Identifying Pesticides with Full Scan, SIM, µECD, and FPD from a Single Injection

Application

Food Safety, Environmental

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Abstract

In this application note, a gas chromatography/mass spectrometry (GC/MS) system capable of providing up to four signals from a single injection is described. When a three-way micro-fluidic splitter is added to the end of the column, two additional signals from GC detectors can be acquired together with the MS data from a single injection. This multi-signal configuration provides: full-scan data for library searching, selective ion monitoring (SIM) data for trace analysis, micro-electron capture detector and flame photometric detector data for excellent selectivity and sensitivity from complex matrices. A combination of element selective detectors, SIM/Scan, and deconvolution reporting software makes a very powerful pesticide analysis system. Examples for trace-level compound quantitation/confirmation or for screening are discussed.

Introduction

Many laboratories in the world are analyzing pesticide residue levels in both foods and the environment to protect human health. The process usually involves homogenizing the sample, extracting the pesticides, and analyzing the target compounds with a Gas Chromatograph (GC) or a Liquid Chromatograph (LC) depending on the nature of the compounds. For GC amenable compounds, the traditional detectors are NPD (Nitrogen Phosphorus Detector), µECD (micro-Electron Capture Detector), and FPD (Flame Photometric Detector) for their excellent sensitivity and selectivity. However, even with dual-column confirmation analysis, these GC detectors cannot be used to verify the identity of the compounds with high confidence.

Full scan mass spectral data and library searching are typically used for final compound verification. However, full-scan analysis has a worse (higher) detection limit (DL) compared to selective detectors on a GC. To improve the DL, the technique selective ion monitoring (SIM) is often used. With SIM, the MS monitors only a few characteristic ions for each target compound within the retention time (RT) range that the target elutes from the column. By monitoring only a few specific ions, the signal-to-noise ratio (S/N) improves significantly. The ions monitored are time programmed in groups corresponding to the RTs of the targets. SIM analyses with closely eluting targets require precise alignment of chromatographic RTs with the time programming of SIM groups. The Retention Time Locking (RTL) technique can be applied to eliminate the need to adjust SIM group time-windows after column maintenance or replacement.

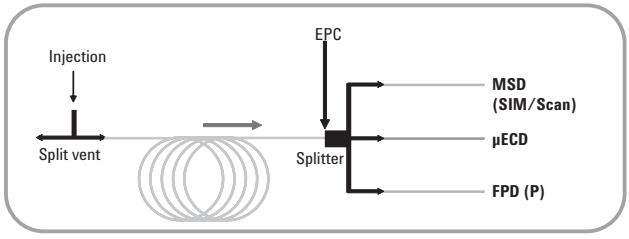
In this application note, a GC/MS system capable of providing up to four signals from a single injection is described. The benefits of the multi-signal detection include:

- Confirmatory information – Full-scan data for library search capability

- Maximum sensitivity – SIM data enables trace analysis
- Excellent selectivity – µECD and FPD detect trace-level hetero-compounds from complex matrices

Experimental

A recent technical note describes "Synchronous SIM/Scan", which takes advantages of the Performance Electronics in the 5975 inert MSD to get both SIM and full-scan signals in a single run without sacrificing performance [1]. The SIM method can be easily developed automatically using the ChemStation's AutoSIM tool [2]. By simply selecting a checkbox in the method, the SIM and full-scan data can be acquired together. The trade-off is giving up some cycles per second but gaining an additional signal (full-scan data or SIM data) for the whole analysis. With properly chosen acquisition parameters, for example, increasing the scan speed, the decrease of cycles per second is usually not significant and does not affect peak quantitation or the quality of results (for example, S/N).



At the end of the column, effluent flow is split three ways according to the length and diameter of the capillary tubing (restrictor) used.

Figure 1. A schematic of the multi-signal configuration. Note: the EPC flow adds to the column flow into the splitter.

Besides the SIM/Scan data, the ChemStation software can simultaneously acquire up to two additional GC detector signals, for example, FPD (in phosphorus- or sulfur- mode) and NPD (nitrogen-phosphorus detector) signals or both P- and S- signals from a dual-wavelength FPD (DFPD). See Figure 1.

