UTILISING THE ENHANCED RESOLUTION OF ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY TO INCREASE THE NUMBER OF TARGET ANALYTES WITHIN MULTI-RESIDUE METHODS

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Abstract
The screening of more than 400 pesticide residue compounds in fruit and vegetables was achieved using ultra performance liquid chromatography combined with tandem quadrupole mass spectrometry (LC/MS/MS) operated in multiple reaction monitoring (MRM) mode. Using a generic extraction, valid for a wide range of compound classes in a representative set of food commodities, the single extract was injected twice using an Ultra Performance Liquid Chromatography (UPLC™) method of ten minutes with two MRM transitions per compound. The limits of determination achieved for the pesticides analysed are well below that required for worldwide surveillance monitoring.

Introduction
Pesticides are widely used in the production of foodstuffs to meet consumer demand for plentiful food, at reasonable prices, all year round. However continued growth in the use of pesticides, 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 governing pesticides. Legislation imposes Maximum Residue Limits (MRLs) for pesticide residues in food products requiring analytical techniques that are sensitive, selective and robust. Multi-residue pesticide analysis is challenging due to the low levels present, the wide variety of pesticides and the very different chemical classes they represent. As there are currently well over 1,000 pesticides in use, laboratories are under increasing pressure to broaden the range of pesticides determined in a single analysis over a shortened run time. The need to meet mandated detection limits, develop sample preparation techniques for complex matrices and the desire to increase sample throughput are the main challenges facing food safety testing laboratories today. The use of a single multi-residue method per instrument can dramatically improve laboratory workflow by removing the need to change detection parameters when determining subsets of a complete compound list; as is often the case when analysing a wide variety of commodities with differing lists of legislated pesticides.
Advances in chromatographic separation and detection technologies have enabled analysts to increase the number of analytes determined in a single run, with tandem quadrupole mass spectrometry offering a highly specific and selective detection technique, which has become the technique of choice[1] within the laboratory. Coupling tandem quadrupole mass spectrometers to a high resolution, rapid separatory technique such as UPLC, enables the analyst to realise the full potential of this combined technology. This technique allows for a significant increase in the number of residues that can be determined within a single injection, offering a 'generic' multi-residue analytical determination.

UPLC has been previously reported[2,3] to offer a significant improvement in chromatographic resolution, whilst also reducing the time taken for each analytical separation. Figure 1 shows the chromatograms obtained from HPLC separation of 81 pesticide residues, having a 40 minute cycle time, along with the resulting method transfer to UPLC. The UPLC separation allows for the separation and detection of 100 pesticide residues with a 13.5 minute cycle time, whilst maintaining or improving chromatographic resolution in all cases. The target compound list could also be increased due to the enhanced separation and slightly different elution order, which allows the MS time windows to be distributed more evenly across the elution range whilst maintaining >10 data point across each chromatographic peak.

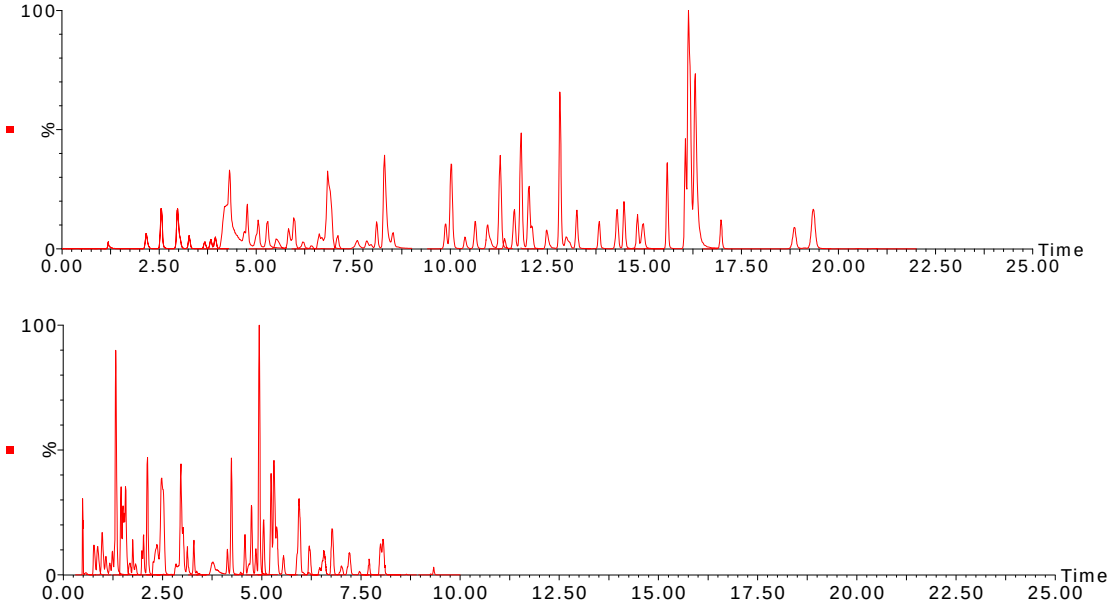

Figure 1, comparison of runtimes and chromatographic resolution for separation of >80 pesticide residues by HPLC (top trace) and after method transfer to UPLC (bottom trace)

