Waters™

Nota applicativa

Application of Elevated Resolution GC-Tof-MS for the Multi-Residue Analysis of Pesticides in Food

Peter Hancock

Waters Corporation



Abstract

This application note demonstrates a method for the targeted screening of pesticide residues in food commodities using the GCT Premier with TargetLynx.

Introduction

The inappropriate or unlawful use of pesticides on agricultural produce can result in unacceptably high levels of their residues in produce destined for human consumption. Food produce that is to be used for this purpose must contain less than the statutory maximum residue limit (MRL) of any given residue.

In the European Union, legislation has recently been established for setting and controlling MRLs in food and feeding stuffs.[1] One key feature of the new legislation is that a default MRL of 0.01 mg/kg will apply to those commodities when no specific MRL is set, unless a different default level is agreed, or until such time as an MRL is set on the basis of data evaluation. The Commission has also announced a recommendation concerning a co-ordinated monitoring programme to ensure compliance with maximum levels of pesticide residues in and on cereals and certain other products of plant origin.[2]

Given that there are over 800 compounds in use to control pests, it is often advantageous to extract and determine as many of them as possible during a single analysis. An extraction, with acetonitrile, followed by dispersive solid phase extraction (SPE) clean up was reported for the analysis of a wide range of pesticides in fruits and vegetables[3] and fatty samples.[4]

Selected ion recording (SIR) or multiple reaction monitoring (MRM) allows for the targeted screening of a finite number of compounds to be achieved. However, much of the chemical information is discarded so full-spectrum techniques are still required in so-called “open” or untargeted screening environments.

To establish a suitable untargeted screening technique there are a number of requirements that would need to be met to extract, detect, locate and identify all components. These include: minimal non-selective sample preparation for a wide range of compound groups with different polarities; simple high resolution GC separation to minimize matrix interference while maintaining resolution of critical pairs; and automated peak detection and deconvolution of all components in the sample.

Exact mass time of flight mass spectrometry (Tof-MS) is a full spectrum technique capable of both the

targeted and the untargeted screening approaches.

A method will be introduced for the targeted screening of pesticide residues in various food commodities down to the legislated concentration of 0.01 mg/kg. The method will also be extended to include untargeted screening results using automatic peak detection, deconvolution and library searching with exact mass confirmation.



Waters GCT Premier Tof-MS System.

Experimental

Methods

Extraction Method

10 g frozen sample was weighed in a centrifuge tube. Acetonitrile (10 mL) was added along with 100 µL of internal standard solution (PCB 138 and TPP) and the tube was shaken for 1 min. $MgSO_4$ (4 g) and NaCl (1g) were added and the solution buffered to pH 5.0–5.5 with citrate buffer. After shaking and centrifugation, an aliquot was transferred to a vial containing PSA sorbent and anhydrous $MgSO_4$. After undergoing further shaking and centrifugation, the extract was acidified to pH 5 to protect base-sensitive residues. The extract was analyzed by GC-Tof-MS.

GC Method

Agilent 6890N GC with 7683B autosampler

Column:	J & W Scientific DB-5MS 20 m x 0.18 mm i.d., 0.18 µm
Flow rate:	1.0 mL/min helium constant flow
Temp. ramp:	40 °C (Hold 2 min) 220 °C @ 30 °C/min 260 °C @ 5 °C/min 280 °C @ 20 °C/min (hold 8 min)
Total run time:	25 min
Injection method:	Cyro cooled PTV in solvent vent mode, 1 µL injected
Vent method:	Vent pressure 5 kPa, Vent flow 20 mL/min for 0.5 min

GC-Tof-MS Method

The Waters GCT Premier orthogonal acceleration time of flight (oa-Tof) mass spectrometer was used in electron ionization (EI+) mode. The ion source was operated at 200 °C with an electron energy of 70eV and a trap current of 200 µA. The temperature of the transfer line was held at 260 °C during the run. Spectra were acquired between 50 and 500 Da in a time of 0.09 s and a delay of 0.01 s (10 spectra/s). Exact mass spectra were obtained using a single-point lock mass (chloropentafluorobenzene, *m/z* = 201.9609) infused into the ion source continuously during the run.

The GCT Premier was tuned so that the resolution was greater than 7000 full width half maximum (FWHM). The pesticide residues analyzed along with their exact masses are listed in Table 1 (first exact mass was used for screening, the second can be used for confirmation, if required, but not in this instance).

