Finding Non-targeted (Unknown) Pesticides Using GC/MS and LC/Q-TOF

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Outline - Workflow for Non-targeted Pesticides Analysis

- Business Issues and Challenges
- *Sample Prep (QuEChERS) – Lehotay, Anastassiades and Mastovska*
- GC/MSD
- *(GC or LC)-QQQ*
- LC/Q-TOF

Business Issues and Challenges

- Global trade increases the number of pesticides to monitor (> several hundreds) **Screen for Unknowns**
- Regulation driving lower detection limits
- Speed: doing more analyses in a day **Faster Data Review and Reporting**
- Need for fast startup **Total Solution Offering**

GC-MSD Workflow

GC/MS (PTV) SIM/Scan
– for known and unknown

(+backflush)

Deconvolution

Final Report

Screen

Confirm

Quantify

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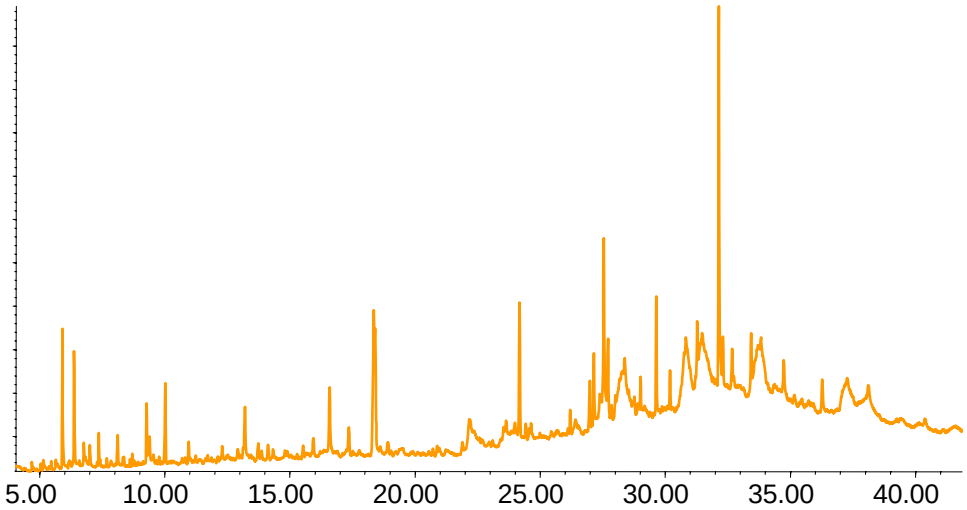
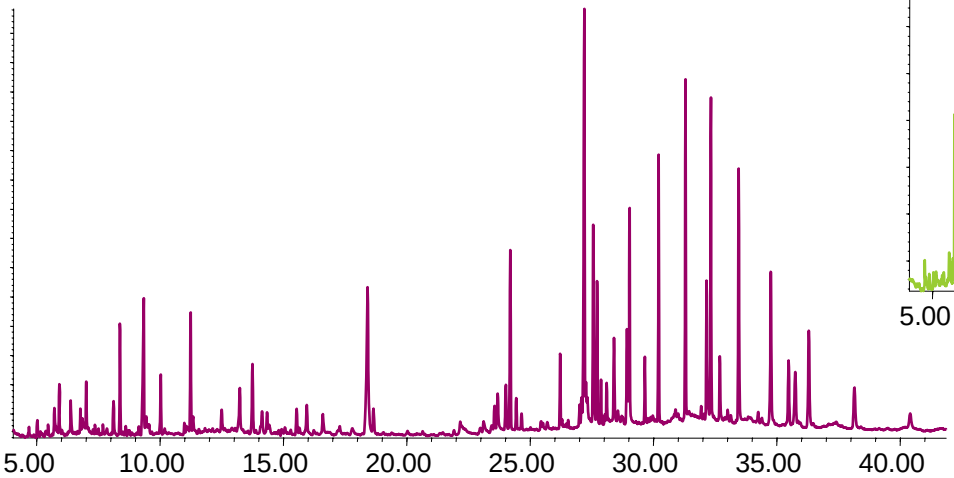
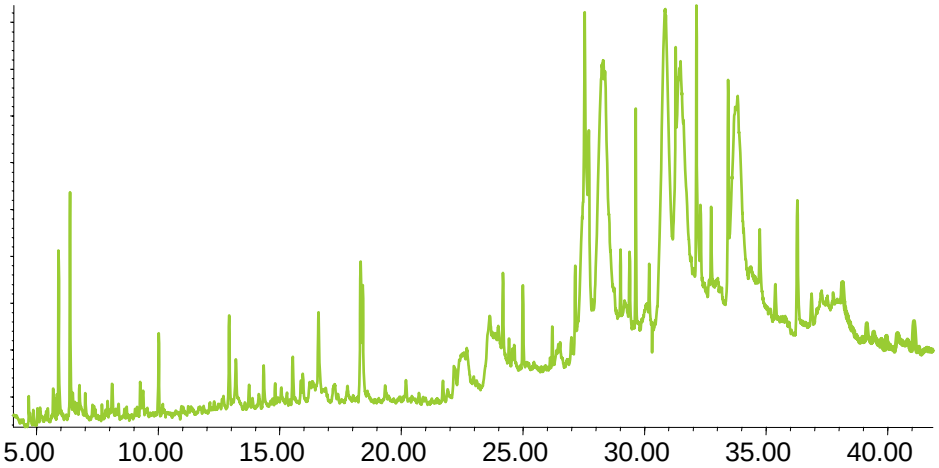
TICs of Surface Water Extracts



How many pesticides (drugs, allergens etc.) are in these samples and how long does it take you to confirm?





17 Surface Water TICs (Scan): Pesticide Analysis Using DRS with Pesticide Database (927 entries)

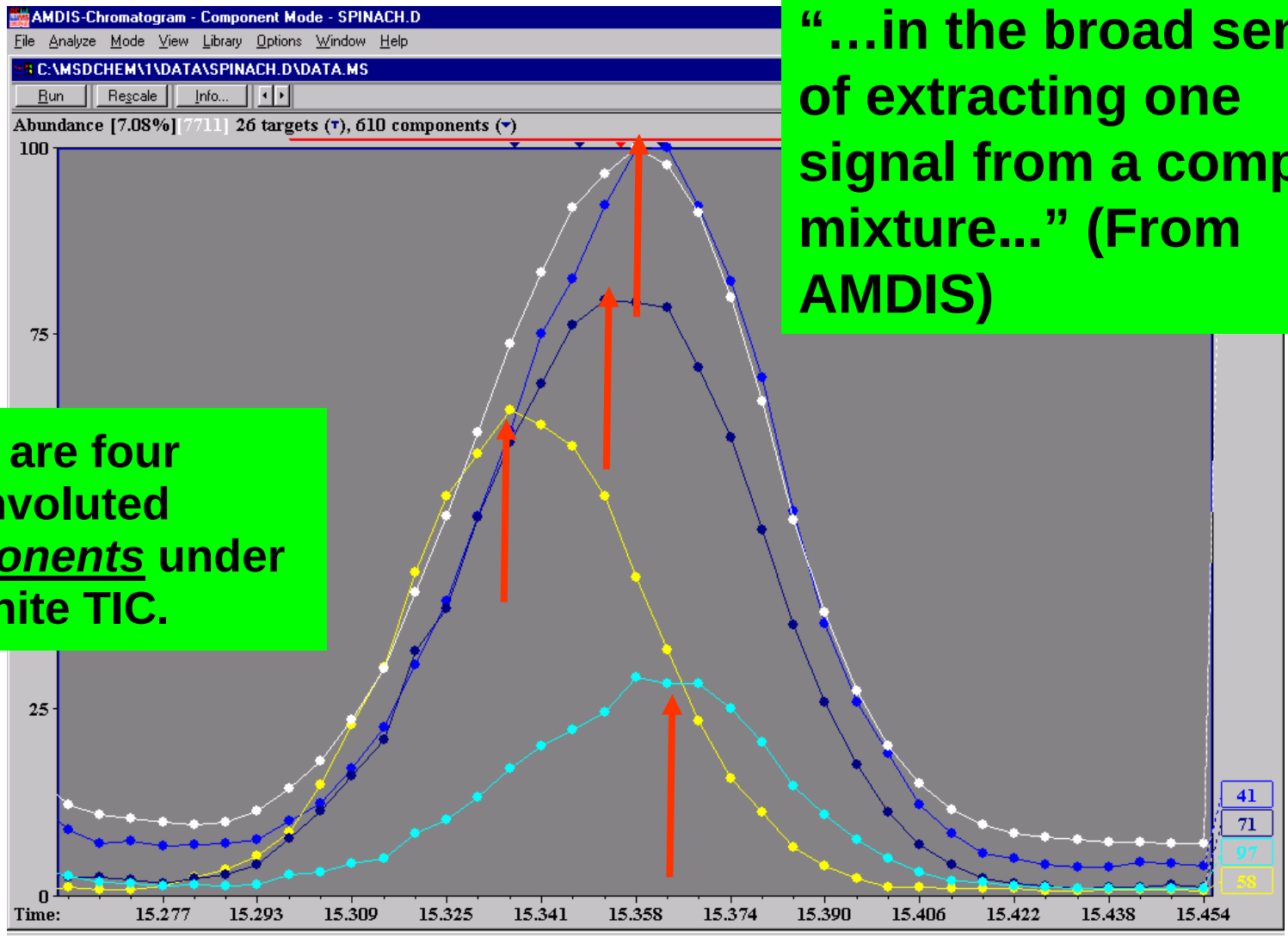
	CDFA*	Agilent DRS
Targets Found (not counting ISTD)	37	Same 37 +99 more
False Positives	1	0
Processing Time	~8 hrs	32 min

DRS: Deconvolution Reporting Software

Saving 7.5 hours

***CDFA is the California Department of Food and Agriculture**

What is Deconvolution?



"…in the broad sense of extracting one signal from a complex mixture..." (From AMDIS)

There are four deconvoluted *components* under the white TIC.

