Food Safety and Testing Application Note Compendium:

Pesticides

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Application Note: 10017

Comparison of GC/MS/MS to GC/MS Analysis of Pesticides in Vegetables

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Key Words

- Comparison
- FS/MS² vs FS/SIM
- Ion Trap GC/MS/MS
- Pesticide
- Single Quad GC/MS

Overview

Purpose

The analysis of chlorinated pesticides has traditionally been performed using an Electron Capture Detector (ECD) with confirmational analysis by GC/MS using Full Scan (FS). FS GC/MS has sensitivity limitations that prohibit it from confirming ECD "hits" at very low levels. To improve sensitivity, Selected Ion Monitoring (SIM) has been used; however, SIM is susceptible to ion interferences from matrix. An alternative approach is tandem mass spectrometry (MS/MS), where a target compound ion is isolated from matrix and then fragmented to generate very unique spectra. A list of chlorinated pesticides was studied in a vegetable matrix by sequential Full Scan (FS) and SIM analysis on the Thermo Scientific DSQ™ (Figure 1), and then by FS and MS/MS analysis on the Thermo Scientific PolarisQ ion trap GC/MS^n (Figure 2). The linearity and precision were examined using internal standards to track any degradation of response.

Methods

The samples and standards were injected using a programmable temperature vaporization injector (PTV) with a cold solvent split injection of 5 µL (Figure 3). The same capillary column, PTV liner, and EI ion volume were used for both studies on the PolarisQ and the DSQ to minimize any chromatographic variables. A spiked onion extract was analyzed to check for detection limits in matrix. A calibration curve was run from 1 pg/µL to 1 ng/µL in methylene chloride. The PolarisQ was set up for sequential FS and MS/MS analysis and the DSQ for sequential FS and SIM analysis.

Results

Both mass spectrometers exhibited good linear range over the concentration studied. The ion trap gave closer numbers for the spiked extract. The ion trap also gave no false positives for the vegetable extract, while the SIM analysis on the DSQ gave eight false positives. The precision for the internal standard was good over the study period. The detection limit was 40 ppb in matrix and 1 pg/µL (5µL) in reagent solution.

Introduction

The analysis of chlorinated pesticides has routinely been done on a semi-specific detector, the Electron Capture Detector (ECD). The identification is strictly by retention time by dual column analysis, utilizing the different elution characteristics of two different stationary phases. The ECD is quite sensitive, but it does not give unequivocal confirmation that may be achieved through analysis by GC/MS by matching retention time and library spectrum. GC/MS has features to enhance specificity, such as Chemical Ionization (CI), Selective Ion Monitoring (SIM,) or MS/MS. Even with SIM, where Multiple Ions are Monitored (MIM), the matrix may contain similar ions at the same retention time, so more stringent selectivity must be invoked to remove the matrix ions from the mass spectrum, which will eliminate false positives and elevated concentration values from matrix interferences. MS/MS does just that by ejecting all but the ion of interest out of the trap. Then a Collision-Induced Dissociation (CID) energy is applied to fragment the ion into a very unique product ion spectrum.

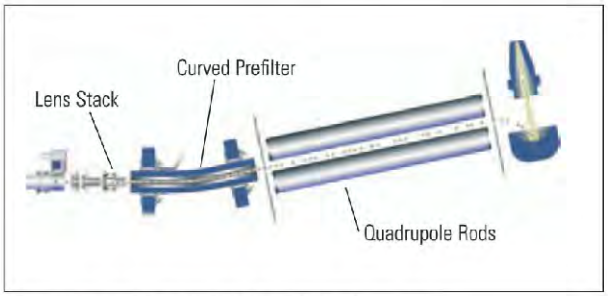

Figure 1: Thermo Scientific DSQ Bent Optics Quadrupole

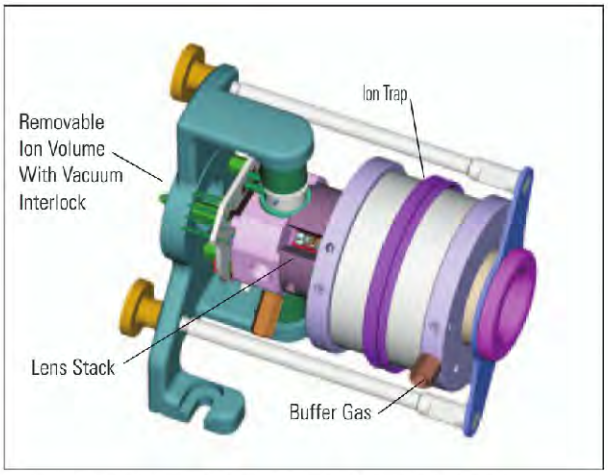

Figure 2: Thermo Scientific PolarisQ External Ionization Ion Trap

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Methods

A cold solvent split injection of 5 µL was made using the PTV, a temperature-programmable injector. The instrument parameters are listed in Table 1. A sample matrix was made by chopping up 5 grams of onion, garlic, broccoli, and tomato and sonicating in 20 mL of methylene chloride. The extract was filtered through glass wool and sodium sulfate to remove water and any particulate. Then the extract was split in half and transferred to 2 x 10 mL volumetric flasks. One was spiked with chlorinated pesticides, and internal standards were added to both. The flasks were then brought to a final volume of 10 mL. The final concentration of the spike was 10 pg/µL (40 ppb in matrix), and the internal standards were 400 pg/µL.

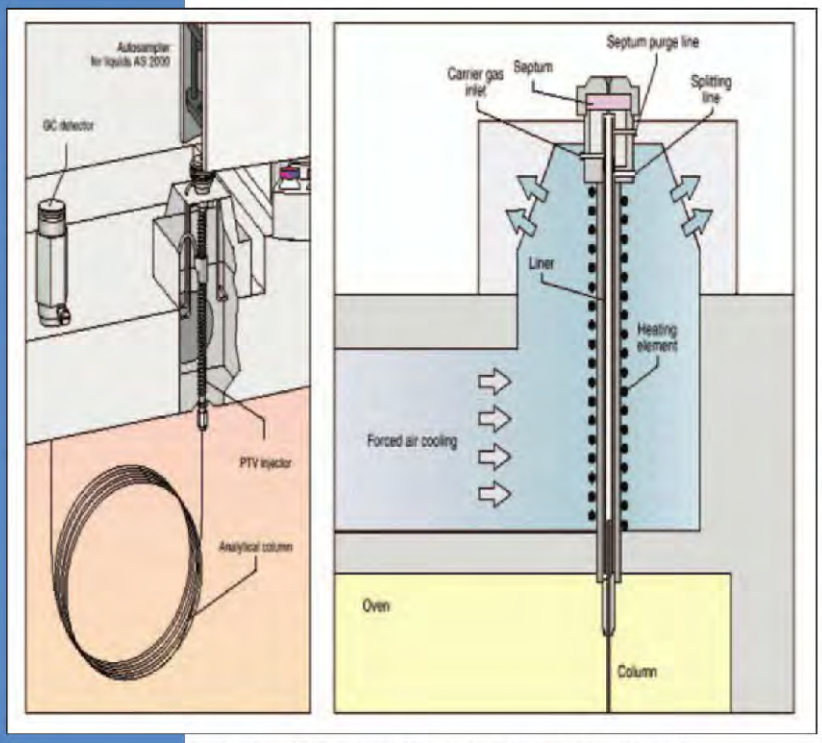

Figure 3: Programmable Temperature Vaporizing Injector (PTV)

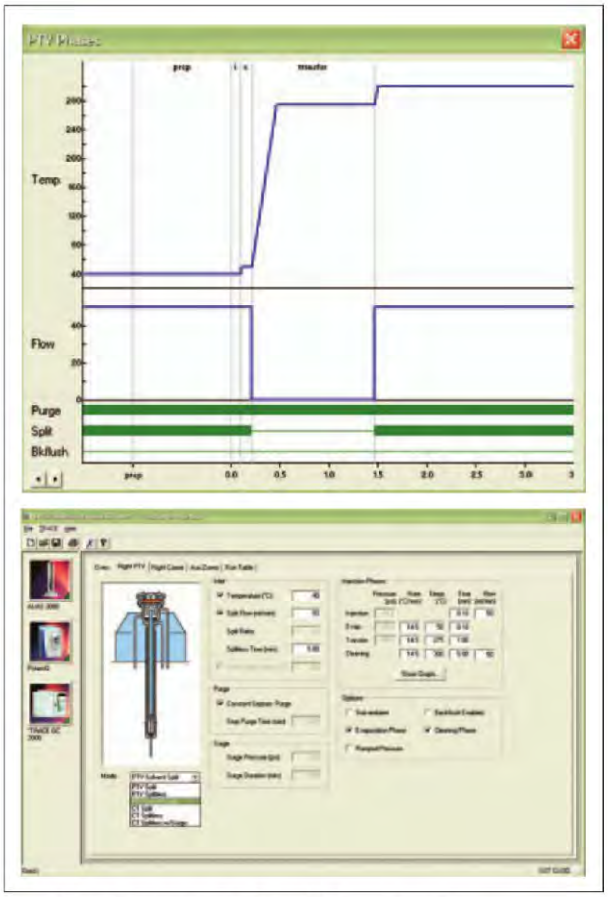

Figure 4: Selective Injection on PTV and Software Control

Method: Optimization of the Injection

A cold solvent split injection of 5 µL was made using the PTV, a temperature-programmable injector, configured with a silanized glass liner with a small wisp of silane-treated glass wool. The extract was injected at 40 °C for 6 seconds and then ramped to 50 °C for 6 more seconds with the split vent open to evaporate the solvent. The split vent was closed, and the pesticides were thermally transferred at 275 °C for one minute into the analytical column, which was at an initial temperature of 40 °C. Since the injector was only programmed to reach the highest temperature required for transfer of the heaviest pesticide, the higher boiling point sample matrix compounds were diverted out of the split vent during the injector cleaning phase. This allowed the run time to be shorter and the final temperature in the oven to be lower (only 275°, rather than 300 °C.) A lower final temperature reduces column bleed, so the ion source stays clean, and the life of the column is extended (Figure 4).

A performance mix containing 5 ng/µL of pentachlorophenol, DFTPP, benzidine, and 4,4'-DDT was run on each system at the start of each study to check for liner and column activity (Figure 5). Then the pesticides were run. A TIC of the standard is shown in Figure 5.

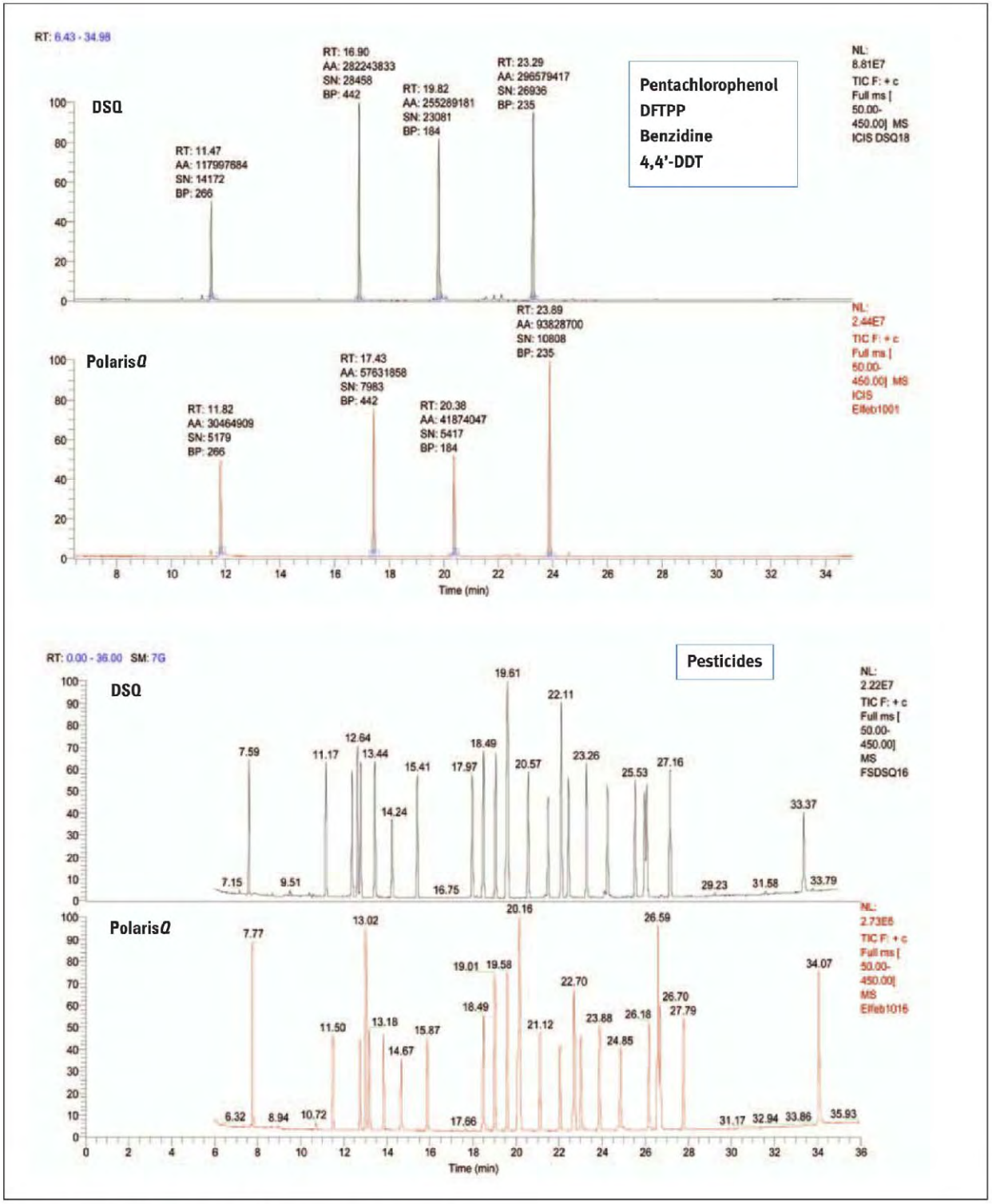

Figure 5: TIC on Polaris*Q* Ion Trap and DSQ Quadrupole of Performance Mix and Chlorinated Pesticides

TRACE GC ULTRA WITH AS3000 AUTOSAMPLER
PTV Cold Solvent Split 5 µL; Inject at 40 °C, evaporate 50 °C,transfer 275 °C, Clean 300 °C
Column: Rtx™-CLPesticides 0.32 mm x 30 m, 0.5 µm; Constant Flow: 1.5 mL/min
Oven: 40 °C, 1 min; 40 °C/min; 150 °C, 0 min; 4 °C/min, 275 °C
DSQ
MS: Source 250 °C; Multiplier: 1100 volts; Emission current: 100 µamp
Sequential FS & SIM Scan:
Full Scan: 50-450 *m/z* 2015 scans/sec
SIM: Dwell time 70-50 milliseconds width 0.2 amu; SIM: Exact mass +/- 0.1 amu (Table 2)
POLARIS*Q*
Trap optional buffer gas control: 2 mL/min helium
Source: 250 °C; Emission current: 250 microamps; Multiplier: 1125 volts
AGC: 50; 1 microscan; Default: Tune parameters: Autotune Tune File
Full Scan: 50 -450 *m/z*; Optimized MS/MS (Table 3)

Table 1: Instrument Parameters

Method: Optimization of the MS/MS

With the external source ion trap, the variable buffer gas control may be changed during the run to the optimal flow for the analyte. The buffer gas actually cools the kinetic energy of the ion to enhance the efficiency of isolation, which results in greater sensitivity. A study was done for a group of pesticides at different buffer gas flows to see what difference varying the helium flow in the trap would make for the overall response in Full Scan and then in the isolation step for MS/MS. The results are shown in Figure 6. The isolation experiment was done with the CID voltage at zero. The response in Full Scan increased with an increase in buffer gas flow. The optimum buffer gas flow for isolation in MS/MS was about 2 mL/min. The PolarisQ MS/MS parameters are listed in Table 3.

The ions are injected into the trap and, within milliseconds, a field is set up to stabilize only the ion of interest in the trap. The ion receives a pulse of CID voltage causing it to fragment into very unique product ions. Finally, these product ions are scanned out to generate a Full Scan spectrum for identification (Figure 7).

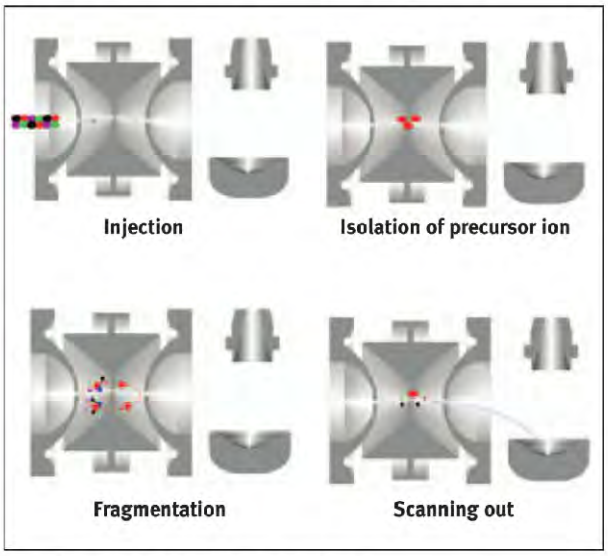

Figure 7: Tandem MS: Generating Product Ions

To illustrate the enhanced sensitivity for identification, Figure 8 shows the TIC in Full Scan versus MS/MS for alpha and gamma chlordane in the vegetable matrix at 40 ppb.

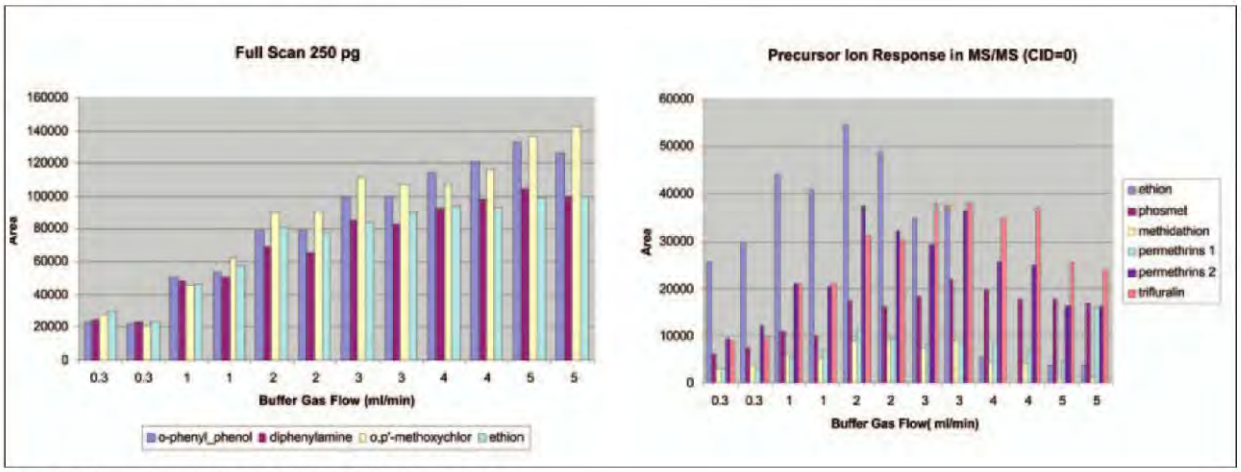

Figure 6: Enhanced Sensitivity with Variable Buffer Gas Control

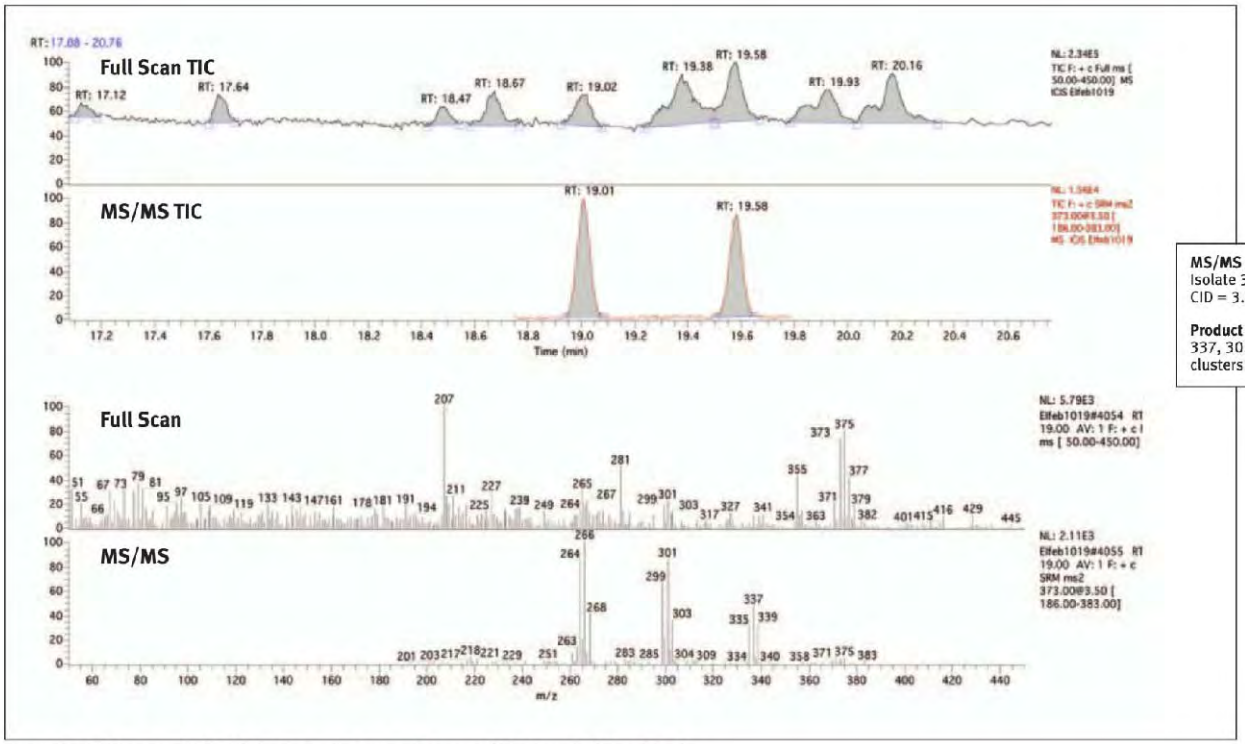

Figure 8: Full Scan and MS/MS Analysis of alpha and gamma Chlordane in Onion Matrix