Figure 1 is a schematic for multi-signal detection. At the end of the column, a three-way micro-fluidic splitter was used to split the column effluent to different detectors [3]. For this study, an FPD and a µECD were installed. Notice on the figure that an Auxiliary Electronic Pneumatics Control (Aux EPC) gas channel was connected to the splitter to maintain the pressure at the end of the column so that the split ratios/flows are kept constant throughout a run. Figure 2 shows a close-up view of the micro-fluidic splitter installed in the GC oven.

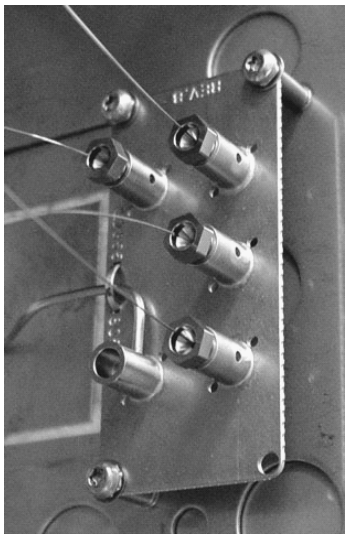


Figure 2. A close-up view of the micro-fluidic three-way splitter in the 6890 GC oven.

The size of the micro-fluidic plate is 1.25 inches (3.2 cm) wide and 2.5 inches (6.4 cm) tall. The device was designed to eliminate the common problems of large thermal mass, excess dead volume, and leaky connection due to oven temperature cycling etc. The splitter's flow paths and connection points are laid out and etched onto a thin, stainless steel plate using photolithography and chem-milling technologies. The plate is diffusion bonded, mounted with column connectors, and surface deactivated, resulting in an integrated and compact micro-fluidic splitter. Metal ferrules are used at the connectors that are leak-free after temperature cycling and will not absorb solvents or sample matrix, improving sensitivity for trace analysis applications.

Deactivated capillary tubing between the splitter and each detector was used as a flow restrictor. Aux EPC pressure and the restrictor dimensions were determined using a spreadsheet-like calculator program to achieve the proper split ratio among all detectors. The three-way splitter can easily turn into a two-way splitter when a connector is capped.

Other advantages of a splitter include back-flushing [3] and quick-swapping. The Aux EPC flow can be run-time programmed to a higher pressure, while at the same time the inlet pressure is lowered to near ambient. This causes the column flow to reverse direction, back-flushing the less volatile materials out of the split vent of the inlet. The Aux EPC on the splitter also allows column changing and inlet maintenance without cooling and venting the MSD. The splitter's flow paths and connection points were designed in such a way

that when the column fitting is removed, the helium gas from the Aux EPC purges the fitting, preventing air from entering the splitter/MSD. See Table 1 for hardware details and settings.

Table 1. Gas Chromatograph, Mass Spectrometer, and Three-Way Splitter Operating Parameters

GC	Agilent Technologies 6890		
Inlet	EPC Split/Splitless		
Mode	Splitless, 1.0 µL injected (7683 ALS)		
Inlet temp	280 °C		
Pressure	~27 psi (chlorpyrifos methyl RT locked to 16.596 min)		
Purge flow	50.0 mL/min		
Purge time	0.75 min		
Total flow	55.3 mL/min		
Gas saver	Off		
Gas type	Helium		
Inlet liner	Siltek Cyclosplitter, 4-mm id, Restek p/n 20706-214.1		
Oven			
Oven ramp	°C/min	Final (°C)	Hold (min)
Initial		70	2.00
Ramp 1	25	150	0.00
Ramp 2	3	200	0.00
Ramp 3	8	280	15
Total run time		46.87 min (last standard elutes around 35 min)	
Equilibration time		0.5 min	
Oven max temp		325 °C	
Column		Agilent Technologies HP 5-ms, p/n 19091S-433	
Length		30.0 m	
Diameter		0.25 mm	
Film thickness		0.25 µm	
Mode		Constant pressure	
Nominal initial flow		2.5 mL/min	
Outlet		Unspecified	
Outlet pressure		3.8 psi (Aux EPC pressure to splitter)	
Front detector (FPD)			
Phosphorus mode		**Sulfur mode**	
Hydrogen flow:	75.0 mL/min	Hydrogen flow:	50.0 mL/min
Oxidizer flow:	100.0 mL/min	Oxidizer flow:	60.0 mL/min
Temperature:	250 °C		
Oxidizer gas type:	Air		
Mode:	Constant makeup flow		
Makeup flow:	60.0 mL/min		
Makeup gas type:	Nitrogen		
Lit offset:	2.00		
Data rate:	5 Hz		

