Waters™

應用手冊

UPLC oa-Tof MS for Rapid Screening of Multiple Pesticide Residues

Daniel McMillan, María Ibañez Martinez

Waters Corporation, Research Institute for Pesticides and Water (IUPA), University Jaume I

Abstract

This application note demonstrates the applicability of Time of Flight (Tof) MS analysis for pesticide screening

and the potential advantages of such a technique in a controlled laboratory environment.

Introduction

Pesticides Analysis Background

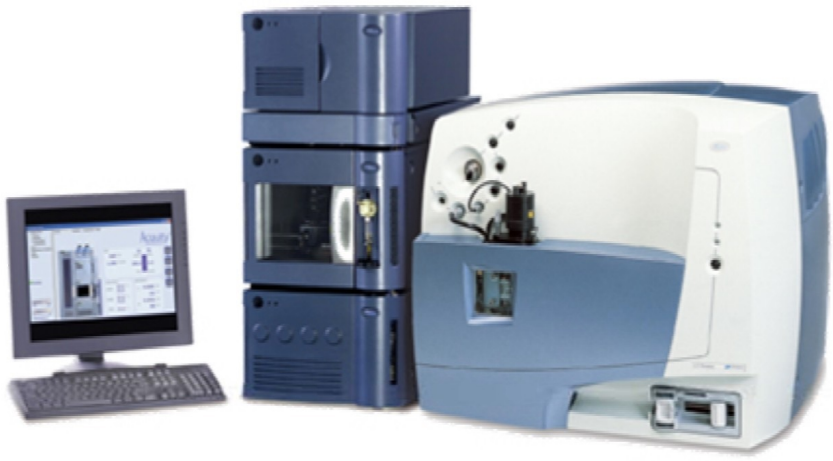
New pesticides are continually being developed and introduced for use in agricultural production. Recent advances in analytical techniques, as well as changes in the types of pesticide compounds and the way in which they are used, have lead to a requirement for a rapid and sensitive, yet generic, pesticide residue screening method.

Traditionally, GC and LC coupled to either single quadrupole MS, or other detectors, have been the most commonly employed analytical techniques. However, due to their relatively low sensitivity and selectivity, they have required large injection volumes or more rigorous sample preparation to reach the required limits of detection. More recently, tandem quadrupole MS/MS analyzers have been used to attain low detection levels and increased selectivity. The superior selectivity is provided by multiple reaction monitoring (MRM) experiments where complex matrices may be analyzed without extensive clean-up. These advantages have made MS/MS the method of choice for low level quantitation and confirmation for a large number of targeted compounds.

Setting up MRM methods for the selected analytes is time consuming and the analysis is inherently targeted towards a limited number of compounds. This has led analysts to consider what other potentially harmful, non-targeted analytes may be in the samples and has resulted in the demand for an analytical method that is sensitive and selective, but not specific.

The Waters Micromass LCT Premier, a time of flight (Tof) mass spectrometer, provides a solution for this dilemma. The LCT Premier Mass Spectrometer couples very high, full-spectral sensitivity with high resolution mass spectra allowing any ionizable component in a sample to be exact mass-measured and its elemental composition calculated or confirmed to <3 ppm.

The Waters ACQUITY UPLC is a novel UltraPerformance Liquid Chromatography System utilizing 1.7 µm stationary phase particles in a high pressure system. This provides a fast, high resolution separation which increases LC-MS sensitivity and mitigates matrix interference arising from minimal sample preparation.



Waters ACQUITY UPLC with Waters Micromass LCT Premier Mass Spectrometer.

Outline of Work

The focus of the experiments presented here is to demonstrate the applicability of Time of Flight (Tof) MS analysis for pesticide screening and the potential advantages of such a technique in a controlled laboratory environment.

Drinking water has recently become the subject of much discussion due to homeland security concerns, where the analysis of unexpected contaminants is a particularly high priority. Although water is a relatively simple medium, the techniques used in such an analysis are applicable to a wide range of different matrices. In order to illustrate this, quantitative results from tomato extracts are also presented. Data generated by the UPLC-MS system is processed with Waters ChromaLynx Application Manager software which de-convolutes the chromatograms and displays the mass-measured spectra from each peak. The spectra can be compared against a library of target analytes or used to help determine the identity of an unknown compound.

Experimental

Methods

Extraction Procedure – *developed by Jeannette Klein and Lutz Alder at FIRA, Berlin.*

- 10 g sample weighed. 5 g for dry sample materials.