Ret. Tm.		MW	SIM Ions			width amu	Dwell Time
7.6	acenaphthene-d10		164.1		FS 50-450		
11.18	alpha-BHC	288	180.8	182.9	108.9	0.2	50
12.39	gamma-BHC	288	181.0	183.0	109.0	0.2	50
12.65	phenanthrene-d10		188.1		FS 50-450		
12.8	beta-BHC	288	181.0	183.0	109.0	0.2	50
13.47	delta-BHC	288	181.0	183.0	109.0	0.2	50
14.26	heptachlor	370	100.0	271.7	273.7	0.2	100
15.43	aldrin	362	262.7	260.8	264.8	0.2	100
17.99	heptachlor epoxide	386	352.7	354.7	350.7	0.2	100
18.51	gamma-chlordane	406	372.7	374.7	271.7	0.2	100
19.07	alpha-chlordane	406	372.7	374.7	271.7	0.2	100
19.63	4,4'-DDE	316	245.9	247.9	176.0	0.2	50
19.56	endosufan I	404	194.9	158.9	169.9	0.2	50
20.59	dieldrin	378	79.0	262.8	278.8	0.2	100
21.5	endrin	378	262.8	81.0	82.0	0.2	100
22.13	4,4'-DDD	318	236.9	234.9	165.0	0.2	100
22.46	endosulfan II	404	194.9	158.9	169.9	0.2	100
23.39	4,4'-DDT	352	234.9	236.7	165.0	0.2	100
24.28	endrin aldehyde	378	67.0	344.8	249.8	0.2	100
25.56	methoxychlor	344	227.0	228.0	152.0	0.2	50
25.99	chrysene-d12		240.1		FS 50-450		
26.1	endosulfan sulfate	420	271.7	386.7	273.7	0.2	50
27.18	endrin ketone	378	316.8	67.0	318.8	0.2	100
33.38	perylene-d12		264.0		FS 50-450		

Table 2: DSQ SIM Parameters

						MSMS, BG = 2.0			
		EI FS Ions			precursor ion	width	CID	Q	product ions scan range
ret. Tm.									
7.77	acenaphthene-d10	164					50-450		
11.51	alpha-BHC	181	183	109	181	6	3	0.45	90-191
12.77	gamma-BHC	181	183	109	181	6	3	0.45	90-191
13.02	phenanthrene-d10	188					50-450		
13.19	beta-BHC	181	183	109	181	6	3	0.45	90-191
13.86	delta-BHC	181	183	109	181	6	3	0.45	90-191
14.67	heptachlor	100	272	274	272	6	3.5	0.45	90-191
15.88	aldrin	263	261	274	263	6	4.5	0.45	131-273
18.5	heptachlor epoxide	353	355	351	353	6	3.5	0.45	176-363
19.03	gamma-chlordane	373	375	272	373	6	3.5	0.45	186-383
19.6	alpha-chlordane	373	375	237	373	6	3.5	0.45	186-383
20.17	4,4'-DDE	246	248	176	246	6	4.5	0.45	123-256
20.1	endosufan I	195	339	341	195	2	3.5	0.45	97-200
21.13	dieldrin	79	263	279	263	6	4.5	0.45	131-273
22.06	endrin	263	81	82	245	4	4	0.45	122-255
22.72	4,4'-DDD	237	235	165	235	6	3.5	0.45	117-245
23.03	endosulfan II	337	339	341	195	2	3.5	0.45	97-205
23.89	4,4'-DDT	235	237	165	235	6	3.5	0.45	117-245
24.87	endrin aldehyde	67	345	250	345	6	2.5	0.45	172-355
26.19	methoxychlor	227	228	152	227	2	3.5	0.45	136-282
26.6	chrysene-d12	240					50-450		
26.71	endosulfan sulfate	272	387	422	272	6	3.5	0.45	136-282
27.8	endrin ketone	317	67	319	317	6	3.5	0.45	158-327
34.05	perylene-d12	264					50-450		

Table 3: PolarisQ MS/MS Parameters

Method: Optimization of the SIM

In order to optimize the sensitivity of SIM on the DSQ, the standard was run in Full Scan to determine the retention time and exact mass of each ion for SIM analysis. The width was set to +/- 0.2 amu with a dwell time as large as possible. The SIM parameters are listed in Table 2. Note the spectra produced by the DSQ in sequential FS and SIM and the spectra from the PolarisQ in sequential FS and MS/MS for Methoxychlor in vegetable matrix at 40 ppb (Figure 9).

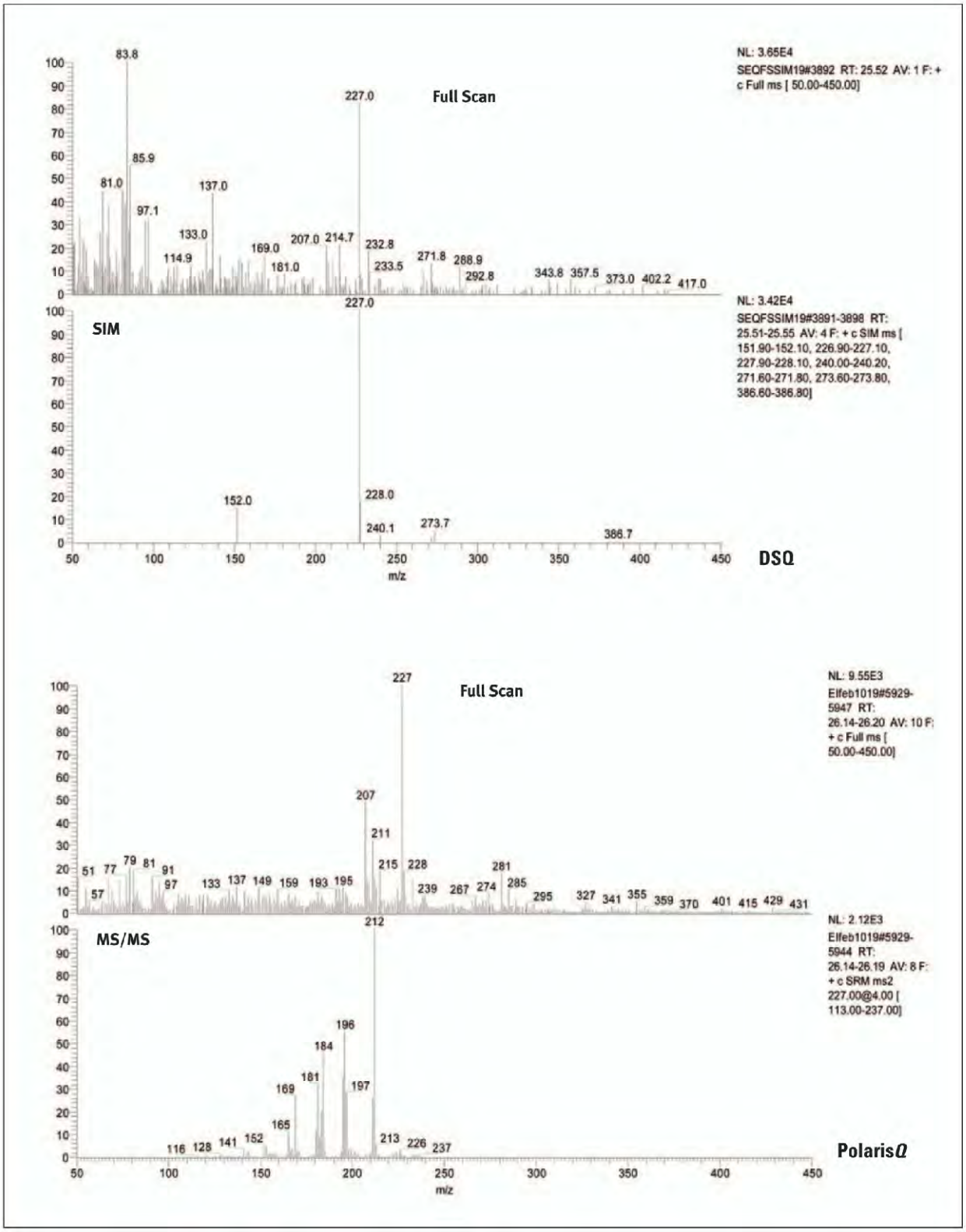

Figure 9: Spectra for Methoxychlor on the DSQ and Polaris*Q* in Matrix (40 ppb)

Results: False Positives and Spike Recoveries

The accuracy of the analysis is best measured in the recovery for the spiked pesticides in matrix and the absence of false positives in the unspiked matrix. Both the PolarisQ and the DSQ showed good linear fits from 1 pg/µL to 1 ng/µL with a 5 µL injection, as shown in Table 4. The PolarisQ gave no false positives and better detection on some compounds with MS/MS than observed in SIM on the DSQ. The tabulation of the data is shown in the chart in Figure 11 and in Table 5.

	1pg to 1 ng/L DSQ SIM R^2	1pg to 1 ng/uL PolarisQ MS/MS R^2
acenaphthene-d10	2.6	5.5
alpha-BHC	0.9988	0.9994
gamma-BHC	0.9983	0.9999
phenanthrene-d10	2.1	6.1
beta-BHC	0.9997	0.9991
delta-BHC	0.9999	0.9995
heptachlor	0.9978	0.9998
aldrin	0.9989	0.9975
heptachlor-epoxide	0.9984	0.9987
gamma-chlordane	0.9998	0.9991
alpha-chlordane	0.9976	0.9996
endosulfan-I	0.9983	0.9993
4,4'-DDE	0.9991	0.9995
dieldrin	0.9986	0.9996
endrin	0.9981	0.9981
4,4'-DDD	0.9997	0.9987
endosulfan-II	0.9998	0.9985
4,4'-DDT	0.9989	0.9927
endrin-aldehyde	0.9992	0.9981
methoxychlor	0.9984	0.9909
chrysene-d12	2.6	3.1
endosulfan-sulfate	0.9995	0.9996
endrin-ketone	0.9996	0.9992
perylene-d12	4.4	7.5

Table 4: Linear Fit Comparison of DSQ SIM and PolarisQ MS/MS

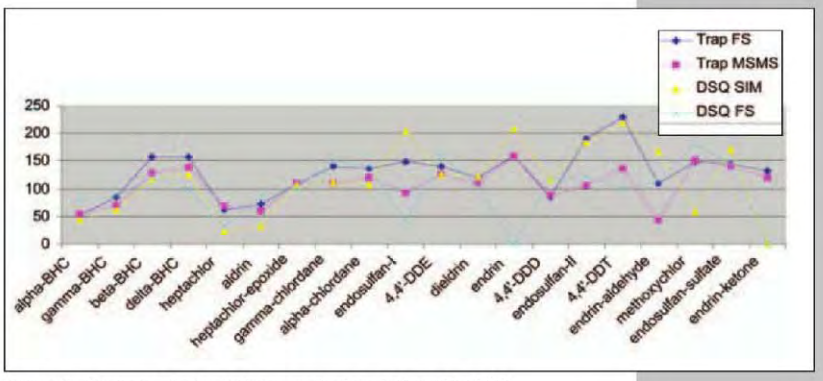

Figure 11: Recovery of 40 ppb Spike on the PolarisQ and the DSQ

	ND	False Positives	Recovery <50%	Recovery >150%
DSQ FS	2	0	3	5
DSQ SIM	1	8	4	6
PolarisQ FS	0	0	0	4
PolarisQ MS/MS	0	0	0	0

Table 5: Tabulation of False Positives and Overall Recovery

Conclusions

The PolarisQ in MS/MS eliminated the adverse effects from the vegetable matrix with spiked recoveries from 50 to 120%. The DSQ in SIM was not able to detect some of the spiked pesticides in matrix and gave elevated recoveries >150% for six compounds, and eight false positives on the unspiked matrix sample. MS/MS was able to eliminate the matrix from the quantitation of the target compounds at a 40 ppb spike in matrix with the variable buffer gas at 2 mL/min. The PolarisQ gave unequivocal confirmation from the product ion spectra in MS/MS.

Acknowledgement

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Application Note: 355

Utility of H-SRM to Reduce Matrix Interference in Food Residue Analysis of Pesticides by LC-MS/MS Using the TSQ Quantum Discovery

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Key Words

- TSQ Quantum Discovery™
- Surveyor™ HPLC
- H-SRM
- Pesticides
- Zero Cross-talk

Introduction

With the recent trend of increased concern about food safety, the number of regulated pesticide residues in food has increased rapidly. In Japan, a new positive list system for monitoring pesticide residues will take effect in 2006. Consequently, an accurate high throughput multi-pesticide screening method which can quantitate high number of pesticide residues during a single analysis is required.

LC-MS/MS is fast becoming the technique of choice for the identification and quantitation of pesticide residues. This is due, in part, to the ease of sample preparation and chromatographic conditions that LC-MS/MS allows, when compared to other techniques such as GC or HPLC with UV absorbance, nitrogen phosphorus detection, or electron capture detection. However, it can be extremely challenging to quantitate multi-pesticide residues in food because of interference from complex sample matrices. Although matrix-related interferences can be decreased by various sample clean up procedures, the analytical instrument used for the quantitation also has to be highly selective and sensitive. The unique Highly-Selective Reaction Monitoring (H-SRM) detection method available with the Thermo Scientific TSQ Quantum has proven to be very useful for this purpose. The analytical results of 35 pesticide residues in food with the H-SRM detection method are reported in this application note.

Goals

- Illustrate the effectiveness of H-SRM for reducing background interference and improving s/n
- Develop a multi-residue LC-MS/MS screening method to detect 35 pesticides, and
- Exhibit the absence of "cross-talk" between co-eluting components.

Experimental Conditions

Sample Analysis

HPLC analysis was performed on the Thermo Scientific Surveyor HPLC System, using a Thermo Scientific HyPURITY™ C18 150 × 2.1 mm 5 µm column. Mobile phase A was water, mobile phase B was methanol, and mobile phase C was water containing 10 mM ammonium acetate. Solvent was pumped at 200 µL/min and analytes eluted using a linear gradient of 20% B to 99% B over 15 minutes, holding at 99% B for 3 minutes, and then returning to 20% B for 5 minutes. Mobile phase C was held at 1% throughout the run.

Mass Spectrometry

Instrument: TSQ Quantum Discovery

Positive ESI

Spray Voltage: 5kV
Sheath/Auxiliary gas: Nitrogen
Sheath gas pressure: 40 (arbitrary units)
Auxiliary gas pressure: 40 (arbitrary units)
Ion transfer capillary temperature: 380 °C
Scan type: SRM or H-SRM
CID conditions: Ar at 1.0 mTorr

Negative ESI

Spray Voltage: 4.25 kV
Sheath/Auxiliary gas: Nitrogen
Sheath gas pressure: 50 (arbitrary units)
Auxiliary gas pressure: 5 (arbitrary units)
Ion transfer capillary temperature: 350 °C
Scan type: SRM or H-SRM
CID conditions: Ar at 1.0 mTorr

MS Instrument Method

Thirty-five pesticide residue compounds were analyzed to find the product ion to be used for quantitation. Three of the compounds were ionized using negative electrospray, while the remaining 32 were ionized using positive electrospray in two different runs. A table of the compounds listing SRM transitions and the optimum collision energy are shown in Table 1.

Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)	Retention Time (min)
Oxamyl	237.17	72.0	15	3.9
Imidacloprid	256.12	209.1	16	6.3
Acetamiprid	223.12	126.0	23	7.3
Aldicarb	208.17	116.0	8	9.0
Propoxur	210.16	111.0	14	10.3
Carbofuran	222.16	165.1	14	10.4
Bendiocarb	224.14	167.0	10	10.4
Carbaryl	202.15	145.0	10	11.0
Ethiofencarb	226.13	107.0	14	11.3
Pirimicarb	239.22	182.1	16	11.5
Methabenzthiazuron	222.12	165.0	17	11.9
MIPC	194.17	95.0	20	11.9
Diuron	233.06	72.1	19	12.4
Azoxystrobin	404.17	372.1	15	12.8
BPMC	208.19	152.0	10	13.1
Siduron	233.20	137.0	17	13.2
Linuron	249.09	182.0	18	13.2
Methiocarb	226.14	169.1	10	13.4
Daimuron	269.21	151.1	14	13.7
Cumyluron	303.14	185.0	14	13.9
Tebufenozide	353.24	133.0	19	14.7
Iprodione	330.07	245.1	15	14.7
Diflubenzuron	311.04	158.0	14	14.8
Etobenzanid	340.08	121.0	36	15.2
Cyprodinil	226.18	93.0	38	15.2
Phoxim	299.08	129.0	12	15.4
Bitertanol	338.21	269.2	10	15.6
Hexythiazox	353.13	228.0	16	16.8
Piperonyl butoxide	356.26	177.1	13	17.2
Flufenoxuron	489.09	158.0	20	17.4
Fenpyroximate	422.26	366.1	15	17.6
Chlorfluazuron	540.03	382.9	20	17.8
Teflubenzuron	379.00	339.0	12	17.08
Hexaflumuron	459.02	439.0	12	16.04
Lufenuron	509.00	326.0	18	16.77

(Positive in Black, Negative in Red)

Table 1: Summary of SRM transitions used for the analysis

Results and Discussion

Figure 1a shows the chromatogram of the 32 pesticides in positive ESI, and Figure 1b shows the three pesticides under negative ESI, all eluting over a chromatographic time scale of 18 minutes. While some compounds co-elute, the specificity of the H-SRM method allows for the individual quantitation and detection of each component, even at very low levels. A summary of the calibration range, linearity, and the reproducibility of each individual compound at 5 ppb (ng/mL) is tabulated in Table 2.

Effect of H-SRM on Detection Limits

H-SRM is an acronym for Highly-Selective Reaction Monitoring (H-SRM), which is a more advanced form of Selective Reaction Monitoring (SRM). Although traditional SRM is a selective technique by itself, it still can not completely eliminate the interference from some food matrix components. Sometimes, it is possible to get incorrect qualitative results or the quantitative analysis can not reach the required detection limits of targeted compounds due to matrix-related interferences. The traditional SRM experiment, using a triple quadrupole instrument, is usually conducted with unit resolution (0.7 FWHM) for the precursor ion. With the more advanced H-SRM, the precursor ion is selected with a peak width of 0.1-0.2 FWHM. The more stringent tolerance accounts for the higher selectivity, which can lower LOQs and increase precision and accuracy at the limits of detection. This can also, in effect help reduce the overall bench time required for sample preparation.

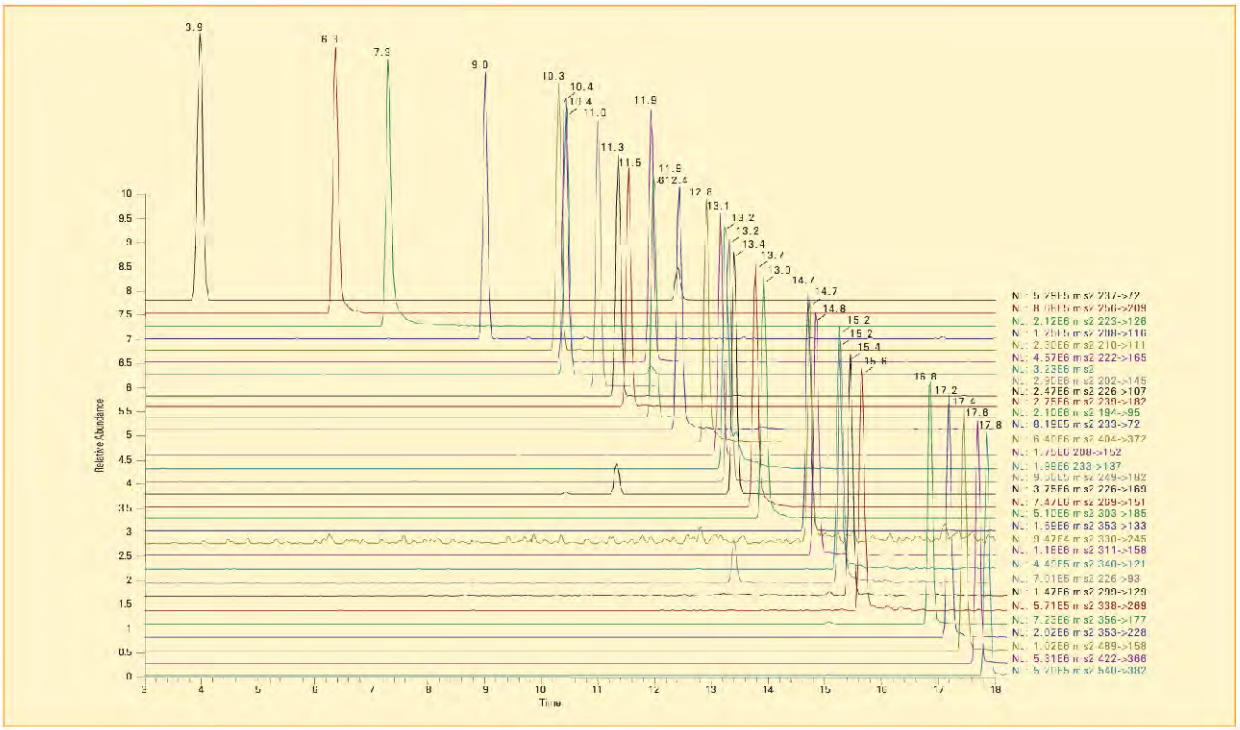

Figure 1a: LC-MS/MS chromatogram of 32 pesticides at 10 ng/mL, positive ESI

Compound	R^2	Range (ppb)	CV(%), n=5
Oxamyl	1.000	0.01-100	1.79
Imidacloprid	0.9994	0.05-100	2.84
Acetamiprid	0.9987	0.05-100	1.17
Aldicarb	0.9993	0.05-100	6.89
Propoxur	0.9997	0.01-100	1.70
Carbofuran	0.9996	0.05-100	0.95
Bendiocarb	0.9992	0.01-100	2.30
Carbaryl	0.9999	0.01-100	1.44
Ethiofencarb	0.9996	0.01-100	2.64
Pirimicarb	0.9995	0.01-100	3.55
Methabenzthiazuron	0.9989	0.01-100	1.73
MIPC	0.9987	0.01-100	1.26
Diuron	0.9987	0.05-100	2.23
Azoxystrobin	0.9989	0.01-100	2.60
BPMC	0.9999	0.05-100	1.57
Siduron	0.9989	0.05-100	1.59
Linuron	0.9989	0.05-100	4.04
Methiocarb	0.9997	0.01-100	1.88
Daimuron	0.9992	0.01-100	3.03
Cumyluron	0.9993	0.01-100	3.17
Tebufenozide	0.9995	0.05-100	1.83
Iprodione	0.9979	0.5-100	6.17
Diflubenzuron	0.9997	0.01-100	2.98
Etobenzanid	0.9997	0.05-100	1.82
Cyprodinil	0.9998	0.1-100	4.49
Phoxim	0.9997	0.05-100	3.14
Bitertanol	0.9996	0.05-100	3.54
Piperonyl butoxide	0.9996	0.01-100	1.65
Hexythiazox	0.9999	0.01-100	2.43
Flufenoxuron	0.9997	0.01-100	3.63
Fenpyroximate	0.9999	0.01-100	2.22
Chlorfluazuron	0.9987	0.01-100	2.77
Teflubenzuron	0.9986	0.01-100	2.35
Hexaflumuron	0.9973	0.01-50	1.58
Lufenuron	0.9998	0.01-10	2.56

Table 2: Calibration range and linearity of each compound, as well as the reproducibility of each compound at 5 ppb

The effects of H-SRM over SRM are clearly illustrated for the three pesticides Iprodione, Biteranol and Etobenzanid in Figures 2 a, b, and c.