Table 1. Gas Chromatograph, Mass Spectrometer, and Three-Way Splitter Operating Parameters (Continued)

Back detector (μECD)	
Temperature:	300 °C
Mode:	Constant makeup flow
Makeup flow:	60.0 mL/min
Makeup gas type:	Nitrogen
Date rate:	5 Hz
Thermal AUX 2	
Use:	MSD Transfer line heater
Initial temp:	280 °C
Pressure AUX 5	
Gas type:	Helium
Initial pressure:	3.80 psi
Initial time:	0.00 min (this value will follow oven ramp)
MSD	Agilent Technologies 5975 inert MSD
Tune file	Atune.U
Mode	Scan
Solvent delay	3.00 min
EM voltage	Atune voltage
Low mass	45 amu
High mass	555 amu
Threshold	100
Sampling	2
A/D Samples	4
Scans/s	2.89
Quad temp	150 °C
Source temp	230 °C
Three-way splitter	Agilent 6890N Option 890, when installed on the GC during factory assembly
Split ratio	10:10:1 MSD:FPD:μECD
MSD restrictor	1.444 m × 0.18-mm id Deactivated fused silica tubing
FPD restrictor	0.532 m × 0.18-mm id Deactivated fused silica tubing
μECD restrictor	0.507 m × 0.10-mm id Deactivated fused silica tubing
Flow to MSD (at 280 °C)	1.53 mL/min
Flow to FPD (at 280 °C)	1.53 mL/min
Flow to μECD (at 280 °C)	0.153 mL/min
Makeup flow (at 280 °C)	1.38 mL/min

Software Used in this Application Note

GC/MSD ChemStation	G1701DA
Deconvolution Reporting Software (DRS)	G1716AA
NIST Library	G1033A
AMDIS (included for free with the NIST library CD)	

Results and Discussion

Figure 3 shows four signals that were simultaneously acquired from a single injection of a pesticide mixture. Due to the high sensitivity of the µECD, the split ratios for the three detectors was set to MSD:FPD:µECD = 10:10:1. This split ratio distributes the sample of a 1-µL splitless injection of a 1-ppm (1000 pg/µL) sample to the different detectors as labeled in Figure 3.

