Nota de aplicación

Determination and Confirmation of Priority Pesticide Residues in Baby Food

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Abstract

This application note demonstrates to utilize the power of the Waters UltraPerformance Liquid Chromatography (UPLC) System combined with the fast MS acquisition rates of the Xevo TQ Mass Spectrometer for rapid determination and confirmation of pesticide residues in baby food.

Introduction

As the population of the world grows, it becomes increasingly important to produce enough food to satisfy the needs of its inhabitants. This escalation in consumer demand has led farmers to use increasing amounts of pesticides to improve their yields and make their operations more cost-effective. This growth in the use of pesticides, coupled with poor agricultural practices and illegal use, can pose significant risks to human health through the presence of pesticide and metabolite residues in food products.

Most countries have strict regulations that govern pesticides. Legislation imposes Maximum Residue Limits[1] (MRLs) for pesticide residues in food products requiring analytical techniques that are sensitive, selective, and robust. The EU baby food directive, 2003/13/EC[2] covers a list of both GC amenable and LC amenable pesticides. These prohibited pesticides have a maximum level in baby food that should not exceed 0.003 mg/kg, or not greater than a limit between 0.004-0.008 mg/kg. This is considered to be among the strictest legislation in the world.

The need to meet mandated detection limits, develop generic sample preparation techniques for complex matrices, and the desire to increase sample throughput are the main challenges that face food safety testing laboratories today. The use of a single multi-residue method per instrument dramatically improves return on investment in a laboratory by removing the need to change method parameters. This is often the case in labs that analyze a wide variety of commodities with various lists of legislated pesticides.

The following application note describes a solution for the rapid analysis of pesticides in fruit- and meat-based baby food extracts which is able to exceed both current European and worldwide legislation.

Experimental

A DisQuE (QuEChERS style dispersive solid-phase extraction) extraction was utilized for this multi-residue method as described below:

Extraction Procedure:[3]

1. Add 15 g of homogenized baby food to the 50 mL DisQuE extraction tube. Add 15 mL of 1% acetic acid in acetonitrile.
2. Shake vigorously for 1 minute and centrifuge > 1500 rcf for 1 minute.
3. Transfer 1 mL of the acetonitrile extract into the 2 mL DisQuE cleanup tube.
4. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
5. Transfer 100 µL of final extract into an autosampler vial. Dilute with 900 µL water, mix, and inject.

Gradient

0.00 min	99% A

5.00 min	1% A
6.00 min	1% A
6.10 min	99% A
8.00 min	99% A

LC Conditions

LC system:	ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm
Column temp:	40 °C
Sample temp:	4 °C
Flow rate:	0.7 mL/min
Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Methanol + 0.1% formic acid
Weak needle wash:	Water + 0.1% formic acid
Strong needle wash:	Methanol + 0.1% formic acid
Total run time:	8 min
Injection volume:	50 µL, full loop injection

MS Conditions

MS system:	Xevo TQ MS

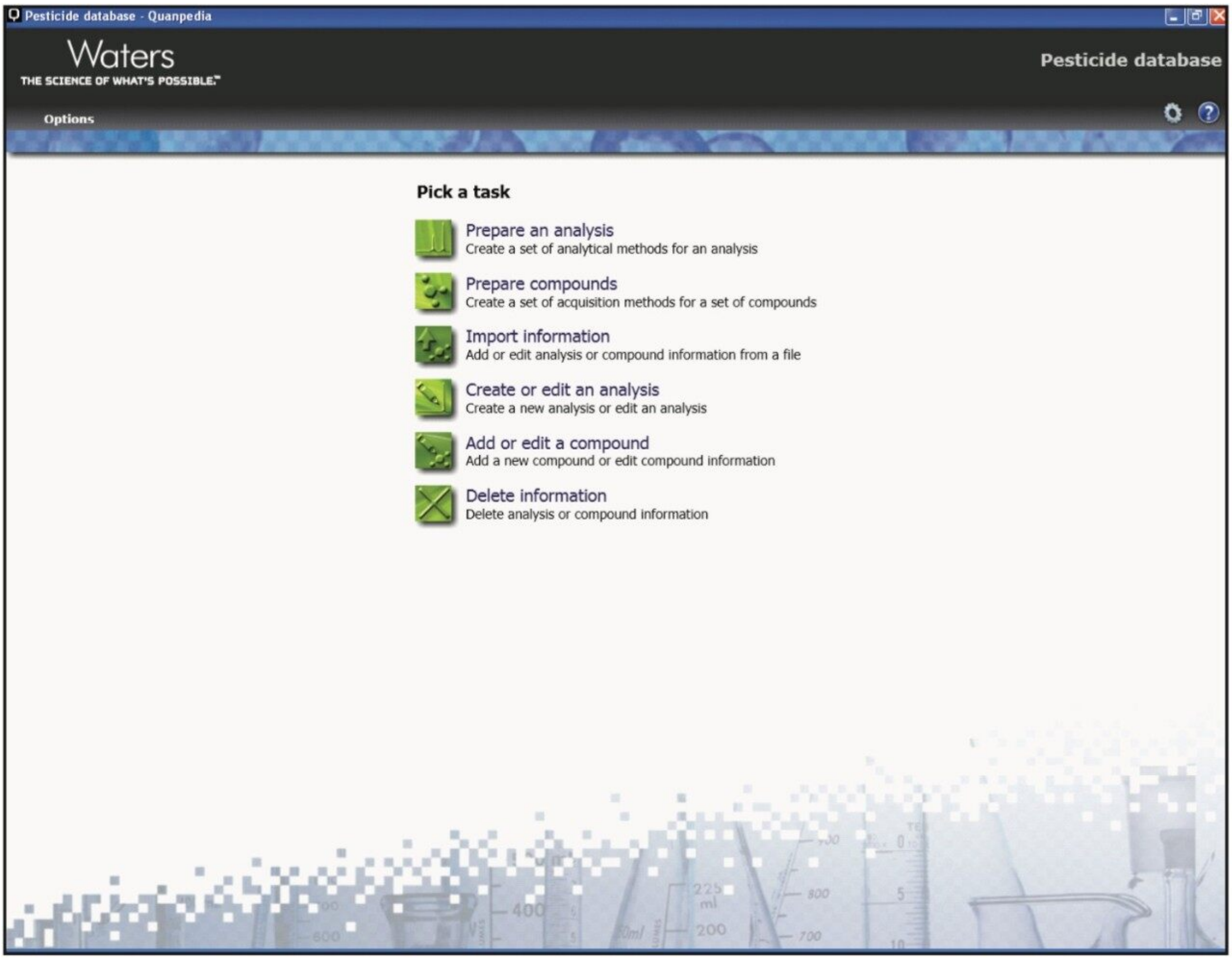
Ionization mode:	ESI +
Capillary voltage:	0.6 kV
Desolvation gas:	Nitrogen, 1000 L/Hr, 400 ˚C
Cone gas:	Nitrogen, 25 L/Hr
Source temp:	120 °C
Acquisition:	Multiple Reaction Monitoring (MRM)
Collision gas:	Argon at 3.5 x 10^{-3} mBar

