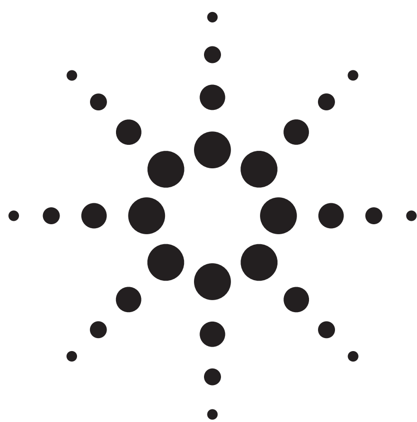

Replacing Multiple 50-Minute GC and GC-MS/SIM Analyses with One 15-Minute Full-Scan GC-MS Analysis for Nontargeted Pesticides Screening and >10x Productivity Gain

Application

Food Safety

Author

Chin-Kai Meng and Mike Szelewski
Agilent Technologies
2850 Centerville Road
Wilmington, DE 19808

Abstract

Pesticide analysis of fruits and vegetables requires finding trace-level residues in complex matrices. Up to now, the typical trade-off is between sensitivity and confirmation. Therefore, multiple injections are needed for screening and confirmation using gas chromatography with mass spectrometry (GC-MS) or GC-MS in combination with GC with element-selective detection. With the recent introduction of hardware and software tools, for example, capillary flow three-way splitter, trace ion detection, and deconvolution, a 15-minute fast analysis can match the results obtained from three injections of approximately 50 minutes each. A table comparing the results from the Food and Drug Administration/Center for Food Safety and Applied Nutrition (FDA/CFSAN) procedure using the traditional multi-instrument approach and the new Agilent single injection approach shows that Agilent's fast analysis is capable of finding all the target analytes in less than one-tenth of the current FDA/CFSAN total analysis time.

Introduction

To have a plentiful food supply, most fruits and vegetables are treated with pesticides (insecticides, fungicides, herbicides, etc.) to protect primarily against insects, molds, and weeds. Therefore, in order to ensure food safety, the food supply is frequently monitored for pesticide residues. Nowadays, the pesticide monitoring is expanding beyond food, for example, to botanical dietary supplements.

The analytical challenge to monitor (identify and quantify) trace multiresidues requires an effective and universal extraction and analysis method for maximum productivity and efficiency. Up to now, the trade-off in analysis has been between sensitivity and confirmation. Element-selective gas chromatograph (GC) detectors, such as the flame photometric (FPD), electron capture (ECD), electrolytic conductivity (ELCD), and halogen selective (XSD) detectors, provide excellent selectivity and sensitivity; however, they lack the capability to identify. On the other hand, mass spectrometry (MS) is capable of identifying an analyte by full-scan library match or multiple target and qualifier ion ratios from selected ion monitoring (SIM). However, MS sometimes lacks the selectivity to

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find target analytes in a complex matrix full of interferences and chemical background. Analyte spectra are sometimes overwhelmed by similiar ions contributed from the coextractives in the matrix that prevent the analyte of interest from being identified or confirmed.

The compromise and the typical approach are to use selective GC detector(s) to flag potential target analytes and use MS SIM for confirmation. For instance, many laboratories screen food samples for semivolatile pesticides using the ECD or ELCD (or XSD) for organohalogen, FPD or pulsed FPD (PFPD) for organophosphorus, and NPD for nitrogen-containing targets [1 – 5]. Any found targets are further confirmed by GC-MS/SIM. In addition, other procedures have used GC-MS/SIM entirely for the screening of pesticides in foods [6 – 8]. In most of these procedures, multiple injections are needed to identify hundreds of compounds at the detection limit in the low parts-per-billion (ppb) levels. To improve the efficiency and increase the productivity of screening for all of these pesticides, the challenge is to reduce the GC-MS or the combination of GC and GC-MS analysis times.

There are several hundred pesticides typically used in the world, and each country has its own pesticide tolerance levels for different agricultural commodities. This presents another analytical challenge in multiresidue monitoring: to develop a nontargeted procedure to identify pesticides at trace levels in different food matrices.

These challenges are met by the recent introduction of hardware and software tools, including GC-MS, capillary flow three-way splitter, trace ion detection, and deconvolution reporting software (DRS). The splitter allows multiple GC as well as MS signals to be acquired from a single injection for productivity gains (from three injections down to one). Trace ion detection minimizes noise on the signal and DRS separates target analyte ions from matrix background ions.

Several sample extracts were analyzed by the current Food and Drug Administration/Center for Food Safety and Applied Nutrition (FDA/CFSAN) multiple injection process and this new Agilent pesticide system. With DRS, the demand for chromatographic resolution is minimum; therefore, the Agilent system was running the analysis at a 3x faster speed (one-third the analysis time) to further increase productivity. A table comparing the results from the current FDA/CFSAN multi-instrument approach and the new Agilent single-injection approach shows that not only is Agilent's fast analysis capable of finding all the target analytes, but it is also accomplished in just one-tenth of the current FDA/CFSAN total analysis time.

Experimental

Sample Preparation

Sample extracts of fresh produce were prepared by FDA based on modifications of the QuEChERS protocols [9, 10]:

- Homogenize 1 to 2 kg of sample
- 15 g sample + 15 mL 1% AcOH/ACN, homogenized
- Add 6 g $MgSO_4$ and 2.5 g NaOAc, shake vigorously for 1 minute, and centrifuge
- Transfer ~15 mL + 0.5 g C-18 + 1.2 g $MgSO_4$, shake, and centrifuge for 5 min at 3,000 rpm
- Transfer ~12 mL + 0.4 g PSA + 0.2 g GCB + 1.2 g $MgSO_4$, vortex
- Add 4 mL toluene, shake, and centrifuge
- Transfer 6 to 8 mL, evaporate and bring to volume with toluene, add I.S.
- Add $MgSO_4$, vortex and centrifuge, transfer to ALS vials
- GC and GC-MS analysis

Sample preparation of dried ginseng powder is similar to that used for fresh produce, but smaller sample sizes (2 g) were used [11].

Capillary Flow Three-Way Splitter

One of the capillary flow devices is a three-way splitter, which consists of two half plates bonded together (diffusion bonding) to form a plate with the etched flow channels inside. The splitter is only 6.5 cm tall and 3 cm wide and is mounted on the side of the oven wall (see Figure 1). The low thermal mass minimizes cold spots and peak broadening. All capillary flow devices use metal column ferrules, have extremely low dead volumes, are inert, and do not leak, even after many oven cycles.

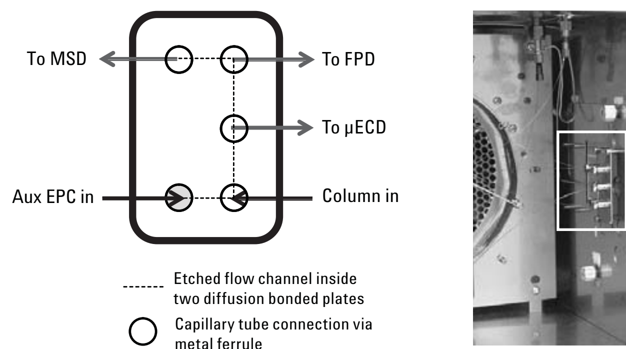


Figure 1. Flow diagram of the three-way splitter. The picture shows the splitter mounted on the oven wall.

The three-way splitter enhances productivity by splitting column effluent *proportionally* to multiple detectors: MSD, dual flame photometric detector (DFPD) and micro-electron capture detector (µECD). Therefore, two GC detector signals can be acquired together with the MS data (both SIM and scan signals if desired) from *one* injection [12]. The exit end of the analytical column is installed into one of the four ports on the splitter using a metal ferrule. The other three ports are connected to three detectors via restrictors (deactivated capillary tubing) of varying diameter and length to set the split ratio among the three detectors. Restrictors are sized for 1:1:0.1 split ratio in favor of MSD and DFPD (µECD has 1/10 of the flow to MSD), with similar hold-up times. The splitter uses auxiliary (Aux) electronic pneumatics control (EPC) for constant pressure makeup flow. The makeup gas (Aux pressure 6) at the splitter is fixed at 3.8 psi to maintain the split ratio throughout the run.

