

# 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**



**Deconvolution**



**(+backflush)**

**Final Report**

MSD Deconvolution Report  
 Sample Name: 0913M704\_CK0P OA  
 Data File: C:\msdchem\1\DATA\0913M704.D  
 Date/Time: 06:05 PM Thursday, Feb 21 2008

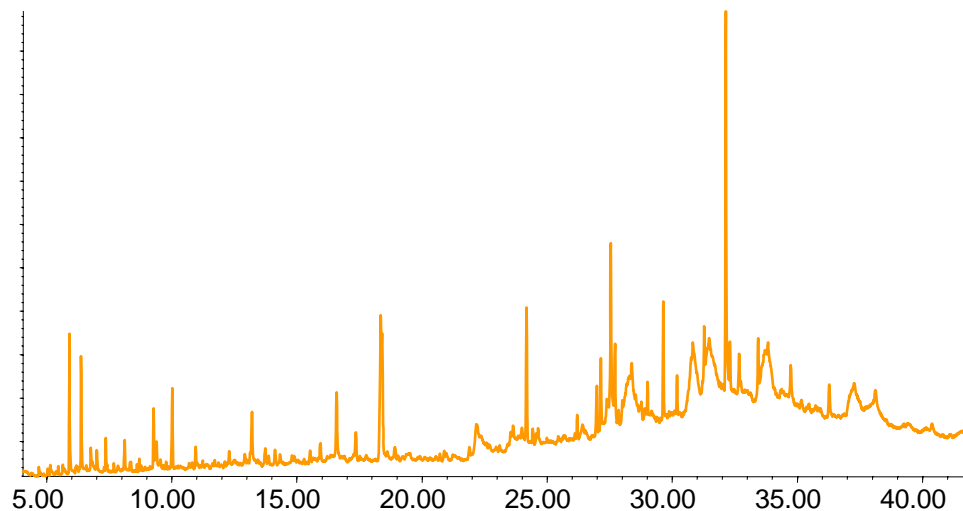
Adjacent Peak Subtraction = 2  
 Resolution = High  
 Sensitivity = High  
 Shape Requirements = Medium

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

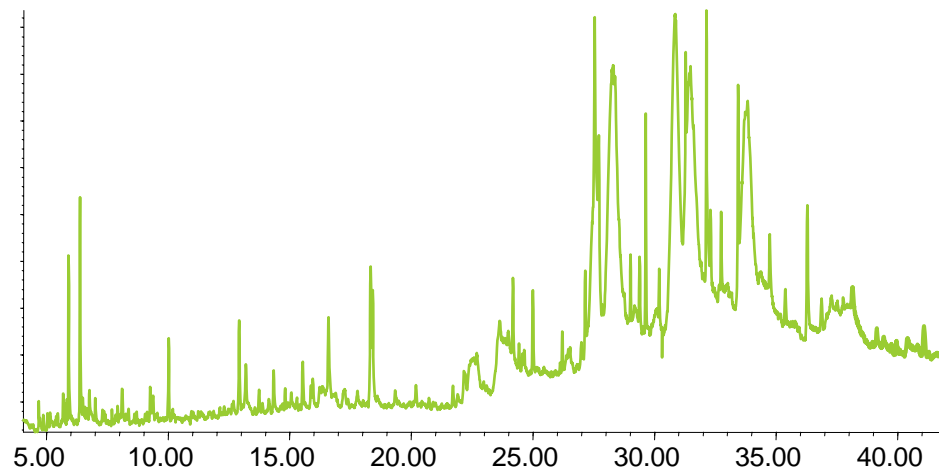
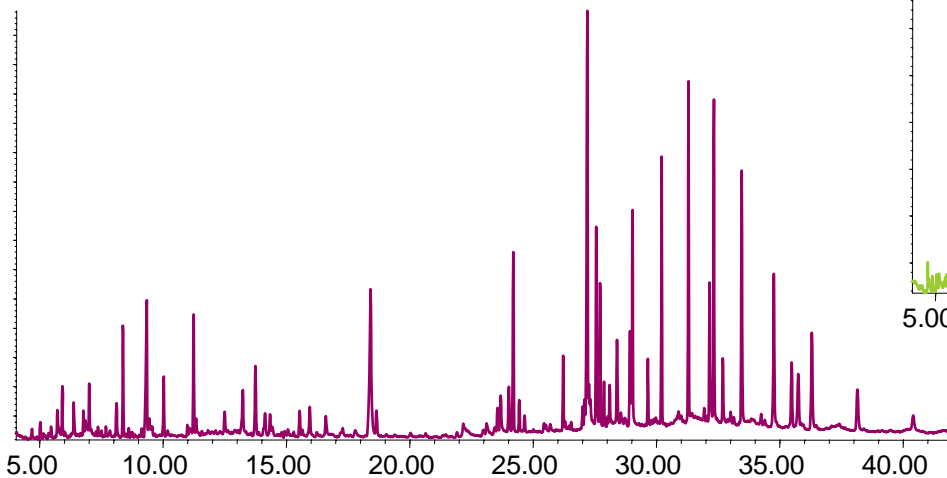
R.T.	Case #	Compound Name	Amount (fmol)	AMDIS	Match	R.T. Diff	Reverse	Hit
							Match	Num
1.156	20215	Lactic acid 2TMS	147.30	50.98	98	-0.3		
1.189	20416	2-Hydroxybutanoic acid 2TMS	6.86	7.23	98	-1.2		
1.441	20141	Glycolic acid 2TMS	19.17	19.18	98	-11.3		
2.008	20520	2-Hydroxypropanoic acid 2TMS	12.23	12.6	97	-7.7		
11.938	114102	Malonic acid 2TMS	12.42	4.67	98	0.0		
12.246	21602	Methylsuccinic acid 2TMS	23.09	23.83	98	-3.2		
13.001	19000342	Acetic acid acid 2TMS (peak2)	14.81	14.79	97	-0.9		
14.043	101752	Ethylsuccinic acid 2TMS	84.09	83.57	97	-3.0		
14.937	110156	Succinic acid 2TMS	86.3	89.4	97	-1.4		
15.249	498215	Methylsuccinic acid 2TMS	3.4	2.29	97	-3.7		
16.747	110178	Formic acid 2TMS	48.89	44.98	99	-2.1		
17.956	110941	Glutaric acid 2TMS	26.12	26.05	97	-1.2		
17.899	126517	3-Methylglutaric acid 2TMS	4.49	3.74	95	-1.1		

**Screen**  
**Confirm**  
**Quantify**

# 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)

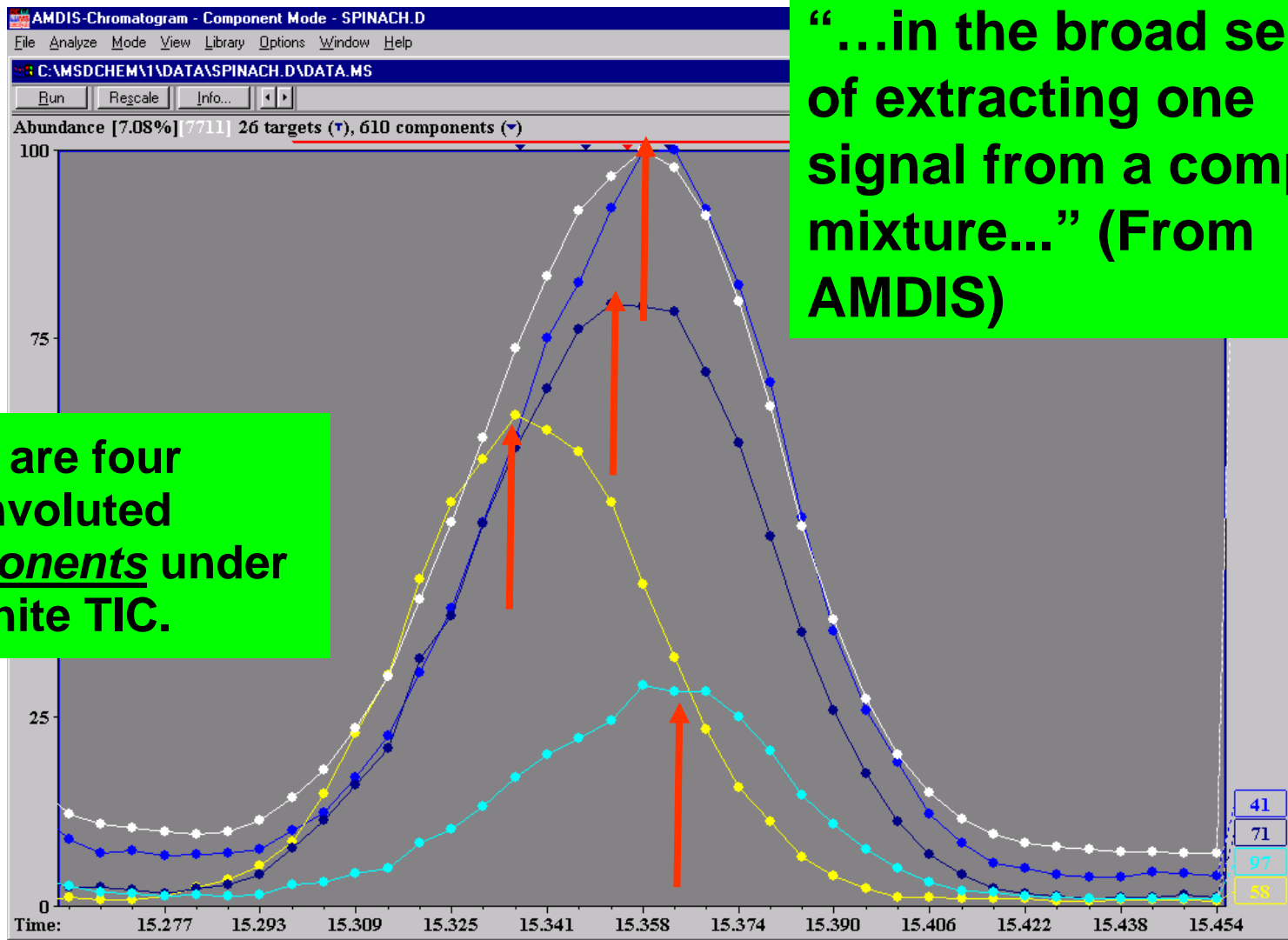
	CDFA*	Agilent DRS
Targets Found (not counting ISTD)	37	Same 37 <b>+99 more</b>
False Positives	1	0
Processing Time	~8 hrs	<b>32 min</b>

