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Applikationsbericht

Determination of Pesticides in Food Using UPLC with Polarity Switching Tandem Quadrupole LC-MS/MS

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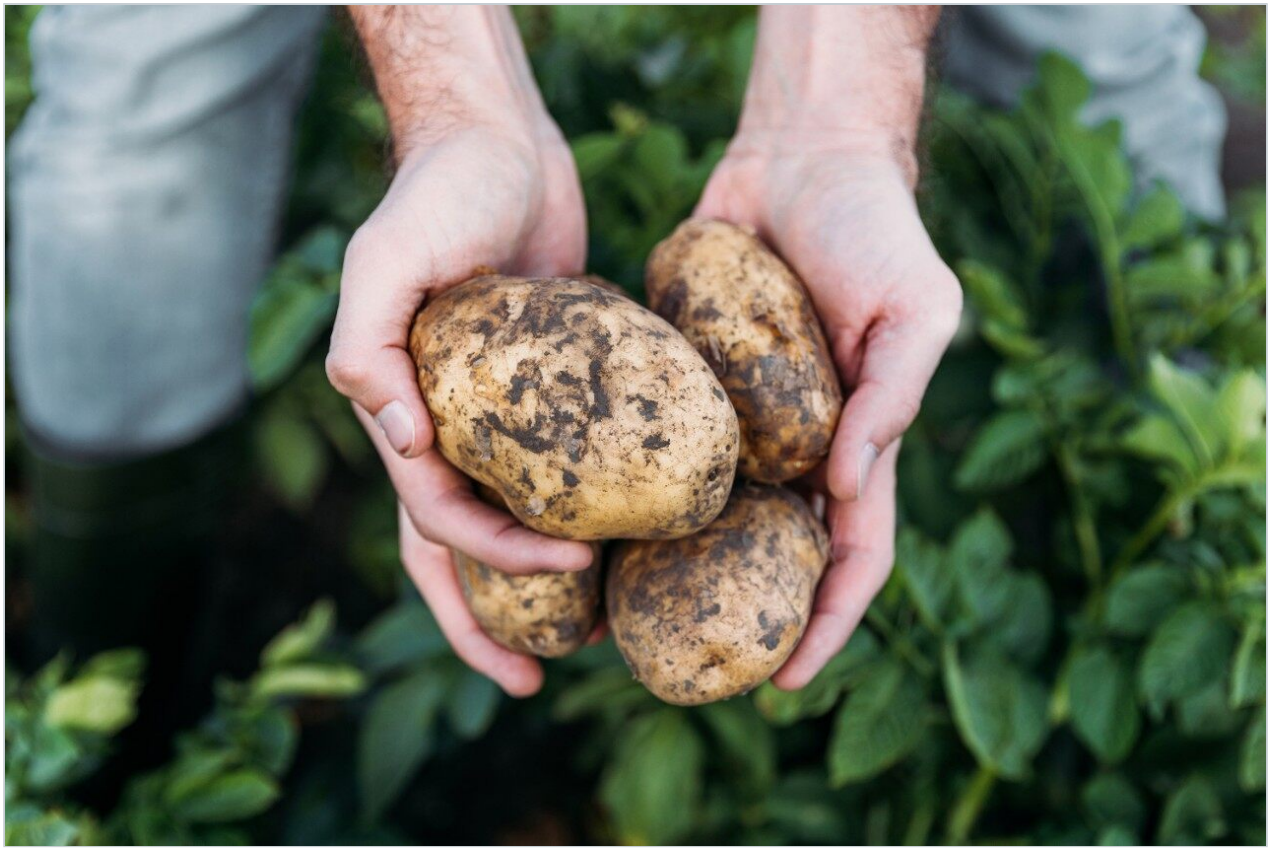

Abstract

The aim of this application note is to evaluate the potential of fast polarity switching, resolution, and speed of analysis of pesticides in potatoes, oranges, and cereal-based baby food using UPLC-MS/MS. Additional pesticides with different polarities, transformation products, and structural isomers (butocarboxim sulfoxide and aldicarb sulfoxide) were included to extend the list of compounds studied.