Refer to Appendix 1 at the end of this document for further MS parameters.

Quanpedia: Database and Method Creation Tool

Method creation has been streamlined with the use of Quanpedia, a searchable database for quantitative LC-MS method information that can be updated with user information.

Each entry in Quanpedia is populated with information that associates the compound name with details of optimal SIR/MRM acquisition methods, acceptable confirmatory ion ratios, appropriate LC methods, and expected peak retention times. For fast-paced, multi-user environments that require quality results the first time, Quanpedia offers a simple and convenient way to rapidly create complete LC-MS data and acquisition methods.



Pick a task

Prepare an analysis
Create a set of analytical methods for an analysis

Prepare compounds
Create a set of acquisition methods for a set of compounds

Import information
Add or edit analysis or compound information from a file

Create or edit an analysis
Create a new analysis or edit an analysis

Add or edit a compound
Add a new compound or edit compound information

Delete information
Delete analysis or compound information

Figure 1. Quanpedia is a simple and easy-to-use method creation tool and MS database.

Acquisition and Processing Methods

These data were acquired using Waters MassLynx Software v. 4.1. Incorporated into MassLynx, the IntelliStart Software automates optimization of MS parameters for the sample and also monitors the health of the MS system, which reduces the time for operatorintensive troubleshooting and upkeep.

Results and Discussion

The analysis of the priority pesticide residues in baby food was achieved by combining the ACQUITY UPLC System with the Xevo TQ MS System — UltraPerformance LC with tandem quadrupole mass spectrometry (UPLC-MS/MS) operated in MRM mode. This tandem quadrupole mass spectrometry offers a highly specific and selective detection technique that has become the technique of choice within the laboratory.[4]

The selectivity given using a tandem quadrupole mass spectrometer (Xevo TQ MS System) shows an advantage over a single quadrupole instrument as it allows co-eluting compounds to be identified and quantified with confidence. Figure 2 shows fensulfothion sulfone and terbufos sulfone that co-elute at 3.32 minutes. All dwell times were optimized to give approximately 12 data points across each peak.

