APPLICATION OF HIGH RESOLUTION TOF-MS FOR MULTIRESIDUE ANALYSIS OF PESTICIDES

Waters

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INTRODUCTION

Pesticide residue analysis is inherently targeted towards a limited number of compounds using techniques such as selected ion recording (SIR) or multiple reaction monitoring (MRM). The use of these selective techniques makes post acquisition reprocessing difficult, and the discovery of unknown contaminants practically impossible. This has led analysts to consider what other, potentially harmful, untargeted residues may be present and has resulted in a demand for analytical methods that are sensitive and selective, but not specific.

Exact mass time of flight mass spectrometry (TOF-MS) is a full spectrum technique capable of both the targeted and the untargeted screening approaches. One distinct advantage of TOF-MS is that methods can be extended to potentially unlimited numbers of residues without loss in sensitivity.

Here the applicability of exact mass TOF-MS was evaluated for targeted and untargeted pesticide residue analysis for both GC and LC amenable compounds. The two techniques were applied to a wide range of pesticides to legislated levels in matrices of differing complexity.

Untargeted screening relies heavily on automatic software processing, therefore, the data was processed using a new software module that automatically peak detects, deconvolutes spectra, and searches the results against a library with exact mass scoring. Some untargeted residues were successfully identified using this method.

METHODS

Extraction Methods

Details in Waters Application Notes 720001437EN / 720001607EN.

LC Method

Waters Acquity UPLC

Column	UPLC BEH C_{18} 2.1x100 mm, 1.7 µm @ 40 °C.
Flow rate	0.45 mL/min.
Mobile phase A/B	5%/95% aqueous MeOH + 2 mM $CH_3CO_2NH_4$
Gradient	0% B → 8.5 min, 100% B (1.5 min).
Injection volume	20 µ L.

LC-MS Method

Waters LCT Premier TOF-MS.

Ionisation mode	+/- Electrospray @ 1000 V.
Source temp.	120 °C.
Desolvation temp.	400 °C.
Gas flow	600 L/hr.
Mass range	50 and 1000 Da.
Acquisition speed	0.25 s per function.
Lock mass	Leucine Enkephalin, + 556.2771, - 554.2615.

GC Method

Agilent 6890N GC with 7683B autosampler.

Column	DB-5ms 20 m x 0.18 mm i.d. x 0.18 µ m.
Flow rate	1.0 mL/min helium constant flow.
Temp. ramp	40 °C (2 min), 220 °C @ 30 °C/min, 260 °C @ 5 °C/min, 280 °C @ 20 °C/min (8 min).
Injection method	Cyro-cooled PTV in solvent vent mode, 1 µL.
Vent method	Pressure 5 kPa, Flow 20 mL/min for 0.5 min.

GC-MS Method

Waters GCT Premier TOF-MS.

Ionisation mode	Electron impact (EI+) @ 70 eV.
Source temp.	200 °C.
Trap current	200 µ A.
Interface temp.	280 °C.
Mass range	50 and 550 Da.
Acquisition speed	10 spectra / s.
Lock mass	2,4,6-tris(trifluoromethyl)-1,3,5-triazine, 284.9949.

Software

MassLynx v4.0 with TargetLynx and ChromaLynx application managers.

RESULTS AND DISCUSSION

Targeted Screening Results

The occurrence of interfering ions with masses close to those of any target analyte is one of the main factors that limits the detection limits of any MS method. Reducing the width of the mass window generally results in a significant elimination of interferences, leading to improvements in the signal to noise (S/N) ratio and, consequently, lower limits of detection (LODs) to be achieved. This is illustrated in Figure 1 with fludioxonil (0.01 mg/kg) in grapefruit. The exact mass chromatogram (0.02 Da, *m/z* 248.0397) shows improved S/N compared to the nominal mass chromatogram (1 Da, *m/z* 248). The mean difference between the nominal and exact mass S/N was a factor of five for all residues.

