Utilising the Enhanced Resolution of UPLC to Increase the Number of Analytes within Multi-Residue Methods

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Keith Worrall, Peter Hancock and James Morphet
Waters Corporation, Manchester, United Kingdom

INTRODUCTION

- **Currently over 1,000 pesticides in use for the production of foodstuffs increasing pressure to broaden the range of pesticides determined in a single analysis over a shortened run time**
- **Legislation imposes Maximum Residue Limits[1] (MRLs) for pesticide residues in food products requiring analytical techniques that are sensitive, selective and robust**
- **A single multi-residue method per instrument can dramatically improve return on investment by removing the need to change method parameters**
- **The following method describes a solution for the rapid analysis of pesticides in mango, avocado and fruit based baby food extracts which is able to exceed current worldwide legislation**

METHODS

Extraction[2]

A QuEChERS-based extraction was utilised for this multi-residue method

1. Add 15 g homogenized sample to the 50 mL extraction tube containing 1.5 g sodium acetate and 6 g magnesium sulfate. Add 15 mL 1% acetic acid in acetonitrile.
2. Add any pre-extraction internal standards.
3. Shake vigorously for 1 minute and centrifuge > 1500 rcf for 1 minute.
4. Transfer 1 mL acetonitrile extract in to the 2 mL centrifuge tube containing 50 mg PSA and 150 mg magnesium sulfate.
5. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
6. Transfer 100 µL of final extract into an autosampler vial. Add any post-extraction internal standards. Dilute with 900µL mobile phase.

Chromatographic Conditions

LC System:	Waters® ACQUITY UPLC® System
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 100 mm, 1.7 µm
Column Temp:	40 °C
Sample Temp:	4 °C
Flow Rate:	0.450 mL/min.
Mobile Phase A:	98:2 water : methanol + 0.1% formic acid
Mobile Phase B:	Methanol + 0.1% formic acid
Gradient:	0.00 min 90% A
	0.25 min 90% A
	7.75 min 0% A
	8.5 min 0% A
	8.51 min 90% A
Cycle time:	10 min
Injection volume:	20 µL, full loop injection

MS Conditions

MS System:	Waters® ACQUITY TQD
Ionization Mode:	ESI positive polarity
Capillary Voltage:	1 kV
Desolvation Gas:	Nitrogen, 800 L/Hr, 400 ˚C
Cone Gas:	Nitrogen, 5 L/Hr
Source Temp:	120 ˚C
Acquisition:	Multiple Reaction Monitoring (MRM)
Collision Gas:	Argon at 3.5 x 10^{-3} mBar

Acquisition and Processing

The data was acquired using Waters MassLynx ™ software version 4.1 and processed using TargetLynx™ Application Manager.

RESULTS AND DISCUSSION

The analysis of 402 pesticide residues in mango, avocado and fruit-based baby food was achieved using ACQUITY TQD: liquid chromatography combined with tandem quadrupole mass spectrometry (UPLC/MS/MS) operated in Multiple Reaction Monitoring (MRM) mode. The rapid determination and confirmation method was achieved in two parts.

Part one was a single injection with one MRM transition per compound, ideal for screening purposes. Figure 1 shows all 402 pesticide residues in one 10 minute run, fully utilising the enhanced speed and resolution of UPLC.

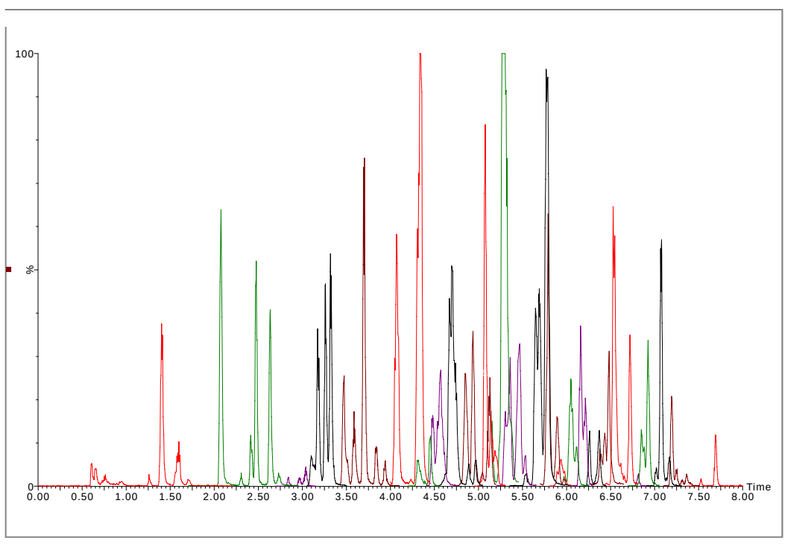


Figure 1. Chromatogram showing all 402 pesticide residues in one 10 minute run.

Part 2, where compounds of interest can be confirmed, was achieved by two separate injections with two MRM transitions per compound. Figures 2 and 3 show the separation of 201 pesticide residues across two run times of 10 minutes each.