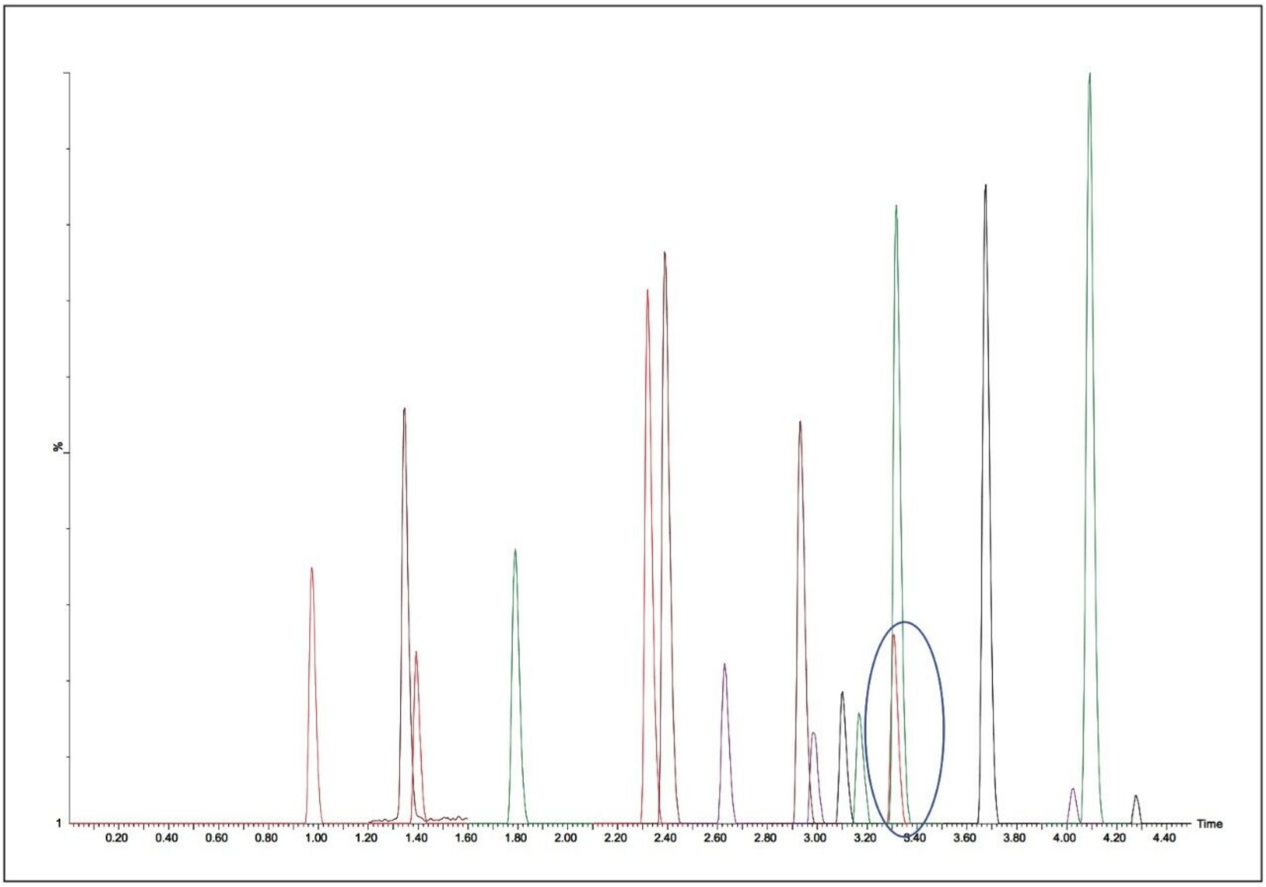


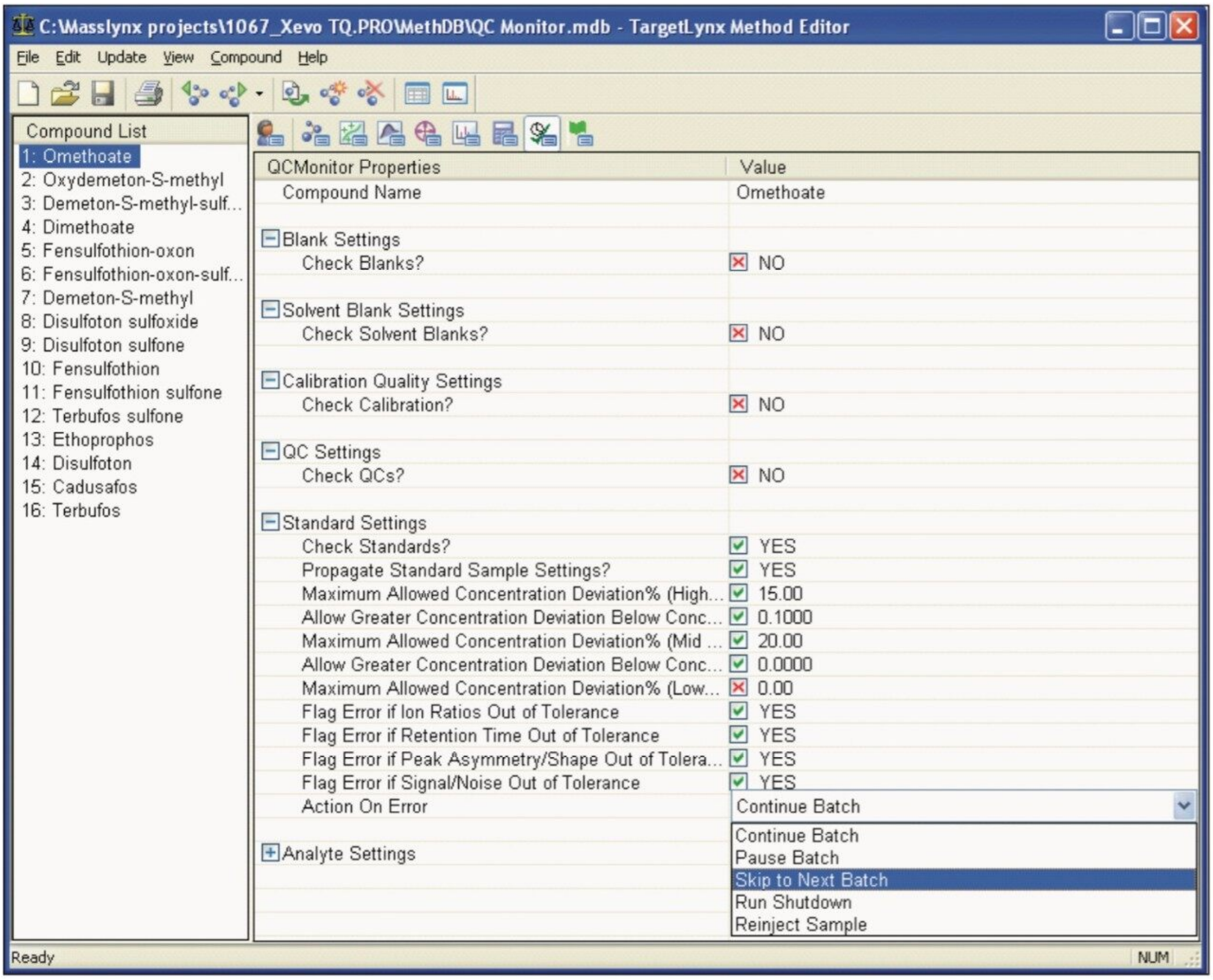
Figure 2. Chromatogram showing all 17 pesticide residues in one injection at 1ng/mL in water

These data were processed using TargetLynx Application Manager. This quantification package from MassLynx Software enables automated data processing and reporting for quantitative data, which incorporate a range of confirmatory checks that identify samples that fall outside user-specified or regulatory thresholds.

