New Tools for Rapid Pesticide Analysis in High Matrix Samples

Application

Food Analysis

Authors

Mike Szelewski and Bruce Quimby
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

Recent developments in GC/MS hardware and software make it possible to analyze samples with high levels of matrix contamination much faster than ever before. New tools such as mass spectral deconvolution, reliable and inert effluent splitters, and column backflushing capabilities can be combined to produce large time savings. By accelerating the chromatographic run, post-run bakeout, and data interpretation steps, analysis times can be shortened by at least three-fold versus conventional methods. These tools are especially useful in analyses with high levels of matrix background, such as the inspection of the food supply for contaminants. In addition to monitoring for pesticide residues, the threat of terrorism has recently raised concerns over deliberate contamination of food with other toxic materials. This article describes a GC/MS system for the rapid screening of foodstuffs for chemical contaminants with a special emphasis on pesticides, organophosphorus, and organosulfur compounds.

Introduction

Techniques for decreasing the analysis time of gas chromatography (GC) methods have been developed in recent years.

Tools like Method Translation [1] have made it straightforward to reduce analysis time by a known factor and maintain the exact relative elution order of the analytes. The use of appropriate shorter and smaller diameter columns can maintain the same resolution while achieving a much shorter analysis time.

One application area where this approach has met difficulty, however, is the gas chromatography/mass spectrometry (GC/MS) analysis of pesticides in complex matrices like food. This application requires that speed-up schemes maintain column capacity in order to handle the large matrix peaks and achieve low detection limits for analytes. Since chromatography is governed by the triangle of speed, resolution, and capacity, resolution must be sacrificed to increase speed at the same capacity. The problem is that chromatographic resolution is also needed to confirm the identity of any target analytes detected in the presence of interferences from the sample. In this note, the reduction in chromatographic resolution in faster analysis is more than adequately compensated for by use of spectral deconvolution [2] and simultaneous element-selective detection for the confirmation step.

The system consists of a GC/MS with a dual-wavelength flame photometric detector (DFPD) for the simultaneous collection of phosphorus, sulfur, and mass spectral data.

The GC column effluent is split between the two detectors in the ratio of 2:1 in favor of the mass selective detector (MSD). The system is retention-time locked to the Agilent pesticide library [1] scaled to threefold faster times, which contains the

retention times and spectra for 567 pesticides used worldwide. Samples are analyzed with MS in full-scan electron-impact ionization (EI) mode. The combination of precise retention times, elemental, and mass spectral data is used to screen for specific target compounds. The flame photometric detector (FPD) data also highlights any non-target, P- or S-containing compounds for identification by MS.

The MS data is screened using the standard quantitation software based on retention time (RT), ion ratios, and spectral cross correlation. The MS data is also processed using spectral deconvolution software, which greatly reduces spectral interferences from the matrix. The deconvoluted spectra are then searched against a table of targets. Any hits are confirmed by searching against the main NIST library. This process is automated by the Agilent Deconvolution Reporting Software (DRS), also providing significant time savings in data interpretation.

The system described here uses column backflushing, a technique used to save large amounts of time with complex samples. Backflushing is done with the splitter hardware. This technique removes heavy residues from the column much faster and at lower temperatures than the conventional bakeout step at the end of the run. This reduces MS source contamination by preventing the higher levels of column bleed and heavy matrix components from entering the MSD. It also increases the column lifetime.

The approach used thus reduces analysis time in three major ways: shortening the chromatographic run time; automating data interpretation; and reducing bakeout time. Other notable advantages are the ability to change columns and/or inlet liners without venting the MSD, and a reduced need for MS source cleaning.

System Configuration

The system configuration used is shown in Figure 1. Key components are:

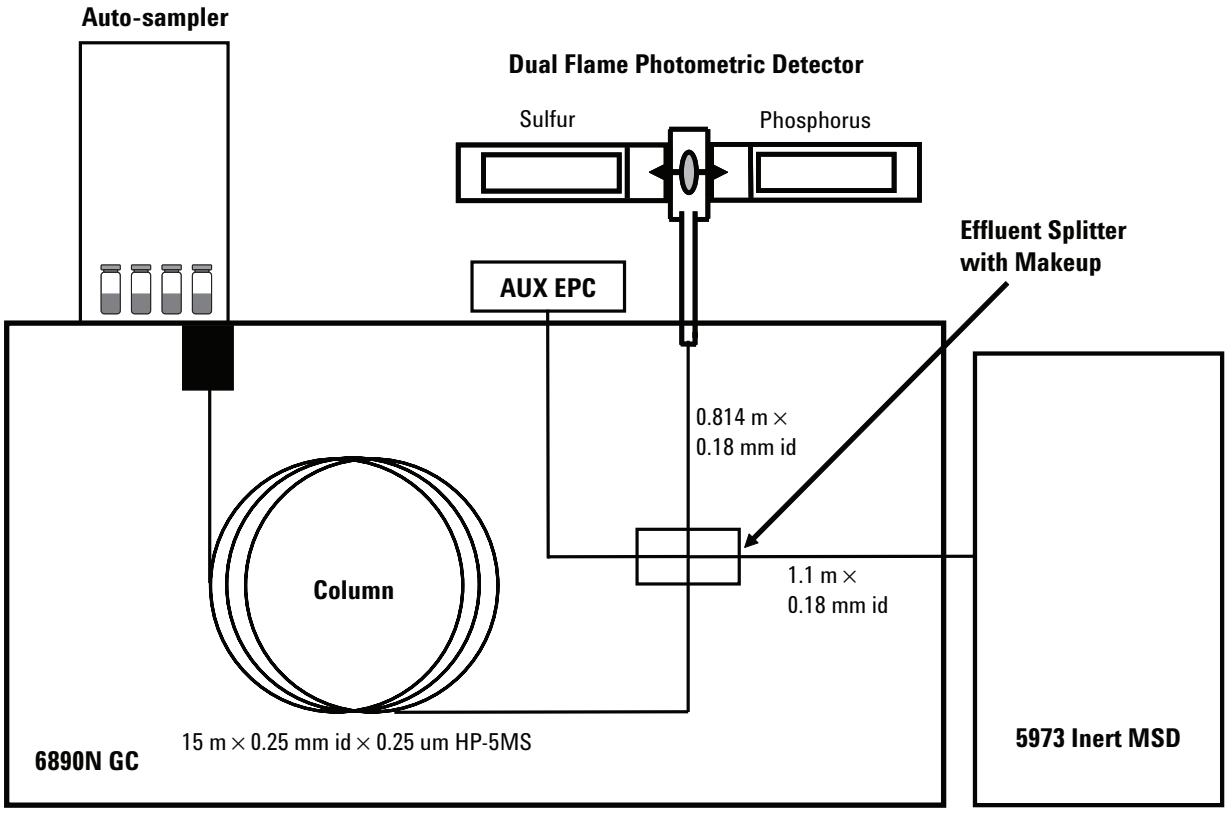


Figure 1. System configuration.

Key Components

Fast Oven With the 6890N 220V oven (option 002), the pesticide analysis method can be run precisely 3 times faster (14 min) using a 15 m HP-MS column. If the 220V GC is further equipped with SP1 2310-0236 (puts MSD interface in back of oven under rear injection port) and the G2646-60500 oven insert accessory (reduces oven volume twofold), the speed can be increased to 4.8 times faster (9 min). The cool-down time of the oven is also reduced.

Dual FPD 6890N Option 241 is a single flame photometric detector with two optical detection channels, one for sulfur and one for phosphorus. The signals from the DFPD are collected, stored, and processed by the MS ChemStation simultaneously with the MS data. The FPD data can be used in several ways. Nontarget organophosphorus compounds like new pesticides or designer nerve agents are highlighted. The presence of an element at the retention time of an identified compound can be used to support confirmation of identity. The response on the FPD can be used for quantitative or semi-quantitative analysis, especially for situations where no calibration standard is available for an identified analyte.

Microfluidic Splitter 6890N Option 889 uses diffusion bonded plate technology combined with metal column ferrules to make an inert, easy-to-use, leak-free, high-temperature column effluent splitter. The splitter uses Auxillary (Aux) electronic pneumatics control (EPC) for constant pressure makeup (6890N Option 301). The Aux EPC makeup can be pressure programmed at the end of the run to higher pressure, while at the same time the inlet pressure is lowered to near ambient. This causes the flow in the column to reverse direction, backflushing heavy materials out the split vent of the inlet. The Aux EPC also allows column changing and maintainance without venting the MSD. When the column fitting is removed from the splitter, helium from the makeup supply purges the fitting, preventing air from entering the MSD. If the column is attached to the splitter but removed from the inlet, helium flows backwards through the column and out the inlet end. Inlet maintainance or column headtrimming can be done without cooling and venting the MSD and air is not introduced into a hot source.

MSD System The 5973N Inert with Performance Electronics and performance turbo (G2579A) EI MSD is used. This configuration provides faster full scan rates while maintaining sensitivity. The scan rates are compatible with the narrower peaks generated by fast chromatography. The performance turbo pump is required to handle the higher flows associated with fast chromatography and backflushing.

DRS Software (G1716AA) Spectral deconvolution of the MS data allows identification of analytes in the presence of overlapped matrix peaks. This significantly reduces chromatographic resolution requirements, allowing much shorter analysis times. DRS utilizes the AMDIS deconvolution program from NIST, originally developed for trace chemical weapons detection in complex samples. DRS presents the analyst with three distinct levels of compound identification: (1) ChemStation, based on retention time and four ion agreement; (2) AMDIS, based on “cleaned spectra” full ion matching and locked retention time; (3) NIST02 search using a >147,000 compound library.