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)

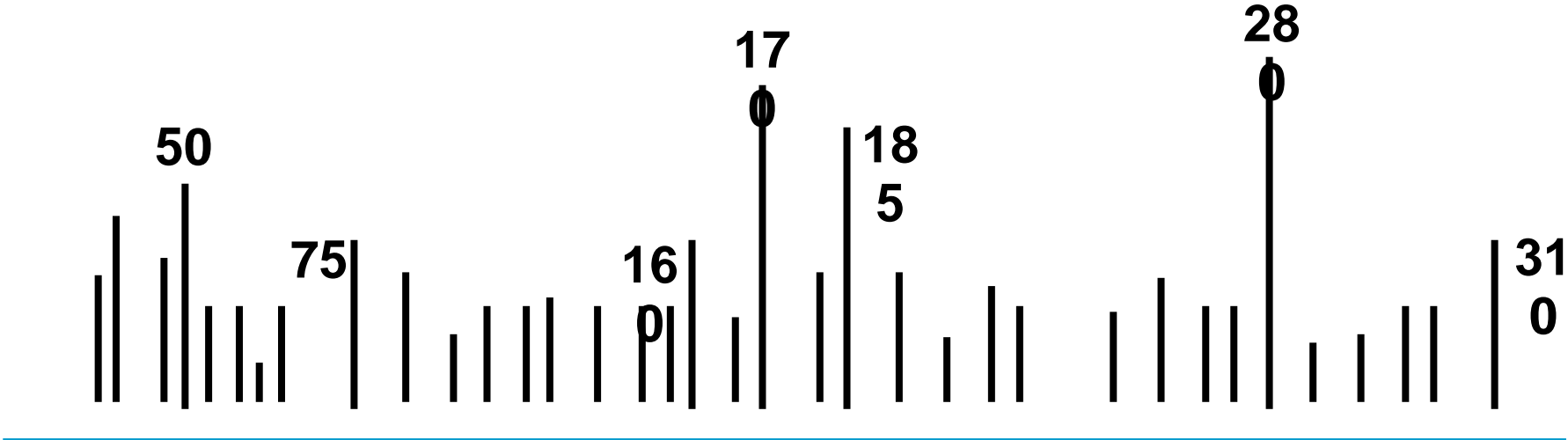
There are four deconvoluted components under the white TIC.

## Automatic Mass spectral Deconvolution and Identification System

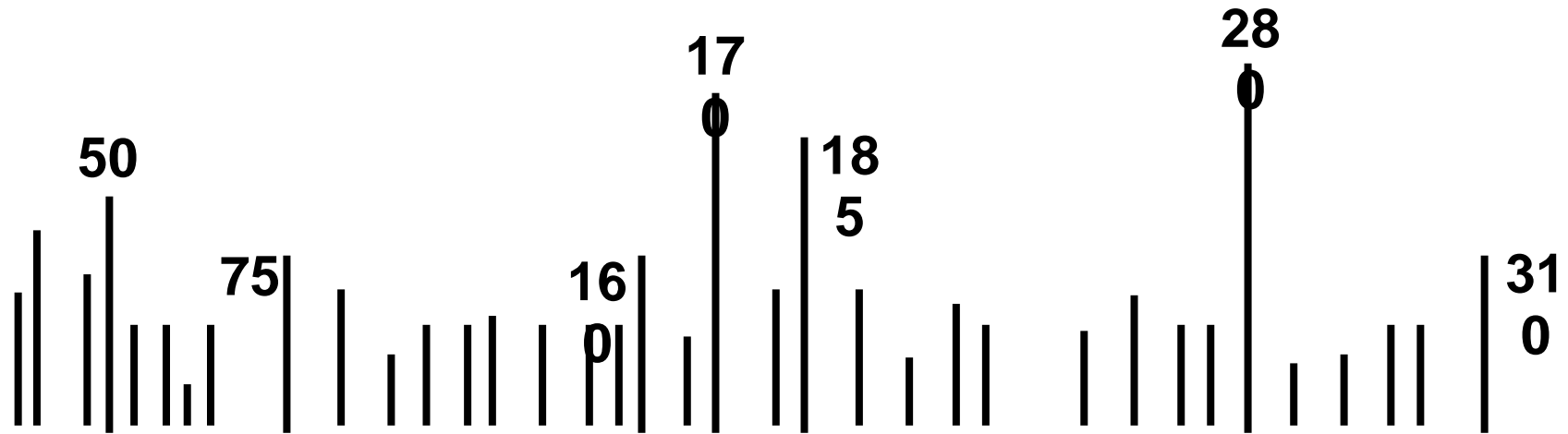
- Developed by the National Institute of Standards and Technology (NIST)
- Developed to detect chemicals in violation of Chemical Weapons Convention (must minimize false positives and false negatives in reporting)
- Used to identify target compounds in complex matrices



# How Does Deconvolution Work?

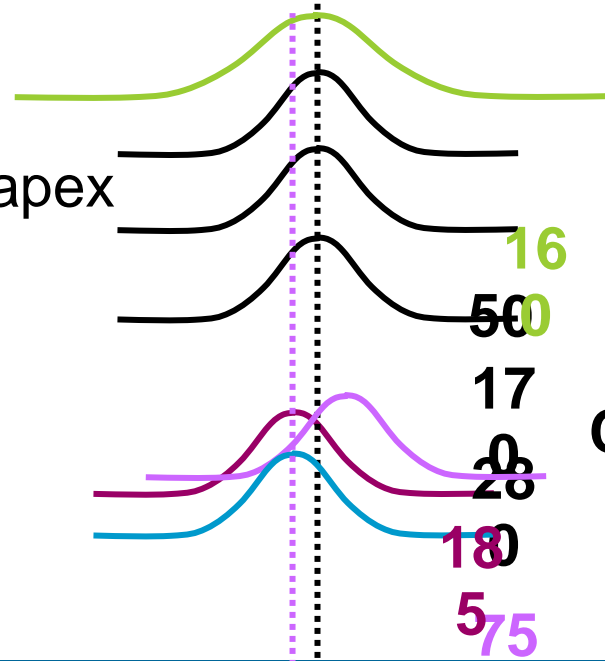


# Eliminate Ions Don't Fit the Criteria



Ion grouping criteria:

1. Same RetTime at apex
2. Same peak width



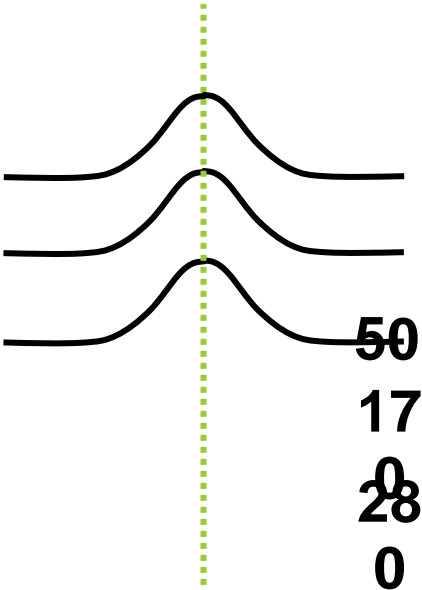
**Extracted Ion Chromatograms (EIC)**

# Spectrum is Deconvoluted/Cleaned

50

17  
0

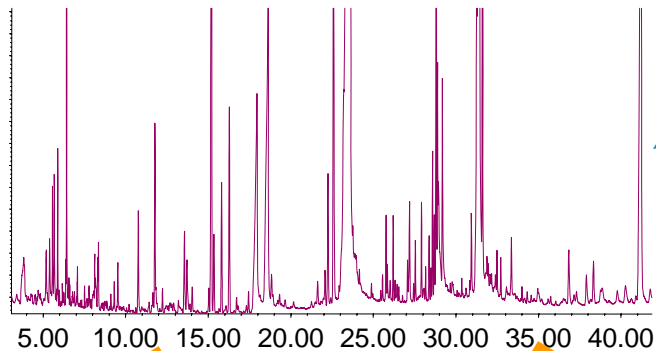
28  
0



A component in  
AMDIS

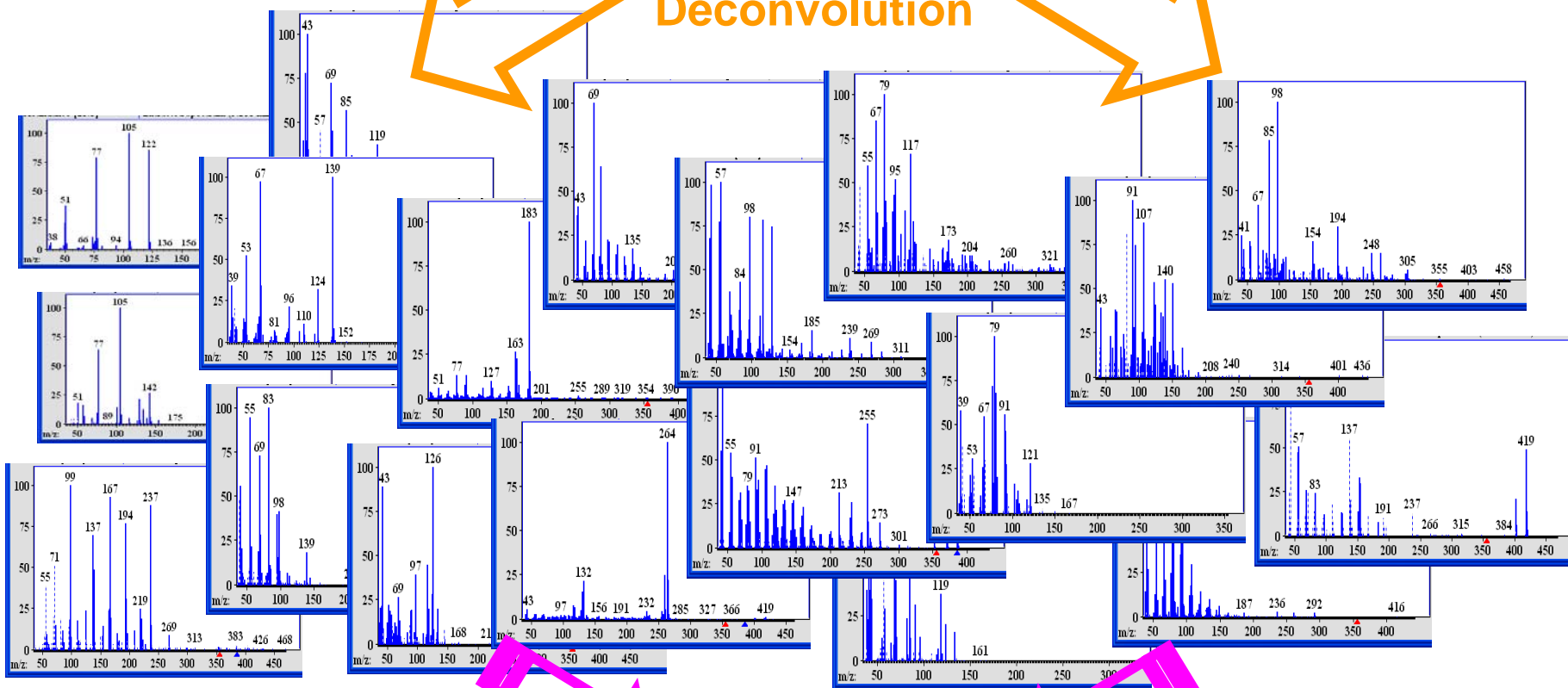
Related ions are  
grouped together as  
a component.

