Column Trapping Method for the Detection and Improved Confirmation of Pesticide Residues in Crude Extracts of Fruit and Vegetables Using LC-MS/MS Detection

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Introduction

The analytical requirement to identify and confirm residues in foodstuffs becomes increasingly challenging as maximum residue levels and consequential reporting limits are decreased, and also to some extent as the range of pesticides included in surveillance broadens. A multi-residue approach, using liquid chromatography with electrospray tandem mass spectrometry[1], has been successfully exploited at SASA for the rapid screening of many pesticides in crude extracts of fruit and vegetables. However, in less favourable cases, difficulties in achieving the relevant reporting limit and/or providing good quality data for confirmation of particular analytes may be experienced. The use of column switching as a trace enrichment technique to enhance or extend the capability of our LCMS approach has been investigated. The potential of the method is illustrated with data for the detection and confirmation of an incurred myclobutanil residue in apple, and for the screening of abamectin in cucumber extracts.

Experimental

Sample Preparation:

Extraction with ethyl acetate, solvent exchange to water/methanol, addition of internal standard (Carbendazim_D4 ring substituted), and filtration.

Liquid Chromatography:

System A:	Agilent 1100 Series
Trapping Column:	Phenomenex Prodigy, 3µ ODS(3), 30 x 4.6mm
Eluent:	H_2O:MeOH (98:2 v/v), 10mM ammonium acetate
Flow:	Isocratic, 0.5ml/min
System B:	Waters 600-MS
Analytical Column:	Phenomenex Hypersil, 3µ C18-BDS, 100 x 4.6mm
Eluent:	H_2O:MeOH (20:80 v/v), 10mM ammonium acetate
Flow:	Isocratic, 0.5ml/min
Switching Valve:	Rheodyne, 2-position, 6 port (MassLynx control)

Mass Spectrometry:

System:	Quattro Ultima (Micromass UK Ltd) with MassLynx V 4.0 data system
Acquisition:	Electrospray +ve/-ve ionisation, multiple reaction monitoring (MRM)
Collision Gas:	Argon

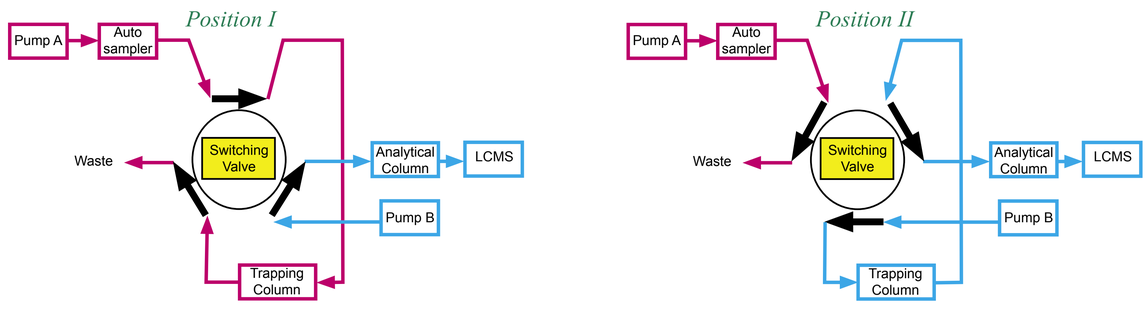


*Figure 1. Schematic of column trapping system. Position I - **Sample loading**, Position II - **Backflushing and analysis**.*

Trapping Method:

Analytes were retained and concentrated on a trapping column using a non-eluting mobile phase while the sample matrix passed to waste. After the concentration step, the switching valve was actuated (1 min after final injection) and analytes backflushed onto an analytical column with an eluting mobile phase. Sample volumes exceeding the capacity of the autosampler (100µl) were loaded using sequential injections.

