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Non-Targeted Analyses for Pesticides Using Deconvolution, Accurate Masses, and Databases – Screening and Confirmation

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1. Introduction

Technical innovations in crop protection have been a key component in the globalization of food production and distribution over the past several decades. The ease of access to foodstuffs from distant growing regions has depended, to a large extent, on the new pesticides that combat the historic foes of food sufficiency: fungi, insects, and weeds. Yet the same public that has come to expect and demand the ready availability of food products also has high expectations of regulators to ensure the safety of their food supply. Regulators in the United States, Europe, and Asia have, therefore, had to grapple with the task of ensuring that exposure levels to agricultural chemicals in the food supply remain within strictly determined parameters. In each region, regulatory agencies have, during the past decade, produced pesticide regulations that are increasingly stringent both in terms of the number of pesticides tracked and the allowable tolerances of those pesticides in the food supply.

Because over a thousand pesticides are used globally to protect crops and improve food production, regulatory stringency translates into new challenges for the refinement of residue data generation and analysis. Because pesticide contamination of foodstuffs and environmental matrices could provide significant risk to consumers, vigilant monitoring is required; but the number of targeted and non-targeted pesticides that must be monitored in a given global region has risen significantly. Therefore, simple, rapid methods for screening hundreds of pesticides at trace levels in various matrices must be established. The widely divergent chemical properties (polarity, thermal stability, etc.) of these non-targeted pesticides call for the application of different analytical methods. Both GC and LC approaches are available, with each using a different technique for data analysis.

1.1 Gas phase analyses

For samples that are amenable to GC analysis, i.e., nonpolar and thermally stable, retention time locking (RTL) can be used. RTL is a technique developed by Agilent Technologies (Santa Clara, CA) that allows analysts to match analyte retention times (RTs) on any Agilent GC instrument in any laboratory in the world, provided that the same nominal GC method and

capillary column are used (Giarocco et al., 2000). Using RTL, Agilent has developed several retention time-locked databases for GC and GC/MS that include the locked retention time, compound name, CAS number, molecular weight, and mass spectrum (RTL URL). The Agilent RTL Pesticide Library contains this information for 927 compounds, including pesticides, metabolites, and endocrine disruptors along with important polychlorinated biphenyls, polybrominated biphenyls, polycyclic aromatic hydrocarbons, synthetic musk compounds, Sudan dyes, and organophosphorus fire retardants. Another database contains all of the analytes specified for GC/MS analysis in the new Japanese "Positive List" regulations.

While data analysis based on retention time is extremely productive and reliable, additional power and speed of analysis is obtained by deconvoluting the spectra. In GC/MS, deconvolution is a mathematical technique that "separates" overlapping mass spectra into "cleaned" spectra of the individual components. As seen at the top left of Figure 1, a total ion chromatogram (TIC) might consist of several overlapping components.

The deconvolution software utilized in the Agilent Deconvolution Reporting Software (DRS) and discussed throughout this article is the Automated Mass Spectral Deconvolution and Identification System (AMDIS) developed by National Institute of Standards and Technology (NIST) (AMDIS URL). In addition, Agilent's RTL Pesticide Library also includes the AMDIS format for use with DRS. A filter can be set in AMDIS that requires the analyte's RT to fall within a user-specified time window at the expected RT.

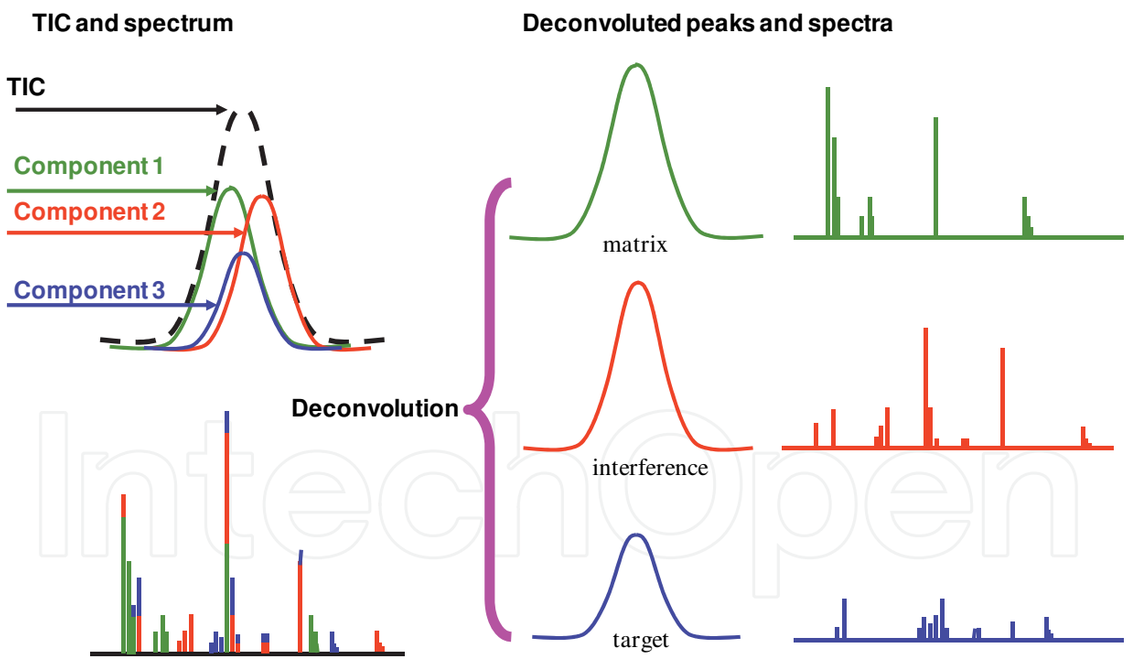


Fig. 1. The mass deconvolution process

The spectra at any particular retention time can be deconvoluted (cleaned) by reporting only those ions whose chromatographic apexes are reached at that particular retention time; all other ions that may elute slightly earlier or later, may be present as general background and are discarded. In addition to the chromatographic apex at a specific RT, peak shape is an additional factor considered in deconvolution. That is, ions with both the same apex location

and similar peak shape are clustered into a component for searching. In this way, the deconvolution process finds those ions whose individual abundances rise and fall together within the spectrum. As illustrated in Figure 2, deconvolution produces "clean" spectra that are the composite of only those ions at the same apex location and with similar peak shape. Deconvolution finds the components (a component is a group of related ions) from a complex TIC. Each component is searched against a retention time locking (RTL) library in AMDIS format. In addition to spectral matching, the locked RT can also be used as a criterion for hits. Depending on the match factor from the search, target compounds can be identified or flagged in a complex TIC.

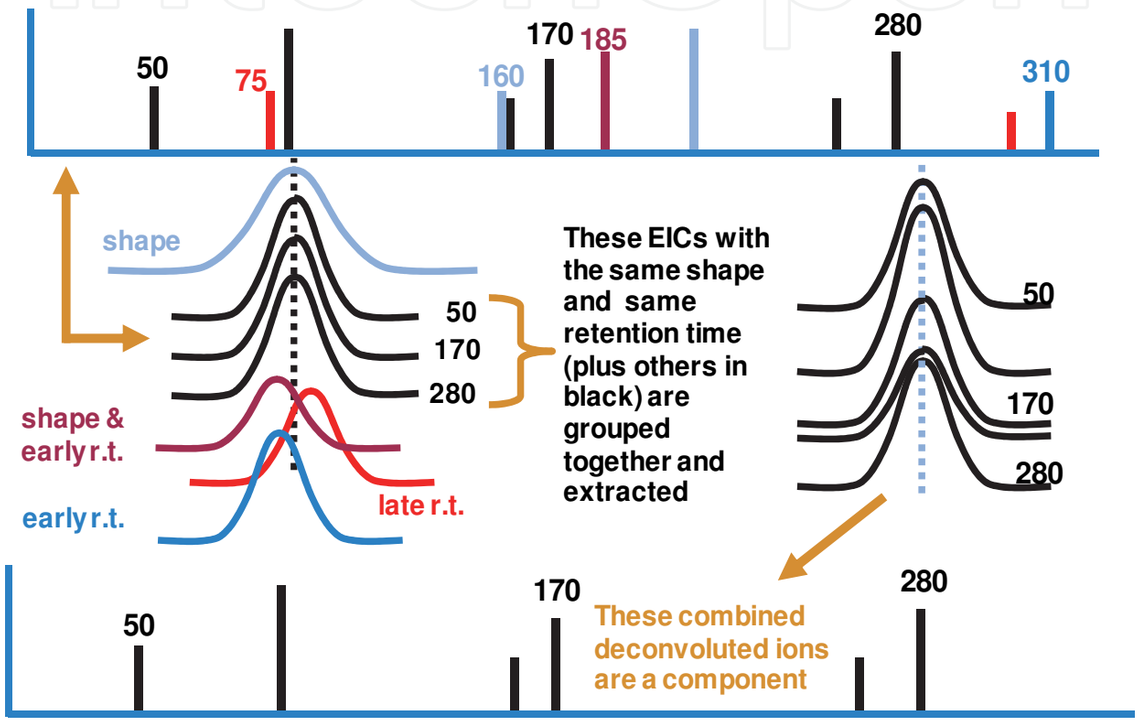


Fig. 2. Graphical representation of the deconvolution process

Because RTL is used to reproduce the RTs with high precision, this window can be quite small—typically 10 s or less. This "RT qualifier" is very useful for blind study/screening (Sandy, 2004). The components identified by deconvolution and Agilent's Pesticide Library can be further confirmed against the NIST library containing pesticides and numerous other compounds. Note that the NIST reverse search is based solely on the ion pattern for the compound and does not incorporate retention time matching.