TIC of Spinach Extract

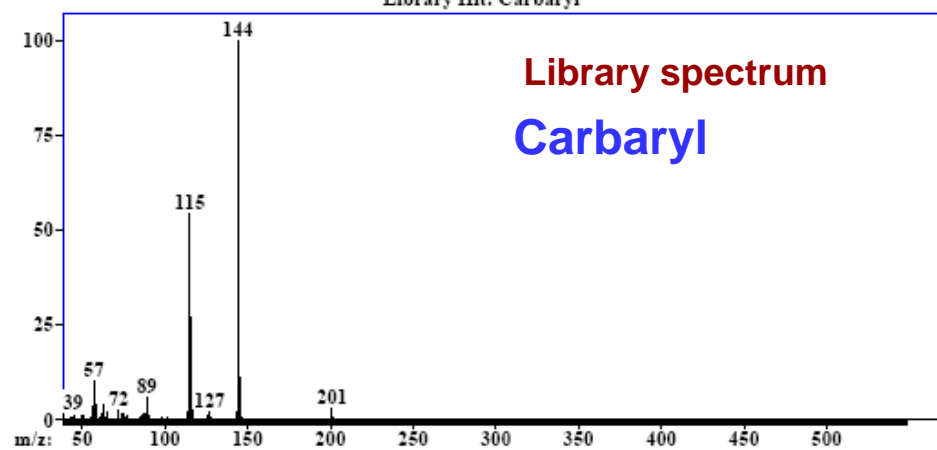
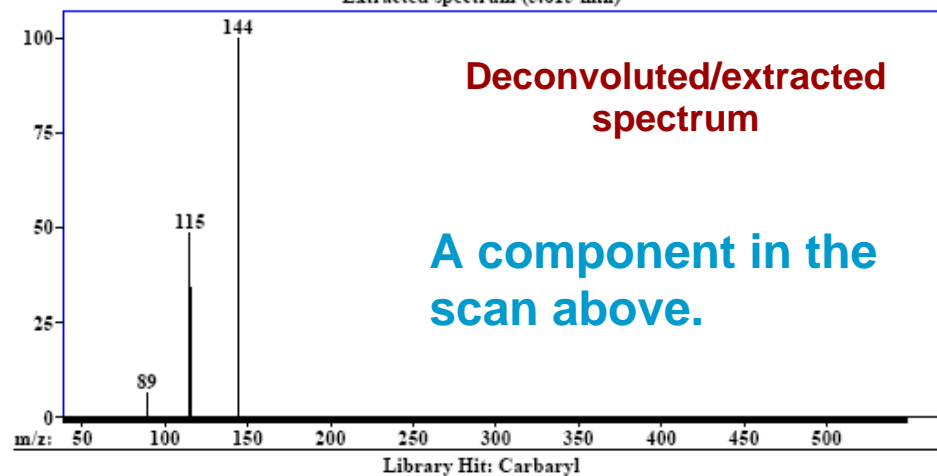
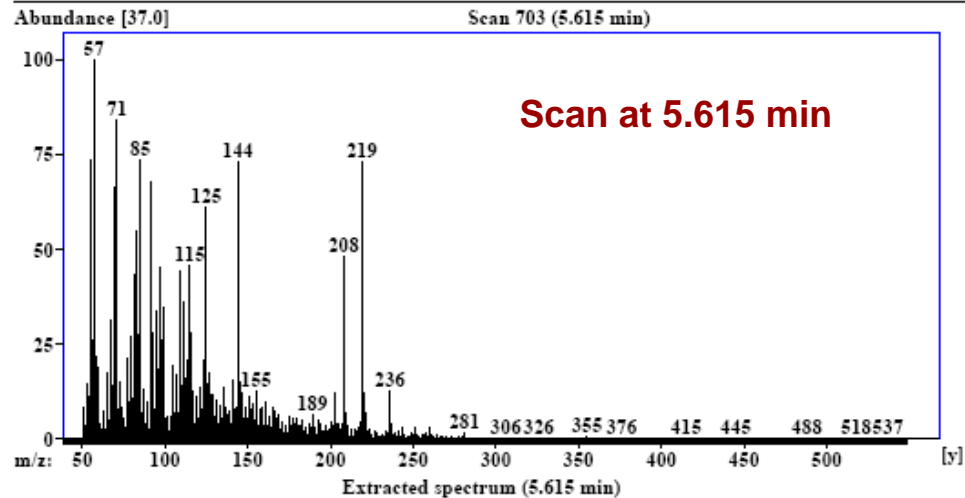


More than 370 peaks found

Deconvolution



Library Search

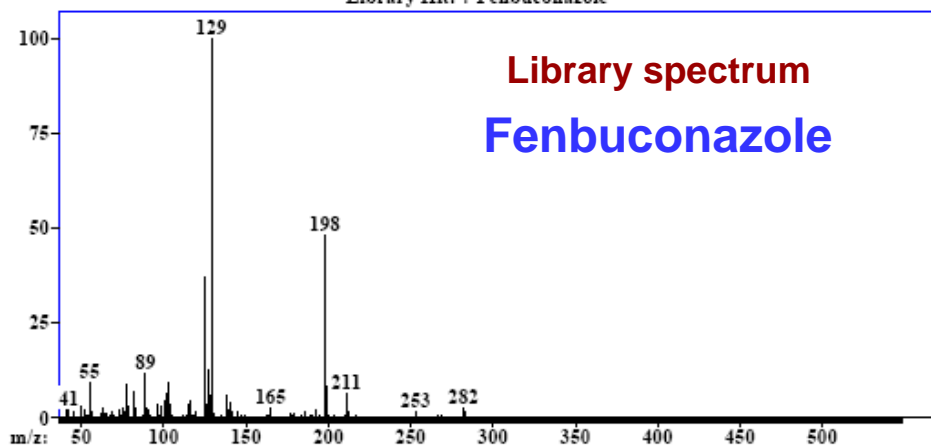
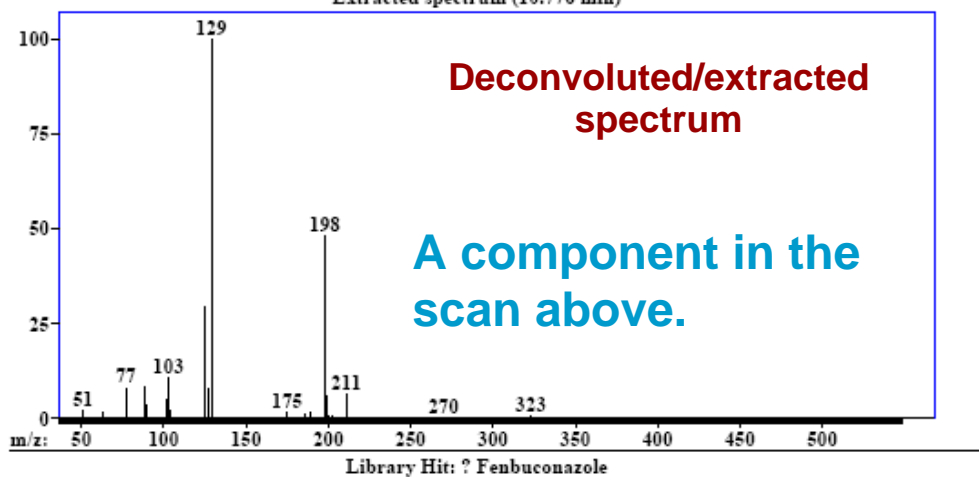
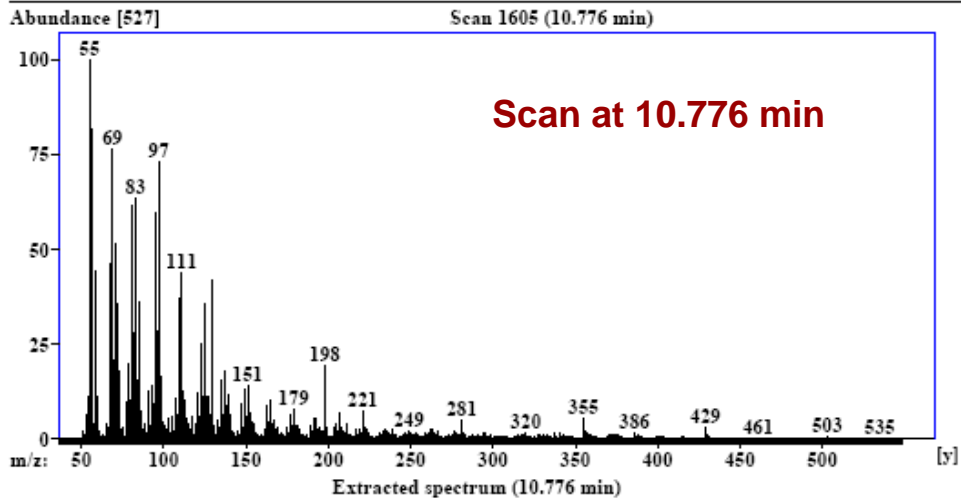


**Peach**



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

# Peach



# DRS Report - Peach

Sample Name: peach

Data File: C:\msdchem\1\DATA\08\_03\_07 FDA\_S\_CI\peach1\_S\_CI.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.