- Water is added to obtain 10 mL as a sum of natural and added water.

- After addition of 20 mL methanol the sample is blended for 2 min.

- 6 mL of extract is mixed with 2 mL of NaCl solution.

- 5 mL is transferred to a ChemElut Column.

- The column is eluted with 16 ml of dichloromethane and evaporated.

- The dry residue is dissolved in 250 µL methanol and 1000 µL water.

- Extract is filtered through 0.45 µm syringe filter.

- Matrix equivalent of 1 g/mL for normal produce or 0.5 g/mL for dry produce.

LC Conditions

LC system:	Waters ACQUITY UPLC
Mobile phase A:	5% aqueous MeOH + 2 mM $CH_3CO_2NH_4$
Mobile phase B:	95% aqueous MeOH + 2 mM $CH_3CO_2NH_4$
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm
Flow rate:	0.45 mL/min
Injection vol.:	20 µL
Column temp:	40 °C
Gradient:	t = 0 min 0% B t = 8.5 min 100% B t = 11 min 100% B t = 11.1 min 0% B t = 13.5 min 0% B

MS Conditions

Instrument:	Waters Micromass LCT Premier
Ion mode:	Electrospray +/-
Capillary voltage:	1000 V
Source temp:	120 °C
Desolvation temp:	400 °C
Gas flow:	600 L/hr
Mass range:	50–1000 Da
Acq. time:	0.25 s/function
Calibration:	$NaCH_2O_2$ in pos. and neg. modes
LockSpray:	Leucine Enkephalin
Reference:	[M+H]+ = 556.2771 Da [M-H]- = 554.2615 Da

Results and Discussion

Figure 1 shows the base peak intensity chromatograms from the analysis of drinking water spiked with 92 pesticide residues at a concentration of 100 ppb. Six of the components ionize exclusively in negative mode, and both traces are shown to illustrate the simultaneous acquisition of all spiked pesticides in positive and negative mode. The chromatographic peaks here are typically ~5 s wide at base.

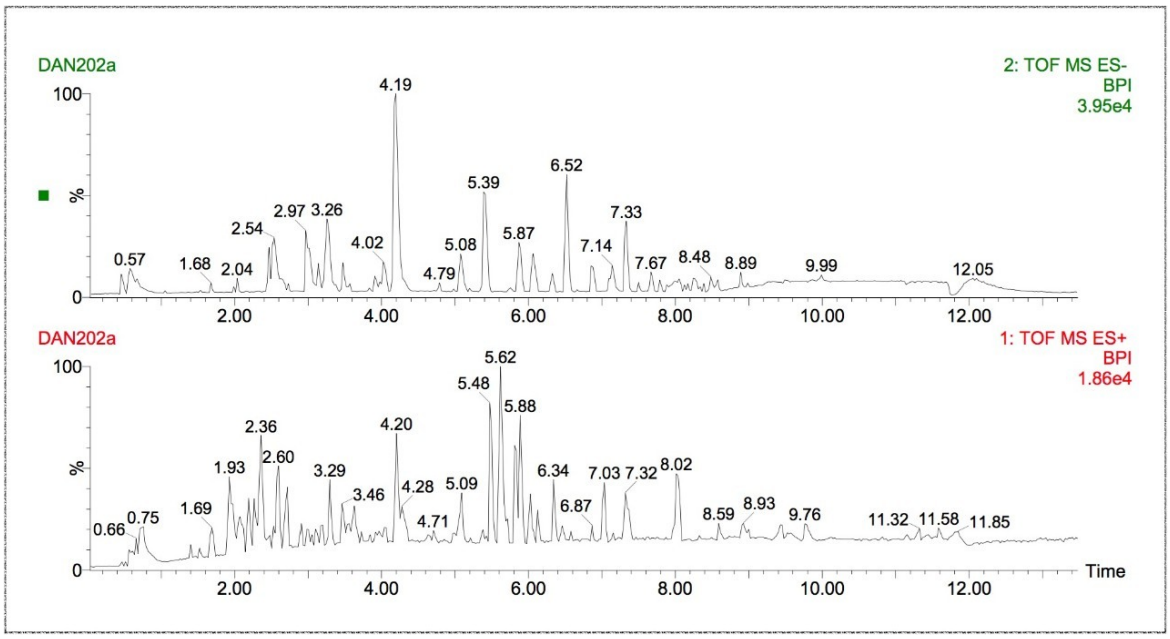


Figure 1. Positive and negative ion BPI chromatograms of drinking water spiked at 100 ppb.