Absence of Cross-talk

In order to quantitate mixtures of many compounds accurately, it is necessary to use short scan speed to ensure sufficient data points for integration. It is important that the system can maintain its sensitivity without cross-talk even at short scan speeds. Cross-talk occurs when ions from one scan event are still present in the collision cell when a second SRM transition is taking place. This leads to signal artifacts in the next transition's chromatogram. This can be especially problematic when different SRM events have the same product ions formed from different precursor ions. Thermo Fisher Scientific's patented design of the orthogonal collision cell used on the TSQ Quantum product line eliminates cross-talk. Figure 3a shows the absence of cross-talk between two different SRM transitions, pirimicarb and linuron. Both yield a product ion of 182, and no artifacts are seen in either chromatogram, even when magnified 100-1000 times. The same effect is shown in Figure 3b for diflubenzuron and flufenoxuron for a common product ion of 158 for dwell times of 20 msec.

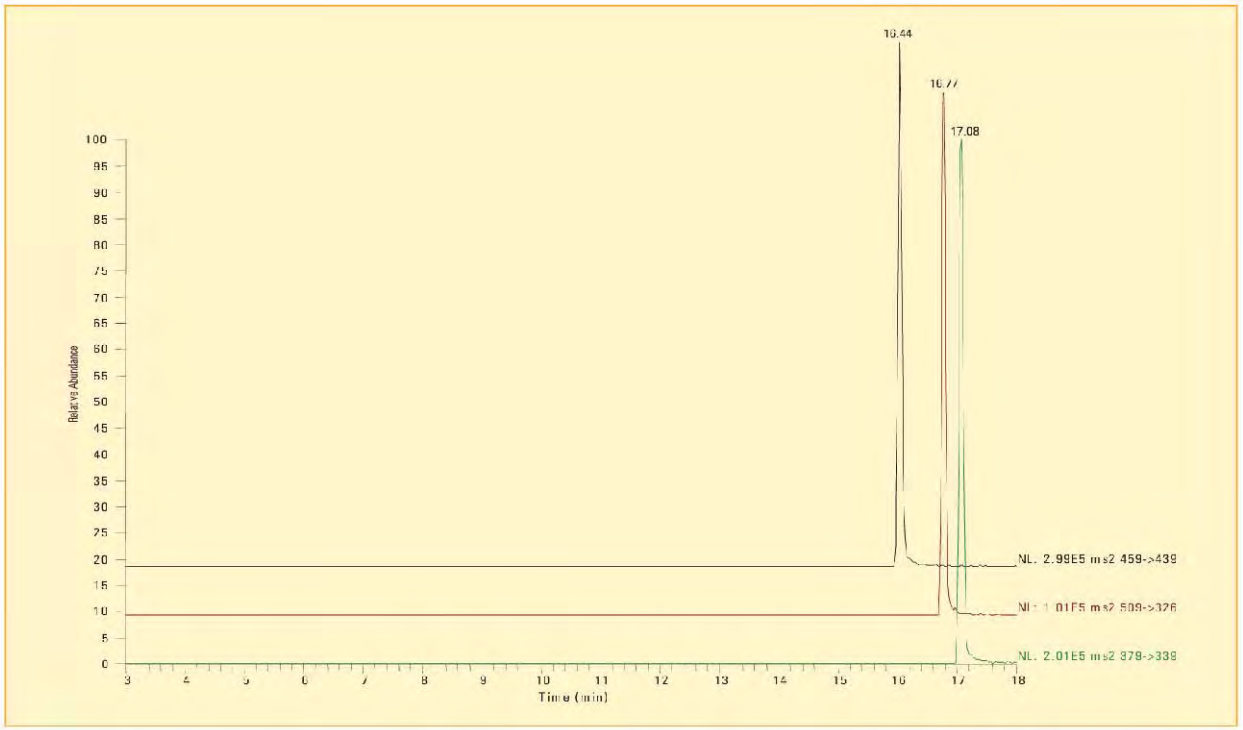

Figure 1b: LC-MS/MS chromatogram of 3 pesticides at 10 ng/mL, negative ESI

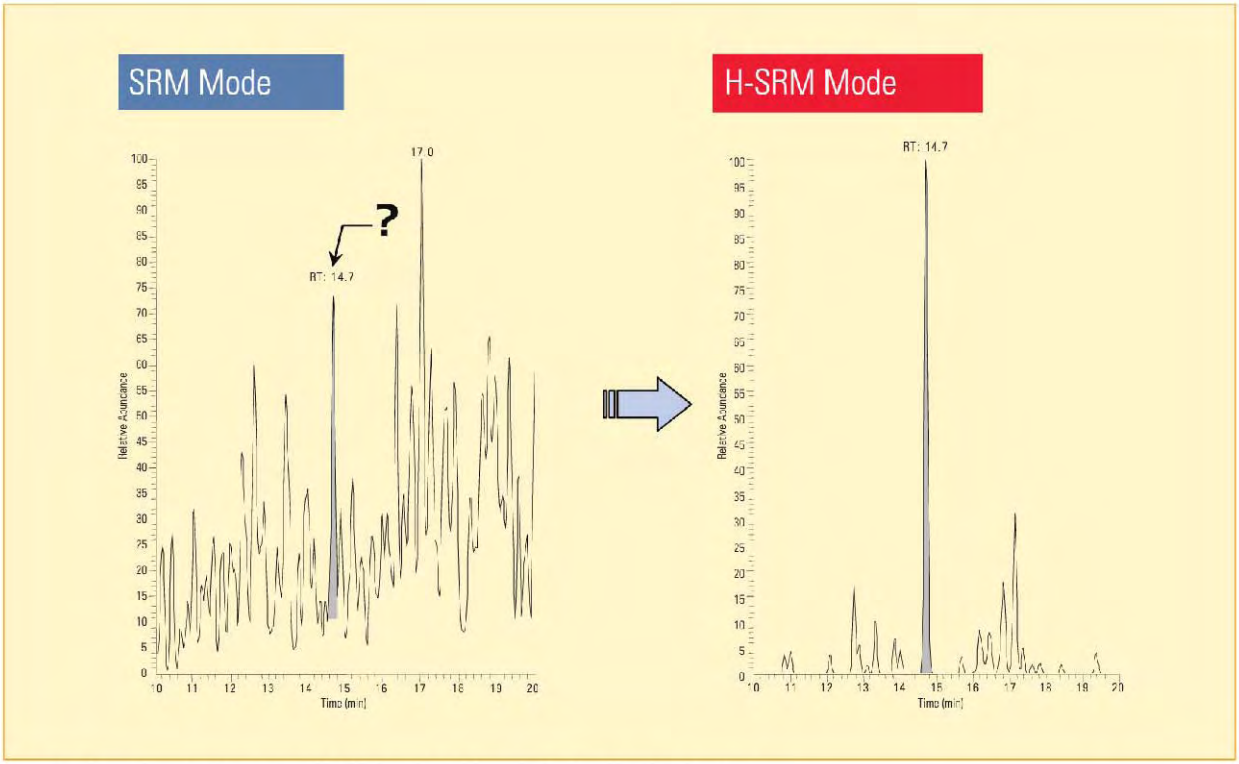

Figure 2a: Comparison of SRM mode and H-SRM mode for the analysis of the fungicide Iprodione

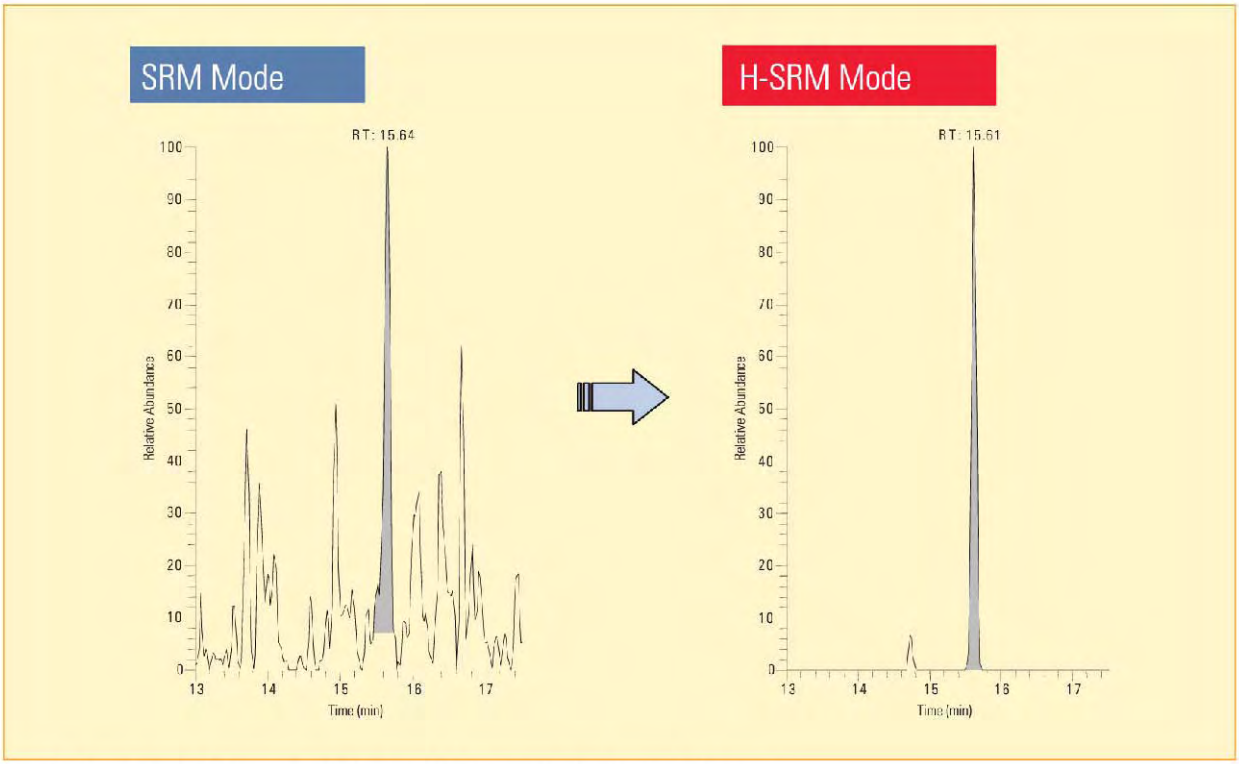

Figure 2b: Comparison of SRM mode and H-SRM mode for the analysis of the fungicide Bitertanol

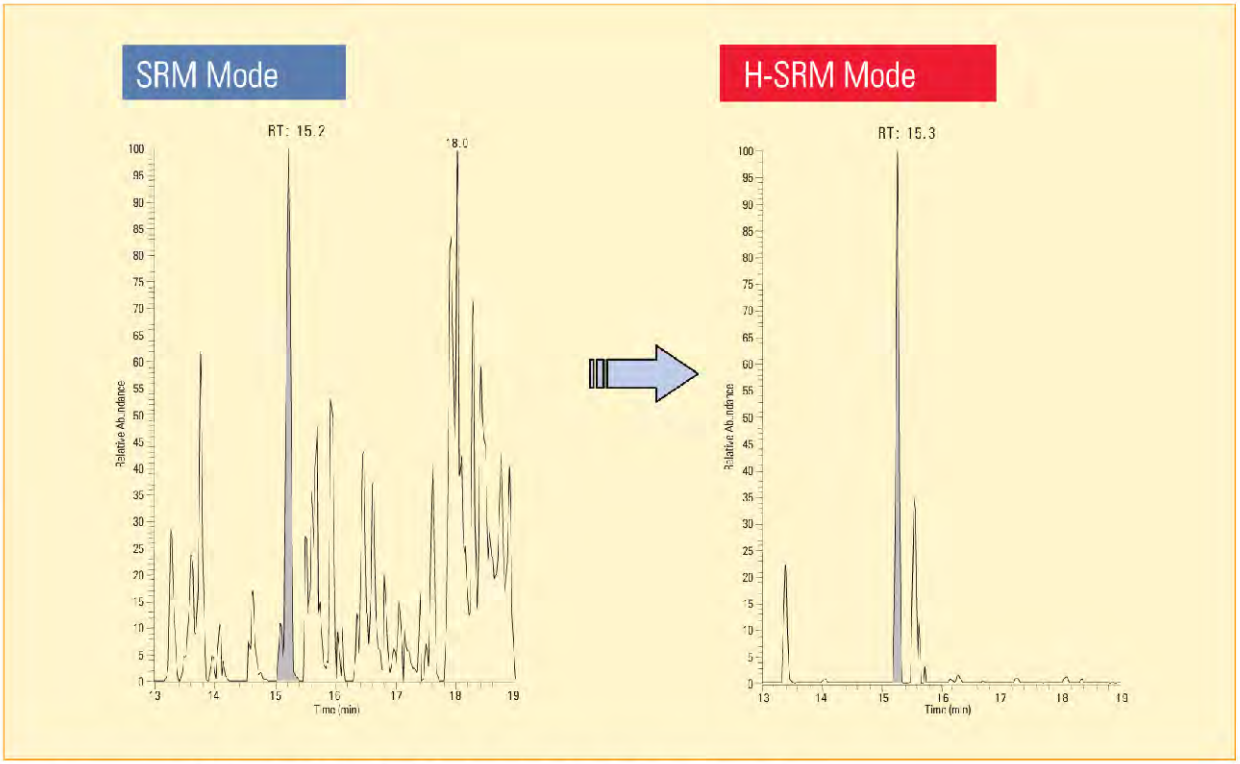

Figure 2c: Comparison of SRM mode and H-SRM mode for the analysis of the herbicide Etobenzanid

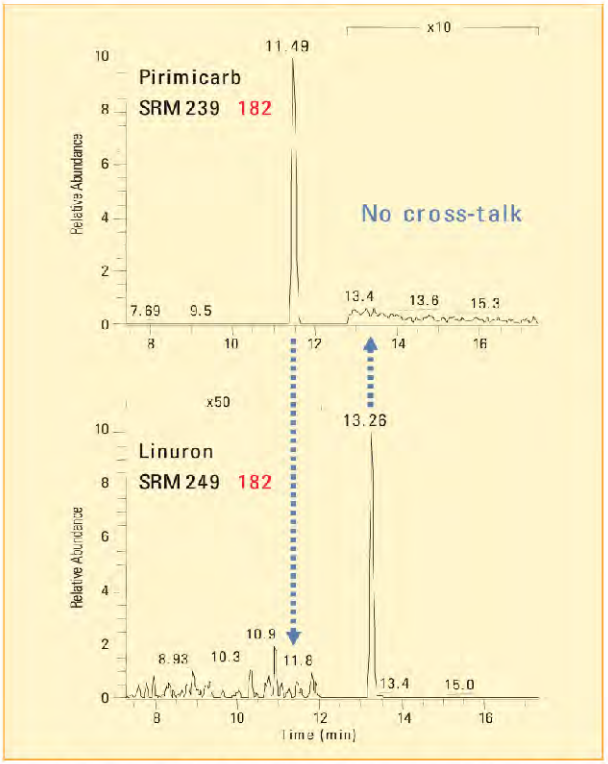

Figure 3a: No cross-talk is observed for the SRM transitions of primicarb and linuron

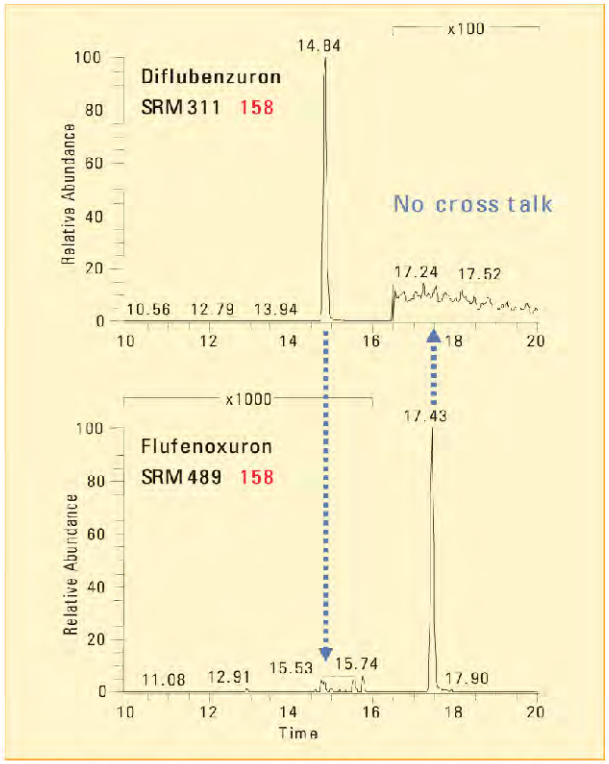

Figure 3b: No cross-talk is observed for the MRM transitions of diflubenzuron and flufenoxuron

Conclusions

An H-SRM LC-MS/MS method to monitor 35 pesticide residues was developed using the TSQ Quantum Discovery. All 35 pesticide residues were quantitated in 18 minutes. Using H-SRM, interferences from the sample matrix background were substantially reduced, leading to improved LOQs. Similarly, no cross-talk issues were detected for any of the tested analytes.

Compared with traditional single pesticide analysis methods, the sample preparation procedures are usually simplified in multi-pesticide analysis methods. This means more interference from the sample matrix may be present making H-SRM the technique of choice for improving detection limits.

Application Note: 10049

Trace Determination of Organo-Phosphorous Pesticides in Olive Oil by GC Analysis through PTV Backflush / FPD

Thermo Fisher Scientific Inc., Milan, Italy

Key Words

- TRACE GC Ultra
- Olive Oil
- Organo-Phosphorus Pesticides
- ppb Levels
- PTV Backflush
- FPD Selectivity

Introduction

Organo-Phosphorous Pesticides (OPP) are widely used in agriculture, due to their relatively low cost, broad spectrum of activity, and high impact on insects compared to other pesticides. However, because the OPPs are well known to cause irreversible effects on the nervous system (reduced activity of neurotransmitters), their possible presence as trace residues in food must be strictly monitored. In this respect, one critical application is the control for OPPs in olive oil.

This class of compounds can effectively be analyzed by Gas Chromatography using a Programmable Temperature Vaporizing (PTV) injector and a Flame Photometric Detector featuring extremely high sensitivity and selectivity for phosphorus containing compounds.

The PTV injector is found to be particularly suitable for samples like edible oils, characterized by the presence of heavy fractions in potentially dirty matrices. The conventional Split-Splitless injector is advantageously able to be kept at a low temperature during the sample introduction phase. This prevents any sample evaporation from the syringe needle, hence eliminating a source of discrimination of higher boiling components. On the other hand, compared to the On-column injector, it allows non-volatile sample by-products to be retained in the vaporization chamber, thus preventing any decay of the column performance in time due to by-products accumulation.

This type of analysis requires high oven temperatures and short columns with a very thin film in order to allow complete elution of the main constituents of vegetable oil, triglycerides. Additionally, the sample must also be very diluted in order to avoid overloading the column with this primary fraction (for quantity) and consequent contamination of the detector. These two factors make trace analysis of contaminants even more complex. To overcome these problems, the heavier fraction is usually completely eliminated with an extended sample preparation step prior to GC analysis.

This paper describes an alternative way to effectively and rapidly analyze OPPs in oils eliminating any interference with the heavy fraction. The use of a special accessory vents the heavier components of the sample when these are not of interest.

Back-flush Device for PTV Injector

The Thermo Scientific TRACE GC Ultra™ equipped with a PTV inlet and a reverse flow device (back-flush) is used for this application. This accessory consists basically of a 3-way solenoid valve (back-flush valve) placed in the carrier gas line, a wide-bore pre-column, a high temperature "T" connector housed in the GC oven connecting the pre-column to the column, and a calibrated flow restrictor (Figure 1).

When the back-flush valve is off (Figure 2), the carrier gas flows in its normal direction through the inlet. A very small flow provided by the restrictor is able to constantly purge the "T" connector between the pre-column, analytical column, and back-flush inlet line. The pre-column consists of a 2 m x 0.53 mm i.d. uncoated fused silica tubing, and the purge flow is about 5 % of the column flow.

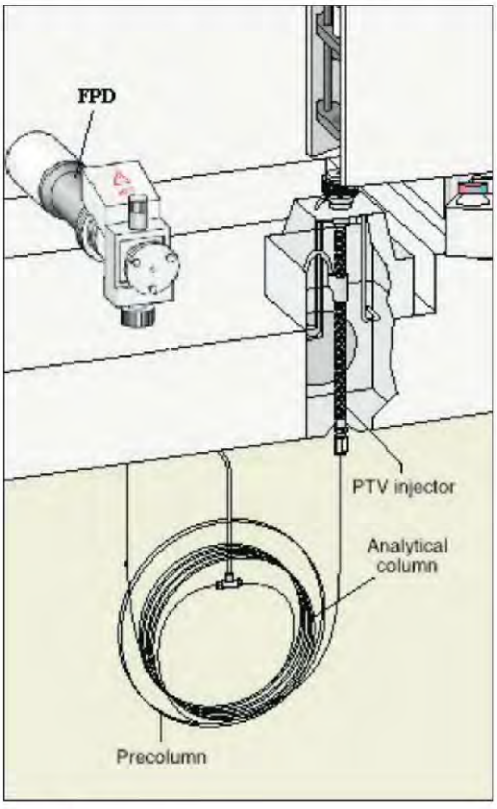

Figure 1: PTV-FPD configuration.

When the back-flush valve is switched on, the system diverts the gas directly to the "T" connection at the end of the pre-column, therefore, sweeping both the latter and the inlet in the opposite direction, with a so called "reverse flow". In this configuration, the carrier gas is able to "flush" anything still in the pre-column or in the injector directly to the vent and through the injector's split line. The small flow provided by the restrictor in the other direction will prevent the back-flushed material to flow through the inlet liner.

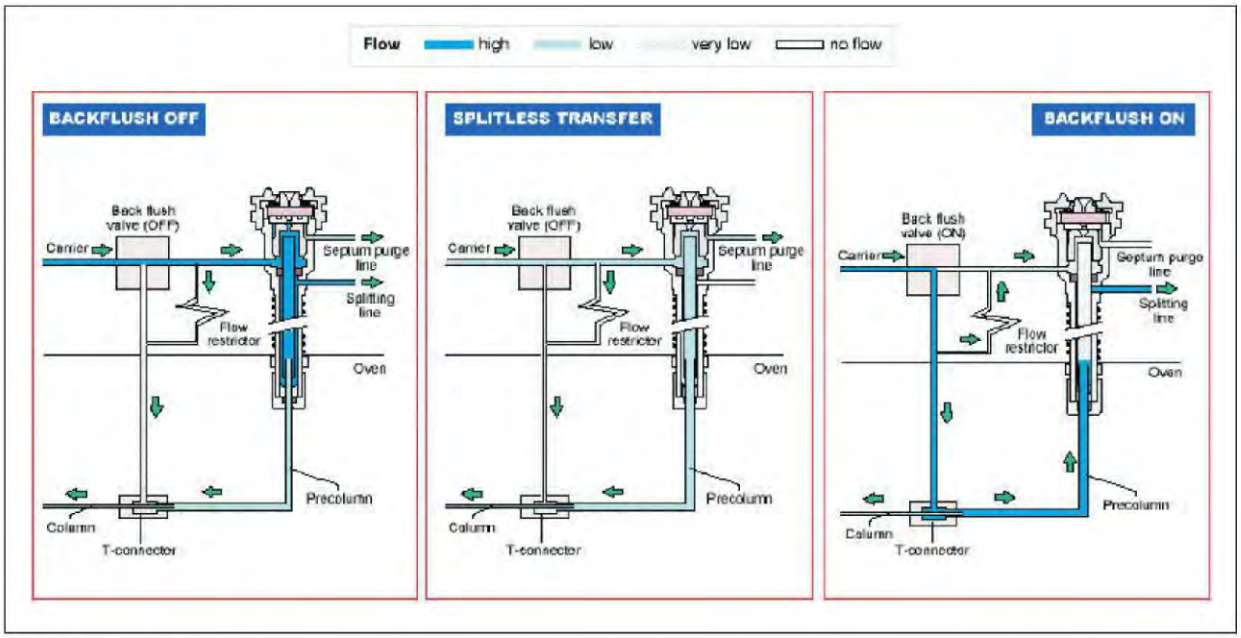

Figure 2: Reverse flow device

In order to clearly demonstrate the effect of the reverse flow device, 2 µL of virgin olive oil diluted 1:10000 in acetone are injected in a TRACE GC Ultra equipped with PTV injector and FID detector. An OV-5, 7 m long, 0.25 mm i.d., 0.25 µm f.t. column is used, together with a 2 m, 0.53 mm i.d. deactivated pre-column. The oven ramp is 60 °C (3 min) to 100 °C at 8 °C/min, then to 380 °C (10 min) at 20 °C/min. The PTV initial Temperature is 80 °C (hold 0.1 min) then ramped at 14.5 °C/sec up to 380 °C (held for all the analysis), with a splitless time of 3 minutes and a split flow of 50 mL/min. Helium is used as carrier gas at constant pressure (55 kPa). Finally the FID detector base body temperature is set at 350 °C.