This multisignal configuration provides full-scan data for library searching, SIM data for trace analysis, DFPD (phosphorus or sulfur mode), and µECD data for excellent selectivity and sensitivity from complex matrices. The trade-off is the decrease of analyte concentration in any detector due to the flow splitting and the additional makeup gas from the splitter. An analyte would have similar retention times in all three detectors. Therefore, the GC data can be used in two ways: first, to confirm the presence of target analytes found by the MSD deconvolution reporting software (DRS), and second, to highlight potential target compounds to be further confirmed by MSD.

With the new 7890A GC software, up to six columns/ restrictors can be configured/assigned to different inlets and outlets. Aux pressure can be either an inlet (for the splitter flow restrictors connected to different detectors) or an outlet (for the analytical column). A graphical user interface makes the configuration easy to set up. Once all the columns and restrictors are configured, the backflush can be executed easily.

Backflush

Traditional bakeout step for removing late eluters could be very time consuming, or even as long as the analysis time depending on the matrix. Backflush is a simple technique to remove high boilers from the column faster and at a lower column temperature to cut down analysis time and increase column lifetime. Capillary flow devices (in this case, a three-way splitter) also provide backflush [13, 14] capability. “Backflush” is a term used for the reversal of flow through a column such that sample components in the column are forced back out the inlet end of the column. By reversing column flow immediately after the last compound of interest has eluted, the long bake-out time for highly retained components can be eliminated. Therefore, the column bleed and ghost peaks are minimized, the column will last longer, and the MS ion source will require less frequent cleaning. The split vent trap may require replacement more frequently than usual.

Figures 2 and 3 are two screen shots from the MSD ChemStation software, providing a summary of the backflush operation. In Figure 2, the column and three restrictor dimensions and respective detectors are shown (the setup came from the column configuration section). For MSD, the user can choose the vacuum pump installed on the system. This information will be used to calculate if the backflush is within the system flow limits. By clicking on the “Evaluate...” button, the screen shown in Figure 3 appears, listing the maximum flow for each detector and the void volumes for a certain backflush time. In this example, Aux pressure is at 60 psi, inlet is at 1 psi, and oven is at 280 °C. The backflushing flow is shown to be 8.66 mL/min, and the void time is shown to be 0.16 min. Therefore, backflushing for 2.5 minutes will send 15.6 void volumes through the column. This is useful for developing the backflush method. Figures 2 and 3 simplify the setup and development of a backflush method.

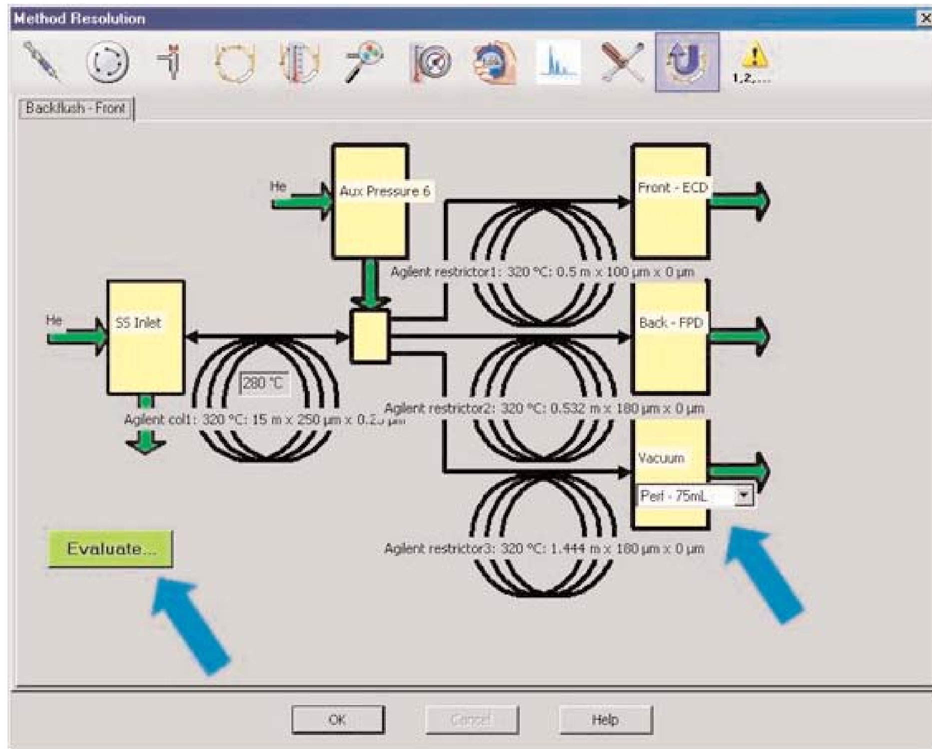


Figure 2. Backflush setup in ChemStation.

Summary of Backflush Calculations

Oven Temperature

280 °C

Detector	Maximum Flow	Allowable Pressure	Flow at Chosen Pressure
Front - ECD	60	204.31	6.7399
Back - FPD	60	57.039	65.277
Vacuum	75	114.7	24.993

Backflush Pressure

60 psi

Inlet Pressure during Backflush: 1 psi

Backflush Flow to Inlet: 8.6566 mL/min

Void Time: 0.16038 min

Void Volumes: 15.588

Backflush Time: 2.5 min

OK Cancel Help

Figure 3. Automated backflush calculations in ChemStation.

Another useful feature in Figure 3 is the "warning," shown as highlighted yellow cells. In this example, setting the backflush pressure to 60 psi sends more than the allowable flow (60 mL/min) to the FPD. Therefore, the backflush pressure setting and the actual flow value to FPD are shown in yellow as "warnings." Although the system will accept the setup, the high flow may cause consequences in the analysis, for example, flameout.

Trace Ion Detection

Trace ion detection [15] is a filtering algorithm to smooth peaks. This filtering is an advanced form of averaging used to remove the noise riding on the signal. The implications from TID are typically a slight loss in peak height and some peak broadening. The default setting in ChemStation for TID is off. It should be turned on for any analysis that uses deconvolution and has more than six sampling points across a peak. TID provides better signal-to-noise ratios and helps deconvolution to confirm target compounds as shown in the Results section.

Deconvolution

In GC/MS, deconvolution is a mathematical technique that separates overlapping mass spectra into deconvoluted spectra of the individual components. Figure 4 is a simplified illustration of this process. Here, the total ion chromatogram (TIC) and apex spectrum are shown on the left. In a complex matrix, a peak may be composed of multiple overlapping components and matrix background ions; therefore, the apex spectrum is actually a composite of these constituents. A mass spectral library search would give a poor match, at best, and certainly would not identify all of the individual components that make up the composite spectrum.

The deconvolution process groups ions whose individual abundances rise and fall together within the spectrum. The deconvolution process first corrects for the spectral skew that is inherent in quadrupole mass spectra and determines a more accurate apex retention time of each chromatographic peak. As illustrated in Figure 4, deconvolution produces a "cleaned" spectrum for each overlapping component. These individual spectra can be library searched with a high expectation for a good match. Deconvolution significantly reduces chromatographic resolution requirements, allowing much shorter analysis times.