	RT	Exact Masses
PCB 138, Int. Std.	14.58	359.8415, 361.8385
TPP, Int. Std.	14.93	326.0708, 325.0630
Biphenyl	7.74	154.0783, 153.0704
OPP	8.35	170.0732, 169.0653
Diphenylamine	8.84	169.0891, 168.0813
Dichloran	9.40	175.9670, 205.9650
Pyrimethanil	9.71	198.1031, 199.1066
Etrimfos	9.75	292.0647, 181.0977
Pirimicarb	9.87	166.0980, 238.1430
Vinclozolin	10.26	212.0034, 284.9959
Metalaxyl	10.39	249.1346, 206.1181
Pirimiphos-methyl	10.53	290.0728, 305.0963
Malathion	10.69	173.0805, 124.9826
Diethofencarb	10.77	267.1471, 225.1001
Chlorpyrifos	10.86	313.9574, 196.9202
Fipronil	11.35	350.9515, 366.9435
Cyprodinil	11.45	224.1188, 225.1266
Procymidon	11.78	283.0167, 285.0137
Mepanipyrim	12.26	222.1050, 221.0953
Fludioxonil	12.49	248.0937, 182.0480
Profenofos	12.60	338.9642, 336.9663
Myclobutanil	12.75	179.0376, 181.0347
Kresoxim-methyl	12.73	206.0817, 116.0473
Buprofezin	12.81	172.1034, 175.0871
Quinoxyfen	14.40	237.0618, 272.0314
Iprodion	15.45	314.0099, 316.0070
Tebufenpyrad	16.06	318.1373, 333.1608
Pyriproxyfen	16.80	136.0762, 226.0994
Fenarimol	17.32	251.0030, 330.0327
Pyridaben	18.24	147.1174, 364.1376
Quizalofop-ethyl	19.61	372.0877, 299.0587
Azoxystrobin	23.26	344.1035, 388.0933

Table 1. Exact mass GC-Tof-MS method parameters.

Acquisition and Processing Methods

The data were acquired using Waters MassLynx Software and processed using either the TargetLynx or

ChromaLynx Application Managers.

Results and Discussion

Targeted Screening Results

Four food commodities were screened: cucumber, sweet pepper, grapefruit and wheat flour. 0.01 mg/kg was chosen to be the reporting level as this is the target MRL for active substances in products for which no specific MRL, is set.[2]

To illustrate the improvement in selectivity offered by exact mass chromatograms, vinclozolin (0.01 mg/kg) was analyzed in cucumber, a relatively simple matrix. The nominal mass chromatogram (1 Da, *m/z* 212), illustrated in Figure 1, contains a number of intense peaks which could lead to interference when using automatic integration. In the exact mass chromatogram (20 mDa, *m/z* 212.0034) vinclozolin has little or no interference, improving the selectivity of the method.

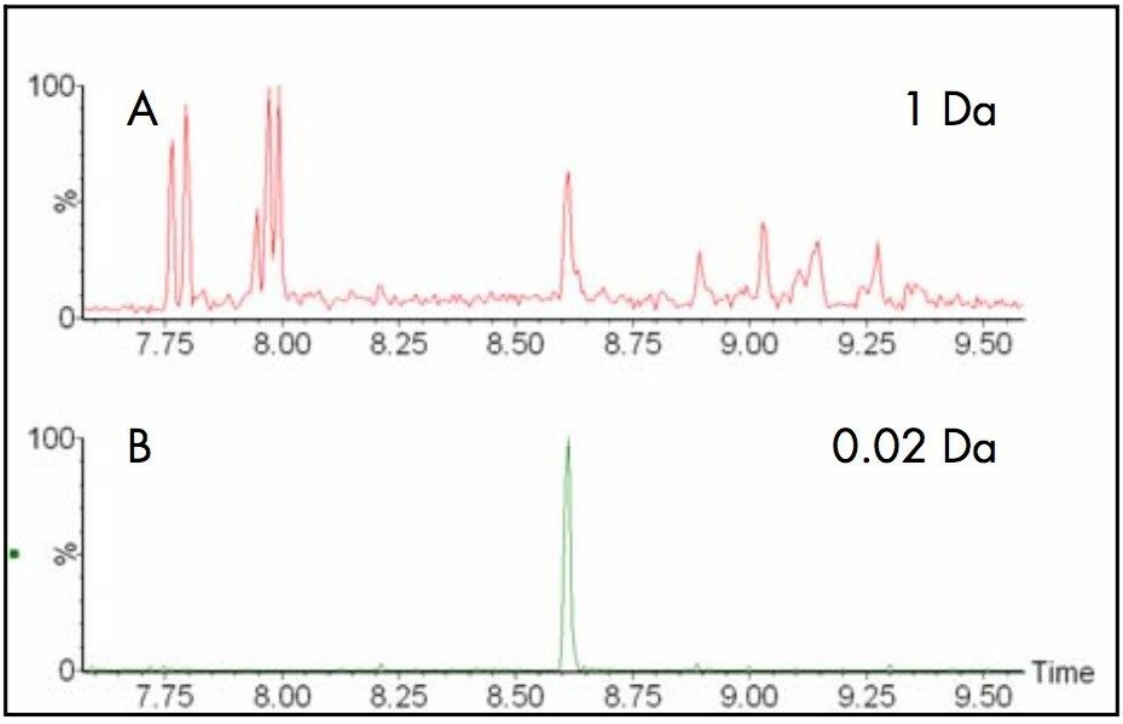


Figure 1. Selectivity offered by nominal mass (A) versus exact mass (B) chromatograms for vinclozolin.

Improving the selectivity can lead to an increase in the signal-to-noise (S/N) ratio which could be the difference between detecting the compound and not detecting it. This is illustrated in Figure 2 with fludioxonil (0.01 mg/kg) in grapefruit. The nominal mass chromatogram (1 Da, *m/z* 248) results in a S/N ratio of 4:1. In the exact mass chromatogram (20 mDa, *m/z* 248.0397) the S/N ratio has increased to 49:1. The mean difference between the nominal and exact mass S/N was a factor of five for all residues.

