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應用手冊

Targeted And Non-Targeted Pesticide Screening In Food Using Elevated Resolution GC-Tof/MS

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Abstract

In this application note, a method will be introduced for the targeted screening of 93 pesticide residues with 106 components in pear, lettuce, and fruit-based baby food to the legislated concentration of 0.01 mg/kg. The method will also be extended to include non-target screening of real samples using automatic peak detection, deconvolution, and library searching with exact mass scoring.

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 (EU) and Japan, legislation has recently been established for setting and controlling MRLs in food.[1,2] One key feature of the legislation is that a Uniform Limit or default MRL of 0.01 mg/kg will apply to those commodities where no MRL is set.

Given that there are approximately 1000 compounds registered worldwide to control pests it is often advantageous to extract and determine as many of them as possible during a single analysis. An extraction method, with acetonitrile, followed by dispersive solid phase extraction (SPE) clean up has been reported for the analysis of a wide range of pesticides in fruits and vegetables[3] and fatty samples.[4]

Targeted detection such as selected ion recording (SIR) or multiple reaction monitoring (MRM) allow for the 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 non-target screening environments.

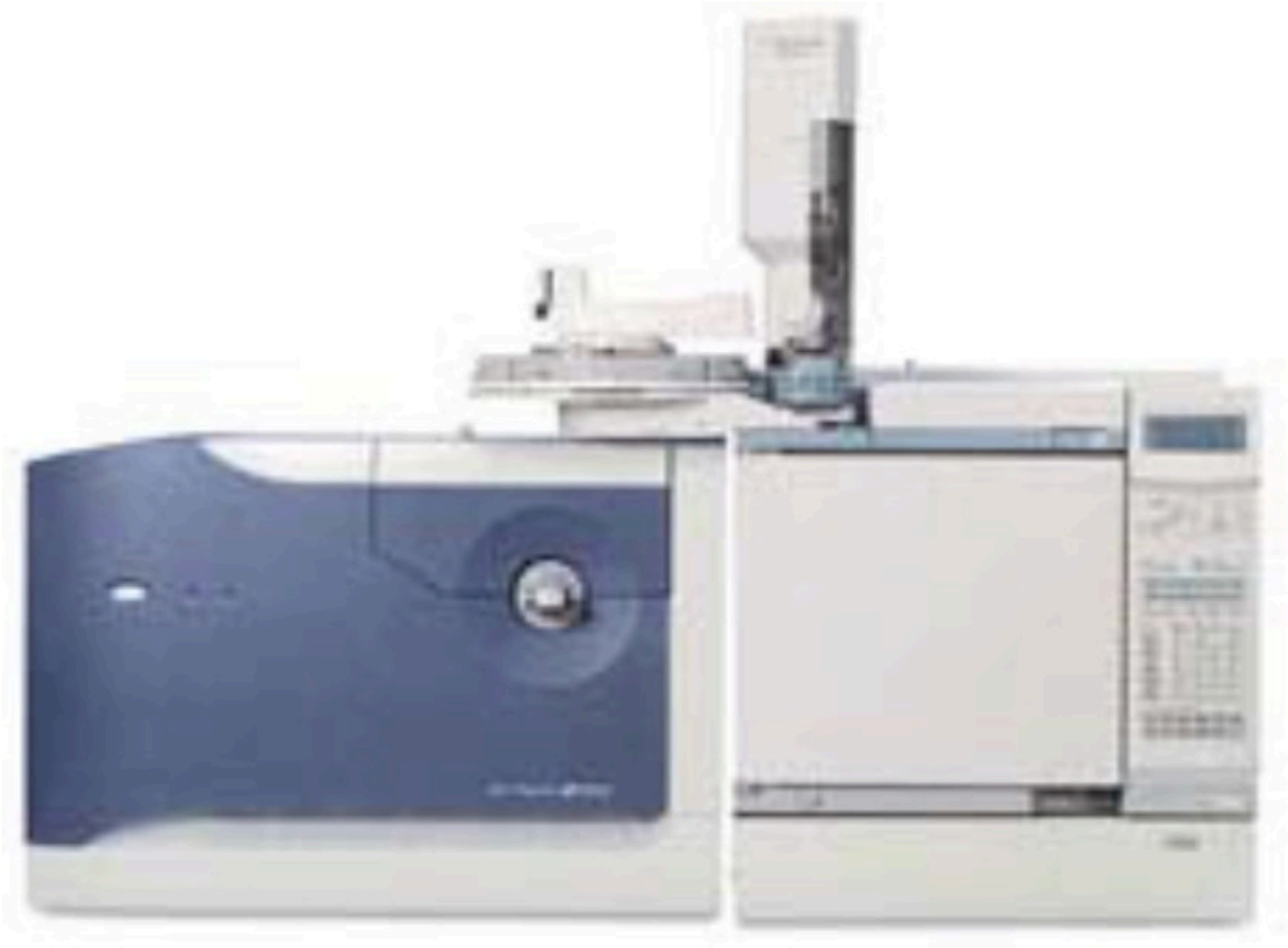
To establish a suitable non-target screening technique the method must be

- Sensitive to achieve the default MRL of 0.01mg/kg.
- Selective to reduce or eliminate matrix interferences.
- Multi-residue so multiple targets can be analyzed in a single run.
- Rugged so complex samples can be analyzed with reduced or no sample clean-up.

However, the method must also be generic so that ‘food scares’ or any changes in legislation do not require updating of methods and re-analysis of samples while non-target components can be detected post acquisition or retrospectively.

Exact mass time of flight mass spectrometry (Tof/MS) is a full spectrum technique capable of both the targeted and the non-targeted approaches. An exact mass Tof/MS method was recently introduced in Waters Application Note 720001607EN[5] and the results described here build on that initial work.

In this application note, a method will be introduced for the targeted screening of 93 pesticide residues with 106 components in pear, lettuce and fruit-based baby food to the legislated concentration of 0.01 mg/kg. The method will also be extended to include non-target screening of real samples using automatic peak detection, deconvolution and library searching with exact mass scoring.



Waters GCT Premier

Experimental

Extraction Method

10 g sample was weighed in a centrifuge tube. Acetonitrile (9.9 mL), acetic acid (0.1 mL), anhydrous $MgSO_4$ (4 g) and sodium acetate (1.66 g) were added and the tube was shaken immediately. After centrifugation at 4300 g for 5 min, an aliquot (1 mL) of the supernatant was transferred to a vial. The aliquot was blown down gently with nitrogen so that 0.2 mL toluene could be added. The 1 mL extract was transferred to a microcentrifuge vial containing 50 mg primary secondary amine (PSA) sorbent, 50 mg carbon sorbent and 150 mg anhydrous $MgSO_4$. The contents were vortex mixed for 30 s and centrifuged at 5000 g for 1 min. The supernatant was transferred to a vial and analysed by GC-Tof/MS.