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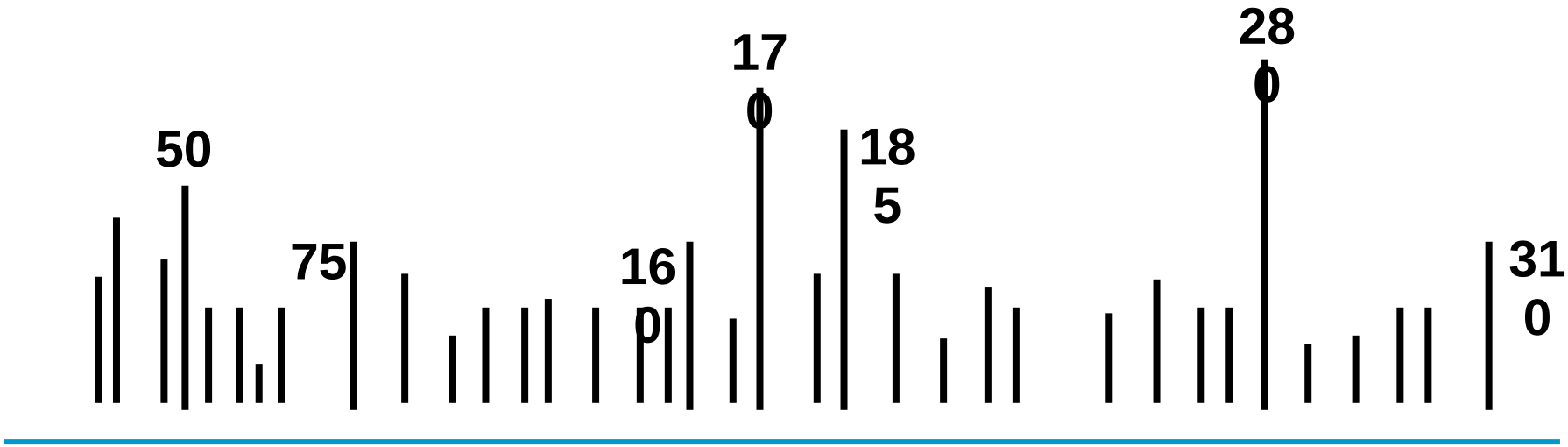
AMDIS

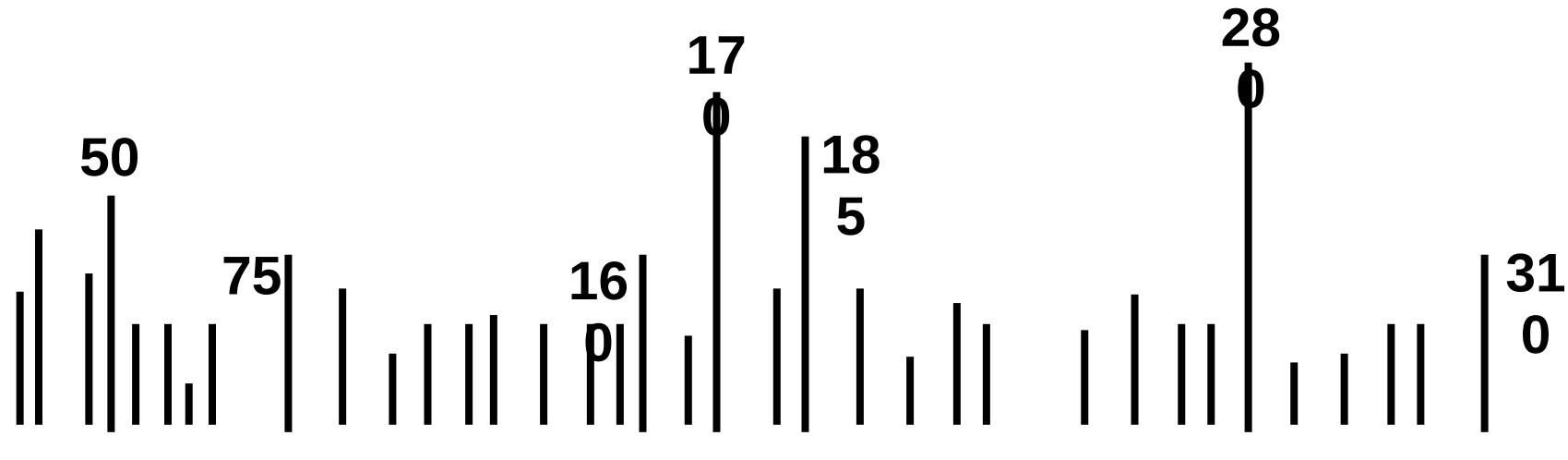
Automatic Mass spectral Deconvolution and Identification System

- Developed by the National Institute of Standards and Technology (NIST)
- Developed to detect chemicals in violation of Chemical Weapons Convention (must minimize false positives and false negatives in reporting)
- Used to identify target compounds in complex matrices

How Does Deconvolution Work?

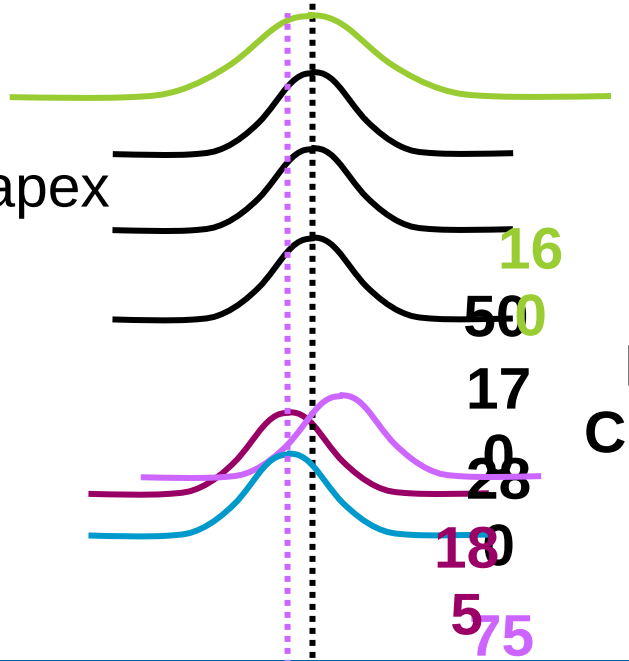


Eliminate Ions Don’t Fit the Criteria



Ion grouping criteria:

1. Same RetTime at apex
2. Same peak width



Extracted Ion Chromatograms (EIC)

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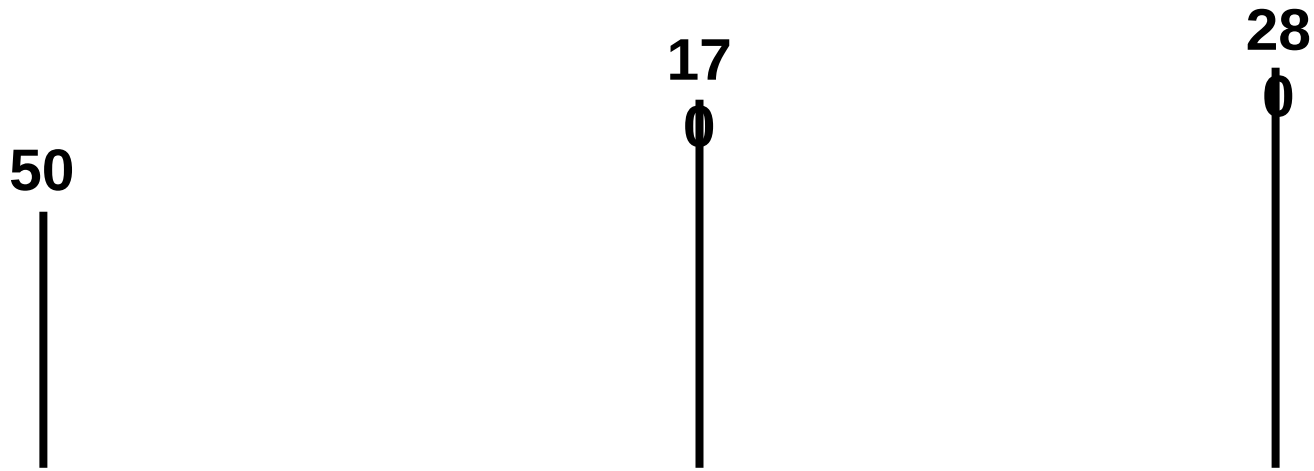
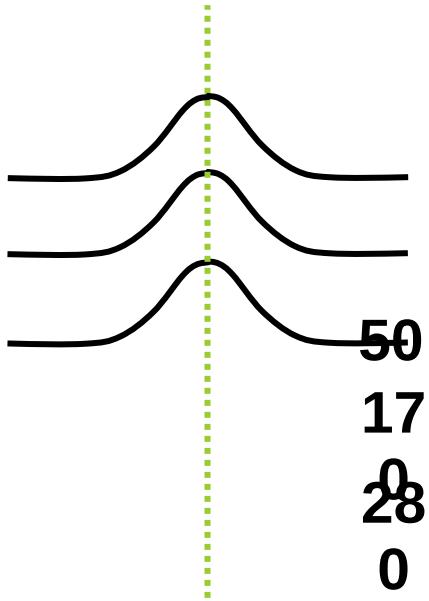
Spectrum is Deconvoluted/Cleaned