R.T.	Cas #	Compound Name	Agilent	AMDIS		NIST	
			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-l		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.

R.T.	Cas #	Compound Name	Agilent	AMDIS		NIST	
			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

Window #8

Abundance

Ion 235.00 (234.70 to 235.70): SPINACH.D\data.ms  
 Ion 237.00 (236.70 to 237.70): SPINACH.D\data.ms  
 Ion 165.00 (164.70 to 165.70): SPINACH.D\data.ms  
 Ion 236.00 (235.70 to 236.70): SPINACH.D\data.ms  
 [GCMS\_PT]\*Amdis extracted peak profile (\*)

26.996  
26.998

27.01

**5 ion overlay**

Window #1

Abundance

Scan 4475 (26.998 min): SPINACH.D\data.ms

9999

4 149 235 289 390 480

m/z--> -15 369

Abundance

[MS\_PT]\*Amdis Extracted Spectrum

9999

1 235

m/z--> -15 369

Abundance

[MS\_PT]\*Amdis Library Spectrum

9999

1 75 165 235 318

m/z--> -15 369

**3 spectra**

Quick Qedit

Start Exit QDel

Configure

#		Compound Name
1	x	13.718 *Phenanthrene-d
2		5.792 Dichlorvos
3		7.565 Mevinphos
4		7.762 Vernolate
5		11.182 Dibrom (naled)
6		11.272 Ethalfuralin
7		11.632 Trifluralin
8		13.028 Prometon
9		13.184 Atrazine
10		13.233 b-BHC
11		13.432 Lindane
12		13.583 Aminocarb
13		14.790 Chlorothalonil
14		16.618 Methyl parathion
15		16.618 Chlorpyrifos Meth
16		16.769 Heptachlor
17		18.508 Bromacil
18	xA	18.445 Di-n-butylphthalat
19		18.832 Malathion
20		19.250 Chlorpyrifos
21		19.538 Carbetamide
22	xA	23.974 Bisphenol A
23		23.864 Dieldrin
24	xA	24.060 p,p'-DDE
25	xA	25.705 p,p'-DDD
26	xA	26.998 p,p'-DDT
27	xA	27.009 Butyl benzyl phth
28		27.424 Hexazinone
29		27.745 Propargite
30	xA	27.928 Piperonyl butoxid
31	xA	29.669 Bis(2-ethylhexyl)p

**"x" and "A" indicators**

[10] Ion 235.00 ...

Abundance

26.996

MSD ion

Time--> 26.83 26.95

[11] [\_GCMS...]

Abundance

26.998

AMDIS ion

Time--> 26.83 26.92

[7] TIC: SPINACH.D\data.ms

(26) p,p'-DDT

26.998min (+0.016) 0.13 ppm m AMDIS: 0.09

response 17838 AMDIS: 12496

Ion	Exp%	Act%
235.00	100	100
237.00	65.40	60.15
165.00	35.60	84.04#
236.00	14.90	26.38#

**MSD & AMDIS areas & amounts**

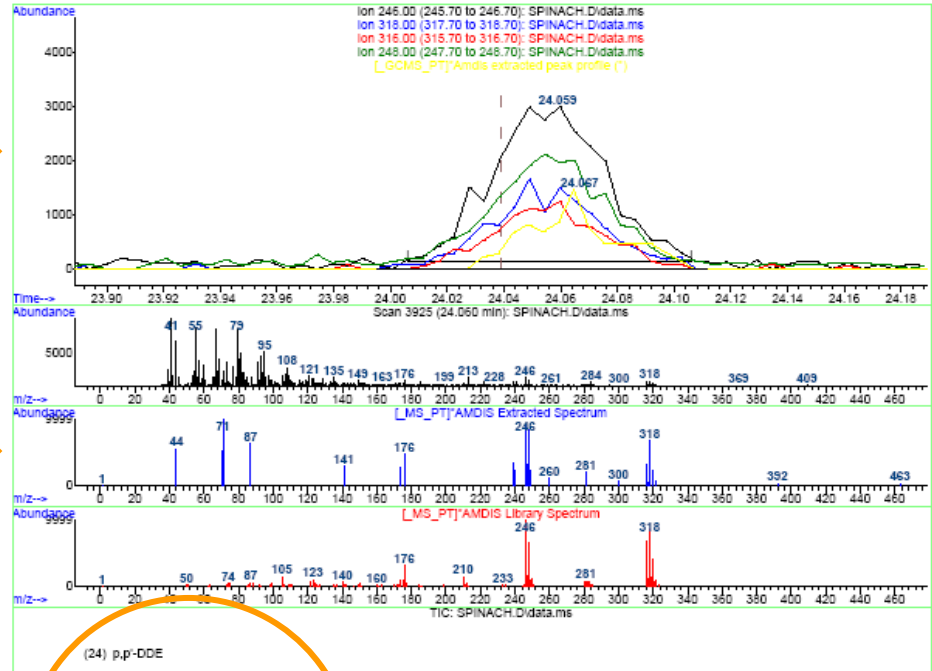
# Graphics Report

MSD and AMDIS  
5-ion overlay

Raw, deconvoluted  
and library spectra

MSD and AMDIS  
areas and amounts

Quantitation Report (Qedit)  
Data Path : C:\msdchem\1\DATA\Trifecta\  
Data File : SPINACH.D  
Acq On : 6 May 2003 13:46  
Operator : Kai  
Sample : + 400 ppb ISTDs, 25 µL PTV  
Misc :  
ALS Vial : 4 Sample Multiplier: 1  
  
Quant Time: Apr 10 07:41:33 2008  
Quant Method : C:\msdchem\1\METHODS\Trifecta\DRS\_DEMO.M  
Quant Title :  
QLast Update : Wed Nov 14 06:39:38 2007  
Response via : Initial Calibration



(24) p,p'-DDE  
24.060min (+0.021) 0.58 ppm m AMDIS: 0.19  
response 79791 AMDIS: 25906

Ion	Exp%	Act%
246.00	100	100
318.00	81.90	49.92#
316.00	57.80	41.50
248.00	61.10	65.81

(24) p,p'-DDE  
24.060min (+0.021) 0.58 ppm m AMDIS: 0.19  
response 79791 AMDIS: 25906

Ion	Exp%	Act%
246.00	100	100
318.00	81.90	49.92#
316.00	57.80	41.50
248.00	61.10	65.81

DRS\_DEMO.M Thu Apr 10 10:06:09 2008



Agilent Technologies

# Summary Quant Report optionally includes both MSD ChemStation and AMDIS amounts

partial report

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
-----					

# 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.

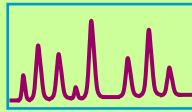
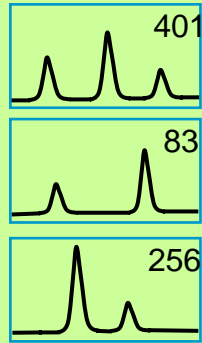
R.T.	Cas #	Compound Name	Amount (ppm)		AMDIS		NIST	
			Chem station	AMDIS	Match	R.T. Diff sec.	Reverse Match	Hit Num.
18.4431	84742	Di-n-butylphthalate	7.18	6.25	95	1.7	92	1
23.974	80057	Bisphenol A	15.39	12.74	97	8.7	91	1
24.060	72559	p,p'-DDE	0.58	0.19	66	2.6	60	2
25.705	72548	p,p'-DDD	0.16	0.13	52	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 (PTV)  
– for known/unknown

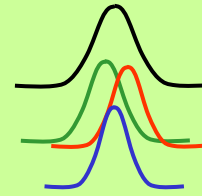


SIM/Scan



(+backflush)

Deconvolution



Library Search

Final Report

MSD Deconvolution Report  
Sample Name: 0107061\_000P\_08  
Data File: C:\msdchem\1\0107061\_08  
Scan Range: 001-100 Thursday, Feb 21, 2008

Adjusted Peak Subtraction = 2  
Resolution = High  
Sensitivity = High  
Shape Requirements = Medium

The MS1 library was not searched for the components that were found in the MSMS target library.