GC Method

Agilent 6890N GC with CTC CombiPal autosampler

Column:	Restek Rxi-5MS 30 m x 0.25 mm i.d., 0.25 µm
Flow rate:	1.0 mL/min helium constant flow
Temp. ramp:	50 °C to 150 °C @ 20 °C/min 150 °C to 280 °C @ 6 °C/min 280 °C (hold 7 min)
Total run time:	34 min illustrated in Figure 1
Injection method:	Cyro cooled PTV in solvent vent mode, 5 µL injected
Vent method:	Vent pressure 5 kPa, Vent flow 20 mL/min for 0.5 min

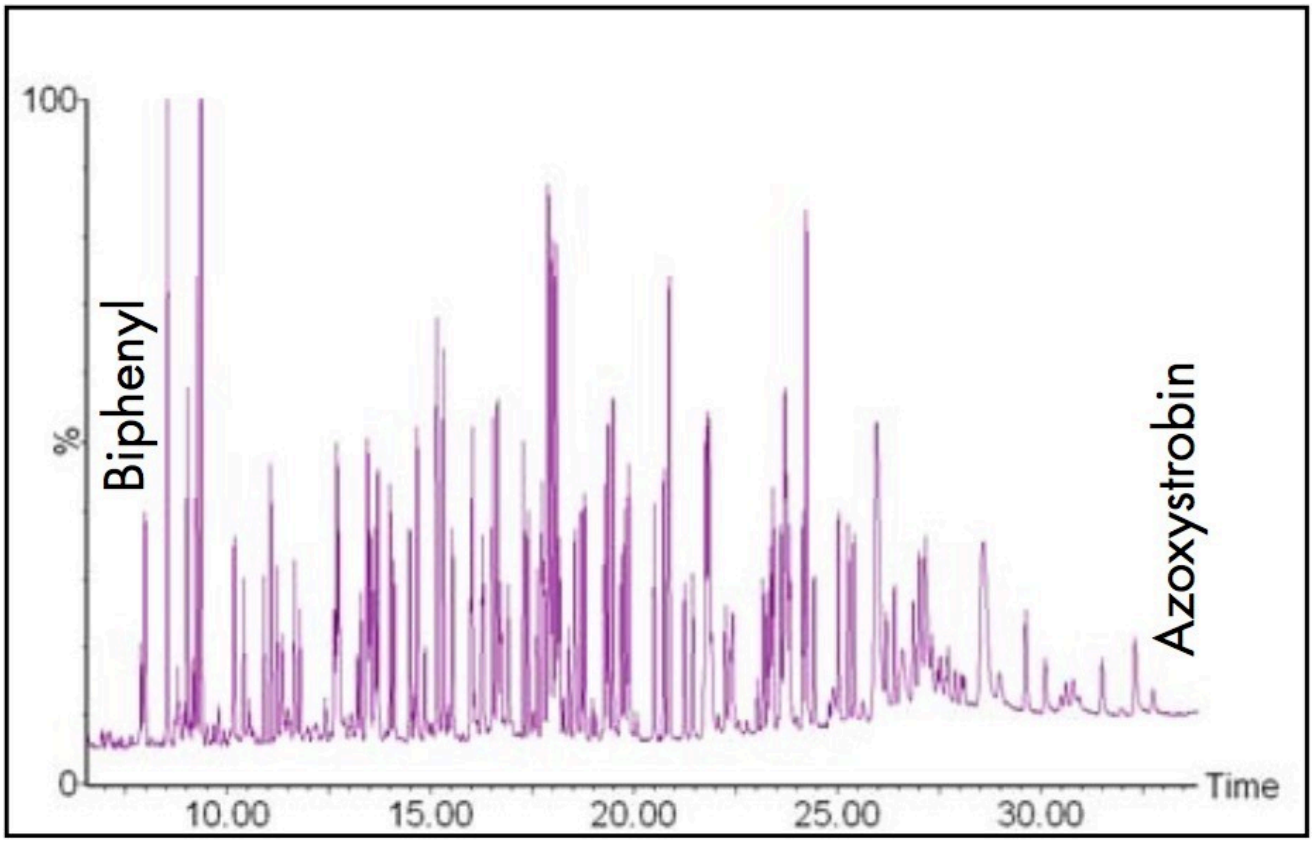


Figure 1. Total ion chromatogram (TIC) for 0.25 µg/mL matrix-matched standard in pear.

GC-Tof/MS Method

The Waters GCT Premier orthogonal acceleration time of flight (oa-Tof) mass spectrometer was used in electron ionisation (EI+) mode. The source was operated at 200 °C with an electron energy of 70 eV and a trap current of 200 µA. The temperature of the transfer line was held at 280 °C during the run. Spectra were acquired between 50 and 500 Da in a time of 0.24 s and a delay of 0.01s (4 spectra/s). Exact mass spectra were obtained using a singlepoint lock mass (Tris[trifluoromethyl]triazine, *m/z* = 284.9949) 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 analysed are listed in Table 1.

Pesticide	Recovery (%RSD)	Pesticide	Recovery (%RSD)	Pesticide	Recovery (%RSD)
Biphenyl	119 (7)	Pendimethalin	85 (4)	Fenhexamid	94 (4)
Heptenophos	114 (6)	Pyrifenox (I)	101 (3)	p,p-DDT	97 (3)
Tecnazene	90 (4)	Chlozolinate	105 (4)	Propiconazole (II)	78 (15)
Diphenylamine	104 (5)	Tolylfluanid	106 (6)	Tebuconazole	92 (7)
Ethoprophos	119 (9)	Chlorfenvinphos-z	114 (2)	Propargite	100 (9)
Trifluralin	112 (5)	Isofenphos	104 (4)	Iprodione	99 (7)
Dichloran	89 (5)	Captan	98 (8)	Pyridaphenthion	94 (4)
Simazine	102 (4)	Mecarbam	104 (6)	Phosmet	84 (3)
Gamma-HCH	106 (9)	Quinalophos	89 (4)	Bromopropylate	99 (4)
Quintozene	64 (5)	Phenthoate	98 (3)	Bifenthrin	95 (3)
Propyzamide	106 (2)	Furalaxyl	106 (1)	Fenpropathrin	102 (6)
Fonofos	115 (8)	Folpet	70 (13)	Tebufenpyrad	91 (3)
Pyrimethanil	59 (5)	Procymidone	104 (3)	Fenazaquin	46 (10)
Diazinon	114 (12)	Methidathion	99 (3)	Tetradifon	89 (6)
Phosphamidon-e	93 (12)	Pyrifenox (II)	99 (9)	Phosalone	73 (4)
Tefluthrin	115 (9)	Paclobutrazol	103 (9)	Cyhalothrin-λ	99 (4)
Chlorothalonil	51 (9)	Tetrachlorvinphos	97 (6)	Fenarimol	99 (6)
Pirimicarb	88 (2)	Endosulfan (I)	105 (7)	Dicofol	99 (2)
Pentachloroaniline	40 (9)	Prothiofos	81 (7)	Pyrazophos	51 (9)
Phosphamidon-z	100 (4)	Fludioxonil	95 (3)	Permethrin cis	88 (6)
Vinclozolin	107 (4)	Profenofos	90 (4)	Permethrin trans	95 (11)
Chlorpyrifos-methyl	97 (8)	p,p'-DDE	102 (4)	Fenbuconazole	78 (7)
Parathion-methyl	103 (5)	Myclobutanil	101 (5)	Cyfluthrin (I)	89 (29)
Carbaryl	106 (5)	Kresoxim-methyl	101 (5)	Cyfluthrin (II)	119 (12)
Tolclofos-methyl	107 (8)	Buprofezin	100 (9)	Cyfluthrin (III-IV)	165 (8)
Metalaxyl	108 (3)	Bupirimate	98 (4)	Cypermethrin (I)	130 (11)
Fenitrothion	98 (5)	Endosulfan (II)	99 (5)	Cypermethrin (II)	103 (22)
Pirimiphos-methyl	102 (5)	o,p-DDT	104 (3)	Cypermethrin (III-IV)	114 (11)
Dichlofluanid	111 (7)	Oxadixyl	98 (5)	Fenvalerate (I)	99 (6)
Malathion	105 (4)	p,p-DDD	102 (7)	Fenvalerate (II)	90 (17)
Chlorpyrifos	85 (5)	Ethion	97 (4)	Difenoconazole (I)	87 (10)
Parathion-ethyl	98 (3)	Triazophos	94 (10)	Difenoconazole (II)	87 (6)
Dicofol BD	105 (9)	Ofurace	100 (4)	Deltamethrin	90 (7)
Pirimiphos-ethyl	97 (6)	Propiconazole (I)	94 (5)	Azoxystrobin	103 (5)
Cyprodinil	52 (2)	Endosulfan-sulfate	103 (5)		
Chlorfenvinphos-e	82 (6)	Trifloxystrobin	100 (6)		

