Application Note: 10017

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

Thermo Fisher Scientific, Austin, TX, USA

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 Polaris*Q* 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 Polaris*Q* 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 Polaris*Q* 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.

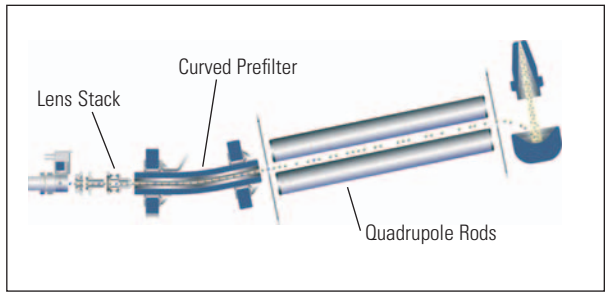

Figure 1: Thermo Scientific DSQ Bent Optics Quadrupole

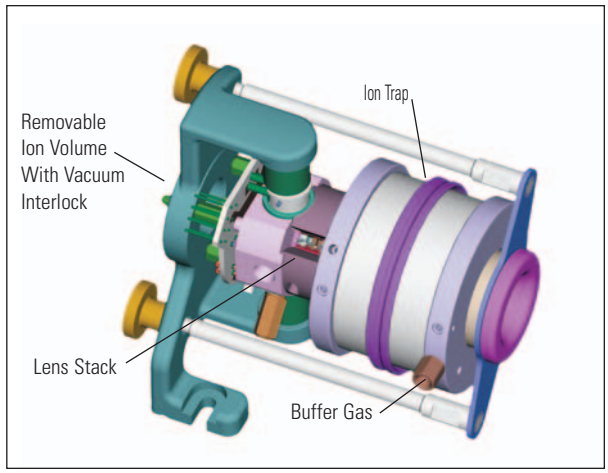

Figure 2: Thermo Scientific Polaris*Q* External Ionization Ion Trap

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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.

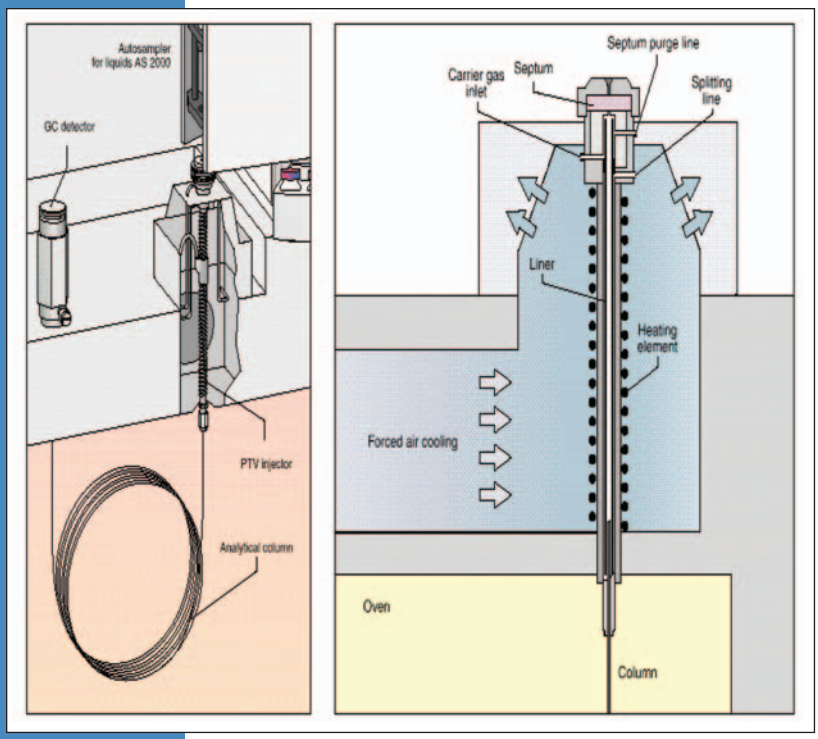


Figure 3: Programmable Temperature Vaporizing Injector (PTV)