The same sample is then injected in the PTV equipped with the back-flush device. Since the heavier fraction is now vented out by the reversed flow, the sample is diluted only 1:1 in acetone.

Sensitivity towards the compounds of interest is simply increased by 4 orders of magnitude, and the absence of the predominant fraction allows both to eliminate the risk of column overloading and to target separation optimization on the lighter components only.

Figure 3 shows the two chromatograms obtained with and without back-flush valve activation respectively. The complete absence of the triglycerides in the second chromatogram proves the effective reliability of the reverse flow enabled after 3 minutes. This timing is proven to be sufficient to allow transfer of the compounds of interest into the analytical column, while diverting any residual heavy fraction into the pre-column for venting.

Analysis of OPPs in Olive Oil

The same equipment is used for the determination of Organo-Phosphorous Pesticides with exception of the detection system. A highly sensitive phosphorous-selective FPD detector is used in place of the FID. Performance and repeatability tests are performed by injecting 2 µL of virgin olive oil spiked with 50/100 ppb of OPPs mixture. Also, in this case, the sample is diluted only 1:1 with acetone, and the optimum conditions for the separation of OPPs are applied. An SE54, 10 m long, 0.25 mm i.d., 0.1 µm f.t. capillary column is used, together with a 2 m, 0.53 mm deactivated pre-column. The GC oven temperature starts with an isotherm at 60 °C (1 min) and is then raised to 350 °C (10 min) at 8 °C/min. The PTV Temperature ranges between 50 °C (0.1 min) and 400 °C (held for all the analysis) at 10 °C/sec, with a splitless time of 1 minute. Helium is used as carrier gas at constant flow (1.5 mL/min), and the FPD detector is set at 300 °C. A 300 mL/min back-flush flow is enabled after 16 minutes.

Figure 4 reports the related chromatogram, together with the repeatability of retention times and peak areas based on 10 consecutive injections, showing excellent separation and sensitivity. Three different commercial olive oils were tested under the same conditions (Figure 5): only Fenthion resulted present in Oil 1 and Oil 3 in different amounts, while Oil 2 was found to be completely destitute of such pesticides. A large number of injections of oil (over 100) were performed without replacing the liner or the pre-column, and no degradation of chromatographic performance was observed.

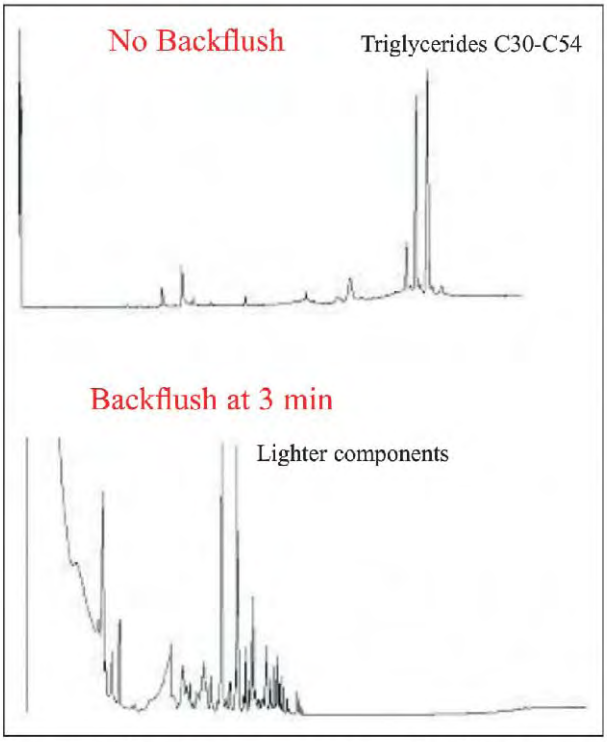

Figure 3: Olive Oil analysis with and without reverse flow; Detector: FID

PEAK NUMBER	SAMPLE COMPOUND	RETENTION TIMES AVERAGE (MIN)	RSD%	PEAK AREAS AVERAGE (COUNTS)	RSD%
1	Dimethoate	16.24	0.08	371681	3.1
2	Parathion-methyl	19.85	0.06	290948	2.6
3	Chlorphiriphos-methyl	18.97	0.05	134474	3.0
4	Malathion	20.04	0.08	174849	5.8
5	Fenthion	20.23	0.04	229989	2.5
6	Chlorphiriphos-ethyl	20.98	0.08	132520	3.7
7	Methidathion	21.89	0.04	826901	3.8

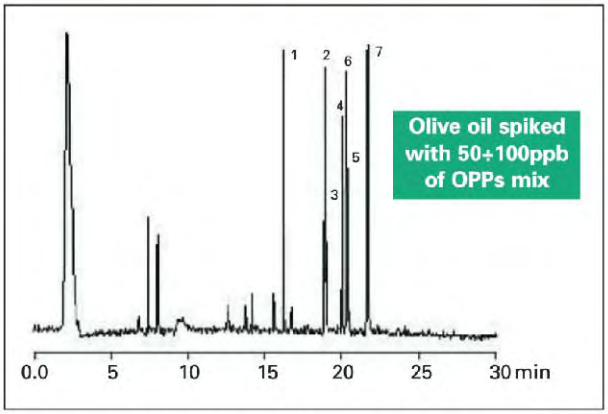

Figure 4: Repeatability Test based on 10 injections; Detector: FPD

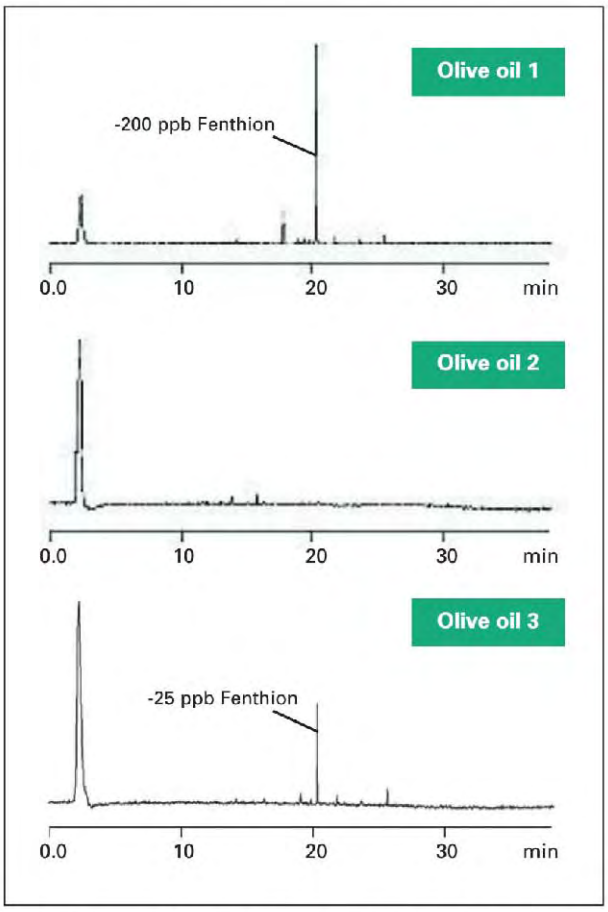

Figure 5: Detection of Fenthion in 3 commercial olive oils; Detector: FPD

Conclusions

OPPs in olive oil matrix can effectively be analyzed with PTV and FPD, provided that the triglycerides are vented out by a reverse flow device. Under these conditions, performance of the PTV injector is found to be greatly improved. The total analysis time is much shorter since no extra waiting time for complete elution of the high boiling components is now required. Sensitivity can be increased by four orders of magnitude (a few ppb) simply through the injection of a more concentrated sample.

Two additional important benefits obtained with the use of the back-flush are the highly extended column lifetime and the strongly simplified sample preparation procedure, which now only requires the dilution of the olive oil with acetone solvent.

Acknowledgement

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Application Note: 425

Determination of Different Classes of Pesticide Residues in Processed Fruits and Vegetables by LC-MS Using the TSQ Quantum Ultra According to EU Directive 91/414 EEC

Eleni Botitsi, Anastasios Economou, Spyros Antoniou, and Despina Tsipi;
General Chemical State Laboratory, Pesticide Residues Laboratory, Athens, Greece

Key Words

- TSQ Quantum Ultra™
- Surveyor™ HPLC System
- H-SRM
- Food Safety
- Pesticide Residues
- Sensitivity

Introduction

A diet rich in fruits and vegetables is thought to reduce the risk of some types of cancer, atherosclerosis, and heart disease. However, commercially grown produce often contains high levels of pesticide residues that can lead to serious health problems when consumed. Due in large part to growing public concern over the amount of pesticide residues in foods, the European Union (EU) has enacted several directives to fix Maximum Residue Limits (MRLs) for different pesticide residues in food of plant origin. MRLs represent the maximum amount of pesticide residues that might be expected in a commodity produced under conditions of good agricultural practice and typically range between 0.01 mg/kg and 10 mg/kg[1]. Although MRLs are not maximum toxicological limits, care is taken to ensure that these maximum levels do not generate toxicological concerns. Thus far, MRLs have been set for approximately 250 active substances. To cover the full variety of agricultural raw commodities (approximately 260 products of plant and animal origin), MRLs must be established for more than 260,000 pesticide/commodity combinations[1,2]

In the EU, pesticides are regulated principally by Directive 91/414/EEC concerning the placing of plant-protection products on the market[3]. According to this legislation, chemical substances or micro-organisms in pesticides are approved for use only if they have undergone a peer-reviewed safety assessment. All foodstuffs intended for human consumption or animal feed in the EU are now subject to a maximum residue limit for pesticides to protect human and animal health. Regulation (EC) 396/2005[4] consolidates in a single act all the limits applicable to various types of food and feed. It establishes MRLs for products of plant and animal origin at the Community level, taking into account good agricultural practices. It was based on several substantial amendments in the Council Directives:

- 76/895/EEC[5], which relates to the fixing of maximum levels for pesticide residues in and on specific fruits and vegetables
- 86/362/EEC[6] for cereals and cereal products
- 86/363/EEC[7] for products of animal origin
- 90/642/EEC[8] for plant products

Additionally, more stringent legislation has been established concerning pesticides in baby food. Since 1999, the EU has introduced the Commission Directives 1999/39/EC[9] and 1999/50/EC[10], which limit all pesticide residues to an MRL value of 0.010 mg/kg in processed cereal-based foods and in fruit and vegetables intended for the production of baby foods. MRLs below 0.010 mg/kg have been established for a few pesticides of higher toxicity, while the use of certain very toxic pesticides has been completely prohibited in the production of baby foods, as underlined in Commission Directives 2003/13/EC[11] and 2006/125/EC[12].

New "active" ingredients entering the market to replace compounds banned by Directive 91/414/ EEC possess considerably different physicochemical properties, and thus demand the development of multi-residue analytical methods. Analytical methodologies used to determine pesticide residues in foods must be capable of quantifying very low levels of residues as well as confirming their identity. This task becomes more difficult as MRLs are decreased and the number of target pesticides and metabolites increases. Therefore, the challenge is to develop a sensitive, cost-effective, multi-residue analytical method that can quickly identify and confirm pesticide residues belonging to various chemical classes in food products. At the same time, the method must accurately quantify these residues at low levels, thus fulfilling the performance criteria described in "Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed," European Commission Document SANCO 2007/3131[13].

Goal

To develop a multi-residue liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method for the detection and quantification of 45 pesticides, including parent compounds and their transformation products from different chemical classes, in various food matrices.

Experimental Conditions

LC-ESI-MS/MS is the analytical technique of choice to assay environmental and food matrices with high sensitivity and selectivity. The technique is especially well-suited for the identification and quantification of polar and thermally labile pesticides and metabolites down to mg/kg levels.

The pesticides included in this study are listed in Table 1.

Thermo SCIENTIFIC

Compound	t_R (min)[a]	Parent ion (*m/z*)	Quantifier ion (*m/z*)	Quantifier ion (*m/z*)
Acephate	2.5	184	143 (10V)	125 (10V)
Aldicarb[b] (Na+)	19.7	213	89 (22 V)	116 (22 V)
Aldicarb-sulfoxide (Na^+)	2.7	229	166 (9V)	109 (15V)
Aldicarb-sulfone (Na^+)	4.1	245	109 (25V)	166 (25V)
Acetamiprid	17.3	223.0	126 (21 V)	90 (35 V)
Azoxystrobin	28.8	404.2	372 (16 V)	344 (26 V)
Carbaryl	24.0	202.1	145 (25 V)	127 (25 V)
Carbofuran	23.1	222.1	123 (24 V)	165 (24 V)
3-hydroxy-carbofuran (-H_2O)	15.8	220	135 (15V)	163 (15V)
Chlorpropham	29.6	214.0	172 (12 V)	154 (19 V)
Carbendazim + benomyl[b]	2.5	192.0	160 (22 V)	132 (31 V)
Cyprodinil	25.3	226.1	93 (40 V)	77 (46 V)
Demeton-S	26.0	259.0	89 (22 V)	116 (22 V)
Demeton-S-methyl	22.8	253.0	61 (40 V)	89 (20 V)
Demeton-S-methyl-sulfone	7.0	263.0	109 (30 V)	169 (20 V)
Demeton-S-methyl-sulfoxide	3.3	247.0	169 (17 V)	109 (29 V)
Dimethomorph A[c] / B[c]	26.8 27.3	388.1	301 (23 V)	165 (35 V)
Disulfoton	33.8	275.0	89 (15 V)	61 (30 V)
Disulfoton-sulfone	27.1	307.0	125 (20 V)	153 (20 V)
Disulfoton-sulfoxide	24.2	291.0	185 (15 V)	157 (25 V)
Ethoprofos	29.2	243.0	131 (21 V)	173 (21 V)
Fenhexamid[d]	29.0	302.0 304.0	97 (22 V)	 97 (26 V)
Flusilazole	29.8	316.1	247 (21 V)	165 (31 V)
Imazalil	21.4	297.0	159 (24 V)	255 (25 V)
Imidachloprid	15.3	256.1	209 (22 V)	175 (22 V)
Kresoxim-methyl	31.8	314.0	222 (14 V)	116 (19 V)
Metalaxyl	24.8	280.1	220 (15 V)	192 (25 V)
Methiocarb	27.6	226.0	169 (11 V)	121 (19 V)
Methiocarb sulfoxide (Na^+)	6.6	185.0	122 (23 V)	170 (23 V)
Methomyl	5.0	163.0	106 (12 V)	88 (12 V)
Myclobutanil	28.7	289.0	125 (35 V)	70 (25 V)
Oxamyl (Na^+)	4.2	242	70 (20V)	121 (20V)
Penconazole	30.0	284.0	159 (35 V)	70 (35 V)
Pirimicarb	7.3	239.1	182 (15 V)	72 (30 V)
Propiconazole	30.8	342.0	159 (31 V)	69 (31 V)
Propoxur	22.7	210.1	111 (17 V)	168 (10 V)
Pyrimethanil	21.2	200.0	182 (35 V)	168 (35 V)
Tetraconazole[d]	29.4	372.0 374.0	159 (38 V)	 161 (31 V)
Thiabendazole	2.5	202.0	131 (36 V)	175 (36 V)
Thiachloprid	21.0	253.0	99 (45 V)	126 (25 V)
Thiodicarb	23.3	355.0	88 (20 v)	108 (20 V)
Thiophanate-methyl	22.5	343.0	151 (23 V)	311 (15 V)
Triadimefon	28.9	294.1	197 (19 V)	225 (19 V)
Triadimenol A[c]/ B[c]	27.1/27.5	296.1	70 (16 V)	99 (16 V)
Triazophos	30.4	314.1	162 (19 V)	119 (33 V)

[a] Retention time

[b] Benomyl was measured as carbendazim[14]

[c] Dimethomorph and triadimenol exist as two isomers with different retention times

[d] For fenhexamid and tetraconazole, the isotopic parent ions were selected due to the lack of a second sound transition

Table 1: Retention times and compound-specific ESI(+)-MS/MS parameters

Sample Preparation

A stock mix solution of all the pesticides was prepared at a concentration of 1 mg/L. Calibration solutions in the concentration range 0.5-100 µg/L were prepared by serial dilution of the stock solution.

Samples were prepared for analysis using extraction with ethyl acetate. Individual samples of fruits and vegetables were first homogenized. After homogenization, a 10.0 g sample was extracted using ethyl acetate and anhydrous sodium sulfate. The mixture was ultra-sonicated for 20 minutes. The mixture was filtrated through a thin layer of anhydrous sodium sulfate and the filtrate was evaporated. The extracts were then reconstituted in 5 mL of methanol. The solution was diluted with water and then filtered through a 0.45 µm syringe filter[14]

HPLC

HPLC analysis was performed using the Surveyor HPLC System (Thermo Fisher Scientific, San Jose, CA). Each 20 µL sample was injected onto a 150×2.1 mm, 3.5 µm, C18 HPLC column equipped with a 10×2.1 mm, 3.5 µm, C18 HPLC guard column. A gradient LC method used mobile phases A (0.1% formic acid) and B (0.1% formic acid in acetonitrile) at a flow rate of 0.2 mL/min. The gradient was: 0–3 min A:B = 90:10 (v/v), 3–31 min A:B = 90:10 (v/v) to A:B = 10:90 (v/v), 31–36 min A:B = 10:90 (v/v), 36–36.5 min A:B = 10:90 (v/v) to A:B = 90:10 (v/v), 36.5–45 min A:B = 90:10 (v/v).

MS

MS analysis was carried out on a TSQ Quantum Ultra triple stage quadrupole mass spectrometer with an electrospray ionization source (Thermo Fisher Scientific, San Jose, CA).

The MS conditions were as follows:
Ion source polarity: Positive
Spray voltage: 4000 V
Sheath gas pressure (N_2): 40 units
Auxiliary gas pressure (N_2): 10 units
Ion transfer tube temperature: 350 °C
Collision gas pressure (Ar): 1.0 mTorr
Q1 resolution: 0.2 FWHM (H-SRM)
Q3 resolution: 0.7 FWHM
Scan Type: H-SRM
Dwell time: 20–50 ms

The LC-MS/MS method was developed according to the scheme shown in Figure 1. The run was divided into four time segments based on the retention times of the target compounds. Multiple scan events were included in each time segment. For each target compound, the protonated molecule $[M+H]^+$ was usually investigated, except in the cases of compounds where the adduct $[M+Na]^+$ was the base peak in the ESI(+) spectra. Two transitions were selected per compound in order to perform quantification and identification simultaneously.

The SRM transitions that were monitored are summarized in Table 1. Identification criteria for the target compounds were based on the LC retention time (t_R) and on the ratio of the two monitored transitions for each compound.[13,14]

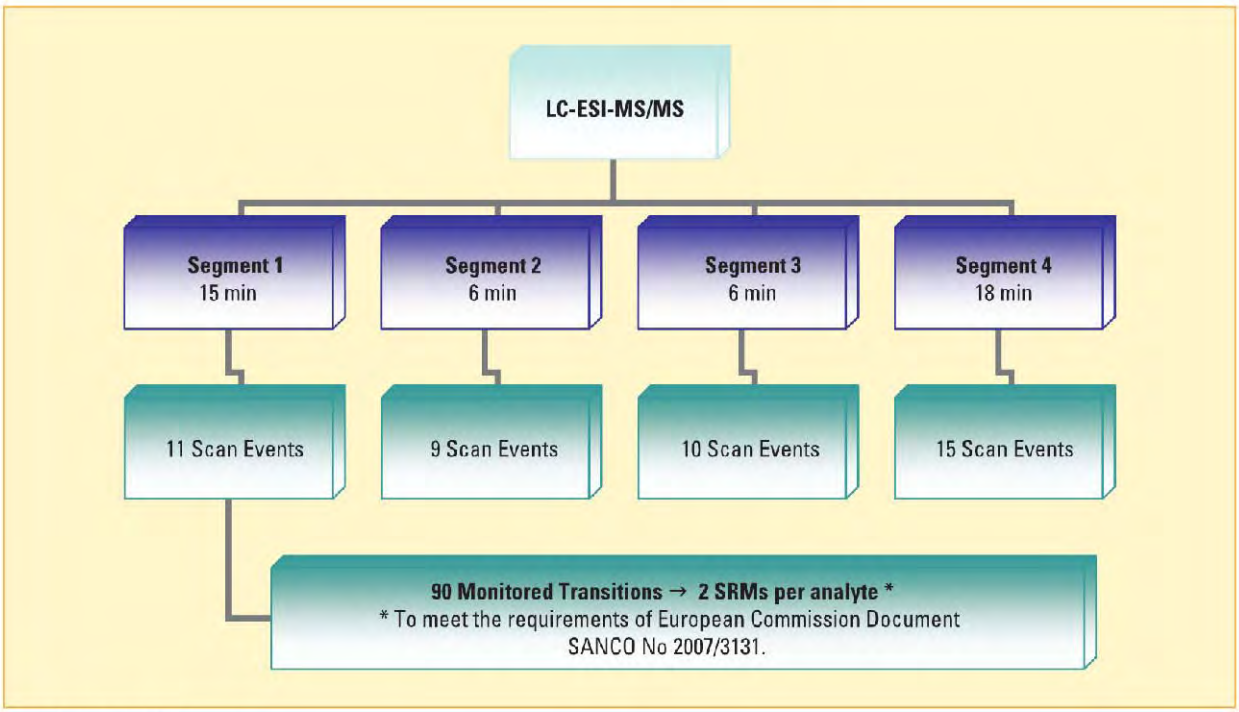

Figure 1: LC-ESI-MS/MS method

Results and Discussion

Although LC-MS/MS is a selective technique, interferences due to isobaric compounds can appear in chromatograms. These isobaric interferences increase the chemical background and can make it difficult to integrate the desired analyte peak reproducibly. Among the compounds included in this study were three sets of isobaric compounds and one set of compounds that share the same fragment ions, which increases the likelihood of cross-talk. Therefore, to eliminate the noise and lower the detection limits, all of the assays in this study were run in the Highly Selective Reaction Monitoring (H-SRM) mode with the Q1 FWHM peak width set at 0.2[14].