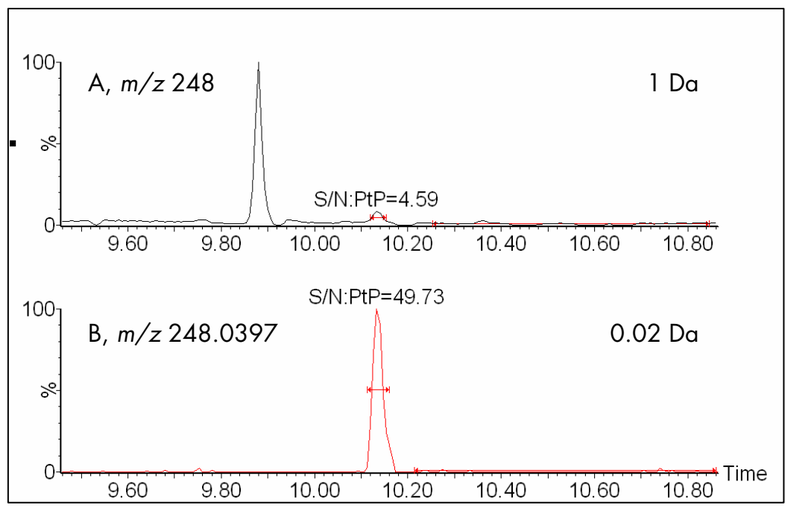

Figure 1. S/N offered by nominal mass (A) versus exact mass (B) chromatograms for fludioxonil.

Increasing the number of ions monitored, as in the case of confirmation or simply increasing the number of residues, on a scanning instrument will lead to a decrease in the overall sensitivity (S/N). Quinoxyfen (0.01 mg/kg) in sweet pepper, shown in Figure 2, illustrates that exact mass TOF-MS does not suffer this effect.

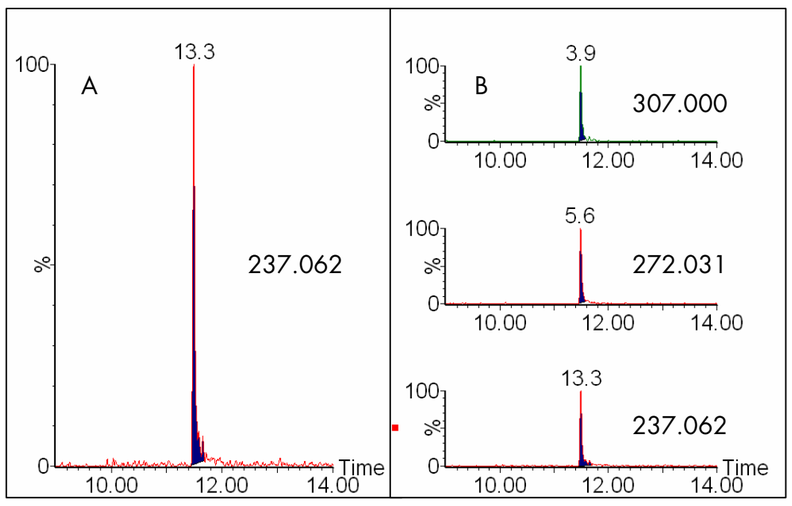

Figure 2. Sensitivity of one ion (A) versus three ions (B) for 0.01 mg/kg quinoxyfen in sweet pepper.

TOF-MS has not previously had sufficient dynamic range to perform quantification functions greater than 3 orders of magnitude. Both exact mass TOF-MS instruments have inbuilt Dynamic Range Enhancement (DRE), extending the dynamic range, which allows quantification to be performed with greater simplicity. Quantification is performed with Waters TargetLynx application manager. A typical TargetLynx browser