Instrument Operating Parameters

The recommended instrument operating parameters are listed in Table 1. These are starting conditions and may have to be optimized.

Split injection was used to match the amount of matrix to the column capacity. Citrus oils cause retention shifts if excess sample is injected. Splitless injection could be used for samples with significantly less matrix. The inlet liner was found to be of low activity, as it does not contain glass wool. Proper mixing for split injections is done by the internal liner geometry.

The 6890 220V oven was needed for the ramps described in Tables 1 and 2. This oven program is necessary for the precise 3× speed increase of the RTLocked pesticide database.

The 15-m HP-5ms column has the same phase ratio as the 30 m column traditionally used for the 1× method. This shorter column allows a flow rate for a 3× precisely scaled faster method. The outlet is listed as “unspecified” because the column connects to the splitter. The splitter pressure is operated at a constant 3.8 psig using an auxillary EPC module.

The 5973 inert Performance Electronics data acquisition sampling rate was set to 1, which is faster than the typical setting of 2. Signal-to-noise is improved over previous systems at faster sampling rates. More data points allows for easier integration and better deconvolution to compensate for the loss in resolution using a shorter column.

The microfluidic splitter parameters are chosen to provide the desired split ratio between detectors

while meeting the flow requirements of the detectors used. A primary consideration with the current system is to make sure that the flow to the MSD does not exceed ~4 mL/min while collecting analyte data. It was also desired to split the effluent 2:1 in favor of the MSD. These parameters were entered into the spreadsheet calculator (included with the splitter), which calculated the lengths and diameters of the detector restrictors

Table 1. Gas Chromatograph and Mass Spectrometer Operating Parameters

GC	Agilent Technologies 6890		
Inlet	EPC Split/Splitless		
Mode	Split, 1.0 µL injected		
Inlet temp	250 °C		
Pressure	23.84 psi		
Split ratio	10:1		
Split flow	44.1 mL/min		
Total flow	48.1 mL/min		
Gas saver	Off		
Gas type	Helium		
Inlet Liner	Siltek Cyclosplitter, 4 mm id, Restek part number 20706-214.1		
Oven	220V		
Oven ramp	°C/min	Next °C	Hold min
Initial		70	0.67
Ramp 1	75	150	0.00
Ramp 2	9	200	0.00
Ramp 3	24	280	3.33 (end of pesticide ramp)
Ramp 4	50	320	50.0 (end of oil elution)
Total run time	13.96 min to elute pesticides		
Total run time	64.76 min to elute heavy components from citrus oils		
Equilibration time	0.5 min		
Oven max temp	325 °C		
Column	Agilent Technologies HP-5MS, p/n 19091S-431		
Length	15.0 m		
Diameter	0.25 mm		
Film thickness	0.25 µm		
Mode	Constant Pressure = 23.84 psi		
Inlet	Front		
Outlet	Unspecified		
Outlet pressure	3.8 psi (aux pressure to splitter)		
Back Detector (FPD)			
Temperature	250 °C		
Hydrogen flow	75.0 mL/min		
Oxidizer flow	100.0 mL/min		
Oxidizer gas type	Air		
Mode	Constant makeup flow		
Makeup flow	60.0 mL/min		
Makeup gas type	Nitrogen		
Flame	On		
Lit offset	5.00		
Photo multiplier	On		

Table 1. Gas Chromatograph and Mass Spectrometer Operating Parameters (Continued)

Signal 1		Signal 2	
Data rate	5 Hz	Data rate:	5 Hz
Type	Back detector	Type:	Front detector
Save data	On	Save data:	On
Zero	0.0 (Off)	Zero:	0.0 (Off)
Range	0	Range:	0
Fast Peaks	Off	Fast Peaks:	Off
Attenuation	0	Attenuation:	0
AUX Pressure 5			
Description			
Gas type	Helium		
Initial pressure	3.80 psi		
Initial time	0.00 min (this value will follow oven ramp)		
MSD	Agilent Technologies 5973 Inert Performance Electronics		
Tune file	Atune.U		
Mode	Scan		
Solvent delay	1.00 min		
EM voltage	Atune voltage		
Low mass	45 amu		
High mass	450 amu		
Threshold	0		
Sampling	1		
Scans/sec	6.68		
Quad temp	150 °C		
Source temp	230 °C		
Transfer line temp	280 °C		
Splitter	Agilent 6890N Option 889		
Split ratio	2:1 MSD:DFPD		
MSD restrictor	1.1 m × 0.18 mm id deactivated fused silica tubing		
DFPD restrictor	0.81 m × 0.18 mm id deactivated fused silica tubing		

Backflush Instrument Operating Parameters

Instrument operating conditions for backflushing are shown in Table 2. These are starting parameters and will have to be optimized depending on sample matrix. Conditions listed here are only those that are different from the Table 1 conditions.