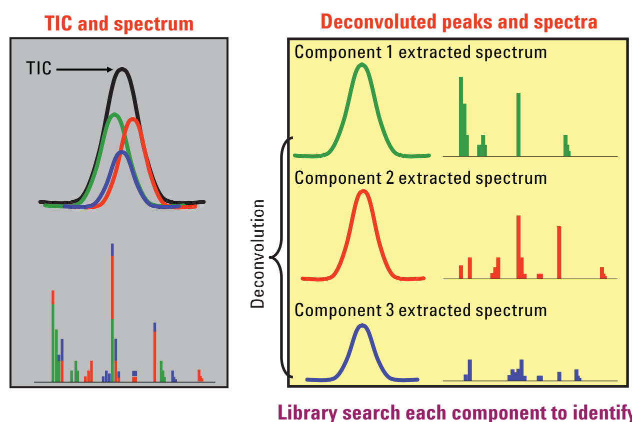


Figure 4. **Deconvolution process of three overlapped peaks.**

Agilent Deconvolution Reporting Software (DRS) utilizes the AMDIS deconvolution program from the National Institute of Standards and Technology (NIST), originally developed for trace chemical weapons detection in complex samples [16]. 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 spectral matching and expected retention time window as a qualifier; and (3) NIST05 search using a >163,000-compound library [17, 18]. In this application, both the ChemStation quantitation database and the AMDIS library have the same 926 entries. These entries include pesticides, numerous metabolites, endocrine disruptors, important PCBs and PAHs, certain dyes, synthetic musk compounds, and several organophosphorus fire retardants [18].

The AMDIS software, shipped with the NIST05 Library CD-ROM, is also capable of deconvoluting selected ion monitoring (SIM) data [19], while previous AMDIS revisions were not. Testing has shown that proper compound identification requires four ions per compound. All Agilent DRS databases are retention time locked and have both full-scan and SIM libraries for AMDIS.

Instrument Method

The system used for this study consists of an Agilent 7890A GC with split/splitless inlet, a three-way splitter, µECD, DFPD, and 5975 MSD. For a detailed description of SIM/scan and the splitter system configuration, please refer to the experimental section of reference [12]. See Table 1 for hardware detail and settings.

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

GC	Agilent Technologies 7890A with 240V fast oven option
Injector	Agilent Technologies 7683
Syringe size	10 µL
Injection volume	1 µL
Solvent A wash	1 (pre), 3 (post)
Solvent B wash	1 (pre), 3 (post)
Sample wash	0
Sample pump	4
Plunger speed	Fast
Inlet	EPC split/splitless
Mode	Splitless
Inlet temperature	250 °C
Pressure	~24.4 psi (chlorpyrifos methyl RT locked to 5.531 min, 3x speed) constant pressure mode
Purge flow	50.0 mL/min
Purge time	2 min
Septum purge flow	3 mL/min
Septum purge mode	Switched
Gas saver	Off
Gas type	Helium
Liner	Helix double taper liner, deactivated, p/n 5188-5398

Oven			
Oven ramp	°C /min	Final (°C)	Hold (min)
Initial		70	0.67
Ramp 1	75	150	0
Ramp 2	9	200	0
Ramp 3	24	280	3.33

Runtime	13.96 min
Oven equilib time	1.0 min
Post-run time	2.5 min
Post-run temperature	280 °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 RT locked to chlorpyrifos methyl at 5.531 min, 3x analysis speed
Nominal initial flow	3.5 mL/min
Outlet	Aux pressure 6
Outlet pressure	3.8 psi (Aux EPC pressure to three-way splitter), helium gas
Backflush (post-run)	
Oven	280 °C
Time	2.5 min
Inlet	1 psi
Aux pressure 6	60 psi (column outlet)
Front detector	µECD
Temperature	300 °C
Const col + makeup	60.0 mL/min
Make gas type	Nitrogen
Data rate	20 Hz
Back detector	Dual FPD
Temperature	250 °C
Hydrogen flow	75.0 mL/min
Air flow	100.0 mL/min
Const Col + Makeup	60.0 mL/min
Make gas type	Nitrogen
Lit offset	2.00
Data rate	20 Hz
Transfer line	250 °C
AUX Thermal 1	MSD transfer line, 280 °C
AUX Pressure 6	Three-way splitter
Gas type	Helium
Initial pressure	3.8 psi
Backflush pressure	60 psi
MSD	Agilent Technologies 5975C MSD
Tune file	Atune.u
Mode	Scan
Solvent delay	1.50 min
EM voltage	Atune voltage
Low mass	50 amu
High mass	550 amu
Threshold	0
Samples	2
Scans/sec	2.91
Quad temp	150 °C
Source temp	230 °C
Three-way splitter	Agilent 7890A Option 890, installed during factory assembly
Pressure	3.8 psi (Aux pressure 6 setting)
Split ratio	1:1:0.1 MSD:DFPD:µECD
MSD restrictor	1.444 m × 0.18-mm id deactivated fused silica tubing, p/n 160-2615-10
DPFD restrictor	0.532 m × 0.18-mm id deactivated fused silica tubing, p/n 160-2615-10
µECD restrictor	0.507 m × 0.10-mm id deactivated fused silica tubing, p/n 160-2635-1
Flow to MSD	3.43 mL/min (at 70 °C), 1.53 mL/min (at 280 °C)
Flow to DFPD	3.43 mL/min (at 70 °C), 1.53 mL/min (at 280 °C)
Flow to µECD	0.343 mL/min (at 70 °C), 0.153 mL/min (at 280 °C)
Makeup (Aux 6)	3.19 mL/min (at 70 °C), 1.52 mL/min (at 280 °C)
Software	
GC/MSD ChemStation	Agilent part number G1701EA (version E.01.00 or higher)
MS Libraries	NIST05a mass spectral library (Agilent part number G1033A) Agilent RTL Pesticide and Endocrine Disruptor Libraries (926 entries) in Agilent and AMDIS formats (part number G1672AA)
Deconvolution software	Automated Mass Spectral Deconvolution and Identification Software (AMDIS_32 version 2.65 Build 116.66)
Library searching software	NIST MS Search (version 2.0d or greater) (comes with NIST'05a mass spectral library – Agilent part number G1033A)
Deconvolution reporting software	Agilent part number G1716AA (version A.03.00 or higher)

Results and Discussion

Backflush Example

Blank runs, made after separate milk analyses with different backflush (BF) times, are shown in Figure 5. The top TIC is a blank run after a milk extract analysis stopped at 42 minutes and the system backflushed for 1 minute. The next TIC is a blank run after another milk extract analysis stopped at 42 minutes and backflushed for 2 minutes and so on for the other five TICs. It is interesting to confirm graphically that the latest eluters disappeared from the TIC earliest in backflushing.

Trace Ion Detection

Figure 6 compares the signals when TID is on and off. Visually, it is obvious that TID smoothes the noise riding on top of the signal. When TID was on, Atrazine was successfully identified by AMDIS. When TID was off, Atrazine was not found by AMDIS and resulted in a false negative. Figure 7 compares TID on and off for two different analytical conditions of the same ginseng extract. On the right, the fast (3x) analysis was a 1-µL splitless injection with TID on. The analyte Diazinon was found by AMDIS with a peak width less than 5 seconds. On the left side, the normal (1x)

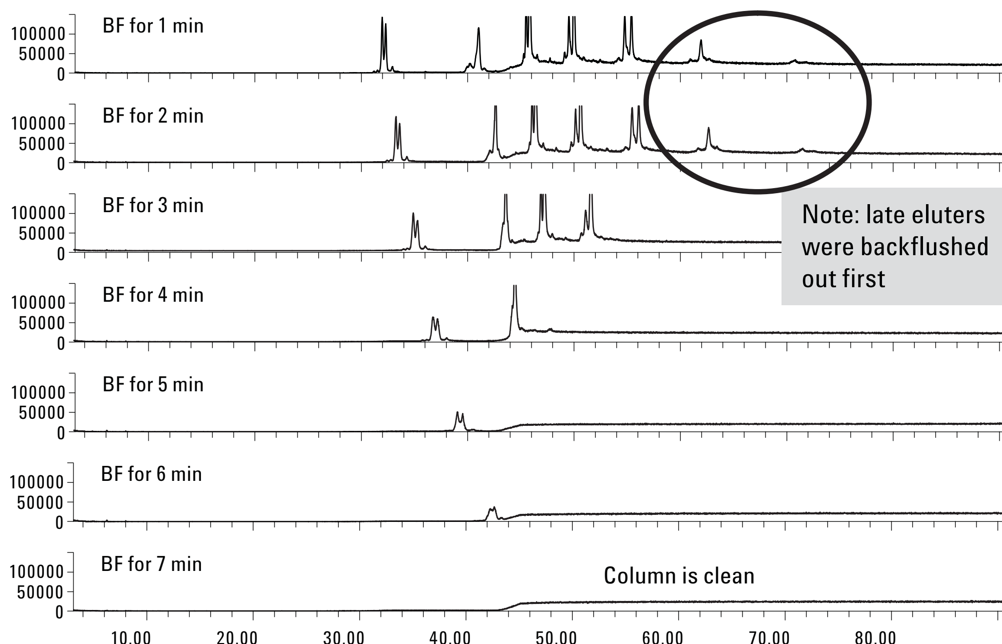


Figure 5. Differences in blank runs as the result of seven different backflush times.