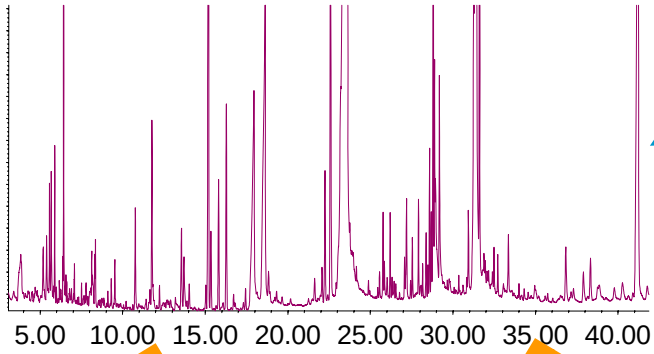




A component in AMDIS

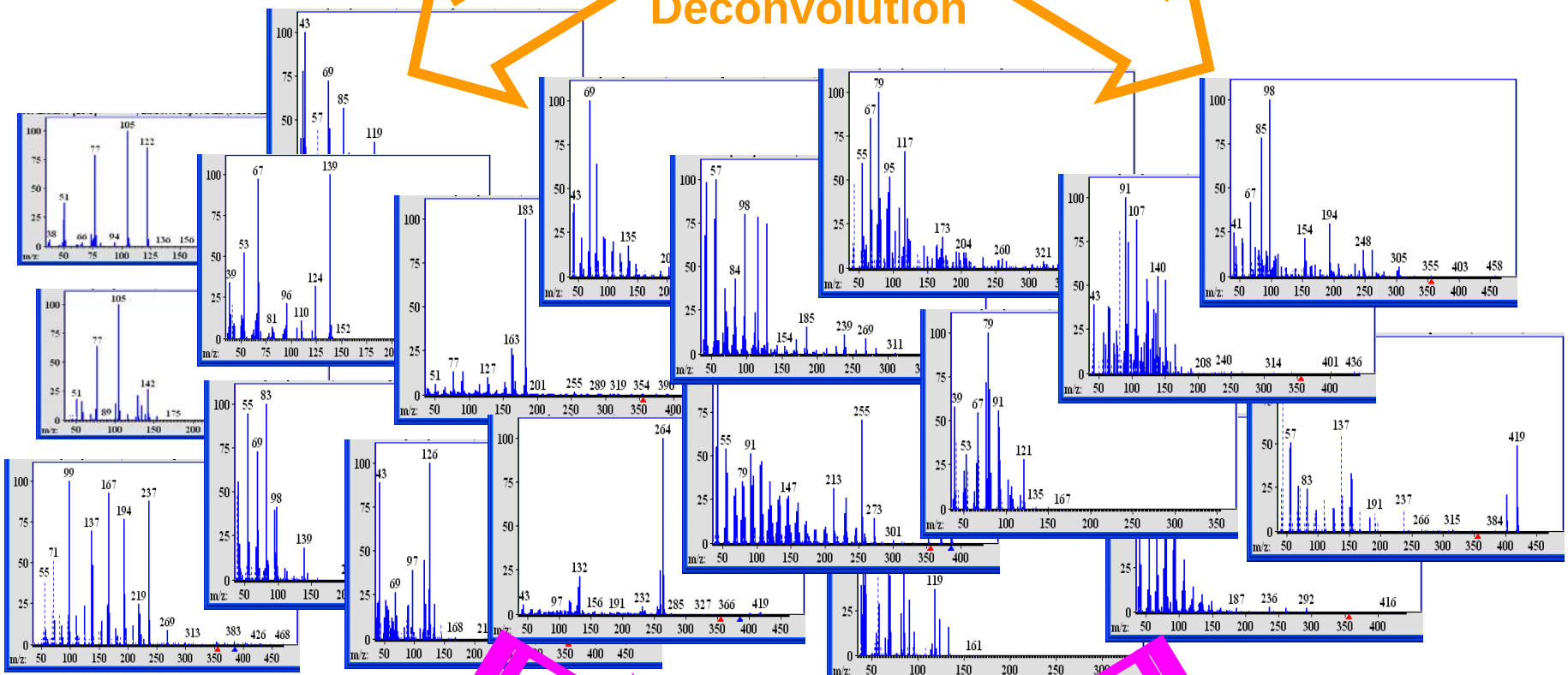
Related ions are grouped together as a component.

TIC of Spinach Extract

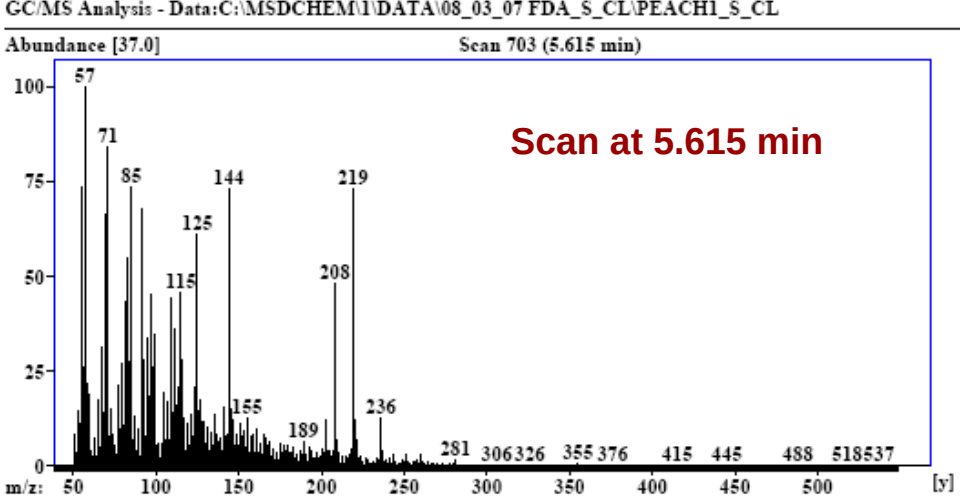


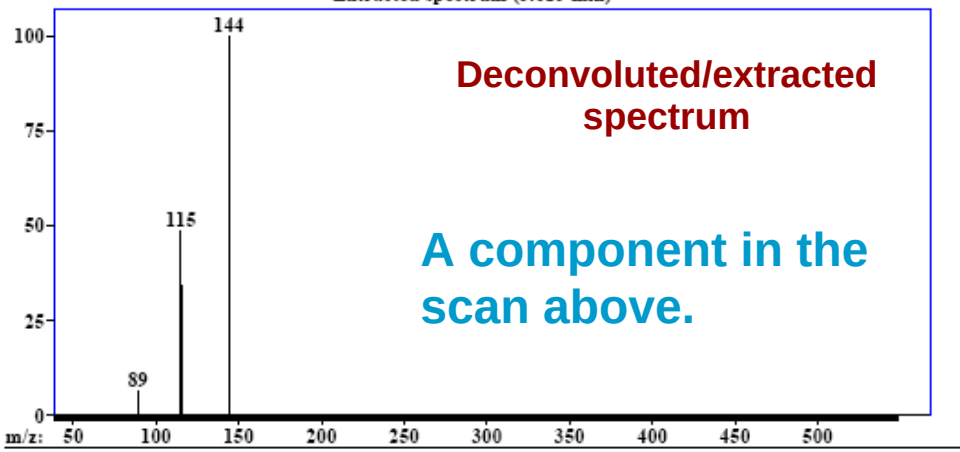
More than 370 peaks found

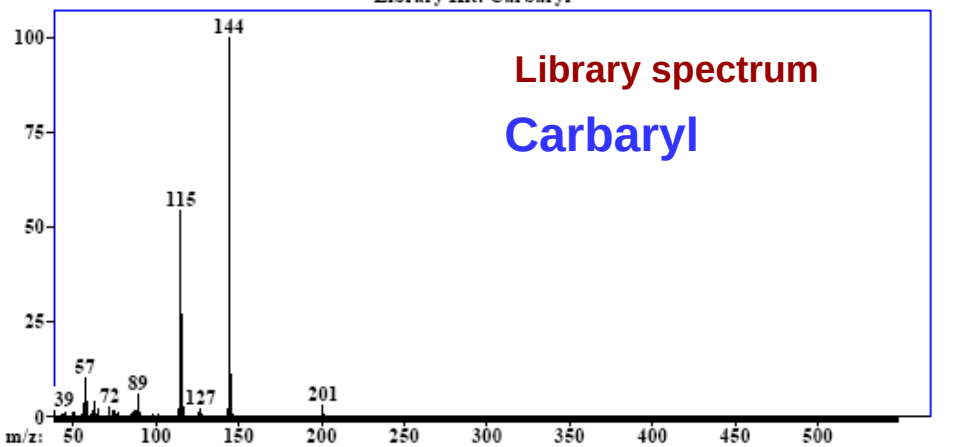
Deconvolution



Library Search

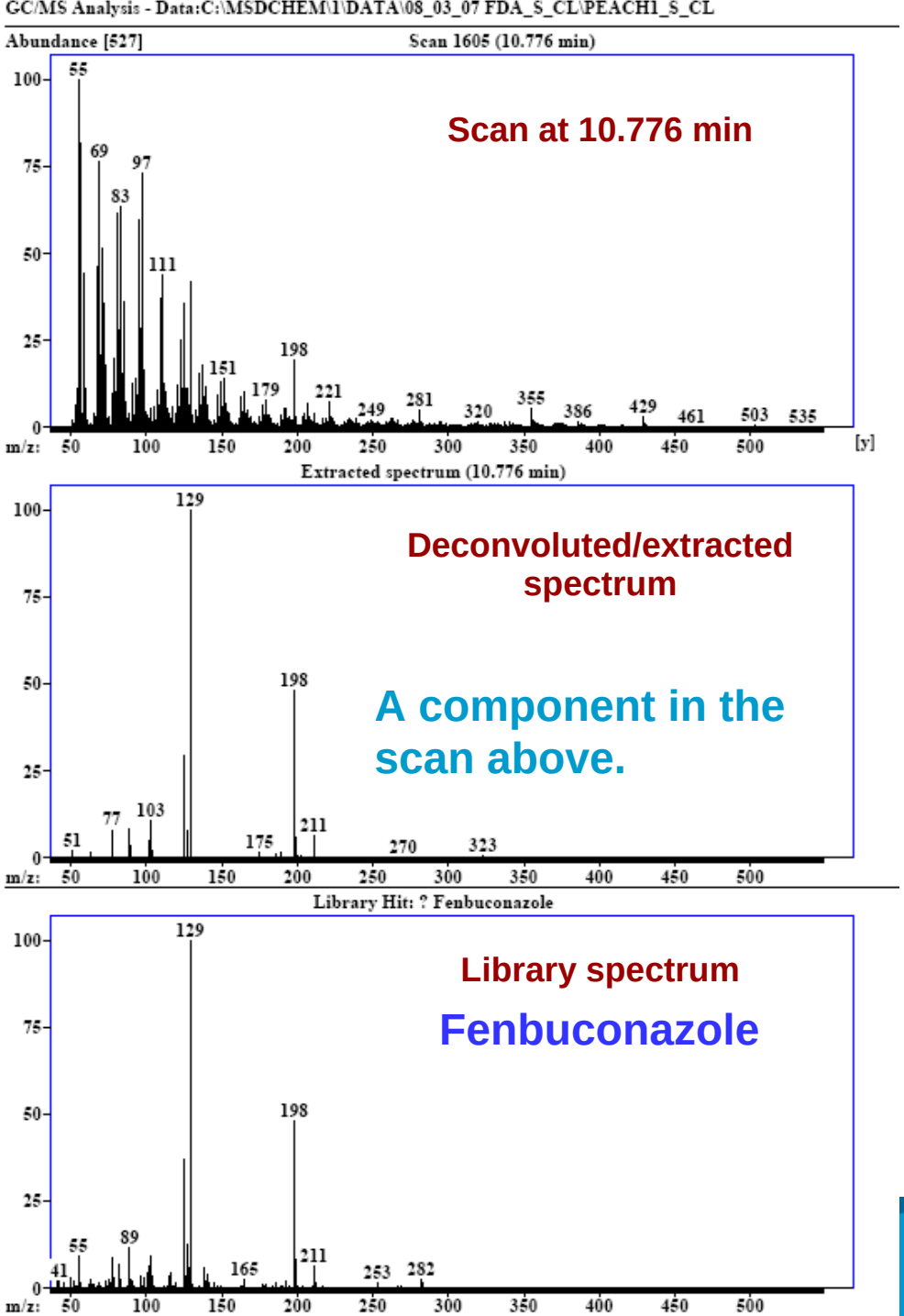






Peach

The power of deconvolution is appreciated while comparing the top two spectra (raw scan and the compound spectrum hidden in the raw scan).