The H-SRM chromatograms of a mix solution of certain pesticides at a concentration of 1 µg/L are shown in Figure 2. Linearity of the method was proven for all cases because the R^2 values were usually greater than 0.99 for the linear regression equations (1/x weighted) in the concentration ranges tested. The instrumental detection limits (IDLs) were, in most cases, below 0.5 µg/L. Figure 3 displays the linearity plots of selected compounds. Linearity data for certain compounds are summarized in Table 2.

Using the H-SRM mode reduced the matrix effects by minimizing the chemical noise caused by co-eluting isobaric compounds. Consequently, the signal-to-noise ratio was enhanced in the complicated food matrices. This effect can be observed in the chromatograms in Figure 4, which show the analysis of a peas sample in the SRM and H-SRM modes. The top two SRM chromatograms illustrate the background in a blank peas extract whereas the bottom two SRM chromatograms show the peaks for methomyl in a peas extract spiked with 1 ppb of methomyl. The narrower window of the Q1 set at 0.2 FWHM in the H-SRM mode improves the selectivity of the analysis and increases the signal-to-noise ratio.

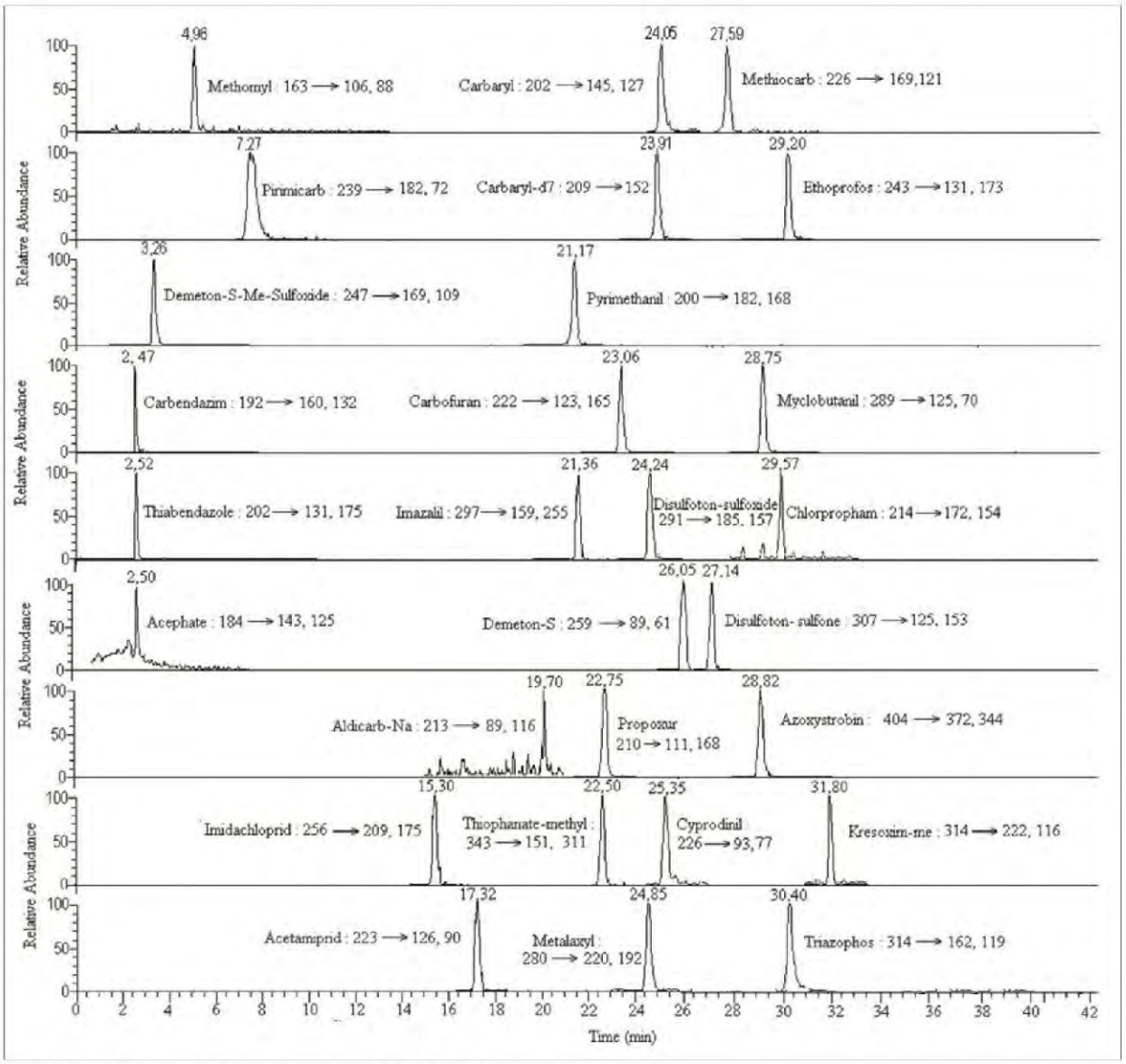

Figure 2: SRM chromatograms for certain pesticides of the standard mix solution

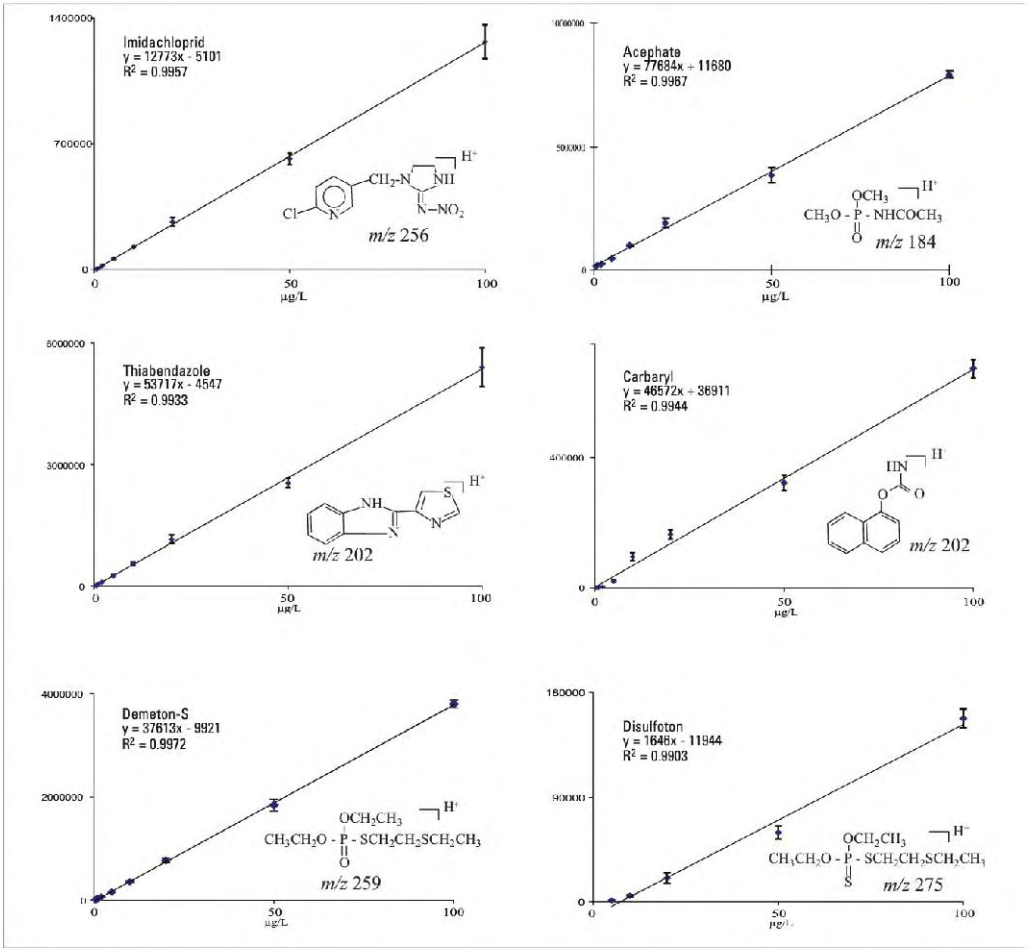

Figure 3: Linearity plots for certain compounds

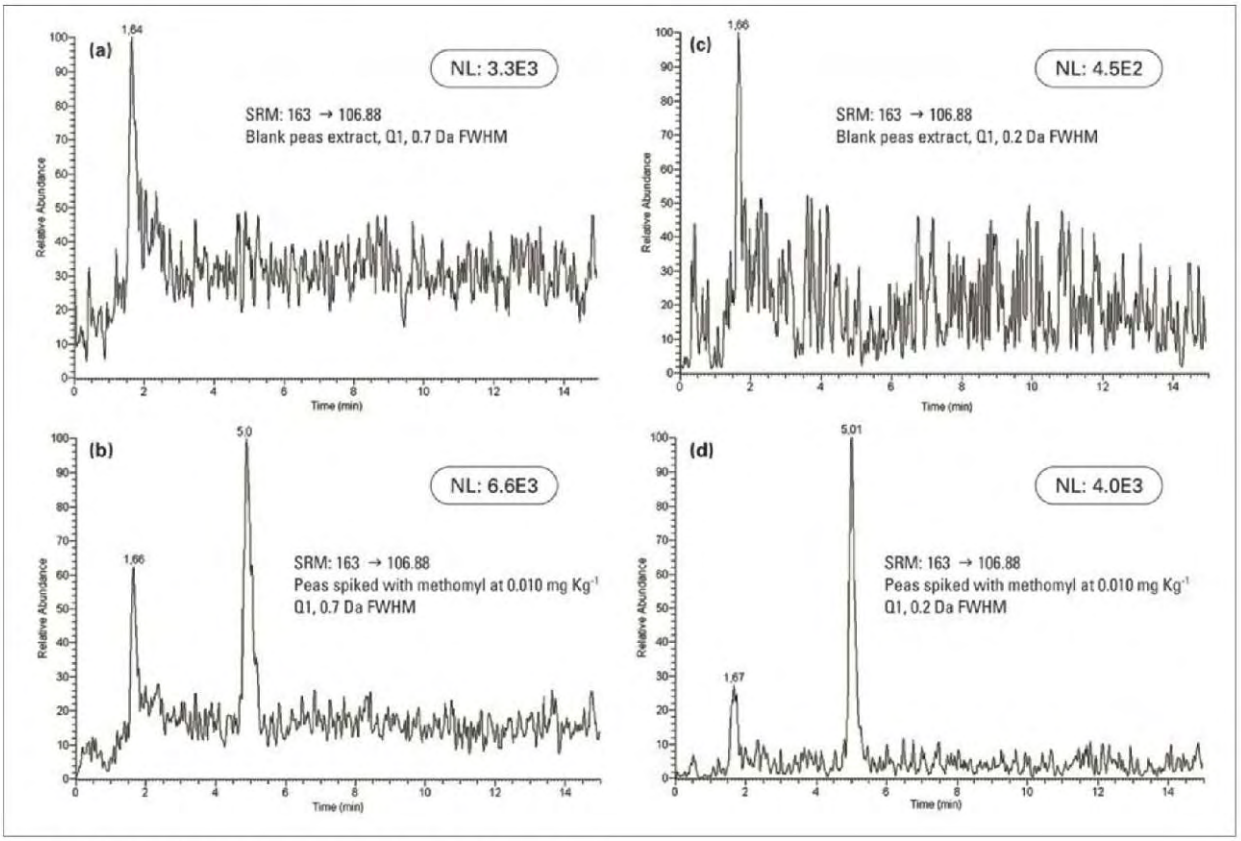

Figure 4: H-SRM and SRM chromatograms of methomyl in pea sample matrix

The matrix-matched calibration curves of methomyl at different Q1 settings are shown in Figure 5 and data of the calibration curves are listed in Table 3. The signal itself is reduced by a factor of two when the Q1 FWHM peak width is changed from 0.7 to 0.2, yet the linearity and accuracy are improved (as demonstrated by the correlation coefficients and back-calculated values of the matrix-matched standards at the low concentration levels, in Table 3).

Some samples were found to contain pesticide residues. Figure 6 displays SRM chromatograms of a sample of frozen peas that contained residues of triazophos and myclobutanil. The confirmation of identity was based on the ion ratio of monitored transitions in the sample and in the standard solution according to the EU Guidelines for pesticide residues monitoring.[13] The concentrations of the residues found in the sample were below the Maximum Residue Limits (MRLs)[1,2].

Compound	Linear regression equations	Concentration range (µg/L)	R^2	IDLs (µg/L)
Acephate	Y=116806+77683.7 X	(1-100)	0.9967	0.5
Aldicarb	Y=-590.6 +1115.4 X	(1-100)	0.9900	0.7
Azoxystrobin	Y=-363884 + 213698 X	(0.5-100)	0.9912	0.2
Carbaryl	Y=36911 + 46572 X	(0.5-100)	0.9903	0.3
Carbendazim	Y=10192 + 211684 X	(0.5-100)	0.9964	0.1
Carbofuran	Y=11107 + 161251 X	(0.5-100)	0.9920	0.2
Chlorpropham	Y=-5289 + 6893.5 X	(1-100)	0.9954	0.6
Cyprodinil	Y=-57425 + 30565.4 X	(0.5-50)	0.9931	0.3
Demeton-S	Y=-9921 + 37615 X	(0.5-100)	0.9972	0.3
Disulfoton	Y=-11944 + 1646.2 X	(5-100)	0.9903	1.5
Disulfoton Sulfoxide	Y=40274 + 141033 X	(0.5-100)	0.9961	0.4
Disulfoton Sulfone	Y=-1633.2 + 8994 X	(0.5-100)	0.9904	0.4
Ethoprofos	Y=-10106 + 40922 X	(0.5-100)	0.9940	0.3
Imidachloprid	Y=-5101.1 +12773.2 X	(0.5-100)	0.9957	0.3
Kresoxim-methyl	Y=-7877.4 + 3056.8 X	(2-100)	0.9900	1.0
Metalaxyl	Y=28427.5 +117245 X	(0.5-100)	0.9964	0.3
Methiocarb	Y=4861 + 48380.4 X	(0.5-100)	0.9921	0.3
Methomyl	Y=-2440.7 +13847.8 X	(0.5-100)	0.9990	0.4
Myclobutanil	Y=-16905.7 +10101.5 X	(0.5-100)	0.9953	0.4
Pirimicarb	Y=23403 +168260 X	(0.5-100)	0.9953	0.2
Propoxur	Y=9181 + 151300 X	(0.5-100)	0.9947	0.2
Pyrimethanil	Y=-4723.7 + 9197.2 X	(0.5-100)	0.9900	0.4
Thiabendazole	Y=-4546.8 + 53716.7 X	(0.5-100)	0.9933	0.3
Triazophos	Y=-18350 +134057 X	(0.5-100)	0.9954	0.3

Table 2: Linearity data and instrumental detection limits (IDLs) for certain pesticides

	Peas Matrix 0.1 g/mL Q1: 0.2 FWHM	Peas Matrix 0.1 g/mL Q1: 0.7 FWHM	Peas Matrix 0.2 g/mL Q1: 0.2 FWHM	Peas Matrix 0.2 g/mL Q1: 0.7 FWHM
1/x	Y = -2469.2 + 10863 X	Y = 4631.3 + 18381.3 X	Y = -3845.1 + 8212 X	Y = 10244 + 19142 X
R^2	0.9966	0.9851	0.9945	0.9861
Accuracy of Matrix-Matched Calibration Curves (1/x)				
1 µg/L	0.91 µg/L (91%)	0.68 µg/L (68%)	0.87 µg/L (87%)	1.24 µg/L (124%)
5 µg/L	4.89 µg/L (97%)	4.74 µg/L (94%)	5.26 µg/L (105%)	4.56 µg/L (91%)
10 µg/L	9.78 µg/L (97%)	11.5 µg/L (85%)	10.5 µg/L (95%)	9.05 µg/L (90%)

Table 3: Linearity and accuracy data for methomyl in pea matrix

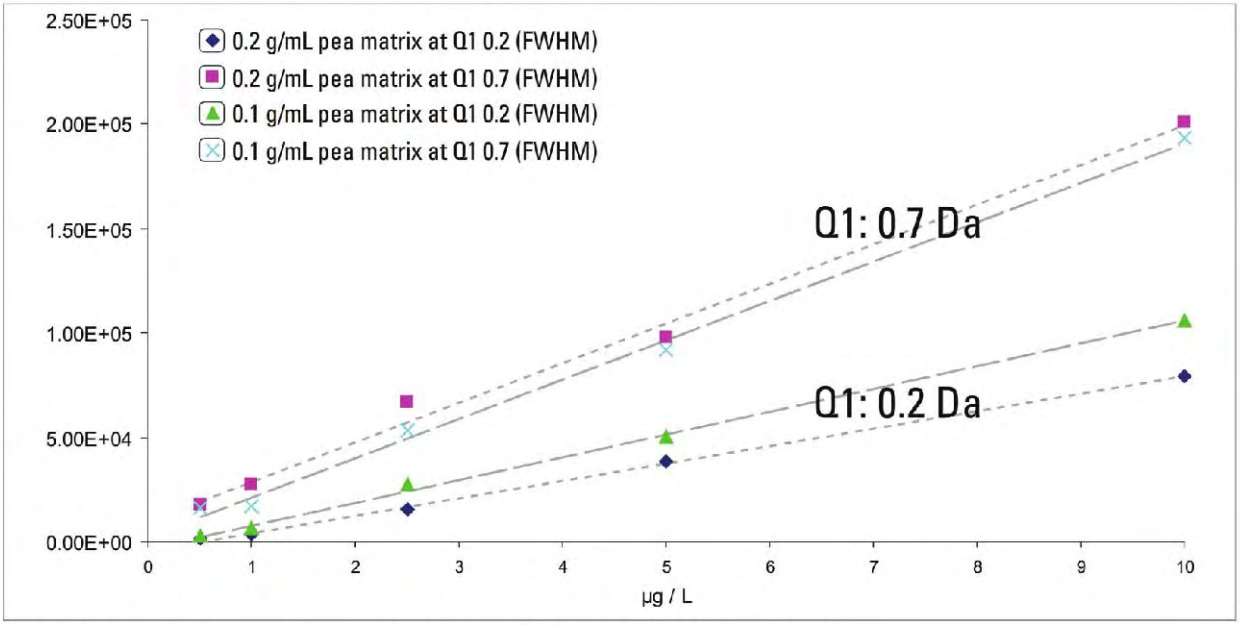

Figure 5: Matrix-matched calibration curves of methomyl in pea extract at Q1: 0.2 (FWHM) and 0.7 (FWHM)

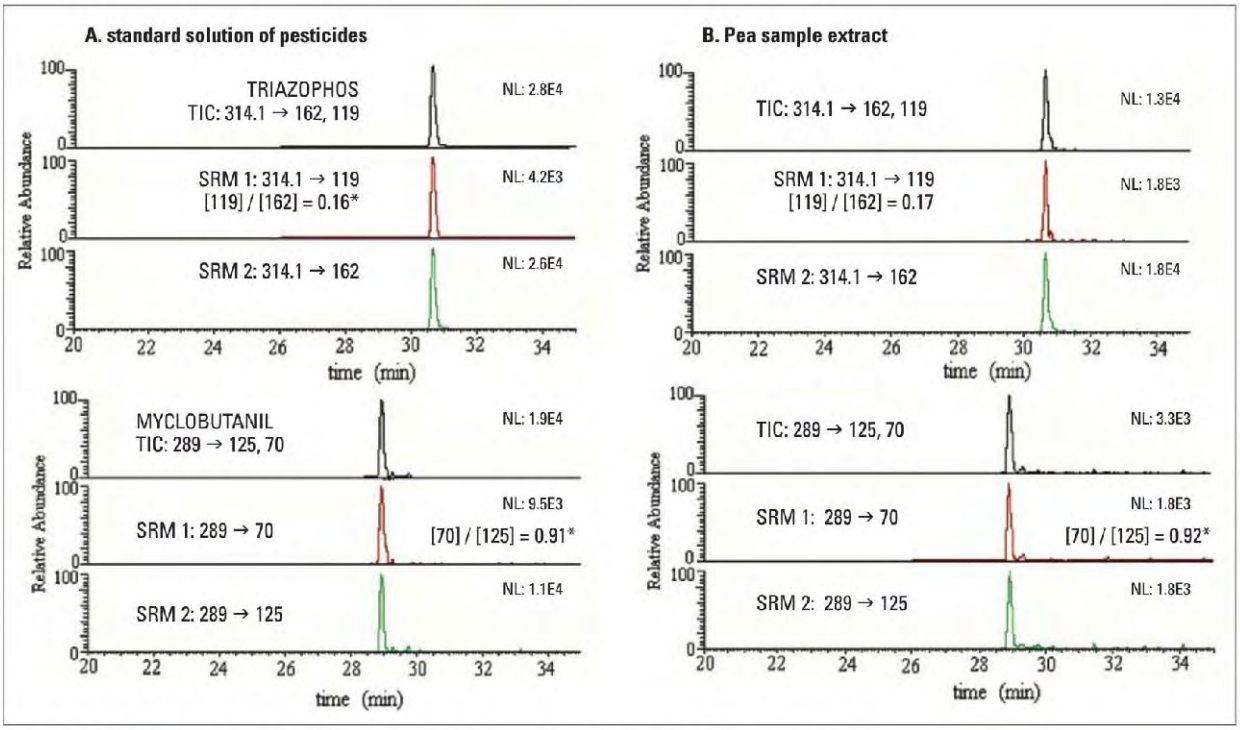

Figure 6: LC-ESI-SRM chromatograms of frozen pea sample extract, with residues of triazophos and myclobutanil

Conclusion

A multi-residue LC-ESI-MS/MS method was developed for the reliable confirmation and quantification of pesticides from different chemical classes at low ppb levels in food matrices. The method uses the Highly Selective Reaction Monitoring (H-SRM) mode of the TSQ Quantum Ultra triple quadrupole mass spectrometer to effectively reduce the background interference and improve the signal-to-noise ratios. For the pesticides investigated, satisfactory precision and accuracy were achieved and Limit of Quantitation (LOQ) values of 0.010 mg/kg were established. The method can be expanded to include more pesticides and their metabolites to improve the range of pesticide residues monitored in food commodities.

References

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[2] European Food Safety Authority (EFSA), www.efsa.europa.eu

[3] Commission Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. *Official Journal of the European Communities* L 230: 1-32.

[4] European Parliament and Council, Regulation (EC) 396/2005 of 23 February 2005, Maximum Residue Levels of Pesticides in Products of Plant or Animal Origin. *Official Journal of the European Communities* L 70: 1-16.

[5] Commission Directive 76/895/EEC of 23 November 1976, relating to the fixing of maximum levels for pesticide residues in and on fruit and vegetables. *Official Journal of the European Communities* L 340: 26-31.

