MULTI-RESIDUE SCREENING OF PESTICIDES IN FRUIT EXTRACTS BY LC/MS - A COMPARATIVE STUDY USING OA-TOF AND TANDEM QUADRUPOLE MASS SPECTROMETRY

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Overview

AIM

- To investigate the applicability of LC/MS methods using oa-TOF and tandem quadrupole mass spectrometry for multi-residue analysis
- Compare the relative performance of each LC/MS technique in terms of quantitative ability (sensitivity, linearity)

METHOD

- Fruit samples (peach) were methanol extracted to produce crude sample
- 15 pesticides targeted for analysis
- LC separation carried out under reversed phase conditions with electrospray ionisation
- Generic MS tuning parameters used for full spectral detection with oa-TOF
- Parent and fragment ions optimised for MRM detection on tandem a quadrupole
- 15 target compounds quantified against matrix matched standards in 2 fruit extracts

RESULTS

- All 15 target pesticides in crude extracts successfully separated by reverse phase HPLC, detected at lowest reporting level and quantified against matrix matched standards using both oa-TOF and tandem quadrupole MS
- oa-TOF provides the facility to generate exact mass measurements for improved specificity of detection
- Generation of exact mass chromatograms comparable to specificity obtained using MRM detection
- Although oa-TOF is a sensitive MS technique, MRM detection using tandem quadrupole allows lower concentrations to be detected

Introduction

Pesticide residue screening in foodstuffs can be extremely challenging sometimes involving in excess of one hundred target compounds. Many pesticides are amenable to analysis by GC-MS with a combination of ionisation and scan techniques. Compounds which are thermally labile or polar in character present the residue analyst with a difficult problem particularly at the low ppb reporting levels demanded by regulatory authorities. Regulation also imposes the necessity of unambiguous confirmation of residues identified in the screening process.

With the advent of robust and sensitive Atmospheric Pressure Ionisation (API) sources, the use of LC-MS has become the method of choice for the screening and confirmation of pesticides which are not amenable to GC-MS. In this paper we compare the applicability of orthogonal acceleration time-of-flight (oa-TOF) and tandem quadrupole mass spectrometry for the analysis of multi-pesticide residues in fruit extracts. The use of oa-TOF for such an application has been previously described[1,2].

Using suitable examples, we will highlight the benefits of both oa-TOF and tandem quadrupole techniques. Oa-TOF provides the facility to acquire full spectral data with on-line exact mass measurement allowing the confirmation of target analytes with a high level of confidence. The enhanced specificity of exact mass chromatograms also provides excellent quantitative linearity by removing matrix-related interferences. Quantitative data obtained with oa-TOF is similar to data obtained using the highly specific Multiple Reaction Monitoring (MRM) detection method with tandem quadrupole MS. The need for complex sample clean-up procedures is therefore removed. Using both MS techniques, low-level detection (ppb) and quantification of target residues against matrix matched standards at concentrations equivalent to 0.5, 0.25, 0.05 and 0.025ng/µL were obtained.

Experimental Methods

Extraction Procedure

- 10g fruit (peach) extracted using ethyl acetate
- Ethyl acetate extract solvent exchanged to methanol
- Solution filtered using Acrodisc 45µM filter prior to analysis

LC Conditions

HPLC:	Waters 2795
Column:	Waters Symmetry C18, 2.1 x 100mm
Column temp:	30°C
Flow rate:	0.2mL/min
Solvent A :	10mM ammonium acetate
Solvent B :	Methanol
Separation:	Isocratic, 30%A / 70%B
Injection Vol:	20µL
Runtime:	15 minutes

MS Conditions

LC/MS:	Micromass LCT with dual ion source
LC/MS/MS:	Micromass Quattro micro
Ion mode:	ESI+ve
ESI Voltage:	500V
Cone:	25V for oa-TOF optimised for each compound for MRM
Collision energy:	Optimised for each compound for MRM

Exact Mass Measurement

Lock Mass:	Leucine enkephalin infused at 10µL/min, 0.25ng/µL solution
Reference spray:	Sampled every 20 seconds
Acquisition mode :	Centroid

Results and Discussion

Figure 1 shows the schematic representation of an oa-TOF mass spectrometer. Ions injected into the TOF pass through a field free region and are separated according to their mass to charge ratio, i.e. the heavier the ion, the longer the flight time. **Figure 2** shows a schematic representation of a tandem quadrupole mass spectrometer. For MRM detection, the precursor ion is selected in Q1, fragmented in Q2 with the product ion selected in Q3.

