

Advantageous of High Resolving LC-MS system (Q-TOF) in food analysis



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Historical Perspective

Food safety has become a very big concern

- Examination of food products for disease
- Caused by organisms, chemical residues, and other hazardous material

Increased food production world wide with increasing population

Increasing import/exportation of food

Exploitation of food production

Drastic reductions in farmland/resources

Regulatory agencies world wide establish guidelines for food safety, within and outside a given country the restrictions vary

- Globalization of the food supply
- Globalization of food safety
- Problems and solutions no longer confined within the boundaries of a single country

Food Safety Testing

Big business with exponential growth

Leading growth market

Affect any and all countries

Testing for many not just one

Food Testing: Segmentation Description

1. **Nutrition:** This segment includes tests that are driven by regulations for what is placed on the food product label. This includes Functional foods(=health Foods) which are required to have scientific data to back up what is claimed on the label.
2. **Safety:** These are tests that are performed to comply with regulations concerning food safety or tests performed to prevent an expensive brand damaging recall due to unsafe food product.
3. **Identity/Traceability:** This is a rapidly changing and/or still developing segment.. GMO is the most established application within this segment, whereas speciation is emerging for example for basmati rice, fish and meat. Also geographic origin tests are part of this segment.
4. **Quality:** This segment includes: raw ingredient testing, process testing, final product quality testing, & compositional control. Also including quality control for ingredient, additives (incl..flavors) and packaging suppliers

Overview of LC-MS Technologies – Which Technique to Use

Sample Prep	Instrument	Screening	Conformation	Quantitation
<p>SPE Or QuEChERS</p> <p>-Extraction - Clean up</p>	<p>LC/QQQ</p> <p>-Target trace Analysis in dirty or complex matrix</p>	<ul style="list-style-type: none"> — Highly selective, sensitive detection of a large number of compounds in a single injection - Low detection limits in complex matrices - Novel Agilent Jet Stream technology improves sensitivity - Back flush tech for GCMS 	<ul style="list-style-type: none"> — MRM provides positive confirmation and low detection limits to meet all regulatory requirements - One-run screening and confirmation 	<ul style="list-style-type: none"> — Routine, high-accuracy MS/MS quantification - Batch-at-Glance for fast view of quantification result

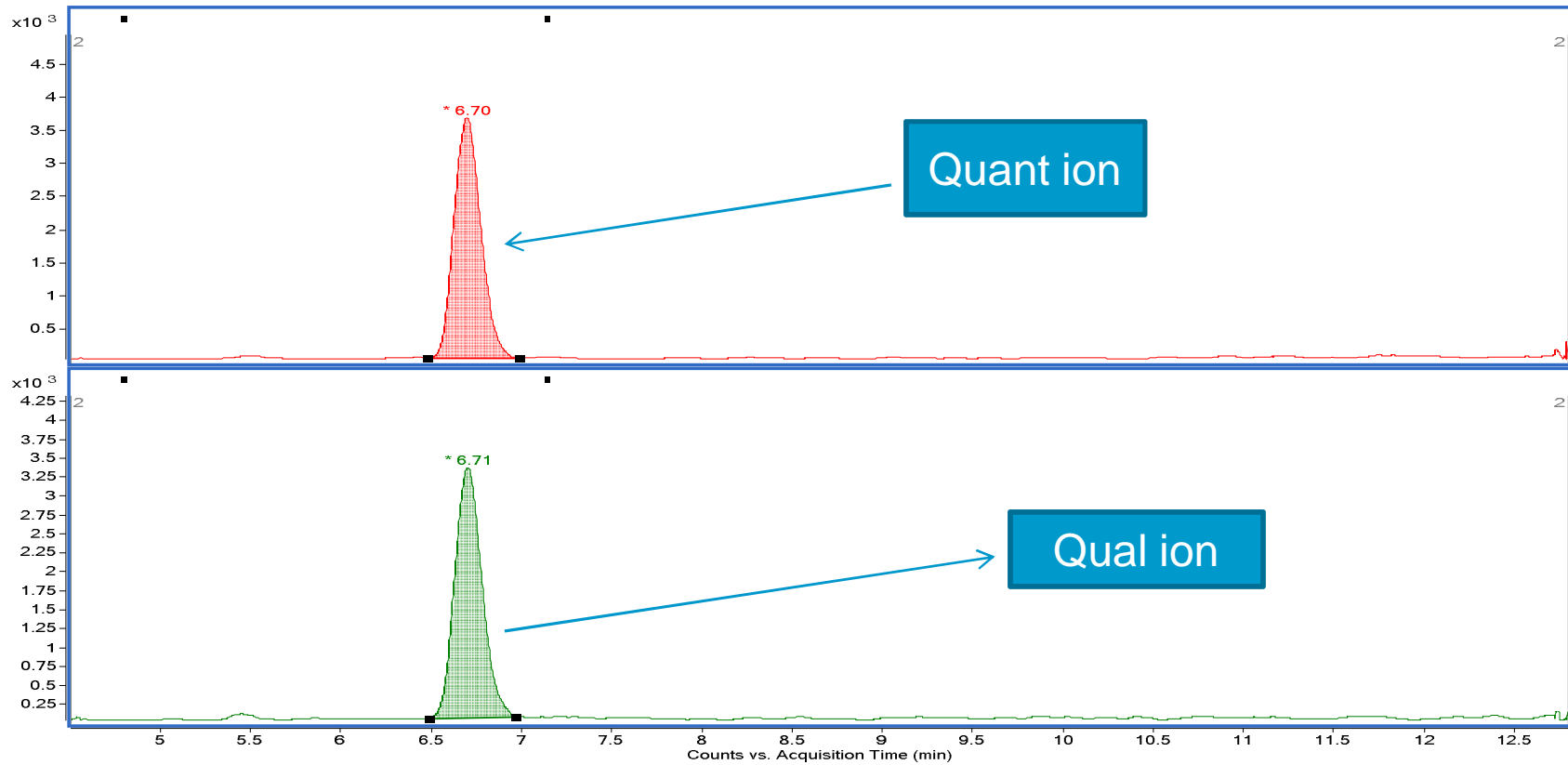
Overview of LC-MS Technologies – Which Technique to Use