GC/MS Analysis - Data:C:\MSDCHEM\1\DATA\08_03_07 FDA_S_CL\PEACH1_S_CL
Abundance [527]
Scan 1605 (10.776 min)
Scan at 10.776 min
Extracted spectrum (10.776 min)
Deconvoluted/extracted spectrum
A component in the scan above.
Library Hit: ? Fenbuconazole
Library spectrum
Fenbuconazole
m/z:
[y]

Peach

DRS Report - Peach

Sample Name: peach
Data File: C:\msdchem\1\DATA\08_03_07 FDA_S_Cl\peach1_S_Cl.D
Date/Time: 03:27 PM Monday, Sep 24 2007

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

			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
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

DRS report from a SIM data file – for Additional Confirmation (4 ions for each target compound)

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.

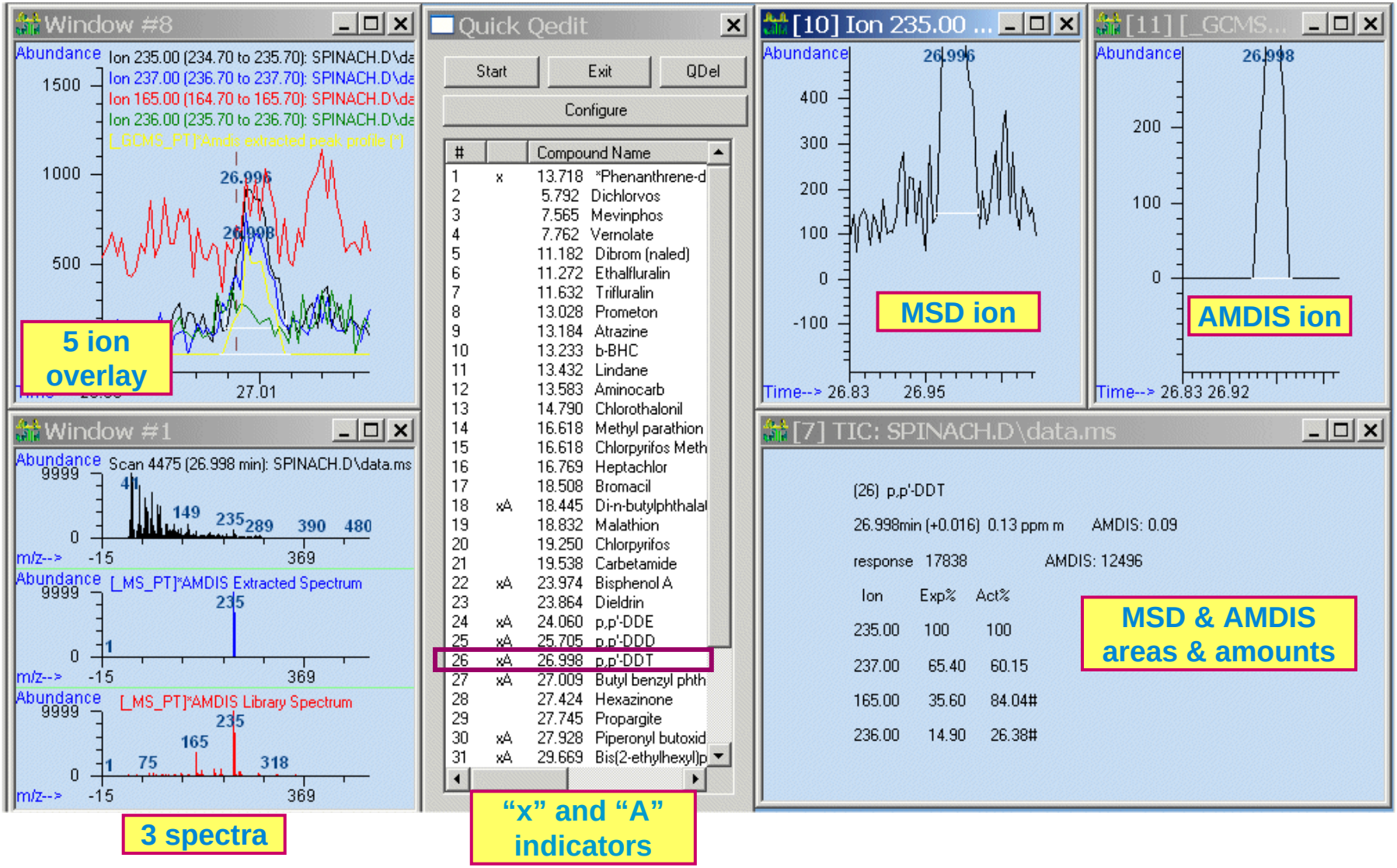
			Agilent	AMDIS		NIST	
R.T.	Cas #	Compound Name	ChemStation Amount (ng)	Match	R.T. Diff sec.	Reverse Match	Hit Num.
5.6148	63252	Carbaryl	0.09	100	2.2		
7.0858	133062	Captan	0.2	99	1.9		
9.0028	60207901	Propiconazole-I	0.03	100	4.4		
9.0727	999048032	Propiconazole-II {CAS #60207-90-1}	0.07	100	4.7		
10.7813	119611006	Fenbuconazole	0.65	100	7.4		

Done My Computer

Can We Quant on Deconvoluted Ions?

- Yes!
- MSD ChemStation (G1701AA) E.02
- Deconvolution Reporting Software, DRS (G1716AA) A.04
- Pesticide RTL Library (G1672AA) – 927 entries

QEdit, p,p'-DDT selected

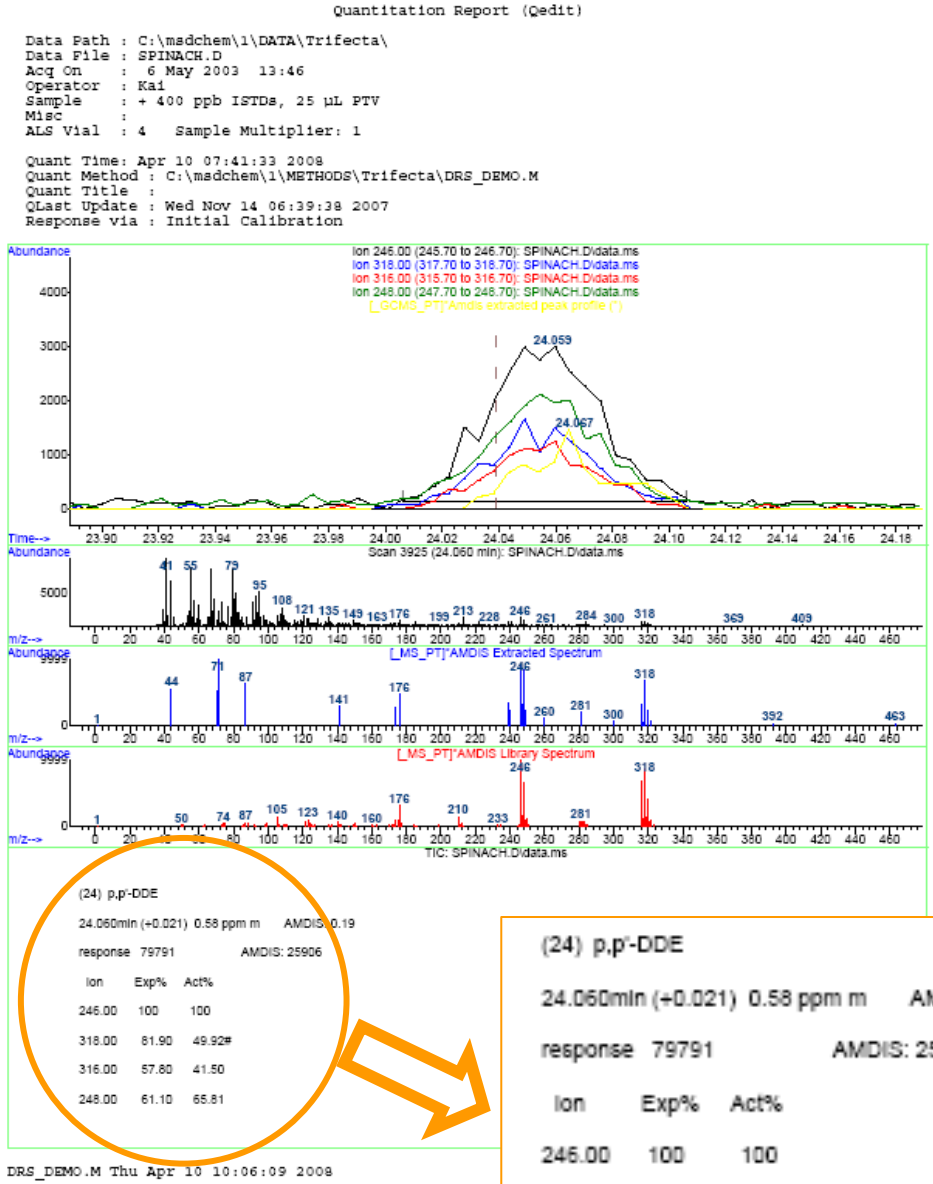


Graphics Report

MSD and AMDIS
5-ion overlay

Raw, deconvoluted
and library spectra

MSD and AMDIS
areas and amounts



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Summary Quant Report optionally includes both MSD ChemStation and AMDIS amounts