The enhanced speed and resolution of UPLC enabled all peaks to elute within 8 minutes. Dwell times of 5ms were used to achieve at least 12 data points across each peak for both quantification and confirmatory ions.

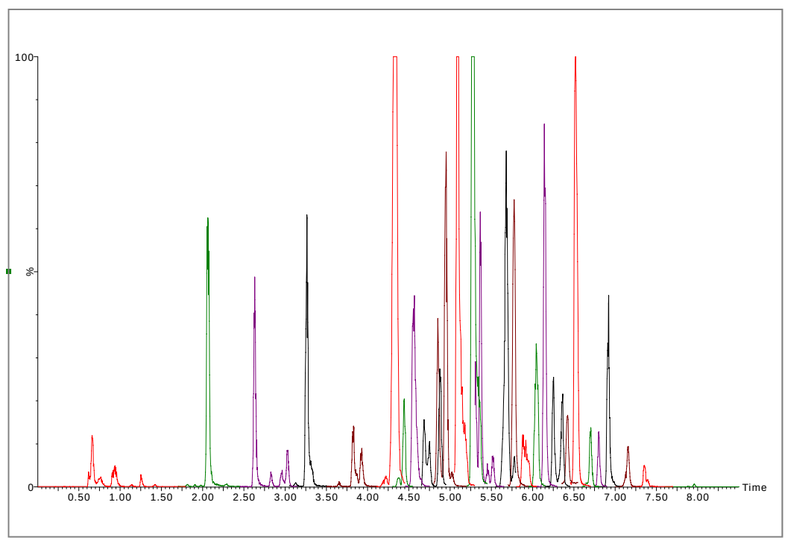


Figure 2: Chromatogram showing first 201 pesticide residues at 10µg/kg in injection solvent.

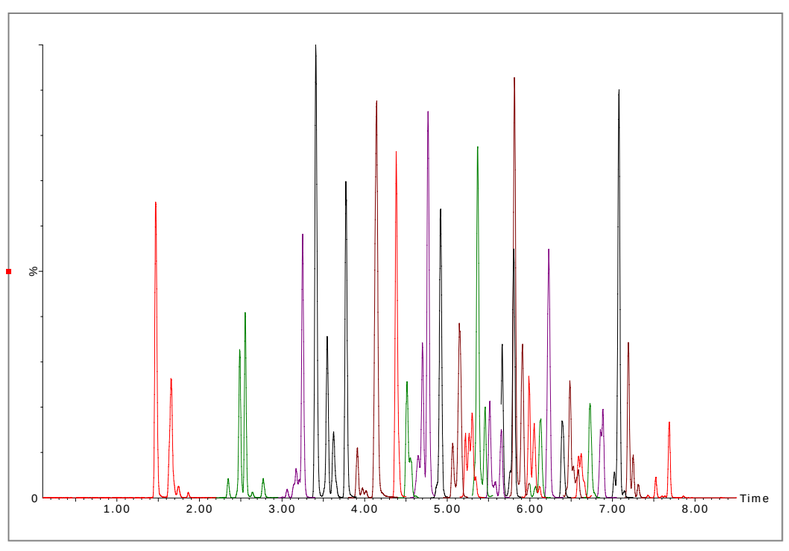
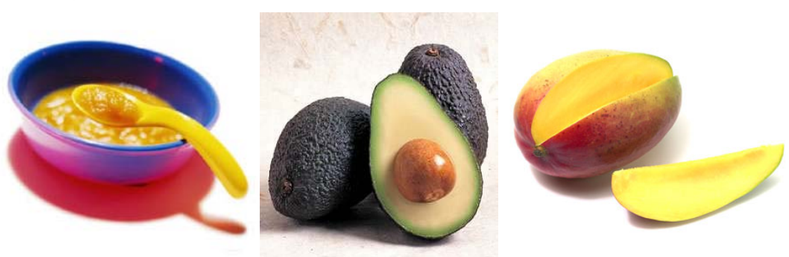


Figure 3: Chromatogram showing second 201 pesticide residues at 10µg/kg in injection solvent.

The selectivity achieved using a tandem quadrupole mass spectrometer (ACQUITY TQD) shows an advantage over a single quadrupole instrument as it allows co-eluting compounds to be identified and quantified with confidence.

For all injections, the same UPLC conditions were used saving analytical time and costs, thus maximising return on investment. This single set up will allow analysts with less experience to run the method as the need for changes to be made in between batches is removed. The Intellistart™ technology provides simple instrument setup and MS method development and therefore easy access even for the most inexperienced MS user.



The 402 pesticide mix was spiked into the three matrices and the extracts analysed. Figures 4 and 5 show pesticides at 10µg/kg, equivalent to the lowest worldwide (EU) legislation, in avocado and baby food extracts, respectively.

