DETERMINATION OF PRIORITY PESTICIDES IN BABY FOODS USING UPLC™ AND TANDEM QUADRUPOLE MASS SPECTROMETRY LC/MS/MS

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Peter Hancock[1], Cristiana C. Leandro[2,3], Brendan J. Keely[2] and Richard J. Fussell[3]

[1]Waters Corporation, Atlas Park, Simonsway, Manchester M22 5PP, UK; [2]University of York, Heslington, York YO10 5DD, UK; [3]Central Science Laboratory, Sand Hutton, York YO41 1LZ, UK

INTRODUCTION

The European Union Baby Food Directive 2003/13/EC[1] designates pesticides as prohibited, in which case they are considered not to have been used if their residue does not exceed 0.003 mg/kg or have maximum residue limits (MRLs) set between 0.004—0.008 mg/kg. Seven pesticides and ten transformation products (e.g. metabolites) listed in the Directive are suitable for multiresidue liquid chromatography—mass spectrometry (LC-MS) analysis.

To be able to enforce the Directive laboratories require multiresidue methods with lower limits of detection (LOD) than those currently available. This necessitates improvements in the extraction, clean up, separation and detection of pesticides in baby food samples.

Ultra performance liquid chromatography (UPLC™)[2] has the potential to provide shorter run times, greater sensitivity and better chromatographic resolution than established high performance liquid chromatography (HPLC) methods.

A sample extraction and preparation method has been developed for the priority pesticides and transformation products specified in baby food. Prior to analysis, co-extractives were removed from acetonitrile extracts using dispersive solid phase extraction with primary secondary amine (50 mg) sorbent[3,4]. Acetonitrile extracts are suitable for direct analysis using liquid chromatography—tandem mass spectrometry (LC/MS/MS).

The aim of this work was to develop a simple and rapid method, suitable for the quantification and confirmation of sixteen priority pesticides and transformation products in different baby foods at a level of 0.001 mg/kg.

METHODS

Extraction Method

- Weigh 10 g homogenised sample into a centrifuge tube
- Add 10 mL acetonitrile and shake vigorously for 1 min
- Add 4 g $MgSO_4$ and 1 g NaCl and vortex immediately
- Centrifuge at 4300 g for 5 min
- Add 150 mg anhydrous $MgSO_4$ + 50 mg PSA to a micro-centrifuge vial
- Transfer 1 mL aliquot of acetonitrile layer to the vial and shake for 30 s
- Centrifuge at 5000 g for 1 min
- Transfer 100 mL into a LC vial and add 900 mL water
- Submit for LC/MS/MS analysis

LC Conditions

LC system	Waters ACQUITY Ultra Performance LC™
Column	Waters Acquity UPLC™ BEH C_{18} 2.1x100 mm, 1.7 µm
Column temperature	40 °C
Flow rate	0.3 mL/min
Mobile phase A	90% Water, 10% MeOH + 20 mM $CH_3CO_2NH_4$
Mobile phase B	10% Water, 90% MeOH + 20 mM $CH_3CO_2NH_4$
Gradient	0 min 100% A → 5 min 100% B → 7 min 100% B
Total run time	10 min
Injection volume	50 µL

MS Conditions

MS system	Waters® Micromass® Quattro Premier™
Ionisation mode	Electrospray in positive ion mode
Cone voltage	Analyte dependent (See table below)
MS/MS	Multiple reaction monitoring (MRM)
Collision energy	Analyte dependent (See table below)
Resolution	Peak width half height < 0.7Da
Collision gas	Argon at a cell pressure of 3.0 x 10^{-3} mBar

Two MRM transitions were acquired for each residue so that quantification and confirmation can be performed with a single injection assuming that the ion ratio between the two transitions is consistent for standards and samples. The confirmation criteria chosen were dependent on the relative abundance of the two transitions in accordance with EU legislation 2002/657/EC[3].