QCMonitor: Automating your Quality Control

The QCMonitor is an automated tool that provides real-time quantitative data quality monitoring to determine whether injections meet tolerances specified by the user. QCMonitor will automatically decide if subsequent samples should be injected, or if more detailed checks are required to ensure the best use of valuable laboratory resources. Injections that fail to meet the acceptance criteria set for calibration curves, QC samples and blanks can be re-injected or, in worst case scenarios, the batch can be skipped over, allowing for other revenue generating batches to be injected. This is especially important during overnight slots when the instrumentation is left unattended. An email facility is also available to send messages if and when deviations occur, alerting chemists to problems as soon as they arise.

Diagnosis of whether co-extracted interferants are the source of QCMonitor flags is also made easier by the ability to acquire high sensitivity full scan data simultaneously with MRM, adding a new dimension to QC for LC-MS/MS analyses. QCMonitor can be found in the TargetLynx Software Method Editor, as shown in Figure 3.



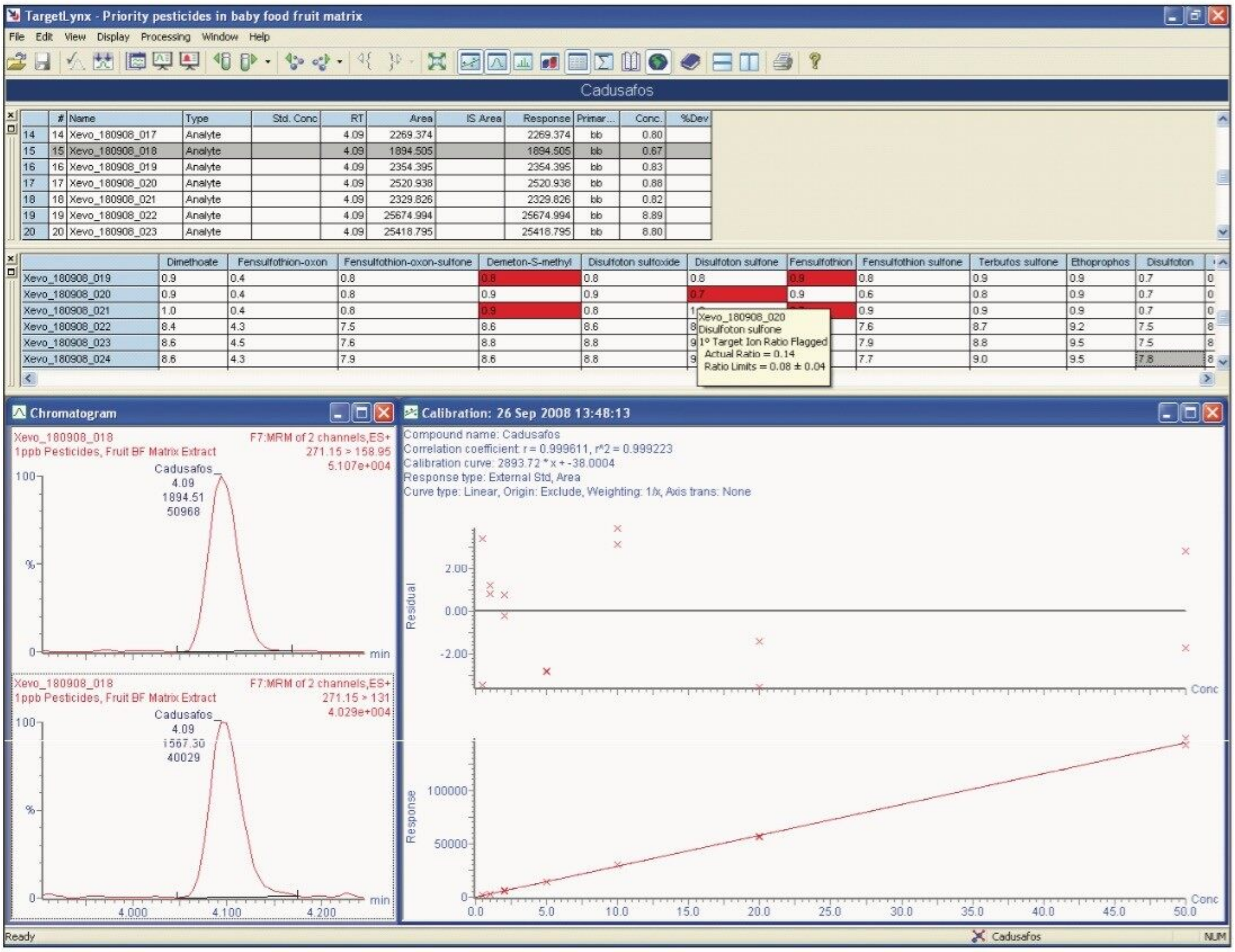
Compound List

1: Omethoate
2: Oxydemeton-S-methyl
3: Demeton-S-methyl-sulf...
4: Dimethoate
5: Fensulfothion-oxon
6: Fensulfothion-oxon-sulf...
7: Demeton-S-methyl
8: Disulfoton sulfoxide
9: Disulfoton sulfone
10: Fensulfothion
11: Fensulfothion sulfone
12: Terbufos sulfone
13: Ethoprophos
14: Disulfoton
15: Cadusafos
16: Terbufos