Sample Prep	Instrument	Screening	Conformation	Quantitation
<p>SPE Or QuEChERS</p> <p>-Extraction - Clean up</p>	<p>LC-TOF And LC/Q-TOF</p>	<ul style="list-style-type: none"> — High resolution reduces noise and matrix interference - Accurate mass provides added compound selectivity and unlimited screening using exact mass databases 	<ul style="list-style-type: none"> — Automated, accurate mass searches against public and private databases - Molecular Formula Generator feature reduces data interpretation time 	<ul style="list-style-type: none"> — Very narrow mass window for extracted ion - Chromatograms provide precise MS and MS/MS quantification - High dynamic range to quantitate a wide range of concentrations

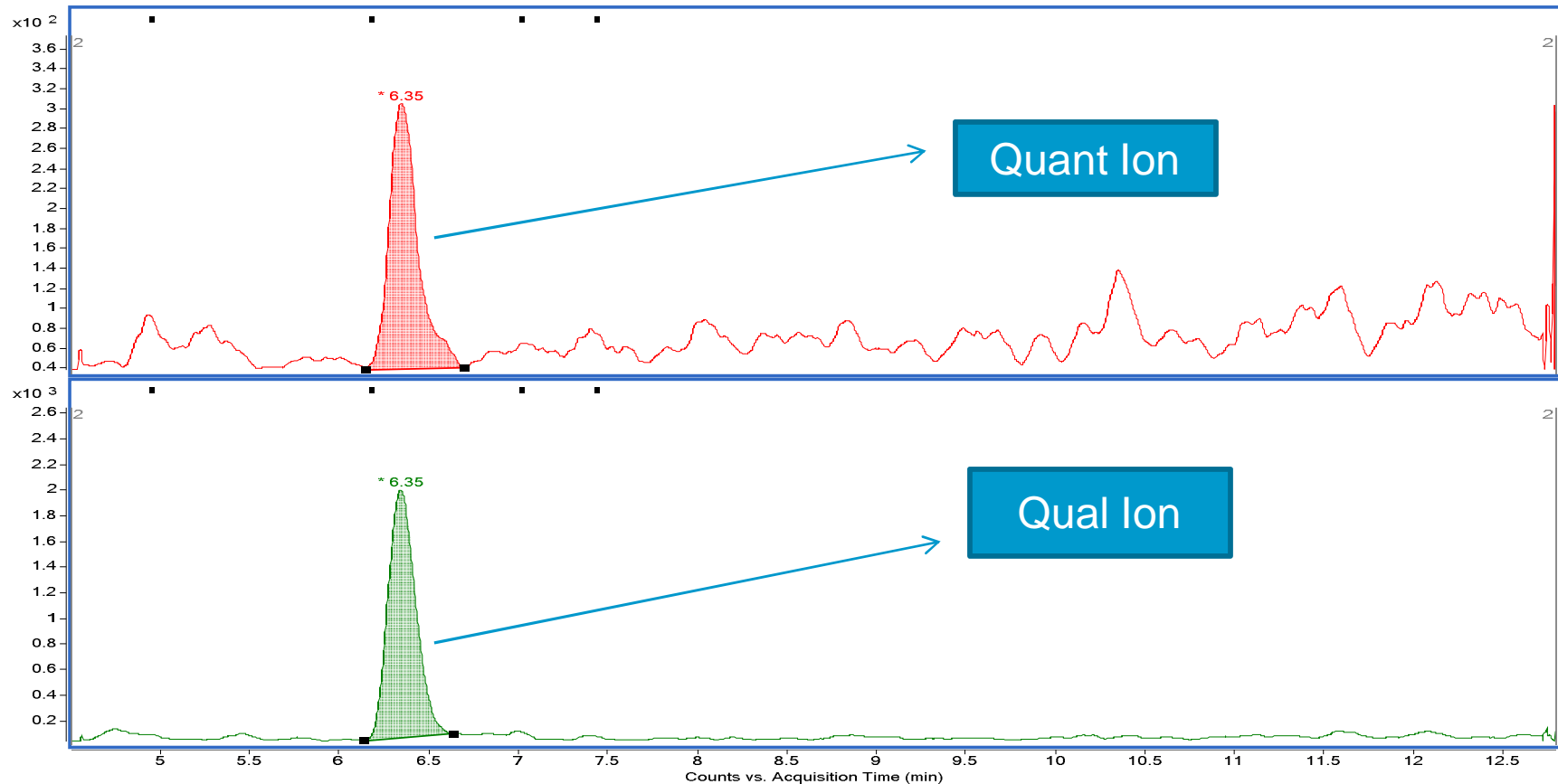
Overview of LC-MS Technologies - Which Technique to Use

Sample Prep	Instrument	Screening	Conformation	Quantitation
SPE Or QuEChERS -Extraction - Clean up	LC- MS (SQ) -screening and Quantification in clean matrix	<ul style="list-style-type: none"> — Selective detection of a large number of compounds in a single injection - Low detection limits in simple matrices - Complementary structure information with DAD and FLD 	<ul style="list-style-type: none"> — Library matching in GCMS 	<ul style="list-style-type: none"> — High dynamic range to quantitate a wide range of concentrations