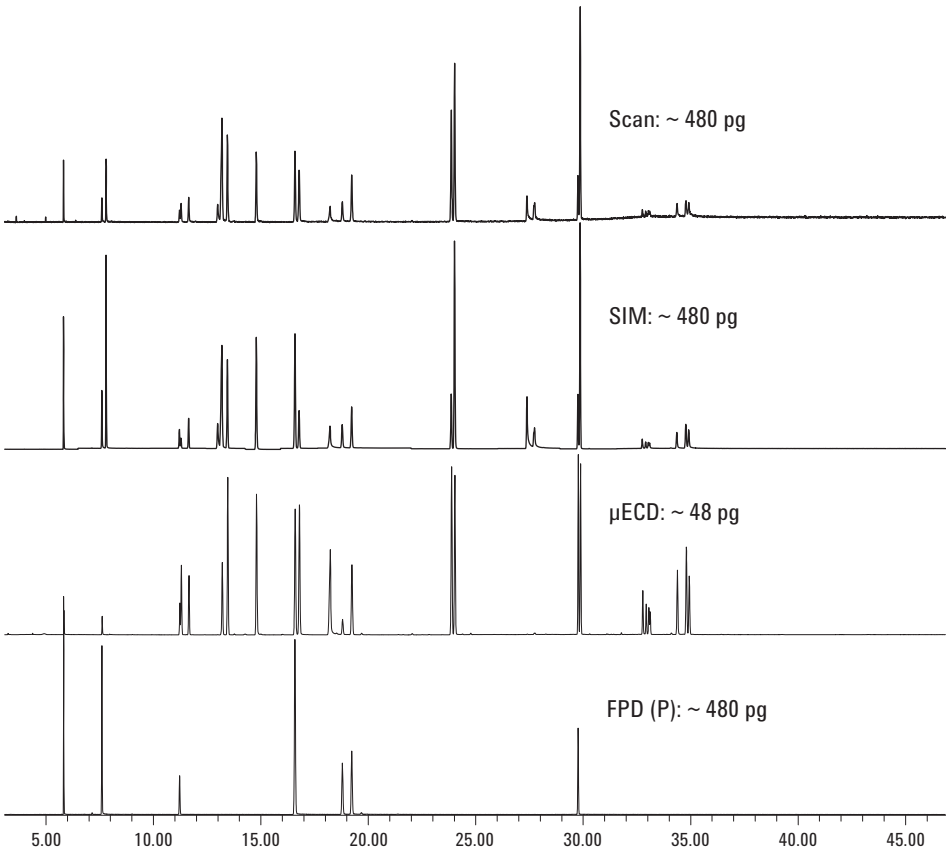


Figure 3. Signals acquired simultaneously from a 1-µL splitless injection of 1-ppm standard. The split ratios were MSD:FPD:µECD = 10:10:1.

Figure 4 shows the signals when the pesticide standard was diluted 100-fold in a produce matrix. The total ion chromatogram (TIC) from full scan was not shown due to the lack of sensitivity. The FPD(P) and µECD were able to detect all the pesticides spiked in this extract. For trace-level target compound analysis, the SIM signal can be used for quantitation and the GC signals used for further confirmation.

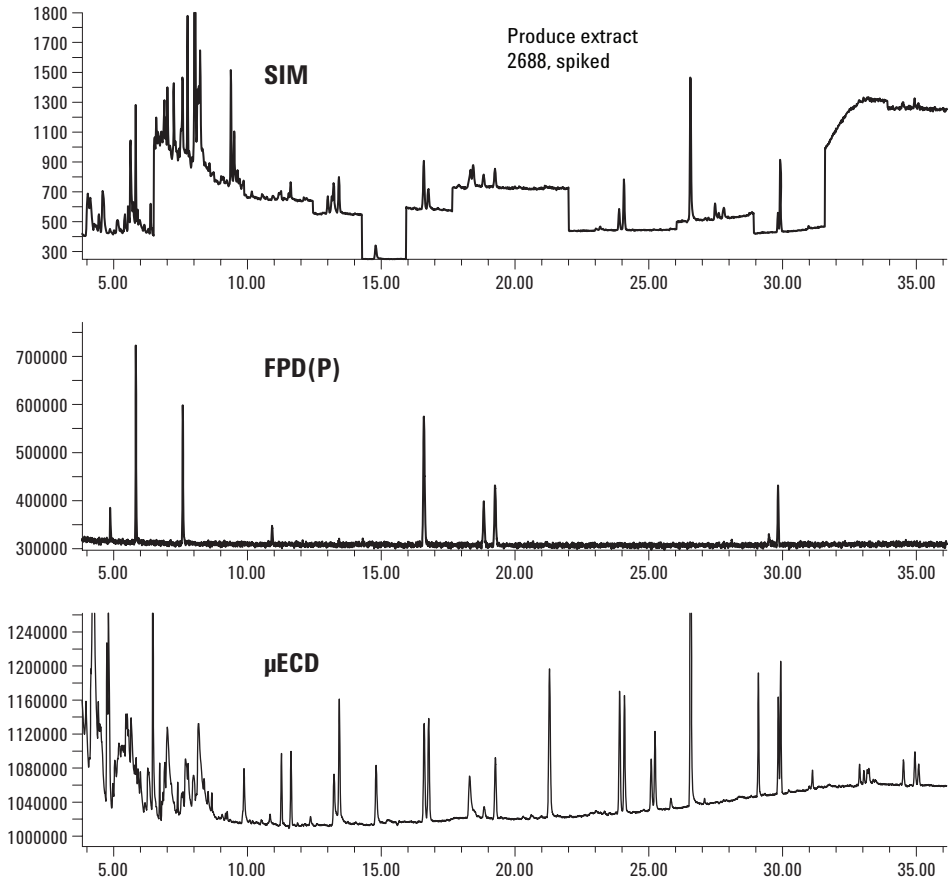


Figure 4. Data of a produce extract spiked at 10 ppb. FPD and µECD were able to detect the respective standards spiked into the extract.