QCMonitor Properties	Value
Compound Name	Omethoate
⊟ Blank Settings	
Check Blanks?	☒ NO
⊟ Solvent Blank Settings	
Check Solvent Blanks?	☒ NO
⊟ Calibration Quality Settings	
Check Calibration?	☒ NO
⊟ QC Settings	
Check QCs?	☒ NO
⊟ Standard Settings	
Check Standards?	☑ YES
Propagate Standard Sample Settings?	☑ YES
Maximum Allowed Concentration Deviation% (High...	☑ 15.00
Allow Greater Concentration Deviation Below Conc...	☑ 0.1000
Maximum Allowed Concentration Deviation% (Mid ...	☑ 20.00
Allow Greater Concentration Deviation Below Conc...	☑ 0.0000
Maximum Allowed Concentration Deviation% (Low...	☒ 0.00
Flag Error if Ion Ratios Out of Tolerance	☑ YES
Flag Error if Retention Time Out of Tolerance	☑ YES
Flag Error if Peak Asymmetry/Shape Out of Tolera...	☑ YES
Flag Error if Signal/Noise Out of Tolerance	☑ YES
Action On Error	Continue Batch
⊞ Analyte Settings	

Figure 3. The features of the TargetLynx Software Method Editor detail the parameters that can be modified within QCMonitor. Highlighted are the settings for the calibration standards, the limits that must be obeyed, and the type of actions that can be applied if the injection fails to meet the set criteria.

A calibration curve was prepared in matrix matched standards and injected. Excellent linearity was achieved using a weighting factor of 1/x with a high coefficient of determination. This is shown in Figure 4.



	#	Name	Type	Std. Conc	RT	Area	IS Area	Response	Primar...	Conc.	%Dev
14	14	Xevo_180908_017	Analyte		4.09	2269.374		2269.374	bb	0.80	
15	15	Xevo_180908_018	Analyte		4.09	1894.505		1894.505	bb	0.67	
16	16	Xevo_180908_019	Analyte		4.09	2354.395		2354.395	bb	0.83	
17	17	Xevo_180908_020	Analyte		4.09	2520.938		2520.938	bb	0.88	
18	18	Xevo_180908_021	Analyte		4.09	2329.826		2329.826	bb	0.82	
19	19	Xevo_180908_022	Analyte		4.09	25674.994		25674.994	bb	8.89	
20	20	Xevo_180908_023	Analyte		4.09	25418.795		25418.795	bb	8.80	

	Dimethoate	Fensulfothion-oxon	Fensulfothion-oxon-sulfone	Demeton-S-methyl	Disulfoton sulfoxide	Disulfoton sulfone	Fensulfothion	Fensulfothion sulfone	Terbufos sulfone	Ethoprophos	Disulfoton	
Xevo_180908_019	0.9	0.4	0.8	0.8	0.8	0.8	0.9	0.8	0.9	0.9	0.7	0
Xevo_180908_020	0.9	0.4	0.8	0.9	0.9	0.7	0.9	0.6	0.8	0.9	0.7	0
Xevo_180908_021	1.0	0.4	0.8	0.9	0.8	1		0.9	0.9	0.9	0.7	0
Xevo_180908_022	8.4	4.3	7.5	8.6	8.6	8		7.6	8.7	9.2	7.5	8
Xevo_180908_023	8.6	4.5	7.6	8.8	8.8	9		7.9	8.8	9.5	7.5	8
Xevo_180908_024	8.6	4.3	7.9	8.6	8.8	9		7.7	9.0	9.5	7.8	8

Xevo_180908_020
Disulfoton sulfone
1° Target Ion Ratio Flagged
Actual Ratio = 0.14
Ratio Limits = 0.08 ± 0.04

Xevo_180908_018
1ppb Pesticides, Fruit BF Matrix Extract
F7:MRM of 2 channels,ES+
271.15 > 158.95
5.107e+004
Cadusafos
4.09
1894.51
50968

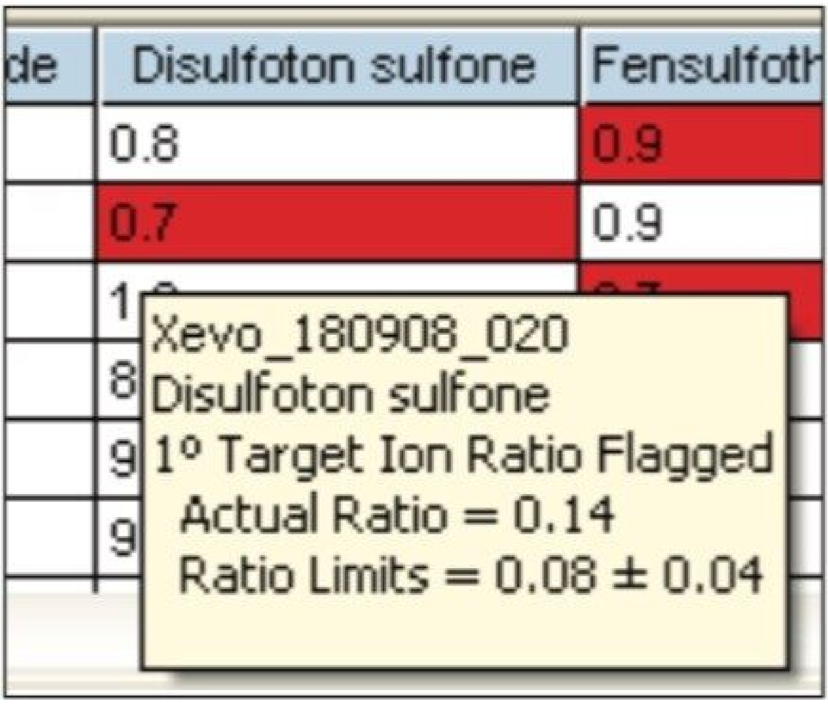
Xevo_180908_018
1ppb Pesticides, Fruit BF Matrix Extract
F7:MRM of 2 channels,ES+
271.15 > 131
4.029e+004
Cadusafos
4.09
1567.30
40029

Compound name: Cadusafos
Correlation coefficient: r = 0.999611, r^2 = 0.999223
Calibration curve: 2893.72 * x + -38.0004
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Figure 4. TargetLynx Software browser view showing cadusafos in fruit-based baby food extract at 0.001 mg/kg. Also highlighted are the red boxes which show ion ratios that are out of tolerance.