Wine Sample analysis, Verrucarol std

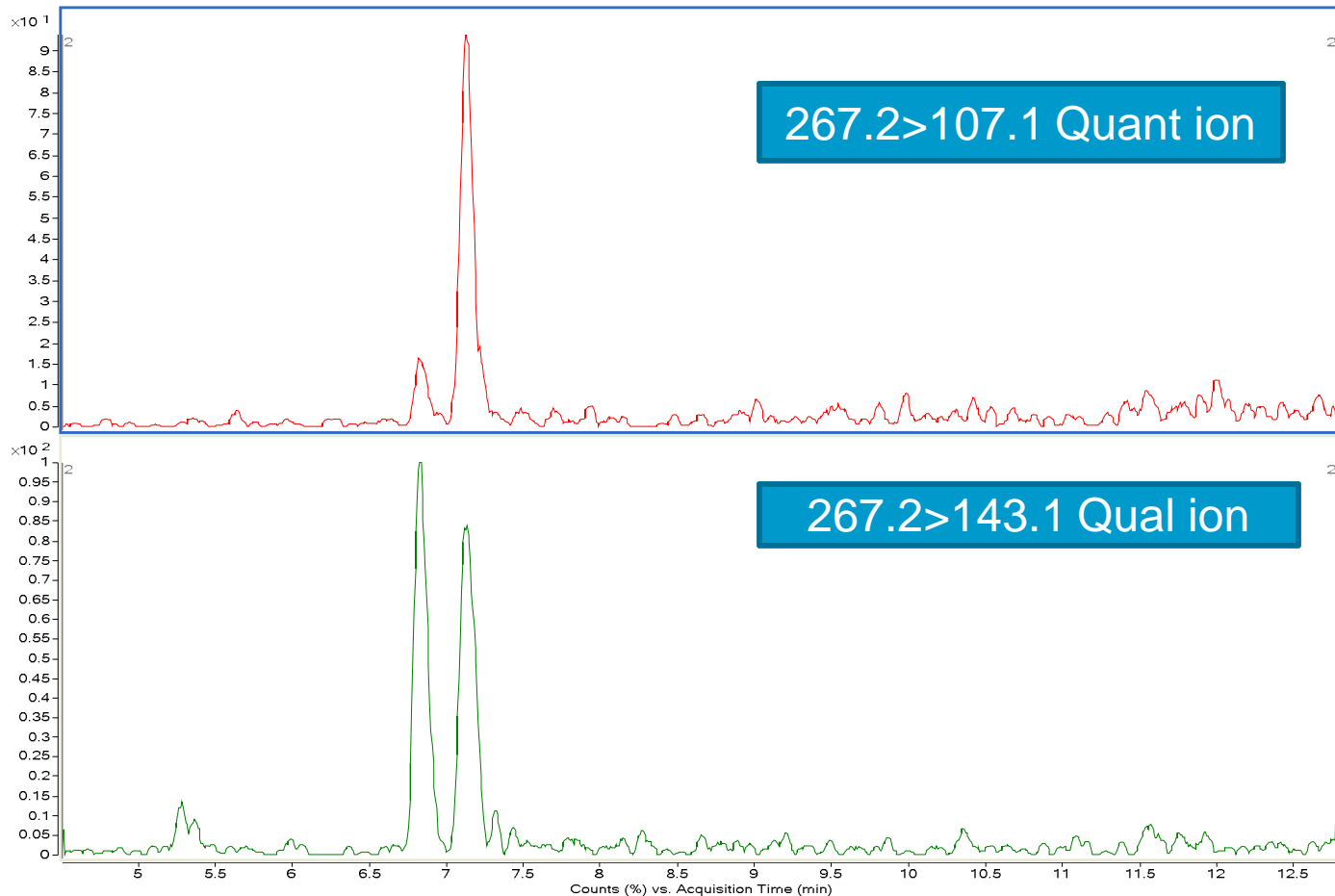


Sample analysis, Verrucarol sample



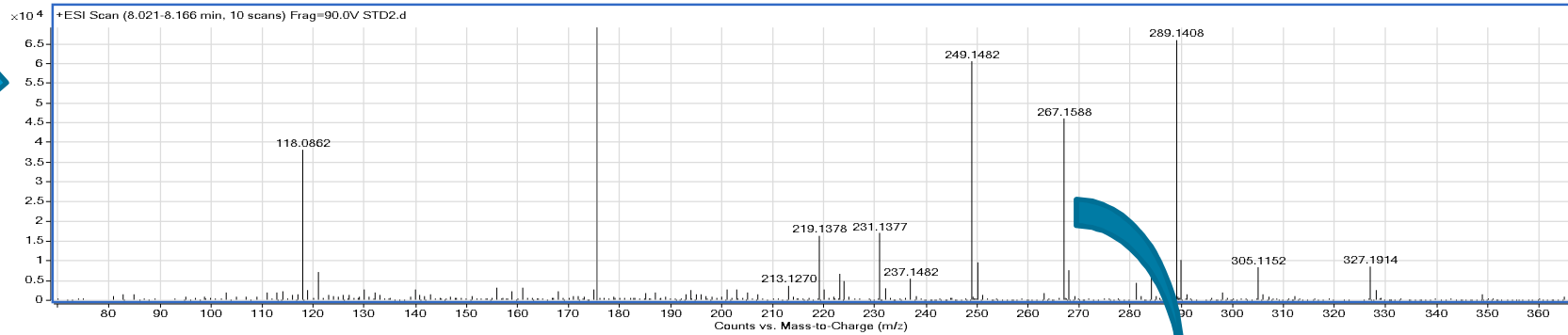
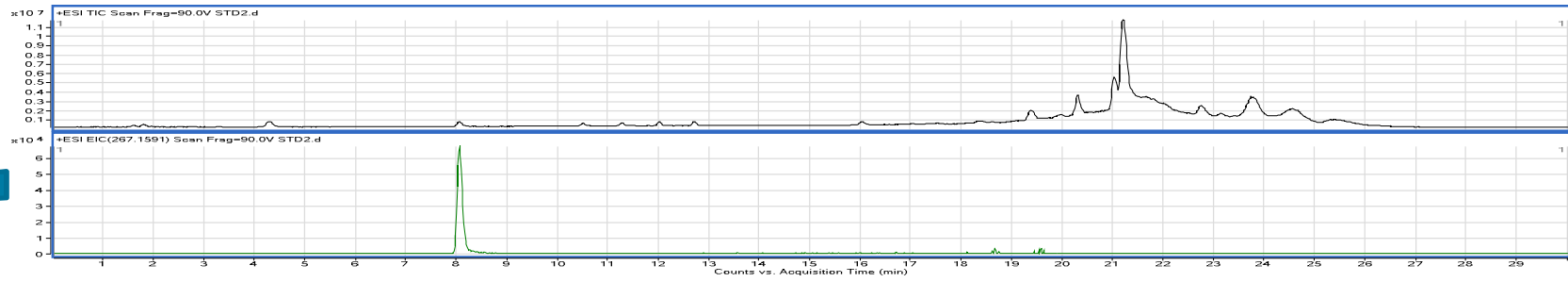