Figure 2 shows a typical combined spectrum of Diuron, together with its elemental composition calculation. It is clear that there are a number of possibilities within 5 ppm of the measured mass, so the observed isotope pattern is compared to a theoretical model using i-FIT Software. In this case, although not the closest match by exact mass (ΔM =1.3 ppm) the correct formula is displayed as the highest rank, since its isotope pattern is the closest match.

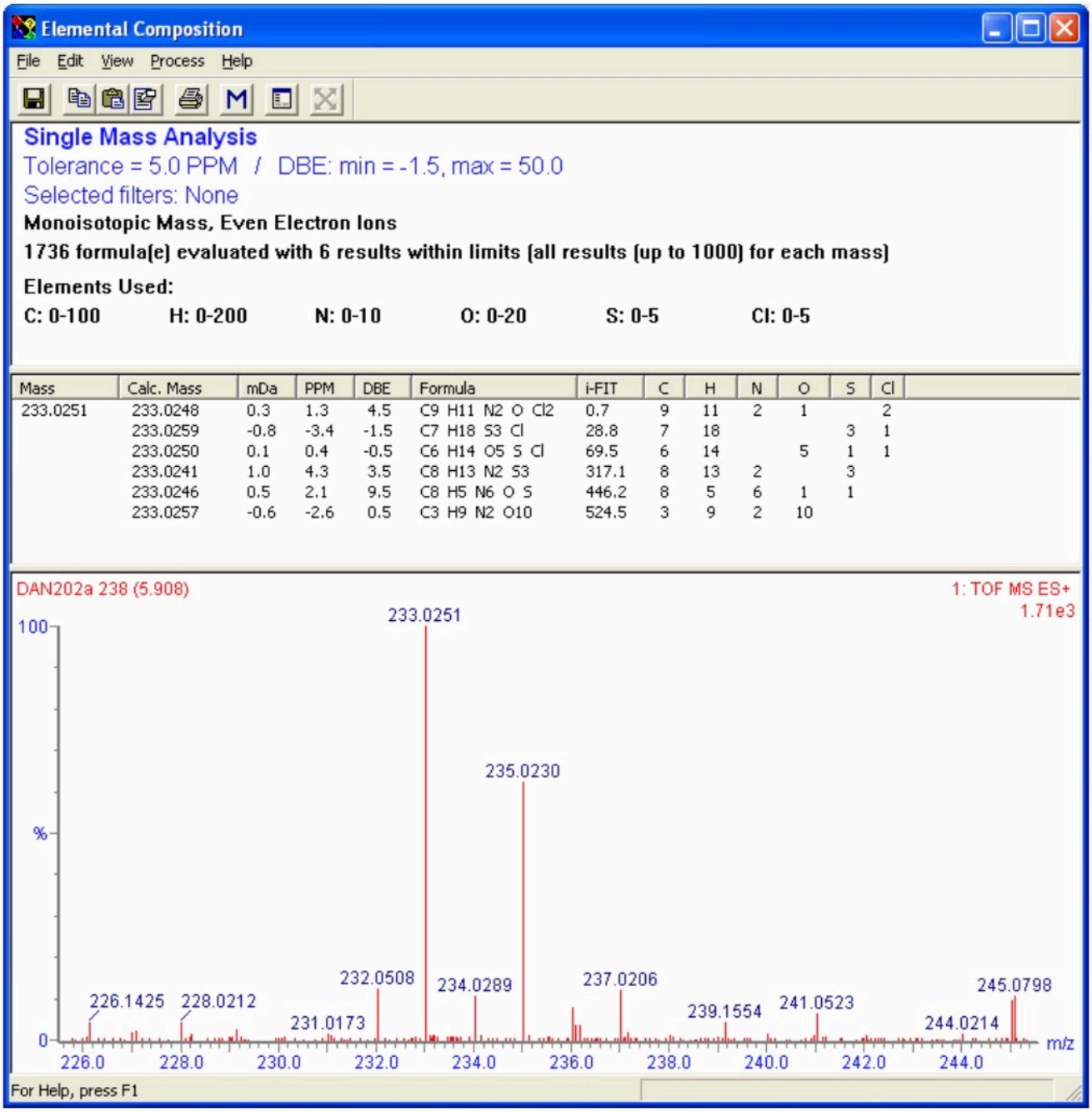


Figure 2. Spectrum and EleComp report for Diuron, ranked by closest isotope ratio fit.