Methods and Materials

All data were acquired using a Waters Acquity UPLC interfaced to a Waters Acquity TQ Detector tandem quadrupole mass spectrometer. Various fruit and vegetable commodities were purchased from local food stores and were extracted using a QuEChERS based method[4]. Each commodity was extracted as received, and spiked with the pesticides at around the MRL level[5] of 10 ppb.

All separations were performed on a BEH C_{18} UPLC column, 100 mm x 2.1 mm x 1.7 µm using the mobile phase gradient and other parameters shown in table 1. Each calibration standard, blank and sample extract was injected twice, with a subset of approximately 200 of the pesticide residues being determined within each injection. Each injection had a total cycle time of 10 mins giving a total run time of 20 mins for the complete group of 400 compounds. The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode, monitoring 2 MRM transitions for each target compound. Prior to extraction the commodities were spiked with the internal standards Simazine D_5, Omethoate D_6 and Quizalofop ethyl D_3.

Sample Temp: 4 ˚C	Column Temp: 40 ˚C	Flow Rate: 0.450 mL min^{-1}.
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
	8.25 min	0% A
	10 min	90% A
Injection volume:	20µL, Full loop injection	

Table 1, LC conditions used for the UPLC separation

Results and discussion

Calibration curves were formed for each of the pesticides to be determined, covering the concentration range 0.5 ppb to 20 ppb with calibration standards containing all 400 target compounds plus the internal standards. Each standard was injected twice, once under each of the gradient conditions. Calibration curve linearity was deemed to be satisfactory, with 1/x weighted curves offering coefficients of determination (r^2) >0.99. Figure 2 below shows a typical acquisition time window from within the calibration curve, for a calibration point at 10 ppb. These chromatograms show the sort of signal that could be expected at a typical MRL level. It can also be observed that the chromatographic peak widths achieved using a 'generic' method are approximately 3 seconds wide at 5% peak height.

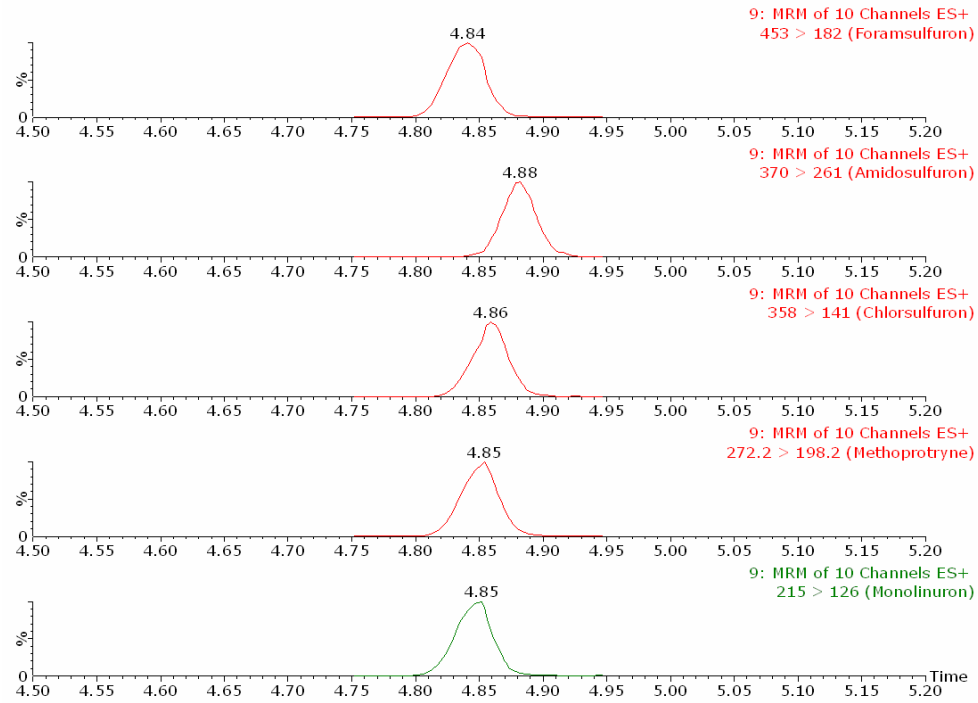

Figure 2, a typical acquisition time window for the 10 ppb calibration standard.