1.2 Liquid phase analyses

For samples that are more amenable to LC analysis, i.e., polar and thermally labile, a triple quadrupole (QQQ) is often used for trace level target analysis of complex matrixes. The multiple reaction monitoring process removes chemical background from the sample matrix, thereby providing superior selectivity and sensitivity for pesticide analysis. But LC-single quadrupole and QQQ instruments cannot readily be used for nontarget

identifications for two reasons. First, due to lack of selectivity and sensitivity, LC-single quadrupole and QQQ instruments do not usually operate in the full-scan mode for pesticide screening. Second, common LC/MS spectral libraries are unavailable due to difficulties in standardizing and reproducing fragmentation energies among instruments from different vendors. Therefore, when screening for a broad range of pesticides, a TOF (time-of-flight) or Q-TOF is the instrument of choice (Mezcua et al, 2009). A high-resolution TOF instrument always acquires full spectra and gives accurate masses.

Recent experience has demonstrated that LC/TOF-MS has the capability and sensitivity to obtain full spectra with a mass accuracy of less than 1 ppm (Ferrer & Thurman, 2005). This level of accuracy in mass allows the analyst to distinguish between compounds whose molecular weights are extremely similar. Table 1 lists several pesticides whose exact mass varies by a fraction of an amu.

Element	**Atomic Number**	**Exact Mass**
H	1	1.007825
C	6	12.000000
N	7	14.003074
O	8	15.994915
Formula	**Exact Mass**	**Compound Name**
$C_6H_6Cl_6$	287.8600665	Lindane
$C_{10}H_{12}N_2O_6S$	288.0416000	Carbasulam
$C_9H_{21}O_2PS_3$	288.0441285	Terbufos
$C_{13}H_{21}O_3PS$	288.0949000	Iprobenfos
$C_{15}H_{17}N_4Cl$	288.1141743	Myclobutanil
$C_{12}H_{21}N_2O_4P$	288.1238937	Diazoxon
$C_{11}H_{20}N_4O_3PS$	288.1256000	Epronaz
$C_{11}H_{21}N_4O_3P$	288.1351000	Pirimetaphos
$C_{16}H_{20}N_2O_3$	288.1473925	Imazamethabenz

Table 1. Exact masses of elements and pesticide compounds

LC/Q-TOF instruments with the capability of measuring 2 ppm (i.e., 0.000576 amu for mass 288) accuracy can easily distinguish between these molecules. A tool called Molecular Feature Extractor (MFE), which is similar to deconvolution used in GC/MS, finds all ions in an LC/TOF data file that represent ions of real compounds in the sample analyzed. Noise and other extraneous ions are excluded. The resulting list of anion or cation masses is then searched against a database of theoretical monoisotopic exact masses of compounds based on their molecular formula and selected adduct ions (H+ or Na+, etc). Then, by comparison to the

Exact Mass Database of hundreds of pesticides, the normally tedious manual process of matching can be done rapidly and reliably (Thurman & Ferrer, 2005) using mass accuracy and RT as the criteria. After identification, the sample can be reanalyzed in the MS/MS mode using a QQQ or Q-TOF instrument to confirm any hits from the database search.

2. Experimental

2.1 GC: Analysis of surface water

The California Department of Food and Agriculture (CDFA) prepared and analyzed surface water samples using an Agilent 6890N gas chromatograph equipped with an Agilent 5973 inert mass selective detector (MSD) (Siegel et al, 2004). The sample collection and preparation procedure is the following: A 1 L water sample is delivered to the laboratory in an amber glass bottle. Samples are stored under normal refrigerated conditions (approximately 4°C) until extraction within 7 days.

2.1.1 Surface water samples

2.1.1.1 Sample preparation

a. Weigh and record the 1 L water sample, including sediments.
b. Pour the water sample, including sediments, into a 2 L separatory funnel. Do not filter since pesticides would stick to humic materials.
c. Spike with 1 mL surrogate spiking solution: 0.5 ng/mL Chlorpyrifos-methyl (0.5 ng). Shake the separatory funnel gently to mix.
d. Add 10–15 g granular Sodium Chloride (NaCl) for salting-out purposes. Shake gently to dissolve salt.
e. Rinse water sample container with 60 mL Methylene chloride and add to the separatory funnel. Weigh and record the empty water sample container. Subtract and record the water sample weight.
f. Shake and release pressure several times. Shake well for 3 min. Let settle until the lower Methylene chloride layer is completely separated from the above water layer. If there is too much emulsion in the funnel, use a sonicator to break up the emulsion.
g. Filter the bottom organic layer through a bed of granular anhydrous sodium sulfate (approximately 20 g) into a 250 mL round-bottom flask. The sodium sulfate is supported on glass wool and is prewashed with 30–40 mL Methylene chloride.
h. Add 60 mL Methylene chloride into the funnel and repeat steps (f) and (g) two more times.
i. The round-bottom flask should now contain about 180 mL methylene chloride. Place the round-bottom flask on a Rotavapor evaporator (at about 100 rpm) and evaporate down to 5–7 mL at 40°C.
j. Transfer the contents of the round-bottom flask to a 15 mL collection tube. Rinse the round-bottom flask with 5 mL methylene chloride and add to the collection tube.
k. Place the 15 mL collection tube on an N_2-Evaporator with water temperature set at 40°C. Evaporate the sample to near dryness.
l. Remove the tube from the N_2-Evaporator and carefully add 1.0 mL methylene chloride and 10 mL 0.5 pg/mL internal standard (ISTD) solution into the collection tube.
m. Vortex and transfer the solution into an autosampler vial.
n. Cap and store the vial in a –5°C freezer until analysis.

2.1.1.2 Reagents and supplies

a. *Methylene chloride.*—Pesticide grade (Sigma-Aldrich, St. Louis, MO).
b. *Anhydrous sodium sulfate.*—Certified American Chemical Society (ACS) 10–60 mesh (Sigma-Aldrich).
c. *Glass wool.*—Pyrex brand fiber glass (Sigma-Aldrich).
d. *Sodium Chloride (NaCl).*—Certified ACS (Sigma-Aldrich).
e. *Nylon filter (0.45* m*m).*—Sigma-Aldrich.
f. *Surrogate spiking solution.*—0.5 ng/mL Chlorpyrifos-methyl in acetone (Ultra Scientific, N. Kingstown, RI).
g. *ISTD solution.*—Anthracene-d10, pyrene-d10, and chrysene-d12 at 0.5 ng/mL (500 ppt); Ultra Scientific ISM-520.

2.1.1.3 Agilent 6890N GC parameters

- *Column.*—30 m x 0.25 mm x 0.25 µm HP-5MS (Agilent Technologies, Santa Clara, CA).
- *Inlet temperature.*—230°C.
- *Injection volume.*—2 µL (splitless).
- *Oven ramp.*—Initial temperature at 70°C, hold for 2 min ramp at 25°/min to 150°C, hold for 0 min, ramp at 3°/min to 200°C, hold for 0 min, ramp at 8°/min to 280°C,hold for 12 min.

2.1.1.4 Agilent 5973 MSD parameters

- Full scan mode.
- Maximum sensitivity Auto Tune.

2.2 Pear and peach samples

2.2.1 Sample preparation

Samples were extracted using the quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method (Anastassiades et al, 2003; Lehotay et al, 2005).

a. 15 g homogenized sample + 15 mL acetonitrile + internal standard
b. Add 1.5 g Sodium Chloride (NaCl) and 6.0 g Magnesium Sulfate Anhydrous ($MgSO_4$)
c. Shake and centrifuge
d. Transfer 9 mL extract to tube containing 0.4 g Primary and Secondary Amine (PSA) + 0.2 g Graphitized Carbon Black (GCB) + 1.2 g Magnesium Sulfate Anhydrous ($MgSO_4$) and vortex
e. Add 3 mL toluene
f. Shake and centrifuge
g. Reduce 6 mL to ~100 µL
h. Add 1.0 mL toluene + QC standard + Magnesium Sulfate Anhydrous ($MgSO_4$) and centrifuge
i. Transfer to Automatic Liquid Sampler (ALS) vials for GC-MS analysis

2.2.2 Agilent 7890 GC parameters

- Autoinjector: 7693A
- Retention gap: 2 m × 0.25 mm id Siltek capillary tubing
- Column: HP-5MS UI (ultra inert), 15 m × 0.25 mm, 0.25 µm (from inlet to Purged Union) Agilent p/n 19091S-431 UI

- Oven ramp: Initial temperature at 100°C, hold for 1.6 min, ramp at 50°/min to 150°C, hold for 0 min, ramp at 6°/min to 200°C, hold for 0 min, ramp at 16°/min to 280°C, hold for 5 min.
- Run time: 20.933 min
- Inlet: Multimode Inlet (MMI) at 17.73 psi (Retention Time Locked), constant pressure mode
- RT locking: Chlorpyrifos-methyl locked to 8.297 min
- Liner: Helix double taper, deactivated (Agilent p/n 5188-5398)
- Injection mode: 2-µL cold splitless (fast injection)
- Inlet temp. Initial temperature at 50°C, hold for 0.01 min ramp at 720°/min to 300°C, hold
- Septum purge: 3 mL/min
- Purged Union: 4 psi (pressure supplied by a pneumatic control module, PCM)
- Split vent: 50 mL/min at 0.75 min
- Gas saver: 20 mL/min after 4 min
- Cryo on: Cryo use temperature 150 °C; time out at 15 min (Liquid CO_2)

2.2.3 Backflush parameters

- Postrun: 5 min
- Oven: 280 °C
- Purged Union: 70 psi
- MMI: 2 psi
- Restrictor: 0.7 m × 0.15 mm deactivated fused silica tubing (from Purged Union to MSD)

2.2.4 Agilent 5975 MSD parameters

Solvent delay: 2.5 min

- EMV mode: Gain Factor = 2
- Mass Range: Full scan, 45-550
- Threshold: 0
- Sample number: 2 A/D Samples 4
- Transfer Line: 280 °C
- Source: 300 °C
- Quad: 200 °C

2.3 LC/Q-TOF: Analysis of grape sample

An acetonitrile extract of a grape sample was prepared by the QuEChERS extraction method and analyzed by 1200 LC and 6510 Q-TOF (Agilent Technologies; 10). The QuEChERS sample extraction and cleanup procedure for both GC and LC is the following:

2.3.1 Extraction

a. Chop samples into small pieces and freeze in a bag overnight before grinding. Dry ice should be added during grinding.
b. Weigh 10 g homogenized sample into a 50 mL Teflon centrifuge tube.
c. Add 10 mL acetonitrile (and ISTD solution, if used).
d. Add 4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate dehydrate, and 0.5 g disodium hydrogen citrate sesquihydrate to the tube.
e. Adjust the pH to 5–5.5 using 5 M sodium hydroxide (NaOH).

f. Shake the sample vigorously for 1 min using a vortex mixer at maximum speed or by hand shaking.
g. Centrifuge for 5 min at 3000 rpm.