Introduction

The European Union residue monitoring program 2005–2007 establishes the need to cover 55 active ingredients in various foods, including potatoes, oranges, and baby foods.[1] Twenty of these pesticides are suitable for multi-residue LC-MS analysis. The majority of this group has a positive polarity in electrospray mode and only one (fludioxonil) has a negative polarity, normally requiring two injections (one in each polarity ion mode). Consequently, compounds with negative polarity are often excluded from monitoring programs. Ideally, these should be determined in a single analysis with polarity switching.

Furthermore, chemists analyzing pesticide residues are under increasing pressure to broaden the range of pesticides determined in a single analysis, to improve limits of detection, precision and quantitation, to increase confidence in the validity of residue data, to provide faster methods, and to reduce usage of hazardous solvents while maintaining or reducing costs. In order to meet these demanding requirements the scope, sensitivity, efficiency, and speed of multi-residue methods of analysis must be improved.

Given that there are many active ingredients used to control pests, it is often advantageous to extract and determine as many of them as possible during a single analysis. An extraction, with acetonitrile, followed by dispersive solid phase extraction (SPE) clean-up has been reported for the analysis of a wide range of pesticides in fruits and vegetables[2] and fatty samples.[3]

Experimental

Extraction Method

A 10 g sample was weighed in a centrifuge tube. Acetonitrile (9.9 mL), acetic acid (0.1 mL), anhydrous $MgSO_4$ (4 g), and sodium acetate (1.66 g) were added and the tube was shaken immediately. After centrifugation at 4,300 g for 5 min, an aliquot (1 mL) of the supernatant was transferred to a microcentrifuge vial containing 50 mg primary secondary amine (PSA) sorbent and 150 mg anhydrous $MgSO_4$. The contents were vortex mixed for 30 s and centrifuged at 5,000 g for 1 min. The supernatant was analyzed by LC-MS/MS after dilution with water (1:9).

UPLC Method

System:	Waters ACQUITY UPLC
Column:	UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm
Column temp.:	40 °C
Flow rate:	0.6 mL/min
Mobile phase A:	Water + 0.1% acetic acid
Mobile phase B:	Methanol + 0.1% acetic acid
Total run time:	7 min
Injection volume:	20 µL

Gradient

Time:	0 min	90%A
Time:	4 min	100%B
Time:	5 min	100%B

LC-MS/MS Method

The Waters Quattro Premier XE Tandem Quadrupole Mass Spectrometer was used in positive and negative

ion electrospray mode switching in 0.02 s. The ion source was operated at 120 °C with a capillary voltage of 1.0 kV. Nitrogen was employed for both the desolvation and cone gases at 800 (400 °C) and 50 L/hr, respectively. The mode of acquisition was multiple reaction monitoring (MRM) at an argon collision gas pressure of 4.0×10^{-3} mBar.

The Quattro Premier XE was tuned so that the precursor and product ions were resolved with a peak width at half height of less than 0.7 Da. The list of pesticide residues and the MRM transitions, along with the retention times, dwell times, cone voltages, and collision energies for the method are listed in Table 1. Pesticide residues listed in red type were acquired in negative ion mode. The dwell times were optimized so that ten to fifteen data points were acquired across each chromatographic peak.

Pesticide	RT	MRM Transitions	Dwell time (s)	Cone Voltage (V)	Collision Energy (eV)
Methamidophos	0.41	142>94	0.015	22	14
		142>125			13
Acephate	0.5	184>143	0.015	16	8
		184>125			18
Omethoate	0.58	214>183	0.015	20	12
		214>155			15
Butocarboxim sulfoxide	0.59	207>75	0.015	17	12
		207>132			6
Pymetrozine	0.61	218>105	0.015	25	17
		218>79			36
Aldicarb sulfoxide	0.64	207>89	0.015	16	14
		207>132			10
Butoxycarboxim	0.68	223>106	0.015	17	10
		223>166			7
Aldicarb sulfone	0.72	223>86	0.015	23	12
		223>76			7
Methomyl	0.85	163>88	0.025	15	8
		163>106			10
Oxydemeton-methyl	0.86	247>169	0.025	20	14
		247>109			28
Demeton-S-methyl sulfone	0.9	263>169	0.025	26	17
		263>121			17
Carbendazim	1.01	192>160	0.025	25	18
		192>132			30
Imidacloprid	1.15	256>209	0.02	22	16
		256>175			20
Thiabendazole	1.18	202>175	0.02	40	25
		202>131			32
Dimethoate	1.27	230>125	0.02	17	20
		230>199			10
Methiocarb sulfoxide	1.26	242>185	0.02	22	13
		242>168			24
Acetamiprid	1.32	223>126	0.02	27	20
		223>56			15
Cymoxanil	1.41	199>128	0.02	17	8
		199>111			18
Methiocarb sulfone	1.4	258>122	0.02	22	20
		258>107			37
Thiacloprid	1.49	253>126	0.02	28	20
		253>90			37
Butocarboxim	1.61	213>75	0.02	24	15
		213>156			10
Aldicarb	1.64	208>116	0.02	7	7
		208>89			7
Carbaryl	2.08	202>145	0.02	18	10
		202>127			28
Thiodicarb	2.19	355>88	0.02	15	16
		355>108			16