Schematic of an oa-TOF MS

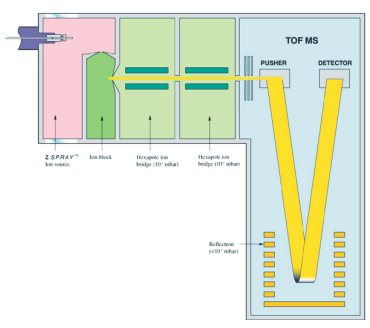

Figure 1

Schematic of a tandem quadrupole MS

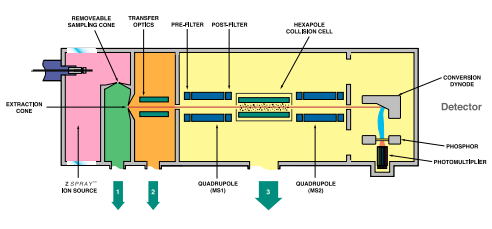

Figure 2

Pesticides in Peach Samples

Table 1 lists the 15 pesticides targeted for analysis.

Pesticide	Formula	M+H	RL (mg/kg)
Pymetrozine	C10H11N5O	218.1042	0.05
Methiocarb sulfoxide	C11H15NSO3	242.0851	0.2
Thiabendazole	C10H7N3S	202.0439	0.05
Bendiocarb	C12H11NO2	224.0923	0.05
Carbaryl	C12H11NO2	202.0868	0.01
Azoystrobin	C22H17N3O5	404.1246	0.05
Methiocarb	C11H15NSO2	226.0902	0.2
Methiocarb sulfone	C11H15NSO4	258.0800	0.2
Myclobutanil	C15H17ClN4	289.1220	0.05
Dichlofluanid	C9H11Cl2FN2S2O2	332.9701	0.05
Fenhexamid	C14H17Cl2NO2	302.0714	0.05
Penconazole	C13H15Cl2N3	284.0721	0.05
Tebuconazole	C16H22ClN3O	308.1529	0.05
Imazalil	C14H14Cl2N2O	297.0561	0.02
Propiconazole	C15H17Cl2N3O2	342.0776	0.05

Table 1. Pesticides targeted for analysis

RL = Reporting level

Using the chromatographic conditions described, all 15 target pesticides in the peach extracts were separated within the 15 minute run time. **Figure 3** shows extracted mass chromatograms obtained using oa-TOF for each of the pesticides in mobile phase solution. **Table 2** lists the exact mass measurements for all the target pesticides, allowing greater confidence in analyte confirmation.

Using oa-TOF, no compound optimisation in terms of sample cone was carried out. For MRM detection, the cone voltage and collision energy were optimised separately for each compound to produce the MS/MS pathway. **Table 3** shows the MRM conditions for each of the 15 pesticides