Another application for this multi-signal system is for screening. In screening, no target list is available for the analysis; therefore, SIM acquisition or MS/MS is not possible. Figure 5 shows three signals (no SIM) from a produce extract.

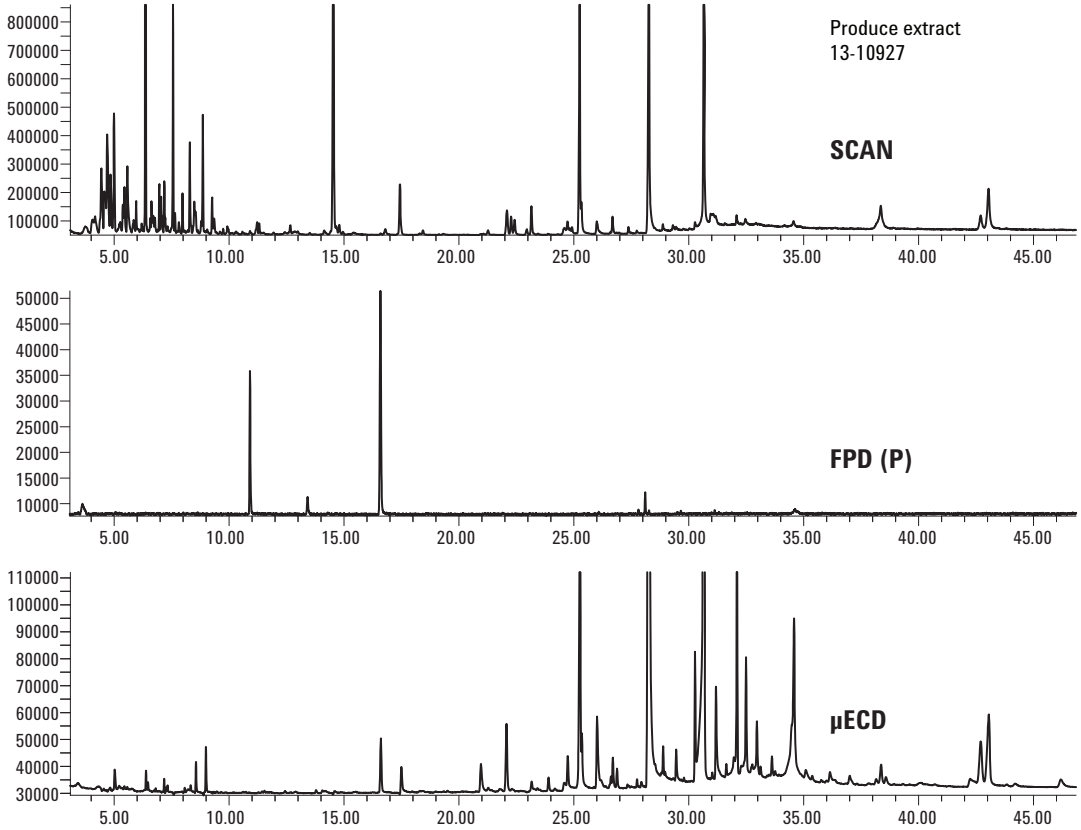
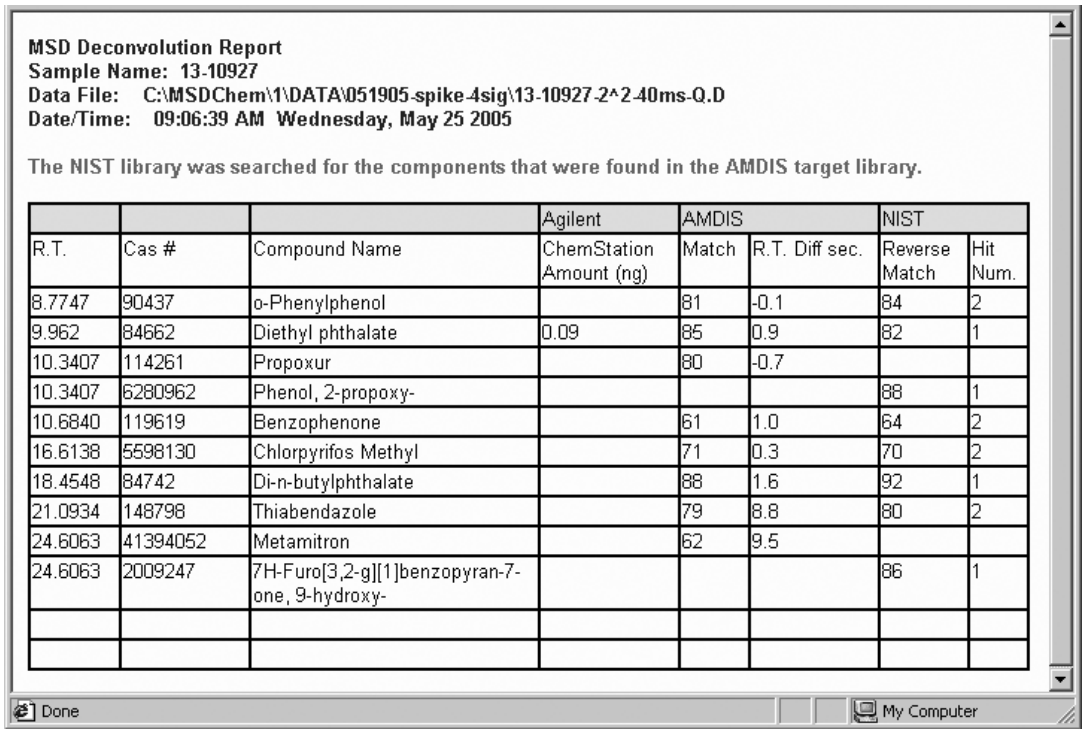