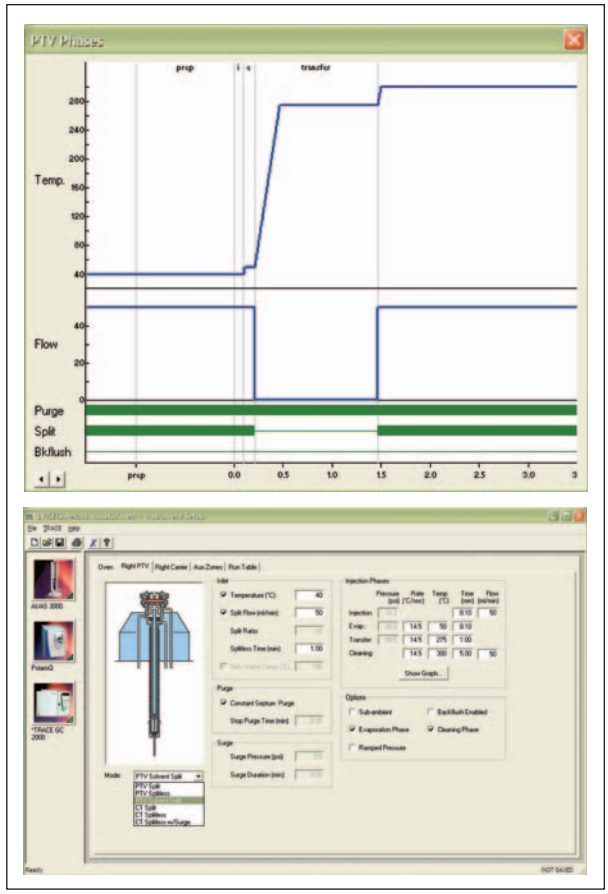


Figure 4: Selective Injection on PTV and Software Control

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.