RT	Comp #	Compound Name	Current Level	MS1	MS2
0.100	1011	Acetic acid (250)	12.28	1011	86
0.100	1012	Formic acid (250)	12.28	1012	86
0.100	1013	Hydrochloric acid (250)	12.28	1013	86
0.100	1014	Sulfuric acid (250)	12.28	1014	86
0.100	1015	Nitric acid (250)	12.28	1015	86
0.100	1016	Phosphoric acid (250)	12.28	1016	86
0.100	1017	Hydrofluoric acid (250)	12.28	1017	86
0.100	1018	Perchloric acid (250)	12.28	1018	86
0.100	1019	Chloroacetic acid (250)	12.28	1019	86
0.100	1020	Dichloroacetic acid (250)	12.28	1020	86
0.100	1021	Trichloroacetic acid (250)	12.28	1021	86
0.100	1022	Tetrafluoroacetic acid (250)	12.28	1022	86
0.100	1023	Pentafluoroacetic acid (250)	12.28	1023	86
0.100	1024	Hexafluoroacetic acid (250)	12.28	1024	86
0.100	1025	Heptafluoroacetic acid (250)	12.28	1025	86
0.100	1026	Octafluoroacetic acid (250)	12.28	1026	86
0.100	1027	Nonafluoroacetic acid (250)	12.28	1027	86
0.100	1028	Decafluoroacetic acid (250)	12.28	1028	86
0.100	1029	Undecafluoroacetic acid (250)	12.28	1029	86
0.100	1030	Dodecafluoroacetic acid (250)	12.28	1030	86
0.100	1031	Tridecafluoroacetic acid (250)	12.28	1031	86
0.100	1032	Tetradecafluoroacetic acid (250)	12.28	1032	86
0.100	1033	Pentadecafluoroacetic acid (250)	12.28	1033	86
0.100	1034	Hexadecafluoroacetic acid (250)	12.28	1034	86
0.100	1035	Heptadecafluoroacetic acid (250)	12.28	1035	86
0.100	1036	Octadecafluoroacetic acid (250)	12.28	1036	86
0.100	1037	Nonadecafluoroacetic acid (250)	12.28	1037	86
0.100	1038	Eicosafluoroacetic acid (250)	12.28	1038	86
0.100	1039	Fluoroacetic acid (250)	12.28	1039	86
0.100	1040	Difluoroacetic acid (250)	12.28	1040	86
0.100	1041	Trifluoroacetic acid (250)	12.28	1041	86
0.100	1042	Tetrafluoroacetic acid (250)	12.28	1042	86
0.100	1043	Pentafluoroacetic acid (250)	12.28	1043	86
0.100	1044	Hexafluoroacetic acid (250)	12.28	1044	86
0.100	1045	Heptafluoroacetic acid (250)	12.28	1045	86
0.100	1046	Octafluoroacetic acid (250)	12.28	1046	86
0.100	1047	Nonafluoroacetic acid (250)	12.28	1047	86
0.100	1048	Decafluoroacetic acid (250)	12.28	1048	86
0.100	1049	Undecafluoroacetic acid (250)	12.28	1049	86
0.100	1050	Dodecafluoroacetic acid (250)	12.28	1050	86
0.100	1051	Tridecafluoroacetic acid (250)	12.28	1051	86
0.100	1052	Tetradecafluoroacetic acid (250)	12.28	1052	86
0.100	1053	Pentadecafluoroacetic acid (250)	12.28	1053	86
0.100	1054	Hexadecafluoroacetic acid (250)	12.28	1054	86
0.100	1055	Heptafluoroacetic acid (250)	12.28	1055	86
0.100	1056	Octafluoroacetic acid (250)	12.28	1056	86
0.100	1057	Nonafluoroacetic acid (250)	12.28	1057	86
0.100	1058	Decafluoroacetic acid (250)	12.28	1058	86
0.100	1059	Undecafluoroacetic acid (250)	12.28	1059	86
0.100	1060	Dodecafluoroacetic acid (250)	12.28	1060	86
0.100	1061	Tridecafluoroacetic acid (250)	12.28	1061	86
0.100	1062	Tetradecafluoroacetic acid (250)	12.28	1062	86
0.100	1063	Pentadecafluoroacetic acid (250)	12.28	1063	86
0.100	1064	Hexadecafluoroacetic acid (250)	12.28	1064	86
0.100	1065	Heptafluoroacetic acid (250)	12.28	1065	86
0.100	1066	Octafluoroacetic acid (250)	12.28	1066	86
0.100	1067	Nonafluoroacetic acid (250)	12.28	1067	86
0.100	1068	Decafluoroacetic acid (250)	12.28	1068	86
0.100	1069	Undecafluoroacetic acid (250)	12.28	1069	86
0.100	1070	Dodecafluoroacetic acid (250)	12.28	1070	86
0.100	1071	Tridecafluoroacetic acid (250)	12.28	1071	86
0.100	1072	Tetradecafluoroacetic acid (250)	12.28	1072	86
0.100	1073	Pentadecafluoroacetic acid (250)	12.28	1073	86
0.100	1074	Hexadecafluoroacetic acid (250)	12.28	1074	86
0.100	1075	Heptafluoroacetic acid (250)	12.28	1075	86
0.100	1076	Octafluoroacetic acid (250)	12.28	1076	86
0.100	1077	Nonafluoroacetic acid (250)	12.28	1077	86
0.100	1078	Decafluoroacetic acid (250)	12.28	1078	86
0.100	1079	Undecafluoroacetic acid (250)	12.28	1079	86
0.100	1080	Dodecafluoroacetic acid (250)	12.28	1080	86
0.100	1081	Tridecafluoroacetic acid (250)	12.28	1081	86
0.100	1082	Tetradecafluoroacetic acid (250)	12.28	1082	86
0.100	1083	Pentadecafluoroacetic acid (250)	12.28	1083	86
0.100	1084	Hexadecafluoroacetic acid (250)	12.28	1084	86
0.100	1085	Heptafluoroacetic acid (250)	12.28	1085	86
0.100	1086	Octafluoroacetic acid (250)	12.28	1086	86
0.100	1087	Nonafluoroacetic acid (250)	12.28	1087	86
0.100	1088	Decafluoroacetic acid (250)	12.28	1088	86
0.100	1089	Undecafluoroacetic acid (250)	12.28	1089	86
0.100	1090	Dodecafluoroacetic acid (250)	12.28	1090	86
0.100	1091	Tridecafluoroacetic acid (250)	12.28	1091	86
0.100	1092	Tetradecafluoroacetic acid (250)	12.28	1092	86
0.100	1093	Pentadecafluoroacetic acid (250)	12.28	1093	86
0.100	1094	Hexadecafluoroacetic acid (250)	12.28	1094	86
0.100	1095	Heptafluoroacetic acid (250)	12.28	1095	86
0.100	1096	Octafluoroacetic acid (250)	12.28	1096	86
0.100	1097	Nonafluoroacetic acid (250)	12.28	1097	86
0.100	1098	Decafluoroacetic acid (250)	12.28	1098	86
0.100	1099	Undecafluoroacetic acid (250)	12.28	1099	86
0.100	1100	Dodecafluoroacetic acid (250)	12.28	1100	86
0.100	1101	Tridecafluoroacetic acid (250)	12.28	1101	86
0.100	1102	Tetradecafluoroacetic acid (250)	12.28	1102	86
0.100	1103	Pentadecafluoroacetic acid (250)	12.28	1103	86
0.100	1104	Hexadecafluoroacetic acid (250)	12.28	1104	86
0.100	1105	Heptafluoroacetic acid (250)	12.28	1105	86
0.100	1106	Octafluoroacetic acid (250)	12.28	1106	86
0.100	1107	Nonafluoroacetic acid (250)	12.28	1107	86
0.100	1108	Decafluoroacetic acid (250)	12.28	1108	86
0.100	1109	Undecafluoroacetic acid (250)	12.28	1109	86
0.100	1110	Dodecafluoroacetic acid (250)	12.28	1110	86
0.100	1111	Tridecafluoroacetic acid (250)	12.28	1111	86
0.100	1112	Tetradecafluoroacetic acid (250)	12.28	1112	86
0.100	1113	Pentadecafluoroacetic acid (250)	12.28	1113	86
0.100	1114	Hexadecafluoroacetic acid (250)	12.28	1114	86
0.100	1115	Heptafluoroacetic acid (250)	12.28	1115	86
0.100	1116	Octafluoroacetic acid (250)	12.28	1116	86
0.100	1117	Nonafluoroacetic acid (250)	12.28	1117	86
0.100	1118	Decafluoroacetic acid (250)	12.28	1118	86
0.100	1119	Undecafluoroacetic acid (250)	12.28	1119	86
0.100	1120	Dodecafluoroacetic acid (250)	12.28	1120	86
0.100	1121	Tridecafluoroacetic acid (250)	12.28	1121	86
0.100	1122	Tetradecafluoroacetic acid (250)	12.28	1122	86
0.100	1123	Pentadecafluoroacetic acid (250)	12.28	1123	86
0.100	1124	Hexadecafluoroacetic acid (250)	12.28	1124	86
0.100	1125	Heptafluoroacetic acid (250)	12.28	1125	86
0.100	1126	Octafluoroacetic acid (250)	12.28	1126	86
0.100	1127	Nonafluoroacetic acid (250)	12.28	1127	86
0.100	1128	Decafluoroacetic acid (250)	12.28	1128	86
0.100	1129	Undecafluoroacetic acid (250)	12.28	1129	86
0.100	1130	Dodecafluoroacetic acid (250)	12.28	1130	86
0.100	1131	Tridecafluoroacetic acid (250)	12.28	1131	86
0.100	1132	Tetradecafluoroacetic acid (250)	12.28	1132	86
0.100	1133	Pentadecafluoroacetic acid (250)	12.28	1133	86
0.100	1134	Hexadecafluoroacetic acid (250)	12.28	1134	86
0.100	1135	Heptafluoroacetic acid (250)	12.28	1135	86
0.100	1136	Octafluoroacetic acid (250)	12.28	1136	86
0.100	1137	Nonafluoroacetic acid (250)	12.28	1137	86
0.100	1138	Decafluoroacetic acid (250)	12.28	1138	86
0.100	1139	Undecafluoroacetic acid (250)	12.28	1139	86
0.100	1140	Dodecafluoroacetic acid (250)	12.28	1140	86
0.100	1141	Tridecafluoroacetic acid (250)	12.28	1141	86
0.100	1142	Tetradecafluoroacetic acid (250)	12.28	1142	86
0.100	1143	Pentadecafluoroacetic acid (250)	12.28	1143	86
0.100	1144	Hexadecafluoroacetic acid (250)	12.28	1144	86
0.100	1145	Heptafluoroacetic acid (250)	12.28	1145	86
0.100	1146	Octafluoroacetic acid (250)	12.28	1146	86
0.100	1147	Nonafluoroacetic acid (250)	12.28	1147	86
0.100	1148	Decafluoroacetic acid (250)	12.28	1148	86
0.100	1149	Undecafluoroacetic acid (250)	12.28	1149	86
0.100	1150	Dodecafluoroacetic acid (250)	12.28	1150	86
0.100	1151	Tridecafluoroacetic acid (250)	12.28	1151	86
0.100	1152	Tetradecafluoroacetic acid (250)	12.28	1152	86
0.100	1153	Pentadecafluoroacetic acid (250)	12.28	1153	86
0.100	1154	Hexadecafluoroacetic acid (250)	12.28	1154	86
0.100	1155	Heptafluoroacetic acid (250)	12.28	1155	86
0.100	1156	Octafluoroacetic acid (250)	12.28	1156	86
0.100	1157	Nonafluoroacetic acid (250)	12.28	1157	86
0.100	1158	Decafluoroacetic acid (250)	12.28	1158	86
0.100	1159	Undecafluoroacetic acid (250)	12.28	1159	86
0.100	1160	Dodecafluoroacetic acid (250)	12.28	1160	86
0.100	1161	Tridecafluoroacetic acid (250)	12.28	1161	86
0.100	1162	Tetradecafluoroacetic acid (250)	12.28	1162	86
0.100	1163	Pentadecafluoroacetic acid (250)	12.28	1163	86
0.100	1164	Hexadecafluoroacetic acid (250)	12.28	1164	86
0.100	1165	Heptafluoroacetic acid (250)	12.28	1165	86
0.100	1166	Octafluoroacetic acid (250)	12.28	1166	86
0.100	1167	Nonafluoroacetic acid (250)	12.28	1167	86