When targeting a specific compound, it is a simple operation to plot an extracted ion chromatogram (XIC) of its exact mass. Because of the high mass accuracy of the Tof data, the selectivity of this technique is greatly enhanced and good signal-to-noise ratios are obtained, as illustrated in Figure 3. However, if multiple residues are to be investigated, it becomes time consuming to target them all individually. In this case, it is useful to process data using the powerful chromatographic deconvolution software provided by the ChromaLynx Application Manager. ChromaLynx automatically plots the XICs of up to the eight most intense ions

at any point in the chromatogram, and if a peak is found to satisfy user-defined parameters, displays its deconvoluted exact mass spectrum. This can then be analyzed to elucidate an elemental composition, or compared against a library of spectra obtained from standards. Each library entry will include mass, formula, retention time and polarity/cone voltage information, all of which can be used to filter the 'hit list' and effectively minimize the occurrence of false positive results. In Figure 4, the ChromaLynx browser indicates the presence of various pesticides in the spiked drinking water sample. Peaks found to match with a high degree of confidence to the library entry are highlighted green, tentative matches in yellow and low 'fits' in red.

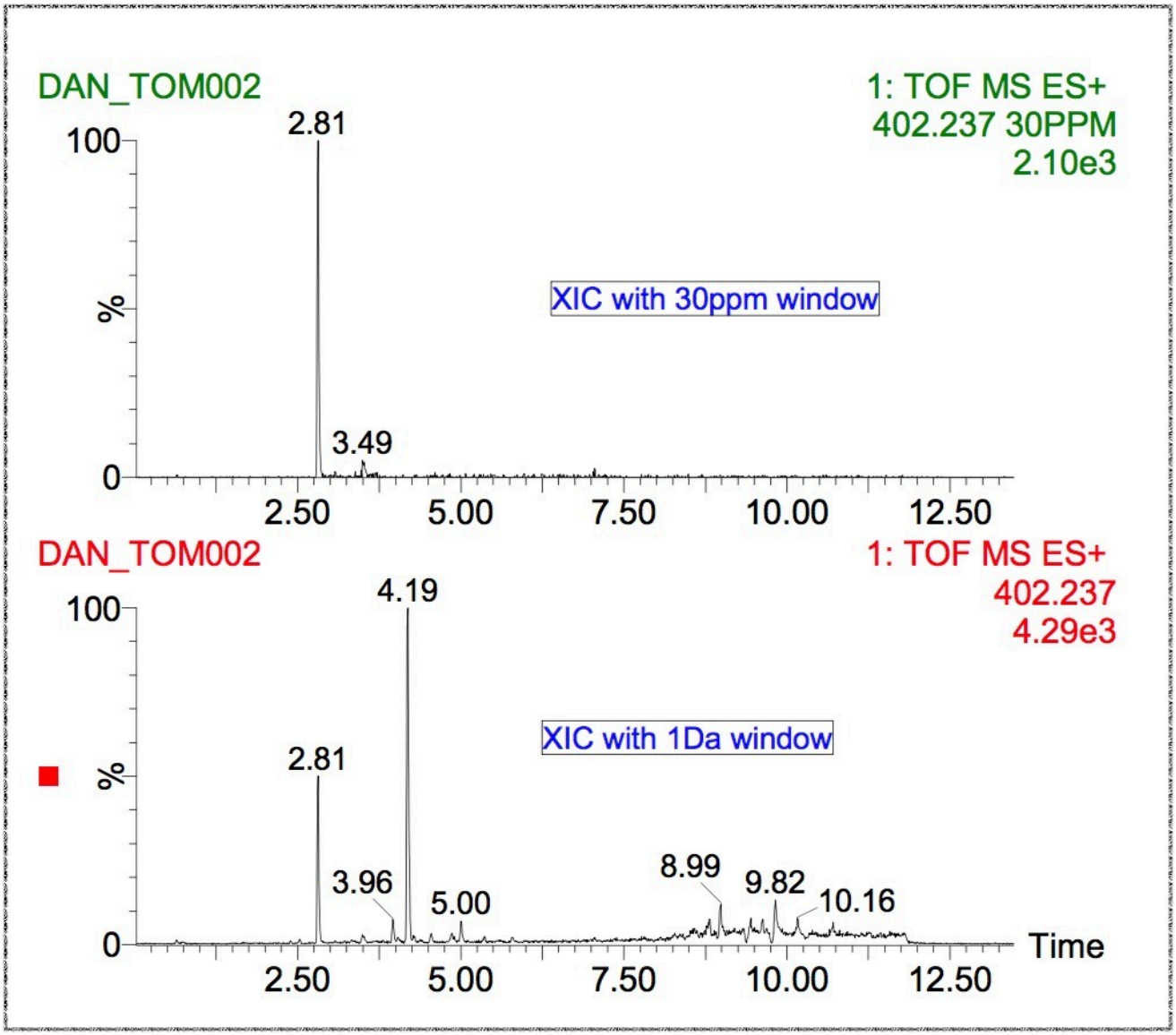


Figure 3. Comparison of different mass extraction windows.