2.3.2 Cleanup

a. Transfer 6 mL supernatant into a 12 mL polypropylene centrifuge tube that contains 150 mg primary-secondary amine adsorbent and 900 mg Magnesium Sulfate Anhydrous ($MgSO_4$).
b. Shake for 30 s.
c. Centrifuge for 5 min at 3000 rpm.
d. Adjust the pH of the cleaned extract to 5.0 for analysis, if necessary.

2.3.3 Agilent 1200 LC parameters

Column	2.1 x 100 mm, 1.8 μm ZORBAX XDB PLUS C18	
Flow Rate	0.3 mL/min	
Injection Volume	10 μL	
Solvent A	0.1% Formic Acid in water	
Solvent B	100% acetonitrile	
Gradient	Time	Solvent B
	0	10%
	20	95%
	25	95%

2.3.4 Agilent 6510 QTOF parameters

Ion Source	ESI			
Drying Gas	325 C			
Drying Gas Flow	10 L/min			
Nebulizer	50 psi			
VCap	4000 V			
Fragmentor	175 V			
Reference Masses	121.050873 and 922.009798			
Acquisition Mode	MS1			
	Min Range	100		
	Max Range	1000		
	Scan Rate	1		
Acquisition Mode	Targeted MS2			
	MS Min Range	100	MS/MS Min Range	100
	MS Max Range	3000	MS/MS Max Range	3000
	MS Scan Rate	1.4	MS/MS Scan Rate	0.7
		Max Time Between MS	10	
		Ramped Collision Energy		
		Slop	5	
		Offset	5	

3. Results and discussion

In a GC/MS scan analysis, it is always very difficult to identify compounds from high matrix background because the matrix ions overwhelm the compound signal. To be certain

of the results, spectral averaging and background subtraction are often practiced. It is therefore a very time-consuming process to confirm compounds in a complex matrix.

3.1 Analysis of GC surface water sample data with DRS

Data files acquired by the CDFA on 17 surface water extracts were compared using two approaches (Wylie et al, 2004). Three example TICs are shown in Figure 3.

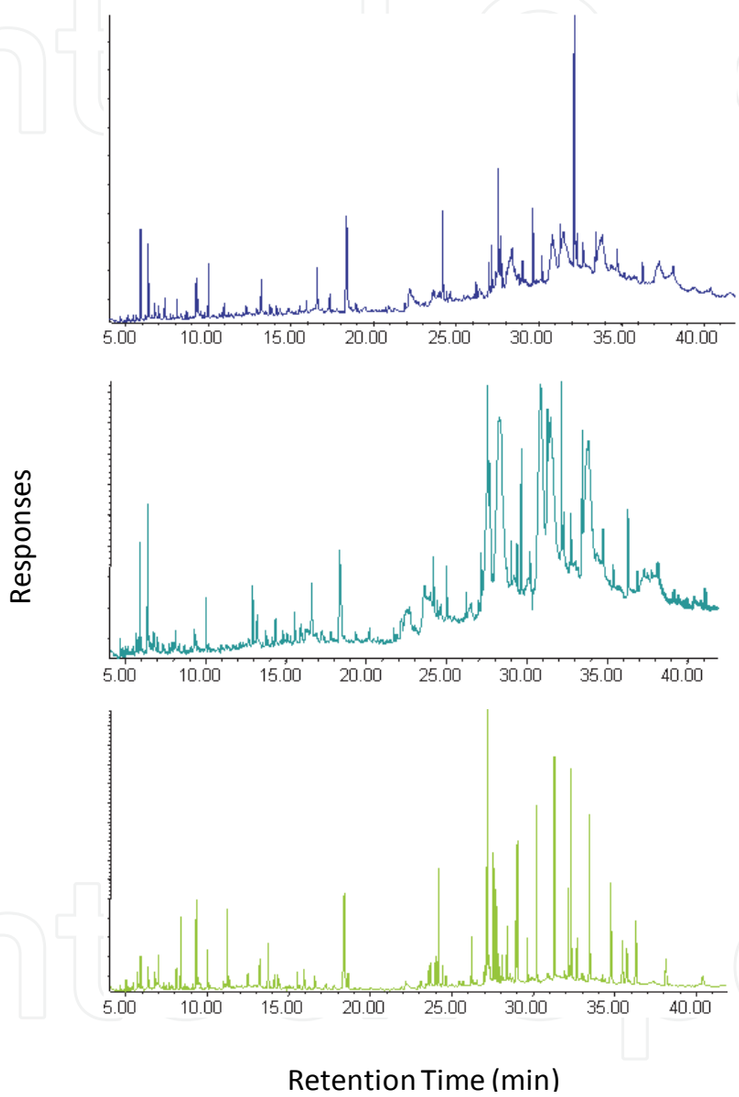


Fig. 3. TICs of surface water extracts showing the complexity of the matrix

The TICs show that the extracts were very complex. Approximately 8 h of a skilled analyst's time were required to process and confirm the results generated by ChemStation and searching the NIST library without the benefit of deconvolution. This workflow resulted in the identification of 37 pesticides plus one false positive. The same data files for the 17 TICs were processed using DRS without re-running the samples. The DRS reports were rapidly generated on all 17 TICs using batch processing. The display screen depicting this option is shown in Figure 4.

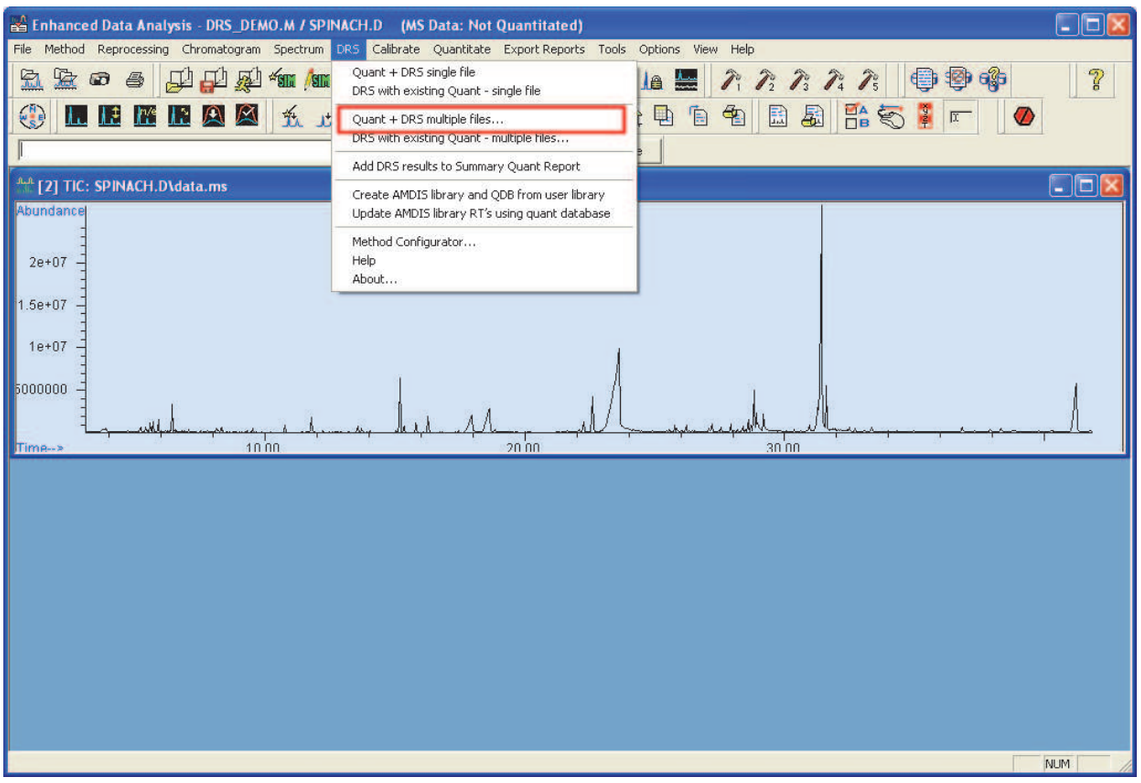


Fig. 4. Selecting simultaneous processing for all TICs in the Deconvolution Reporting Software (DRS)

Figures 5 and 6 show an example chromatogram and the corresponding DRS report for that chromatogram, respectively. The compounds listed in the report are only compounds in the specific DRS library. Therefore, the non-targeted compounds found in any sample are