[6] Commission Directive 86/362/EEC of 24 July 1986 on the fixing of maximum levels for pesticide residues in and on cereals. *Official Journal of the European Communities* L 221: 37-42.

[7] Commission Directive 86/363/EEC of 24 July 1986 on the fixing of maximum levels for pesticide in and on foodstuffs of animal origins. *Official Journal of the European Communities* L 221: 43-47.

[8] Commission Directive 90/342/EEC of 27 November 1990 on the fixing of maximum levels for pesticide residues in and on certain products of plant origin, including fruit and vegetables. *Official Journal of the European Communities* L 350: 71-79.

[9] Commission Directive 1999/39/EC of 6 May 1999 Amending Directive 96/5/EC on processed cereal-based foods and baby foods for infants and young children. *Official Journal of the European Communities* L 124: p.8-10.

[10] Commission Directive 1999/50/EC of 25 May 1999 Amending Directive 91/321/EEC on infant formulae and follow-on formulae. *Official Journal of the European Communities* L 139: 29-31.

[11] Commission Directive 2003/13/EC of 10 February 2003 Amending Directive 96/5/EC on processed cereal-based foods and baby foods for infants and young children. *Official Journal of the European Communities* L 41: 33-35.

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[14] Helen Botitsi, Anastasios Economou and Despina Tsipi. "Development and validation of a multi-residue method for the determination of pesticides in processed fruits and vegetables using liquid chromatography–electrospray ionization tandem mass spectrometry," *Anal BioAnal Chem* 2007, 389, 1685-1695.

Application Note: 10262

Analysis of PCBs in Food and Biological Samples Using GC Triple Quadrupole GC-MS/MS

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Key Words

- TSQ Quantum XLS
- dl-PCBs
- Food Safety
- Isotope Dilution
- PCBs
- SRM
- WHO-PCBs

Introduction

Polychlorinated biphenyls (PCBs) are a class of extremely persistent industrial chemicals manufactured for use in electrical transformers, capacitors, inks, paints, pesticides, dust control or insulating fluids. Estimates have put the total global production of PCBs on the order of 1.5 million tons. Between 1930 and 1977, the United States was the single largest producer with over 600,000 tons produced. The European region follows with nearly 450,000 tons through 1984.[1,2]

PCBs include 209 distinct chemical forms (congeners), each having different health effects. Although production of PCBs was banned in the United States in 1977, PCB products are still in use. Because of their persistence in the environment, they have been transported around the globe via wind and air currents. PCBs contaminate the bodies of every animal and human being on earth.

The international Stockholm Convention on Persistent Organic Pollutants (POPs) recognizes PCBs among twelve of the world's most dangerous chemicals known to be detrimental to human health and the environment. In spite of the slow but steady decrease of dioxin body burdens, which shows the results of the combined efforts to prevent further distribution, levels of PCBs are expected to stay unaffected globally (Dioxin Conference 2007 Tokyo).[3] Monitoring PCB levels as a part of ongoing programs for the Stockholm Convention will continue for years, with numerous sample requests, particularly for dangerous dioxin-like (dl) PCBs. In particular, coplanar dl-PCBs – non-ortho-substituted PCBs – are the focus of food safety controls due to having a toxicity similar to 2,3,7,8-TCDD. dl-PCBs also contribute significantly to the sample toxic equivalents (TEQ) value.

This application details a fast, reliable and highly selective trace level screening method for the quantitation of PCBs in environmental, food and biological samples, using triple stage quadrupole mass spectrometry with the Thermo Scientific TSQ Quantum XLS. The analytical strategy is analogous to the well-established United States Environmental Protection Agency (USEPA) Method 1668A.[4]

Due to the different analytical response, each chlorination degree is measured against its own isotopically labeled internal standard. This allows for optimal analytical precision and compound similarity. The internal standard compounds are labeled with ^{13}C on the biphenyl backbone, for a total of 12 labels on the biphenyls. The ^{13}C-labeled PCBs are spiked into each sample, which enables accurate identification and correction for the concentration of the native (unlabeled) compounds in the analytical process. This is generally termed "Isotope Dilution Quantitation." A suffix of "L" behind the IUPAC congener number is used to denote the labeled compound; for example, 101L indicates the labeled analogue of the pentachlorobiphenyl congener 101.

Experimental Conditions

Instrument Configuration

Sample analyses were carried out using the TSQ Quantum XLS™ GC-MS/MS system, equipped with a Thermo Scientific TRACE GC Ultra gas chromatograph. The TRACE GC Ultra™ was configured with split/splitless injector, and sample introduction was performed using the Thermo Scientific TriPlus AS liquid autosampler. The capillary column was a Thermo Scientific TRACE TR-Dioxin 5MS column (5% phenyl film) of 30 m length, 0.25 mm inner diameter and 0.10 µm film thickness. Table 1 describes selected instrumental conditions for the GC, autosampler, and mass spectrometer.

TRACE GC Ultra	
Injector:	Split/splitless, 260 °C, 1.2 min splitless
Carrier:	He, constant flow, 0.8 mL/min
Temp. Program:	90 °C, 4 min 15 °C/min, 160 °C 4 °C/min, 225 °C 7 °C/min, 290 °C
Total Run Time:	32.00 min
Transfer Line:	260 °C

TriPlus™ Autosampler	
Injection Volume:	1.0 µL
Pre-Injection Delay (s):	0.2
Post-Injection Delay (s):	0.2

TSQ Quantum XLS	
Source Temp:	240 °C
Ionization:	EI, 40 eV
Emission Current:	100 µA
Q1 Resolution:	0.7 Da
Q3 Resolution:	0.7 Da
Collision Gas:	Argon, 2.0 mTorr
Collision Gas Energy:	22 eV

Table 1: Selected instrument settings for the TSQ Quantum XLS, TRACE GC Ultra, and TriPlus Autosampler

Thermo SCIENTIFIC

Sample Measurements

USEPA Method 1668 describes a method for the determination of PCB congeners.

> ...[Method 1668] was developed by the U.S. Environmental Protection Agency's (EPA's) Office of Science and Technology for congener-specific determination of the polychlorinated biphenyl (PCB) congeners designated as toxic by the World Health Organization. Revision A of Method 1668 has been expanded to include congener-specific determination of more than 150 chlorinated biphenyl (CB) congeners. The toxic PCBs and the beginning and ending level-of-chlorination CBs are determined by isotope dilution high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The remaining CBs are determined by internal standard HRGC/HRMS. Method 1668A is applicable to aqueous, solid, tissue, and multi-phase matrices.[4]

Commercially available EPA 1668 standards (Wellington, Guelph, ON, Canada) were employed for this application. 68A-CVS is a series of calibration solutions typically used for USEPA Method 1668, Rev. A for HRGC/HRMS. All internal standards (ISTD) were the 12-fold ^{13}C labeled analogues for each PCB chlorination degree. The treatment of samples, internal standards and analytical strategy complied fully with EPA Method 1668A.

TSQ Quantum XLS SRM Settings

While USEPA Method 1668 requires the analytes to be "...separated by the GC and detected by a high-resolution (R 10,000) mass spectrometer, [with] two exact *m/z* values ...monitored at each level of chlorination (LOC) throughout a pre-determined retention time window", the method described in this application employs a triple quadrupole mass spectrometer equipped with hyperbolic quadrupole rods for increased selectivity, as an alternative approach to HRMS.[4] According to the EU Commission Directive 96/23/EC concerning the performance ranking of analytical methods, the number of identification points of GC-MS/MS methods can be similar or even superior to HRMS, especially for MS/MS techniques using independent product ion transitions (Table 2).[5]

Technique	Number of Ions	Identification Points
GC-MS (EI or CI)	n	n
GC-MS (EI and CI)	2 (EI) + 2 (CI)	4
GC-MS/MS	1 precursor and 2 product ions	4
GC-MS/MS*	2 precursor ions, each with 1 product ion	5
HRMS	n	2n

Table 2: Examples of the number of identification points earned for analytical GC/MS techniques, (n = integer).[3]

** denotes method described here.*

According to the EU Commission Directive 96/23/EC, suitable confirmatory methods for organic residues or contaminants are required to be either full scan techniques or methods that use "...at least 4 identification points (PCBs, dioxins, furans) for techniques that do not record the full mass spectra", which are the common target compound multiple ion detection (MID) methods.[5] By using MS/MS transitions from two PCB precursor ions and detecting individual product ions for each chlorination degree, the measurement scheme in this application follows the EU Commission Directive 96/23/EC and provides five identification points for each PCB. The monitored ion transitions are based on the molecular precursor ions ($^{12}C_{12}H_{10-x}{}^{35}Cl_x$) relative to the mono ^{37}Cl isotopes thereof ($^{12}C_{12}H_{10-x}{}^{35}Cl_{x-1}{}^{37}Cl$) to form the product ions with a loss of 2 chlorine during the collision induced dissociation (CID) fragmentation process (Table 3). The internal standards follow the same scheme; however, they show a shift of 12 Da due to the 12-fold ^{13}C-labeling.

PCB	Precursor 1 *m/z*	Precursor 2 *m/z*	Product 1 *m/z*	Product 2 *m/z*
MoCB	188.04	190.04	153.04	153.04
MoCB ISTD	200.08	202.08	165.10	165.10
DiCB	222.00	224.00	152.06	152.06
DiCB ISTD	234.04	236.04	164.10	164.10
TrCB	255.96	257.96	186.02	186.02
TrCB ISTD	268.00	270.00	198.02	198.02
TeCB	289.92	291.92	219.98	219.98
TeCB ISTD	301.96	303.96	232.02	232.02
PeCB	323.90	325.90	253.95	255.95
PeCB ISTD	335.92	337.92	265.99	267.99
HxCB	357.80	359.80	287.90	289.95
HxCB ISTD	369.90	371.90	299.51	301.95
HpCB	391.80	393.80	321.90	323.90
HpCB ISTD	403.80	405.80	333.90	335.90
OcCB	427.80	429.80	357.80	357.80
OcCB ISTD	439.80	441.80	369.90	369.90
NoCB	461.70	463.70	391.80	393.80
NoCB ISTD	473.80	475.80	403.80	405.80
DeCB	495.70	497.70	425.80	427.80
DeCB ISTD	507.70	509.70	437.80	439.80

Table 3: SRM data acquisition scheme for PCBs using one precursor ion with the MID detection of two product ions each. The PCB nomenclature is from EPA Method 1668 and reflects level of chlorination.

When choosing precursor ions it should be noted that only the molecular ion M^+, e.g. *m/z* 357.80 $C_{12}H_4{}^{35}Cl_6$ of the monoisotopic HxCB, gives rise to a unique product ion. The next ion of the isotope cluster, *m/z* 359.80, carries one ^{37}Cl which statistically leads to two product ions, one of which gets the ^{37}Cl substitution. This isotope effect leads to lower product ion intensities as the chlorination degree increases.

The analysis sequence in selected reaction monitoring (SRM) mode uses six (6) retention time windows with overlapping masses for all 10 levels of chlorination (LOC). Except for Segment 1, two chlorination degrees were always monitored in parallel. This is due to the staggered elution order of the individual PCB congeners with adjacent chlorine substitution. The high number of masses taken into each SRM analysis segment demonstrates the speed and capacity of the TSQ Quantum XLS for parallel multi-component detection. Tables 4 and 5 detail the SRM segments and settings.

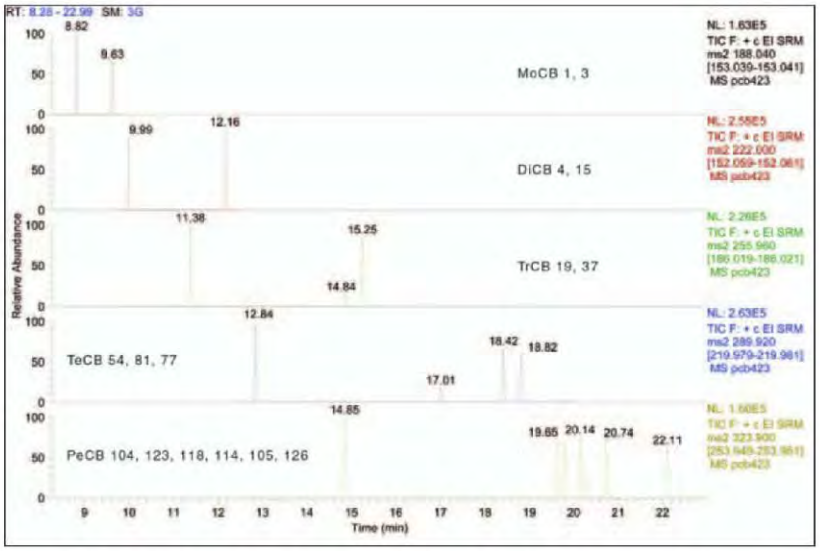

Figure 1: Extracted ion chromatogram showing the congeners with chlorination degrees of mono to penta PCB of a PCB standard (1 pg on-column)

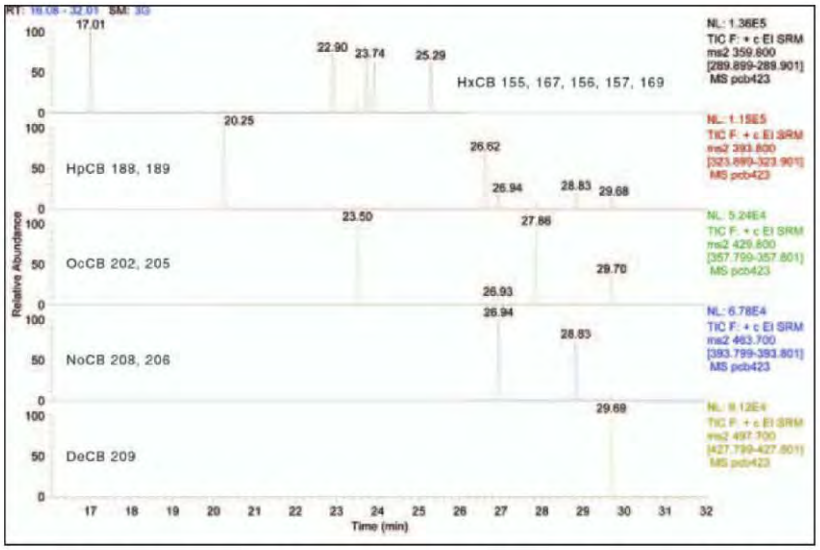

Figure 2: Extracted ion chromatogram showing the congeners with chlorination degrees of hexa to deca PCB of a PCB standard (1 pg on-column)

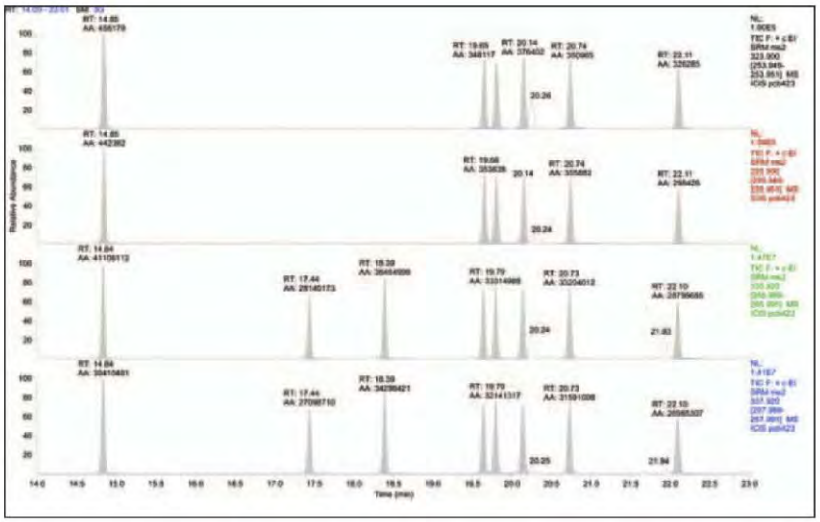

Figure 3: Pentachloro-PCB congeners in standard using two independent SRM transitions each for native (top) and labeled (bottom) PCB congeners 104, 123, 118, 114, 105, 126. The ISTD traces also show the components 101L, 111L.

Results and Discussion

Method Development

All PCB congeners at each chlorination degree were detected using two independent SRM transitions. Each transition used a different precursor ion from the chlorine isotope cluster of the molecular ion region. Data acquisition was performed using the detailed SRM settings described in Tables 4 and 5. MS/MS results for the TSQ Quantum XLS are shown in Figures 1 and 2. Figure 1 illustrates chlorination degrees from mono- to pentachloro-biphenyls, while Figure 2 displays the hexa- to decachloro-biphenyl chlorination range. These results were generated using the SRM transitions as described in Table 3. The mass chromatograms in Figures 1 and 2 use the most intense precursor ion for each compound to show the sequence of chlorination degrees. All congeners can be detected at a high response for each SRM transition. The observed decrease in intensity is due to the statistical decrease of the individual isomer concentration as a part of the molecular PCB cluster when injected at 1 pg on-column.

Figure 3 compares the two independent SRM transitions for one chlorination degree. The upper mass chromatograms represent precursors *m/z* 323.90 and *m/z* 325.90 from the native pentachloro-PCB congeners, while the bottom mass chromatograms show the labeled internal standard (precursors of *m/z* 335.92 and 337.92). This comparison demonstrates the excellent consistency between the SRM traces, which allows for confident confirmations of the PCBs.

These SRM mass chromatograms from the TSQ Quantum XLS triple quadrupole MS operated in standard resolution mode (0.7 Da peak width) show very good correlation to data achieved using gas chromatography and high resolution mass spectrometry (GC-HRMS). With two independent transitions based on two different precursor ions, the TSQ Quantum XLS method meets the high certainty required by the EU directives, as shown for the pentachloro-PCBs in Figure 3. The high speed of the TSQ Quantum XLS analyzer also provides an average of 6 to 8 data points across a chromatographic peak, even while monitoring two chlorination degrees in each SRM window. This allows for reliable peak integration and quantitation.

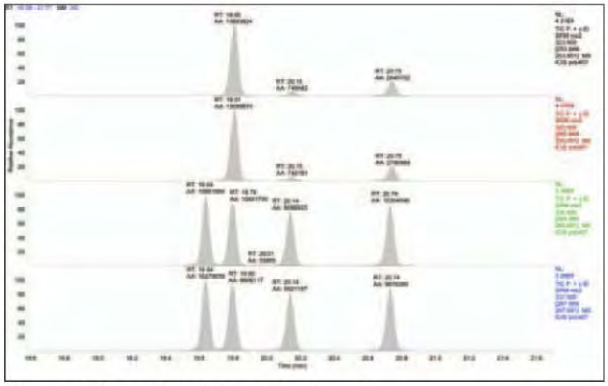

Figure 4: dl-Pentachloro-PCBs in a blood sample

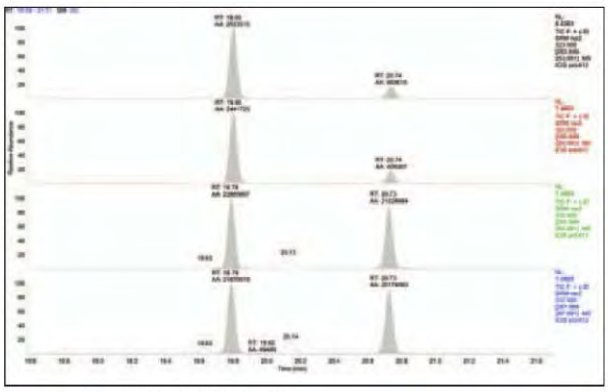

Figure 5: dl-Pentachloro-PCBs in milk

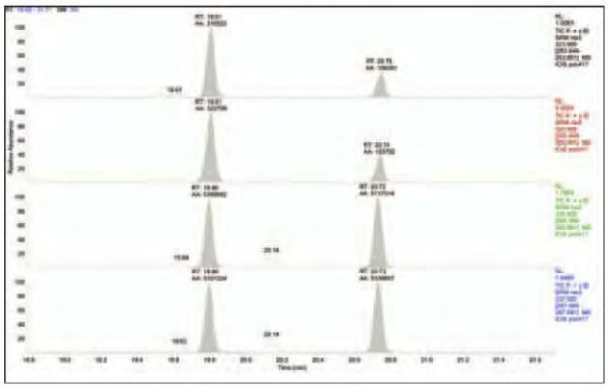

Figure 6: dl-Pentachloro-PCBs in egg yolk

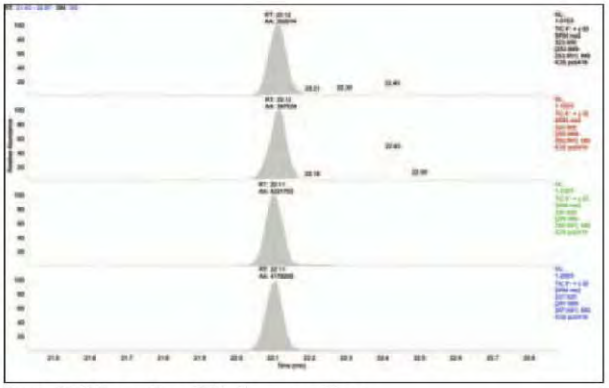

Figure 7: dl-Pentachloro-PCBs in green cabbage

Performance with Complex Matrices

To test the chromatographic and mass acquisition methods with matrix samples, a number of challenging sample types were prepared. The TSQ Quantum XLS demonstrated excellent sensitivity, selectivity and robustness with these samples, as shown in Figures 4 through 7. These results allow for comparison of the results achieved for the pentachloro-PCBs in matrices covering blood, milk, egg yolk and green cabbage. The TSQ Quantum XLS provided clean and background-free mass traces for all types of matrix studied. This selectivity is particularly evident when comparing the matrix samples to the standard samples shown in Figures 1 through 3. Even in very complex samples such as blood (Figure 4) and green cabbage (Figure 7), no increase in the level of background can be observed.

Compared to the standard runs, the PCB concentrations in sample range from a mid-femtogram (fg) to the low picogram (pg) level. PCB concentrations were measured at 0.2 and 1.0 pg/µL for native PCBs and at 100 pg/µL for all added ^{13}C-labeled internal standards. The selectivity of the TSQ Quantum XLS virtually eliminates matrix interference, allowing for low detection limits, enhanced confidence in quantitative results, and accurate identification of these compounds.

Conclusion

The Thermo Scientific TSQ Quantum XLS facilitates the screening and quantitation of PCBs at low levels in difficult matrix samples and provides results with high certainty. The analytical setup complies with USEPA Method 1668A, following an isotope dilution quantitation protocol. The added ^{13}C-labeled internal standard components were detected with high reliability as demonstrated in different samples with complex matrix background.

Confirmatory methods provide information on the chemical structure of the analyte. The TSQ Quantum XLS with its unique hyperbolic quadrupole technology offers superior and uniform selectivity for low level PCB samples in different complex matrices including egg, milk, cabbage and blood. Using the TSQ Quantum XLS in H-SRM mode, the PCB pattern that is typical when using high resolution mass spectrometry, such as magnetic sector, can be detected.

The proposed MS/MS measurement scheme using two precursor ions and SRM detection of individual product ions is a valuable solution for screening for PCBs in various complex matrices at the relevant levels. For the fast control of food samples, GC-MS/MS with the TSQ Quantum XLS exceeds the current EU directives for a minimum of four (4) identification points, in that the method described here offers five (5) identification points.