Table 1.

Mean recovery and % RSD for 0.01 mg/kg recovery samples (n = 5) from fruit-based baby food.

Acquisition and Processing Methods

The data were acquired using Waters MassLynx software version 4.1 and processed using either the TargetLynx

or ChromaLynx Application Managers.

Results and Discussion

Target Screening Results

Three food commodities were screened; pear, lettuce, and fruit-based baby food. To test the extraction method described, five recovery experiments were performed in fruit-based baby food, spiked at 0.01 mg/kg. The mean recovery and relative standard deviation (% RSD) in parenthesis of each analyte are listed in Table 1.

Satisfactory recoveries were obtained for the majority of the pesticides in fruit-based baby food with 81% of the residues recovered in the accepted 70–110% range as specified in the AQC guidelines.[6] The % RSDs were less than 30% for all residues, with 87% residues giving % RSDs less than 10%. Consistent low recoveries for a few (quintozene, pyrimethanil, chlorothalonil, pentachloroaniline, fenazaquin, and pyrazophos) are attributed to losses during the dispersive SPE clean-up step.

Methidathion at a concentration of 0.01 mg/kg in fruit-based baby food was chosen to illustrate the improved selectivity and signal-to-noise (S/N) ratio of exact mass chromatograms. The nominal mass chromatogram (1 Da, *m/z* 145), illustrated in Figure 2, has a S/N ratio of 6:1, close to the limit of detection (LOD). In the exact mass chromatogram (20 mDa, *m/z* 145.0072) the S/N ratio is now 48:1 just by reducing the mass window by which the chromatogram is drawn. Improved selectivity and S/N reduce the likelihood of interference when using automatic integration packages.

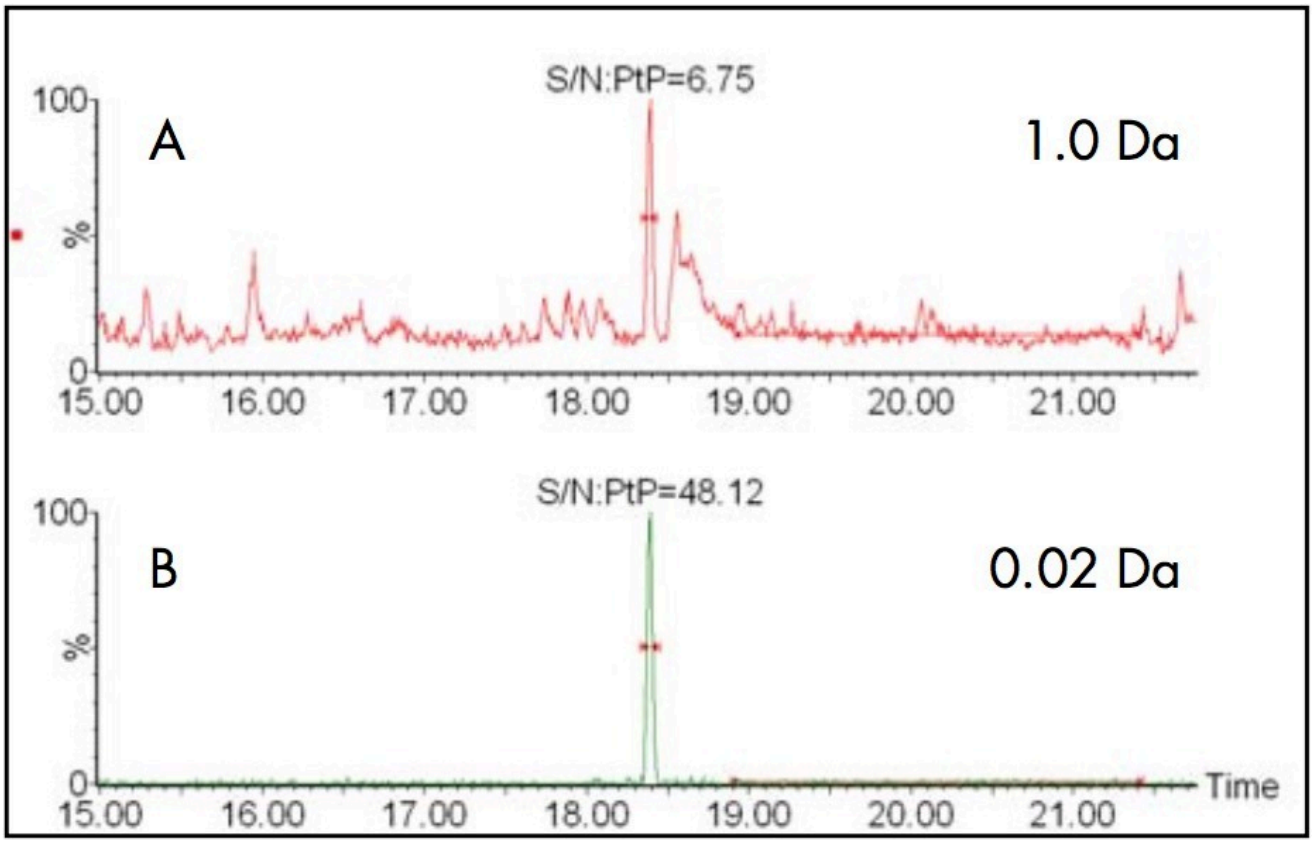


Figure 2. S/N ratio offered by nominal mass (A) versus exact mass (B) chromatograms for methidathion.