# LC/MS Workflow

LC/Q-TOF or TOF  
Full Spectrum

– for **unknown compounds**



Exact Mass  
Database Search

Name	RT	MW	MS/MS	DB Name	DB Ref
Compound 180	11.142	214.12016	93.01		
Gal 102 (Chlorine)	8.963	213.0476	98.00	CAS104412	0381
Gal 117 (Chlorine)	9.846	207.0576	97.00	CAS123901	0380
Compound 188	11.240	222.0924	99.01		
Gal 98 (Chlorine)	8.963	220.0676	98.00	CAS104412	0381
Gal 106 (Chlorine)	9.254	222.0924	104.01	CAS104412	0381
Compound 187	8.912	222.0924	148.01		
Compound 142	7.201	222.0924	168.01		
Gal 88 (Chlorine)	8.963	220.0676	98.00	CAS104412	0381
Gal 105 (Chlorine)	10.000	222.0924	138.01	CAS104412	0381
Compound 174	10.000	222.0924	138.01		
Compound 176	10.000	222.0924	168.01		
Gal 122 (Chlorine)	12.146	220.0676	97.00	CAS104412	0381
Compound 178	11.142	222.0924	168.01		
Compound 172	12.146	222.0924	168.01		

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

# 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

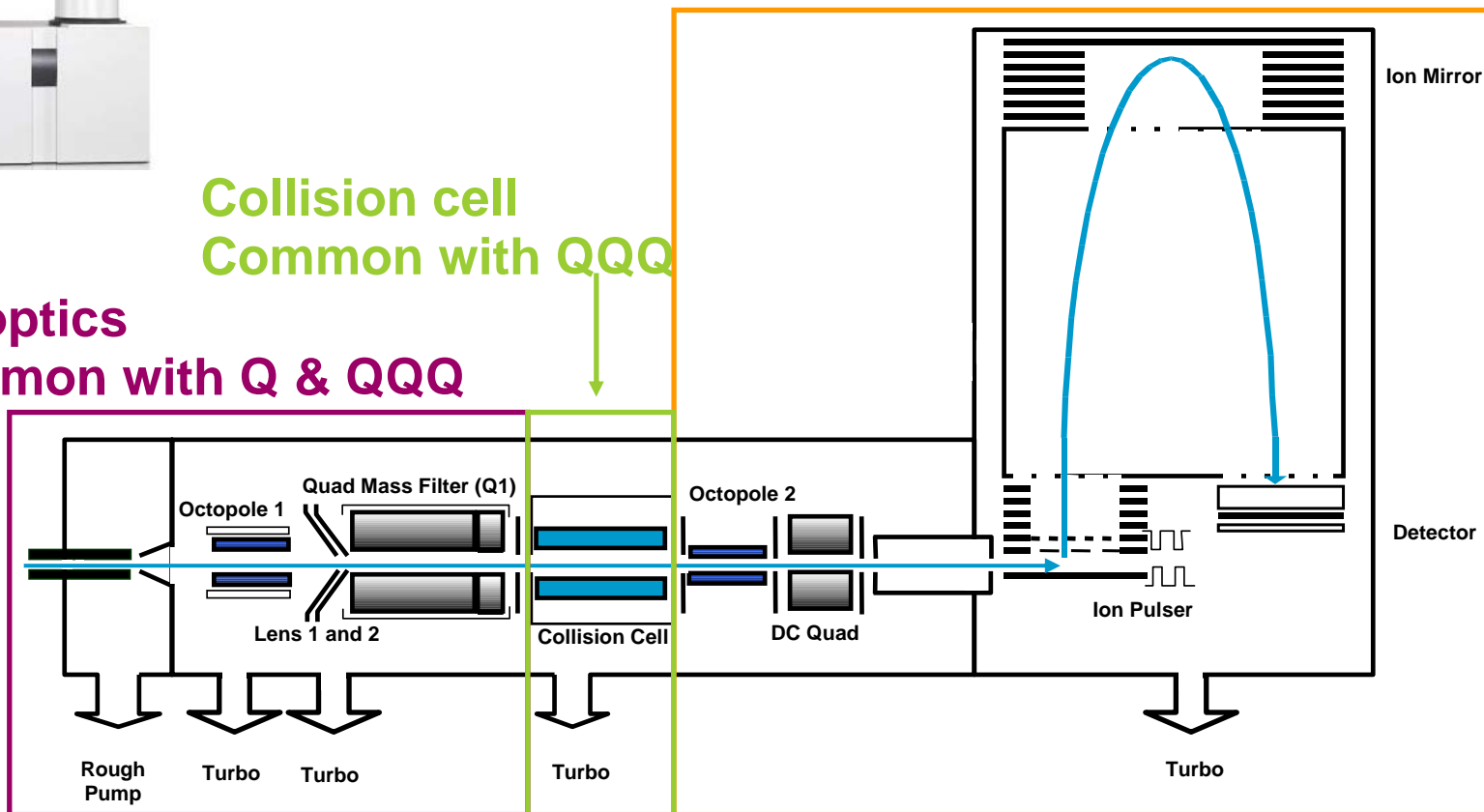
Exact Mass  
Mass Accuracy (ppm)



Flight tube  
Common with TOF

Collision cell  
Common with QQQ

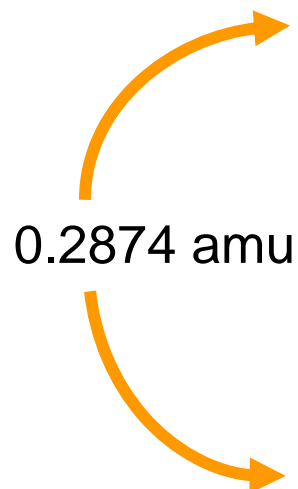
Ion optics  
Common with Q & QQQ



# What does “Exact Mass” mean?

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

0.2874 amu



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	Primetaphos
C16H20N2O3	288.1473925	Imazamethabenz

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

# Mass Analysis for TOF

$$v = d/t$$

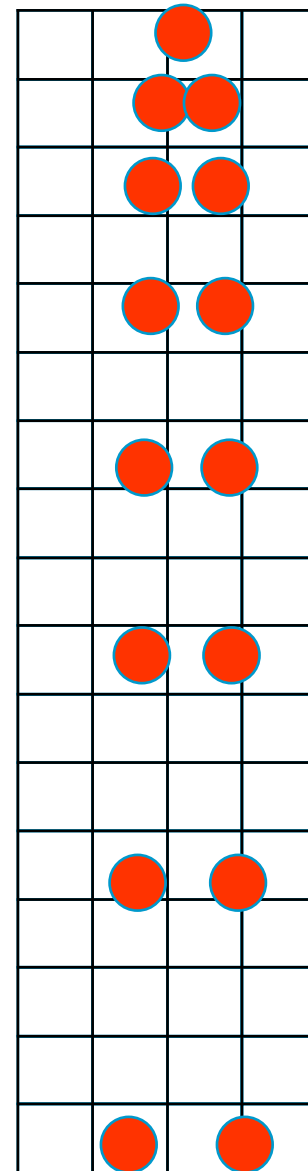
$$E = \frac{1}{2} m v^2 = \frac{1}{2} m (d/t)^2$$

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

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<sup>+</sup>) of Reserpine

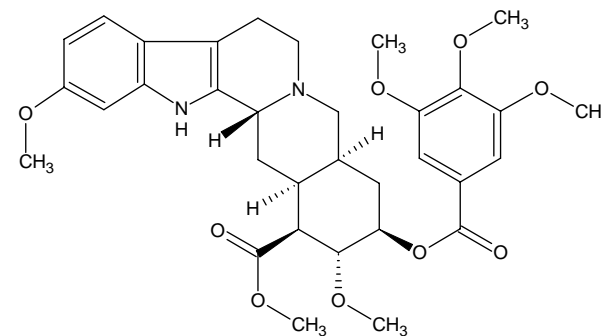
Atom	Mass of Atom	# of Atoms	Sum
Hydrogen	1.00783	40	<b>40.31300</b>
Carbon	12.00000	33	<b>396.00000</b>
Nitrogen	14.00307	2	<b>28.00615</b>
Oxygen	15.99492	9	<b>143.95424</b>
<b>Total</b>			<b>608.27338</b>
<b>Plus H</b>	1.00783	1	<b>1.00783</b>
<b>Total</b>			<b>609.28121</b>
<b>Minus e-</b>	0.00055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

Calculated = exact

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

**0.9027038 ppm**

**Error if the electron was not omitted!**



**Reserpine (C<sub>33</sub>H<sub>40</sub>N<sub>2</sub>O<sub>9</sub>)**

# How Much Accuracy is Needed?

Reserpine ( $C_{33}H_{40}N_2O_9$ ) has a protonated ion at 609.28066 (MH<sup>+</sup>)

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

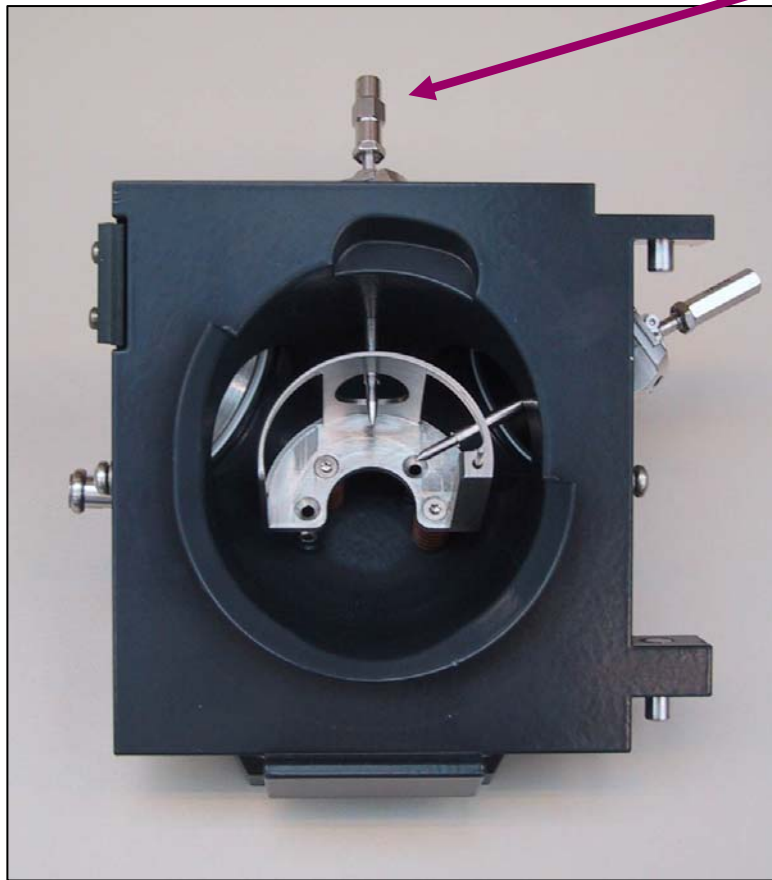
$$(0.1/609.28 \times 10e6 = 164.128 \text{ ppm})$$

