



PESTICIDE RESIDUE ANALYSIS OF FRUIT AND ANIMAL EXTRACTS USING LIQUID CHROMATOGRAPHY-ORTHOGONAL ACCELERATION TIME-OF-FLIGHT MASS SPECTROMETRY (LC-*oa*-TOFMS)

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GC-MS

LC-MS

LC-MS-MS

MALDI-MS

ICP-MS

LC-ICP-MS

IR-MS



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Overview

AIM

- To investigate the application of LC-*oa*TOFMS for screening pesticide residues in fruit and bird of prey extracts.
- Highlight *oa*-TOF benefits including full spectral MS sensitivity for low level analyte detection, exact mass measurement for compound confirmation, selectivity of exact mass chromatograms and quantitation of target compounds.

METHOD

- Organic grape and 2 bird of prey samples methanol extracted.
- Dual API electro spray ion source for dedicated exact mass measurement used to detect and identify 17 target pesticides from crude grape extract and suspect poison in bird of prey liver samples.
- Generic MS tuning parameters used for all analyses.
- Target compounds quantified against matrix matched standards.

RESULTS

- All 17 target pesticides in crude grape extracts successfully separated by reverse phase HPLC, detected at lowest reporting level and quantified against matrix matched standards.
- Exact mass measurements better than 1.8mDa obtained.
- Selectivity of exact mass chromatograms improved analyte detection by removing matrix related peaks.
- Suspect poison in bird of prey liver extracts identified and confirmed as chloralose.

Introduction

Pesticide residue screening in foodstuffs, to meet statutory requirements, 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 describe a novel screening method for 17 pesticides (including metabolites) in fruit extracts using elevated resolution with exact mass measurement on an LC-orthogonal acceleration time-of-flight mass spectrometer (LC-*oa*TOFMS). To perform 'on-line' exact mass measurement of the chromatographic peaks as they eluted, the MS was equipped with a dual electro-spray ion source^(1,2). The source allows the introduction of a single point lock mass into the mass spectrometer via a dedicated reference spray which is kept separate from the analyte spray. The design and use of such an ion source overcomes practical problems associated with exact mass measurement of LC peaks using post-column addition of lock mass into eluent stream e.g. variation of reference signal intensity with LC gradients, mass interferences with analytes having same nominal mass and suppression of reference compound with high concentrations of analyte response.

Using suitable examples, we will show the applicability of *oa*-TOF MS to pesticide screening studies. Benefits include confirmation of target compounds and identification of unknown analytes by exact mass measurement using a single lock mass, improved selectivity of exact mass chromatograms relative to nominal mass data and low level detection (ppb) and quantification against matrix matched standards at 3 reporting levels (0.1, 0.05 & 0.02mg/kg).

Experimental Methods

The organic grape and two separate bird of prey liver samples (Red Kite and Buzzard) were solvent extracted using methanol to produce crude extracts. The samples were then subjected to exact mass LC-MS analysis using the following conditions:

LC Conditions

HPLC : Waters 2790
Column : Waters Symmetry C18, 2.1x100mm
Flow rate : 0.3mL/min
Solvent A : 10mM ammonium acetate
Solvent B : Methanol
Gradient : 0min=95%A
2min=75%A
8min=2%A
12min=2%A
13min=95%A and re-equilibrate
Injection Vol : 10µL

MS Conditions

MS : Micromass LCT with dual ion source
Ion mode : ESI+ve (for grape extracts)
ESI-ve (for bird of prey extracts)
Capillary : 3000V (ESI+ve)
2500V (ESI-ve)
Cone : 20V
Source temp : 120°C
Desolv. Temp : 350°C

Exact Mass Measurement

Lock Mass : Sulphadimethoxine (C₁₂H₁₄N₄O₄S) infused at 10µL/min
Acquisition time : 1 second
Inter-spray time : switching time every 10 seconds
Acquisition mode : Centroid

Results

Dual Electro spray Design and Operation

The dual electro spray ion source is mounted directly onto the Micromass LCT mass spectrometer (see Figure 1). The separate analyte and reference electro spray inlets are positioned around the sampling cone at 90° to each other (see Figure 2). To sample the sprays independently and sequentially, a baffle arrangement driven by an external stepper motor is used to admit ions from one spray at a time to the sampling cone region of the mass spectrometer. With both positions being indexed via optical sensors,

the data from both the analyte and reference spray are stored as separate functions within the same datafile. During an analytical experiment, the reference spray is sampled typically once every 5-10 seconds maximising the time spent sampling data from the analyte stream. Subsequent exact mass measurements are made on the analyte data by applying a calibration correction factor calculated from the reference data. Both sprayers are held at the same electro spray voltage and have an equal flow of nitrogen nebuliser gas. The analyte sprayer has a directed flow of heated desolvation gas but as the reference sprayer is typically operated at low flow rates (<20µL/min) additional desolvation gas is not required.