The reconstructed total ion chromatograms are shown in figure 3 for the complete separation of all 400 compounds, with both injections displayed.

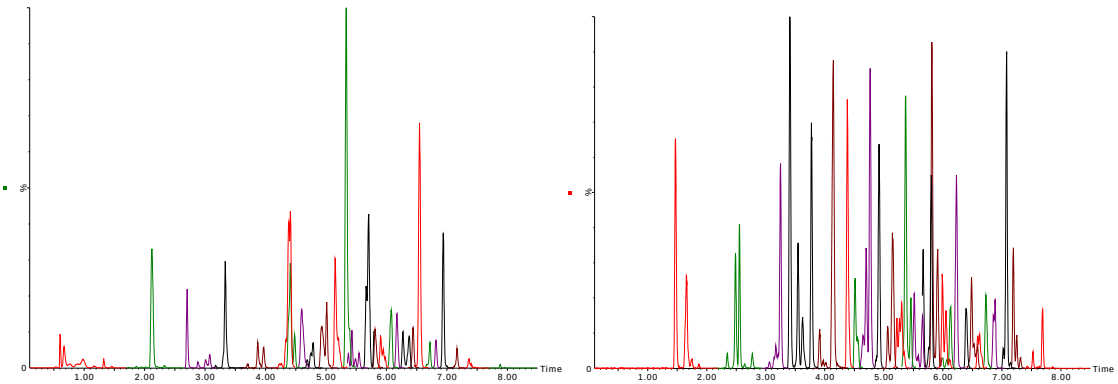

Figure 3, reconstructed total ion chromatograms for all 400 pesticides determined, with run 1 on the left and run 2 on the right.

All food extracts were then injected under both sets of MS conditions, allowing for determination of all 400 pesticide residues within a 20 minute timeframe. All data was processed using TargetLynx^{TM} software that allowed for the entire analytical sequence (2 injections per vial) to be viewed within a single results file. This allows for rapid evaluation of results and generation of a single sample report for both analytical runs.

Analysis of the sample extracts using this method allowed screening and confirmation at the required MRL levels, positive samples could be confirmed according to current QC/QA and confirmation recommendations[6]. Figure 4 shows an example of the detection and confirmation of two residues, spiked at 10 ppb in Mango and baby food.

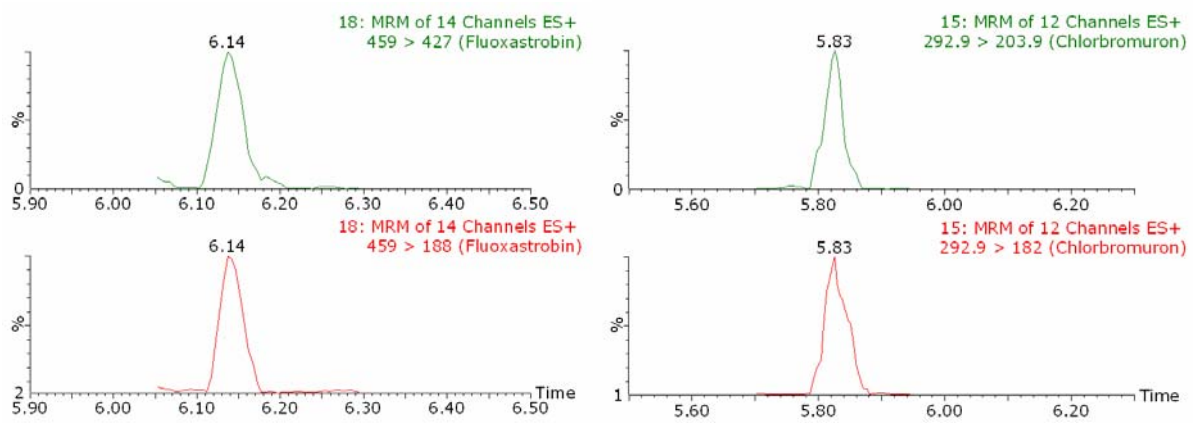

Figure 4, Fluoxastrobin detected in Mango (left) and Chlorbromuron detected in baby food (right)

Conclusions

The enhanced resolution of UPLC allows the rapid screening and confirmation of >400 pesticide residues in 20 minutes. This offers the capability for a single analytical method to be used on an instrument irrespective of the target compounds that must be analysed, reducing the possibility for errors when creating multiple analytical sequences.

References

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