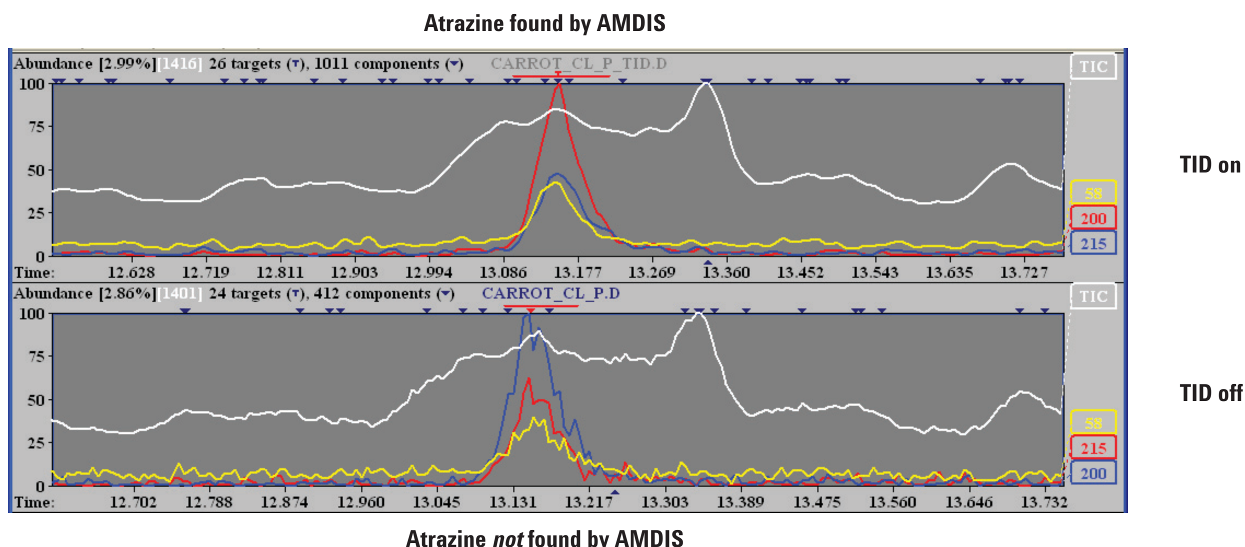


Figure 6. The power of deconvolution with TID for atrazine, from AMDIS.

analysis was a 5-µL cold splitless injection using a PTV with TID off. The 9-second-wide Diazinon was not found by AMDIS, also a false negative. Both examples show that TID is a very useful feature for trace target analysis.

Benefits of TID:

- Improves the signal-to-noise ratio
- AMDIS is more thorough in identifying components, resulting in fewer false positives
- Improves library match quality
- Improves area repeatability, resulting in more reliable quantitation

DRS and Splitter

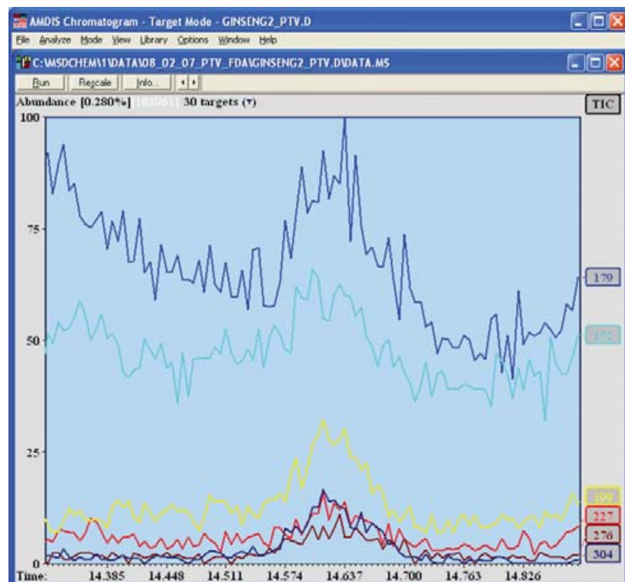
Figures 8 and 9 are DRS reports for ginseng and peach extracts with pesticides highlighted (for a detailed explanation of the report, please refer to references [17] and [20]). Figures 10 and 11 show simultaneously collected GC and MS signals (RT locked) for the corresponding ginseng and peach extracts from a three-way splitter. The presence of the GC peaks from the µECD and FPD (P) helps confirm the targets reported by DRS. Each run is finished at 15 minutes using the 3x speed and a 240V oven. With deconvolution, less peak resolution is required for compound identification. A 4-minute backflush is added after the run to make sure that the column is clean to maintain the next run's locked RTs for all peaks.

Deconvolution

Figures 12 through 15 show the results from AMDIS. There are three spectra for each target compound found by AMDIS. The top window shows the spectrum (scan) from the TIC. This is the only spectrum that would be available for library searching without deconvolution – obviously quite useless. The middle window shows the deconvoluted spectrum and the bottom window is the target compound's spectrum in the library. The compound confirmation can be done easily and with confidence by visually comparing the bottom two spectra. The power of deconvolution is appreciated while comparing the top two spectra (the raw scan and the spectrum hidden in the raw scan).

It is easy to further confirm the hits found by deconvolution. In Figure 9, four pesticides found by AMDIS in the peach extract have a match factor of about 80 or lower. The four pesticides are Cabaryl, Captan, Propiconazole, and Fenbuconazole. A SIM method of these compounds was set up to analyze the peach extract. By selecting the proper AMDIS library (full-scan or SIM), DRS can process full-scan as well as SIM data files [19]. Figure 16 is the DRS report of the peach SIM analysis. The high match factor (99 or higher) and the small RT difference of all targets found by AMDIS confirm the presence of all compounds.

PTV, 1x speed, 5 µL injection
No TID, Diazinon not found (false negative)



Splitless, 3x speed, 1 µL injection
with TID, Diazinon found

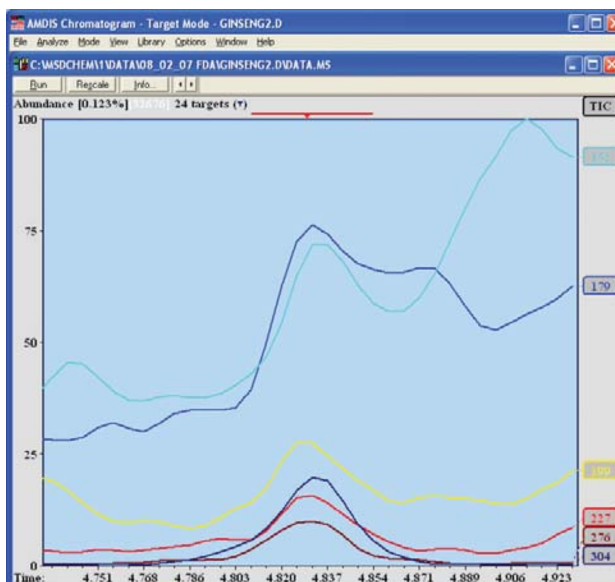


Figure 7. The power of deconvolution with TID for diazinon.

