 (21)	8.90
Dimethoate	230.2	125.3 (21)	170.7 (13)	6.43
Dimethomorph A&B	388.1	300.9 (21)	164.9 (31)	8.12/8.34
Ediphenfos	310.8	283.1 (11)	111.2 (19)	8.80
Fenazaquin	307.2	161.2 (16)	57.2 (21)	10.18
Fluazifop P	384.3	282.1 (18)	254.2 (27)	9.29
Fluzilazol	316.1	165.1 (27)	247.1 (18)	8.66
Hexithaizox	353.1	228.1 (14)	167.8 (24)	9.66
Imazalil	296.9	159.1 (23)	176.2 (20)	7.50
Imidacloprid	256.1	209.2 (15)	175.2 (17)	6.16
d4-Imidacloprid	259.9	213.1 (17)	179.1 (20)	6.24
Isoproturon	207.1	72.1 (18)	165.3 (14)	7.73
d6-Isoproturon	213.2	78.3 (19)	171.1 (14)	7.71
Isoxaben	333.1	165.1 (20)	149.9 (38)	8.15
Lactofen	479.1	462.1 (5)	344.2 (15)	9.35
Malathion	347.9	330.7 (5)	99.4 (29)	8.22
Metalaxyl	279.9	220.2 (13)	192.1 (18)	7.63
Methomyl	163.1	106.1 (10)	88.1 (8)	5.95
Metribuzin	215.2	187.1 (16)	74.1 (34)	7.21
Myclobutanyl	289.1	70.3 (18)	124.9 (30)	8.38
Omethoate	214.2	125.1 (22)	155.2 (15)	5.58
Oxadyxil	296.2	279.2 (5)	219.3 (15)	6.80
Oxamyl	236.9	72.2 (14)	90.3 (5)	5.75
d10-Parathion-ethyl	302.1	238.1 (17)	270.1 (11)	8.83
Pethoxamid	296.1	131.1 (20)	250.2 (12)	8.48
d6-Primicarb	245.2	185.1 (16)	78.3 (28)	6.86
Profenofos	374.8	304.9 (17)	222.8 (31)	9.37
Promecarb	225.2	207.9 (7)	151.2 (6)	8.29
Propoxur	210.1	111.1 (14)	168.2 (7)	7.12
Pymetrozin	218.0	105.2 (23)	78.3 (37)	5.53
Pyperonil-butoxide	356.0	177.1 (13)	147.1 (29)	9.49
Pyrimethanyl	200.1	181.2 (35)	168.1 (28)	8.00
Quinoxifen	307.9	196.8 (31)	214.1 (33)	9.68
Spirodiclofen	410.9	313.1 (9)	71.1 (12)	9.83
Tebuconazol	308.2	70.2 (22)	124.9 (33)	8.88
Thiacloprid	253.1	126.1 (19)	90.1 (33)	6.55
Triadimefon	294.1	197.1 (15)	69.4 (20)	8.32
Trifloxistrobin	409.5	186.3 (17)	206.4 (13)	9.24