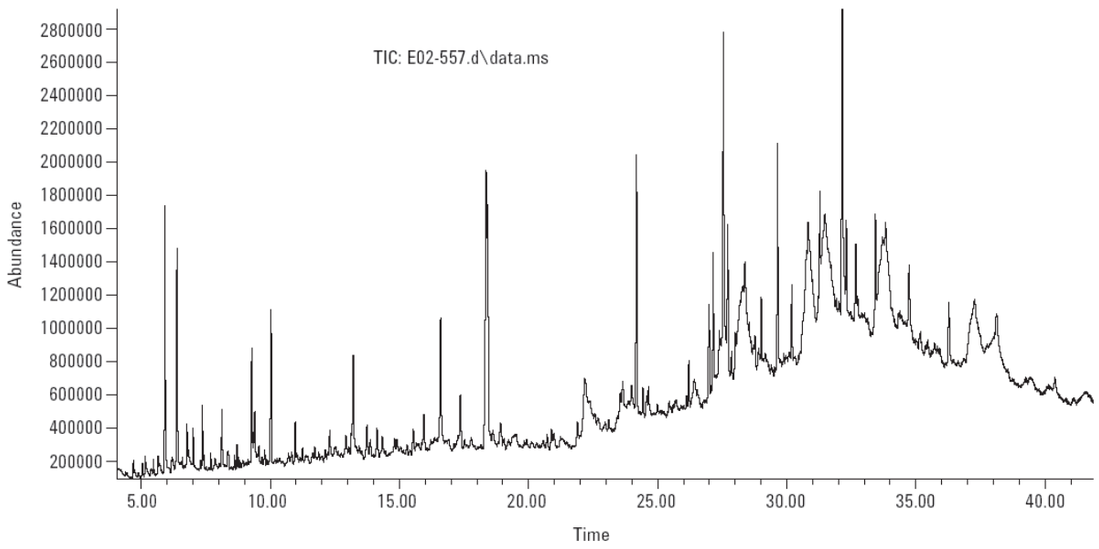


Fig. 5. Example total ion chromatogram (TIC) for pesticide analysis using GC/MS and DRS

			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
4.8840	104121	4-Chlorophenyl isocyanate		86	-1.8	86	2
6.3888	102363	Diuron Metabolite [3,4-Dichlorophenyl isocyanate]		99	3.1	95	1
6.8136	54115	Nicotine		72	2.9	64	3
6.8357	759944	EPTC		84	2.0	85	1
7.6988	95761	3,4-Dichloroaniline		93	2.1	89	2
7.9342	131113	Dimethylphthalate		64	1.7	81	4
8.0431	85416	Phthalimide		72	3.8	81	1
8.1112	25013165	Butylated hydroxyanisole		63	-7.7	59	1
8.941	29878317	Tolyltriazole		74	5.9	68	4
9.7859	134623	N,N-Diethyl-m-toluamide		82	2.0	85	2
10.0019	84662	Diethyl phthalate		98	2.6	92	1
10.7109	119619	Benzophenone		88	2.6	85	2
10.9684	126738	Tributyl phosphate		96	3.0	92	1
11.6465	1582098	Trifluralin		84	0.6	76	1
12.9290	122349	Simazine		87	1.2	86	2
13.4460	115968	Tris(2-chloroethyl) phosphate		83	1.9	84	1
13.7451	1517222	Phenanthrene-d10		93	1.2	85	1
14.8285	4147573	Aziprotryn metabolite		64	7.1	79	1
15.3906	58082	Caffeine		61	0.7	78	1
15.9474	84695	Diisobutyl phthalate		89	3.2	89	2
16.5988	5598130	Chlorpyrifos Methyl		97	0.4	91	1
17.3680	7287196	Prometryn		90	1.7	84	1
18.4213	84742	Di-n-butylphthalate		99	0.4	93	1
18.9223	51218452	Metolachlor		94	0.8	91	1
20.5633	121552612	Cyprodinil		72	-0.1	64	1
26.4194	23576241	Norflurazon, Desmethyl-		86	-4.8	73	2
26.9700	27314132	Norflurazon		88	1.5	82	1
27.0010	85687	Butyl benzyl phthalate		94	-0.4	93	1
27.3984	51235042	Hexazinone		89	0.8	83	1
28.0171	78513	Tris(2-butoxyethyl) phosphate		76	3.6	82	1
29.6537	117817	Bis(2-ethylhexyl)phthalate		98	0.3	90	3
13.739		Phenanthrene-d10	10				

Fig. 6. MSD Deconvolution Report for the chromatogram in Figure 5

associated to the specific DRS library used (commercially available or user-built). Using DRS in conjunction with the 927 compound Agilent Pesticide Database, the same 37 pesticides were identified along with an additional 99 new identifications and no false positives. In addition to the improved results, the speed of the analysis was reduced from 8 h to 32 min, thus representing a 15-fold gain.

3.2 Analysis of pear data with DRS: DRS following the GC/MS analysis

The power of deconvolution is appreciated while comparing the top two spectra in Figure 7. The raw scan or original nondeconvoluted scan is shown on top. The clean scan, that is the deconvoluted component, is shown in the middle. The bottom scan is the identified compound in the AMDIS library.

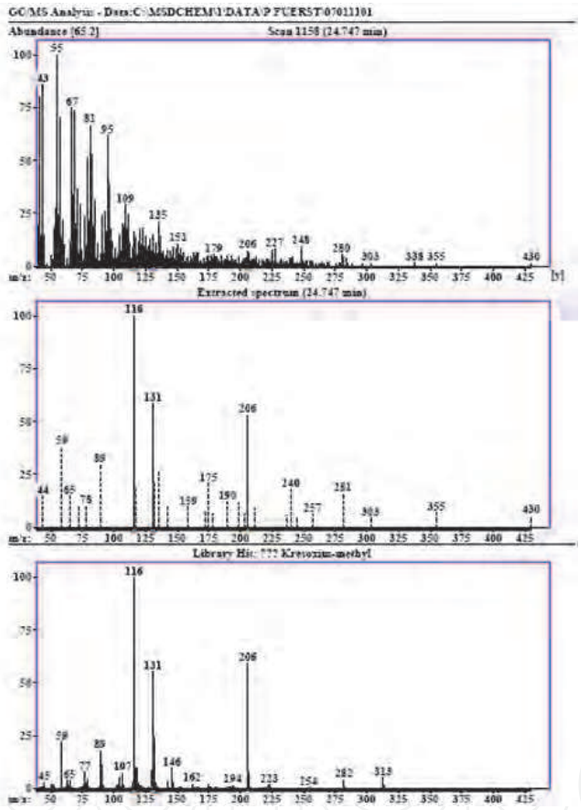


Fig. 7. Analysis results of pear extract using GC and deconvolution (DRS)

Without deconvolution, the analyst would visually compare the background subtracted raw scan and library scans for confirmation. It would be very difficult, if not impossible, to say that Kresoxim-methyl, the target compound in this example, is present using that type of comparison. The top mass spectrum is the raw scan from the TIC at 24.747 min and is clearly unsuited for library-searching purposes. In contrast, the middle spectrum (dashed lines are uncertain ions) presents the deconvoluted scan extracted from the raw scan. This deconvoluted (cleaned) scan is now easily and confidently matched against the library spectrum for Kresoxim-methyl presented in the bottom panel. For discussion on deconvolution parameter settings and additional advantages of deconvolution, reference the Agilent Application Note (Meng & Szelewski, 2010)

3.3 Analysis of peach data with DRS: DRS following the GC/MS analysis

An example DRS report for a peach extract is shown in Figure 8. As shown, Carbaryl appears with an AMDIS match factor of 74 versus the theoretical best match score of 100.

MSD Deconvolution Report
Sample Name: peach
Data File: C:\msdchem\1\DATA\FDA\08_03_07 FDA_S_CI\peach1_S_CI.D
Date/Time: 10:54:30 AM Monday, July 27, 2009

Adjacent Peak Subtraction = 1
Resolution = Medium
Sensitivity = High
Shape Requirements = Medium

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

			Amount (ng)		AMDIS		NIST	
R.T.	Cas #	Compound Name	Chem station	AMDIS	Match	R.T. Diff sec.	Reverse Match	Hit Num
2.2748	97530	Eugenol		0.09	77	2.5	72	7
3.3036	86737	Fluorene			76	0.5	63	16
3.3242	84662	Diethyl phthalate	0.2	0.17	89	1.4	90	1
3.5084	877098	2,4,5,6-Tetrachloro-m-xylene	0.36	0.33	97	3.1	91	1
3.558	119619	Benzophenone		0.06	73	0.9	81	2
3.6458	126738	Tributyl phosphate	1.29	1.09	88	1.5	91	1
5.316	84695	Diisobutyl phthalate		1.59	99	3.1	89	8
5.609	63252	Carbaryl		0.07	74	1.1	81	10
6.142	84742	Di-n-butylphthalate		2.31	97	0.6	93	1
7.083	133062	Captan		0.11	82	1.5	85	1
7.5447	959988	Endosulfan (alpha isomer)	0.04	0.04	91	-0.0	86	2
9.0198	85687	Butyl benzyl phthalate		0.07	81	3.5	85	1
9.070	999048032	Propiconazole-II		0.03	75	4.3		
9.0702	60207901	1H-1,2,4-Triazole, 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-					75	1
9.528	732116	Phosmet		1.33	96	5.4	93	1
10.7788	119611006	Fenbuconazole		0.42	76	6.9		
10.7788	0000	Piperazine-2,5-dione, 3-hydroxy-6-isopropyl-3-trifluoromethyl-					59	1

Fig. 8. DRS report for analysis of peach extract in full scan mode.