			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
2.1437	3228033	Promecarb artifact [5-isopropyl-3-methylphenol]		68	6.1		
2.1437	1450722	Ethanone, 1-(2-hydroxy-5-methylphenyl)-				89	1
2.2759	97530	Eugenol		78	2.7	80	2
3.5107	877098	2,4,5,6-Tetrachloro-m-xylene	2.02	93	3.5	80	2
3.6664	126738	Tributyl phosphate		81	5.2	86	2
4.6128	120127	Anthracene		81	-12.2	91	8
4.8290	333415	Diazinon		69	1.3	72	2
5.3286	84695	Diisobutyl phthalate		91	5.3	88	6
6.1577	84742	Di-n-butylphthalate		94	3.4	91	3
6.4993	1861321	Chlorthal-dimethyl	0.22	88	3.9	79	3
9.3157	115866	Triphenyl phosphate		78	13.8	92	2
9.9290	117817	Bis(2-ethylhexyl)phthalate		82	8.9	79	6
9.931	84617	Dicyclohexyl phthalate	7.12				
11.2520	84764	Di-n-nonyl phthalate		72	-11.6	80	1
12.2997	131860338	Azoxystrobin		77	17.1	61	4

Figure 8. DRS report for ginseng with pesticides highlighted.

			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
1.823	91203	Naphthalene	0.05				
2.2748	97530	Eugenol		78	2.5	76	3
3.3036	86737	Fluorene		76	0.5	89	5
3.3242	84662	Diethyl phthalate		89	1.4	89	2
3.5084	877098	2,4,5,6-Tetrachloro-m-xylene	0.36	98	3.1	87	2
3.6423	126738	Tributyl phosphate	1.3	90	0.9	87	2
5.3160	84695	Diisobutyl phthalate	2	99	3.1	92	2
5.6130	63252	Carbaryl		81	1.8	84	7
6.1423	84742	Di-n-butylphthalate		97	0.6	92	2
7.0835	133062	Captan		83	1.5	75	2
7.5430	959988	Endosulfan (alpha isomer)		91	-0.3	80	3
8.0082	80057	Bisphenol A		80	11.8	76	4
9.0198	85687	Butyl benzyl phthalate		80	3.5	76	3
9.0713	60207901	Propiconazole-I		76	16.8	72	2
9.5285	732116	Phosmet		96	5.4	85	2
10.7788	119611006	Fenbuconazole		76	6.9		
10.7788	0000	Piperazine-2,5-dione, 3-hydroxy-6-isopropyl-3-trifluoromethyl-				59	1

Figure 9. DRS report for peach with pesticides highlighted.

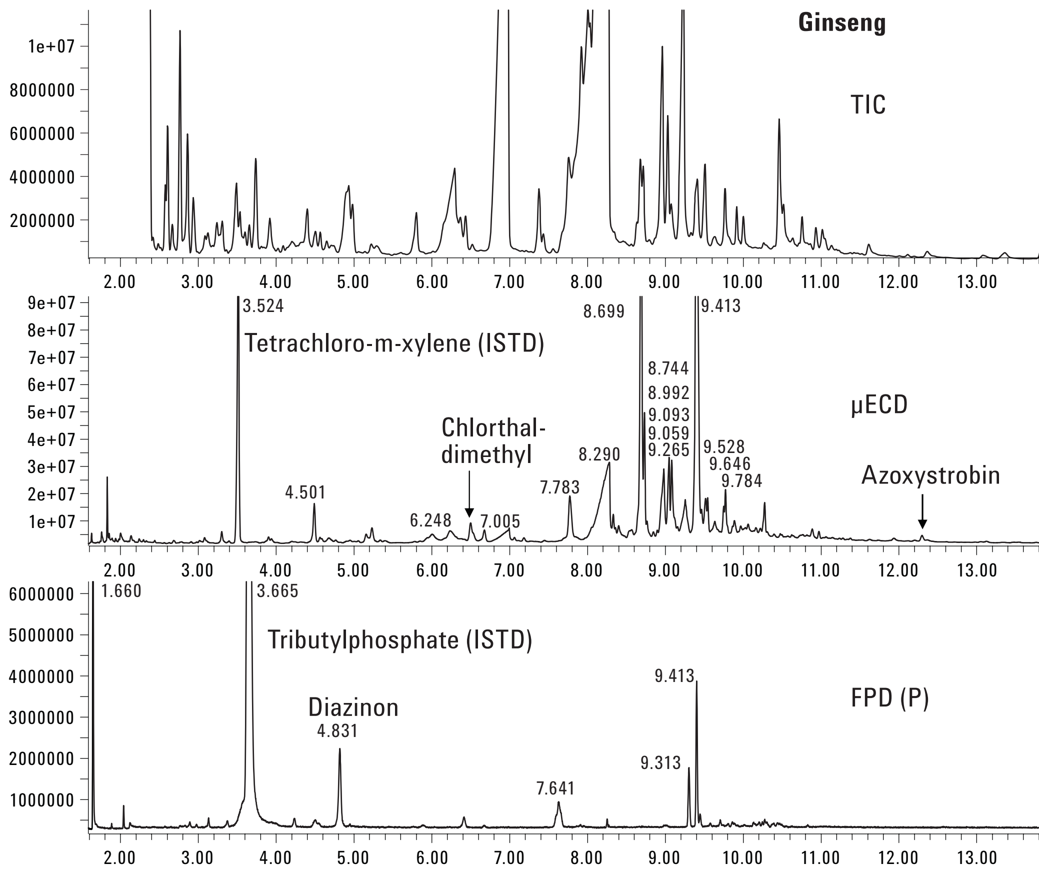


Figure 10. Simultaneous display of MSD and GC selective detector signals for ginseng.

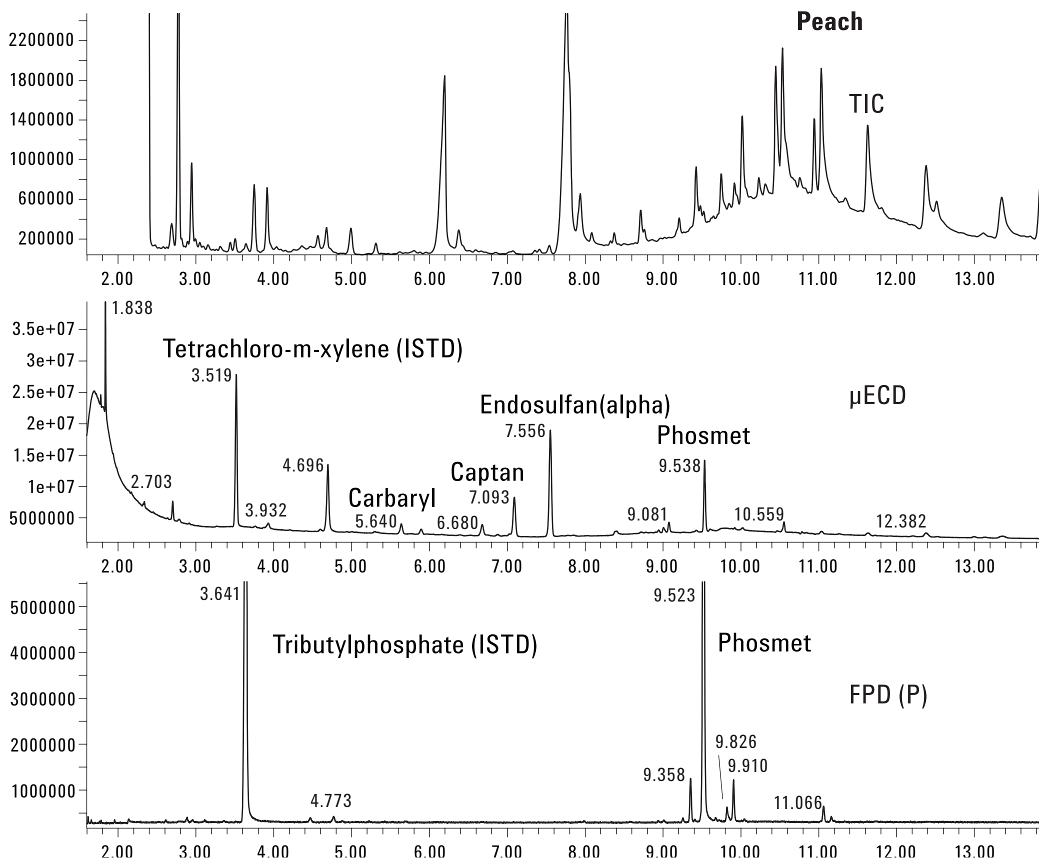


Figure 11. Simultaneous display of MSD and GC selective detector signals for peach.