Table 2: Ion transitions for SRM setting

	Grape [Rec %] (%RSD)			Baby Food [Rec %] (%RSD)			Wheat Flour [Rec %] (%RSD)		
Analyte	**10 ng/g**	**100 ng/g**	**250 ng/g**	**10 ng/g**	**100 ng/g**	**250 ng/g**	**10 ng/g**	**100 ng/g**	**250 ng/g**
Abamectin	66 (17)	64 (18)	71 (11)	68 (19)	76 (5)	76 (4)	89 (17)	99 (5)	101 (7)
Ametryn	111 (16)	99 (18)	118 (9)	111 (8)	115 (5)	125 (5)	108 (16)	111 (4)	109 (7)
Azinphos-me	111 (9)	121 (19)	110 (11)	105 (5)	100 (4)	112 (5)	85 (13)	92 (6)	124 (4)
Azoxystrobin	105 (15)	69 (8)	104 (9)	86 (4)	90 (5)	88 (2)	87 (5)	118 (3)	117 (2)
Bifenazate	90 (14)	88 (5)	96 (9)	101 (5)	106 (5)	113 (4)	121 (5)	112 (4)	108 (3)
Carbaryl	69 (8)	86 (8)	90 (8)	98 (5)	111 (6)	120 (4)	110 (4)	110 (3)	107 (3)
Carbendazim	93 (14)	108 (5)	104 (8)	122 (7)	89 (5)	97 (3)	73 (14)	123 (6)	116 (3)
Carfentrazone-ethyl	85 (14)	74 (11)	84 (11)	92 (6)	102 (5)	104 (3)	112 (7)	119 (4)	114 (2)
Chlormequate	LOD	90 (12)	77 (17)	74 (16)*	90 (10)	89 (10)	LOD	106 (7)	100 (7)
Clofentezin	78 (18)*	71 (9)	84 (6)	71 (18)	73 (12)	82 (10)	123 (10)*	110 (7)	94 (13)
Cymoxanil	110 (13)	93 (14)	114 (13)	96 (19)	80 (17)	78 (7)	89 (19)	101 (15)	83 (12)
Cypermethrin	121(13)*	84 (17)	74 (11)	122 (12)	79 (12)	87 (9)	123 (13)*	115 (9)	114 (11)
Dazomet	106 (19)	107 (18)	117 (9)	80 (17)	114 (5)	118 (5)	84 (7)	102 (5)	99 (5)
Diazinon	80 (15)	75 (5)	87 (10)	87 (9)	99 (6)	103 (4)	122 (3)	108 (2)	105 (3)
Dimethoate	90 (4)	88 (10)	95 (4)	106 (3)	114 (4)	117 (3)	73 (7)	118 (4)	112 (4)
Dimethomorph A	70 (15)	84 (8)	74 (8)	81 (5)	85 (4)	86 (4)	112 (4)	98 (3)	98 (2)
Dimethomorph B	89 (11)	71 (4)	77 (4)	86 (4)	91 (4)	89 (4)	110 (8)	114 (5)	118 (4)
Ediphenfos	94 (14)	72 (7)	90 (8)	109 (6)	110 (5)	114 (4)	105 (11)	111 (8)	110 (6)
Fenazaquin	101 (6)	88 (12)	78 (4)	78 (4)	83 (7)	85 (8)	104 (10)	81 (12)	73 (16)
Fluazifop P	101 (17)	72 (16)	86 (13)	101 (8)	100 (7)	103 (6)	116 (5)	107 (4)	106 (4)
Fluzilazol	87 (12)	69 (9)	89 (9)	91 (9)	102 (6)	107 (5)	122 (5)	110 (3)	106 (5)
Hexithaizox	75 (17)	82 (15)	93 (15)	93 (15)	119 (8)	120 (12)	102 (5)*	94 (11)	91 (14)
Imazalil	79 (8)	82 (11)	85 (8)	88 (5)	95 (8)	102 (6)	85 (19)	81 (5)	77 (12)
Imidacloprid	86 (8)	93 (6)	97 (5)	111 (4)	117 (3)	124 (2)	107 (3)	112 (3)	110 (3)
Isoproturon	95 (8)	74 (10)	86 (7)	104 (5)	109 (4)	101 (4)	123 (18)	109 (4)	114 (3)
Isoxaben	84 (14)	74 (5)	87 (7)	95 (4)	103 (4)	103 (3)	115 (5)	121 (3)	114 (2)
Lactofen	91 (17)	70 (15)	81 (12)	104 (7)	108 (5)	116 (9)	131 (7)	111 (6)	109 (7)
Malathion	117 (9)	83 (13)	75 (10)	103 (6)	91 (4)	88 (5)	104 (9)	94 (5)	112 (4)
Metalaxyl	79 (9)	76 (9)	80 (5)	88 (5)	98 (5)	97 (5)	74 (8)	123 (4)	115 (3)
Methomyl	75 (9)	68 (8)	81 (10)	73 (12)	81 (4)	87 (5)	99 (10)	96 (10)	89 (10)
Metribuzin	89 (11)	73 (6)	87 (4)	106 (10)	112 (5)	113 (7)	103 (13)	112 (4)	107 (3)
Myclobutanyl	90 (17)	75 (11)	90 (10)	102 (8)	104 (5)	110 (4)	105 (3)	119 (4)	117 (3)
Omethoate	70 (20)*	72 (8)	76 (9)	76 (18)	78 (7)	81 (11)	71 (16)*	75 (14)	70 (6)
Oxadyxil	71 (9)	72 (7)	87 (5)	84 (4)	101 (4)	100 (4)	87 (6)	123 (4)	117 (2)
Oxamyl	69 (9)	71 (9)	69 (7)	74 (8)	78 (5)	79 (6)	96 (11)	95 (10)	88 (7)
Pethoxamid	74 (10)*	70 (6)	77 (8)	89 (5)	88 (8)	91 (6)	123 (3)	115 (3)	108 (2)
Profenofos	112 (17)	72 (12)	95 (11)	109 (6)	115 (4)	120 (4)	115 (8)	106 (3)	105 (2)
Promecarb	90 (10)	86 (5)	94 (5)	104 (6)	114 (3)	115 (4)	128 (4)	122 (3)	112 (2)
Propoxur	84 (6)	87 (6)	84 (7)	98 (6)	106 (4)	108 (4)	91 (6)	115 (4)	110 (4)
Pymetrozin	101 (8)	94 (4)	121 (14)	101 (4)	112 (5)	113 (3)	89 (3)	117 (3)	110 (2)
Pyperonil-butoxide	78 (17)	93 (9)	86 (9)	95 (4)	102 (4)	109 (4)	115 (10)	113 (6)	111 (3)
Pyrimethanyl	120 (13)	121 (7)	108 (13)	80 (14)	114 (5)	101 (4)	94 (10)	106 (5)	110 (6)
Quinoxifen	90 (19)	78 (20)	104 (6)	87 (10)	99 (8)	105 (7)	98 (12)	90 (7)	86 (9)
Spirodiclofen	83 (11)	79 (17)	78 (17)	89 (16)	102 (6)	103 (7)	83 (4)	98 (5)	96 (5)
Tebuconazol	83 (15)	79 (8)	83 (6)	94 (4)	93 (6)	98 (4)	121 (7)	115 (4)	117 (3)
Thiacloprid	95 (8)	80 (10)	89 (8)	109 (5)	113 (5)	109 (3)	69 (8)	124 (6)	116 (4)
Triadimefon	69 (12)	68 (8)	83 (5)	96 (8)	104 (6)	109 (4)	118 (8)	115 (3)	114 (3)
Trifloxistrobin	82 (5)	76 (8)	81 (11)	99 (6)	97 (6)	104 (4)	109 (4)	98 (5)	92 (4)