In general, an AMDIS match factor of 80 or higher is enough to confirm a hit. The RT difference of 1.1 s between the actual and expected RTs also indicates excellent agreement, i.e., high confidence in the results. RTL and a suitable time window (e.g., ±10 s) provide this added qualification function in screening. Some missing quantitation results in the ChemStation column were due to some out-of-range qualifier ion ratios. Qualifiers out-of-range are very typical for complex matrixes. The last two columns of the DRS report show the results from searching all the AMDIS hits against the NIST08 mass spectral library, which contains information on mass spectra only (not on RT). When the NIST library search finds a compound in the top 100 matches (a user-settable value) that agrees with the AMDIS results, its match factor is listed in the Reverse Match column. The Hit Number is shown in the last column, with 1 being the compound with the best match (highest match factor) in the NIST database. The compounds listed in the figure are all in the top 20 hits, with many as the number one hit. The further confirmation of hits found by DRS can easily be done with another injection in selected ion monitoring (SIM) mode or using GC detectors. In Figure 8, four compounds of interest with AMDIS match factors close to or less than 80 (Carbaryl, Captan, Propiconazole-II, and Fenbuconazole) were highlighted and further confirmed using SIM and GC detectors. Figure 9 is a DRS report of the same peach extract analyzed in SIM mode.

MSD Deconvolution Report
Sample Name: peach
Data File: C:\msdchem\1\DATA\FDA\08_03_07
FDA_S_C\peach_SIM.D
Date/Time: 10:35:43 AM Monday, July 27, 2009

Adjacent Peak Subtraction = 1
Resolution = Medium
Sensitivity = High
Shape Requirements = Medium

The NIST library was not searched for the components that were found in the AMDIS target library.

			Amount (ng)		AMDIS		NIST	
R.T.	Cas #	Compound Name	Chem station	AMDIS	Match	R.T. Diff sec.	Reverse Match	Hit Num
5.616	63252	Carbaryl	0.1	0.07	94	2.5		
7.0882	133062	Captan	0.2	0.14	96	2.3		
9.003	60207901	Propiconazole-I	0.03	0.02	93	4.6		
9.073	999048032	Propiconazole-II	0.07	0.04	97	5.1		
10.7836	119611006	Fenbuconazole	0.68	0.5	94	7.8		

Fig. 9. DRS report for analysis of peach extract in SIM mode.

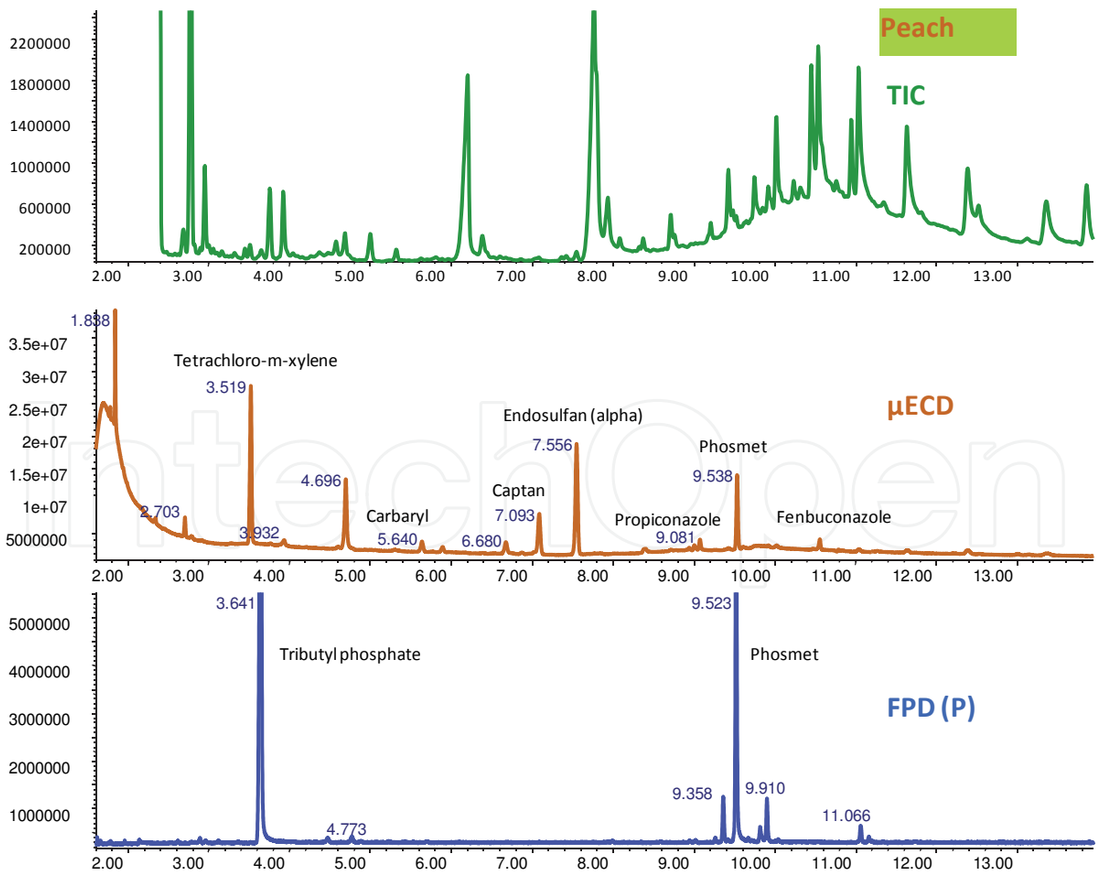


Fig. 10. Simultaneous display of MSD and GC selective detector signals for peach extract.

All of the compounds of interest in Figure 8 were found with AMDIS match factors over 90. Noticeably, the AMDIS quantitation results in Figures 8 (full scan) and 9 (SIM) were very comparable. These quantitation results are actually "semi-quant" results based on one average response factor for all of the 927 compounds in the DRS quantitation method. Figure 10 shows another approach for compound confirmation.
The figure shows simultaneously collected MSD TIC and GC element-selective detector signals [μECD (micro electron capture detector) and FPD (flame photometric detector) in the phosphorus (P) mode] for peach extract. The results were obtained from a single injection with column flow split into three detectors (Meng & Szelewski, 2007). All compounds of interest were shown in the μECD chromatogram at the expected RT. Both SIM and GC element-selective detector analyses are easy and useful approaches to confirm hits found in the DRS screening process.

3.4 Analysis of grape sample data with MFE (Molecular Feature Extractor) and exact mass search (Meng et al, 2009)

Figure 11 shows the raw TIC of grape sample obtained from the LC/Q-TOF.

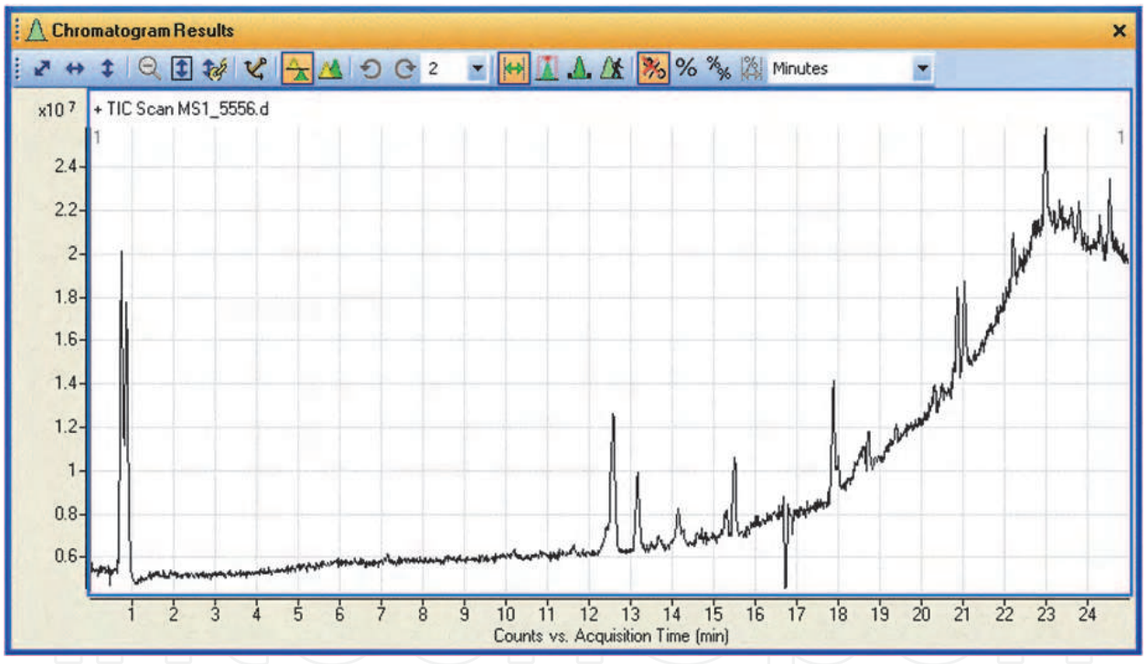


Fig. 11. Raw full spectrum TIC obtained from LC/Q-TOF analysis of grape sample.