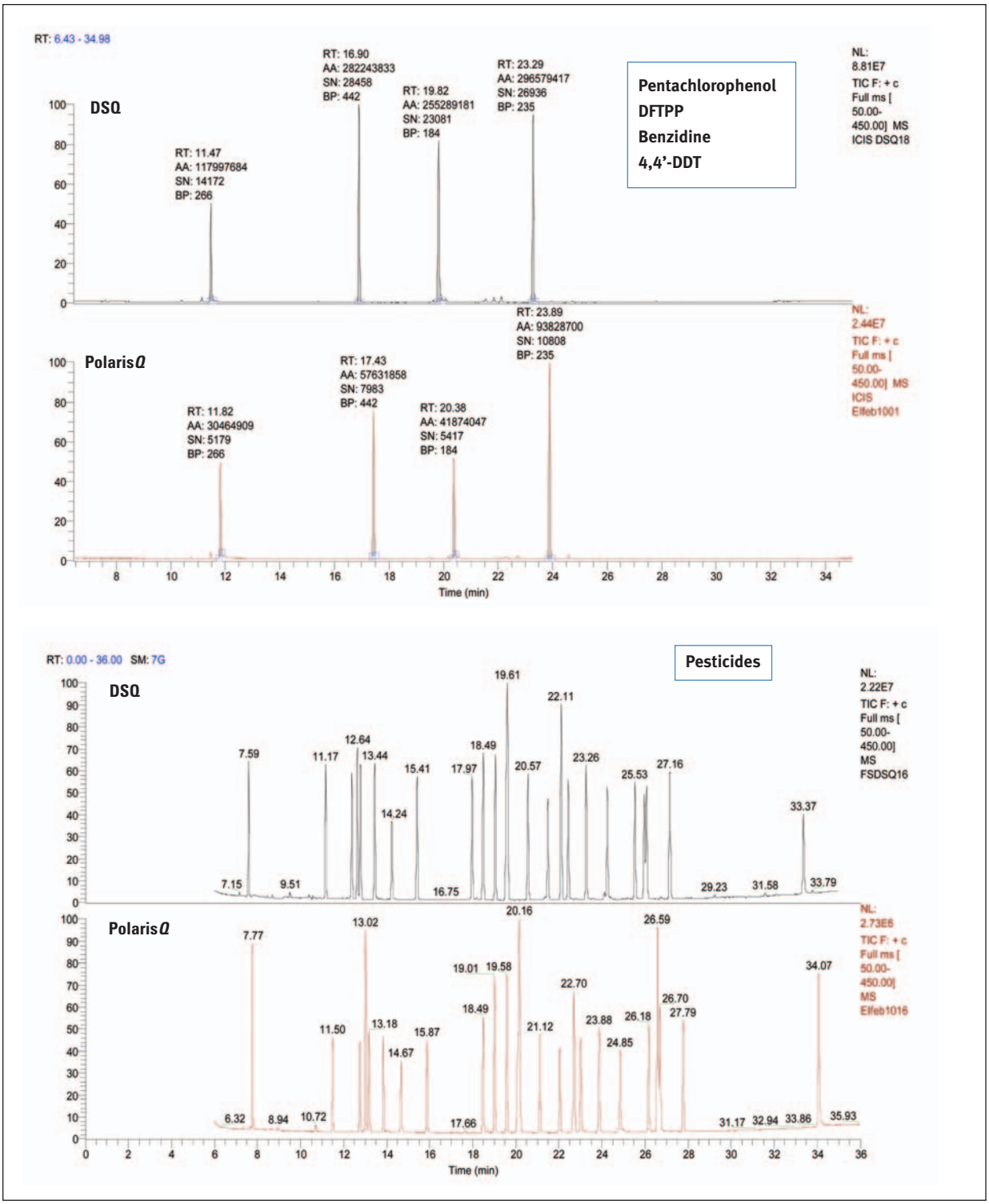


Figure 5: TIC on Polaris*Q* Ion Trap and DSQ Quadrupole of Performance Mix and Chlorinated Pesticides

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).

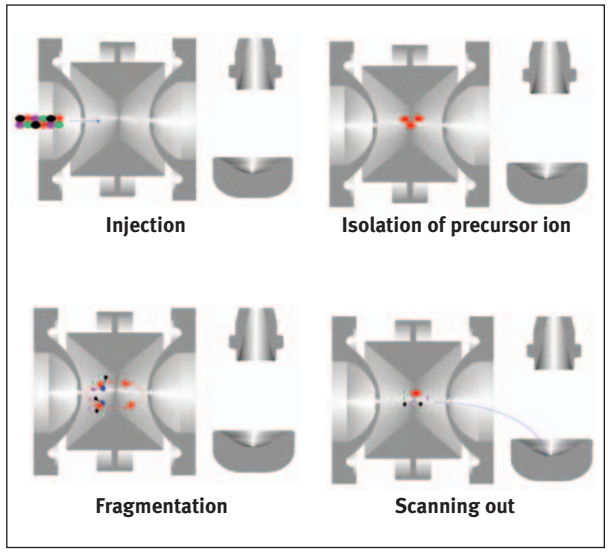


Figure 7: Tandem MS: Generating Product Ions

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.