Figure 5. Full-scan, FPD(P), and µECD data for extract 13-10927.

The Deconvolution Reporting Software (DRS) [3, 4] found several pesticides in the TIC as shown in Figure 6.



MSD Deconvolution Report
Sample Name: 13-10927
Data File: C:\MSDChem\1\DATA\051905-spike-4sig\13-10927-2^2-40ms-Q.D
Date/Time: 09:06:39 AM Wednesday, May 25 2005

The NIST library was searched for the components that were found in the AMDIS target library.

			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
8.7747	90437	o-Phenylphenol		81	-0.1	84	2
9.962	84662	Diethyl phthalate	0.09	85	0.9	82	1
10.3407	114261	Propoxur		80	-0.7		
10.3407	6280962	Phenol, 2-propoxy-				88	1
10.6840	119619	Benzophenone		61	1.0	64	2
16.6138	5598130	Chlorpyrifos Methyl		71	0.3	70	2
18.4548	84742	Di-n-butylphthalate		88	1.6	92	1
21.0934	148798	Thiabendazole		79	8.8	80	2
24.6063	41394052	Metamitron		62	9.5		
24.6063	2009247	7H-Furo[3,2-g][1]benzopyran-7-one, 9-hydroxy-				86	1

Done My Computer

Figure 6. Report for extract 13-10927 generated from DRS.

The possible pesticides in the sample were benzophenone, chlorpyrifos methyl, and thiabendazole. Propoxur and metamitron were not confirmed by both AMDIS and NIST; therefore, they were most likely false positives.

Due to the complexity of the sample matrix and other interferences, it is sometimes difficult to get a high library match factor from peaks in the TIC, even after background subtraction. Therefore, element selective detectors would be very useful in providing the supporting information for compound confirmation. The multi-signal system was retention time locked, therefore, from the RT and the aligned peaks from the FPD(P) and the µECD responses, chlorpyrifos methyl ($C_7H_7Cl_3NO_3PS$) was confirmed.