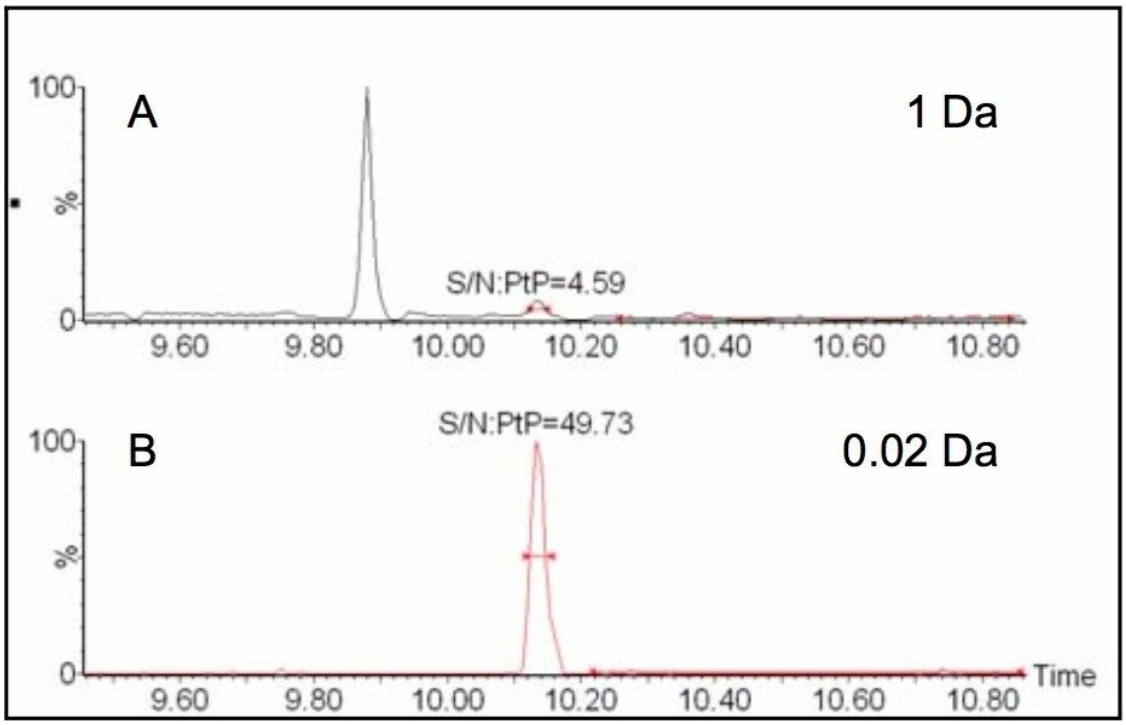


Figure 2. S/N offered by nominal mass (A) versus exact mass (B) chromatograms for fludioxonil.

The sensitivity of the method is illustrated in Figure 3, showing that quinoxyfen can be screened to a level below 0.01 mg/kg in sweet pepper. With a scanning instrument increasing the number of ions, as in the case of confirmation or increasing the number of residues, will decrease the overall sensitivity (S/N). With exact mass Tof, increasing the number of ions does not affect the sensitivity, as can be seen by the two peak areas for quinoxyfen (13.3).

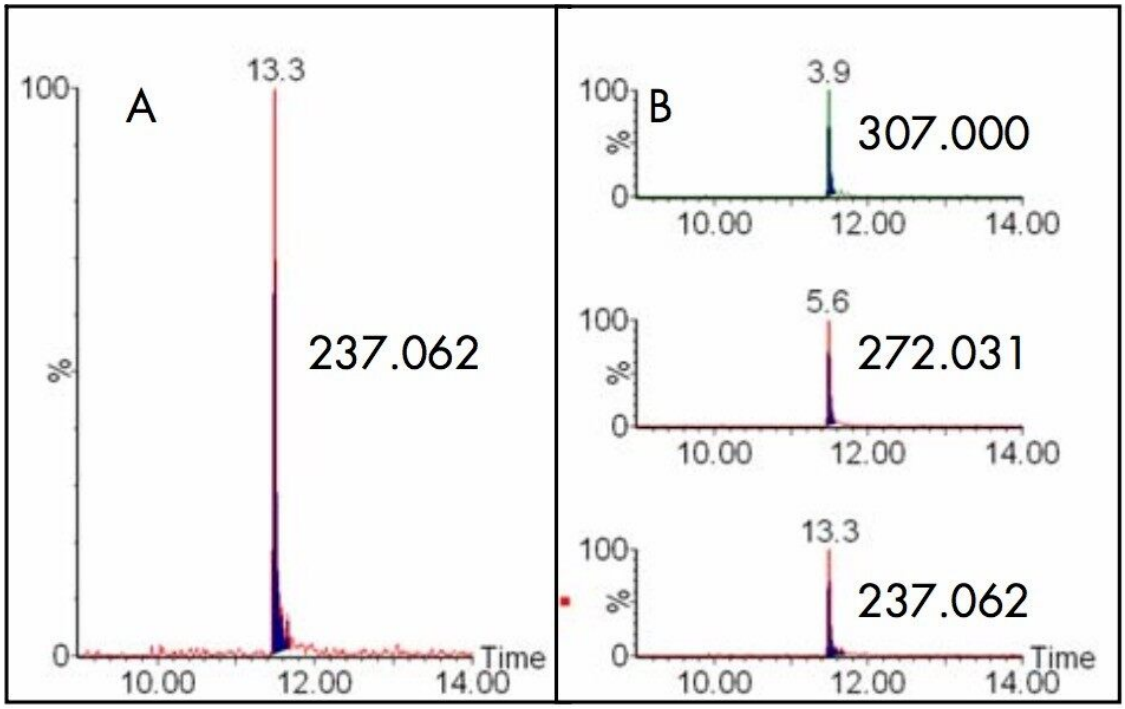


Figure 3. Sensitivity of one ion (A) versus three ions (B) for 0.01 mg/kg quinoxyfen in sweet pepper.