Table 1. MRM method parameters.

Pesticide	RT	MRM Transitions	Dwell time (s)	Cone Voltage (V)	Collision Energy (eV)
Phorate sulfoxide	2.25	277>97	0.02	18	32
		277>143			20
Lenacil (-ve)	2.3	233>151	0.03	44	24
		233>107			32
Phorate sulfone	2.29	293>97	0.02	18	30
		293>115			24
Azinphos-methyl	2.46	318>160	0.02	14	8
		318>261			8
Imazalil	2.52	297>159	0.02	30	20
		297>69			20
Linuron	2.56	249>160	0.02	28	16
		249>182			15
Methiocarb	2.63	226>169	0.02	16	10
		226>121			19
Azoxystrobin	2.6	404>372	0.02	22	15
		404>329			30
Fludioxonil (-ve)	2.65	247>180	0.03	45	28
		247>126			35
Triadimefon	2.77	294>69	0.015	22	21
		294>197			15
Iprovalicarb	2.84	321>119	0.015	15	18
		321>203			8
Methiocarb sulfone	1.4	258>122	0.02	22	20
		258>107			37
Thiacloprid	1.49	253>126	0.02	28	20
		253>90			37
Butocarboxim	1.61	213>75	0.02	24	15
		213>156			10
Aldicarb	1.64	208>116	0.02	7	7
		208>89			7
Carbaryl	2.08	202>145	0.02	18	10
		202>127			28
Thiodicarb	2.19	355>88	0.02	15	16
		355>108			16
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		318>261			8
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		249>182			15
Methiocarb	2.63	226>169	0.02	16	10
		226>121			19

Table 1. MRM method parameters. (continued)

Pesticide	RT	MRM Transitions	Dwell time (s)	Cone Voltage (V)	Collision Energy (eV)
Azoxystrobin	2.6	404>372	0.02	22	15
		404>329			30
Fludioxonil (-ve)	2.65	247>180	0.03	45	28
		247>126			35
Triadimefon	2.77	294>69	0.015	22	21
		294>197			15
Iprovalicarb	2.84	321>119	0.015	15	18
		321>203			8
Triadimenol	2.85	296>70	0.015	14	10
		296>99			16
Dichlofluanid	2.86	333>123	0.015	22	24
		333>224			10
Fenhexamid	2.86	302>97	0.015	35	25
		302>55			35
Flufenacet	2.88	364>152	0.015	17	20
		364>194			10
Cyprodinil	2.95	226>93	0.015	45	33
		226>108			25
Diflubenzuron (-ve)	2.96	309>156	0.1	20	11
		309>289			9
Fenoxycarb	3	302>88	0.015	21	20
		302>116			12
Spiroxamine	3.04	298>144	0.015	32	20
		298>100			32
Tolylfluanid	3.06	347>137	0.015	19	28
		347>238			10
Zoxamide	3.11	336>187	0.015	25	24
		336>159			41
Phorate	3.15	261>75	0.015	11	12
		261>97			32
Hexaflumuron (-ve)	3.31	459>276	0.02	22	22
		459>175			39
Teflubenzuron (-ve)	3.47	379>196	0.02	18	25
		379>339			15
Fluazinam	3.5	463>416	0.02	26	21
		463>398			17
Lufenuron (-ve)	3.49	509>175	0.02	22	40
		509>326			22
Flucycloxuron (-ve)	3.58	482>156	0.02	34	14
		482>462			13
Flufenoxuron (-ve)	3.62	487>156	0.02	27	16
		487>329			22

Table 1. MRM method parameters. (continued)

Acquisition and Processing Methods

The data were acquired using Waters MassLynx Software and processed using TargetLynx Application Manager.