Instead of baking the column at 320 °C for 50 min, the heavy matrix material is backflushed through the column inlet, out the split vent. This is accomplished in 5 min at 300 °C, saving 45 min of run time, preserving column life, and shortening cool down time.

The column inlet pressure is reduced to 1.1 psi by using the ramped pressure feature of the EPC. At the end of the backflush time, it is ramped back to initial conditions.

Simultaneous with ramping the inlet pressure down to 1.1 psig, the Aux EPC splitter pressure is ramped up to 23 psig. This increase in pressure at the column outlet, along with the decrease in inlet pressure, backflushes the column. The backflush pressure for the Aux EPC is set to limit the flow to the MSD to < 8 mL/min. This is calculated using the Agilent Flow Calculation software program, also supplied with the splitter kit. The calculator is used to find the pressure which gives the desired flow through the MSD splitter restrictor (1.1 m × 0.18 mm id) at the backflushing temperature 300 °C. The result was 23.6 psig which would produce a flow of 8 mL/min, so 23 psig was used.

The backflush time is determined empirically and depends on the sample matrix. The process is to try a backflush run followed by a blank run with the conventional long-hold to see if the heavy materials

are completely removed. If not, the process is repeated with a longer backflush time. As a very rough guide, start with a backflush time of five void times for the backwards flow. Using the Agilent Flow Calculation software with the “inlet pressure” at 23 psig, the “outlet pressure” at 1.1 psig, and the temperature at 300 °C, the hold-up time (void time) is 0.423 min. The rough guide says that the column should be backflushed for 2.12 min. This works for removing most heavies, but 5 min is required in this case to remove them all.

At the end of the backflush time, the Aux EPC is ramped back to initial conditions.

The MSD is time-programmed to collect data over the time range of 1 to 13.96 min. All of the pesticides elute during this time range.

Table 2. Backflush Gas Chromatograph and Mass Spectrometer Operating Parameters. Use Table 1 Conditions Except for These Differences

Backflush Oven Conditions			
Oven ramp	°C/min	Next °C	Hold min
Initial		70	0.67
Ramp 1	75	150	0.00
Ramp 2	9	200	0.00
Ramp 3	24	280	3.33 (end of pesticide ramp)
Ramp 4	50	300	5.40 (end of oil backflush)
Total run time	13.96 min to elute pesticides		
Total run time	19.76 min to elute heavy components from citrus oils		
Backflush Column Conditions			
Mode	Ramped Pressure		
Press ramp	psi/min	Next psi	Hold min
Initial		23.84	13.96 (end of pesticide ramp)
Ramp 1	99	1.1	5.57 (backflush time)
Ramp 2	99	23.84	0.00
Backflush AUX 5 Conditions			
Press ramp	psi/min	Next psi	Hold min
Initial		3.8	13.96 (end of pesticide ramp)
Ramp 1	99	23.0	5.61 (backflush time)
Ramp 2	99	3.8	0.00
Backflush MSD Conditions			
Timed MS detector entries			
Time (min)	State (MS on/off)		
13.96	Off		

Results

A mixture of 25 pesticides was run at 1×, 3×, and 4.8× speeds. The Total Ion Chromatograms (TICs) are shown in Figure 2.

There is some loss in resolution, but the loss is limited because the shorter columns are run at flows closer to the optimum. The resolution losses are mitigated by using the faster scanning capabilities of the performance electronics together with DRS.