The selectivity and S/N ratio improvement obtained from using exact mass chromatograms depends on the complexity of the final sample extract. This will ultimately depend on the complexity of the original food commodity and the sample preparation method used. Figure 3 illustrates that when nominal mass windows (1 Da, *m/z* 248) are used, the detection of endosulfan sulfate at 0.01 mg/kg is affected by matrix interference but this differs from matrix to matrix. However, when exact mass windows (20 mDa, *m/z* 248.0397) are used, the likelihood of matrix interference is significantly reduced. The two peaks that can be seen preceding endosulfan sulfate in the exact mass chromatogram are endosulfan I and II.

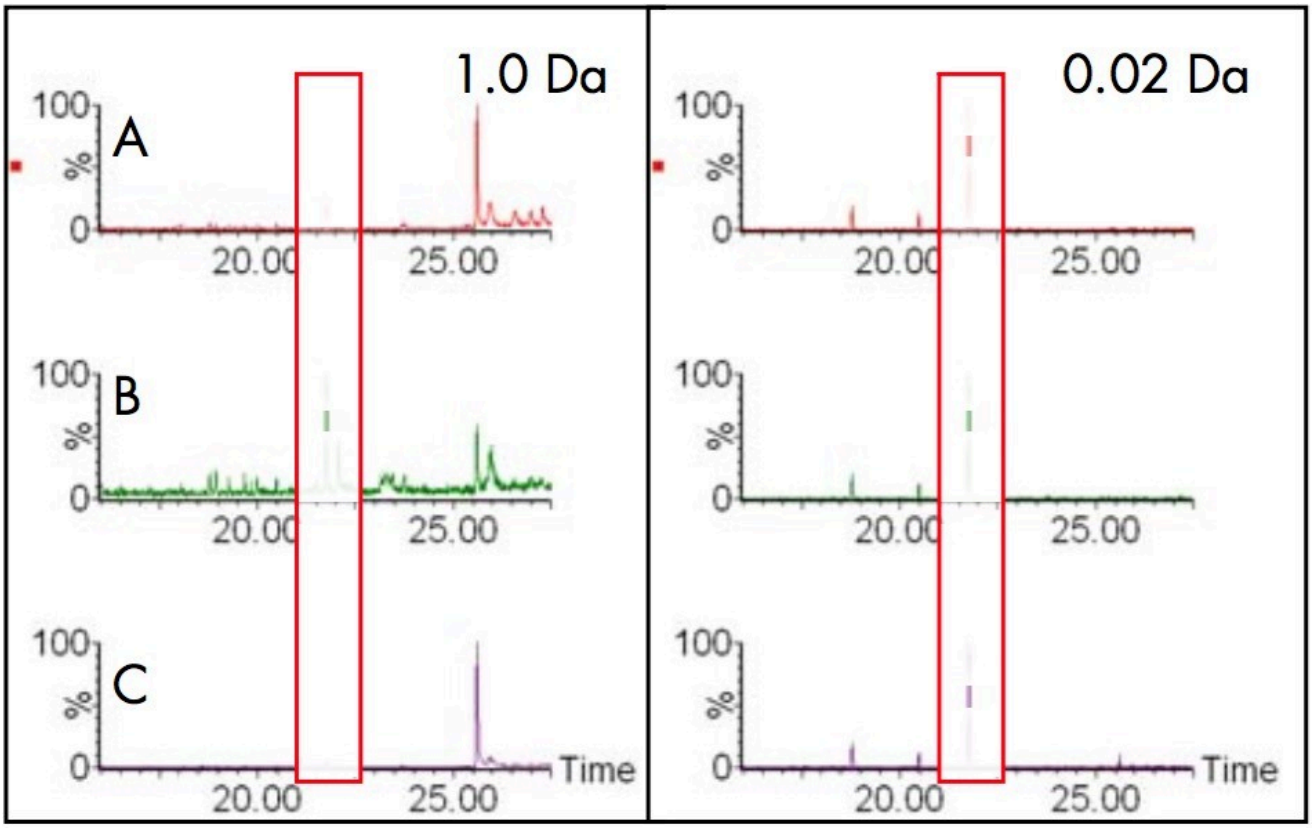


Figure 3. Selectivity offered by nominal mass versus exact mass chromatograms for endosulfan sulfate in pear (A), lettuce (B) and fruit-based baby food (C).

The sensitivity of the method is illustrated in Figure 4, showing that propyzamide can be screened to a level below 0.01 mg/kg in pear. Increasing the number of ions, as in the case of confirmation or increasing the number of residues, on a scanning instrument will decrease the overall sensitivity (peak area and S/N) in SIR mode due to the reduced duty cycle. With Tof increasing the number does not decrease the sensitivity, as can be seen by the two peak areas (29) when moving from one ion acquired to three ions acquired. Therefore, if there is interference for one mass, processing can be moved to a different mass without re-injection. The number of confirmation ions or residues can be increased without effect.