Table 3: Average method recovery [%] and %RSD [%] values at 3 different spike levels in the investigated matrices (n=6)

*LOD: spike level at or below LOD, * spike level at or below LOQ*

		Grape		Baby Food		Wheat Flour	
Analyte	**Spike Level [ng/g]**	**Mean within day precision [%RSD]**	**Between day precision [%RSD]**	**Mean within day precision [%RSD]**	**Between day precision [%RSD]**	**Mean within day precision [%RSD]**	**Between day precision [%RSD]**
Abamectin	100	11	14	6	11	10	11
Ametryn	100	11	19	9	12	8	16
Azinphos-me	100	12	15	5	6	9	11
Azoxystrobin	100	14	22	7	10	6	6
Bifenazate	100	10	17	7	9	6	9
Carbaryl	100	16	19	7	16	8	17
Carbendazim	100	8	11	7	12	7	9
Carfentrazone-ethyl	100	9	17	10	15	8	10
Chlormequate	100	12	15	10	15	8	10
Clofentezin	100	14	21	11	15	9	11
Cymoxanil	100	16	19	14	21	11	15
Cypermethrin	100	12	16	12	16	10	12
Dazomet	100	15	20	13	20	15	21
Diazinon	100	9	17	6	16	8	12
Dimethoate	100	12	17	9	17	10	13
Dimethomorph A	100	11	17	7	16	8	10
Dimethomorph B	100	6	10	7	11	7	14
Ediphenfos	100	10	11	7	7	6	6
Fenazaquin	100	12	21	9	13	13	13
Fluazifop P	100	9	14	8	8	11	10
Fluzilazol	100	9	19	6	10	5	8
Hexithaizox	100	8	19	9	18	15	19
Imazalil	100	10	18	10	15	10	17
Imidacloprid	100	7	8	5	6	14	16
Isoproturon	100	15	21	6	12	7	12
Isoxaben	100	12	17	7	9	7	7
Lactofen	100	12	17	7	20	12	15
Malathion	100	7	19	8	17	5	17
Metalaxyl	100	12	19	6	11	8	8
Methomyl	100	12	18	7	14	10	20
Metribuzin	100	8	16	7	8	8	9
Myclobutanyl	100	10	14	8	10	8	14
Omethoate	100	18	19	14	16	13	14
Oxadyxil	100	12	18	4	10	6	13
Oxamyl	100	10	19	7	15	9	15
Pethoxamid	100	8	19	8	16	5	10
Profenofos	100	8	19	5	19	11	11
Promecarb	100	10	20	4	5	12	14
Propoxur	100	7	19	6	8	8	9
Pymetrozin	100	11	16	6	10	9	10
Pyperonil-butoxide	100	6	19	6	15	6	15
Pyrimethanyl	100	14	20	6	8	9	11
Quinoxifen	100	9	18	9	10	10	13
Spirodiclofen	100	9	18	8	18	10	13
Tebuconazol	100	8	13	9	10	6	6
Thiacloprid	100	16	17	9	13	9	13
Triadimefon	100	9	19	8	11	7	8
Trifloxistrobin	100	13	18	8	11	10	13