Compound	R.T.	QIon	Response	Conc	Units
Internal Standards					
1) Phenanthrene-d10	13.718	188	4953294	10.00	ppm
Target Compounds					
18) Di-n-butylphthalate	18.445	149	989364m	7.18	ppm
22) Bisphenol A	23.974	213	2119577m	15.39	ppm
24) p,p'-DDE	24.060	246	79791m	0.58	ppm
25) p,p'-DDD	25.705	235	21489m	0.16	ppm
26) p,p'-DDT	26.998	235	17838m	0.13	ppm
27) Butyl benzyl phthalate	27.009	149	35766m	0.26	ppm
30) Piperonyl butoxide	27.928	176	5208006m	37.80	ppm
31) Bis(2-ethylhexyl)phtha...	29.669	149	429941m	3.12	ppm
34) Permethrin II	31.614	183	29430854m	213.63	ppm
AMDIS Imported Quantitation Results					
18) Di-n-butylphthalate	18.443	149	861673	6.25	ppm
22) Bisphenol A	23.975	213	1754970	12.74	ppm
24) p,p'-DDE	24.065	246	25906	0.19	ppm
25) p,p'-DDD	25.715	235	17508	0.13	ppm
26) p,p'-DDT	26.993	235	12496	0.09	ppm
27) Butyl benzyl phthalate	27.010	149	21499	0.16	ppm
30) Piperonyl butoxide	27.927	176	4563220	33.12	ppm
31) Bis(2-ethylhexyl)phtha...	29.665	149	377661	2.74	ppm
34) Permethrin II	31.613	183	27779700	201.65	ppm

partial report

DRS A.04 Report after importing AMDIS results

QEdit reviewed with manual integrations

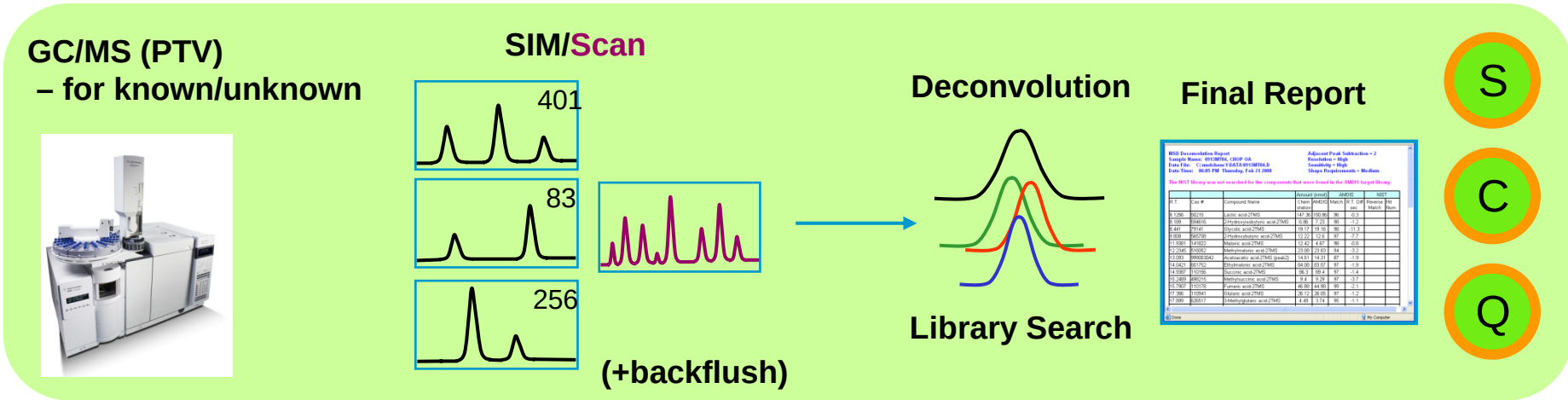
MSD Deconvolution Report
Sample Name: + 400 ppb ISTDs, 25 µL PTV
Data File: C:\msdchem\1\DATA\Trifecta\SPINACH.D
Date/Time: 07:41 AM Thursday, Apr 10 2008

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

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

			Amount (ppm)		AMDIS		NIST	
R.T.	Cas #	Compound Name	Chem station	AMDIS	Match	R.T. Diff sec.	Reverse Match	Hit Num.
18.4431	84742	Di-n-butylphthalate	7.18	6.25	95	1.7	92	1
23.974	80057	Bisphenol A	15.39	12.74	97	8.7	91	1
24.060	72559	p,p'-DDE	0.58	0.19	66	2.6	60	2
25.705	72548	p,p'-DDD	0.16	0.13	52	1.8	65	2
26.9932	50293	p,p'-DDT	0.13	0.09	53	0.7	43	6
27.009	85687	Butyl benzyl phthalate	0.26	0.16	54	0.2	57	25
27.9265	51036	Piperonyl butoxide	37.8	33.12	96	1.6	94	1
29.6648	117817	Bis(2-ethylhexyl)phthalate	3.12	2.74	94	1.0	86	3
31.6131	52645531	Permethrin II	213.63	201.65	90	3.8	91	3
13.718		Phenanthrene-d10	10					

GC-MSD Workflow Summary



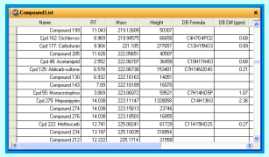
- GC/MS in full scan mode combined with deconvolution reporting software (DRS) enables unknown pesticide analysis at 10 µg/kg (ppb) in various food commodities from one injection
- Significant time saved on data analysis (screen and quant on 927 pesticides), from hours to minutes

Application Note 5989-7670EN: Replacing Multiple 50-Minute GC and GC-MS/SIM Analyses with One 15-Minute Full-Scan GC-MS Analysis for Non-targeted Pesticides Screening and >10x Productivity Gain

LC/MS Workflow

LC/Q-TOF or TOF
Full Spectrum
– for unknown compounds

Exact Mass
Database Search



Molecular
Formula Generation

C16 H19 N3 P Cl
C15 H25 O P S Cl
C18 H21 O P Cl

Another injection for MS/MS (QQQ or QTOF)

Screen

Quantify

Confirm

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Why Use (Q-)TOF?

QQQ

- Routine **target** compound screening/quantitation in a single injection
- **MRM** detection limit meets all regulatory requirements

TOF/Q-TOF

- Always **full spectrum**
- Un-limited number of routine **target and non-target** compound screening (using exact mass databases)

Q-TOF

- Full scan **accurate MS/MS spectra** for added confidence for total unknowns

All systems share SAME software platform, ion source, and collision cell – 100% workflow portability

Agilent Q-TOF Fundamentals

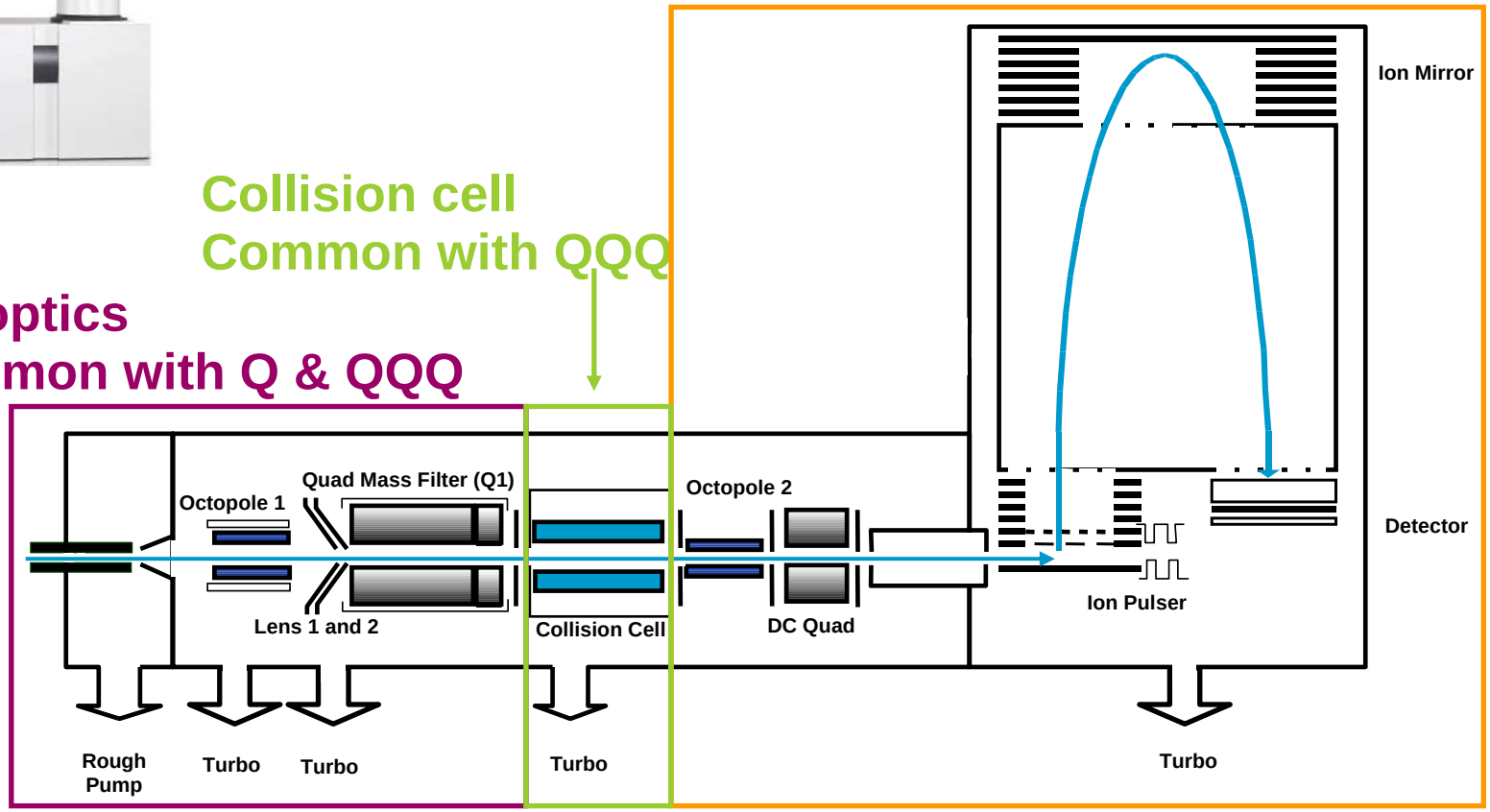
Exact Mass

Mass Accuracy (ppm)

Flight tube
Common with TOF

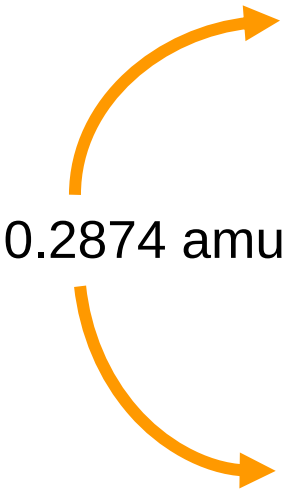
Collision cell
Common with QQQ

Ion optics
Common with Q & QQQ



What does “Exact Mass” mean?