It usually takes less than 3 minutes to turn off the FPD photomultiplier, swap the P-filter with the S-filter, and turn the photomultiplier back on. After the swap, adjust the detector gas flows to optimize the response in either P- or S- mode. A new injection of the same extract was made in FPD(S) mode. The FPD(S) result is shown with previously acquired signals in Figure 7. Two major peaks were seen on the FPD(S) chromatogram. From the peak RTs, they supported the presence of chlorpyrifos methyl and thiabendazole ($C_{10}H_7N_3S$) respectively. Note that the full-scan TIC barely showed a peak for either compound, which made it impossible for traditional data analysis to identify both compounds. The FPD(S) mode is very selective, but it is not as sensitive as the FPD(P) mode. Although the µECD is very sensitive, it is not as selective as the FPD. A combination of GC detectors, SIM/Scan, and DRS makes a very powerful pesticide analysis system.

Conclusion

The Synchronous SIM/Scan provides users with library searchable full-scan spectra as well as trace level SIM data in a single analysis. When a three-way micro-fluidic splitter is added to the end of the column, two additional signals from element selective detectors can be acquired together with the MS data from a single injection. This configuration makes it very attractive for the analysis of trace-level pesticide residues in foods or environmental samples.

This multi-signal configuration provides: full-scan data for library searching, SIM data for trace analysis, µECD and FPD data for excellent selectivity and sensitivity from complex matrices. In this application note, examples of µECD signal and FPD signal (P- or S- mode) were acquired together with the SIM/Scan data from a single injection for trace-level compound quantitation/confirmation, or for screening.

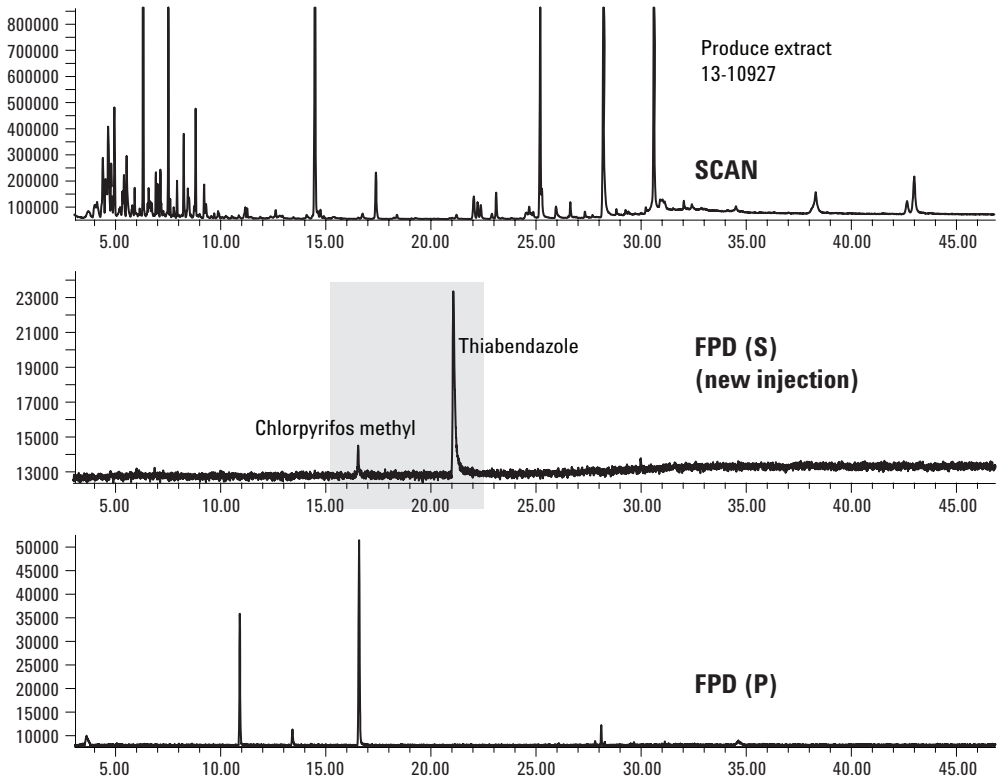


Figure 7. Full-scan, FPD(S), and FPD(P) data for extract 13-10927.

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Printed in the USA
July 6, 2005
5989-3299EN

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