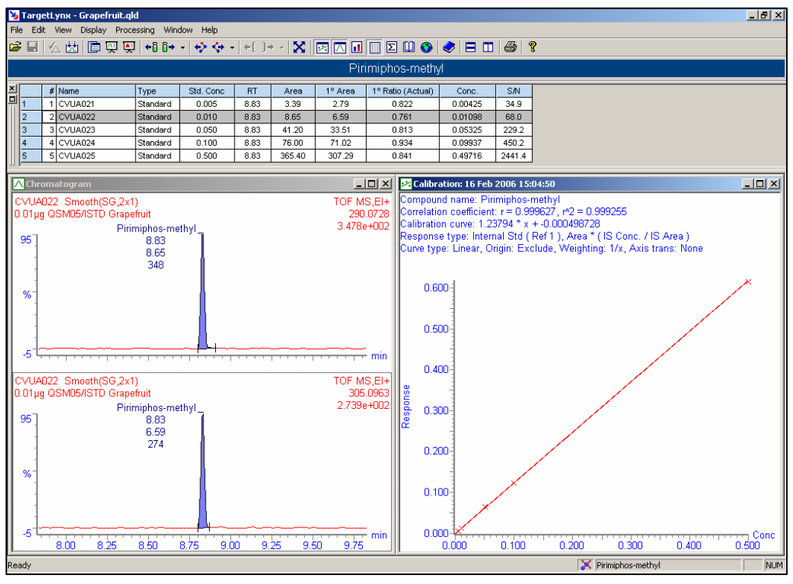

Figure 3. Example TargetLynx browser for pirimiphos-methyl at a concentration of 0.01 mg/kg in grapefruit.

Untargeted Screening Results

In the untargeted screening environment, there may be hundreds of peaks that need to be located and identified, which would be very time consuming if performed manually. Here automatic processing is essential using a package such as Waters ChromaLynx application manager.

ChromaLynx automatically plots the RICs of the eight most intense ions at any point in the chromatogram. If a peak is found to satisfy user-defined parameters the software will display its deconvoluted mass spectrum. The spectrum is then submitted to an automatic library search routine with the ability to confirm by exact mass scoring of the "n" most intense ions.

ChromaLynx processing of the 0.05 mg/kg spiked cucumber extract located 550 components in the chromatogram, of which 30 residues were spiked. An example of one of the untargeted compounds, etrimfos, that was found is illustrated in Figure 4. Etrimfos was confirmed with three ions within 1.1 mDa of their expected exact masses.

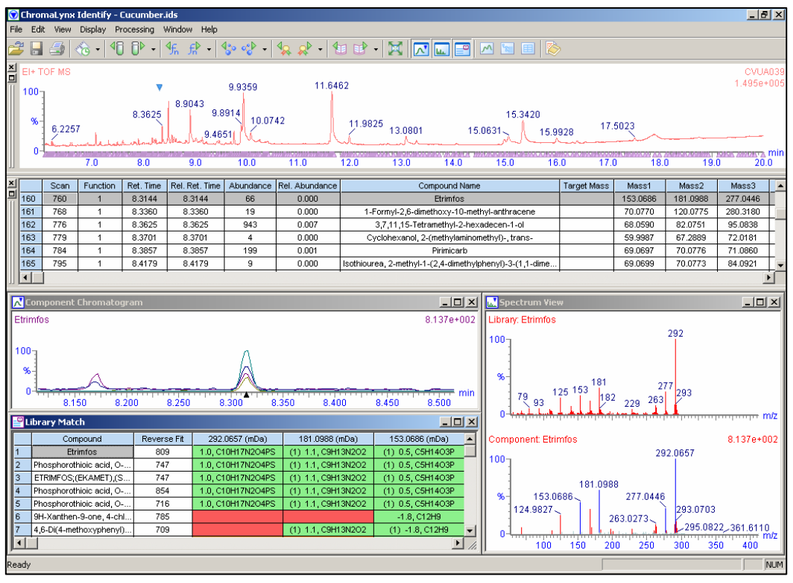

Figure 4. Example ChromaLynx browser for an untargeted pesticide residue in spiked cucumber (0.05 mg/kg).

Moving to a more complex matrix such as grapefruit, more than 1500 components were located using ChromaLynx. An example of an unexpected residue located and identified using this method is illustrated in Figure 5. In the example, enilconazole or imazalil, a fungicide commonly used on citrus fruit was identified.

Figure 5. Example ChromaLynx browser for an unexpected pesticide residue in grapefruit.

CONCLUSIONS

- **Methods have been presented for the targeted screening of pesticide residues in food commodities using both the GCT Premier and LCT Premier with TargetLynx.**
- **The residues can be screened to concentration levels of 0.01 mg/kg or less in various matrices with the use of exact mass chromatograms.**
- **The methods can be extended to larger numbers of residues without loss in sensitivity due to the full spectrum approach provided by exact mass TOF instruments.**
- **The saved data files can also be used to screen for untargeted residues using ChromaLynx.**
- **ChromaLynx enables automatic peak detection, deconvolution, library searching and exact mass scoring.**

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