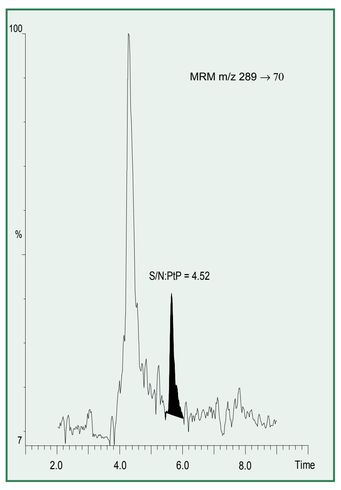
Results and Discussion

Myclobutanil

After optimisation of chromatographic parameters, tests with reference compounds demonstrated that sequential injections could be loaded with no degradation in chromatographic performance. Subsequent tests, with matrix-matched standards, confirmed that a linear increase in response was obtained over an extended sample volume range (25 - 500µl). Calibration curves derived for 500µl loadings of matrix-matched standards were linear over a range 0.00005 to 0.0125 µg/ml. The limit of determination (S/N =3) of approximately 0.0001mg/kg achievable for myclobutanil in apple is illustrated by analysis of a matrix-matched standard (Fig. 2). A marked improvement in the signal to noise ratio (S/N) was obtained when column trapping was utilised, and it was possible to use additional structurally significant MRM transitions to confirm an incurred residue in apple puree (Fig. 3). Data obtained for this residue using the column trapping method compare well with those generated without trace enrichment (Table 1). This incurred residue was near the lower limit of the original LCMS method; analysis of residues below this level would necessitate use of the enhanced method.

	Original LCMS Method *		Column Trapping Method **			
MRM	m/z 289 - 70	m/z 291 - 70	m/z 289 - 70	m/z 291 - 70	m/z 289 - 125	m/z 291 - 127
Extract 1	0.0080	0.0076	0.0065	0.0065	0.0064	0.0065
Extract 2	0.0083	0.0078	0.0074	0.0076	0.0080	0.0076

Table 1. Data for a myclobutanil residue (mg/kg) in apple puree generated with, and without, column trapping. (10 µl injection, ** 500 µl sample loading)*



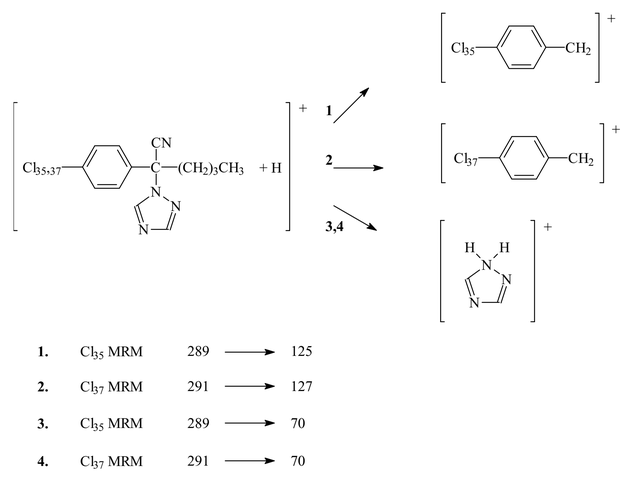


Figure 2. Myclobutanil in a 0.00005 µg/ml matrix-matched standard (500µl loading).