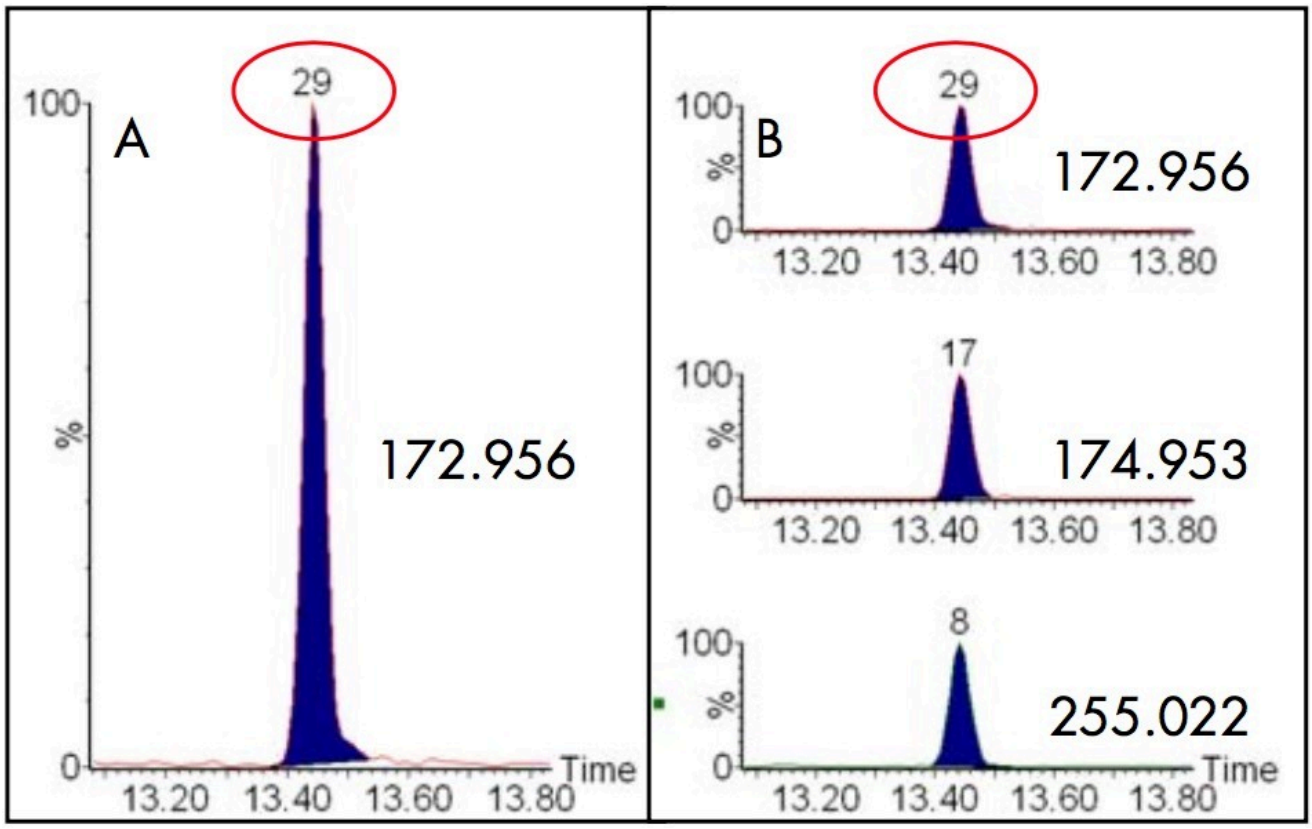
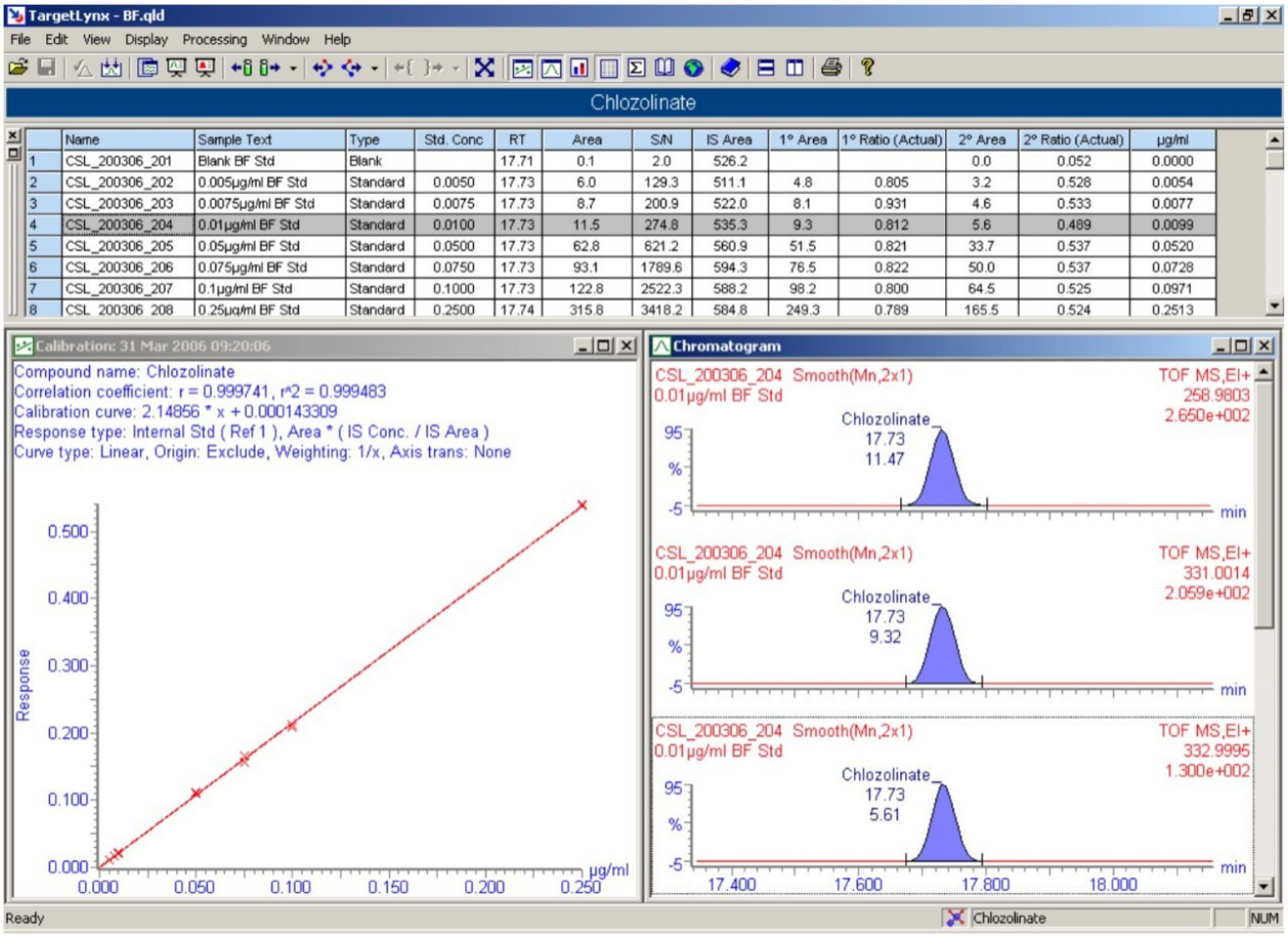


Figure 4. Sensitivity of one ion (A) versus three ions (B) for 0.01 mg/kg propyzamide in pear.

The TargetLynx browser for chlozolinate at a spiked concentration of 0.01 mg/kg in fruit-based baby food is illustrated in Figure 5. 93 residues with 106 components could be screened using this method in pear, lettuce, and fruit-based baby food to a concentration of 0.01 mg/kg. This number is not absolute because more residues could be added as there will no effect on sensitivity. The results show that the GCT Premier can be used in a targeted screening environment.



	Name	Sample Text	Type	Std. Conc	RT	Area	S/N	IS Area	1° Area	1° Ratio (Actual)	2° Area	2° Ratio (Actual)	µg/ml
1	CSL_200306_201	Blank BF Std	Blank		17.71	0.1	2.0	526.2			0.0	0.052	0.0000
2	CSL_200306_202	0.005µg/ml BF Std	Standard	0.0050	17.73	6.0	129.3	511.1	4.8	0.805	3.2	0.528	0.0054
3	CSL_200306_203	0.0075µg/ml BF Std	Standard	0.0075	17.73	8.7	200.9	522.0	8.1	0.931	4.6	0.533	0.0077
4	CSL_200306_204	0.01µg/ml BF Std	Standard	0.0100	17.73	11.5	274.8	535.3	9.3	0.812	5.6	0.489	0.0099
5	CSL_200306_205	0.05µg/ml BF Std	Standard	0.0500	17.73	62.8	621.2	560.9	51.5	0.821	33.7	0.537	0.0520
6	CSL_200306_206	0.075µg/ml BF Std	Standard	0.0750	17.73	93.1	1789.6	594.3	76.5	0.822	50.0	0.537	0.0728
7	CSL_200306_207	0.1µg/ml BF Std	Standard	0.1000	17.73	122.8	2522.3	588.2	98.2	0.800	64.5	0.525	0.0971
8	CSL_200306_208	0.25µg/ml BF Std	Standard	0.2500	17.74	315.8	3418.2	584.8	249.3	0.789	165.5	0.524	0.2513

Figure 5. Example TargetLynx browser for chlozolinate in fruit-based baby food (0.01 mg/kg).