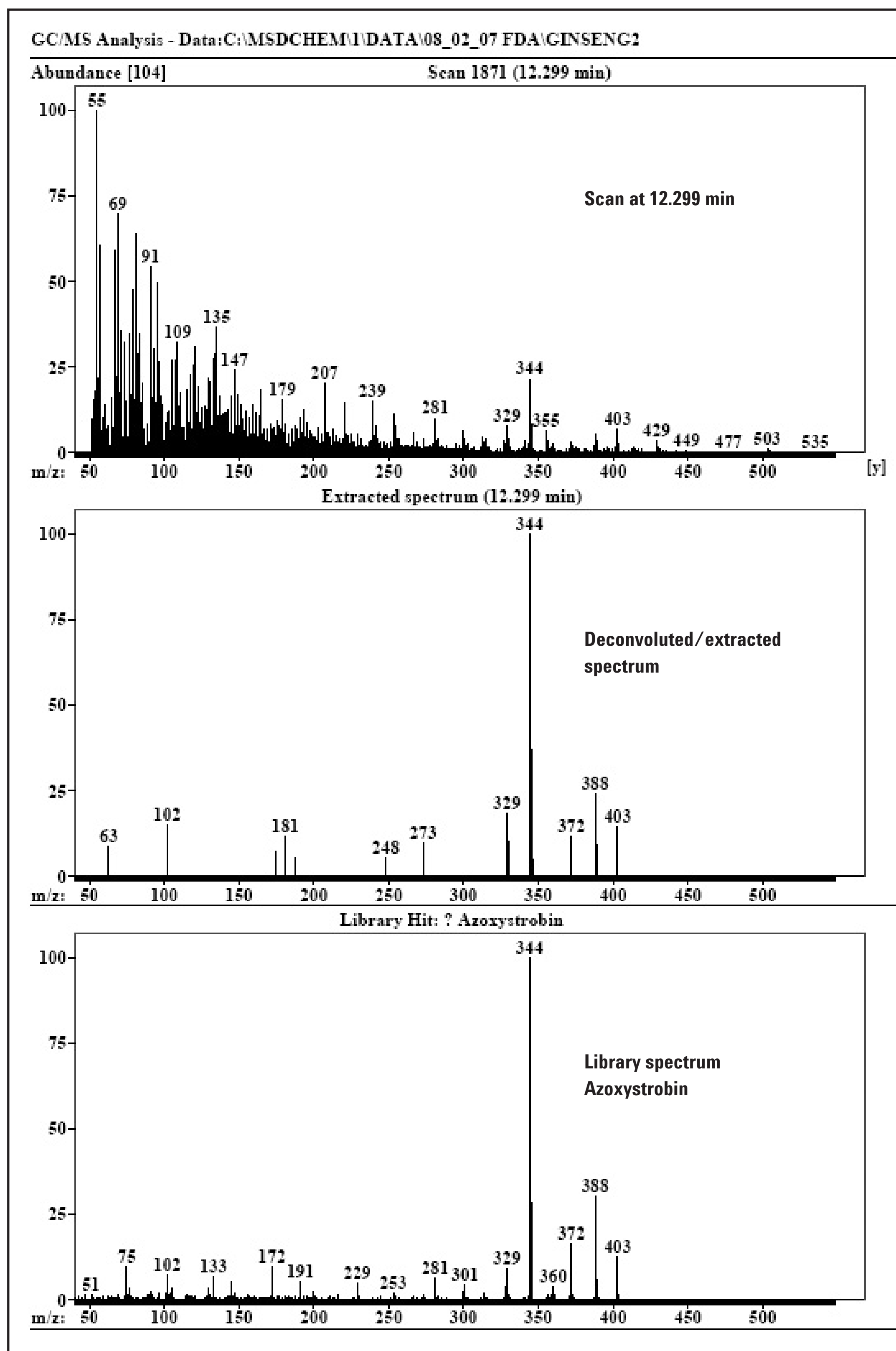


Figure 12. Raw (dirty) spectrum, deconvoluted (clean) spectrum, and library spectrum of azoxystrobin found in ginseng, from AMDIS.

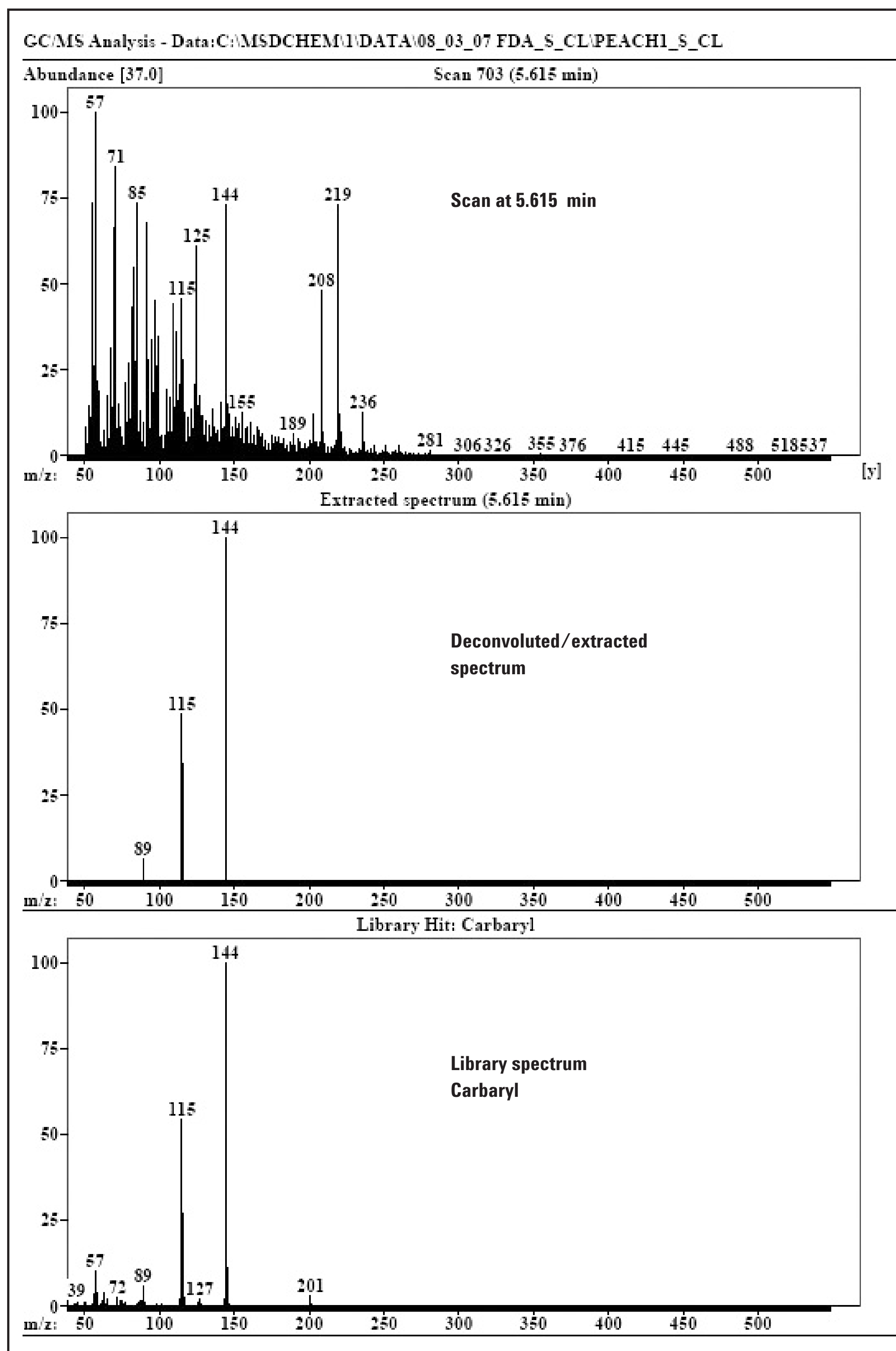


Figure 13. Raw (dirty) spectrum, deconvoluted (clean) spectrum, and library spectrum of cabaryl found in peach, from AMDIS.