With TargetLynx Software as standard, the process of quantification is simpler than ever. The results browser and report generator clearly indicate when samples contain residues that are above minimum reporting levels.

The advantage of using the ACQUITY UPLC System with the Xevo TQ MS System is that ion ratio confirmation is also possible. Figures 4 and 5 show an ion ratio for Disulfoton sulfone that fails to meet the criteria required. The TargetLynx Software Method Editor can be easily manipulated by the user to set pass and fail criteria for each compound with regard to ion ratio. The ability of each injection to meet these criteria is then shown with a red or a white box. This injection shows a concentration of 0.7 ng/mL (0.007 mg/kg for the sample), but the ratio between the areas of the primary and secondary MRM transition traces do not lie within the limits set by the chemist. Within the EU, ion ratio confirmation is important for pesticide analysis as documented in SANCO/2007/3131.[5] When the mouse is positioned over this red box, a further message is displayed detailing the problem. In this instance the actual ion ratio does not lie within the limits specified by the chemist. This feature automatically performs these calculations, which allows chemists to use their time more cost effectively and improve laboratory workflow.



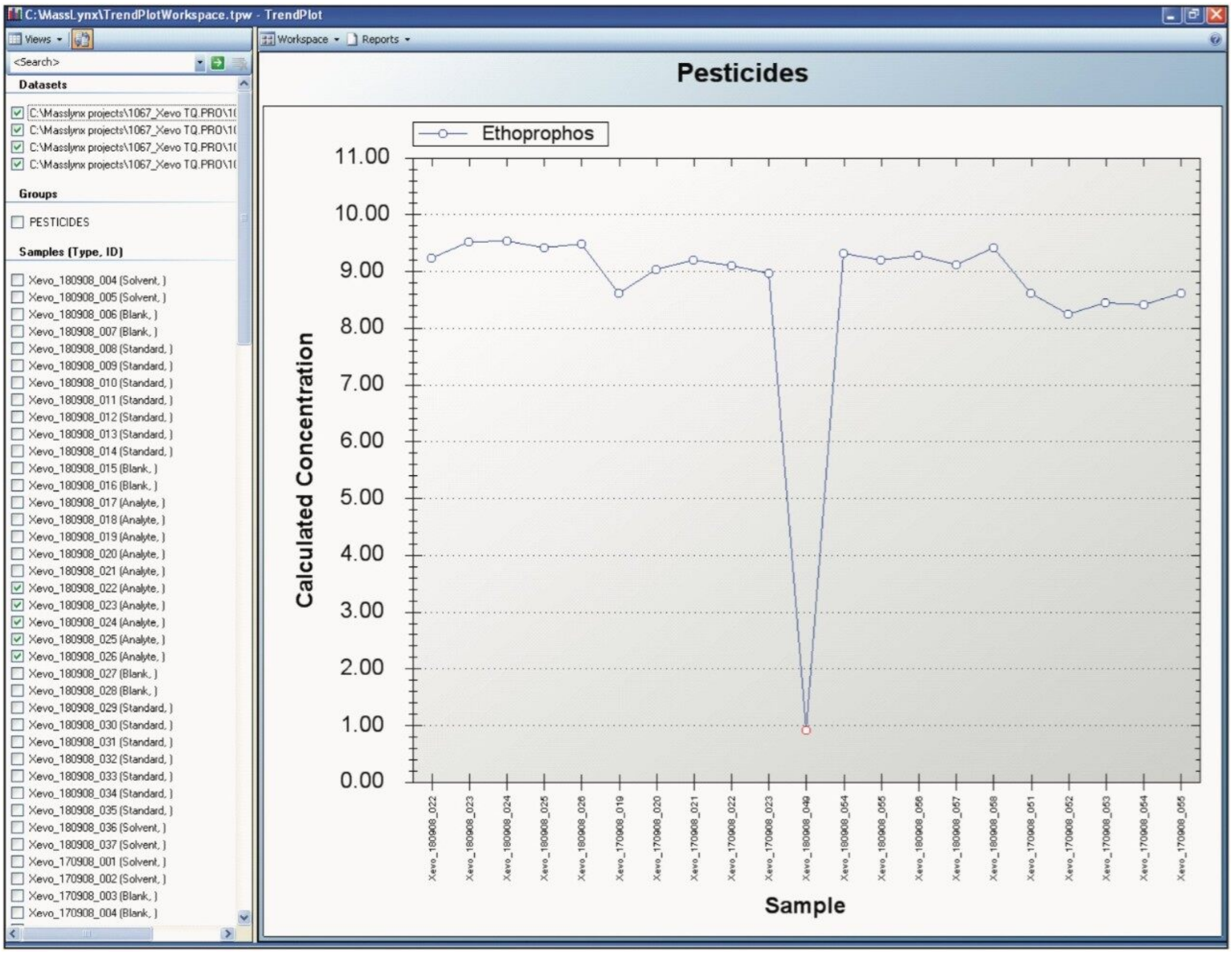
de	Disulfoton sulfone	Fensulfoth
	0.8	0.9
	0.7	0.9
	1	
	8	
	9	
	9	

Xevo_180908_020
Disulfoton sulfone
1° Target Ion Ratio Flagged
Actual Ratio = 0.14
Ratio Limits = 0.08 ± 0.04

Figure 5. Closeup view showing the type of user message TargetLynx Software produces to flag an out-of-range ion ratio result.