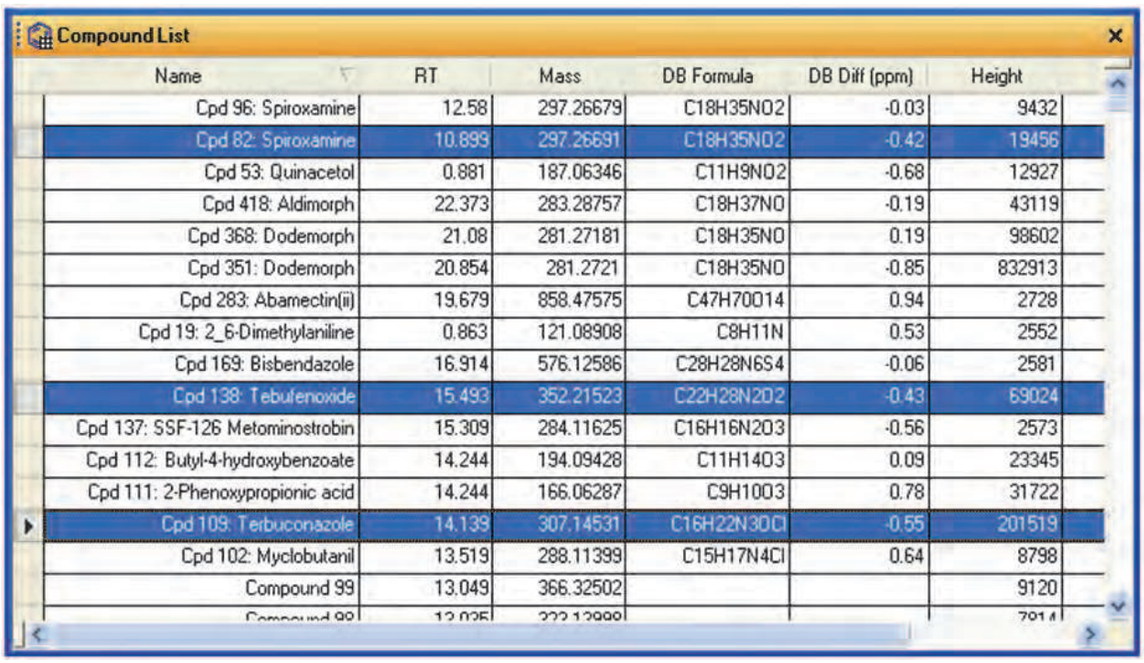
The MFE was used to find the masses of interest within the TIC based on user-defined settings (*see* Table 2 for settings).
The result was 510 potential compounds. The accurate mass measured for each of the 510 compounds was then searched against an Exact Mass Database, using search parameters described in Table 3.
The RT, an optional entry in the database, can be used as a qualifier. Out of the 510 potential compounds, 15 had a match of exact mass with a compound in the database within 3 ppm accuracy. As an example, in the Compound List shown in Figure 12, compound 82 was identified as Spiroxamine.

Extraction	Ion Species	Charge State	Compound Filters	Mass Defect	Results
Peak filter: Use peaks with height ≥ 1000	Positive ions: H, Na, K, NH4	Peak spacing tolerance: 0.0025 m/z, plus 7.0 ppm Limit assigned charge states to a maximum of 1	Relative height ≥ 0.2%	Filtering not used	Delete previous compounds Highlight all compounds

Table 2. Settings for Molecular Feature Extractor (MFE)

Search Criteria	Database	Peak Limits	Positive Ions	Search Results
Match mass only with 3.00 ppm tolerance	Exact Mass Compound database	10	H. Na, K, NH4 Charge state range 1–2 No neutral losses	Limit to the best 5 hits

Table 3. Settings for Database Search



Name	RT	Mass	DB Formula	DB Diff (ppm)	Height
Cpd 96: Spiroxamine	12.58	297.26679	C18H35NO2	-0.03	9432
Cpd 82: Spiroxamine	10.893	297.26691	C18H35NO2	-0.42	19456
Cpd 53: Quinacetol	0.881	187.06346	C11H9NO2	-0.68	12927
Cpd 418: Aldimorph	22.373	283.28757	C18H37NO	-0.19	43119
Cpd 368: Dodemorph	21.08	281.27181	C18H35NO	0.19	98602
Cpd 351: Dodemorph	20.854	281.2721	C18H35NO	-0.85	832913
Cpd 283: Abamectin(ii)	19.679	858.47575	C47H70O14	0.94	2728
Cpd 19: 2_6-Dimethylaniline	0.863	121.08908	C8H11N	0.53	2552
Cpd 169: Bisbendazole	16.914	576.12586	C28H28N6S4	-0.06	2581
Cpd 138: Tebufenoxide	15.493	352.21523	C22H28N2O2	-0.43	69024
Cpd 137: SSF-126 Metominostrobin	15.309	284.11625	C16H16N2O3	-0.56	2573
Cpd 112: Butyl-4-hydroxybenzoate	14.244	194.09428	C11H14O3	0.09	23345
Cpd 111: 2-Phenoxypropionic acid	14.244	166.06287	C9H10O3	0.78	31722
Cpd 109: Terbuconazole	14.139	307.14531	C16H22N3OCl	-0.55	201519
Cpd 102: Myclobutanil	13.519	288.11399	C15H17N4Cl	0.64	8798
Compound 99	13.049	366.32502			9120

Fig. 12. A portion of the compound list of grape sample from MFE and Exact Mass Search.

The hits from the database search can easily be confirmed using MS/MS analysis available on the Q-TOF. The three highlighted compounds were selected for targeted-MS/MS analysis. The "DB Diff (ppm)" column in the figure presents the difference in ppm (not amu) between the experimental mass found and the database-listed mass, and shows the excellent mass accuracy of the instrument.
As shown in Figure 13, the grape sample was analyzed again in the LC/Q-TOF-MS/MS mode where both the MS1 full spectrum and the MS/MS full spectrum were acquired.

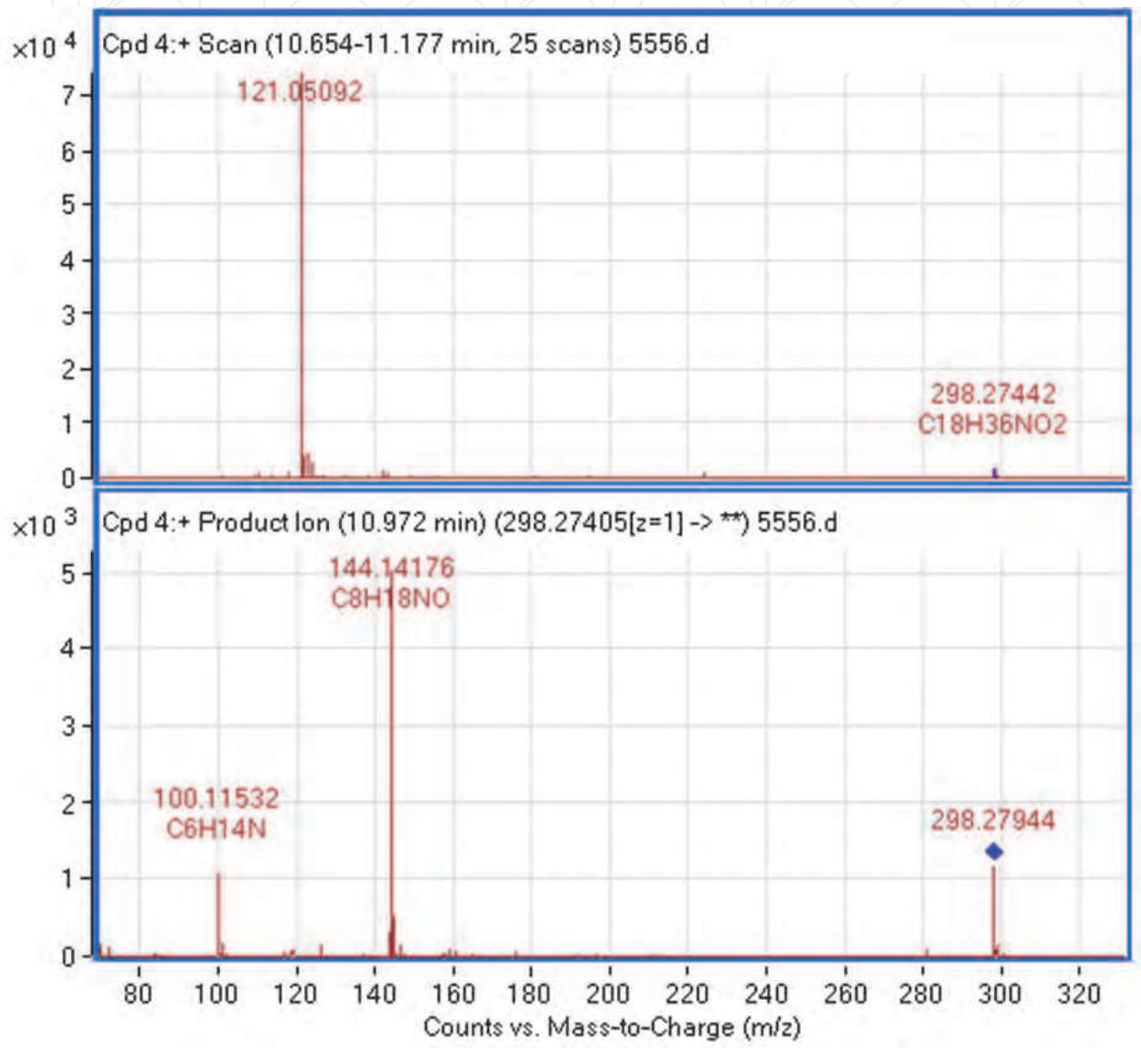


Fig. 13. Analysis of grape sample by LC/Q-TOF full spectrum and MS/MS.

The ion 121.05092 seen in the MS1 mode is the reference ion used for real-time mass axis calibration. The Spiroxamine precursor ion at 298.27442 is found in this scan at RT 10.884 min. The MS/MS full spectrum scan shows the precursor ion 298.27944 and two fragment ions at 100.11532 and 144.14176 as predicted from Spiroxamine's molecular structure. Criteria for finding compounds in the resulting MS/MS chromatogram are listed in Table 4. This additional injection in the targeted MS/MS mode confirmed the hits from the database search.

Integrator	Processing	Cpd TIC Peak Filters	Peak Spectrum	Results
MS/MS Integrator	Maximum chromatogram peak width 0.25 min	Filter on peak area Limit (by height) to the largest 10 peaks	Spectra to include average scans > 10% of peak height Exclude TOF spectra anywhere if above 40.0% of saturation MS/MS peak spectrum background: None	Delete previous compounds Highlight all compounds Extract MS/MS chromatogram Extract MS/MS spectrum

Table 4. Software settings for "Find Compounds by Targeted MS/MS".