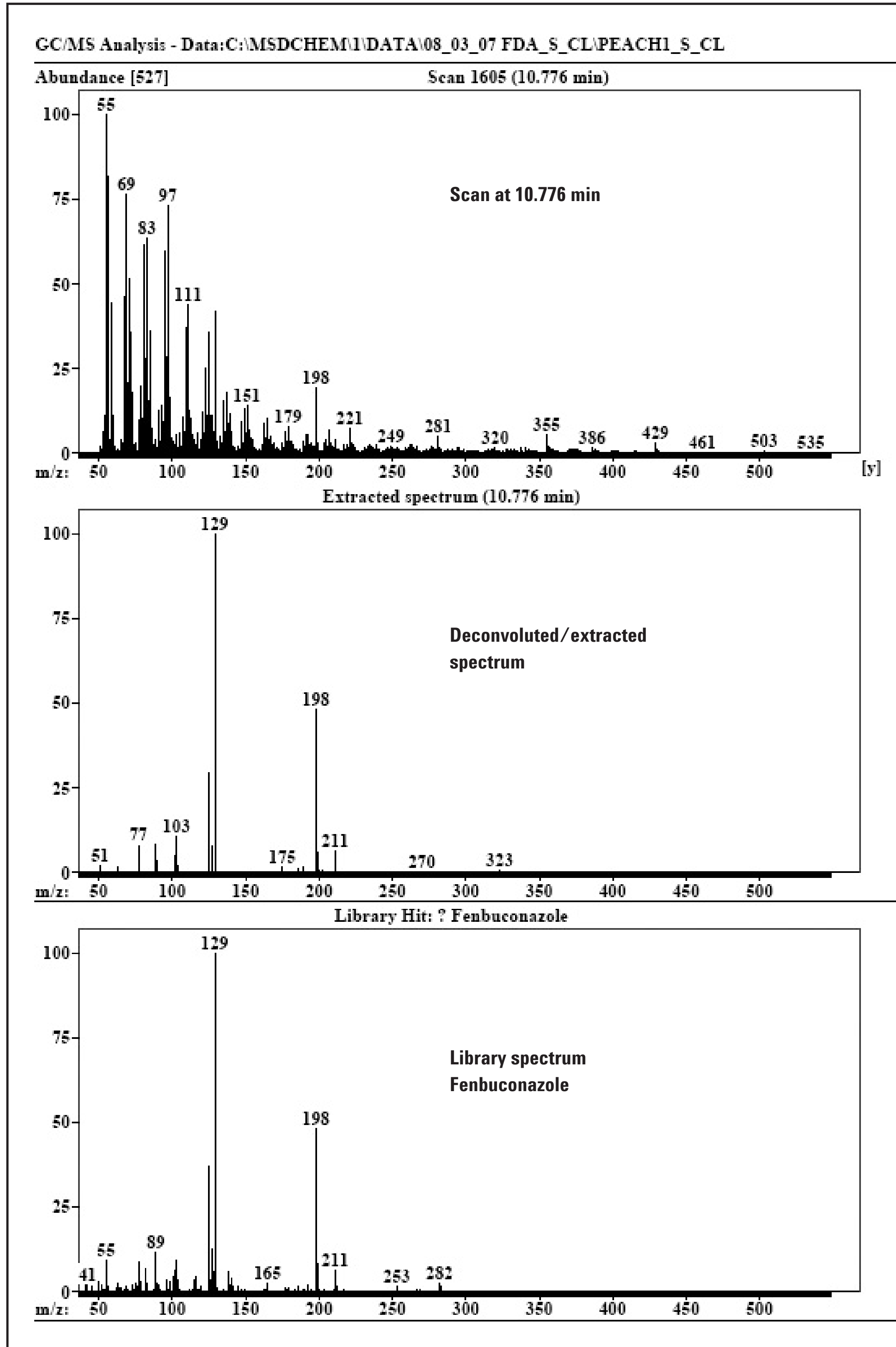


Figure 14 Raw (dirty) spectrum, deconvoluted (clean) spectrum, and library spectrum of fenbuconazole found in peach, from AMDIS.

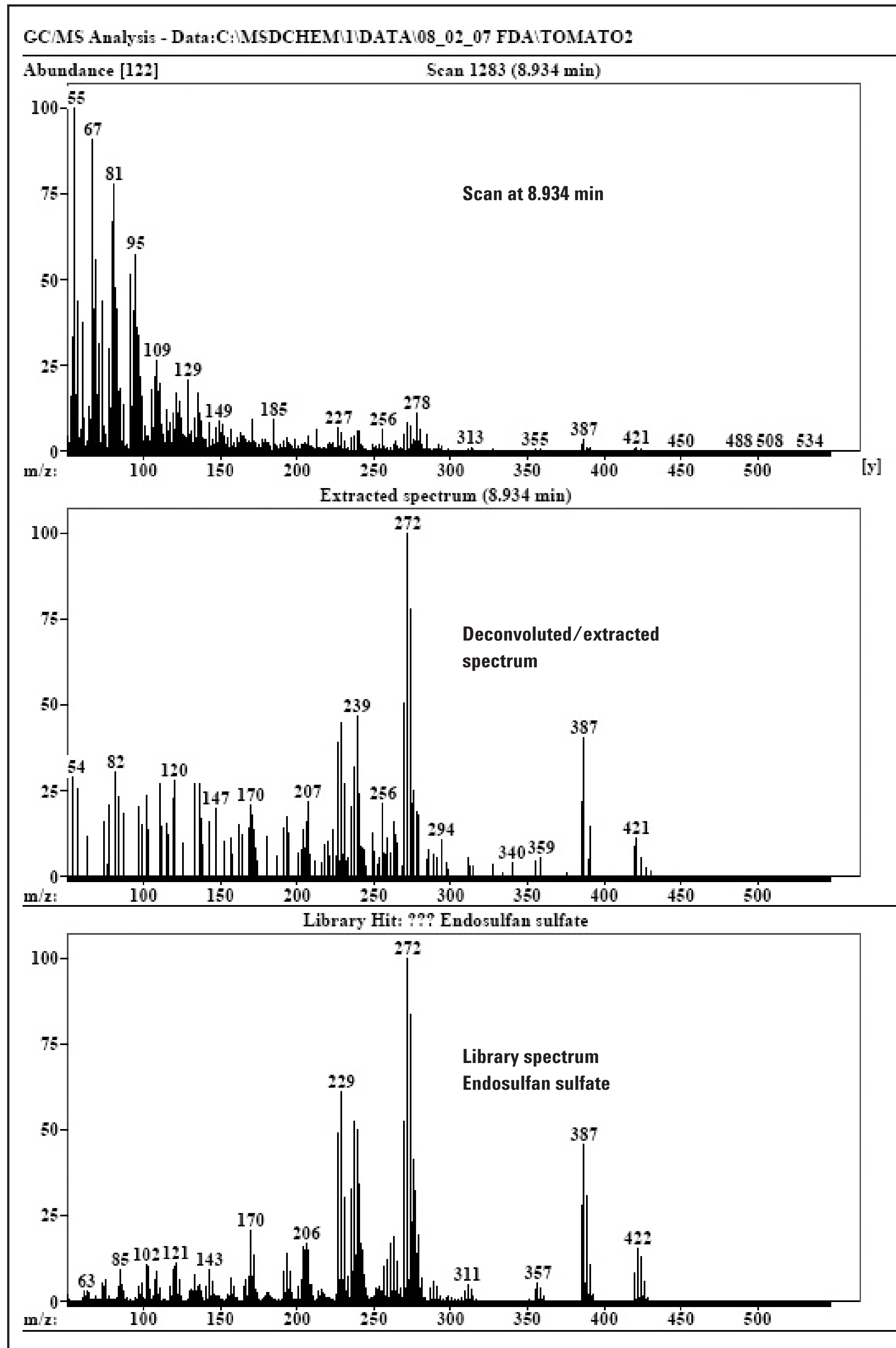
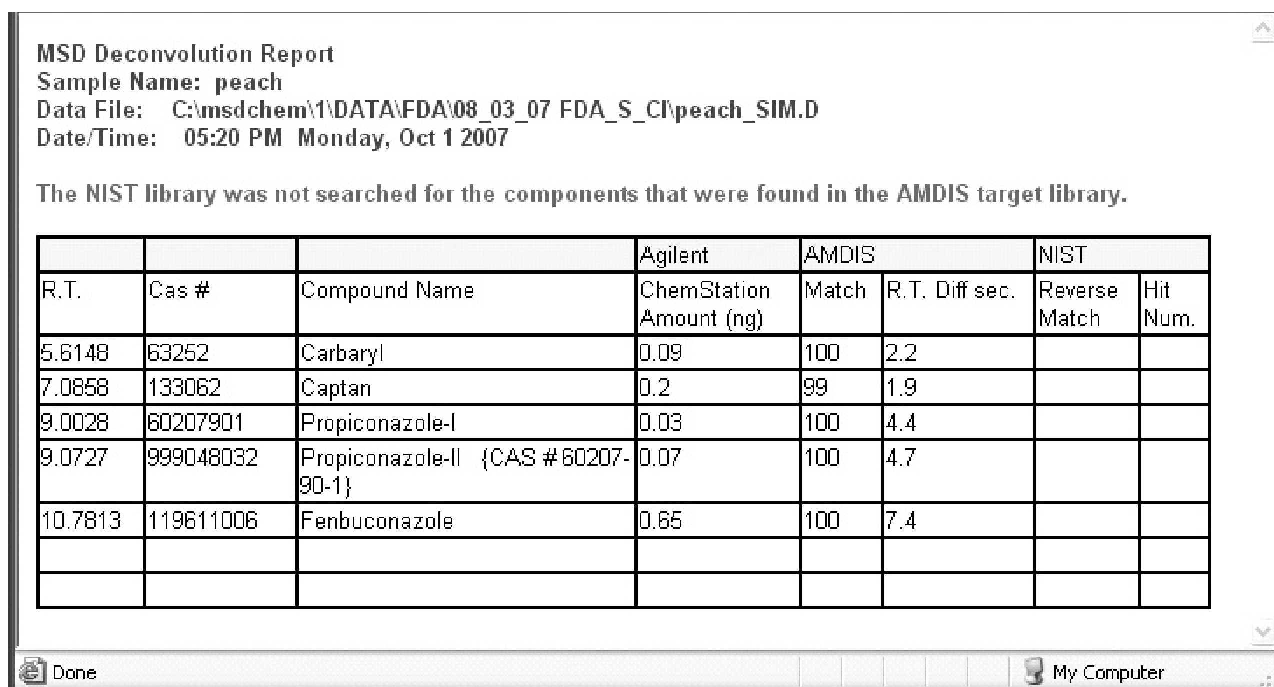