Matrix-matched standards were prepared in the concentration range 5–500 pg/µL, equivalent to 0.005–0.5 mg/kg. PCB 138 was used as an internal standard to correct for any volumetric errors. The resulting data was processed using Waters TargetLynx Application Manager. A representative curve for pyrimethanil in wheat flour with a correlation coefficient of $r^2 = 0.9994$ is illustrated in Figure 4.

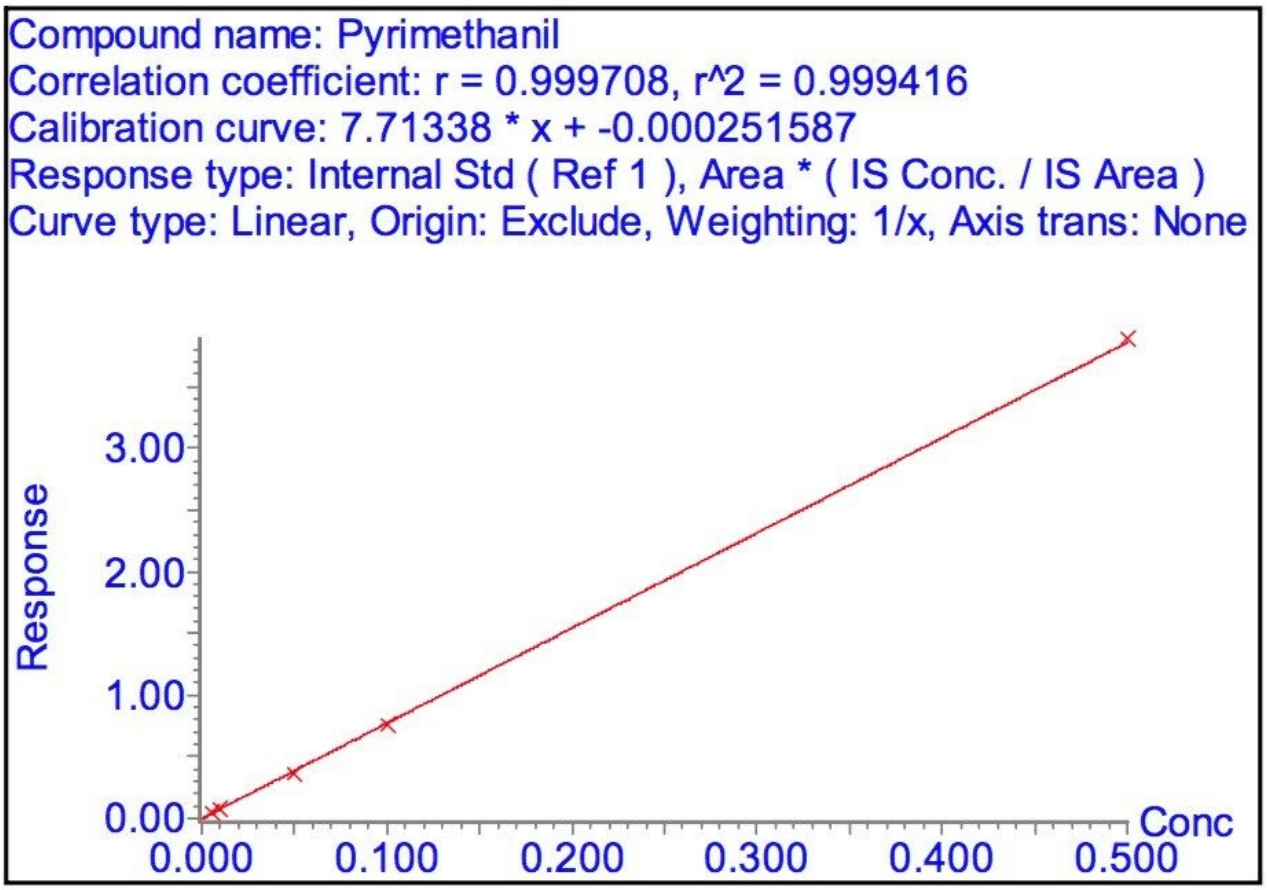


Figure 4. Calibration curve for pyrimethanil.

The TargetLynx browser for pirimiphos-methyl at a spiked concentration of 0.01 mg/kg in grapefruit is illustrated in Figure 5. Thirty residues could be screened using this method in all matrices to a concentration of 0.01 mg/kg. This number is not absolute because more residues could be added as there will no affect on sensitivity. The results show that the GCT Premier can be used in a targeted environment.