Pesticide	Quantification Transition	Confirmation Transition	Cone Voltage
Omethoate	214 > 183 (12 eV)	214 > 155 (16 eV)	20 V
Oxydemeton-s-methyl	247 > 169 (14 eV)	247 > 109 (28 eV)	20 V
Demeton-s-methylsulfone	263 > 169 (17 eV)	263 > 121 (17 eV)	26 V
Dimethoate	230 > 125 (20 eV)	230 > 171 (15 eV)	13 V
Fensulfothion-oxon	293 > 237 (19 eV)	293 > 265 (14 eV)	28 V
Fensulfothion-oxon-sulfone	309 > 253 (16 eV)	309 > 175 (27 eV)	27 V
Demeton-s-methyl	231 > 89 (12 eV)	231 > 61 (30eV)	12 V
Disulfoton sulfoxide	291 > 185 (14 eV)	291 > 97 (31 eV)	18 V
Disulfoton sulfone	307 > 97 (29 eV)	307 > 115 (24 eV)	23 V
Fensulfothion	309 > 281 (15 eV)	309 > 157 (25 eV)	29 V
Fensulfothion sulfone	325 > 269 (16 eV)	325 > 297 (11 eV)	26 V
Terbufos sulfone	321 > 171 (12 eV)	321 > 115 (29 eV)	21 V
Terbufos sulfoxide	305 > 187 (11 eV)	305 > 131 (28 eV)	14 V
Ethoprophos	243 > 131 (20 eV)	243 > 173 (15eV)	20 V
Disulfoton	275 > 89 (10 eV)	275 > 61 (33 eV)	9 V
Cadusafos	271 > 159 (15 eV)	271 > 131 (23 eV)	18 V
Terbufos	289 > 103 (9 eV)	289 > 233 (5 eV)	12 V

RESULTS AND DISCUSSION

To test the extraction method described, seven recovery experiments were performed in fruit-based, potato-based and cereal-based baby foods, spiked at 0.001 mg/kg. The mean recovery and relative standard deviation (% RSD) of each analyte are listed below.

Pesticide Residue	Fruit	Potato	Cereal
Cadusafos	105 (4)	100 (4)	105 (2)
Demeton-s-methyl	113 (5)	104 (4)	98 (5)
Demeton-s-methylsulfone	104 (4)	105 (6)	103 (3)
Dimethoate*	107 (2)	106 (7)	104 (4)
Disulfoton	89 (17)	100 (13)	106 (13)
Disulfoton sulfone	106 (6)	103 (1)	102 (3)
Disulfoton sulfoxide	105 (4)	106 (4)	103 (3)
Ethoprophos	107 (2)	103 (3)	103 (4)
Fensulfothion	107 (5)	98 (5)	106 (3)
Fensulfothion-oxon	106 (2)	102 (5)	103 (4)
Fensulfothion-oxon-sulfone	109 (4)	102 (4)	105 (4)
Fensulfothion sulfone	105 (4)	102 (5)	104 (2)
Omethoate	98 (2)	90 (2)	97 (3)
Oxydemeton-s-methyl	105 (6)	101 (4)	110 (3)
Terbufos	108 (14)	85 (10)	98 (6)
Terbufos sulfone	108 (4)	103 (5)	107 (5)
Terbufos sulfoxide	110 (3)	99 (4)	106 (4)

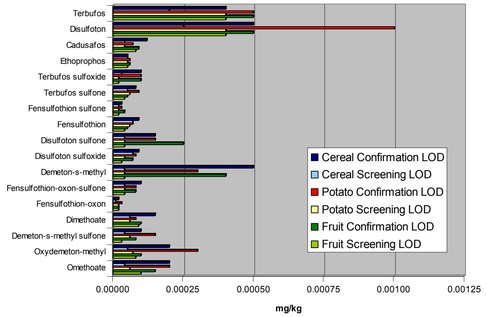
*Dimethoate was only included in the compound list as a possible precursor for omethoate

The extraction method was further tested for ten different baby foods ranging from simple, low fat (fruit-based) to complex, high fat (meat-based). The mean recovery and RSD for each analyte with two determinations in the ten different baby foods, spiked at 0.001 mg/kg, are listed below.