The method was also applied to a blind study of 11 pear samples and 12 lettuce samples containing incurred residues. Matrix-matched calibration standards was used for quantification purposes. The TargetLynx Application Manager was used to provide automatic quantification with two exact mass chromatograms (0.02 Da) processed for each residue. For illustration purposes, the reporting level was chosen to be 0.005 mg/kg while the maximum residue limit was chosen to be 0.01 mg/kg.

A TargetLynx browser showing three exact mass chromatograms for incurred chlorpyrifos is illustrated in Figure 6. In this example, the results indicate a screened concentration of 0.023 mg/kg in pear, not exceeding the UK/EU MRL of 0.500 mg/kg. The correlation between the elevated resolution GC-Tof/MS method and the established single quadrupole SIR method was good.

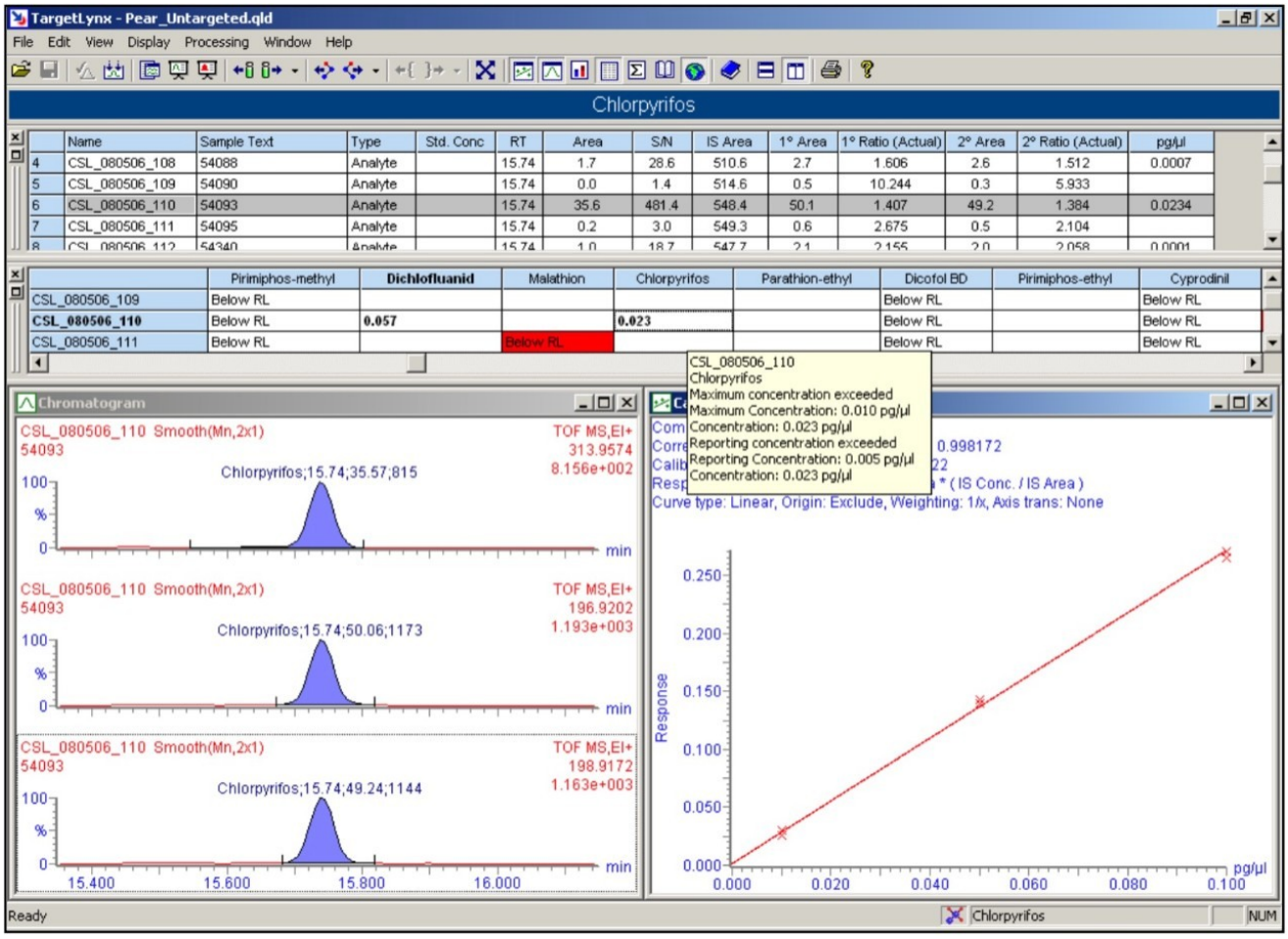


Figure 6. Example TargetLynx browser for pear containing incurred chlorpyrifos at 0.023 mg/kg.

ChromaLynx automatically plots the reconstructed ion chromatograms (RICs) of up to 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.

Deconvolution is important because as the number of residues and/or matrix peaks increase, the probability of peaks co-eluting also increases. In target screening, this is not important unless the co-eluting peaks have the same exact masses but in non-target screening being able to obtain a “clean” spectrum for each component is the basis of any result. The importance of deconvolution for non-target screening can be observed in Figure 7. Given the data shown an analyst is likely to conclude there are five components in this section of the chromatogram at 17.61, 17.73, 17.78, 17.87, and 17.97 min.