Verrucarol shows similar MRM transitions in std and sample, only difference is of RT and Ion ratio of Quant & Qual

Sample analysis with spiked Verrucarol std



Verrucarol shows similar MRM transitions in std and sample, only difference is of RT and Ion ratio of Quant & Qual

Verrucarol std spectra using Q-TOF

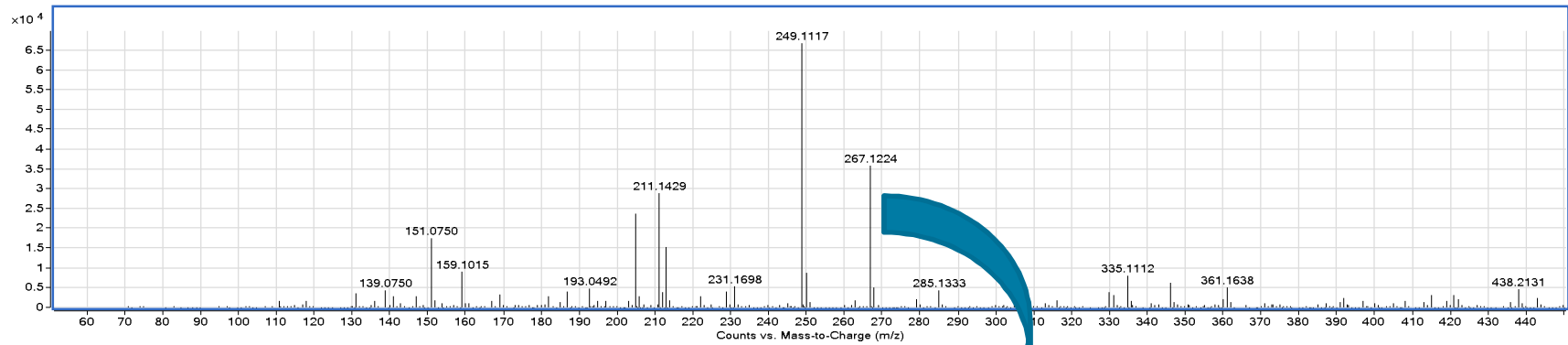
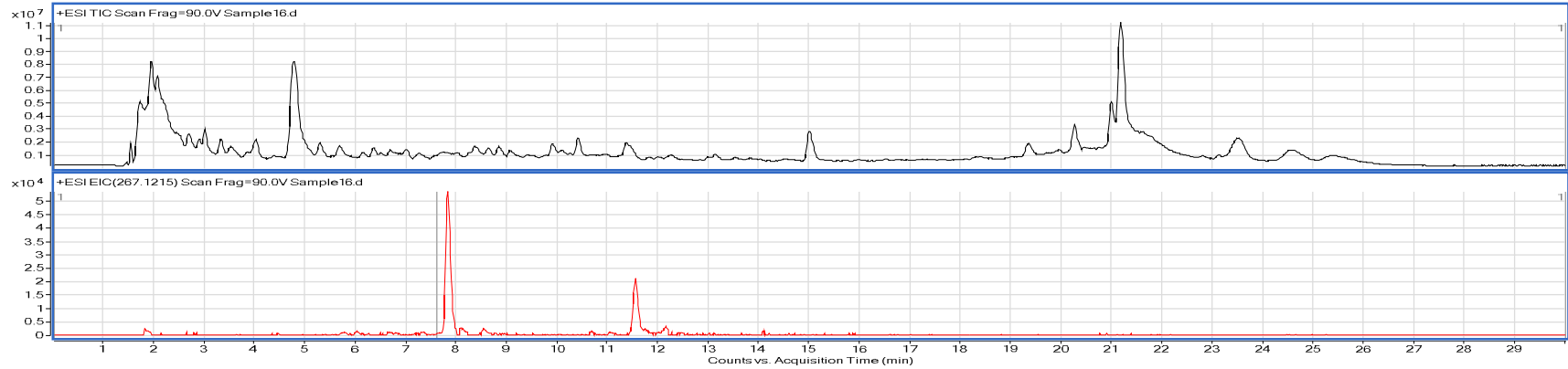