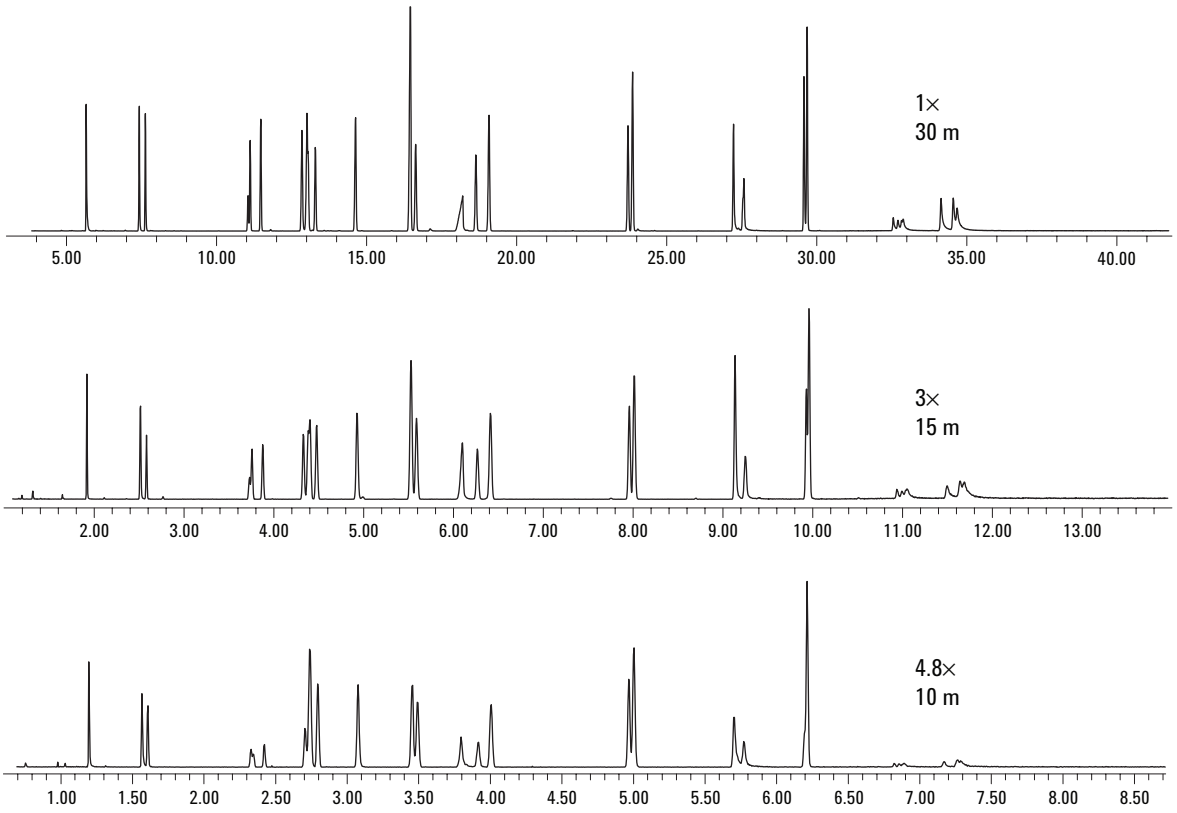


Figure 2. TICs of pesticide test mix at three different scaled speeds. All columns have the same phase ratio.

A neat lemon oil was analyzed using the 3× speed conditions. The TIC is shown in Figure 3 with the DFPD phosphorus (P) and sulfur (S) data channels. The ChemStation software can simultaneously acquire two signals of GC data with the MSD data. The pesticides elute within the 14 min window shown, but the matrix continues to elute for an additional 50 min at 320 °C.