Extracted mass chromatograms of target pesticides

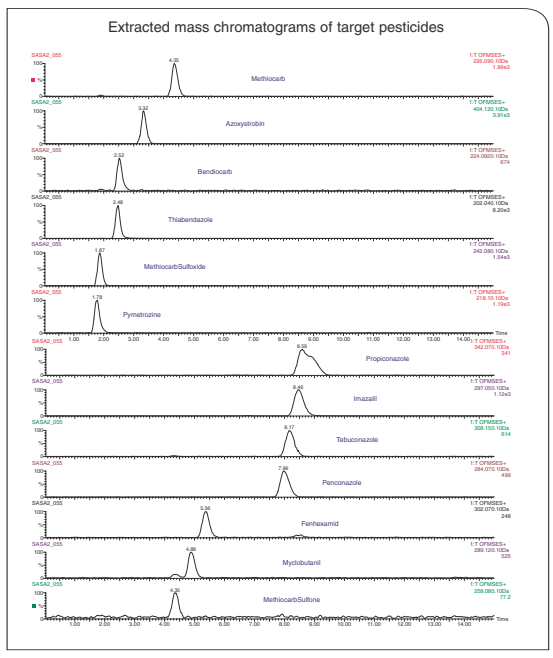

Figure 3

Pesticide	Theoretical M+H	Measured M+H	mDa Error	ppm Error
Pymetrozine	218.1042	218.1050	0.8	3.7
Methiocarb sulfoxide	242.0851	242.0847	-0.4	-1.7
Thiabendazole	202.0439	202.0433	-0.6	-3
Bendiocarb	224.0923	224.0914	-0.9	-4
Carbaryl	202.0868	nd		
Azoystrobin	404.1246	404.1227	-1.9	-4.7
Methiocarb	226.0902	226.0914	1.2	5.3
Methiocarb sulfone	258.0800	258.0818	1.8	6.9
Myclobutanil	289.1220	289.1214	-0.6	-2.1
Dichlofluanid	332.9701	nd		
Fenhexamid	302.0714	302.0717	0.3	1
Penconazole	284.0721	284.0716	-0.5	-1.8
Tebuconazole	308.1529	308.1534	0.5	1.6
Imazalil	297.0561	297.0550	-1.1	-3.7
Propiconazole	342.0776	342.0775	-0.1	-0.3

Table 2. Exact mass measurements.

n/d - no molecular ion observed

Compound	MRM Transition	Cone Voltage	Collision Energy
Pymetrozine	218 > 104	25	20
Methiocarb sulfoxide	242 > 185	25	15
Thiabendazole	202 > 175	25	20
Bendiocarb	224 > 166.8	20	10
Carbaryl	202 > 145	25	20
Azoystrobin	404 > 372	20	15
Methiocarb	226 > 169	20	10
Myclobutanil	289 > 68	25	20
Dichlofluanid	333 > 224	25	20
Fenhexamid	302 > 224	25	20
Penconazole	284 > 159	25	20
Tebuconazole	308 > 68.3	25	20
Imazalil	297 > 158.5	25	20
Propiconazole	342 > 158.5	25	20

Table 3. MRM Conditions.

Quantitative Linearity of oa-TOF and Tandem Quadrupole

Using thiabendazole as a suitable example, **Figure 4** shows the calibration graphs obtained using oa-TOF for (a) the methanol standard and (b) the matrix matched standard. **Figure 5** shows the calibration graphs obtained using MRM for (a) methanol standard and (b) matrix matched standard. Good quantitative linearity is obtained (better than 0.99) is obtained using both techniques for all compounds.

Calibration graphs for (a) methanol and (b) matrix matched standards using oa-TOF