TrendPlot: Monitor Long-Term System Health

The TrendPlot tool provides confirmation that the Xevo TQ MS System results generated by your laboratory are consistently of the highest quality. It is possible to choose specific injections by charting both short-term intra-batch and long-term inter-batch trends in your analytical performance. In this example, ethoprophos samples have been plotted with the outlier easily seen in Figure 6.



Datasets

- ☑ C:\Masslynx projects\1067_Xevo TQ.PRO\1
- ☑ C:\Masslynx projects\1067_Xevo TQ.PRO\1
- ☑ C:\Masslynx projects\1067_Xevo TQ.PRO\1
- ☑ C:\Masslynx projects\1067_Xevo TQ.PRO\1

Groups

- ☐ PESTICIDES

Samples (Type, ID)

- ☐ Xevo_180908_004 (Solvent,)
- ☐ Xevo_180908_005 (Solvent,)
- ☐ Xevo_180908_006 (Blank,)
- ☐ Xevo_180908_007 (Blank,)
- ☐ Xevo_180908_008 (Standard,)
- ☐ Xevo_180908_009 (Standard,)
- ☐ Xevo_180908_010 (Standard,)
- ☐ Xevo_180908_011 (Standard,)
- ☐ Xevo_180908_012 (Standard,)
- ☐ Xevo_180908_013 (Standard,)
- ☐ Xevo_180908_014 (Standard,)
- ☐ Xevo_180908_015 (Blank,)
- ☐ Xevo_180908_016 (Blank,)
- ☐ Xevo_180908_017 (Analyte,)
- ☐ Xevo_180908_018 (Analyte,)
- ☐ Xevo_180908_019 (Analyte,)
- ☐ Xevo_180908_020 (Analyte,)
- ☐ Xevo_180908_021 (Analyte,)
- ☑ Xevo_180908_022 (Analyte,)
- ☑ Xevo_180908_023 (Analyte,)
- ☑ Xevo_180908_024 (Analyte,)
- ☑ Xevo_180908_025 (Analyte,)
- ☑ Xevo_180908_026 (Analyte,)
- ☐ Xevo_180908_027 (Blank,)
- ☐ Xevo_180908_028 (Blank,)
- ☐ Xevo_180908_029 (Standard,)
- ☐ Xevo_180908_030 (Standard,)
- ☐ Xevo_180908_031 (Standard,)
- ☐ Xevo_180908_032 (Standard,)
- ☐ Xevo_180908_033 (Standard,)
- ☐ Xevo_180908_034 (Standard,)
- ☐ Xevo_180908_035 (Standard,)
- ☐ Xevo_180908_036 (Solvent,)
- ☐ Xevo_180908_037 (Solvent,)
- ☐ Xevo_170908_001 (Solvent,)
- ☐ Xevo_170908_002 (Solvent,)
- ☐ Xevo_170908_003 (Blank,)
- ☐ Xevo_170908_004 (Blank,)

Figure 6. TrendPlot Tool shows outliers within a batch or performance of the instrument when data from several batches is added together.

Conclusion

A rapid multi-residue method was developed for the determination and confirmation of LC amenable priority pesticides. The analysis of pesticides in fruit- and meat-based baby food extracts exceeds current worldwide legislated limits.

Improved efficiency and increased sample throughput was realized through the combination of powerful UPLC and fast MS acquisition technologies. ACQUITY UPLC combined with the Xevo TQ MS (as shown in Figure 7) offers:

- Enhanced chromatographic resolution and short analysis times.
- Incorporation of confirmatory MRM traces.
- Complies with legislative regulations such as SANCO.
- IntelliStart technology that is designed to reduce the burden of complicated operation, training new users, time-intensive troubleshooting, and upkeep.
- The compact features of the ACQUITY UPLC and Xevo TQ MS Systems will give any laboratory an advantage as it gives high-end performance with a benchtop footprint.
- Automated system setup and quality control system checks for simple access.

Figure 7. ACQUITY UPLC with Xevo TQ MS.