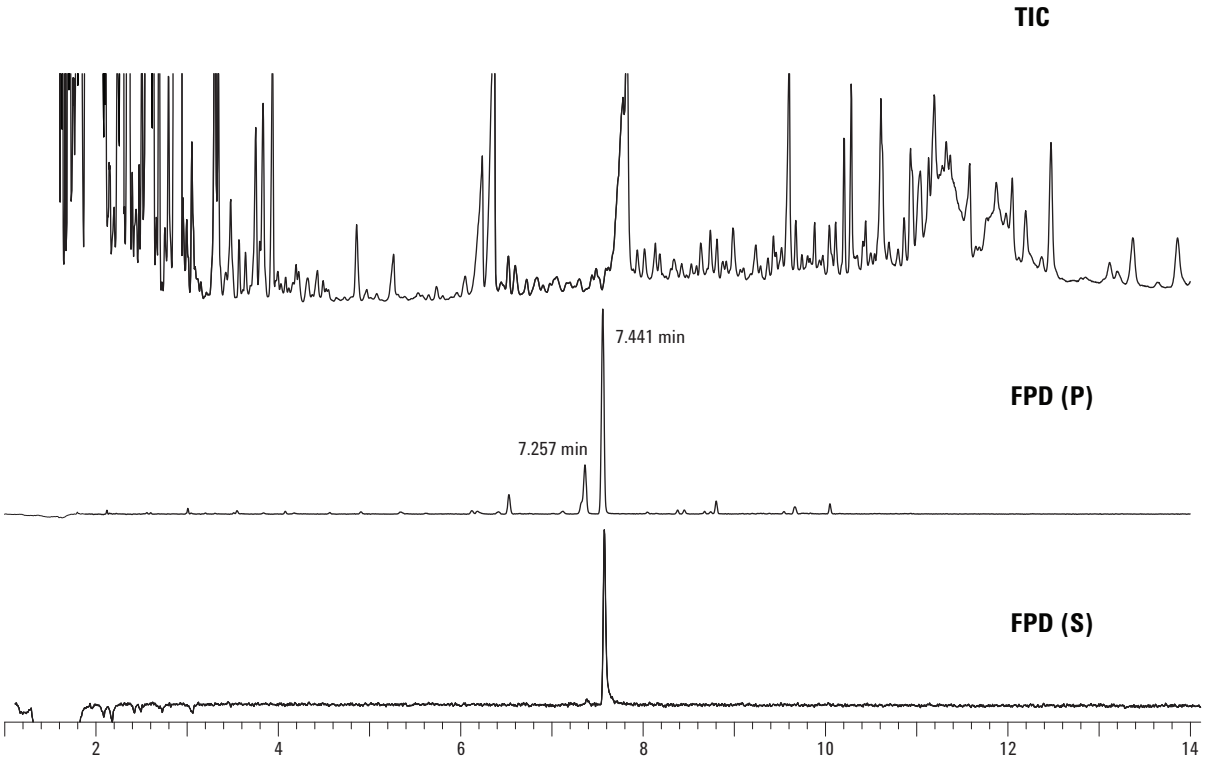


Figure 3. MS and DFPD data from lemon oil analyzed with 3x method.

The P and S chromatograms indicate the possible presence of numerous pesticides. The largest P peak, 7.441 min, also contains S. A PBM reverse Library search identified the peak as Methidathion ($C_6H_{11}N_2O_4PS_3$) with a match quality of 45. It was also identified using a target ion and three qualifier ions.

The raw apex spectrum of the P peak at 7.257 min is shown in the top of Figure 4. No match was found for a P-containing compound in the top 20 library search results. It was also not identified by the ChemStation target and qualifier ion criteria due to out-of-range ratios.

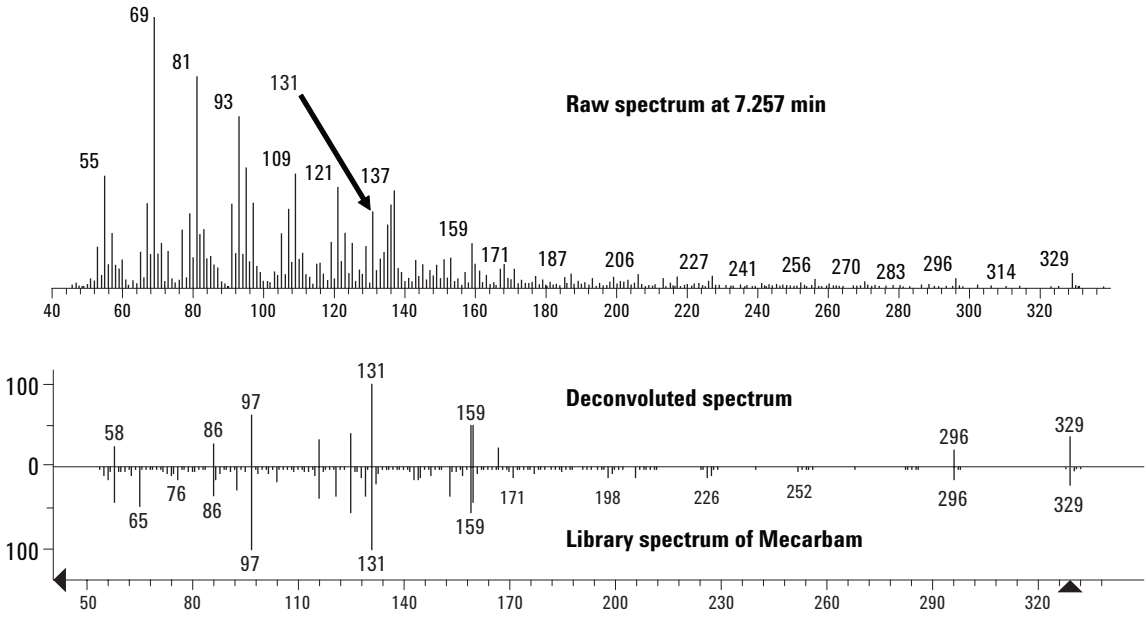
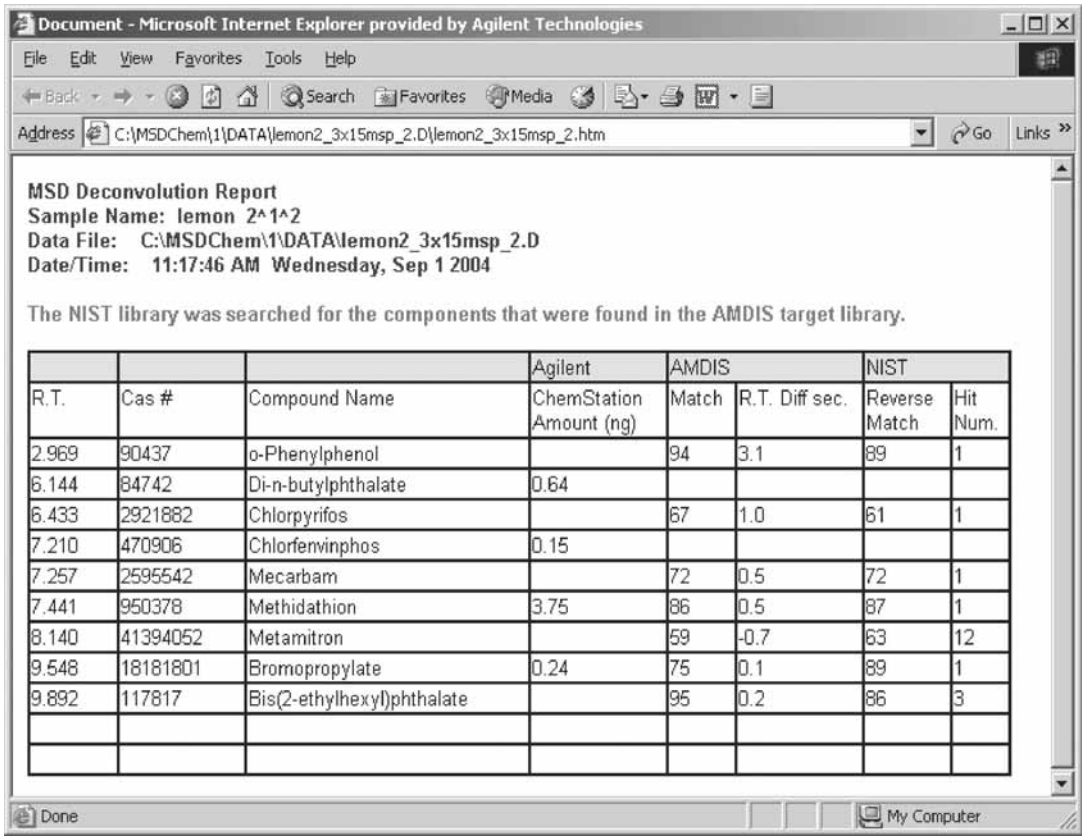