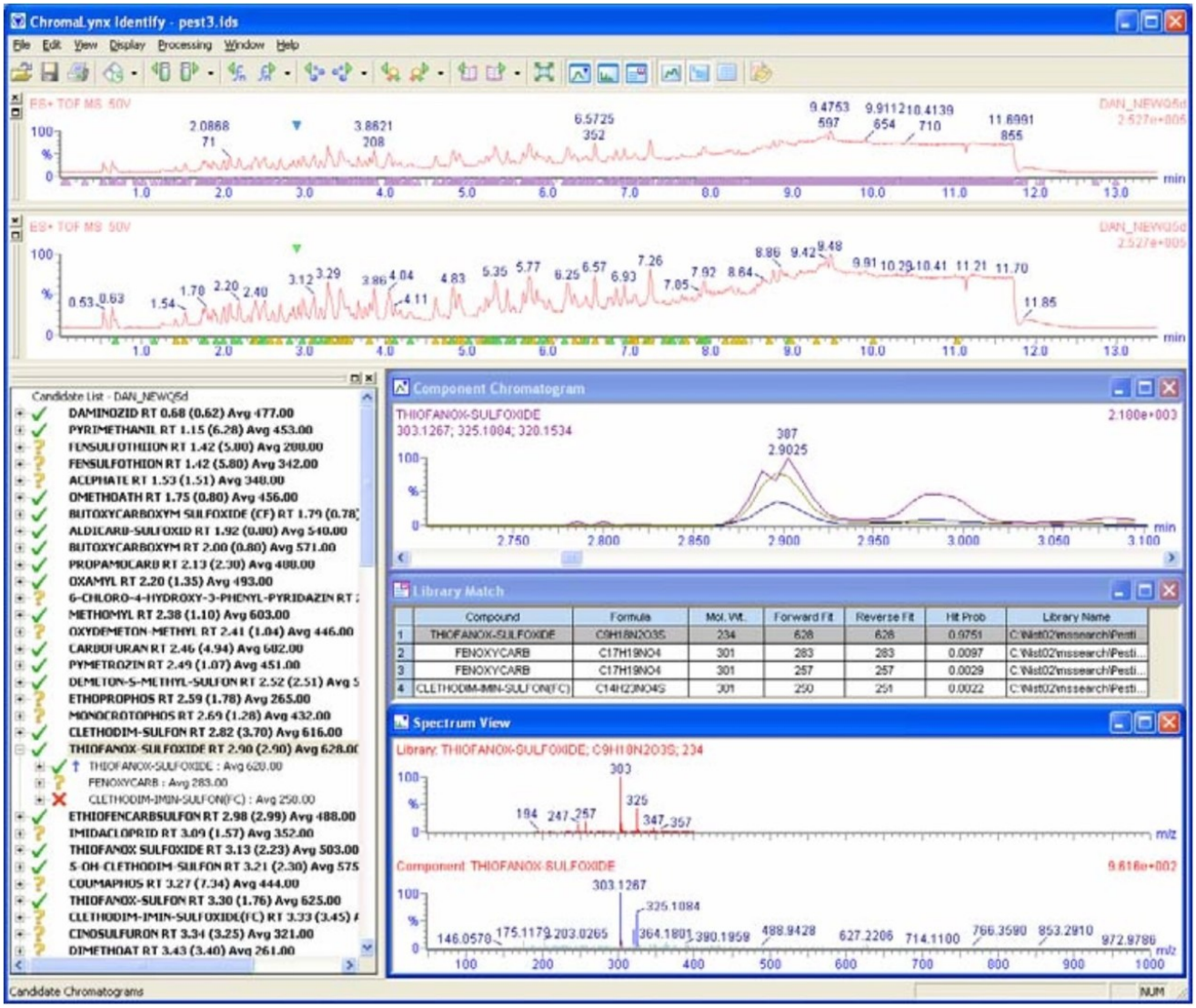


Figure 4. ChromaLynx browser window showing screening results.