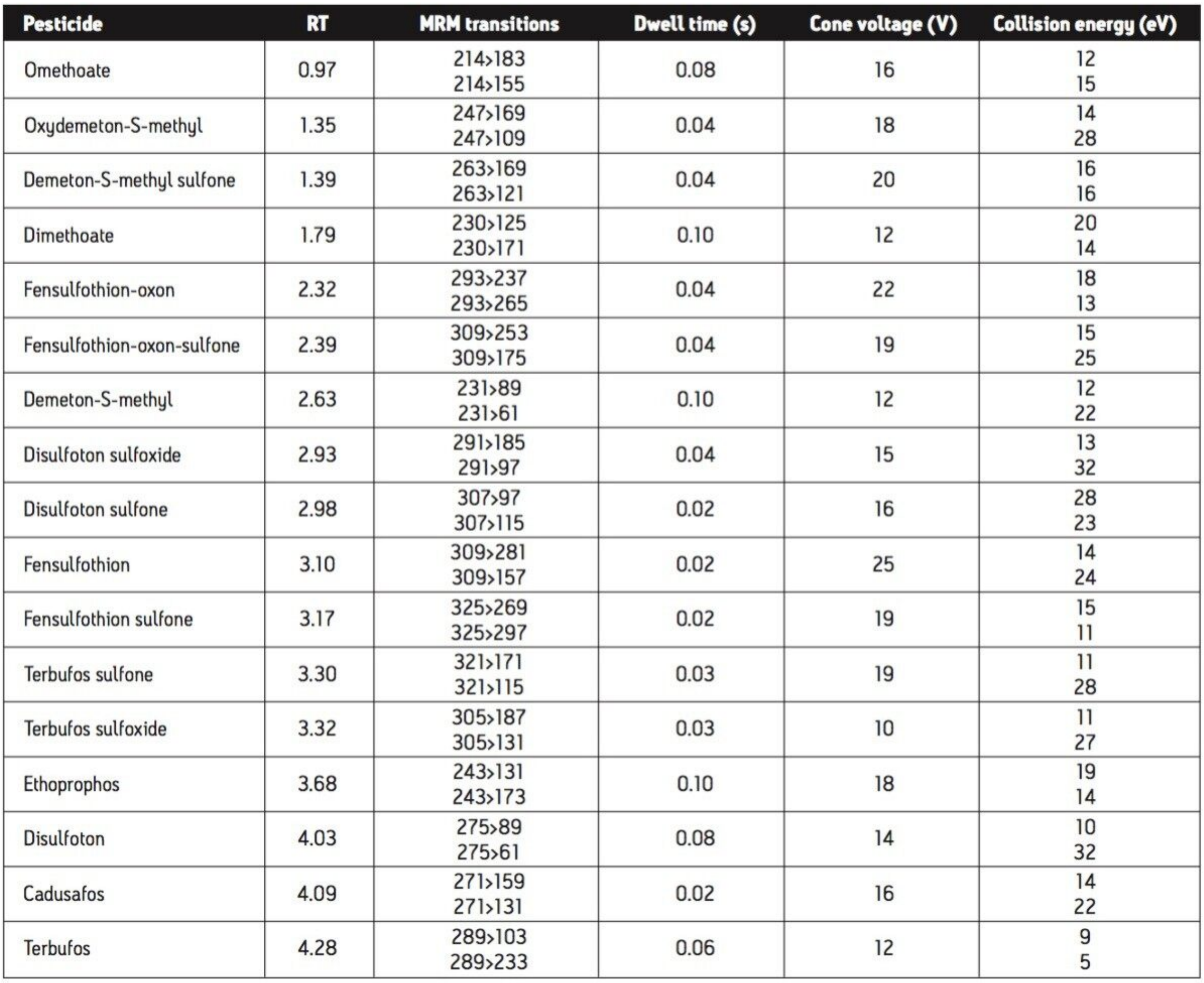
The benefits of this Waters UPLC-MS/MS solution for a revenue conscious laboratory can be realized through increased efficiency by analytical time savings, a decreased need for sample retesting, and increased lab productivity. Cost savings can be made by lowering the use of lab consumables with the environmental impact of solvent usage also being reduced.

The sensitivity achieved for a large number of pesticide residues in real food matrices indicates this UPLC-MS/MS method is the ideal basis for rapid analysis of pesticides in a wide range of food samples.

References

1. Commission of the European Communities EC 396/2005, OJ 2005; L70:1.
2. Commission of the European Communities EC 2003/13, L41:33-36.
3. DisQuE Dispersive Sample Preparation Kit Care and Use Manual, P/N 715001888 <https://www.waters.com/webassets/cms/support/docs/715001888.pdf> .
4. Leandro CC, Hancock P, Fussell RJ, Keely BJ. J. Chrom A. 2007; 1144:161.
5. Method Validation and Quality Control Procedures for Pesticide Residues in Food and Feed. Document No SANCO/2007/3131 <https://ec.europa.eu/food/plant/pesticides_en> .

Appendix 1. Xevo TQ MS parameters.



Pesticide	RT	MRM transitions	Dwell time (s)	Cone voltage (V)	Collision energy (eV)
Omethoate	0.97	214>183 214>155	0.08	16	12 15
Oxydemeton-S-methyl	1.35	247>169 247>109	0.04	18	14 28
Demeton-S-methyl sulfone	1.39	263>169 263>121	0.04	20	16 16
Dimethoate	1.79	230>125 230>171	0.10	12	20 14
Fensulfothion-oxon	2.32	293>237 293>265	0.04	22	18 13
Fensulfothion-oxon-sulfone	2.39	309>253 309>175	0.04	19	15 25
Demeton-S-methyl	2.63	231>89 231>61	0.10	12	12 22
Disulfoton sulfoxide	2.93	291>185 291>97	0.04	15	13 32
Disulfoton sulfone	2.98	307>97 307>115	0.02	16	28 23
Fensulfothion	3.10	309>281 309>157	0.02	25	14 24
Fensulfothion sulfone	3.17	325>269 325>297	0.02	19	15 11
Terbufos sulfone	3.30	321>171 321>115	0.03	19	11 28
Terbufos sulfoxide	3.32	305>187 305>131	0.03	10	11 27
Ethoprophos	3.68	243>131 243>173	0.10	18	19 14
Disulfoton	4.03	275>89 275>61	0.08	14	10 32
Cadusafos	4.09	271>159 271>131	0.02	16	14 22
Terbufos	4.28	289>103 289>233	0.06	12	9 5

Featured Products

ACQUITY UPLC System <https://www.waters.com/514207>

MassLynx MS Software <https://www.waters.com/513662>

TargetLynx <https://www.waters.com/513791>

Quanpedia <https://www.waters.com/10148049>

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