Two MRM transitions were acquired for each residue so that quantification and confirmation could 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 Quality Control Procedures for Pesticide Residue Analysis.[4]

Results and Discussion

To test the extraction method described, five recovery experiments were performed in cereal-based baby food, spiked at 0.01 mg/kg. The mean recovery and relative standard deviation (% RSD) in parenthesis of each analyte are listed in Table 2.

Pesticide	% Recovery (% RSD)	Pesticide	% Recovery (% RSD)
Methamidophos	79 (4)	Phorate sulfone	106 (5)
Acephate	89 (6)	Azinphos-methyl	113 (19)
Omethoate	88 (8)	Linuron	104 (3)
Butocarboxim sulfoxide	90 (5)	Imazalil	105 (6)
Pymetrozine	76 (7)	Methiocarb	103 (5)
Aldicarb sulfoxide	93 (6)	Azoxystrobin	104 (5)
Butoxycarboxim	101 (6)	Fludioxonil	107 (5)
Aldicarb sulfone	103 (8)	Triadimefon	104 (9)
Methomyl	100 (3)	Iprovalicarb	100 (7)
Oxydemeton-methyl	91 (5)	Triadimenol	93 (6)
Demeton-S-methyl sulfone	96 (2)	Dichlofluanid	73 (14)
Carbendazim	97 (4)	Fenhexamid	93 (8)
Imidacloprid	100 (9)	Flufenacet	99 (8)
Thiabendazole	87 (4)	Cyprodinil	95 (6)
Dimethoate	103 (3)	Diflubenzuron	100 (11)
Methiocarb sulfoxide	95 (4)	Fenoxycarb	102 (7)
Acetamiprid	97 (4)	Spiroxamine	98 (7)
Cymoxanil	93 (11)	Tolylfluanid	85 (13)
Methiocarb sulfone	100 (6)	Zoxamide	100 (8)
Thiacloprid	101 (3)	Phorate	96 (6)
Butocarboxim	106 (6)	Hexaflumuron	124 (9)
Aldicarb	104 (7)	Teflubenzuron	107 (6)
Carbaryl	101 (5)	Fluazinam	99 (4)
Thiodicarb	99 (3)	Lufenuron	109 (5)
Phorate sulfoxide	102 (6)	Flucycloxuron	105 (9)
Lenacil	94 (3)	Flufenoxuron	114 (3)

Table 2. Mean recovery and % RSD for 0.01 µg/mL recovery samples (n = 5) from cereal-based baby food.

Good recoveries in the range 73 (dichlofluanid) - 124% (hexaflumuron) with % RSDs of less than 19% (azinphos-methyl) were obtained for all the pesticides spiked at the 0.01 µg/mL levels in cereal-based baby food.

The separation of the pesticides was optimized by changing the pH of the mobile phase. For multi-residue methods, the pH needs to accommodate different chemical properties, e.g. thiabendazole is a very basic compound and prefers low pH conditions. 5 mM ammonium acetate was originally used, however, this compromised the peak shape for thiabendazole. Acetonitrile with 0.1% acetic acid improved the peak shape for this compound, however, compounds such as tolylfluanid were retained on the column.

The final mobile phase contained methanol with 0.1% acetic acid, which gave a good peak shape for thiabendazole and allowed analysis of all analytes without compromising the response or the peak shape for the remaining pesticides. Dilution of the acetonitrile extracts with water also improved the peak shape and reduced any matrix effects in the extracts.

Using the UPLC method developed, the 52 pesticides of interest were eluted in less than four minutes (Figure 1) without a loss in resolution. An increase in the speed of the chromatographic separation by more than a factor of 10 was achieved using the described method compared to a typical HPLC separation time for approximately 50 pesticides of 50–60 minutes.