3.5 Analysis of strawberry sample data with MFE (Molecular Feature Extractor) and exact mass search (Meng et al, 2009)

A strawberry sample was also analyzed similar to the grape sample above. The MFE produced 822 potential compounds. Figure 14 shows the TIC, the hyperlinked extracted compound chromatogram (ECC), and the mass spectrum for one of these compounds.

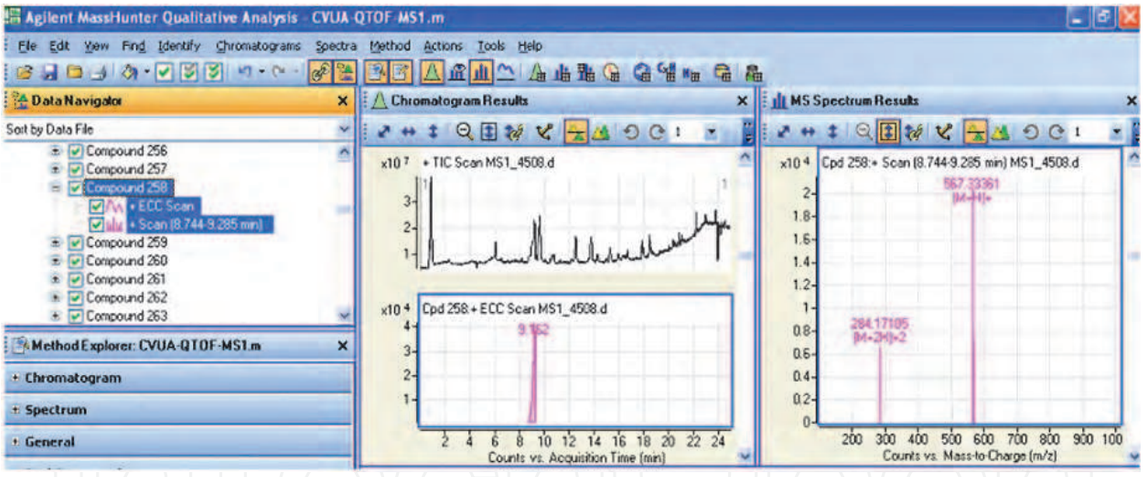


Fig. 14. The ECC and mass spectrum are shown for one of 822 compounds found using the MFE software along with the TIC.

Since ECCs are created when one of the MFE "Find Compounds" algorithms is run, the ECC consists of all the related ions and no chemical noise. The accurate mass of each of these compounds was subsequently searched against a working exact mass database of 1600 pesticides [MassHunter Personal Pesticide Database (G6854AA)]. The criteria used in this search are shown in Table 3. Twenty-six of the 822 compounds had mass matches (3 ppm tolerance) with pesticides in the database. Three plausible exact-mass match compounds, cyprodinil, azoxystrobin, and boscalid were then selected for further confirmation using MS/MS (Q-TOF) analysis with the same instrument. The ECC and mass spectrum for each of the three are provided in Figure 15 along with the database search results showing a difference of less than 1 ppm in experimental and database masses.

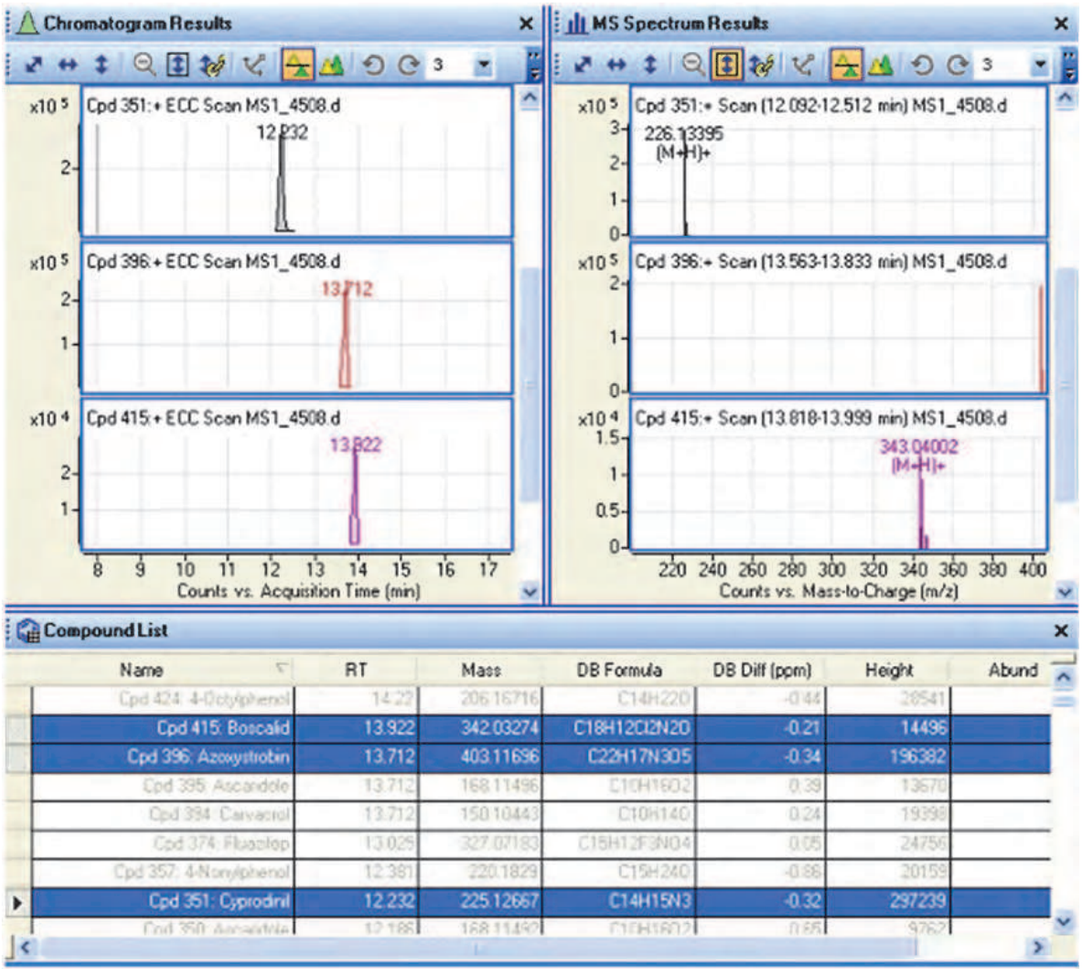


Fig. 15. The hyperlinked ECC and mass spectrum for three positive pesticides, cyprodinil, azoxystrobin, and boscalid, found in the strawberry extract, are shown along with the exact mass database search results (a portion of the results is shown).

The precursor ion (M + H)+ masses chosen for the MS/MS analysis of the strawberry extract were exact masses from the database: 226.13395, 404.12410, and 343.03995 for cyprodinil, azoxystrobin, and boscalid, respectively. Criteria for finding compounds in the resulting MS/MS chromatogram are listed in Table 4. Using the accurate MS/MS masses for the fragment ions, formulas were generated for each compound found in this step.

The confirmation process for azoxystrobin will be further discussed as an example. In Figure 16, the best-fit (with mass accuracy of 0.26 ppm and isotopes) formula generated from the Targeted MS/MS analysis for one of the compounds was $C_{22}H_{17}N_3O_5$, the formula for azoxystrobin.

The two associated fragment masses for this peak had less than 1 ppm difference in mass (0.31 and 0.2 ppm) when compared to the database masses for fragments expected from the $C_{22}H_{17}N_3O_5$ parent formula. In addition, the three isotope masses for the molecular ion all differed by less than 1 ppm. The table outlined in Figure 16 shows that the experimental isotope abundances of the three isotopes match well with the calculated (theoretical) abundances. The boxes in Figure 17 surrounding the isotopes represent the theoretical isotope abundances.