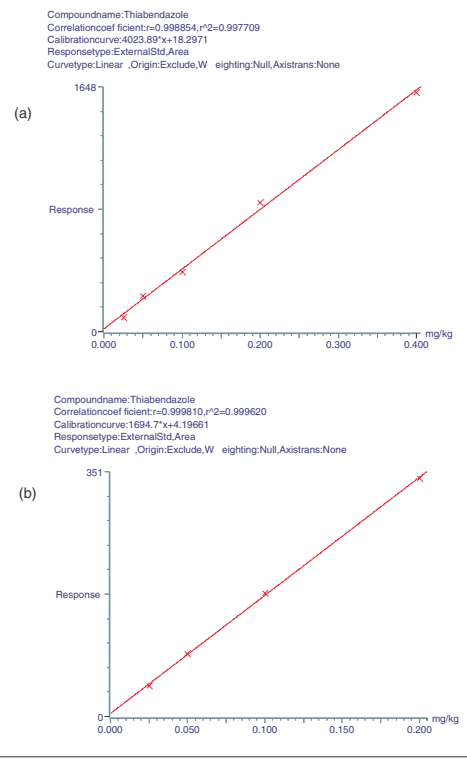

Figure 4

Calibration graphs for (a) methanol and (b) matrix matched standards using MRM

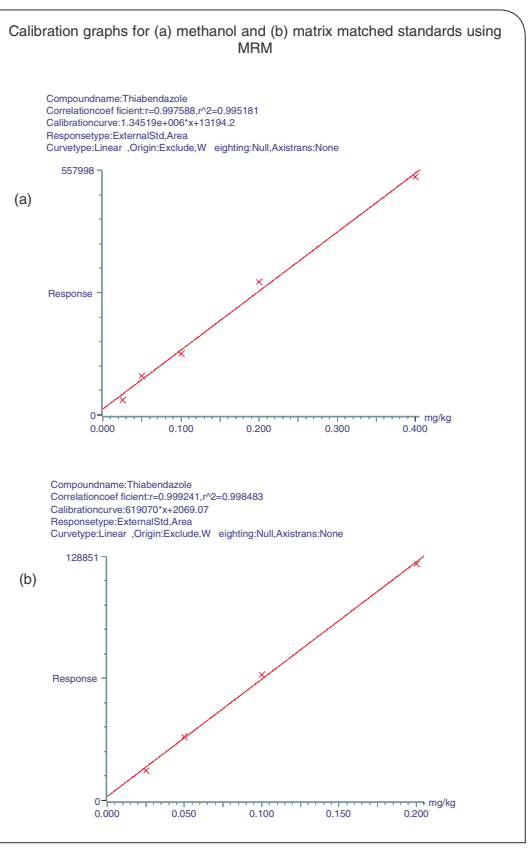

Figure 5

Signal to Noise Comparison

From the signal to noise ratios calculated for both the oa-TOF and MRM data, it has been found that the highly specific technique of MRM is typically 4-25 times more sensitive than using oa-TOF. In this study, two sets of 5 and a set of 4 MRM channels were acquired.

Figure 6 shows the calculated signal to noise ratios for thiabendazole at the 1RL on both instruments. With MRM detection (5 simultaneous channels) the signal to noise is approximately 4 time better by tandem MS. **Figure 7** shows the calculated signal to noise ratios for pymetrozine at the 1RL . Again, MRM detection (4 simultaneous channels), the signal to noise is approximately 30 times better.

Signal to Noise comparison for thiabendazole

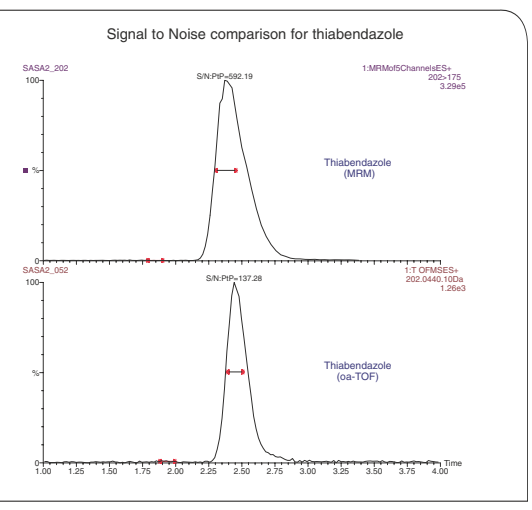

Figure 6

Signal to Noise comparison for pymetrozine

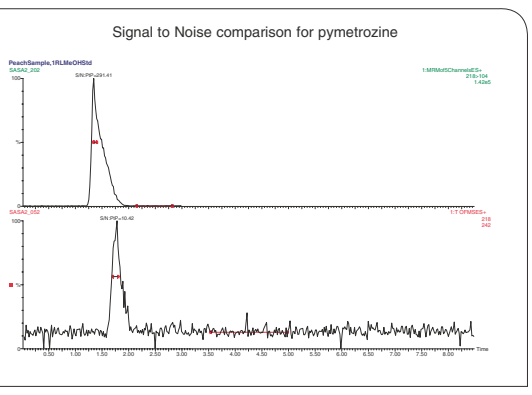

Figure 7

Conclusion

- LC-MS is routinely used in the screening of pesticide residues
- Both oa-TOF and tandem quadrupole MS technology can be used to quantify pesticide residues in environmental samples at low ppb concentrations due to the inherent sensitivity.
- oa-TOF provides elevated spectral resolution allowing exact mass measurement and full MS spectral sensitivity for low level analyte detection.
- Real time exact mass measurement of LC peaks allows confirmation of target compounds and confidence when determining unknown analytes.
- Similar quantitation results of residues in peach extracts were obtained using oa-TOF and tandem quadrupole.
- MRM detection is a more sensitive technique than full scan oa-TOF, allowing lower limits of detection.

References

1. Sage AB et al, Pesticide Residue Analysis of Fruit and Animal Extracts using Liquid Chromatography-orthogonal acceleration Time-of-Flight Mass Spectrometry (LC-oa-TOFMS), Poster presented at the 49th American Society for Mass Spectrometry Conference, Chicago, Illinois, May 2001
2. Sage AB *et al*, Micromass Application Note AN260.

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