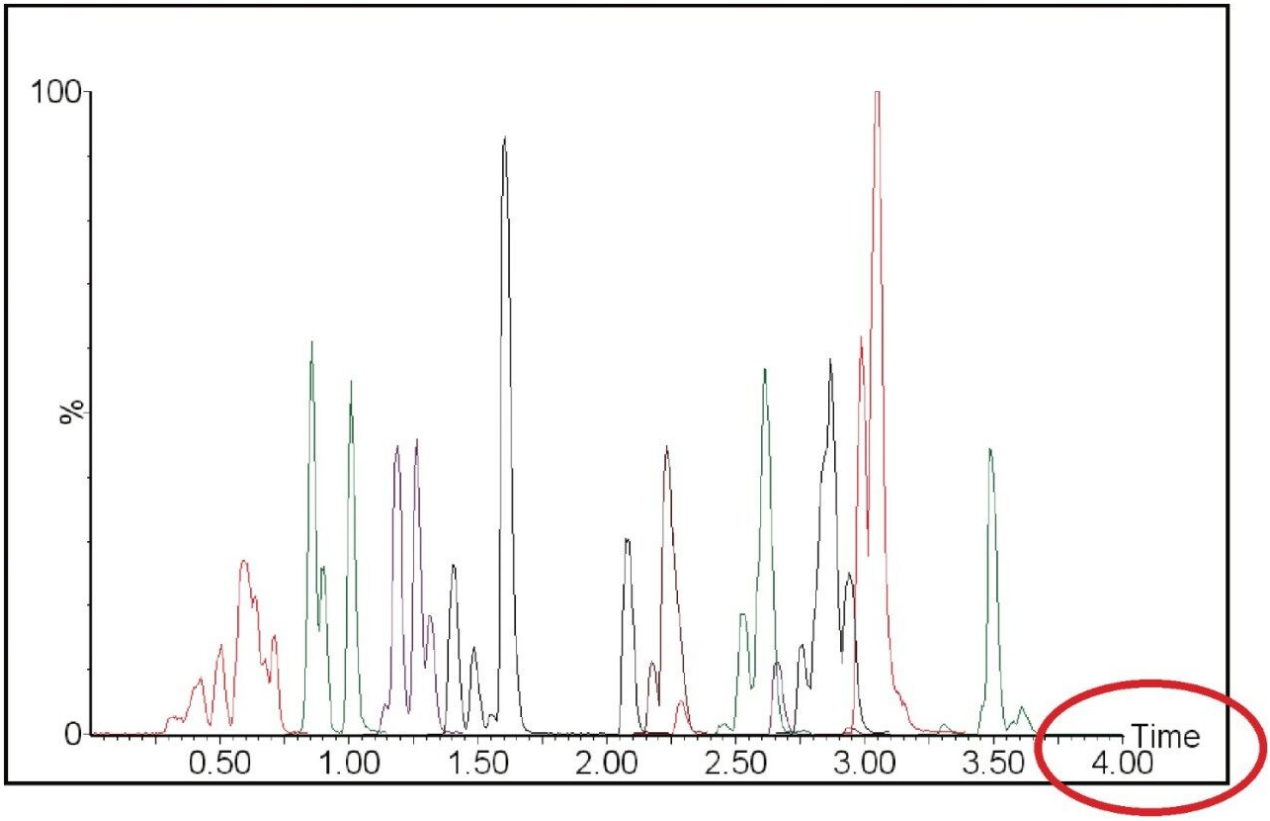

Figure 1. Total ion chromatogram (TIC) for 0.1 µg/mL matrix-matched standard in cereal-based baby food.

Butocarboxim sulfoxide and aldicarb sulfoxide are structural isomers that share one confirmation MRM transition (*m/z* 207>132) but differ in the quantification transition. However, one transition is not enough for confirmation. If they co-elute, chromatographic resolution is critical. Figure 2 illustrates the improved resolution achieved using UPLC (R_s = 1.3) compared to HPLC (R_s = 0.9) between the critical pair even though the gradient time is much shorter (20 min compared to 4 min). It is possible to obtain better resolution of the isomers using optimized conditions with HPLC but it then reduces the applicability of the method to such a broad range of compounds. The improved resolution offered by UPLC enables analysts to confidently confirm the identity of these two pesticides.