Traditional Tof MS instruments suffer from detector saturation at relatively low concentration levels, thus limiting the quantitative capability of an analysis. The LCT Premier utilizes novel ion optics to provide the Dynamic Range Enhancement (DRE) function which enables precise and easy quantification comparable to that of more conventional mass analyzers. Figure 5 shows an example of the quantification of loxynil performed in positive/negative switching mode with DRE.

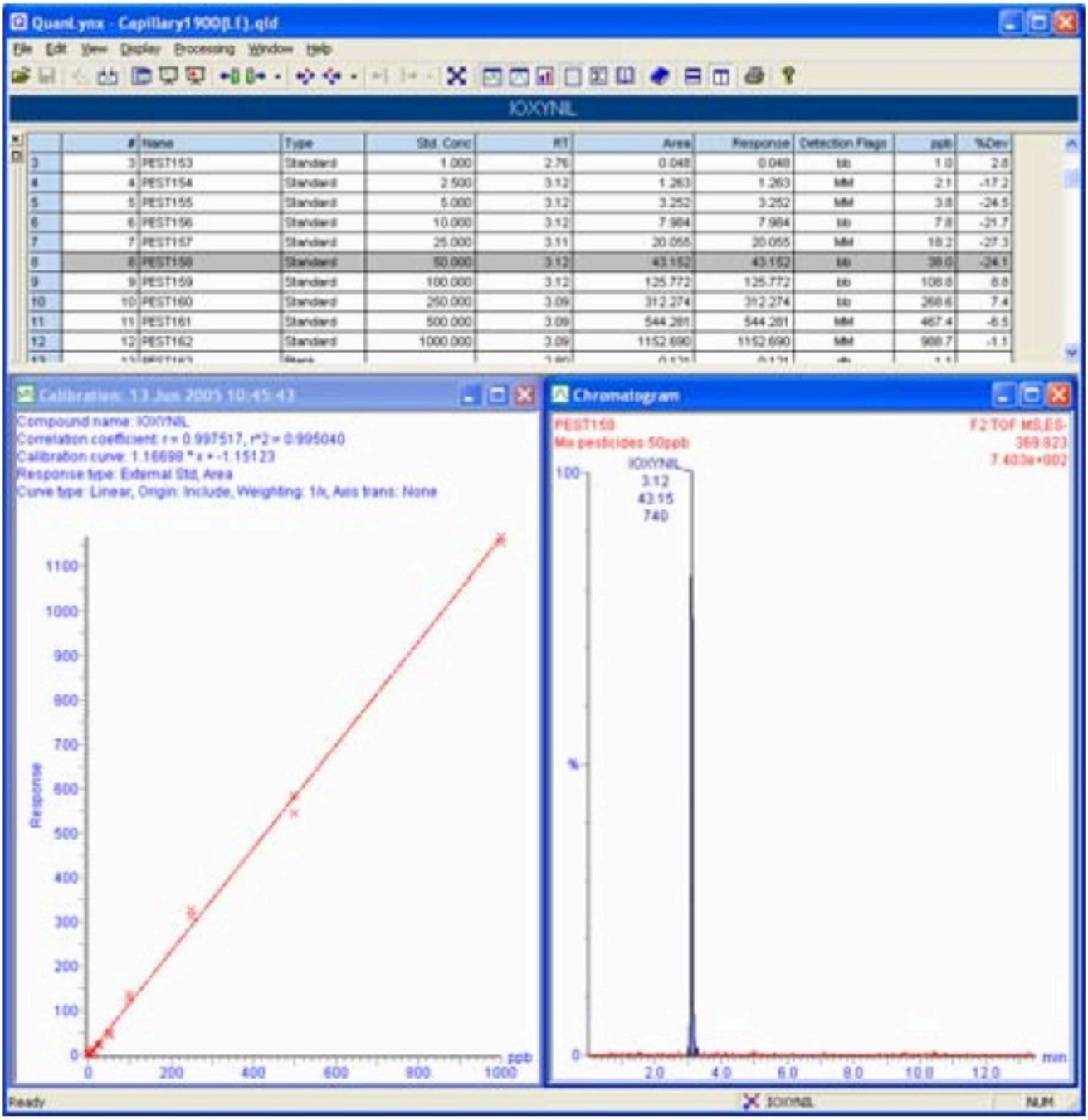
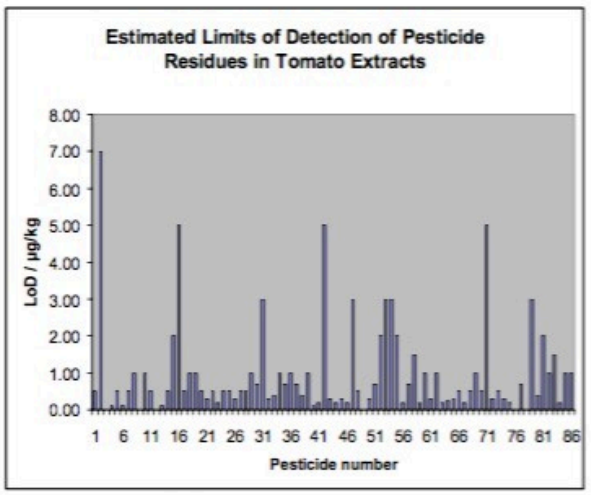