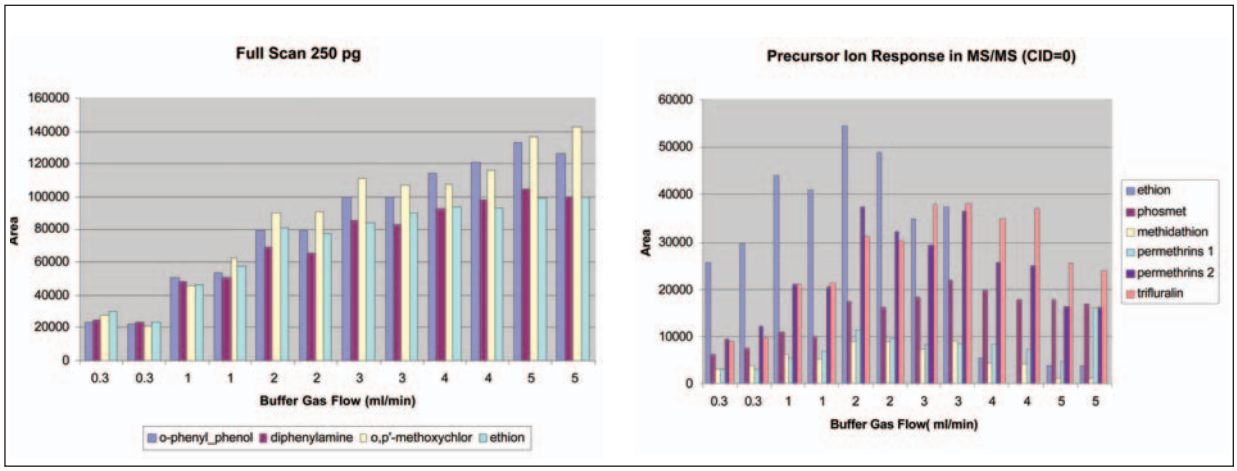
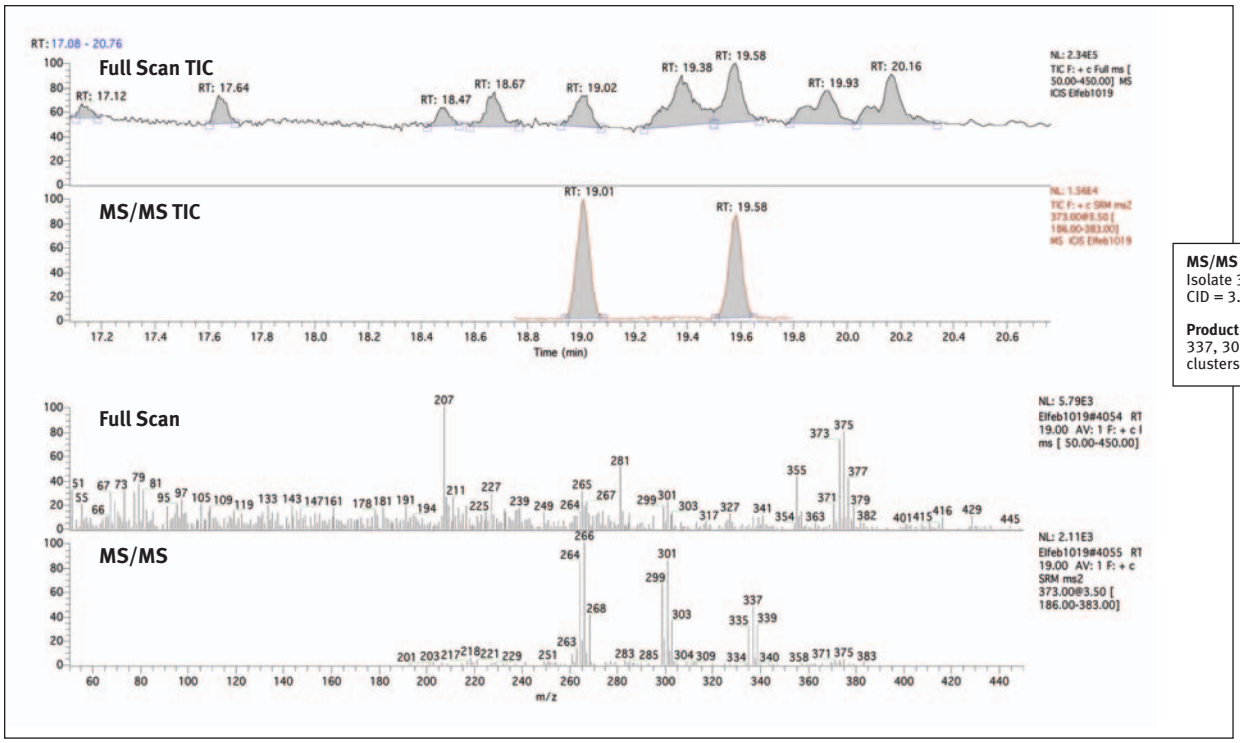


Figure 6: Enhanced Sensitivity with Variable Buffer Gas Control



MS/MS of Chlordane:
Isolate 373 m/z width 6 amu
CID = 3.5

Product Ions:
337, 301, and 266 chlorine clusters

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: Polaris*Q* 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 Polaris*Q* in sequential FS and MS/MS for Methoxychlor in vegetable matrix at 40 ppb (Figure 9).