Element	Atomic Number	Exact Mass
H	1	1.007825
C	6	12.000000
N	7	14.003074
O	8	15.994915



C6H6Cl6	287.8600665	Lindane
C10H12N2O6S	288.0416000	Carbasulam
C9H21O2PS3	288.0441285	Terbufos
C13H21O3PS	288.0949000	Iprobenfos
C15H17N4Cl	288.1141743	Myclobutanil
C12H21N2O4P	288.1238937	Diazoxon
C11H20N4O3PS	288.1256000	Epronaz
C11H21N4O3P	288.1351000	Pirimetaphos
C16H20N2O3	288.1473925	Imazamethabenz

Uses accurate mass on TOF/Q-TOF to identify all of them.

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Mass Analysis for TOF

$v = d/t$

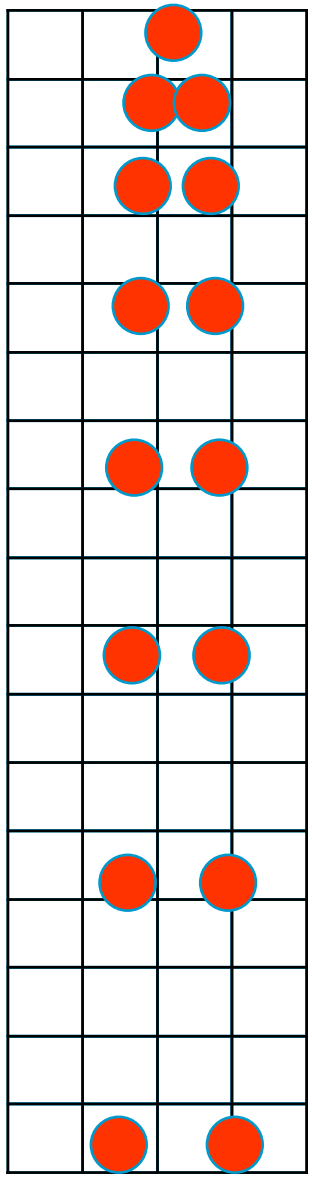
$E = \frac{1}{2} m v^2 = \frac{1}{2} m (d/t)^2$

$m = (2E/d^2)\ t^2$

Energy (E) and Distance (d) are fixed

The measured mass is proportional to the flight time (time-of-flight).

The Key for getting useful TOF results is **good mass accuracy**.



Calculation of Exact Mass and Error in Measured Mass (MH^+) of Reserpine

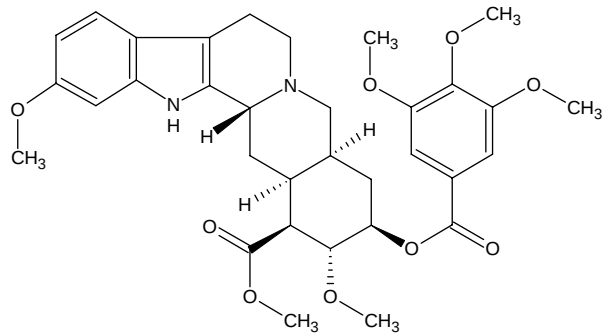
Atom	Mass of Atom	# of Atoms	Sum
Hydrogen	1.00783	40	**40.31300**
Carbon	12.00000	33	**396.00000**
Nitrogen	14.00307	2	**28.00615**
Oxygen	15.99492	9	**143.95424**
Total			**608.27338**
Plus H	1.00783	1	**1.00783**
Total			**609.28121**
Minus e-	0.00055	1	**0.00055**
			609.28066

Calculated = exact

$$\frac{\text{(Measured - Calculated)}}{\text{Calculated}} \text{ X 1,000,000 = ppm}$$

0.9027038 ppm

Error if the electron was not omitted!



Reserpine ($C_{33}H_{40}N_2O_9$)

How Much Accuracy is Needed?

Reserpine ($C_{33}H_{40}N_2O_9$) has a protonated ion at 609.28066 (MH^+)

Single quad reports mass to +/- 0.1 = 165 ppm

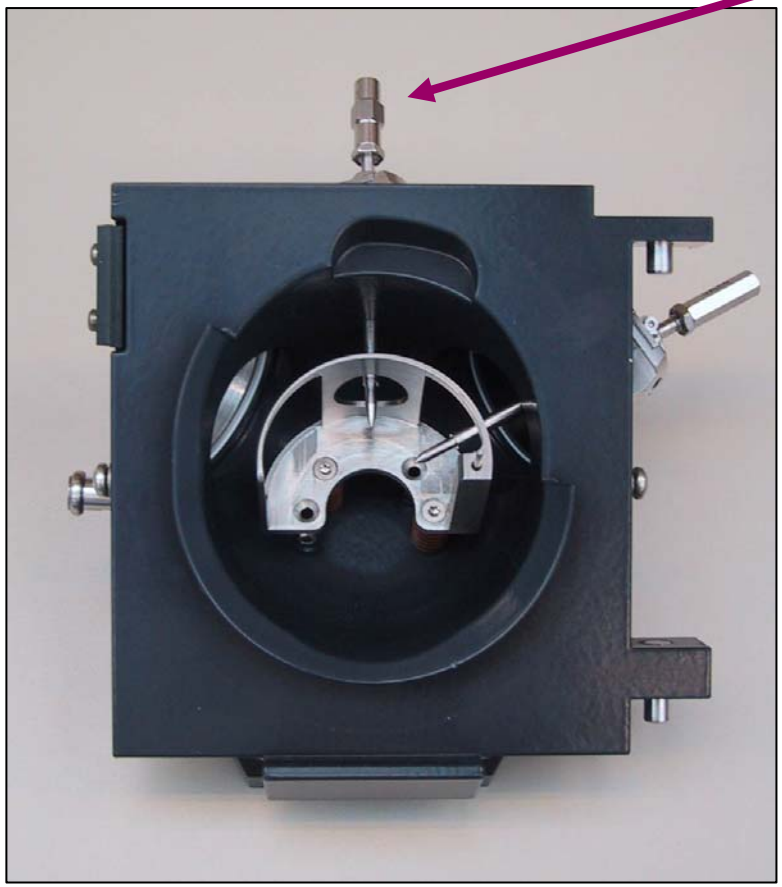
(0.1/609.28 x 10e6 = 164.128 ppm)

Number of possible formulas using only C, H, O & N:

Accuracy	Mass Difference	Number of Formulas
165 ppm	**0.1 amu** (609.18 - 609.38)	**209**
10 ppm	**0.061 amu**	**13**
5 ppm	**0.030 amu** (609.25 - 609.31)	**7**
3 ppm	**0.0018 amu**	**4**
2 ppm	**0.0012 amu** (609.2795 - 609.2819)	**2**

Measured mass accuracy can **narrow the number of hits** in a TOF database search, therefore reduce risk of investing effort on the wrong molecule.

Automatic Internal Referencing (Easy-to-use)



Analytical Sprayer

Reference Sprayer

Dual Spray ESI source and Calibration Delivery System (CDS) → automates the introduction of reference masses