For contract and governmental control labs, the TSQ Quantum XLS provides a high productivity solution with increased sample throughput even for complex matrix samples. The TSQ Quantum XLS delivers ultimate performance in PCB trace analysis with the added economic advantage of using reduced clean-up methods.

References

1. General information about PCBs, see *www.wikipedia.org*
2. Fiedler, H., Polychlorinated Biphenyls (PCBs): Uses and Environmental Releases, UNEP Persistent Organic Pollutants. *www.chem.unep.ch/pops/POPs_Inc/proceedings/bangkok/FIEDLER1.html*
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Note

The following abbreviations were used in this application note:

MoCB = Monochlorobiphenyl
DiCB = Dichlorobiphenyl
TrCB = Trichlorobiphenyl
TeCB = Tetrachlorobiphenyl
PeCB = Pentachlorobiphenyl
HxCB = Hexachlorobiphenyl
HpCB = Heptachlorobiphenyl
OcCB = Octachlorobiphenyl
NoCB = Nonachlorobiphenyl
DeCB = Decachlorobiphenyl

A suffix "L" following the congener number indicates a labeled compound.

Segment	1	2	3	4	5	6
Duration (min)	9.85	2.60	3.30	4.33	6.03	5.89
Start Time (min)	0	9.85	12.65	15.75	20.08	26.11

Table 4: TSQ Quantum XLS H-SRM analysis segments

Segment 1:	Precursor (*m/z*)	Product (*m/z*)	Width (*m/z*)	Time (s)
	188.04	153.04	0.002	0.150
	190.04	153.04	0.002	0.150
	200.08	165.10	0.002	0.030
	202.08	165.10	0.002	0.030

Segment 2:	Precursor (*m/z*)	Product (*m/z*)	Width (*m/z*)	Time (s)
	222.00	152.06	0.002	0.080
	224.00	152.06	0.002	0.080
	234.04	164.10	0.002	0.030
	236.04	164.10	0.002	0.030
	255.96	186.02	0.002	0.080
	257.96	186.02	0.002	0.080
	268.00	198.02	0.002	0.030
	270.00	198.02	0.002	0.030

Segment 3:	Precursor (*m/z*)	Product (*m/z*)	Width (*m/z*)	Time (s)
	255.96	186.02	0.002	0.080
	257.96	186.02	0.002	0.080
	268.00	198.02	0.002	0.030
	270.00	198.02	0.002	0.030
	289.92	219.98	0.002	0.080
	291.92	219.98	0.002	0.080
	301.96	232.02	0.002	0.030
	303.96	232.02	0.002	0.030
	323.90	253.95	0.002	0.080
	325.90	255.95	0.002	0.080
	335.92	265.99	0.002	0.030
	337.92	267.99	0.002	0.030

Segment 4:	Precursor (*m/z*)	Product (*m/z*)	Width (*m/z*)	Time (s)
	289.92	219.98	0.002	0.080
	291.92	219.98	0.002	0.080
	301.96	232.02	0.002	0.030
	303.96	232.02	0.002	0.030
	323.90	253.95	0.002	0.080
	325.90	255.95	0.002	0.080
	335.92	265.99	0.002	0.030
	337.92	267.99	0.002	0.030
	357.80	287.90	0.002	0.080
	359.80	289.90	0.002	0.080
	369.90	299.95	0.002	0.030
	371.90	301.95	0.002	0.030

Segment 5:	Precursor (*m/z*)	Product (*m/z*)	Width (*m/z*)	Time (s)
	323.90	253.95	0.002	0.070
	325.90	255.95	0.002	0.070
	335.92	265.99	0.002	0.030
	337.92	267.99	0.002	0.030
	357.80	287.90	0.002	0.070
	359.80	289.90	0.002	0.070
	369.90	299.95	0.002	0.030
	371.90	301.95	0.002	0.030
	391.80	321.90	0.002	0.070
	393.80	323.90	0.002	0.070
	403.80	333.90	0.002	0.030
	405.80	335.90	0.002	0.030
	427.80	357.80	0.002	0.070
	429.80	357.80	0.002	0.070
	439.80	369.90	0.002	0.030
	441.80	369.90	0.002	0.030

Segment 6:	Precursor (*m/z*)	Product (*m/z*)	Width (*m/z*)	Time (s)
	391.80	321.90	0.002	0.070
	393.80	323.90	0.002	0.070
	403.80	333.90	0.002	0.030
	405.80	335.90	0.002	0.030
	427.80	357.80	0.002	0.070
	429.80	357.80	0.002	0.070
	439.80	369.90	0.002	0.030
	441.80	369.90	0.002	0.030
	461.70	391.80	0.002	0.070
	463.70	393.80	0.002	0.070
	473.80	403.80	0.002	0.030
	475.80	405.80	0.002	0.030
	495.70	425.80	0.002	0.070
	497.70	427.80	0.002	0.070
	507.70	437.80	0.002	0.030
	509.70	439.80	0.002	0.030

Table 5: Individual SRM descriptors for acquisition segments 1 through 6

Original data acquired using the Thermo Scientific TSQ Quantum GC. Performance of the Thermo Scientific Quantum XLS typically meets or exceeds these results.

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Application Note: 477

Screening for 250 Pesticides in Orange Oil and Ginseng Extract by LC-MS/MS Using TraceFinder Software

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Key Words

- TraceFinder software
- TSQ Vantage
- Triple Quadrupole
- Food safety
- Pesticides

Introduction

Orange oil is widely used for its fragrance and flavoring in consumer products such as cosmetics, medications, and processed foods. In addition, as consumers demand more environmentally friendly cleaning options, orange oil is used increasingly in household cleaning products. Orange oil is derived from the outermost part of the orange; therefore, pesticide contamination is a concern.

Ginseng, an herb used to stimulate the adrenal gland and increase energy, has been used in various systems of medicine for centuries. The root of the ginseng plant contains active chemical components called ginsenosides, which are believed to be responsible for the medicinal properties of the herb. Therefore, the root is commonly dried and made into tablets, extracts, and teas to be taken internally or made into creams for external use. Pesticide contamination in these products is also a concern because the ginseng plant may carry residuals of environmentally persistent pesticides.

Analyzing orange oil and ginseng for pesticides is challenging in part because of the extensive exporting and importing of produce. Pesticides that are approved in one country may be banned in another, and approved pesticides may have different restrictions on the permissible levels of exposure.

Thermo Scientific TraceFinder, a software program with built-in workflows, has been developed to assist routine analysis in environmental and food residue applications. It includes a methods database pre-loaded with the appropriate *m/z* and optimized parameters of contaminants commonly encountered in environmental and food samples, which can be customized by the user to include unique compounds. An LC-MS/MS library of commonly found contaminants, organized in National Institute of Standards and Technology (NIST) format, helps to confirm the compounds being analyzed. Data collection, analysis, and report generation can be performed using the same software program. To demonstrate the software capabilities, a mixture of 250 pesticides spiked into orange oil samples and ginseng extract samples were analyzed using both negative and positive ionization modes on a Thermo Scientific TSQ Vantage Extended Mass Range (EMR) mass spectrometer.

Goal

To develop a quick and efficient LC-MS/MS method for screening pesticides in orange oil and ginseng extract using TraceFinder™ software.

Experimental Conditions

Method

Orange oil and ginseng extract were spiked with a mixture of 250 pesticides (Table 1) to give solutions containing 1 ppb and 10 ppb of each pesticide. A 5 µL sample of the spiked orange oil or ginseng extract was injected directly onto the HPLC column. A simple gradient was used with a retention time of 18 minutes. Using the TraceFinder software, Timed-Selective Reaction Monitoring (T-SRM) was used to create the instrument method, collect and process the data. In a T-SRM experiment, using prior knowledge of the retention times of the compounds, the method is set to look for specific transitions only during the expected retention-time window. This increases the number of SRM transitions that can be monitored effectively per experiment. It also increases the dwell time and duty cycle for monitoring individual compounds per experiment. The result is more accurate and sensitive quantitation.

Sample Preparation

Samples were prepared by a modified QuEChERS procedure.[1] Mixtures of 250 pesticides were prepared in acetonitrile at concentrations of 20 ppb and 200 ppb. For the 10 ppb experiment, a solvent standard was made by mixing 50 µL of the 200 ppb pesticide mixture, 150 µL of acetonitrile, and 800 µL of buffer. The 10 ppb spiked sample was prepared by adding 50 µL of the 200 ppb pesticide mixture, 50 µL of acetonitrile, and 800 µL of water to orange oil or ginseng that has been extracted with 100 µL of acetonitrile. The sample was filtered with a 0.2 µm nylon membrane to remove any particulates.

Similarly, for the 1 ppb experiment, the solvent standard was prepared by mixing 50 µL of the 20 ppb pesticide mixture, 150 µL of acetonitrile, and 800 µL of buffer. The 1 ppb spiked sample was prepared by adding 50 µL of the 200 ppb pesticide mixture, 50 µL of acetonitrile, and 800 µL of water to orange oil or ginseng that has been extracted with 100 µL of acetonitrile. The sample was filtered to remove any particulates.

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Table 1. 250 pesticides and SRM transitions

Pesticide Name	Precursor Ion [M+H]+	Product Ions
Methamidophos	142.0	95.0, 125.0
Naphthol, 1-	143.2	115.1, 143.2
Methomyl	163.1	88.1, 106.1
Fenuron	165.0	46.3, 72.1
o-phenylphenol	169.0	115.3, 141.3
Phropham	180.0	120.0, 138.0
Acephate	184.1	95.2, 143.0
Fuberidazole	185.1	130.1, 157.0
Propamocarb	189.0	102.1, 144.0
Tricyclazole	190.1	136.1, 163.1
Carbendazim	192.1	132.1, 160.1
Isoprocarb	194.1	95.0, 137.0
Cymoxanil	199.1	111.1, 128.1
Cycluron	199.1	72.2, 89.1
Pyrimethanil	200.1	82.0, 107.0
Diamidafos (Nellite)	201.1	82.4, 107.2
Thiabendazole	202.0	131.0, 175.0
Carbaryl	202.1	12.0, 145.0
Dinotefuran	203.2	114.0, 129.0
Aldicarb_Sulfoxide	207.0	89.0, 132.0
Isoproturon	207.1	72.0, 165.2
Promecarb	208.1	109.0, 151.0
Aldicarb+NH_4	208.1	89.2, 116.1
Butocarboxin	208.1	91.4, 109.2
Aminocarb	209.1	137.1, 152.1
Propoxur	210.1	111.1, 168.1
Acibenzolar-S-methyl	211.1	136.0, 140.0
Chlortoluron	213.1	140.0, 168.0
Omethoate	214.1	155.0, 183.0
Simetryne	214.1	96.0, 124.0
Monolinuron	215.1	99.0, 126.0
Metribuzin	215.1	131.0, 187.1
Pymetrozine	218.0	79.0, 105.0
Pyracarbolid	218.2	96.9, 124.9
Thidiazuron	221.1	94.2, 102.1
Formetanate	222.1	120.0, 165.0
Bufencarb	222.1	77.2, 95.2
Methabenzhiazuron	222.1	150.0, 165.0
Carbofuran	222.1	123.1, 165.1
Acetamiprid	223.1	90.2, 126.1
Butoxycarboxin	223.1	86.2, 106.1
Mexacarbate	223.2	151.0, 166.0
Monocrotophos	224.1	127.0, 193.1
Dioxacarb	224.1	123.1, 167.1
Mepanipyrim	224.1	77.0, 106.0
Bendiocarb	224.2	106.0, 109.1
Aldicarb sulfoxide + NH_4	224.2	89.0, 131.7
Mevinphos	225.1	127.1, 192.8
Cyprodinil	226.0	93.0, 108.0
Methiocarb	226.1	121.0, 169.0
Ethiofencarb	226.1	107.0, 106.0
Secbumeton	226.2	99.9, 169.9
Prometon	226.2	141.9, 184.0
Terbumeton	226.2	113.9, 169.9
Ametryn	228.2	96.0, 185.9
Tebuthiuron	229.2	116.1, 172.1
Dimethoate	230.1	125.1, 199.1
Flonicamid	230.1	174.1, 203.1
Fluometuron	233.1	46.3, 72.1
Diuron	233.1	46.3, 72.0
Siduron	233.1	94.0, 137.0
Carboxin	236.0	87.0, 143.0
Thiofanox + NH_4	236.1	57.2, 76.1
Oxamyl + NH_4	237.1	72.1 90.1
Carbetamide	237.1	118.1, 192.0
Carbofuran-3-hydroxy	238.1	181.1, 220.1
Dicrotophos	238.1	112.1, 193.1

Pesticide Name	Precursor Ion [M+H]+	Product Ions
Bentazone_neg	239.1	132.0, 197.0
Pirimicarb	239.1	72.0, 182.0
Butoxycarboxin + NH_4	240.1	86.2, 106.1
Aldicarb sulfone + NH_4	240.1	86.2, 148.0
Prometryn	242.2	157.9, 199.9
Terbutryn	242.2	91.0, 185.9
Ethoprophos	243.1	97.1, 131.1
Cyanophos	244.0	125.1, 212.0
Fonophos	247.0	109.1, 137.1
Fludioxinil	247.1	126.0, 180.0
Forchlorfenuron	248.1	93.0, 129.0
Linuron	249.1	160.0, 182.0
Clothianidin	250.1	132.1, 169.1
Thiacloprid	253.1	90.2, 126.1
Imidacloprid	256.1	175.1, 209.1
Thiobencarb	258.1	100.2, 125.0
Demeton-S	259.0	61.2, 89.2
Metobromuron	259.1	148.0, 170.0
Phorate	261.0	75.1, 143.0
Parathion-methyl	264.0	109.1, 124.9, 232.1
Diethofencarb	268.2	180.1, 226.0
Thiometon + Na	268.9	61.1, 89.1
Mepronil	270.1	119.0, 228.0
Nitenpyram	271.2	225.0, 237.0
Methoprotryne	272.2	198.0, 240.0
Disulfoton	274.9	61.3, 89.3
Neburon	275.1	57.2, 88.0
Bromoxynil	276.1	79.0, 81.0
Fenitrothion	278.0	108.8, 125.1, 246.0
Fenthion	279.0	169.1, 247.0
Oxadixyl	279.0	132.0, 219.0
Metalaxyl	280.1	192.1, 220.1
Propetamphos	282.0	138.1, 156.0
Penconazole	284.1	70.1, 159.0
Ethofumesate	287.0	120.9, 258.9
Vamidothion	288.1	118.1, 146.0
Terbufos	289.0	57.5, 103.1
Iprobenfos	289.0	91.2, 205.0
Myclobutanil	289.1	70.2, 125.0
Chloroxuron	291.1	46.2, 72.2
Parathion	292.0	97.0, 236.0
Uniconazole	292.1	70.2, 125.0
Cyproconazole	292.1	93.2, 125.0
Thiamethoxam	292.2	132.0, 211.1
Amitraz	294.1	122.2, 163.1
Paclobutrazole	294.1	70.0, 125.0
Triadimefon	294.2	197.1, 225.1
Triadimenol	296.1	70.0, 99.0
Imazalil	297.2	159.0, 201.0
Spiroxamine	298.2	100.0, 144.0
Quinalphos	298.9	163.1, 243.0
Mefenacet	299.2	120.1, 148.0
Ditalimfos	300.1	144.2, 145.3
Phenmedipham	301.2	136.0, 168.0
Bifenazate	301.2	152.0, 170.0
Fenhexamid	302.1	55.0, 97.0
Furalaxyl	302.1	95.0, 242.1
Flutriafol	302.2	70.1, 123.0
Fenoxycarb	302.2	88.0, 116.0
Methidathion	302.9	85.2, 144.9
Clofentezine	303.1	102.0, 138.0
Fenamiphos	304.0	217.0, 234.0
Fenpropimorph	304.4	130.1, 147.1
Diazinon	305.0	153.1, 169.1
Pirimiphos-methyl	306.0	108.2, 164.1
Buprofezin	306.2	116.0, 201.0
Fenazaquin	307.2	57.2, 160.9

Table 1. 250 pesticides and SRM transitions (continued)

Pesticide Name	Precursor Ion [M+H]+	Product Ions
Quinoxyfen	307.9	161.9, 196.8
Tebuconazole	308.2	70.2, 125.0
Diflubenzuron	308.9	156.0, 289.0
Fensulfothion	309.2	163.0, 251.0
Edifenphos	311.0	109.1, 283.0
Fenamidone	312.2	236.2, 264.2
Triazophos	314.0	119.2, 162.1
Kresoxim-methyl	314.1	222.1, 267.1
Hexaconazole	314.1	70.2, 159.0
DEF	315.0	169.0, 259.1
Nuarimol	315.1	81.0, 251.9
Flusiazole	316.2	165.0, 247.1
Bupirimate	317.3	108.1, 166.1
Phosmet	317.9	133.1, 160.1
Azinphos-methyl	317.9	125.0, 261.0
Triticonazole	318.1	70.0, 125.0
Desmedipham + NH_4	318.2	136.0, 182.0
Tebupirimfos	319.1	166.1, 210.2
Metconazole	320.2	70.1, 124.9
Phenthoate	320.9	79.3, 247.0
Iprovalicarb	321.2	119.0, 203.0
Pyriproxyfen	322.2	96.0, 185.3
Sulprofos	322.9	218.9, 247.0
Sulfotep-ethyl	323.2	219.0, 247.1
EPN	324.0	157.0, 296.0
Flutolanil	324.2	242.0, 262.0
Cyazofamid	325.2	108.0, 261.0
Famphur	326.0	217.0, 281.0
Diniconazole	326.2	70.2, 148.2
Benalaxyl	326.2	148.0, 208.0
Dimoxystrobin	327.1	116.0, 205.0
Diclobutrazol	328.1	70.2, 159.0
Etaconazole	328.2	123.0, 159.0
Epoxiconazole	330.2	121.0, 123.0
Malathion	330.9	99.2, 285.0
Fenarimol	331.1	81.0, 268.0
Pirimiphos ethyl	334.1	182.1, 198.1
Ipconazole	334.1	70.2, 125.0
Tebufenpyrad	334.2	117.0, 145.2
Zoxamide	336.2	159.0, 187.0
Fenbuconazole	337.0	70.4, 125.1
Bitertanol	338.1	99.0, 269.0
Mesotrione	340.2	185.9, 228.0
Pyridaphenthion	341.1	189.0, 205.0
Prothioconazole	342.0	100.0, 306.0
Propiconazole	342.2	69.2, 159.0
Thiophanate-methyl	343.2	151.1, 311.2
Boscalid	343.2	271.0, 307.0
Azinphos-ethyl	346.0	132.1, 160.1
Isofenfos	346.0	217.0, 245.0
Triflumizole	346.1	73.0, 278.1
Tebufenozide	353.1	133.0, 297.0
Hexythiazax	353.2	168.1, 228.2
Piperonyl butoxide	356.2	119.0, 177.0
Triflumuron	359.1	139.0, 156.0
Clethodim	360.2	164.0, 268.0
Etoxazole	360.2	141.0, 177.1
Isoxaflutole	360.2	220.0, 251.0
Topramezone	364.2	124.9, 333.9
Flufenacet	364.2	152.0, 194.0
Benzoximate	364.4	105.2, 199.2
Pyridaben	365.2	147.0, 309.1
Methoxyfenozide	367.3	105.0, 149.0
Propargite	368.2	174.9, 231.0
Picoxystrobin	368.2	145.0, 205.1
Loxynil	369.9	127.0, 215.0, 242.9
Spiromefesin	371.3	255.3, 273.3

Pesticide Name	Precursor Ion [M+H]+	Product Ions
Tetraconazole	372.2	70.0, 159.0
Famoxadone	373.1	282.4, 329.6
Pyrazophos	374.0	194.0, 222.1
Fluquinconazole	376.2	307.0, 349.2
Prochloraz	376.2	266.0, 308.0
Bromuconazole 46	378.0	70.2, 159.0
Teflubenzuron	379.2	196.0, 339.0
Benthiavalicarb	382.1	116.0, 180.0
Furathiocarb	383.2	195.0, 252.0
Ethion	384.9	97.1, 143.0
Dimethomorph	388.1	165.0, 301.0
Pyraclostrobin	388.2	163.0, 194.0
Famoxadone + NH_4	392.1	238.0, 331.2
Rotenone	395.3	192.1, 213.2
Ethiprole	397.1	255.0, 351.0
Flucarbazone	397.1	115.0, 129.9
Alanycarb	400.3	91.0, 238.0
Pinoxaden	401.2	57.1, 317.0
Sulfentrazone	404.0	307.0, 387.0
Azoxystrobin	404.1	329.1, 372.1
Difenoconazole	406.2	111.0, 251.0
Trifloxystrobin	409.3	186.0, 206.1
Spirodiclofen	411.0	213.1, 313.1
Benfuracarb	411.1	195.1, 252.0
Mandipropamid	412.1	327.9, 355.9
Carfentrazone-ethyl	412.2	366.2, 384.0
Fenpyroximate	422.2	214.0, 366.0
Fipronil	437.2	330.2, 368.0
Hexaflumuron	458.9	175.0, 439.0
Fluoxastrobin	459.2	188.0, 427.1
Fluazinam	463.2	398.0, 416.0
Temephos	466.9	405.1, 419.1
Dioxathion	474.0	153.0, 271.1
Flufenoxuron	487.2	156.0, 304.0
Novaluron	491.2	305.0, 471.0
Butafenacil + NH_4	492.3	180.0, 331.0
Novaluron	493.3	141.0, 158.0
Hydramethylnon	495.3	150.9, 323.0
Lufenuron_neg	509.2	175.0, 326.0
Lufenuron	511.3	141.0, 158.0
Milbemycin A3	511.4	475.2, 493.2
Milbemycin A4 - H_2O	525.4	489.2, 507.2
Noviflumuron	527.0	193.0, 344.0
Indoxacarb	528.3	203.0, 293.0
Chlorfluazuron	539.7, 541.9	383.0, 385.0
Milbemycin A4 + NH_4	560.4	507.2, 525.2
Moxidectin	640.2	498.5, 528.5
Spinosyn A	732.5	98.0, 142.0
Spinosyn D	746.5	98.0, 142.0
Emamectin B1b	872.4	158.2, 302.3
Avermectin B1b + NH_4	876.5	145.0, 291.0
Emamectin	886.7	158.0, 302.0
Avermectin B1a + NH_4	890.4	305.3, 307.0, 567.4
Ivermectin B1a + NH_4	892.5	307.0, 569.0
Avermectin B1a + Na	895.4	183.1, 751.5
Doramectin	916.4	331.4, 593.5
Eprinomectin B1a	936.5	352.1, 490.2

HPLC

Chromatographic analysis was performed using the Thermo Scientific Accela HPLC pump and Accela™ autosampler. The chromatographic conditions were as follows:

Column:	Thermo Scientific Hypersil GOLD PFP (100 mm × 2.1 mm, 1.9 µm)		
Injection volume:	5 µL		
Column temperature:	45 °C		
Mobile phase A:	5 mM ammonium formate in water		
Mobile phase B:	5 mM ammonium formate in methanol		
Flow rate:	0.3 mL/min		
Gradient:	Time (min)	A%	B%
	0	95	5
	2	75	25
	30	0	100
	35	0	100

MS

MS analysis was carried out on a TSQ Vantage EMR™ triple stage quadrupole mass spectrometer with a HESI-II heated electrospray ionization source.