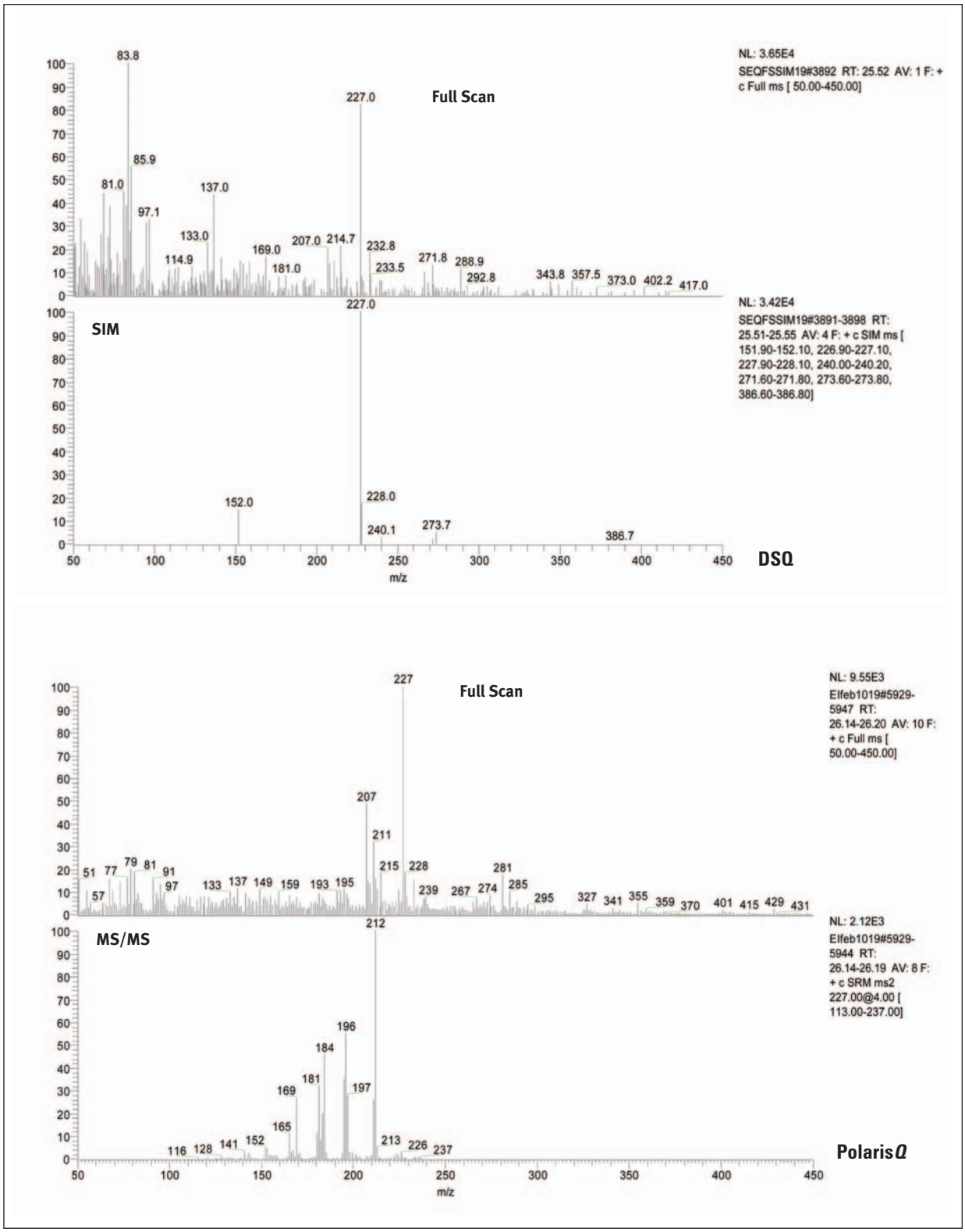


Figure 9: Spectra for Methoxychlor on the DSQ and Polaris*Q* in Matrix (40 ppb)

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

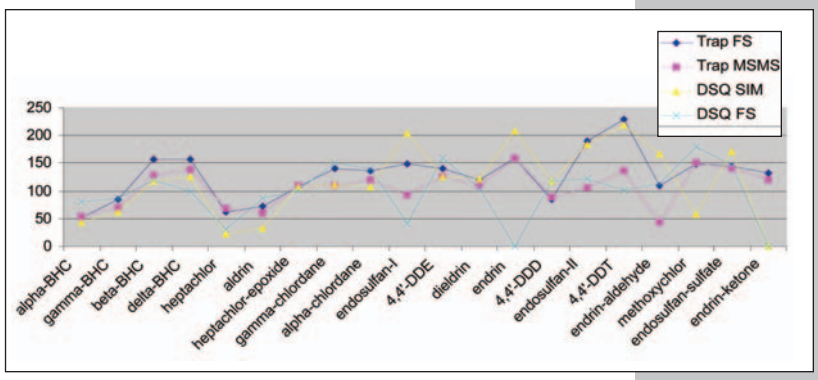


Figure 11: Recovery of 40 ppb Spike on the PolarisQ and the DSQ

	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

Authors: Jessie Butler and Meredith Conoley

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Australia
+61 2 8844 9500
Austria
+43 1 333 50340
Belgium
+32 2 482 30 30
Canada
+1 800 530 8447
China
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