Figure 4. Top - Raw mass spectrum of peak at 7.257 min in lemon oil. Bottom - Deconvoluted spectrum of 7.257 min peak compared to NIST02 library spectrum of Mecarbam.

The DRS report is shown in Figure 5. The peak at 7.257 min is clearly identified as Mecarbam by the DRS software. The deconvoluted spectrum has a match factor of 72, compared to both the Agilent Pesticide AMDIS database and the NIST02 library. Additionally, the DRS report shows the elution time to be only 0.5 s from expected. Further confirmation is the presence of P with S barely visible. The deconvoluted spectrum for the peak at 7.257 min is shown at the bottom of Figure 4, together with the NIST library spectrum of Mecarbam.

The DRS report displays the amount for compounds found by the normal ChemStation quant process. The compounds must be properly calibrated to have a meaningful value. In this lemon oil, the ChemStation found four compounds. The AMDIS portion of DRS found two of the same compounds and an additional five compounds missed by the ChemStation. The NIST02 library search confirmed all of the compounds found by AMDIS using the NIST02 >147,000 compound library. The DRS results show good match quality at the locked retention times for seven target compounds.

No single software package can produce this same confidence level in compound identification. An experienced analyst would take 1–4 hours to process this complex sample manually with each of the three software packages used by DRS. DRS produced this report is less than two minutes.



			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
2.969	90437	o-Phenylphenol		94	3.1	89	1
6.144	84742	Di-n-butylphthalate	0.64				
6.433	2921882	Chlorpyrifos		67	1.0	61	1
7.210	470906	Chlorfenvinphos	0.15				
7.257	2595542	Mecarbam		72	0.5	72	1
7.441	950378	Methidathion	3.75	86	0.5	87	1
8.140	41394052	Metamitron		59	-0.7	63	12
9.548	18181801	Bromopropylate	0.24	75	0.1	89	1
9.892	117817	Bis(2-ethylhexyl)phthalate		95	0.2	86	3

Figure 5. DRS Report for lemon oil.

Backflushing

Citrus oils contain significant amounts of material that elute after the last pesticide. This requires a 150-min hold at 320 °C to elute all of the heavy material with a 1× method. The total run time for the 1× method is therefore 195 min, as shown in Table 3.

Table 3. Method Run Time Comparison

Column	30 m	15 m	10 m
Speed	1×	3×	4.8×
Run time	min	min	min
Pesticides	42	14	8.75
No backflush matrix	195	65	40.6
With backflush matrix	n/a	20	12.5

The 3× method requires a 50-min hold at 320 °C, as shown at the top in Figure 6, resulting in a 65-min run time. With backflushing, all of this heavy material is removed in 5 min at 300 °C, as shown in the bottom of Figure 6. This is a 9-fold reduction in analysis time compared to the 1× method.