Figure 5. Quantification of Ioxynil in +/- switching mode with Dynamic Range Enhancement.

In order to verify the method on samples in a more complex matrix, organic tomatoes were purchased locally, spiked at various concentrations around the typical Maximum Residue Level (MRL) and extracted as outlined in the procedure above. Limits of Detection (LoDs) as shown in Figure 6 were estimated by extrapolating the calibration curve to the concentration which would give a signal-to-noise ratio of 3:1. It should be noted that the lowest MRL currently standing for tomatoes is 20 µg/kg in the cases of acephate, daminozide, oxydemeton-methyl, dimethoate and prosulfuron. All others, where set, are 50 µg/kg or higher. Figure 7 overleaf shows an

example of the quantification of Triasulfuron in tomato extracts.



Number	Compound	Number	Compound
1	Acephate	44	Metolachlor
2	Aldicarb	45	Pymetrozin
3	Amidosulfuron	46	Pyrimethanil
4	Azoxystrobin	47	Thiofanox
5	Bendiocarb	48	Thiofanox-sulfoxide
6	butoxycarboxym	49	Butocarboxim
7	Carbaril	50	Clethodim-imin-sulfoxide
8	Carbendazim	51	Ethiofencarb
9	pyridazin	52	Fenhexamid
10	Chlorsulfuron	53	Imidacloprid
11	Clethodim	54	Triflumuron
12	Daminozide	55	Isoproturon
13	Fenoxycarb	56	Metamitron
14	Fenpropimorph	57	Methamidophos
15	Flufenoxuron	58	Methiocarb
16	Pyridate	59	Metsulfuron-methyl
17	Aldoxicarb	60	monocrotophos
18	Atrazine	61	Oxydemeton-methyl
19	Butoxycarboxim-sulfoxid	62	Quizalofop-ethyl
20	Carbofuran	63	Tebuconazole
21	Cinosulfuron	64	Thiacloprid
22	Clethodim-sulfon	65	Trisulfuron-methyl
23	Demethon-S-methyl-sulfon	66	Fluazifop-P-butyl
24	Desmedipham	67	Omethoate
25	Ethiofencarbsulfon	68	Pirimicarb
26	Imazalil	69	Propamocarb
27	Linuron	70	Quinmerac
28	Oxamyl	71	Tebufenozide
29	Aldicarb-sulfoxid	72	Thifensulfuron-methyl
30	Bensulfuron-methyl	73	Thiofanox-sulfon
31	Carbofuran-3-OH	74	Thiophanate-methyl
32	Clethodim-sulfoxid	75	triasulfuron
33	Diuron	76	Dimethoat
34	Furathiocarb	77	Nicosulfuron
35	Methomyl	78	Thiodicarb
36	Promecarb	79	3,4,5-trimethacarb
37	propoxur	80	5-OH-clethodim-sulfon
38	Thiabendazol	81	Haloxyfop-ethoxyethyl
39	diflubenzuron	82	Flazasulfuron
40	Clethodim-imin-sulfon	83	Prosulfuron
41	Cyprodinil	84	Vamidothion
42	Haloxyfop-methyl	85	Isoxaflutole
43	Metalaxyl	86	Spiroxamine

Figure 6. Graph of estimated LoDs and list of pesticides analyzed in tomato extracts.