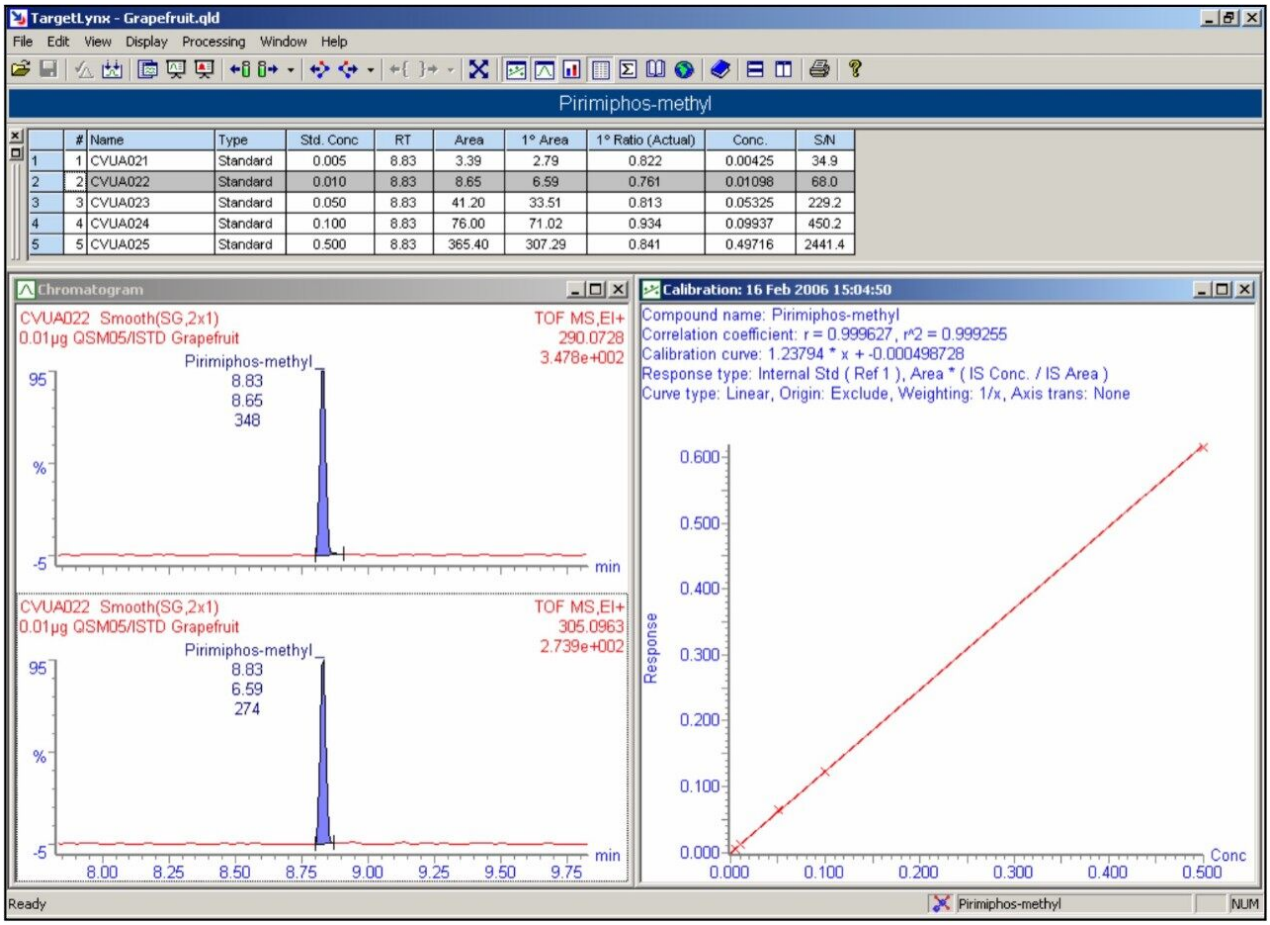


Figure 5. Example of TargetLynx browser for pirimiphos-methyl at a concentration of 0.01 mg/kg in grapefruit.

The MRM method was also applied to “real” samples containing incurred pesticide residues.

Five different matrices were supplied to further test the method; orange, cherry tomato, grapes, kiwi and strawberry. The cucumber matrix-matched calibration curve was used for quantification purposes. The TargetLynx Application Manager was used to provide automatic quantification and confirmation with two exact mass chromatograms (0.02 Da) processed for each residue.

An example TargetLynx browser containing 0.067 mg/kg chlorpyrifos in grapes is illustrated in Figure 6. This correlates well with the targeted Quattro micro GC Tandem Quadrupole MS/MS method where the concentration was found to be 0.066 mg/kg.[5]