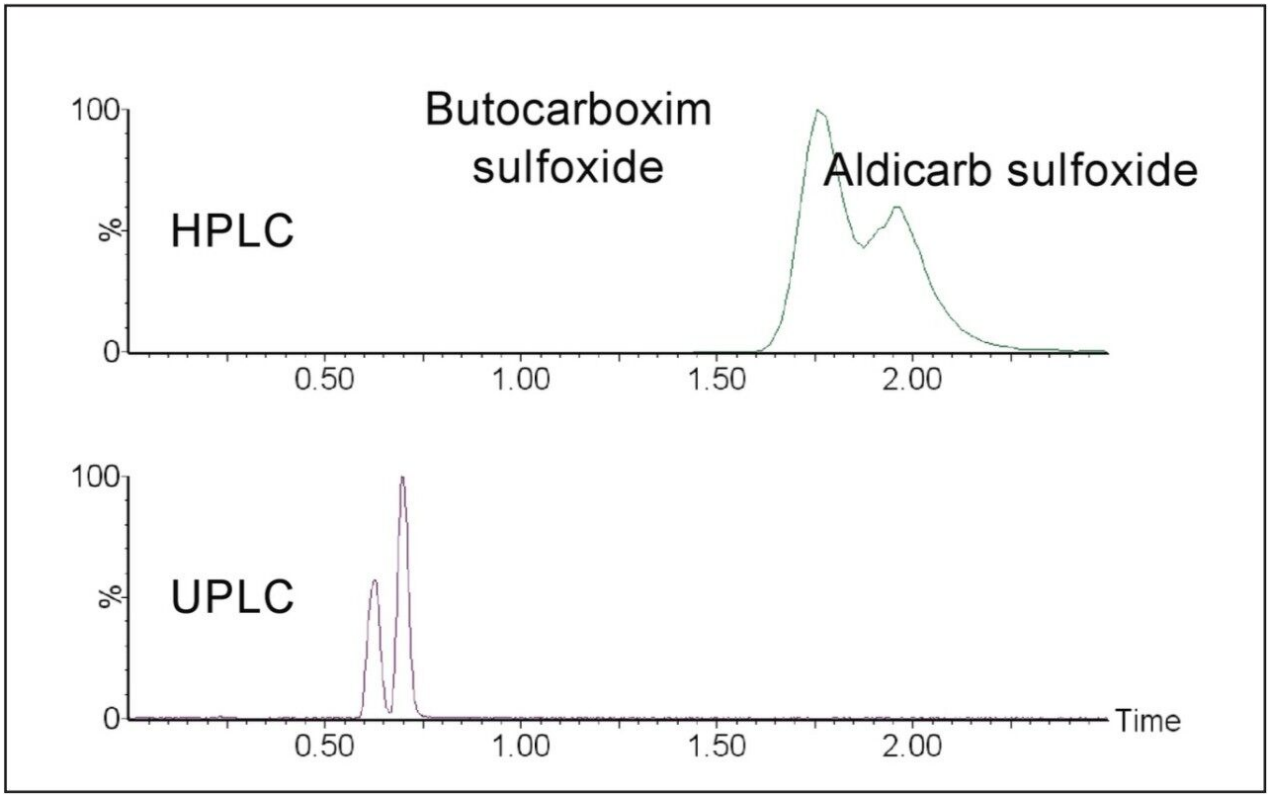

Figure 2. Comparison of HPLC and UPLC for the resolution of structural isomers.

Using the UPLC method described, phorate sulfone and phorate sulfoxide (both positive ion compounds) co-elute with lenacil (a negative ion compound) in a time window of 9 s (Figure 3). To get 15 data points across each peak with two MRM transitions per compound, the overall cycle time for each transition, including dwell time, inter-scan delay, and inter-channel delay would need to be 100 ms. Older instruments require at least 200 ms just to switch, so the number of data points or the peak shape would have to be compromised to perform positive/negative switching in the same experiment.