Table 4: Method (intermediate) precision values for all matrices

	Baby Food		Grape		Wheat Flour	
Compound	**LOD [ng/g]**	**LOQ [ng/g]**	**LOD [ng/g]**	**LOQ [ng/g]**	**LOD [ng/g]**	**LOQ [ng/g]**
Abamectin	2.4	7.2	2.0	6.0	3.1	9.3
Ametryn	2.5	7.5	2.5	7.5	1.4	4.2
Azinphos-Me	1.1	3.3	1.1	3.3	1.2	3.6
Azoxystrobin	0.9	2.7	0.9	2.7	0.9	2.7
Bifenazate	2.8	8.4	2.7	8.1	2.9	8.7
Carbaryl	1.5	4.5	1.6	4.8	1.2	3.6
Carbendazim	1.3	3.9	1.4	4.2	2.6	7.8
Carfentrazone-ethyl	1.5	4.5	1.5	4.5	2.1	6.4
Chlormequate	6.0	18.0	10.3	31.0	9.2	27.7
Clofentezin	3.2	9.6	4.1	12.3	4.5	13.5
Cymoxanil	3.3	9.9	3.1	9.3	3.2	9.6
Cypermethrin	3.0	9.0	5.0	15.0	4.5	13.5
Dazomet	1.4	4.3	1.3	4.0	1.2	3.6
Diazinon	1.1	3.3	1.0	3.0	1.3	3.9
Dimethoate	1.2	3.6	1.2	3.6	1.2	3.6
Dimethomorph	1.0	3.0	1.0	3.0	2.0	6.0
Edifenphos	1.2	3.6	1.1	3.3	1.2	3.6
Fenazaquin	2.0	6.0	2.5	7.5	2.2	6.6
Fluazifop P	1.0	3.0	1.2	3.6	1.8	5.4
Fluzilazol	1.0	3.0	1.0	3.0	1.5	4.5
Hexithiazox	3.0	9.1	3.4	10.2	4.0	12.0
Imazalil	1.2	3.6	1.4	4.2	1.5	4.5
Imidacloprid	1.1	3.3	1.2	3.6	1.2	3.6
Isoproturon	1.7	5.1	1.8	5.4	1.3	4.0
Isoxaben	1.0	3.0	1.0	3.0	1.1	3.3
Lactofen	1.4	4.2	1.9	5.7	2.5	7.5
Malathion	3.0	9.0	1.8	5.4	1.6	4.8
Metalaxyl	0.9	2.7	0.9	2.7	2.1	6.3
Methamyl	1.6	4.8	1.4	4.2	1.7	5.1
Metribuzin	1.5	4.5	1.6	4.8	1.9	5.7
Myclobutanyl	2.0	6.0	1.4	4.2	1.5	4.5
Omethoate	3.0	9.0	3.5	10.5	3.6	10.8
Oxadyxil	1.8	5.4	1.7	5.1	2.5	7.5
Oxamyl	2.5	7.5	3.3	9.9	2.9	8.7
Pethoxamid	2.7	8.1	3.5	10.5	2.9	8.7
Profenofos	1.9	5.7	1.9	5.7	2.5	7.5
Promecarb	1.8	5.4	1.7	5.1	1.9	5.7
Propoxur	1.6	4.8	1.5	4.5	1.2	3.6
Pymetrozin	1.1	3.3	1.4	4.2	1.1	3.3
Pyperonil-butoxide	0.8	2.4	0.8	2.4	0.8	2.4
Pyrimethanil	1.9	5.7	2.3	6.9	3.1	9.2
Quinoxifen	1.5	4.5	1.8	5.4	2.0	6.0
Spirodiclofen	2.5	7.5	2.6	7.8	3.2	9.6
Tebuconazol	1.3	3.9	1.8	5.4	2.2	6.6
Thiacloprid	1.0	3.0	1.0	3.0	1.4	4.2
Triadimefon	1.4	4.2	1.5	4.5	2.9	8.7
Trifloxistrobin	1.2	3.6	1.6	4.8	1.6	4.8