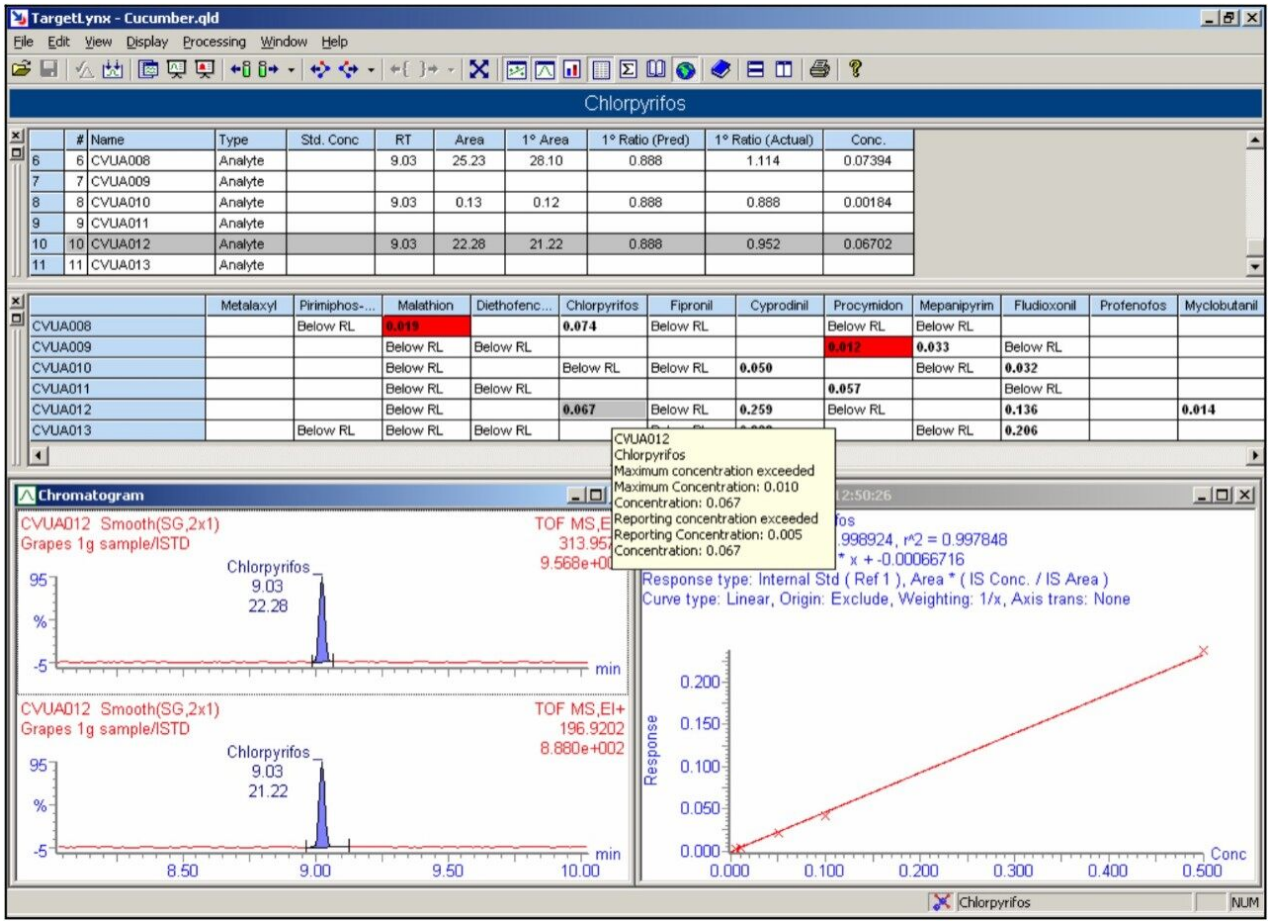


Figure 6. Example TargetLynx browser for grapes containing incurred chlorpyrifos.