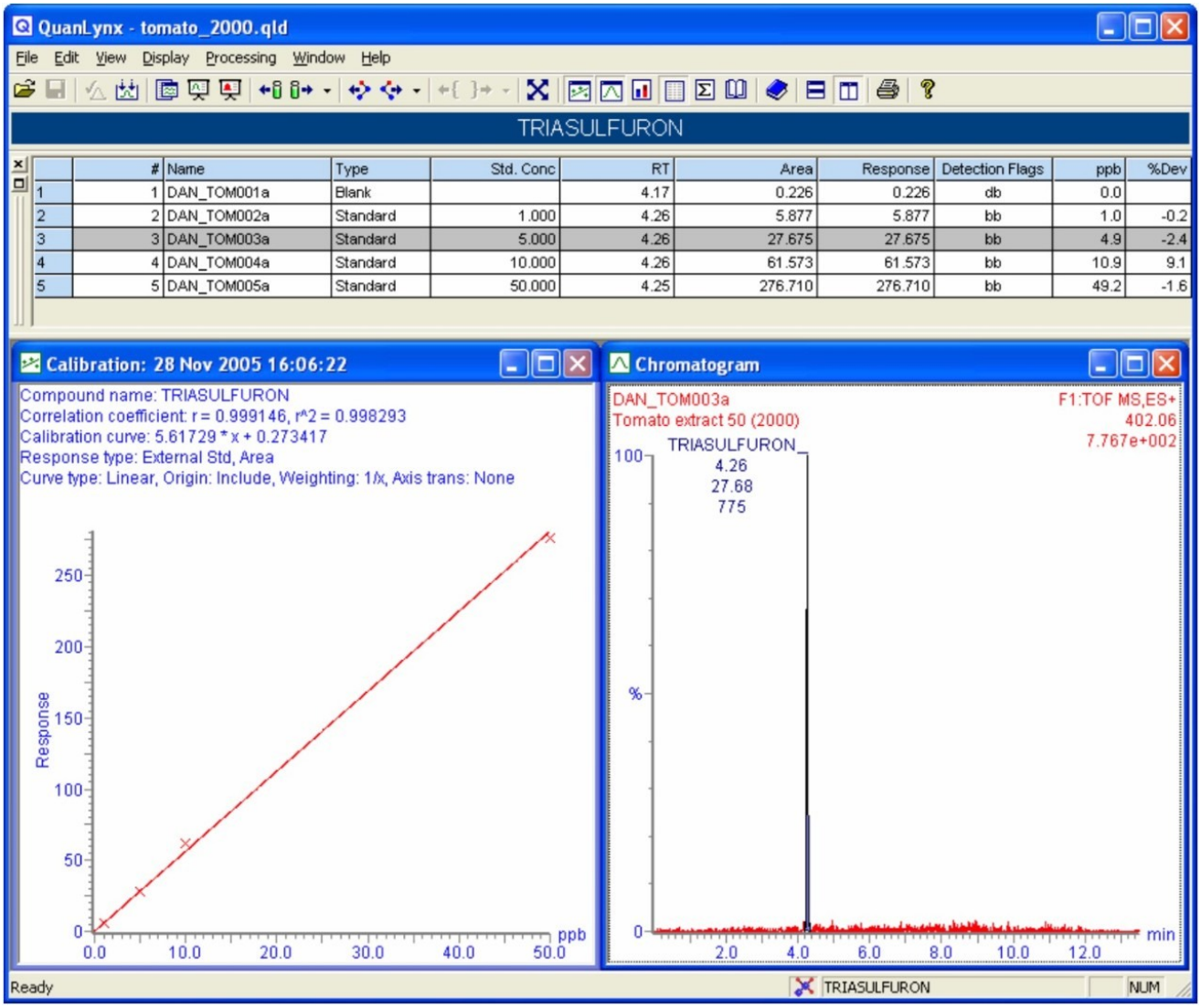


Figure 7. Quantification of Triasulfuron in tomato extracts. The MRL for this compound in tomatoes is 50 µg/kg.

Conclusion

- The method is shown to provide a rapid and sensitive automated screening analysis that is applicable to both simple and complex matrices.
- The ACQUITY UPLC System provides a fast chromatographic run with good resolution so as to minimize interference from co-eluting peaks.
- High mass-accuracy MS spectra provided by the LCT Premier Mass Spectrometer allows confirmation of targeted compounds and helps identify unknowns.

- ChromaLynx Application Manager Software performs automated de-convolution of complex chromatographic data to provide simplified results.
- Further work should extend the library to contain as many contaminants as possible, and investigate the use of exact-mass fragments formed by in-source CID for confirmation purposes.

Featured Products

- ACQUITY UPLC System <https://www.waters.com/514207>

720001437, January 2006