Dual Electro spray Ion Source Mounted onto LCT *oa*-TOF MS

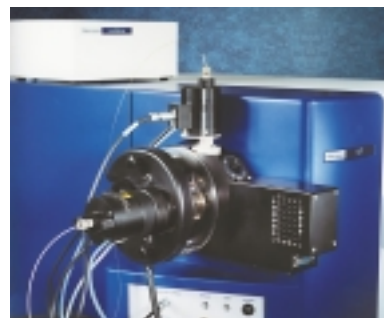


Figure 1

Dual Electro spray Schematic

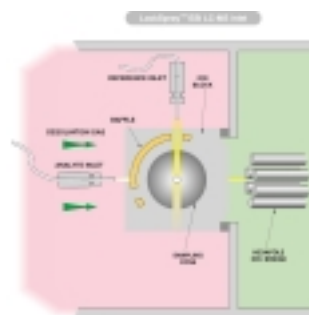


Figure 2

Grape Extract Results

Using the chromatographic conditions described, all 17 target pesticides in the grape extracts were separated within the 16 minute run time. Figure 3 shows a typical BPI chromatogram obtained from standard compounds dissolved in mobile phase solvent. As the actual grape samples were crude extracts subjected to minimal sample clean up (methanol extraction), some signal suppression was observed due to matrix. Figure 4 highlights the effect of matrix on MS signal and the complexity of determining the actual target compounds from matrix related material. Although some ion suppression was observed, all 17 pesticides were detected at the lowest level matrix matched standard and in the extracts.

Table 1 lists the 17 pesticides targeted in this experiment along with the exact mass measurements obtained using the dual electro spray ion source. All mass measurements were better than 1.8mDa allowing confirmation of the target compound.

Typical Chromatographic Separation

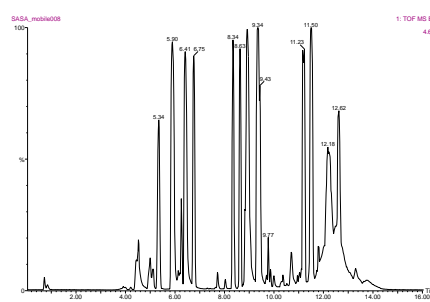


Figure 3

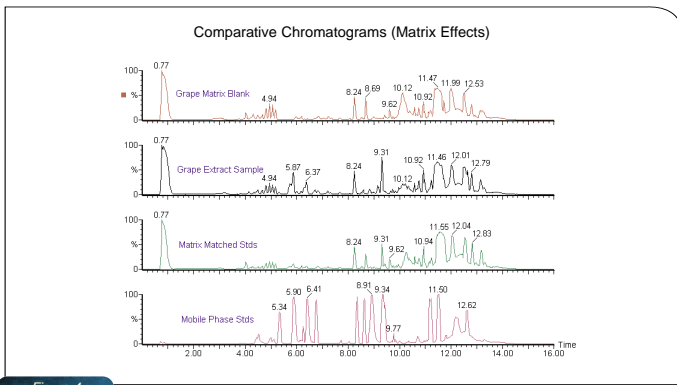


Figure 4

Pesticide	Formula	Theoretical M+H	Measured M+H	mDa Error
3-hydroxycarbofuran	C12H15NO4	238.1079	238.1095	1.6
Carbendazim	C9H9N3O2	192.0773	192.0758	1.5
Carbofuran	C12H15NO3	222.113	222.1116	1.4
Carbosulfan	C20H32N2O3S	381.2212	381.2210	0.2
Fenarimol	C17H12Cl2N2O	331.0405	331.0401	0.4
Fenbutatin-oxide	C60H78OSn2	517.2129	517.2126	0.3
Imazalil	C14H14Cl2N2O	297.0561	297.0566	0.5
Methiocarb	C11H15NO2S	226.0902	226.0910	0.8
Methiocarbsulfone	C11H15NO4S	258.08	258.0810	1.0
Methiocarbsulfoxide	C11H15NO3S	242.0851	242.0849	0.2
Methomyl	C5H10N2O2S	163.0541	163.0538	0.3
Myclobutanil	C15H17ClN4	289.122	289.1222	0.2
Penconazole	C13H15Cl2N3	284.0721	284.0735	1.4
Propiconazole	C15H17Cl2N3O2	342.0776	342.0794	1.8
Pyrimethanil	C12H13N3	200.1187	200.1174	1.3
Tebuconazole	C16H22ClN3O	308.1529	308.1541	1.2
Thiabendazole	C10H7N3S	202.0439	202.0426	1.3

Table 1 Exact Mass Measurement Results

As all samples analysed were 'on-line' exact mass measured, exact mass chromatograms could be plotted using a narrow window (0.05Da) around the mass of interest. Using carbosulfan as an example (M+H=381.2212), Figure 5 clearly demonstrates the improved selectivity of exact mass chromatograms over nominal mass chromatograms (1Da) by removing matrix material at the same nominal mass. This improved selectivity offers lower limits of determination with oa-TOF as signal to noise is often improved (Figure 6).