AutoTune

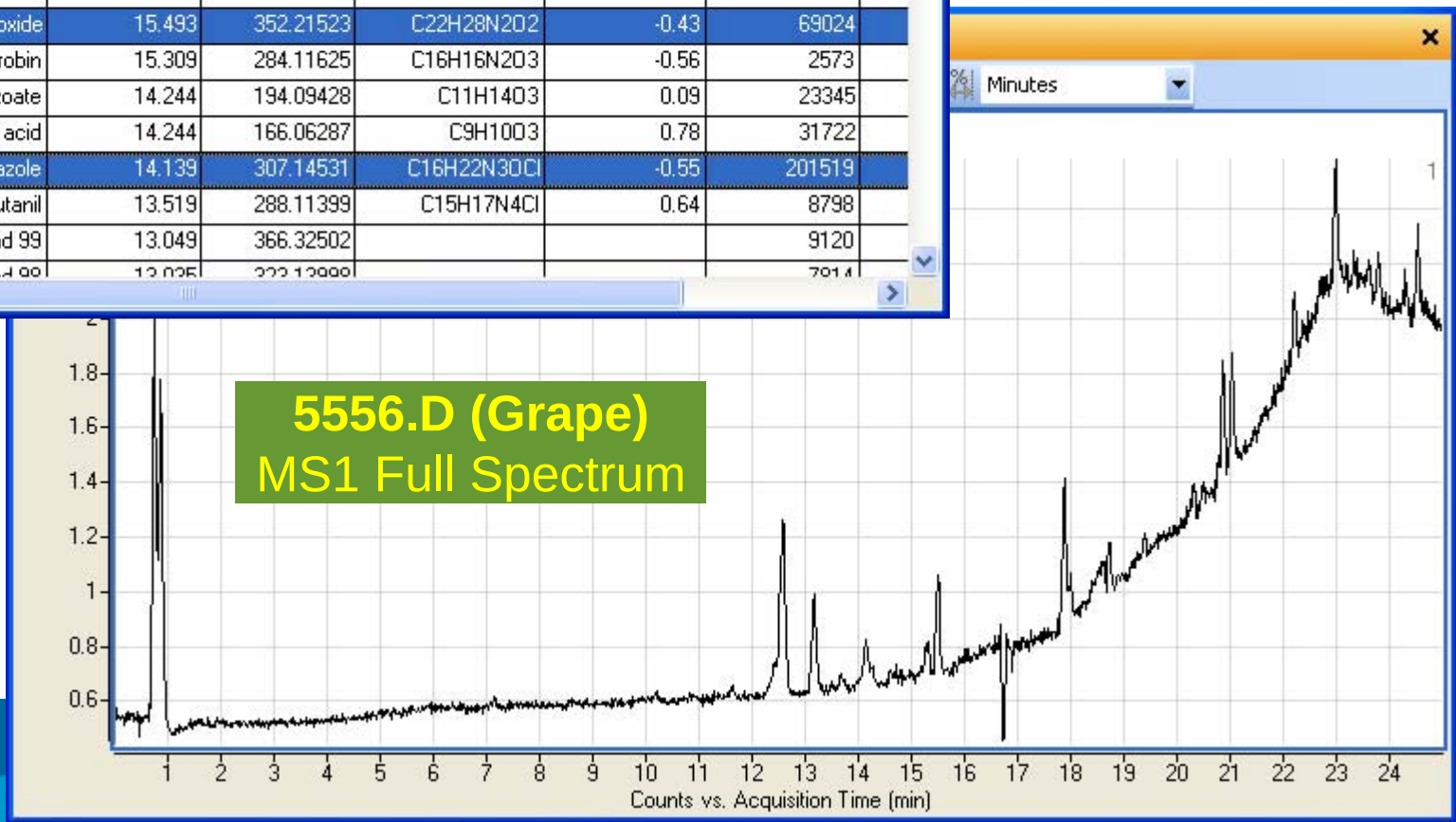
TOF/Q-TOF Unlimited Number of Compound Screening

Compound List

Name	RT	Mass	DB Formula	DB Diff (ppm)	Height
Cpd 96: Spiroxamine	12.58	297.26679	C18H35NO2	-0.03	9432
Cpd 82: Spiroxamine	10.899	297.26691	C18H35NO2	-0.42	19456
Cpd 53: Quinacetol	0.881	187.06346	C11H9NO2	-0.68	
Cpd 418: Aldimorph	22.373	283.28757	C18H37NO	-0.19	
Cpd 368: Dodemorph	21.08	281.27181	C18H35NO	0.19	
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

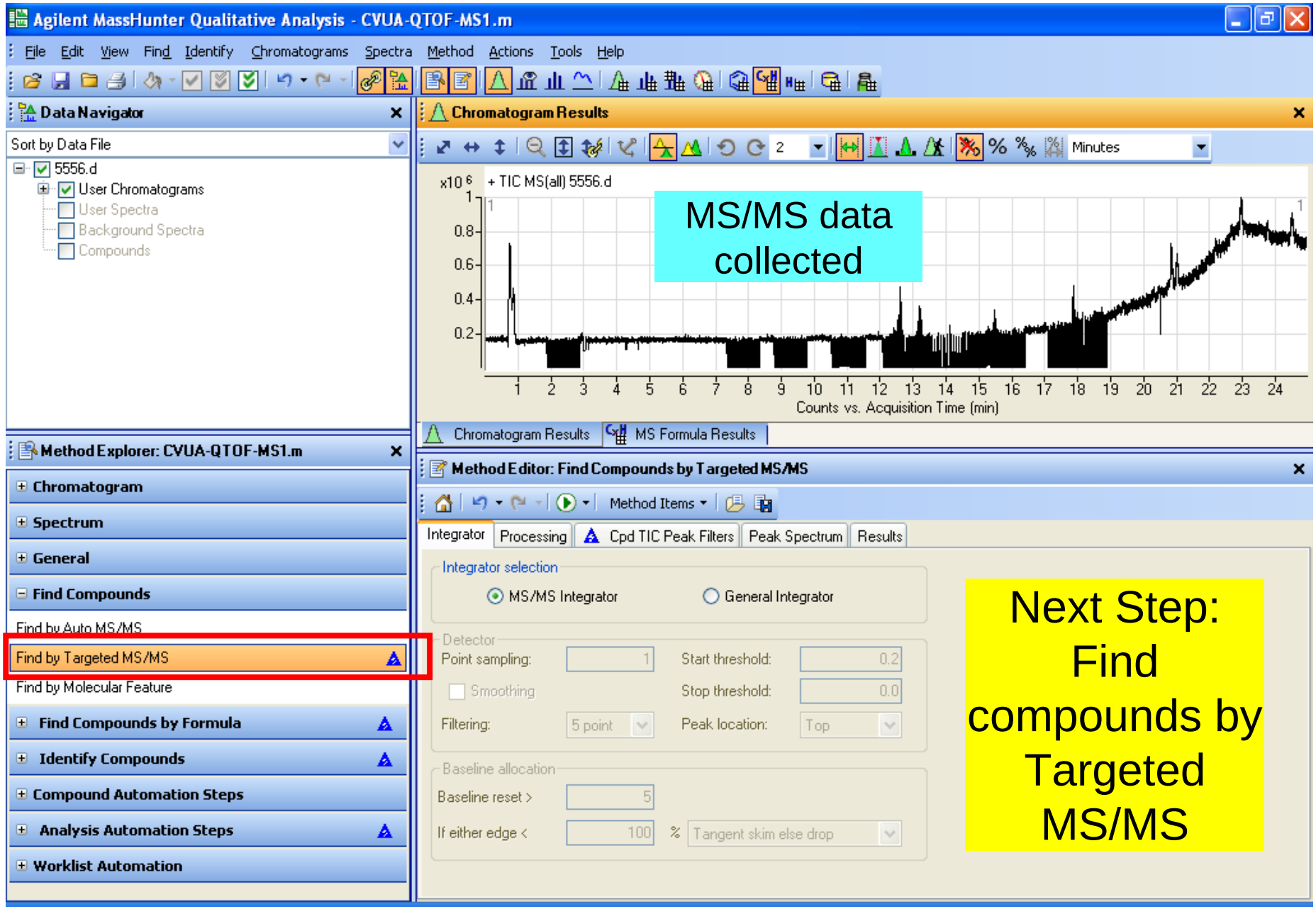
Molecular Feature Extractor

15 out of 510 compounds had hits from database search.

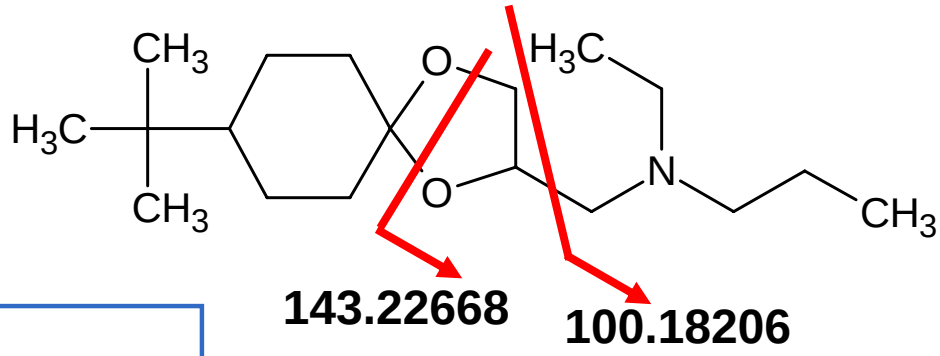


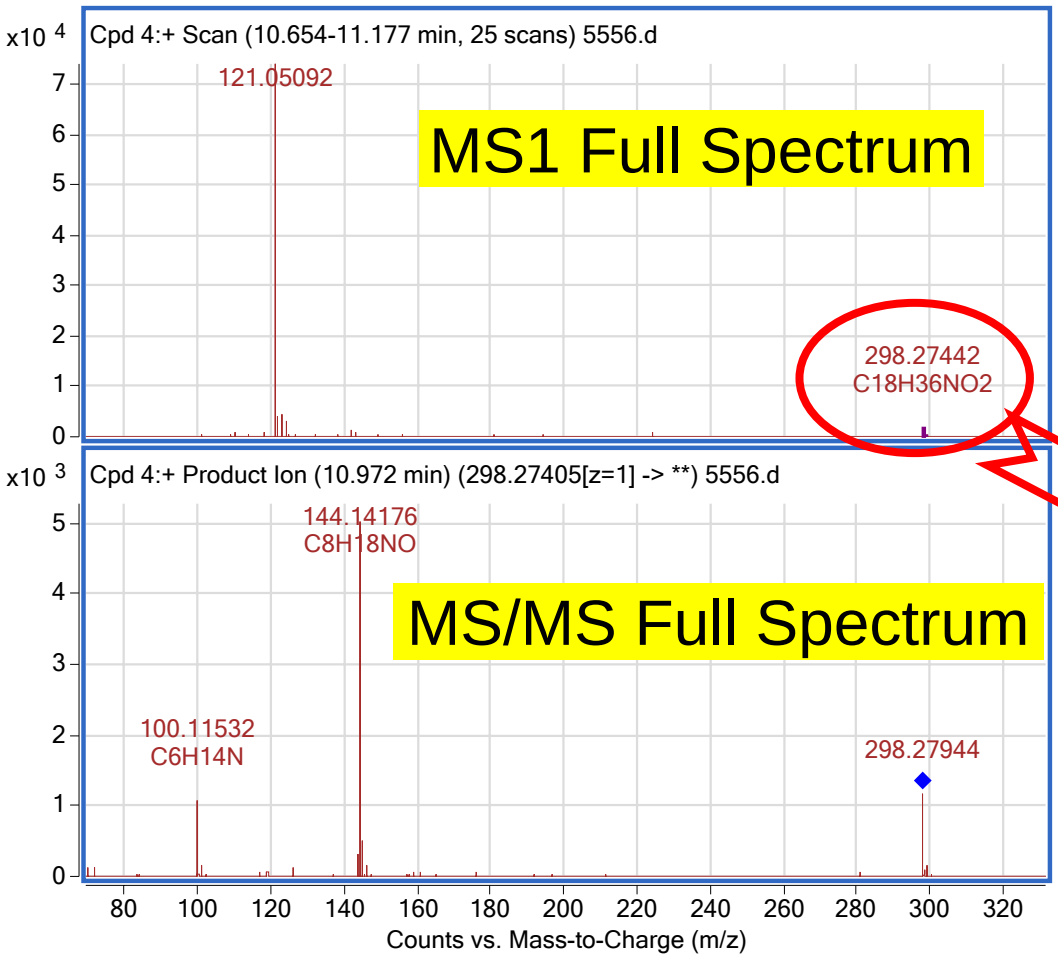
Run Another Analysis in MS/MS (Targeted) Mode to Confirm Hits Found in MS1 Mode