Figure 15 Raw (dirty) spectrum, deconvoluted (clean) spectrum, and library spectrum of endosulfan sulfate found in tomato, from AMDIS.



MSD Deconvolution Report
Sample Name: peach
Data File: C:\msdchem\1\DATA\FDA\08_03_07 FDA_S_CI\peach_SIM.D
Date/Time: 05:20 PM Monday, Oct 1 2007

The NIST library was not 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.
5.6148	63252	Carbaryl	0.09	100	2.2		
7.0858	133062	Captan	0.2	99	1.9		
9.0028	60207901	Propiconazole-I	0.03	100	4.4		
9.0727	999048032	Propiconazole-II {CAS #60207-90-1}	0.07	100	4.7		
10.7813	119611006	Fenbuconazole	0.65	100	7.4		

Done My Computer

Figure 16. DRS report from the SIM analysis of peach. Please refer to reference 18 for the explanation of the fictitious CAS number assigned to Propiconazole-II (999048032).

Comparison of Incurred Samples

The current approach at FDA/CFSAN is to find a wide suite of organohalogen and organophosphorus pesticide residues. This requires four injections (GC-MS/SIM and GC-ELCD for organohalogen and GC-MS/SIM and GC-FPD for organophosphorus screening) of approximately 50-minutes runtime each (total runtime = 200 minutes). Table 2 shows that FDA found several target compounds in three extracts as well as quantitation results from both GC and MS. In comparison, using the new tools (splitter, TID, and deconvolution) found as many target compounds and a few more in just one short (15-minute) full-scan analysis. The three-way splitter was used to get selective GC signals (µECD and FPD) for confirmation purposes. Due to column effluent splitting to three detectors (1:1:0.1), the MSD is getting less than half of the amount injected. FDA/CFSAN GC and GC/MS/SIM analyses for organohalogen monitor-

Table 2. Comparison of the Agilent Pesticide System Results with the FDA Results

	Agilent DRS (full scan/TID)	FDA (FPD, ELCD, SIM)	GC-FPD or ELCD	GC-MS/SIM
Ginseng	Diazinon Chlorthal-dimethyl Azoxystrobin	Diazinon (FPD, SIM)	25 ± 3 ppb	25 ± 2 ppb
Peach	Carbaryl Captan Endosulfan (alpha) Phosmet Propiconazole I and II Fenbuconazole	Phosmet (FPD, SIM)	320 ± 37	230 ± 23
Tomato	Chlorothalonil Endosulfan (alpha) Endosulfan (beta) Endosulfan sulfate	Chlorothalonil (ELCD, SIM) Endosulfan (alpha) (ELCD, SIM) Endosulfan (beta) (ELCD, SIM) Endosulfan sulfate (ELCD, SIM)	205 ± 10 16 ± 2 34 ± 4 14 ± 2	153 ± 47 26 ± 4 47 ± 5 21 ± 6

1 15-min injection (splitter) found these

2 50-min injections found these

FDA quant results

ing found endosulfan sulfate at 14/21 ppb (pg/µL) in tomato. Agilent MSD/DRS also found this compound in full-scan mode with less than half the amount reported by FDA/CFSAN available at the MSD due to the split. Several other target compounds that did not contain any halogens or the organophosphorus moeity at the low ppb concentrations were also identified by DRS, such as carbaryl ($C_{12}H_{11}NO_2$) in peach and azoxystrobin ($C_{22}H_{17}N_3O_5$) in ginseng. The two FDA/CFSAN procedures for organohalogen and organophosphorus pesticides never would have been able to detect these additional nitrogen-containing pesticides. This shows that deconvolution of data acquired with TID is capable of identifying compounds below 10 pg on column in full-scan mode.

Conclusions

The trade-off in trace-level pesticide residue analysis is sensitivity versus confirmation. Therefore, the common practice is to use element-selective GC detectors to screen the extracts and use MS/SIM to confirm hits found by GCs. This can take as many as four injections to have a complete residue analysis from a sample extract.

Recent introduction of hardware and software tools, which include the capillary flow three-way splitter, trace ion detection, and deconvolution reporting software, can increase productivity dramatically. With deconvolution the demand for chromatographic resolution is lowered; therefore, the Agilent system can run the analysis at a 3x faster speed to further increase productivity. A single-injection approach even at the 3x fast speed can replace the three-injection approach.

A table comparing the results from the current FDA/CFSAN multi-instrument approach and the new Agilent single-injection approach shows that not only is Agilent's fast analysis capable of finding all the target analytes, but it can also do it in just one-tenth of the current FDA/CFSAN total analysis time.

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Acknowledgements

The authors would like to thank Jon Wong, FDA/CFSAN, for the sample extracts and results used in this study as well as valuable feedback on this application.

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Printed in the USA
December 18, 2007
5989-7670EN

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