MS Formula Results: + Scan (8.021-8.166 min)

m/z	Ion	Formula	Abundance
267.1588	(M+H) ⁺	C ₁₅ H ₂₃ O ₄	45916.9

Best	Formula (M)	Ion Formula	Score	Cross Score	Calc m/z	Diff (ppm)	Mass Match	Abund Match	Spacing Match
<input checked="" type="checkbox"/>	C ₁₅ H ₂₂ O ₄	C ₁₅ H ₂₃ O ₄	99.61		267.1591	1.05	99.36	99.73	99.95

Sample analysis using Q-TOF



The screenshot shows a software interface with a table of mass spectrometry data. The table has columns for m/z, Formula, and Abundance. The selected row shows m/z 249.1117 and Formula C₁₂H₁₅N₃O₂.

m/z	Formula	Abundance
249.1117	C ₁₂ H ₁₅ N ₃ O ₂	65000

Scan	Formula (M)	Ion Formula	Score	Score Score	Calc. m/z	Diff. (ppm)	Mass Match	Abund. Match	Spacing Ratio
1	C ₁₂ H ₁₅ N ₃ O ₂	C ₁₂ H ₁₅ N ₃ O ₂	100	100	249.1117	0.0	100%	100%	1.0

Results- Verrucarol

1-Peak at RT 8.15 min shows formula $C_{15}H_{22}O_4$ (267.1591 m/z) with 1ppm mass accuracy which is matching with Verrucarol std

2- Peak at RT 7.85 min shows formula $C_{14}H_{18}O_5$ (267.1224 m/z) with 1 ppm mass accuracy

By using Q-TOF it is possible to differentiate between Verrucarol and similar mass. As both ions shows similar fragmentation (MRM) it is not possible to differentiate by using QQQ. Also RT difference between both is just 0.3 min



Why Q-TOF?

Sometimes the combined power of
LC Resolution

+

MS Resolving Power

Is not enough based upon:
Small Δ Mass Defect (Nature)

+

Intense Matrix Ions (Food Sample)