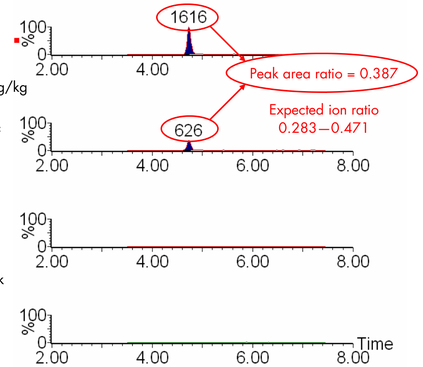
Pesticide Residue	Mean Recovery	%RSD
Cadusafos	103	6
Demeton-s-methyl	97	5
Demeton-s-methylsulfone	106	5
Dimethoate	102	6
Disulfoton	114	19
Disulfoton sulfone	103	7
Disulfoton sulfoxide	99	6
Ethoprophos	104	4
Fensulfothion	103	5
Fensulfothion-oxon	103	5
Fensulfothion-oxon-sulfone	102	6
Fensulfothion sulfone	108	7
Omethoate	90	4
Oxydemeton-s-methyl	124	5
Terbufos	100	20
Terbufos sulfone	102	7
Terbufos sulfoxide	103	4

Matrix-matched calibrations curves were linear over the concentration range 0.0005 – 0.0100 mg/kg, with correlation coefficients of greater than 0.995 obtained for all the pesticide residues in all three matrices. The data were processed using Waters TargetLynx™ application manager.

The instrumental limits of detection (LODs) were determined for each residue from the matrix-matched calibration curves for quantification (1 MRM transition) and confirmation (2 MRM transitions). The LOD was defined as signal-to-noise (S/N) ratio of at least 3:1. All the EU MRLs for the compounds analysed are greater than 0.00075 mg/kg in baby food. Therefore, this confirmatory method is able to surpass the current legislation.



To illustrate that quantification and confirmation can be performed in a single run, demeton-s-methylsulfone at the 0.001mg/kg concentration level is compared to the matrix blank. The expected ion ratio from solvent standards is 0.377. For confirmation, any concentration in the extracts must have a ratio between 0.283 and 0.471 (± 25 %). The 0.001mg/kg



Ion ratios are important as they provide the basis of confirmation. The ion ratio statistics were calculated for the 21 recovery experiments across the fruit-based, potato–based and cereal–based baby foods along with the 20 recovery experiments from the ten different baby foods, all spiked at 0.001 mg/kg. Using the method described, all residues can be confirmed to EU guidelines at the 0.001mg/kg concentration level.

Pesticide Residue	Fruit, Potato and Cereal n = 21	10 Matrices n = 20	EU legislation 2002/657/EC
Cadusafos	0.526 (5)	0.542 (6)	20
Demeton-s-methyl	0.130 (7)	0.126 (8)	30
Demeton-s-methylsulfone	0.390 (7)	0.396 (9)	25
Dimethoate	0.757 (9)	0.767 (12)	20
Disulfoton	0.090 (17)	0.078 (22)	50
Disulfoton sulfone	0.102 (7)	0.097 (8)	50
Disulfoton sulfoxide	0.615 (7)	0.605 (6)	20
Ethoprophos	0.893 (4)	0.896 (4)	20
Fensulfothion	0.931 (6)	0.940 (6)	20
Fensulfothion-oxon	0.942 (4)	0.955 (5)	20
Fensulfothion-oxon-sulfone	0.884 (9)	0.896 (4)	20
Fensulfothion sulfone	0.408 (7)	0.397 (8)	25
Omethoate	0.729 (9)	0.732 (8)	20
Oxydemeton-s-methyl	0.541 (6)	0.541 (5)	20
Terbufos	0.310 (11)	0.323 (12)	25
Terbufos sulfone	0.502 (6)	0.496 (6)	25
Terbufos sulfoxide	0.360 (5)	0.363 (4)	25

CONCLUSIONS

A method has been described for the determination and confirmation of seven priority pesticide residues with sixteen components in different baby foods.

The QuEChERS extraction method yielded very good recoveries and precision at the low concentration levels required by legislation.

The sensitivity of ACQUITY UPLC and Quattro Premier allows the method to meet the challenge set by the EU Baby Food Directive 2003/13/EC[1].

REFERENCES

1. Commission Directive 2003/13/EC amending Directive 96/5/EC on processed cereal-based foods and baby foods for infants and young children, *Official Journal of the European Communities* No. L41/33.
2. M.E. Swartz, *LC GC Europe*, Separation Science, June (2005) 5.
3. M.E. M. Anastassiades, S. Lehotay, D. Stajnbaher, F. Schenck, *J. AOAC Int.* 86 (2003) 412.

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