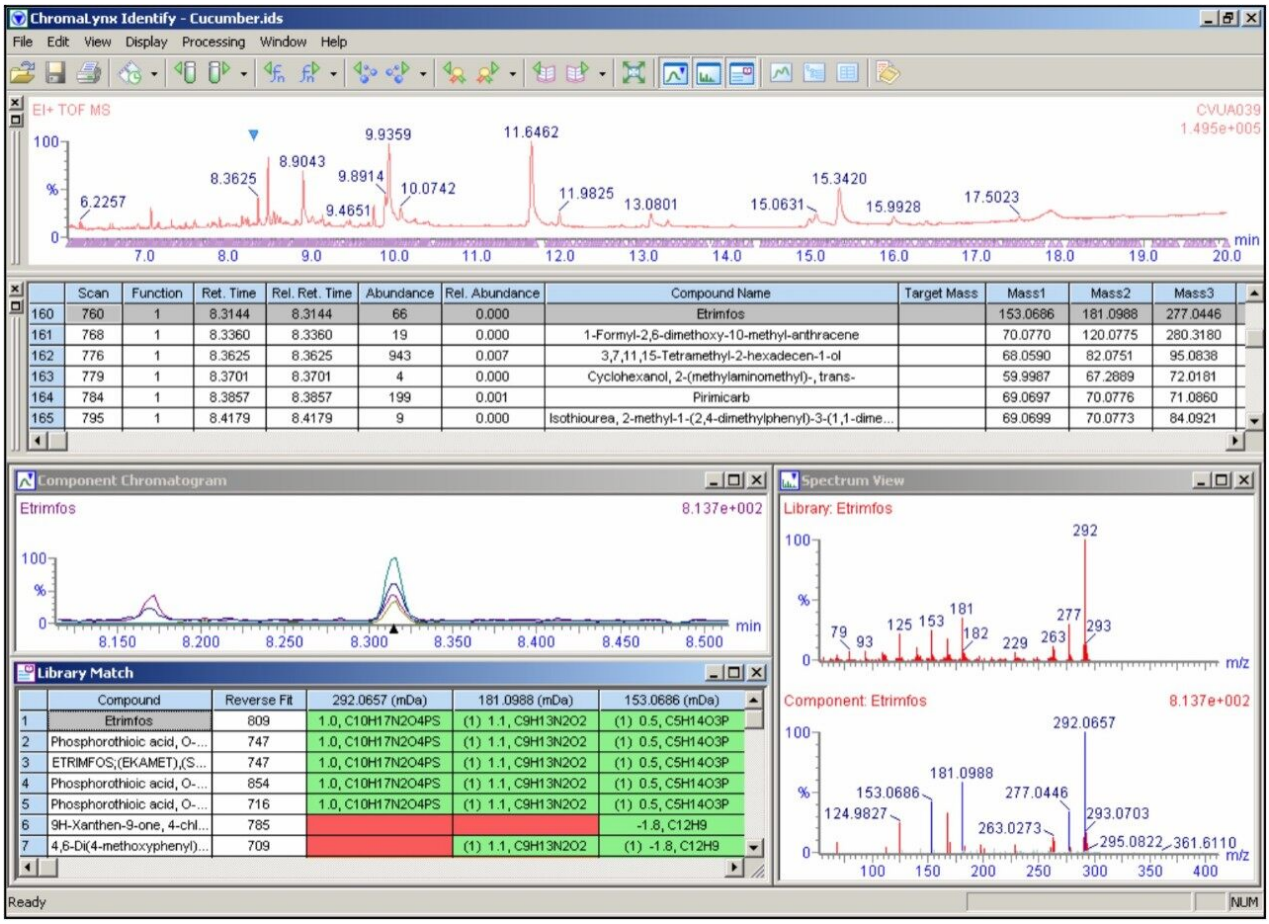
Untargeted Screening Results

In the untargeted screening environment, there may be hundreds of peaks that need to be located, which would be very time consuming if performed manually. In this case, it would be useful to process automatically using a deconvolution package such as Waters ChromaLynx Application Manager.

ChromaLynx automatically plots the reconstructed ion chromatograms (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 can then be submitted to an automatic library search routine with the ability to confirm by exact mass scoring.

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 7. Etrimfos was confirmed with three ions within 1.1 mDa of their expected exact masses. Ninety-one percent of the untargeted residues at 0.05 mg/kg were

located and identified using this method.



	Scan	Function	Ret. Time	Rel. Ret. Time	Abundance	Rel. Abundance	Compound Name	Target Mass	Mass1	Mass2	Mass3
160	760	1	8.3144	8.3144	66	0.000	Etrimfos		153.0686	181.0988	277.0446
161	768	1	8.3360	8.3360	19	0.000	1-Formyl-2,6-dimethoxy-10-methyl-anthracene		70.0770	120.0775	280.3180
162	776	1	8.3625	8.3625	943	0.007	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		68.0590	82.0751	95.0838
163	779	1	8.3701	8.3701	4	0.000	Cyclohexanol, 2-(methylaminomethyl)-, trans-		59.9987	67.2889	72.0181
164	784	1	8.3857	8.3857	199	0.001	Pirimicarb		69.0697	70.0776	71.0860
165	795	1	8.4179	8.4179	9	0.000	Isothiourea, 2-methyl-1-(2,4-dimethylphenyl)-3-(1,1-dime...		69.0699	70.0773	84.0921

	Compound	Reverse Fit	292.0657 (mDa)	181.0988 (mDa)	153.0686 (mDa)
1	Etrimfos	809	1.0, C10H17N2O4PS	(1) 1.1, C9H13N2O2	(1) 0.5, C5H14O3P
2	Phosphorothioic acid, O-...	747	1.0, C10H17N2O4PS	(1) 1.1, C9H13N2O2	(1) 0.5, C5H14O3P
3	ETRIMFOS;(EKAMET),(S...	747	1.0, C10H17N2O4PS	(1) 1.1, C9H13N2O2	(1) 0.5, C5H14O3P
4	Phosphorothioic acid, O-...	854	1.0, C10H17N2O4PS	(1) 1.1, C9H13N2O2	(1) 0.5, C5H14O3P
5	Phosphorothioic acid, O-...	716	1.0, C10H17N2O4PS	(1) 1.1, C9H13N2O2	(1) 0.5, C5H14O3P
6	9H-Xanthen-9-one, 4-chl...	785			-1.8, C12H9
7	4,6-Di(4-methoxyphenyl)...	709		(1) 1.1, C9H13N2O2	(1) -1.8, C12H9

Figure 7. Example of ChromaLynx browser for an untargeted pesticide residue in spiked cucumber (0.05 mg/kg).

ChromaLynx automatically performs exact mass confirmation of the library search. The formula from the library hit is submitted to elemental composition and the “n” most intense ions are confirmed/rejected by exact mass. Green boxes indicate a good exact mass match, amber boxes indicate a tentative exact mass match and red boxes indicate no match.

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 8. In this example, enilconazole or imazalil was identified. Imazalil is a conazole fungicide commonly used on citrus fruit. In the European Union it has an MRL of 5 mg/kg.