4.8x Method

Using the 220V oven, SP1 2310-0236, and oven insert accessory, the method can be scaled to 4.8× faster, as shown in Table 3. There is a practical limit to the amount of matrix that can be tolerated with the reduced resolution using a 10-m column. However, for matrices less complex than a citrus oil, the 4.8× method can be successfully used to save even more time. The Performance Electronics allows running at faster scan speeds while maintaining signal/noise ratio. Sufficient points across a peak are maintained even with faster chromatography.

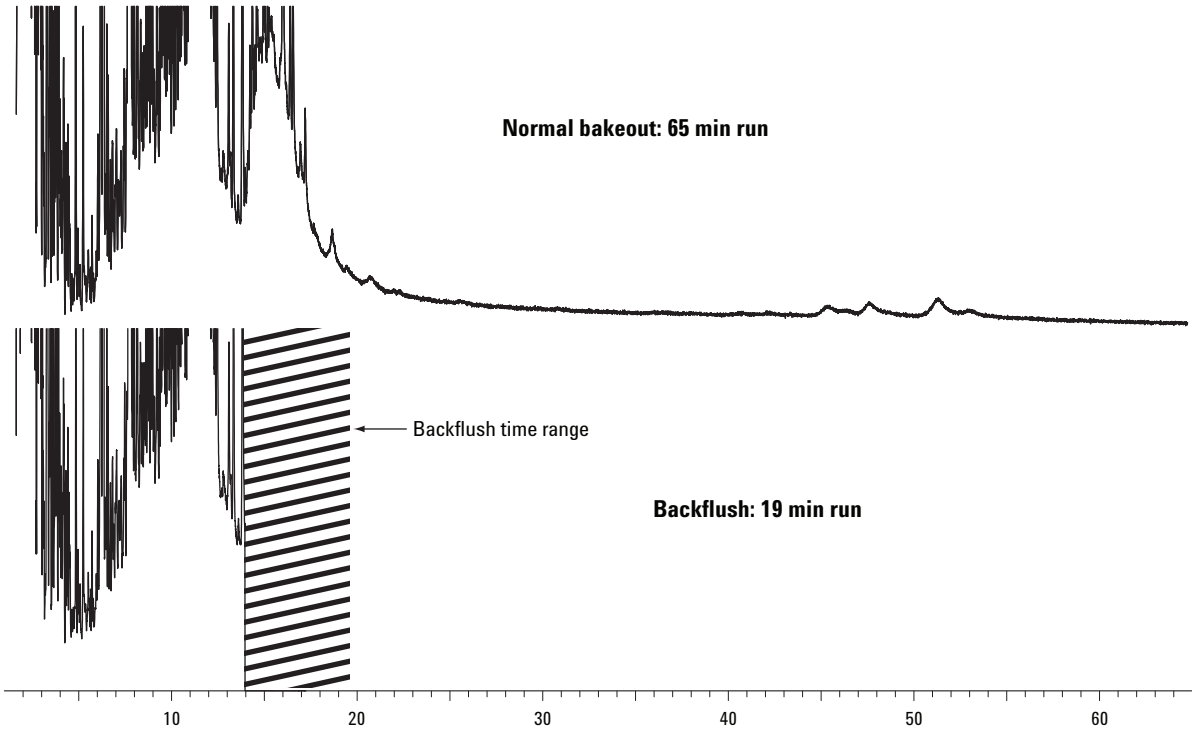


Figure 6. Top - 3× lemon oil analysis with 50 min bakeout at 320 °C. Bottom - 3× lemon oil analysis with 5 min backflush at 300 °C.

Conclusions

New tools are available for the analysis of pesticides in complex matrices. These tools can be combined to significantly reduce analysis and data processing time.

- Fast oven - programming rates needed for faster runs
- Shorter column - 3× faster runs with sufficient resolution
- Microfluidic splitter - confidence in results using selective detection simultaneous with MS data
- Backflush - additional 3× reduction in run time with lower column temperatures for extended lifetime
- Performance Electronics - maintain signal/noise at faster sampling rates
- DRS - three levels of target analyte identification in less than two minutes

Using the above tools, the run time for analysis of lemon oil was reduced from 195 minutes to 20 minutes (nine-fold). DRS reduced the data analysis from hours to minutes.

References

1. B. D. Quimby, L. M. Blumberg, M. S. Klee, and P. L. Wylie “Precise Time-Scaling of Gas Chromatographic Methods Using Method Translation and Retention Time Locking” Agilent Technologies publication 5967-5820E www.agilent.com/chem
2. P. Wylie, M. Szelewski, and C. K. Meng “Comprehensive Pesticide Screening by GC/MSD using Deconvolution Reporting Software” Agilent Technologies publication 5989-1157EN www.agilent.com/chem

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

Printed in the USA
October 13, 2004
5989-1716EN

Agilent Technologies