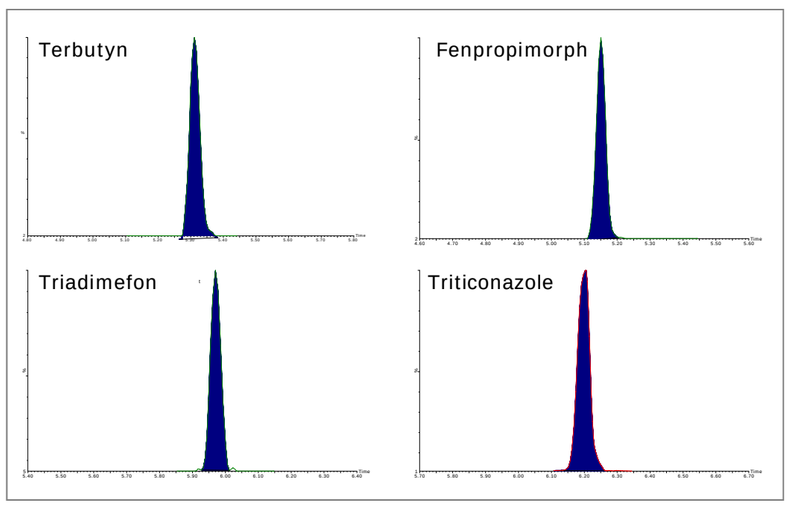


Figure 4. Four pesticides in avocado extract at 10µg/kg.

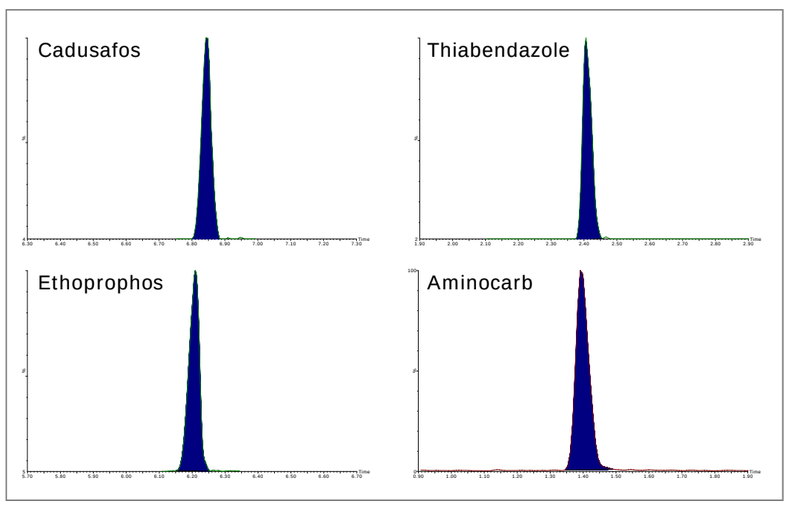


Figure 5. Four pesticides in baby food extract at 10µg/kg.

The advantage of using Acquity TQD is that ion ratio confirmation is also possible. This is used to confirm the identity of any pesticide that was presumptive positive from the screening method. Within the EU, ion ratio confirmation is important for pesticide analysis as documented in SANCO/2007/3131[3]. In the confirmatory runs, all 402 pesticides were determined with both primary (quantitation) and secondary (confirmation) MRM transitions present.

CONCLUSION

- **A rapid multi-residue method was developed for the screening of over 400 pesticides one 10 minute run with one MRM transition per pesticide**
- **For confirmation, two 10 minute runs are required with two MRM transitions per pesticide**
- **The analysis of pesticides in mango, avocado and fruit based baby food extracts was able to exceed current worldwide legislated limits**
- **Improved efficiency and increased sample throughput was realized through the combination of powerful UPLC and fast MS acquisition technologies. The Waters® ACQUITY TQD offers:**
  - **Enhanced chromatographic resolution and short analysis times**
  - **Incorporation of confirmatory MRM traces to comply with legislative regulations such as SANCO**
  - **IntelliStart technology which is designed to reduce the burden of complicated operation, training new users, time-intensive troubleshooting and upkeep**
- **The benefits of this Waters® UPLC/MS/MS solution for a revenue conscious laboratory can be realized through increased efficiency through:**
  - **Analytical time saving, decreased need for sample retesting, resulting in increased lab productivity**
  - **Cost savings through 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 an ideal basis for the rapid analysis of pesticides in a wide range of food samples.**

ACKNOWLEDEMENTS

The authors would like to thank Central Science Laboratory (CSL), Sand Hutton, York, UK and VWA, Amsterdam, The Netherlands for kindly supplying MRM transitions and standard solution mixes that were analysed in this project. Furthermore, University of Jaume I Castellon, Spain, Waters UK, Waters US and Nihon Waters, Japan demonstration laboratories are all thanked for their contributions in supplying MRM transitions.

References

1. Commission of the European Communities EC 396/2005, OJ 2005; L70:1
2. Lehotay, *J.AOAC Int.* **90**(2) 2007, 485-520.
3. http://ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf

50 YEAR LEGACY
VISION ■ CONTRIBUTION ■ ADVANCEMENT

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS

720002778EN