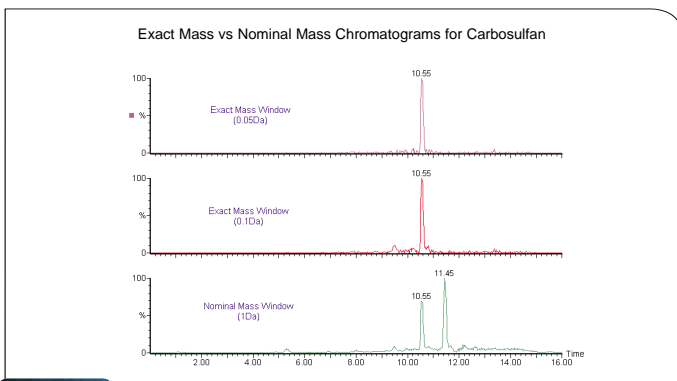


Figure 5

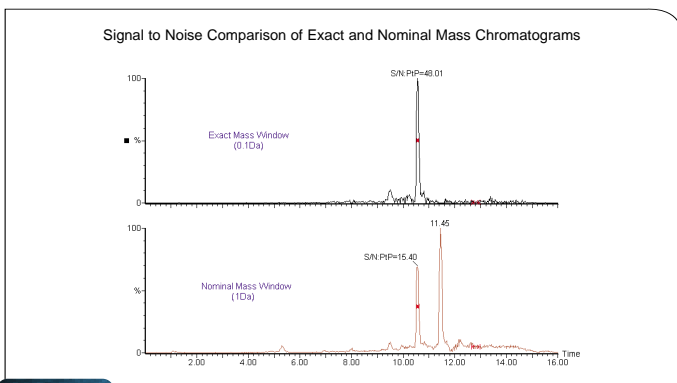


Figure 6

Using the matrix matched standards to generate calibration curves, the 17 target pesticides listed in Table 1 were quantified in 2 grape samples at the 3 different reporting levels (0.1, 0.05 & 0.02mg/kg). Linear calibration lines were applied with no fit weighting and excluding the origin. Using carbosulfan and fenarimol as examples, Figure 7a & b show the typical calibration graphs obtained.

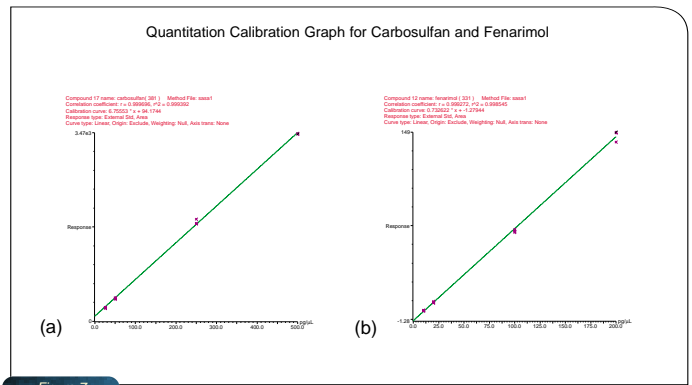


Figure 7

Bird of Prey Extract Results

The livers from a Red Kite and Buzzard were methanol extracted to produce a crude sample for LC-MS analysis. The two birds were suspected of being poisoned by an unknown compound. The use of oa-TOF with the exact mass measurement allowed the identification if the compound as chloralose (C₈H₁₁Cl₃O₆, M-H=306.9543 Da). Chloralose is a narcotic rodenticide used for poisoning avine pests. The exact mass measurements were:

Red Kite sample = 306.9555 (1.2 mDa, 3.9ppm)
 Buzzard sample = 306.9546 (0.3 mDa, 1ppm)

Figure 8 shows a exact mass measured spectrum of chloralose from the Buzzard sample along with generated elemental compositions.

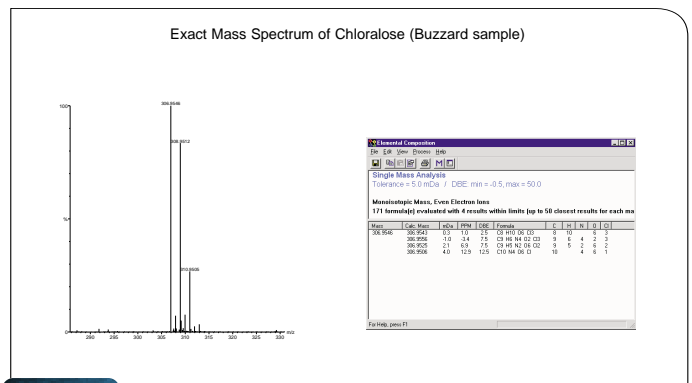


Figure 8

Conclusion

- LC-*oa*-TOF MS has been used to screen pesticide residues in environmental samples at low ppb concentrations.
- *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.
- Exact mass measurement also allows exact mass chromatograms (0.05Da) to be generated enhancing selectivity over nominal mass chromatograms (1Da) by removing matrix interferences.
- 17 target pesticides quantified at 3 reporting levels in organic grape extracts.
- Suspect poison in avine samples identified by exact mass measurement as chloralose.

References

1. Eckers, C.; Wolff, J.-C.; Haskins, N.J.; Sage, A.B.; Giles, K. and Bateman, R.; Analytical Chemistry, 2000, Vol 72 (16), 3683-3688.
2. Sage, A.B. and Giles, K.; Micromass Application Note AB24, June 2000.

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