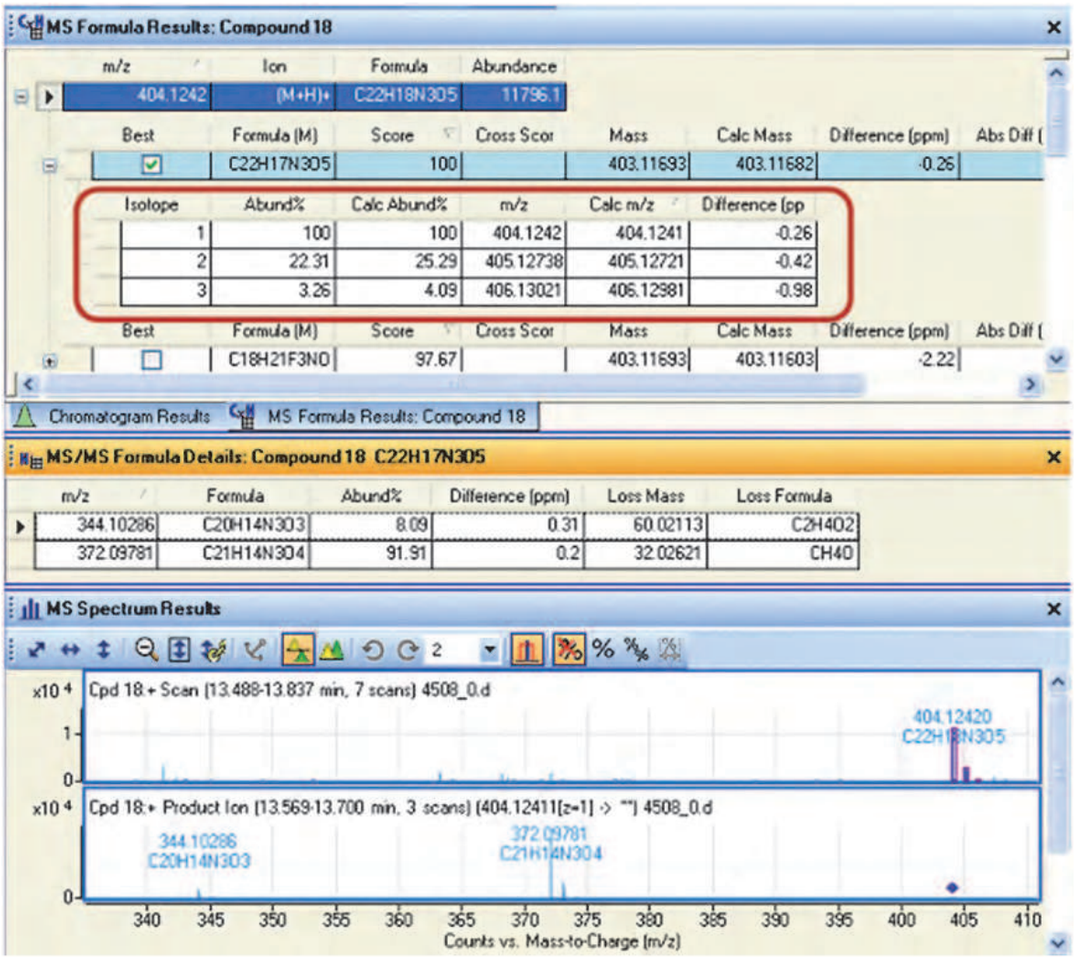


Fig. 16. Results from the formula analysis of MS and MS/MS data of a compound (Azoxystrobin) in the strawberry extract.

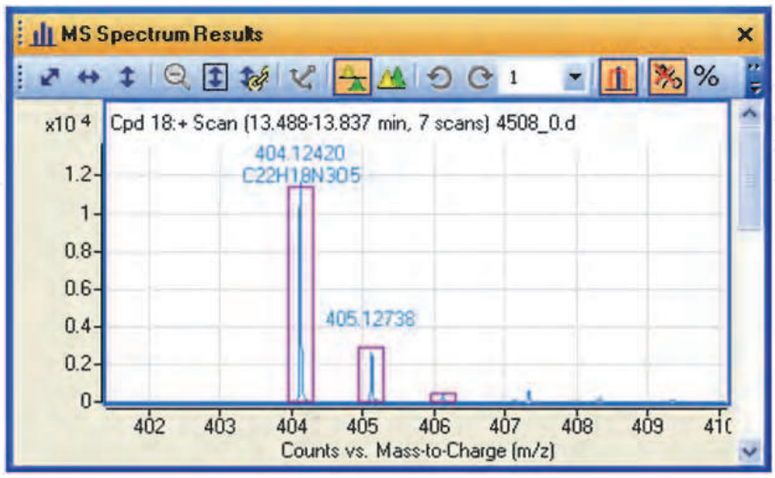


Fig. 17. The experimental isotope abundances of the three isotopes match well with the theoretical abundances (outlined in boxes).

Final confirmation of the structure is obtained by comparing the experimental fragment masses to likely theoretical fragment ion masses. Analysis of the structural formula, as seen in Figure 18, shows two likely fragments, with masses of 344.10351 and 372.09843 that match closely in mass to the two fragment ions found in the MS/MS data: 344.10286 and 372.09781 amu.

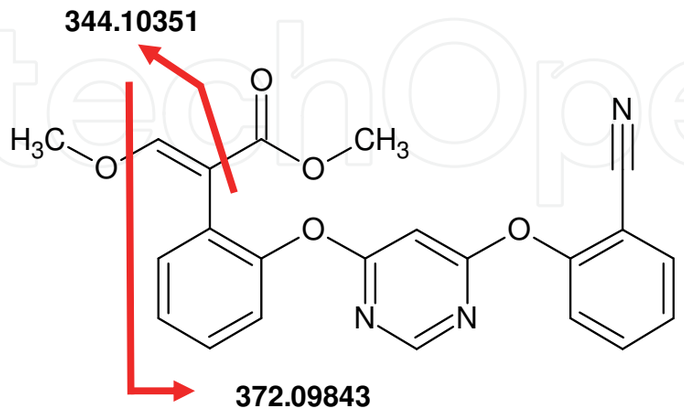


Fig. 18. Structural analysis for Azoxystrobin fragments.

The difference between experimental and calculated is 0.31 and 0.20 ppm, respectively. The accuracy of this comparison, along with the MS data identification and MS/MS formula generation results all strongly suggest the presence of Azoxystrobin in the analyzed strawberry extract.
A diagram depicting this "screen and confirm" workflow is shown in Figure 19.

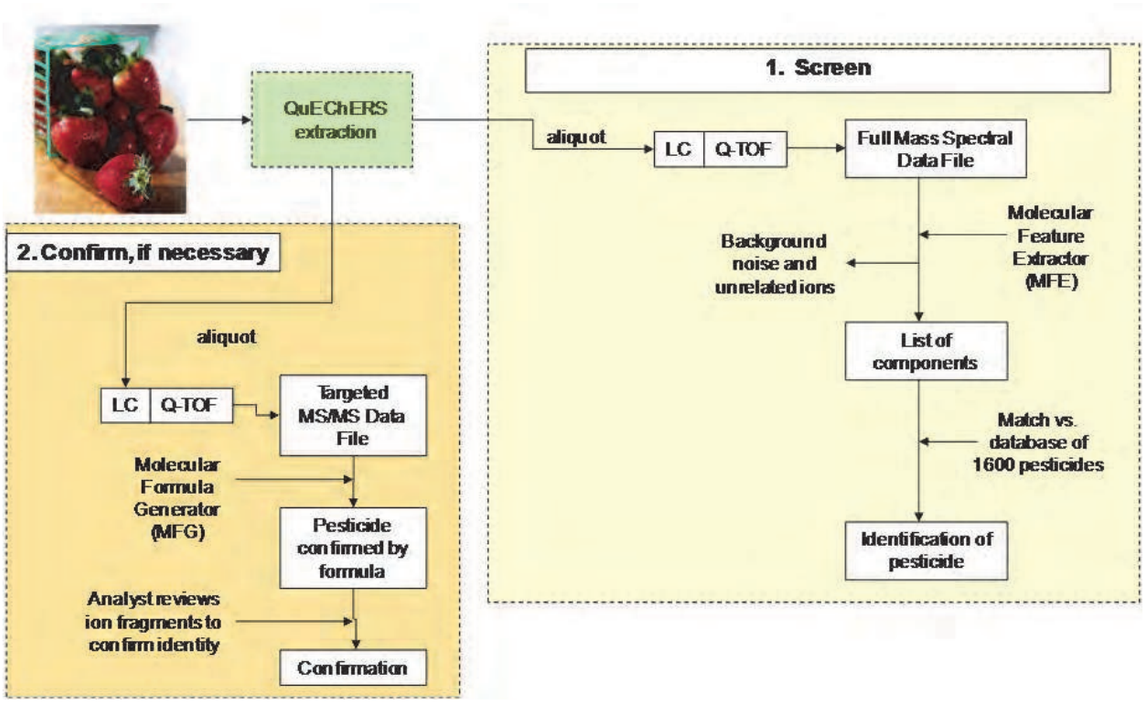


Fig. 19. Screen and Confirm - LC/Q-TOF analysis and software workflow.

4. Conclusion

GC/MS in the full scan mode combined with deconvolution enables unknown pesticide screening down to the 50 µg/Kg level in various food commodities in a single injection. Both GC/MS and LC/TOF (Q-TOF) screening have the following unique advantages: Crops can be screened for an unlimited number of compounds (depending on the number of compounds in the DRS library or exact mass compound database), and sensitivity is the same regardless of number of compounds screened (unlike QQQ). The hits from the GC/MS screening can be confirmed with an additional injection in the SIM mode or using GC element-selective detectors. LC/Q-TOF also provides the accurate mass MS/MS for confirmation of hits and structure elucidation, thereby frees the analyst from labor-intensive manual comparison of fragmentation patterns. The workflow of "Screen, Confirm, and Quantify" in non-targeted pesticide analysis is explained and illustrated with real samples.

5. Acknowledgement

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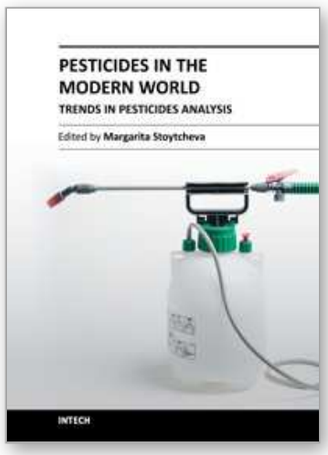

Pesticides in the Modern World - Trends in Pesticides Analysis

Edited by Dr. Margarita Stoytcheva

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The book offers a professional look on the recent achievements and emerging trends in pesticides analysis, including pesticides identification and characterization. The 20 chapters are organized in three sections. The first book section addresses issues associated with pesticides classification, pesticides properties and environmental risks, and pesticides safe management, and provides a general overview on the advanced chromatographic and sensors- and biosensors-based methods for pesticides determination. The second book section is specially devoted to the chromatographic pesticides quantification, including sample preparation. The basic principles of the modern extraction techniques, such as: accelerated solvent extraction, supercritical fluid extraction, microwave assisted extraction, solid phase extraction, solid phase microextraction, matrix solid phase dispersion extraction, cloud point extraction, and QuEChERS are comprehensively described and critically evaluated. The third book section describes some alternative analytical approaches to the conventional methods of pesticides determination. These include voltammetric techniques making use of electrochemical sensors and biosensors, and solid-phase spectrometry combined with flow-injection analysis applying flow-based optosensors.

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