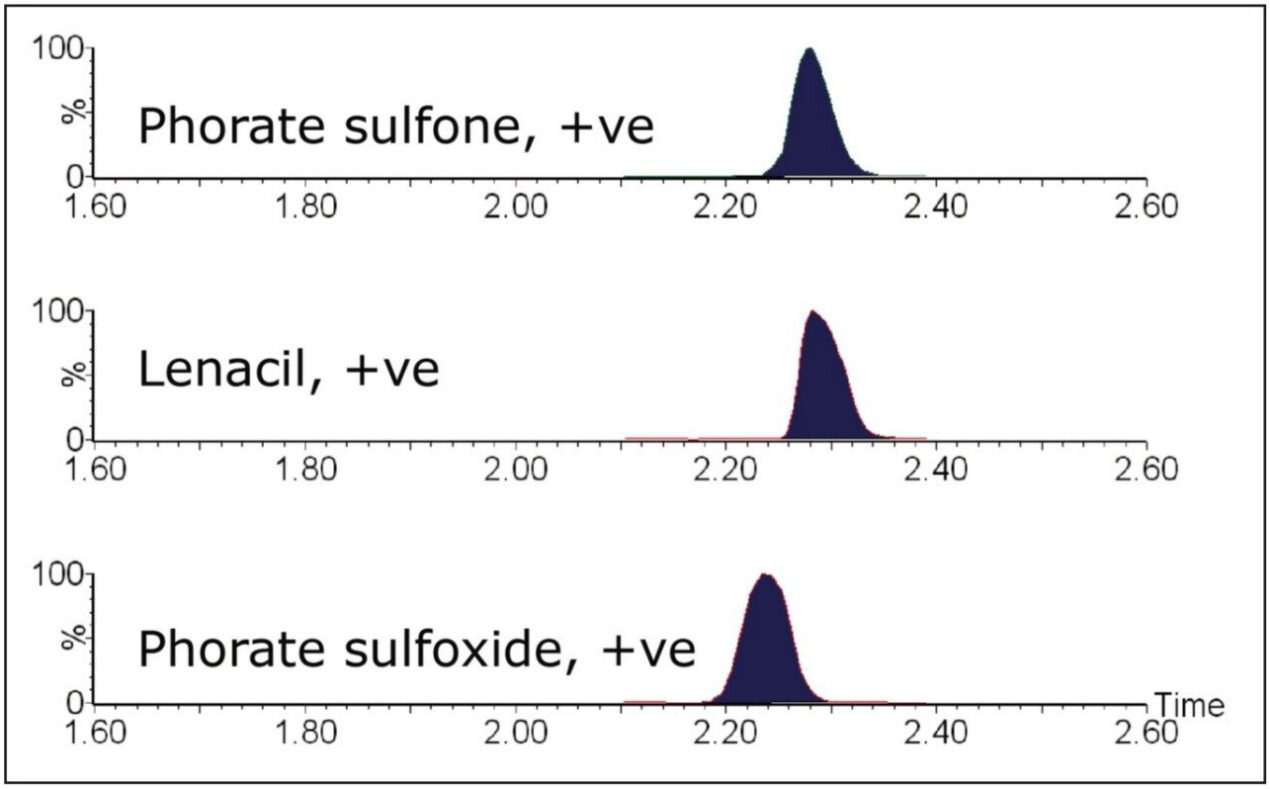

Figure 3. Positive/negative switching for 0.01 µg/mL matrix-matched standard in cereal-based baby food.

The ability to switch between positive and negative ion modes using a 20 ms interscan delay can be tested by observing the linearity of the calibration curve produced from a number of different concentration levels. The three matrix-matched curves for phorate sulfone, phorate sulfoxide and lenacil in cereal-based baby food between 0.005 and 0.250 µg/mL (equivalent to 0.005–0.250 mg/kg) are illustrated in Figure 4. Good correlation coefficients were obtained for all three compounds indicating that positive/negative switching can be achieved on the Quattro Premier XE using a 20 ms inter-scan delay.