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**



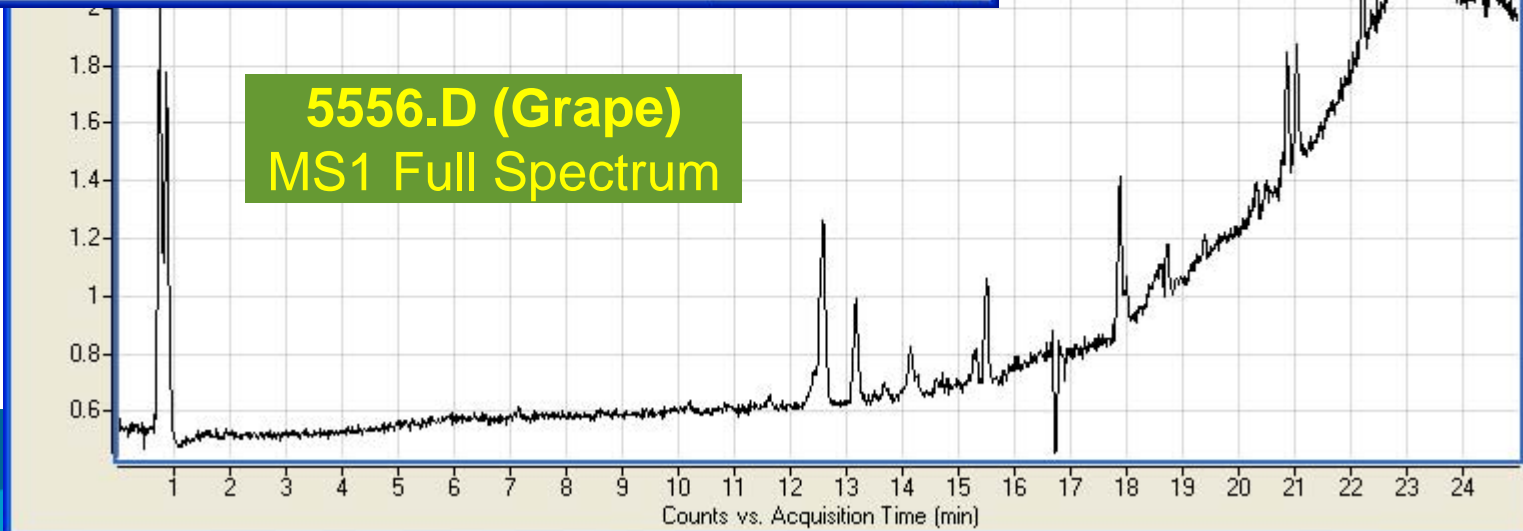
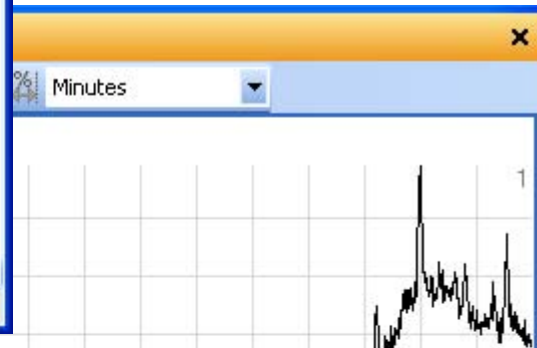
**AutoTune**

# TOF/Q-TOF Unlimited Number of Compound Screening

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 Metaminostrobin	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
Compound 99	12.025	222.13999			7014

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) <sup>+</sup>
Spiroxamine	10.884	298.27406
Terbuconazole	14.139	308.15242
Tebufenoxide	15.492	353.22236



Data Navigator

Sort by Data File

- 5556.d
  - User Chromatograms
  - User Spectra
  - Background Spectra
  - Compounds

Method Explorer: CVUA-QTOF-MS1.m

- Chromatogram
- Spectrum
- General
- Find Compounds

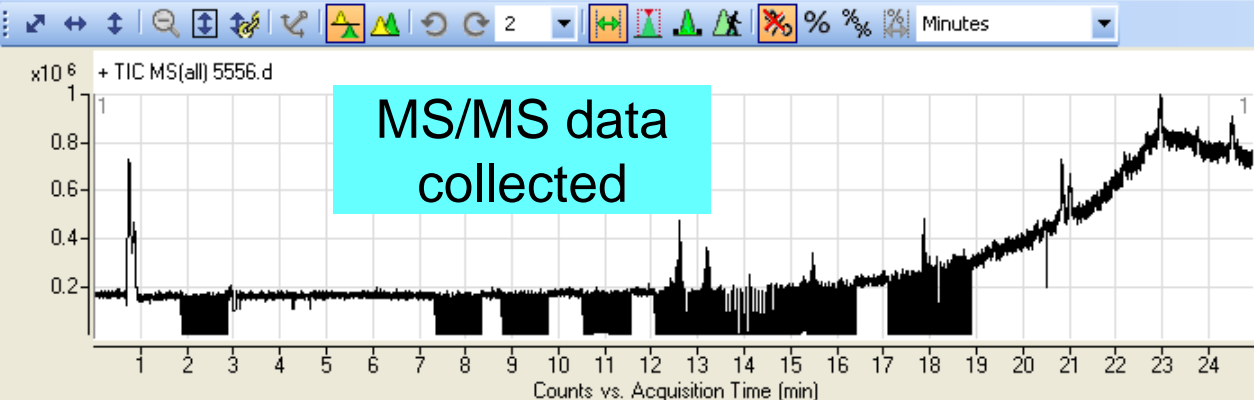
Find by Auto MS/MS

Find by Targeted MS/MS

Find by Molecular Feature

- Find Compounds by Formula
- Identify Compounds
- Compound Automation Steps
- Analysis Automation Steps
- Worklist Automation

Chromatogram Results



Chromatogram Results MS Formula Results

Method Editor: Find Compounds by Targeted MS/MS

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:

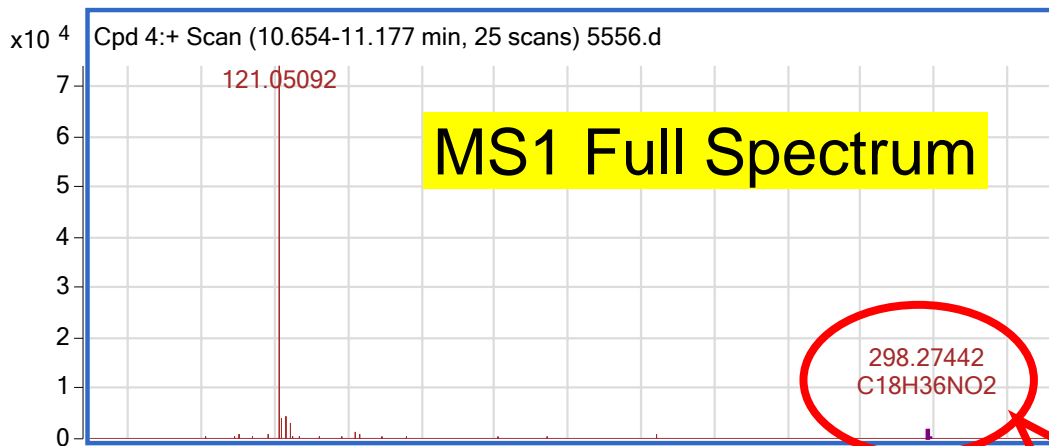
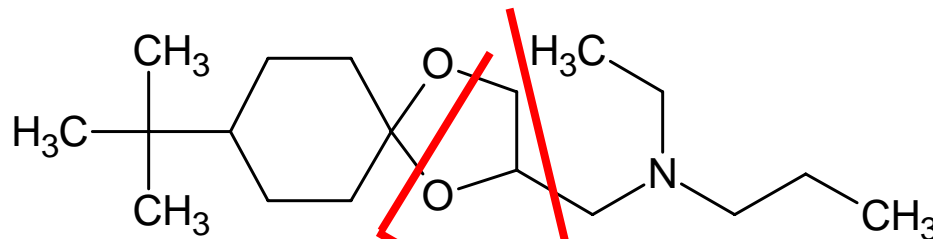
Baseline allocation

Baseline reset >

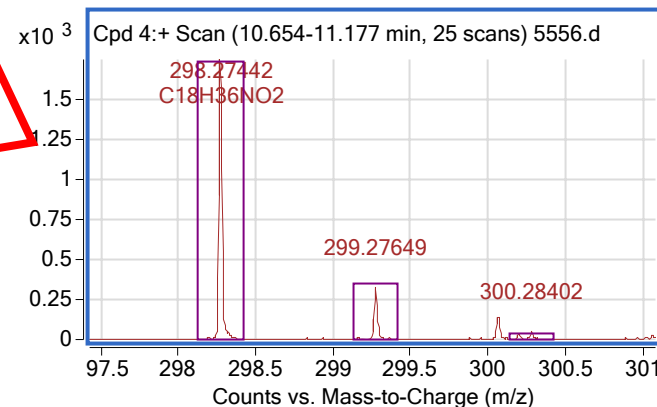
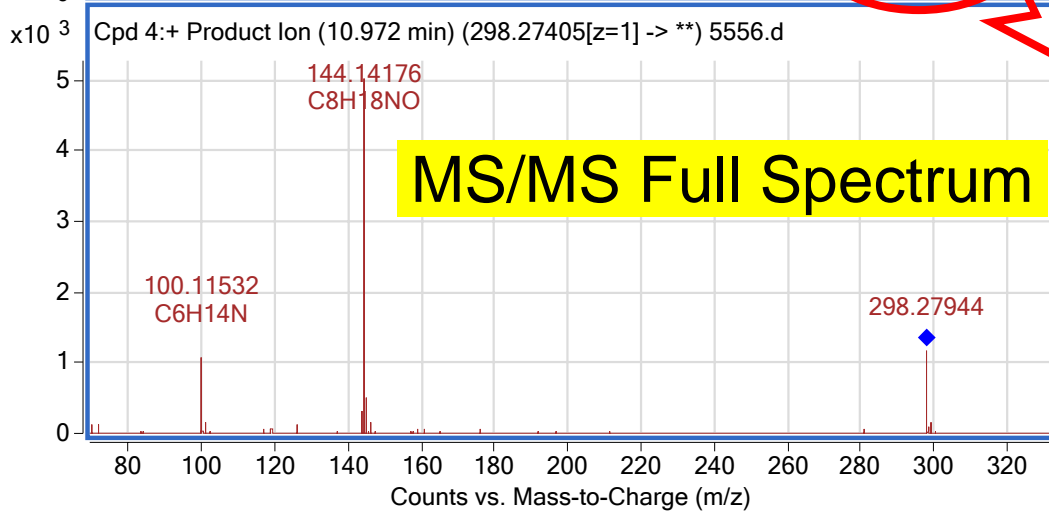
If either edge <  %