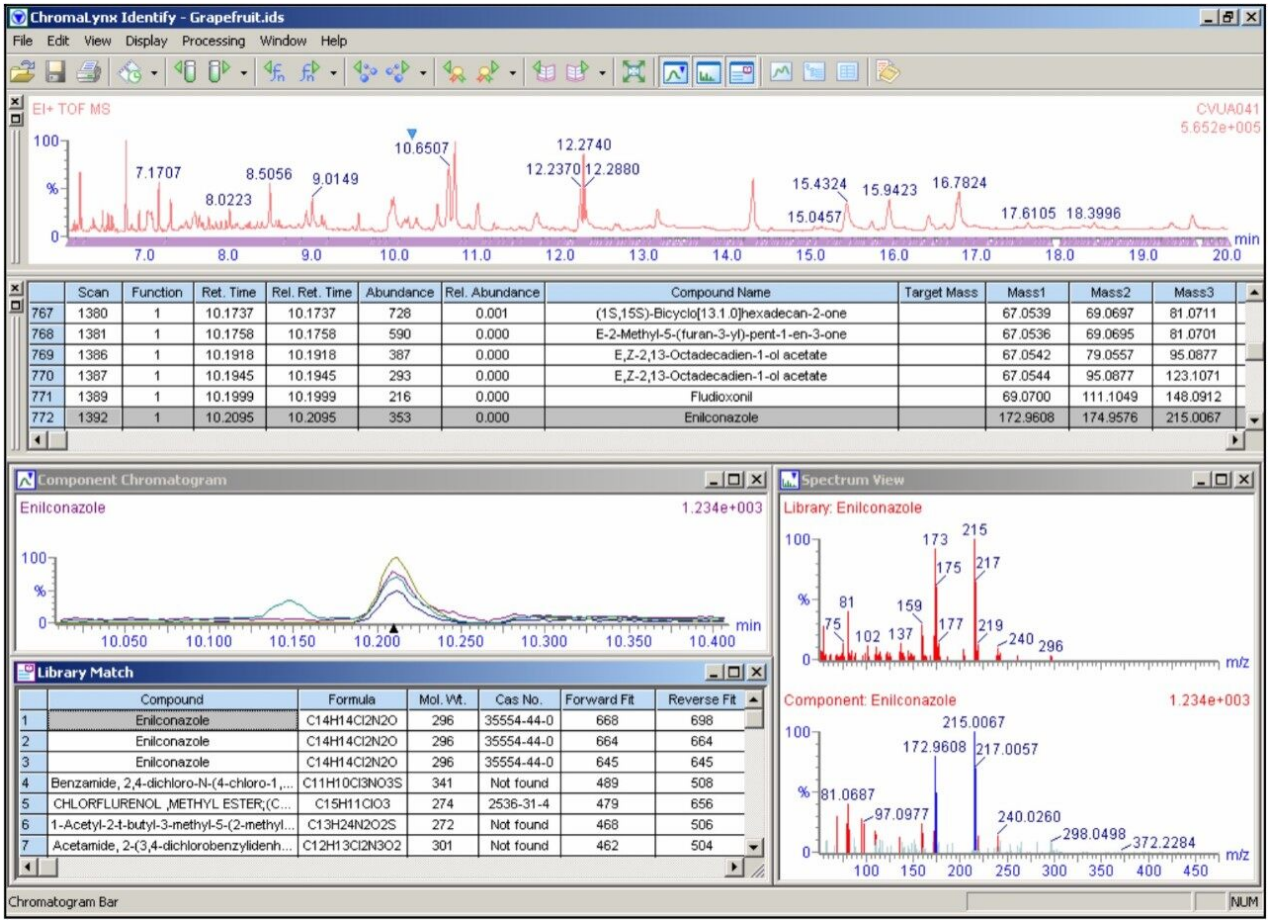


Figure 8. Example ChromaLynx browser for an unexpected pesticide residue in grapefruit.

Conclusion

A method has been presented for the targeted screening of pesticide residues in food commodities using the GCT Premier with TargetLynx.

The residues can be screened to concentration levels of 0.01 mg/kg or less in cucumber, sweet pepper, grapefruit and wheat flour with the use of exact mass chromatograms.

The method can also be extended to larger numbers of residues without loss in sensitivity due to the full-spectrum approach provided by exact mass Tof instruments.

The method was tested on incurred samples, giving results that correlated well with the previously described

Quattro micro GC tandem quadrupole method.

The single injection can also be used to screen for untargeted residues in the same extract using ChromaLynx.

ChromaLynx enables automatic peak detection, deconvolution, library searching and exact mass confirmation.

References

1. Regulation (EC) NO 396/2005 of 23 February 2005, *Off. J. of the European Union* No. L70/1.
2. Recommendation of 18 January 2006, *Off. J. of the European Union* No. L19/23.
3. M. Anastassiades, S. Lehotay, D. Stajnbaher, F. Schenck, *J. AOAC Int*. 86 (2) (2003) 412.
4. S.J. Lehotay, K. Maštovska, S.J. Yun, *J. AOAC Int*. 88 (2) (2005) 630.
5. P. Hancock, J. Dunstan, C. Wauschkuhn, D. Fuegel, M. Anastassiades, Waters Application Note, 720001439EN <https://www.waters.com/webassets/cms/library/docs/720001439en.pdf> (2005).

Acknowledgements

The author would like to thank Carmen Wauschkuhn, Diane Fuegel and Michelangelo Anastassiades at CVUA Stuttgart, Fellbach 70736, Germany for supplying the sample extracts that were analyzed in this project.

Featured Products

MassLynx MS Software <https://www.waters.com/513662>

TargetLynx <https://www.waters.com/513791>

ChromaLynx <https://www.waters.com/513759>

720001607, April 2006

© 2021 Waters Corporation. All Rights Reserved.