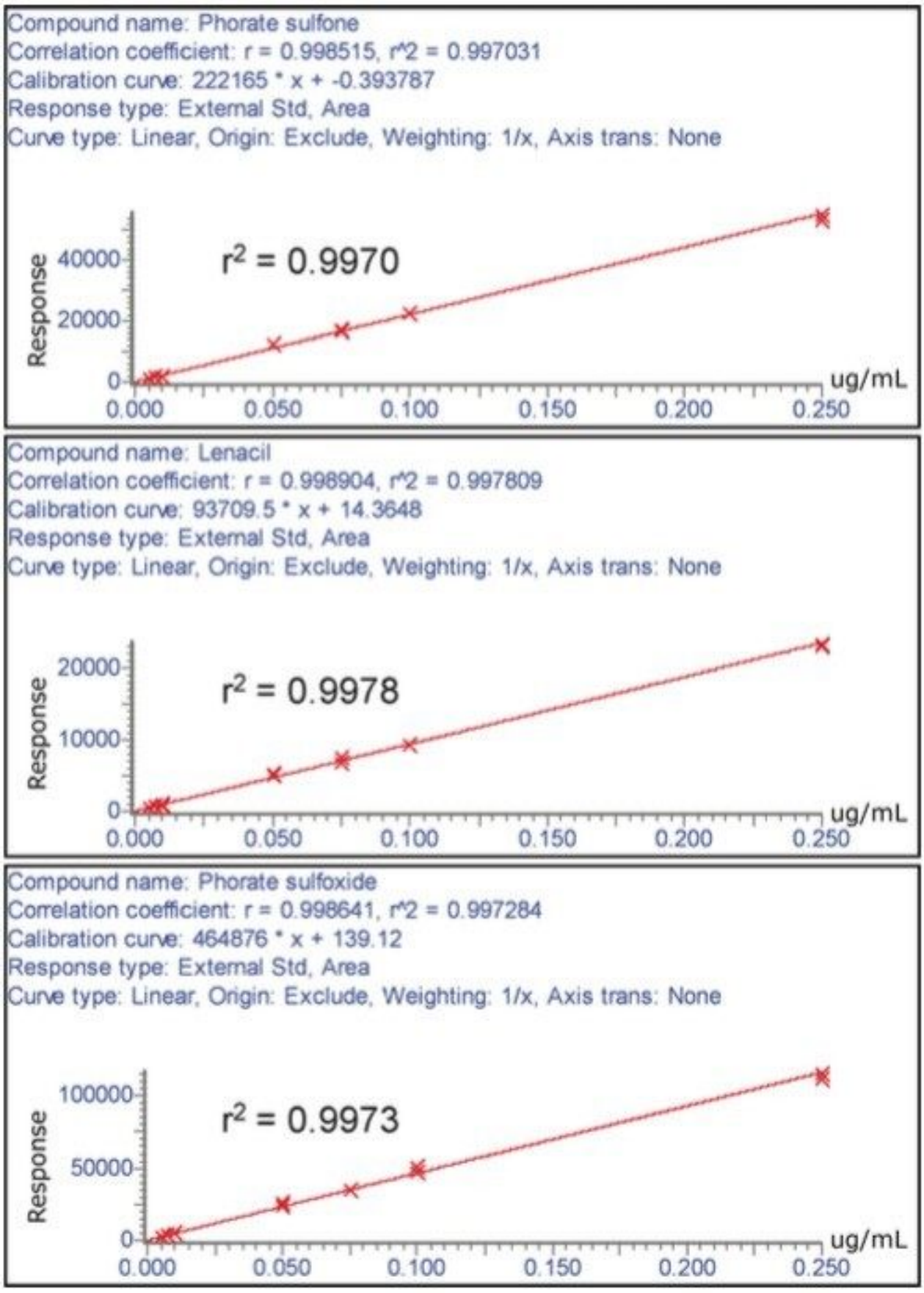

Figure 4. Linearity during positive/negative switching using a 20 ms inter-scan delay.

The TargetLynx Application Manager was used to provide automatic quantification and confirmation with t

2. M Anastassiades, S Lehotay, D Stajnbaher, F Schenck, *J. AOAC Int.* 86 (2003) 412.

3. S J Lehotay, K Mastovska, S J Yun, *J. AOAC Int.* 88 (2005) 630.

4. Quality Control Procedures for Pesticide Residues Analysis of 24 March 2006, Document Number SANCO/10232/2006.

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