Compound	RT	Precursor Mass $(MH)^+$
Spiroxamine	10.884	298.27406
Terbuconazole	14.139	308.15242
Tebufenoxide	15.492	353.22236


Agilent MassHunter Qualitative Analysis - CVUA-QTOF-MS1.m
File Edit View Find Identify Chromatograms Spectra Method Actions Tools Help
Data Navigator
Sort by Data File
5556.d
User Chromatograms
User Spectra
Background Spectra
Compounds
Chromatogram Results
+ TIC MS(all) 5556.d
MS/MS data collected
Counts vs. Acquisition Time (min)
Chromatogram Results
MS Formula Results
Method Explorer: CVUA-QTOF-MS1.m
Chromatogram
Spectrum
General
Find Compounds
Find by Auto MS/MS
Find by Targeted MS/MS
Find by Molecular Feature
Find Compounds by Formula
Identify Compounds
Compound Automation Steps
Analysis Automation Steps
Worklist Automation
Method Editor: Find Compounds by Targeted MS/MS
Method Items
Integrator
Processing
Cpd TIC Peak Filters
Peak Spectrum
Results
Integrator selection
MS/MS Integrator
General Integrator
Detector
Point sampling:
Start threshold:
Smoothing
Stop threshold:
Filtering:
Peak location:
Baseline allocation
Baseline reset >
If either edge <
Tangent skim else drop
Next Step: Find compounds by Targeted MS/MS

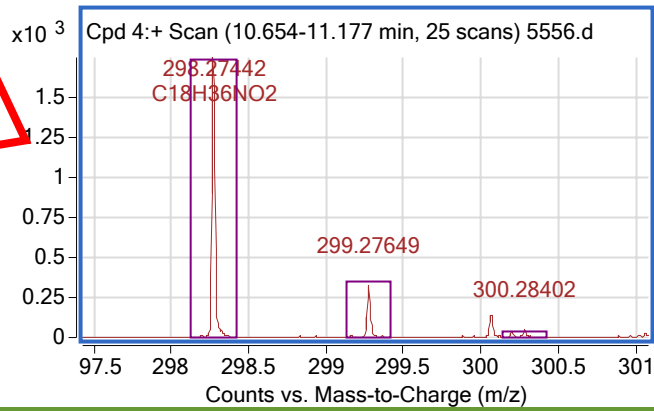
Spiroxamine





The compound name Spiroxamine came up in the MS1 mode.

Use formula results and MS/MS results to confirm the hit.

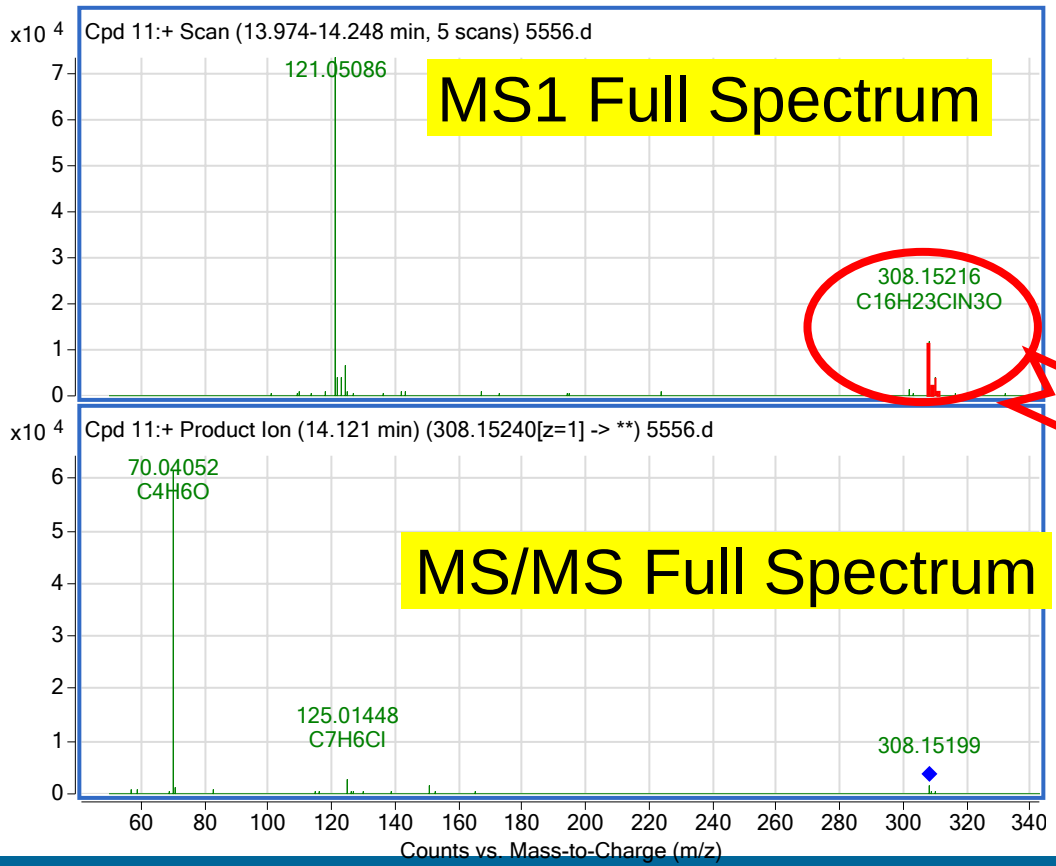


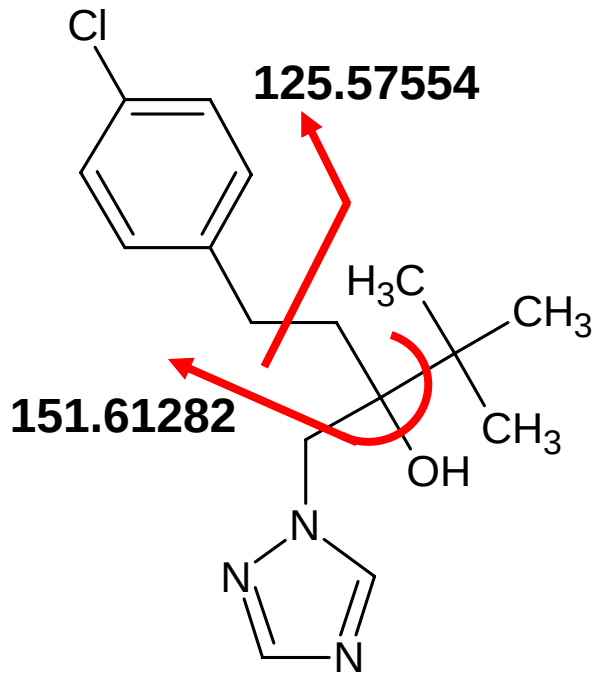
Boxes represent theoretical isotope ratios

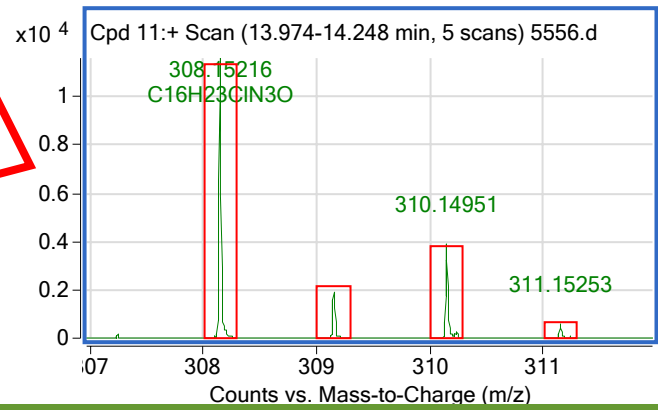
Terbuconazole

The compound name Terbuconazole came up in the MS1 mode.

Use formula results and MS/MS results to confirm the hit.







Boxes represent theoretical isotope ratios

Screen Pesticides with LC-TOF/Q-TOF

- Accurate Mass provides added compound selectivity
- Higher resolution provides added interference selectivity
- Always full spectral data
- Unlimited number of compounds can be screened (search exact mass compound database for identification)
- Sensitivity is the same regardless of number of compounds screened*
- MS/MS (Q-TOF) assists compound confirmation

*Triple quadrupole (QQQ) mass spectrometer can be more sensitive up to a limited number of compounds. That limit has not been definitively determined.

Pesticide Workflow: Screen S, Confirm C and Quantify Q

Representative Sample

QuEChERS

Extraction

Clean-up

1/3

1/3

1/3

GC/MS (PTV) SIM/Scan – for known and unknown

(+backflush)

Deconvolution Final Report

S C Q

GC/QQQ MRM – for known targets

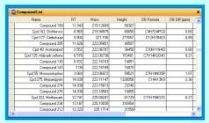
S C Q

LC/QQQ MRM – for known targets

S C Q

LC/QTOF or TOF Full Spectrum – for unknown compounds

Exact Mass Database Search



Molecular Formula Generation

S Q

C16 H19 N3 P Cl
C15 H25 O P S Cl
C18 H21 O P Cl

Another injection for MS/MS (QQQ or QTOF)

C

Agilent Technologies

Summary

- GC/MS and Deconvolution to Screen, Confirm and Quantify
- QQQ for Targeted analysis in complex matrix
  - Sensitivity unsurpassed
  - Complex matrix with less clean-up
- TOF/Q-TOF for Targeted and Unknown Screening
  - Sensitive full scan analysis
  - Searching exact mass database leads to identification
  - Quantitative
  - Accurate mass MS/MS for identification of fragments and structure elucidation