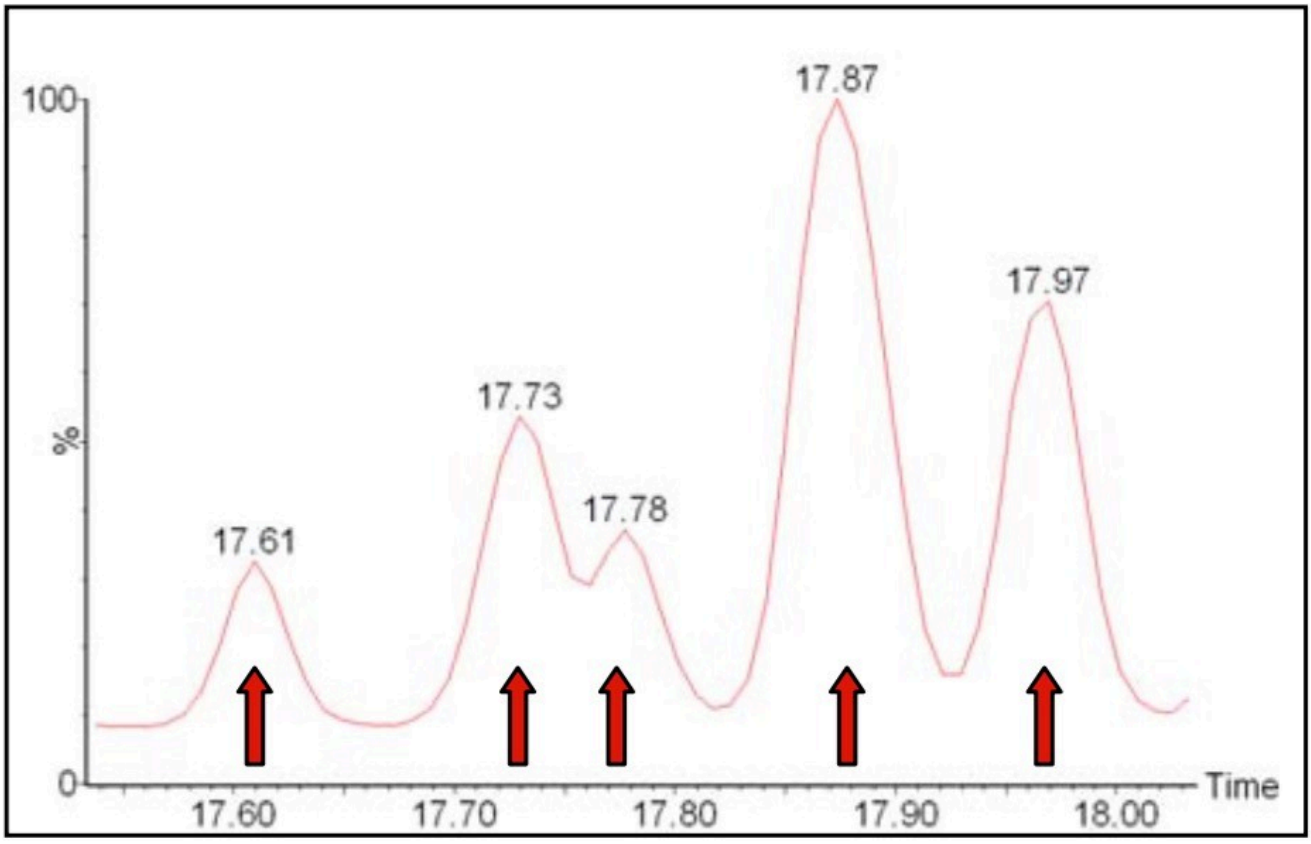


Figure 7. How many components are present in the spiked lettuce sample?

However, submitting the same section of the chromatogram to ChromaLynx results in the example browser displayed in Figure 8. Here eight components, indicated by a pink triangle, were found with three peaks co-eluting under the peak at 17.87 min and two peaks co-eluting under the peak at 17.97 min.

	Scan	Function	Ret. Time	Rel. Ret. Time	Abundance	Rel. Abundance	Compound Name	Target Mass	Mass1	Mass2	Mass3
5	1697	1	17.6093		2387	0.023	PENDIMETHALIN;[PENOXALIN];(PROWL),(STOMP),(HERB...		161.0849	162.0833	191.0738
6	1712	1	17.7312		4292	0.042	CHLOZOLINATE;(SERINAL)		185.9889	186.9624	187.9765
7	1718	1	17.7775		2779	0.027	Tolyfluanid		62.9698	97.9391	106.0654
8	1730	1	17.8720		9423	0.092	CHLORFENVINPHOS (E);[CVP];(BIRLANE),(SUPONA),(S...		58.0625	121.0249	184.9995
9	1731	1	17.8845		9192	0.090	ISOFENPHOS;(OFTANOL),(AMAZE)		58.0641	95.9670	96.9508
10	1732	1	17.8904		4381	0.043	MECARBAM;(MURFOTOX)		78.9404	96.9510	124.9834

	Compound	Formula	Mol. Wt.	Forward Fit	Reverse Fit	Hit Pro
1	Tolyfluanid	C10H13Cl2F...	346	736	745	0.960
2	3,5-XYLYL METHYLCARBAMATE;[XMC];(MACBAL)	C10H13NO2	179	308	363	0.009
3	METOLACHLOR;(DUAL)	C15H22ClNO2	283	286	393	0.003
4	ETHIOFENCARB;(CRONETON)	C11H15NO2S	225	285	317	0.003
5	SIMETRYN;(GY-BON)	C8H15N5S	213	272	327	0.002

Figure 8. Example ChromaLynx browser for a section of the spiked lettuce sample.

Figure 9 illustrates each component under the peak at 17.87 min as an exact mass chromatogram and indicates overlapping peaks with slightly different peak centres.