The MS conditions were as follows:

Ion source polarity:	Positive and negative ion mode
Spray voltage:	3500 V
Vaporizer temperature:	400 °C
Ion sweep gas:	2.0 units
Ion transfer tube temperature:	200 °C
Sheath gas pressure (N_2):	55 units
Auxiliary gas pressure (N_2):	15 units
Resolution:	0.7 amu (FWHM) on Q1 and Q3
Scan Width:	0.002 Da
Chrom Filter:	10.0 ms
Collision Gas Pressure:	1.5 mTorr
Scan Type:	Timed SRM (T-SRM)
Cycle Time:	0.4 s

Two SRM transitions per pesticide were monitored for confirmation (Table 1).

Software

Data collection and processing was handled by TraceFinder environmental and food safety software. TraceFinder includes several methods applicable to the environmental and food safety markets, as well as a comprehensive Compound Datastore (CDS). The CDS includes SRM transitions and collision energies for several hundred pesticides, herbicides, personal care products, and pharmaceutical compounds that are of interest to the environmental and food safety industries. A user can select one of the included methods in TraceFinder or quickly develop new or modified methods by using the pre-existing SRM transition information in the CDS, thus eliminating time-consuming compound optimizations.

Results and Discussion

Method Development

The method development section of the software allows the user to choose the compounds that will be analyzed. In this experiment, the appropriate SRMs for the 250 pesticides were chosen from the CDS (Figure 1) and inserted into the instrument method for detection (Figure 2). No compound optimization is necessary for compounds already in the CDS.

Additionally, the calibration levels, QC levels, and peak detection settings are defined in the method development section. Results can be flagged based on user-defined criteria. For example, a flag can be set for a compound whose calculated concentration is beyond the upper limit of linearity, above a defined reporting limit, or below a limit of detection. This allows for faster reviewing of the data after collection; positive samples can be quickly identified.

Acquisition

The Acquisition section provides a step-by-step process to acquire data. The overall progress is followed in an overview section on the left side of the screen (Figure 3). A green check box indicates that the step has been completed and that there are no errors. The steps include template selection (pre-defined sample lists, which are helpful in routine analysis), method selection, sample list definition, report selection, and instrument status.

A final status page summarizes the method and all of the samples to be run. In addition, it gives an overall summary of the status of the instrument (Figure 4). Three colored dots are shown: green indicates an "ok" status; yellow indicates that the attached device is in standby; and red indicates that the attached device is not ready. From the final status page, the batch can be acquired or saved to be run at a later date. A previously saved calibration curve can be used, so that a calibration need not be run every day.

Data Review

The targeted screening analysis of 250 pesticides in a ginseng extract sample was reviewed in the Data Review section of TraceFinder software. In this section, calibration lines, ion ratios, peak integration, and MS spectra (if applicable) can all be viewed (Figure 5). In addition, the Data Review section can flag samples that meet certain user-set criteria. For example, if a tolerance is specified for the ion ratio, a green flag means that the criteria has been met, while a red or yellow flag indicates that it has not. As another example, flags can be used to alert for the presence of carry-over in a blank sample. A red flag indicates that there is a significant issue with the blank sample. In this experiment, the two-point calibration was sufficient to show the calculated amount of the different pesticides found in ginseng extract.

The Data Review section allows user adjustments, such as peak reintegration. The effects of the changes on the results are instantly updated in the results grid.

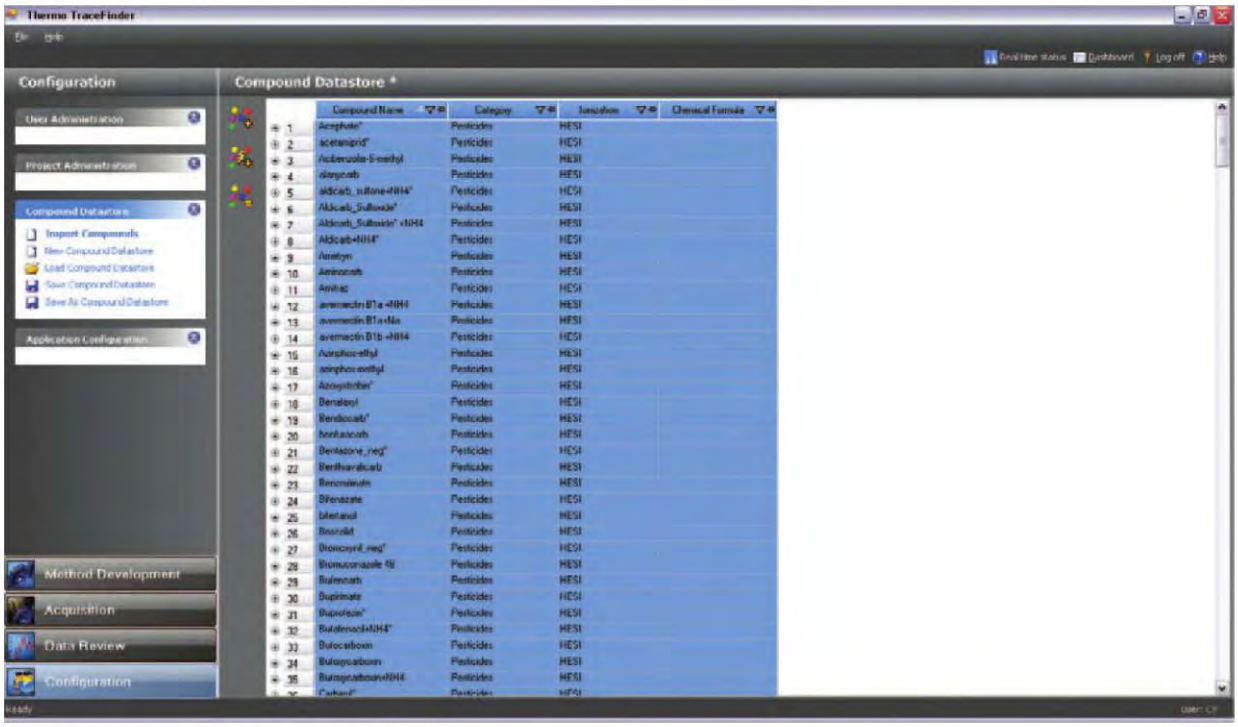

Figure 1. TraceFinder Compound Datastore (CDS)

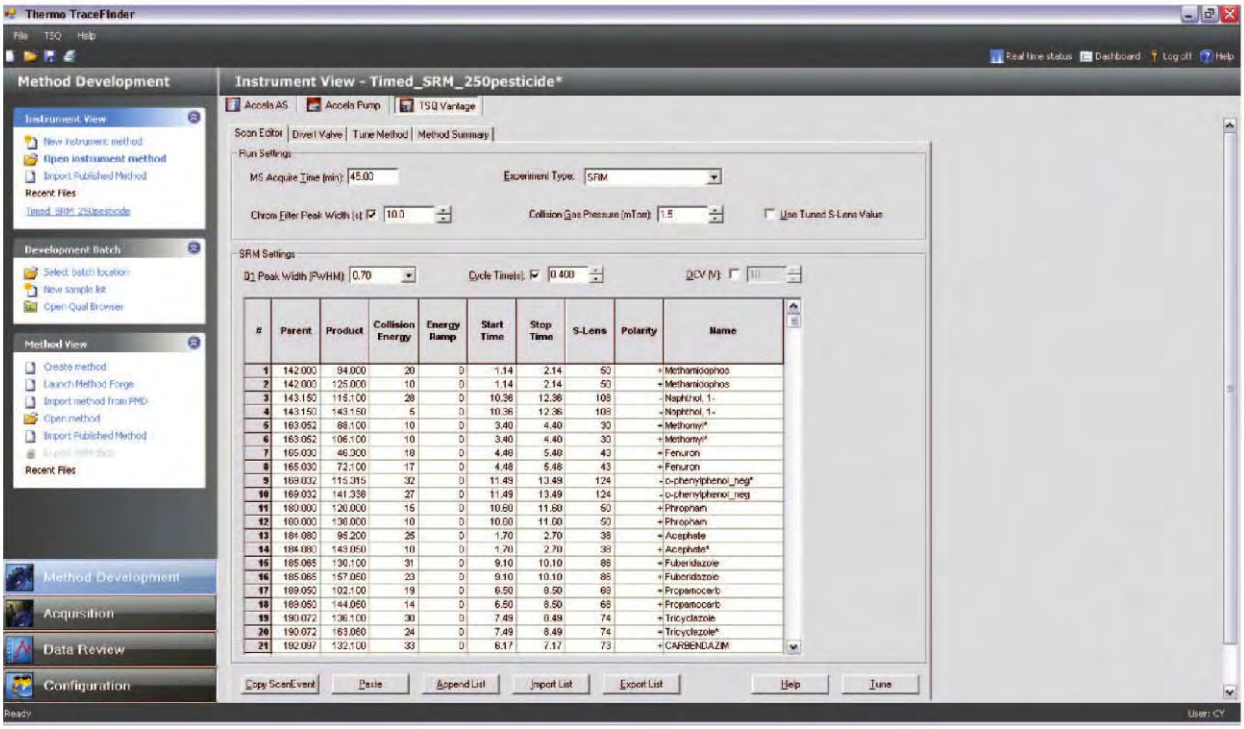

Figure 2. The Compound Datastore is easily inserted into the Instrument Method page.

Reporting

A large number of report templates are available in TraceFinder software. The user has the option of creating PDF reports, printing reports directly to the printer, or saving reports in an XML format, which is useful with laboratory information management systems (LIMS). The user can decide which reports are most applicable to each particular method. In this manner, a supervisor or lab director can set up methods and reports, lock the method, and make it non-editable by technicians. In this way, the integrity of a method is preserved, which is especially useful in controlled environments.

Two examples of the reports generated by TraceFinder software are shown in Figures 6 and 7. This view shows

the on-screen preview function. Figure 6 shows the Calibration Density Report, which displays calibration curves for each compound on one page. Figure 7 shows the Quantitation Report for 1 ppb level in ginseng extract. In this report, the sample summary is provided at the top of the page, and the quantified results follow beneath the chromatogram. TraceFinder can generate results for the entire batch with one click, or the user can view reports individually and print only those of interest.

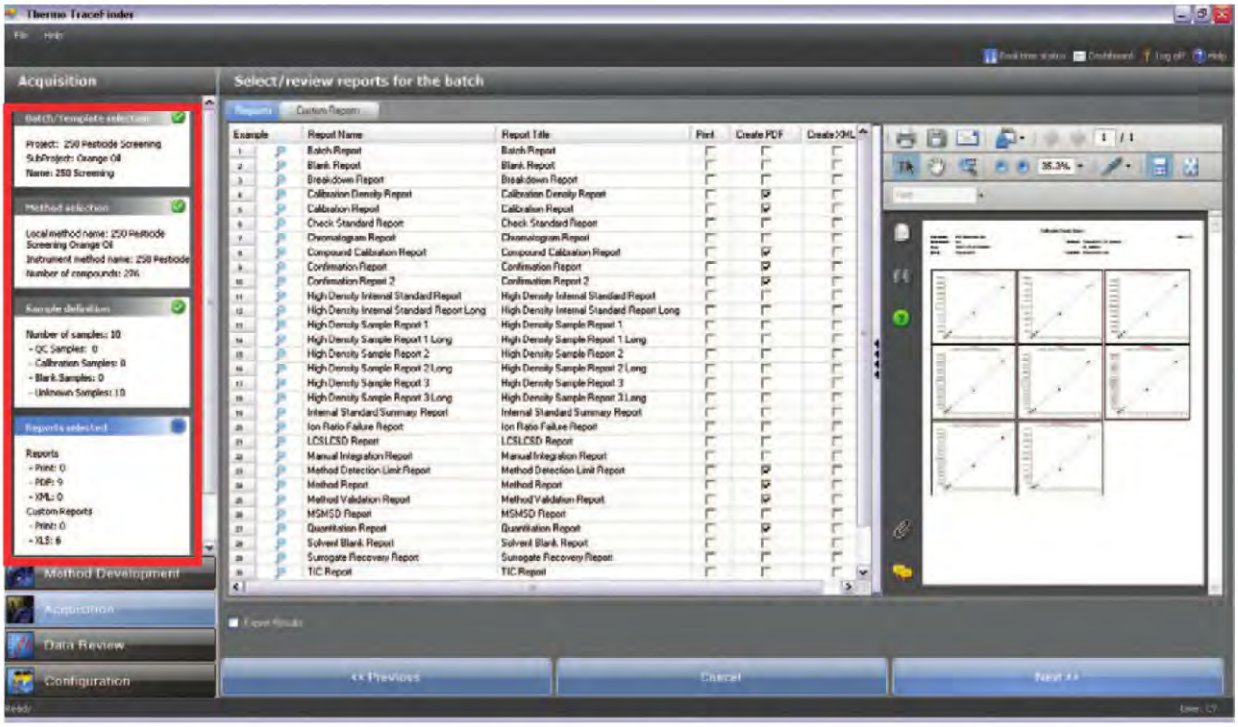

Figure 3. Acquisition section, showing the reporting templates and report preview. The red box at the left outlines the overall progress.

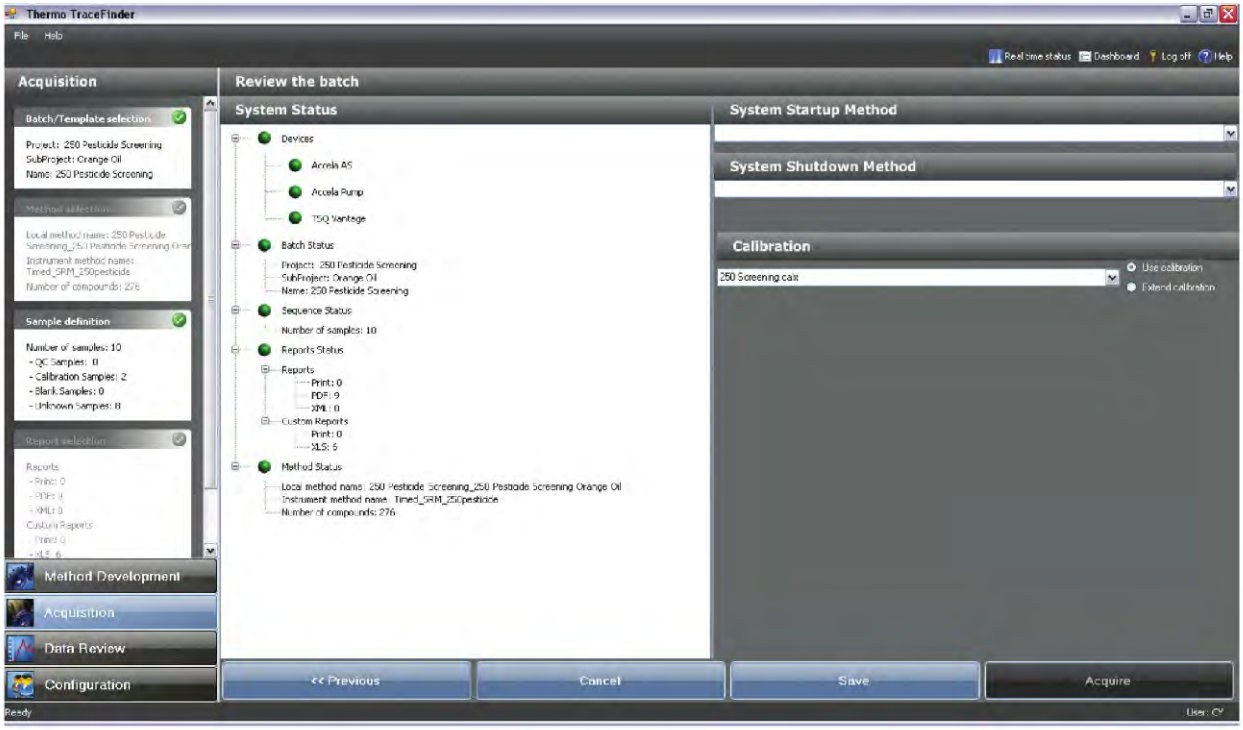

Figure 4. TraceFinder Acquisition status page. This is the final view before submitting a batch for analysis, providing the user instant instrument and method feedback.

Figure 5. TraceFinder Data Review section. The red and yellow flags indicate that there are certain issues with the compound. For example, the ion ratio may be off or the value may be below the specified limit of detection.

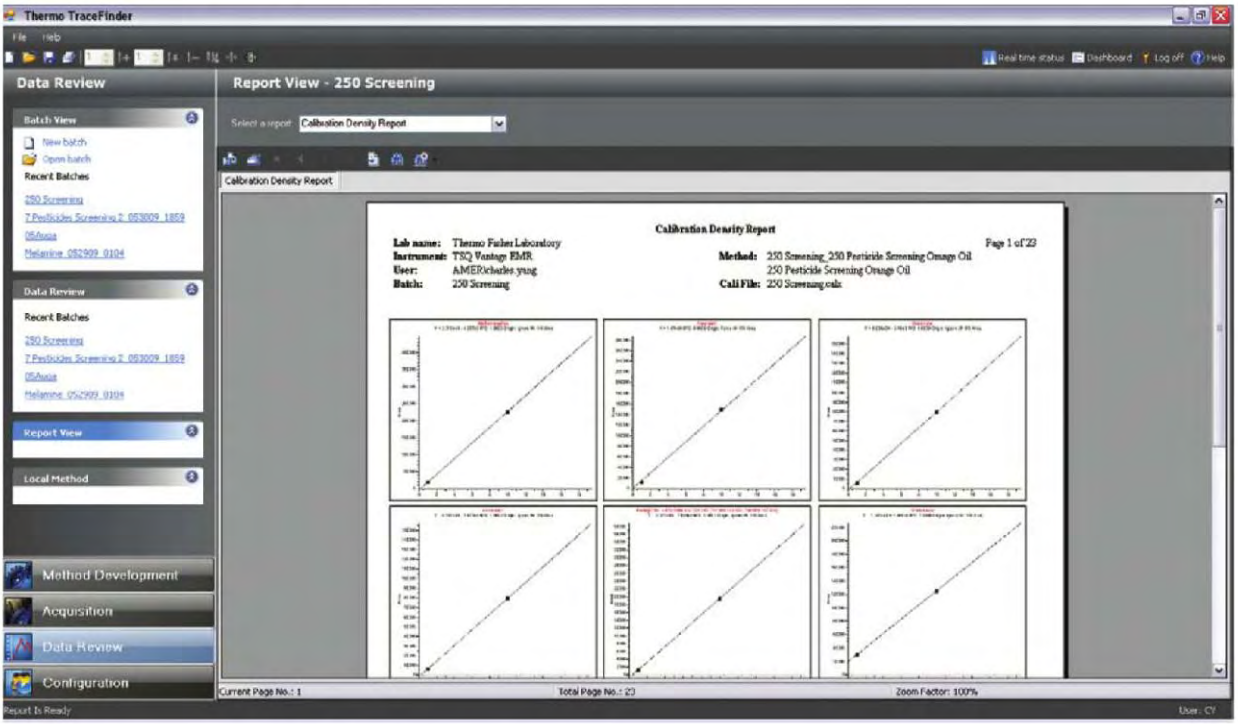

Figure 6. Report View section of TraceFinder, showing calibration curves.

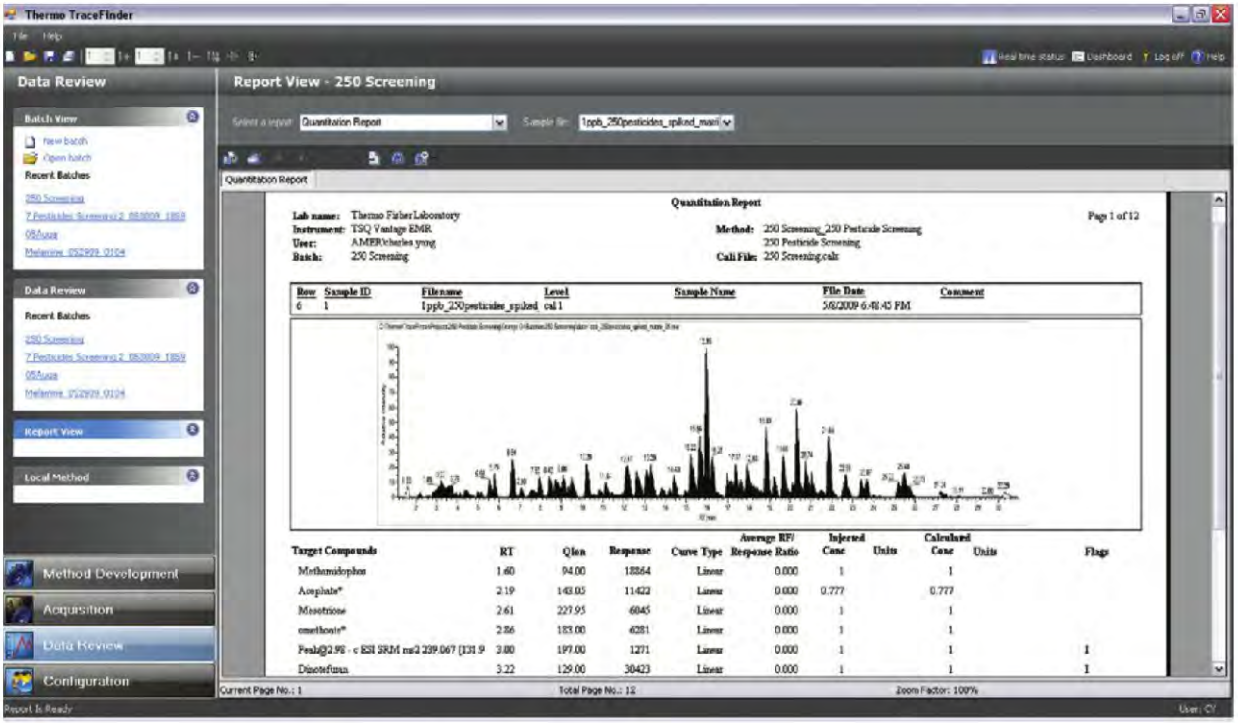

Figure 7. Report View section of TraceFinder showing quantitation results of ginseng.

Conclusion

A new software package, TraceFinder, with an extensive menu of preconfigured methods and report formats, was used to simplify method development for the screening of 250 pesticides in orange oil and ginseng extract. The results from this experiment show positive confirmation of approximately 220 pesticides in orange oil and 250 pesticides in ginseng extract based on the tolerances set in the method for quantitation and confirmation. The method development capabilities and Compound Datastore of TraceFinder software allowed for the quick creation of a method for the analysis of these compounds. In addition, the ability to flag problematic samples in the data review section helped to reduce the overall analysis time by filtering out samples that did not meet predefined criteria.

References

1. Wong, J.W., Hennessy, M.K., Hayward, D.G., Krynitsky, A.J., Cassias, I., Schenck, F.J. (2007) J. Agric. Food Chem. 55, 1117-1128.

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