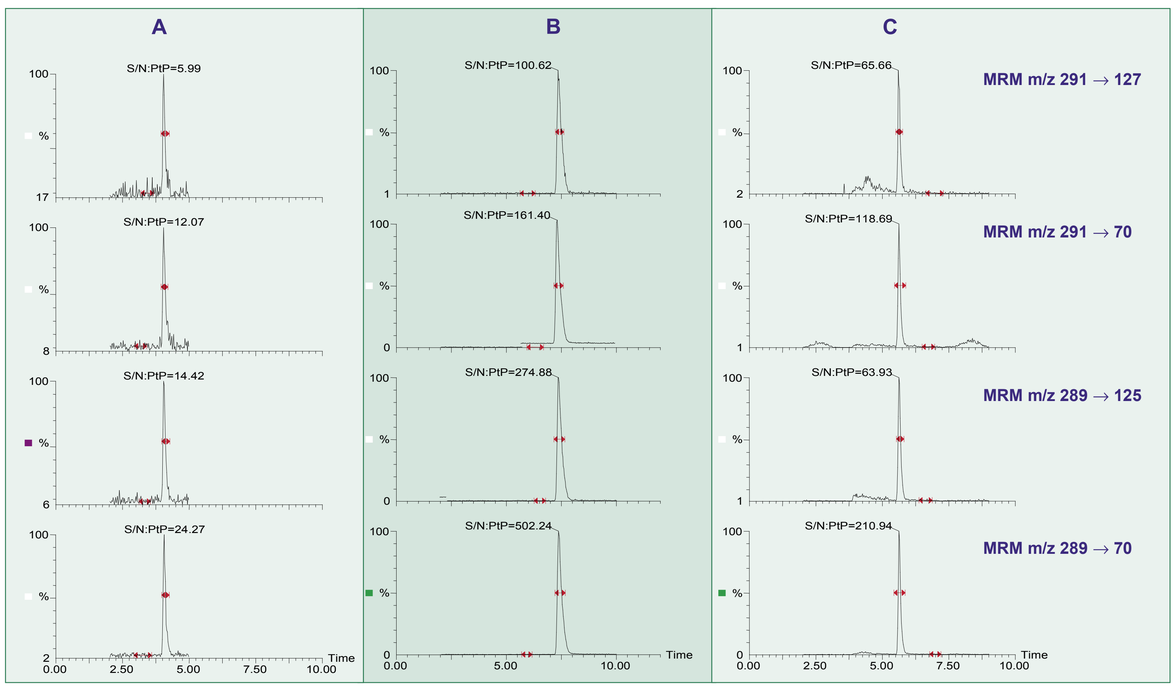
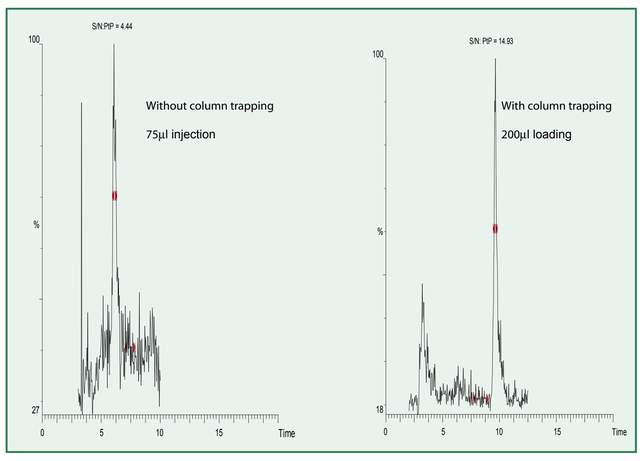
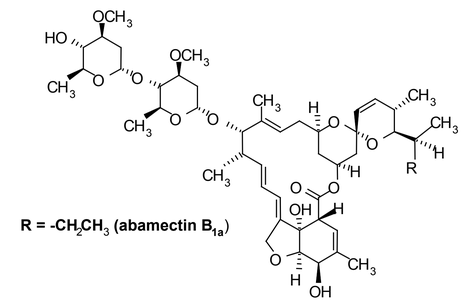


Figure 3. Signal/noise ratios, (A) 10 µl injection of myclobutanil (0.0125 µg/ml) in apple matrix without column trapping; (B) 500 µl loading of myclobutanil (0.0125 µg/ml) in apple matrix with column trapping; (C) 500 µl loading of apple puree extract (≡ 0.5g/ml) with column trapping, residue ~ 0.007 mg/kg.

Abamectin

A reporting limit (RL) of 0.01mg/kg had been applied to abamectin in recent surveillance of cucumbers. Ideally the analytical method should be capable of achieving at least half this concentration, suggesting a LOD of 0.0025 µg/ml (≡ 0.5RL). The routine LOD obtained, for this less favourable analyte, using the original LCMS method approaches that value. Column trapping with a sample loading of 200µl successfully enhanced the quality of screening data (Fig. 4).





ES –ve
MRM m/z 931.6 → 229.2

Figure 4. Abamectin (0.0025µg/ml) in cucumber matrix.

Conclusions

- Enhanced sensitivity attainable.
- No degradation in chromatographic performance or sensitivity with sample amounts equivalent to 0.25g of crude extract.
- Volume range of autosampler can be extended by multiple injection.
- Calibration curves linear (0.0001 - 0.025 mg/kg).
- Use of less intense MRM transitions realised for confirmation purposes.
- Screening data improved for less favourable analytes.

Reference: 1. Taylor M.J., Hunter K., Hunter K.B., Lindsay D., Le Bouhellec S., Journal of Chrom A, 98 (2002) 225-236