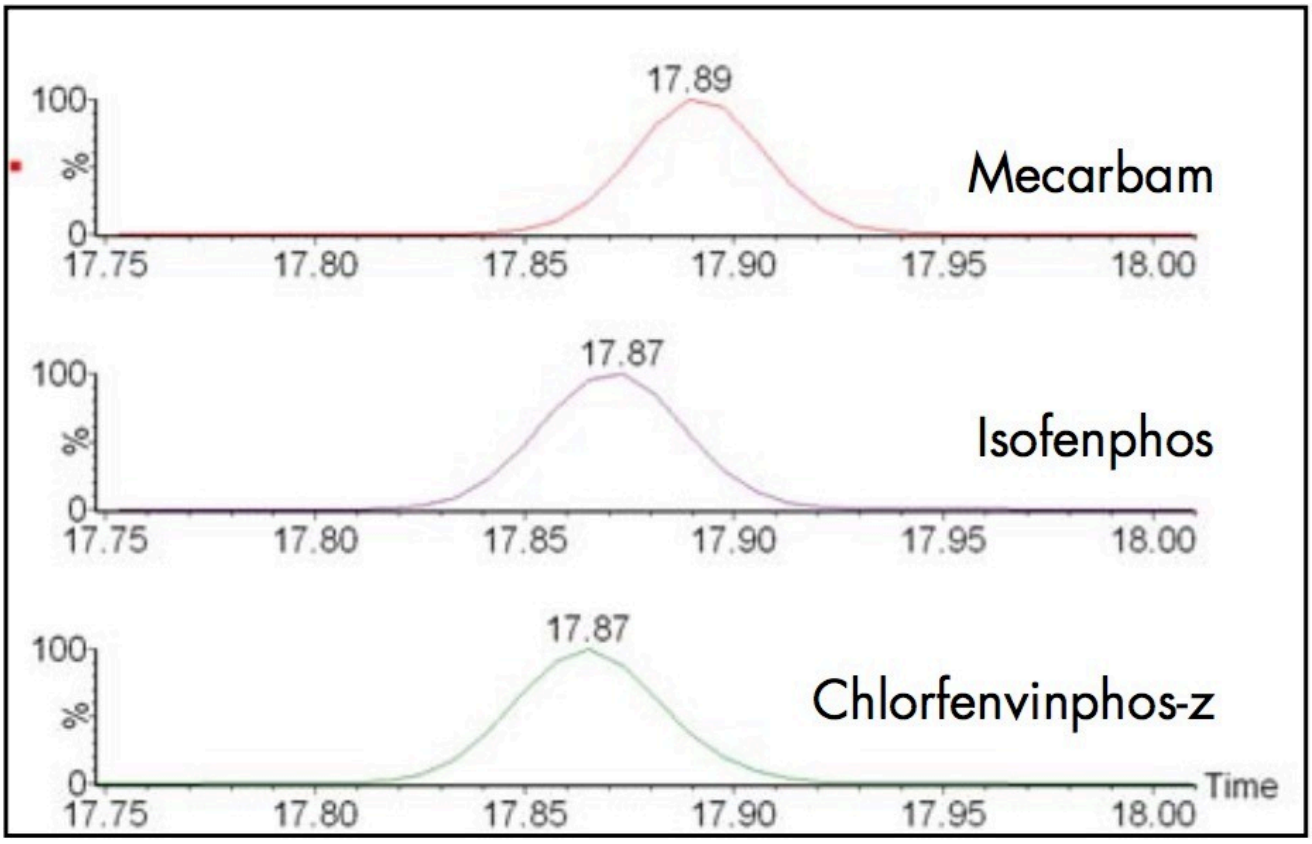


Figure 9. Extracted mass chromatograms for the three components eluting at 17.87 min in lettuce.

ChromaLynx processing of a real lettuce extract located more than 500 components in the chromatogram. Components can be reduced to a list of possible candidates by using the fit factor from the mass library search. ChromaLynx automatically performs exact mass scoring 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.

An example of one of the non-target residues, DCPA or chlorothal-dimethyl, that was found is illustrated in Figure 10. In this example, DCPA was scored with two ions within 0.8mDa of their expected masses.

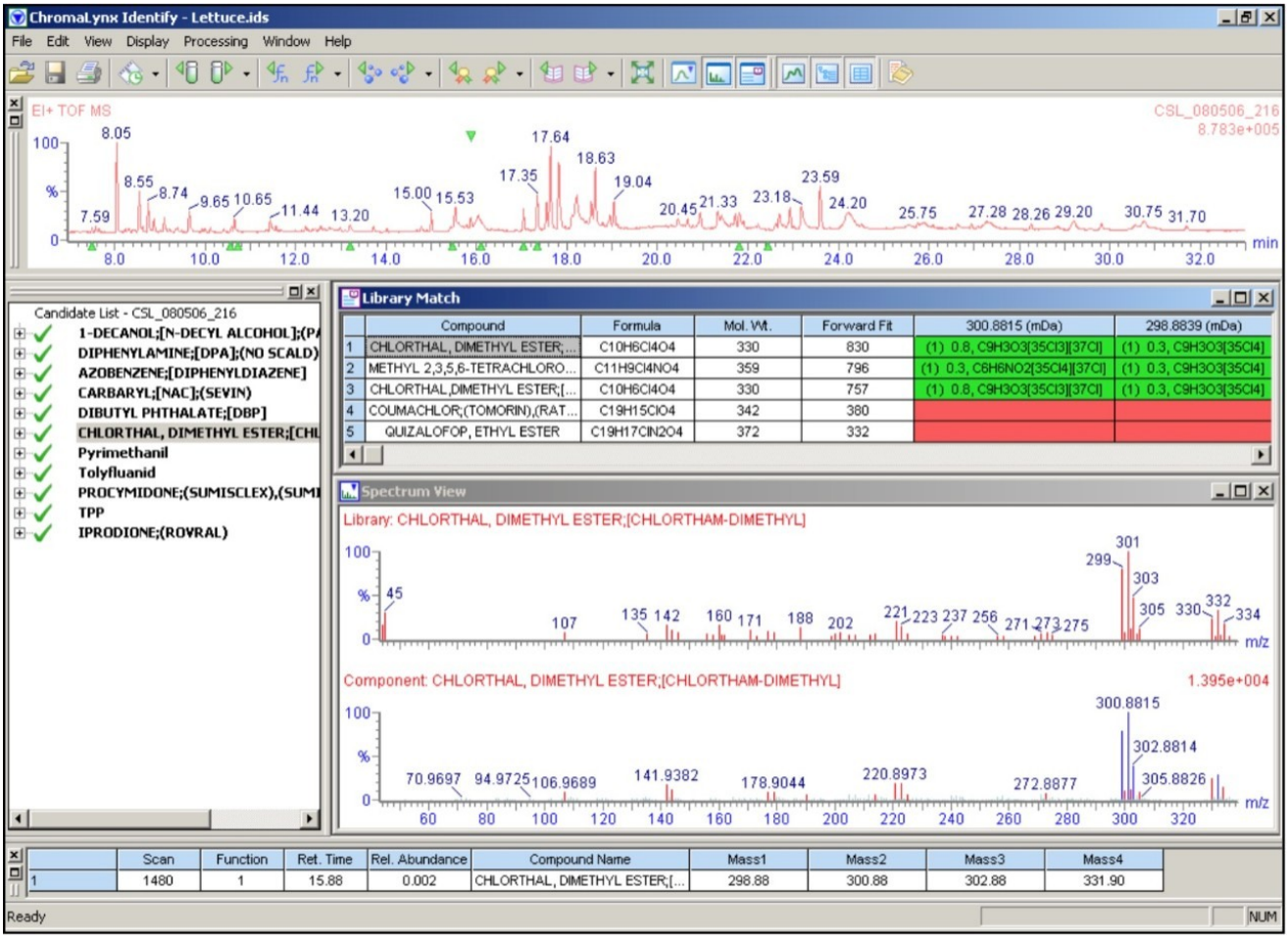


Figure 10. ChromaLynx browser of a non-target residue in lettuce.

Following the non-target screening of DCPA in lettuce, CSL re-extracted the sample and quantified using their established single quadrupole method.

The validation recoveries at 0.01 mg/kg were 95–103% (n=5). The confirmation of identity was based on three ions (*m/z* 301, 299, and 332). DCPA was confirmed at 0.07 mg/kg indicating that non-target residues can be screened successfully using the GCT Premier together with ChromaLynx.

Conclusion

An exact mass GC-Tof/MS method has been developed for the quantification of approximately 100 pesticide residues.

The residues can be screened to concentration levels of 0.01 mg/kg or less in pear, lettuce, and fruit-based baby

foods with the use of exact mass chromatograms.

Good linearity and satisfactory recoveries for the majority of pesticides were obtained.

A targeted method using TargetLynx and a nontargeted method using ChromaLynx were then employed for residue screening.

The targeted method correlated well with previous results obtained from an established single quadrupole MS method.

This joint approach led successfully to the identification of incurred residues in pear and lettuce samples.

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