Table 5: Limits of detection and limits of quantification (LODs and LOQs) of the method for different matrices

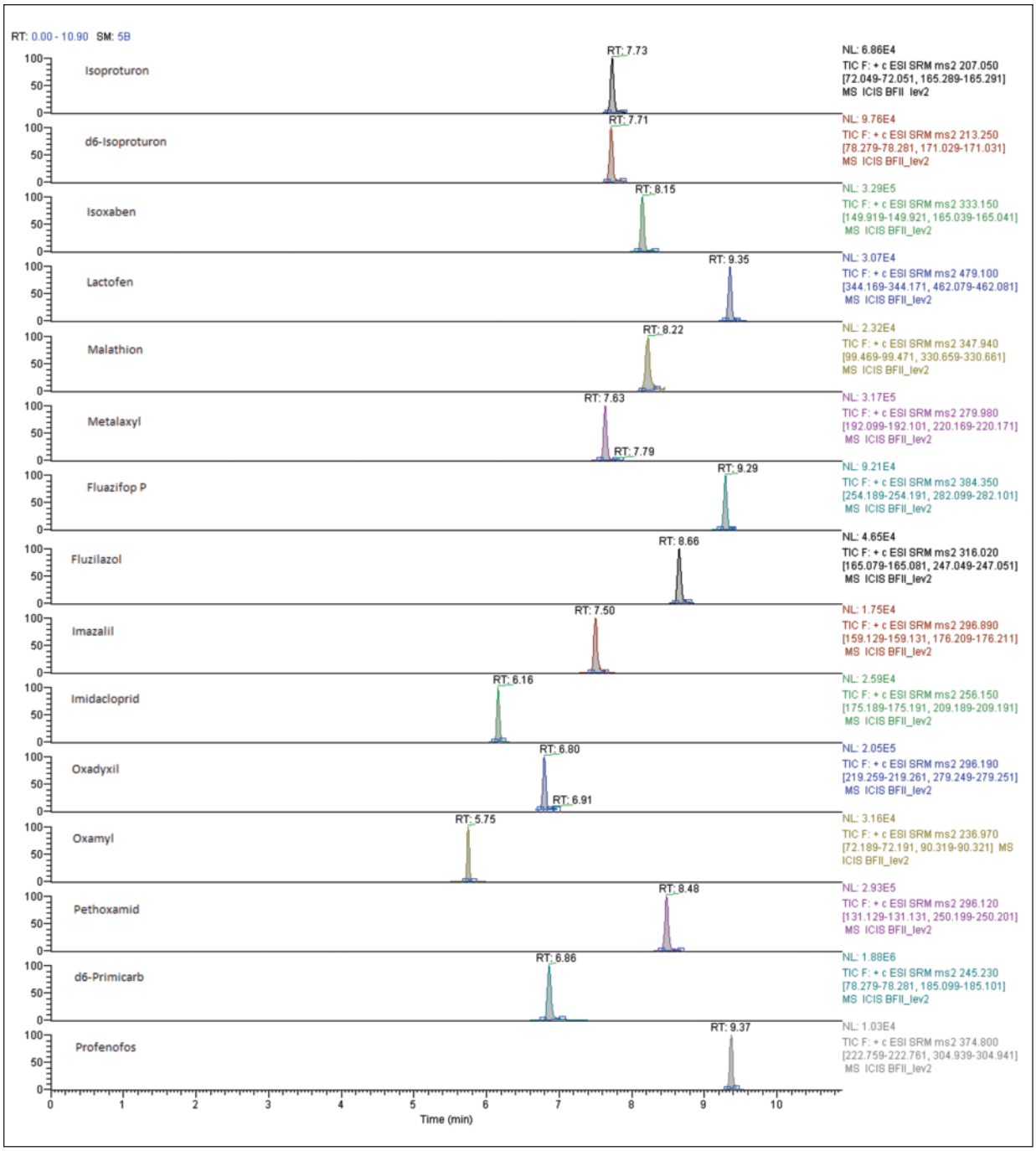


Figure 2: Illustration of selected target substance peaks and internal standards in baby food matrix spiked at legislation limit 10 ng/g

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