Next Step:  
Find  
compounds by  
Targeted  
MS/MS

# Spiroxamine



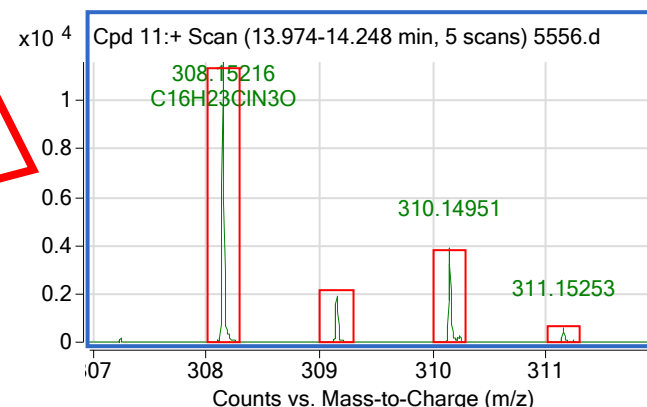
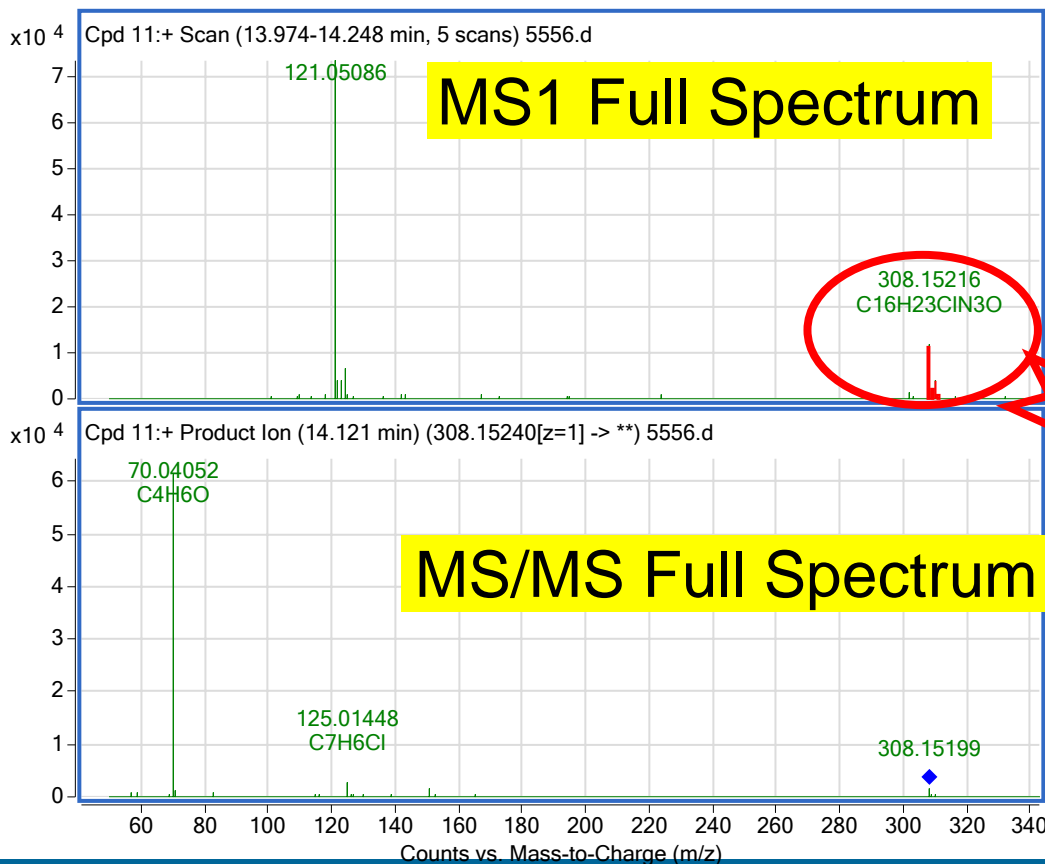
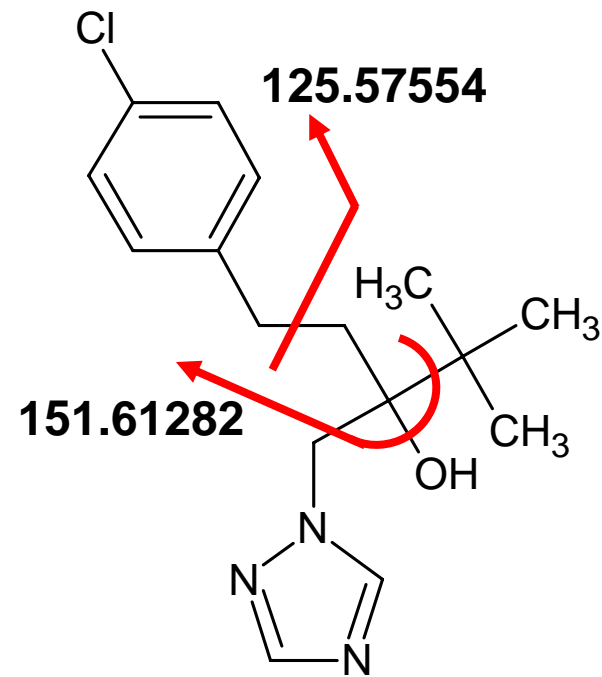
The compound name Spiroxamine came up in the MS1 mode. Use formula results and MS/MS results to confirm the hit.



# Terbuconazole

The compound name Terbuconazole came up in the MS1 mode.

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



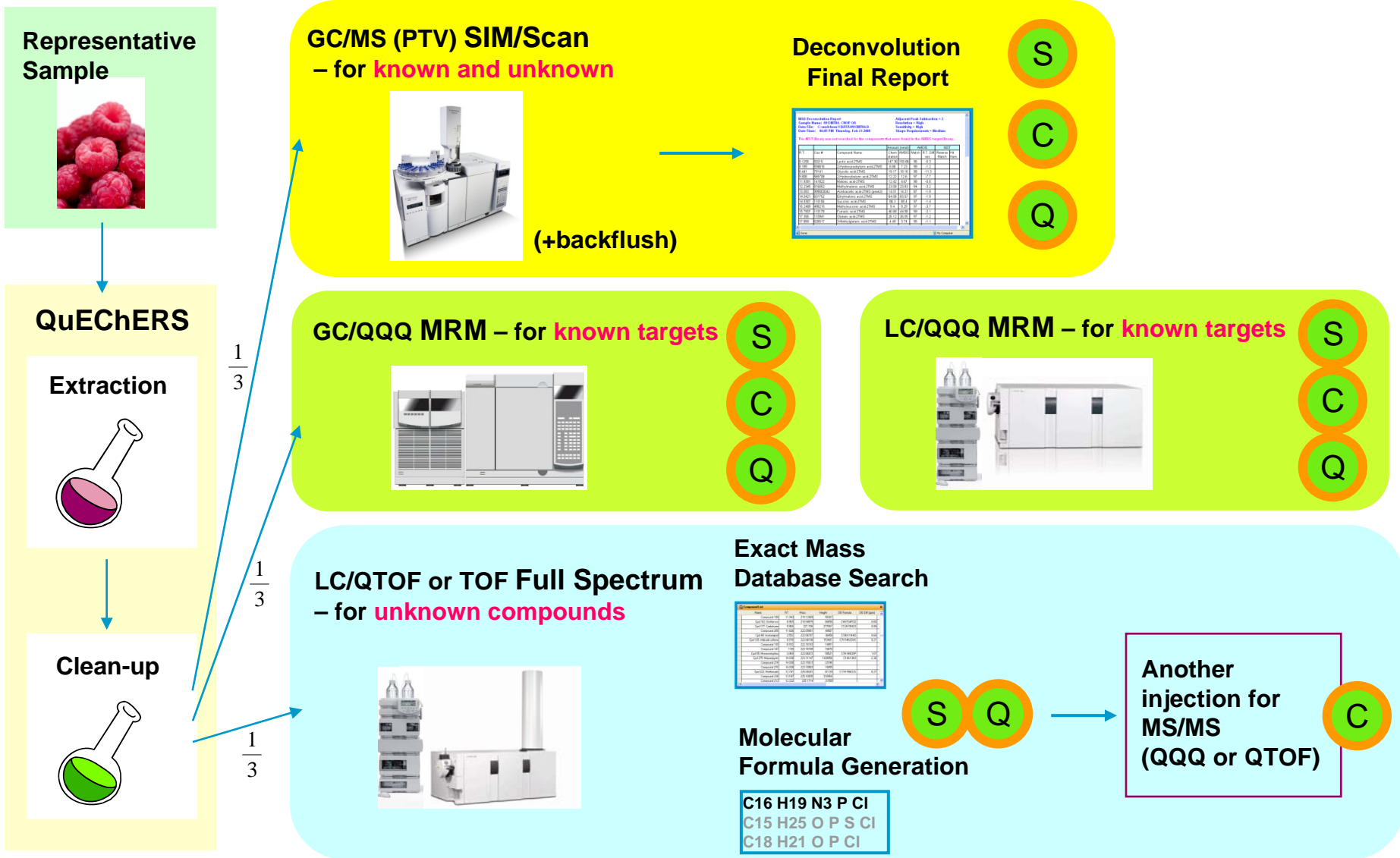
Boxes represent theoretical isotope ratios

# 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**



# 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