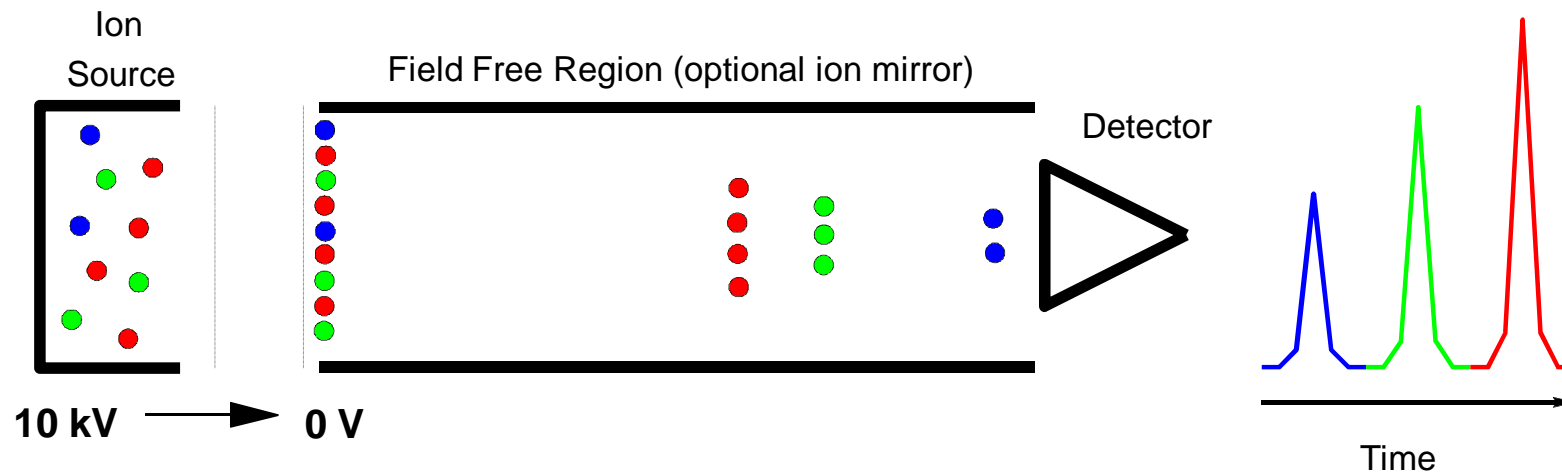
High resolution MS/MS can solve some of these problems

Performance Parameters Mass Spectrometer

- Mass accuracy
- Resolution
- Sensitivity
- Dynamic range

Time-of-Flight Mass Spectrometer

The Concept



$$E = \frac{1}{2} m v^2 \quad v = \sqrt{\frac{2E}{m}} \quad t = \frac{\text{length}}{\sqrt{\frac{2E}{m}}} = \frac{2.0}{\sqrt{\frac{2(10000)}{3200}}} = 80 \text{ usec}$$

When leaving the ion source, ions get accelerated to the same kinetic energies. Ions of different m/z move at different velocities and arrive at the detector at different times.

Mass accuracy



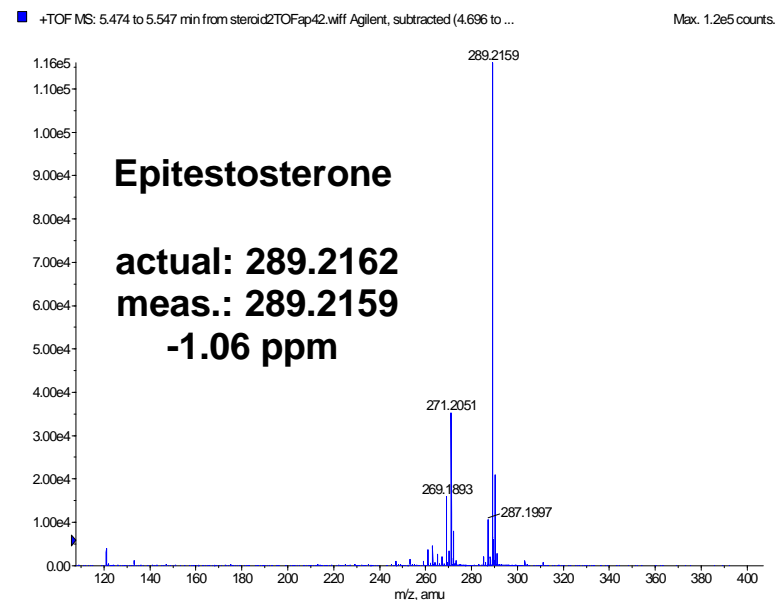
Formulae for Mass accuracy

$$\text{Mass accuracy} = \left(\frac{\text{measured} - \text{actual}}{\text{actual}} \right) \times 10^6$$

$$(1000.001 - 1000.000) / 1000 \times 10^6 = 1 \text{ ppm}$$

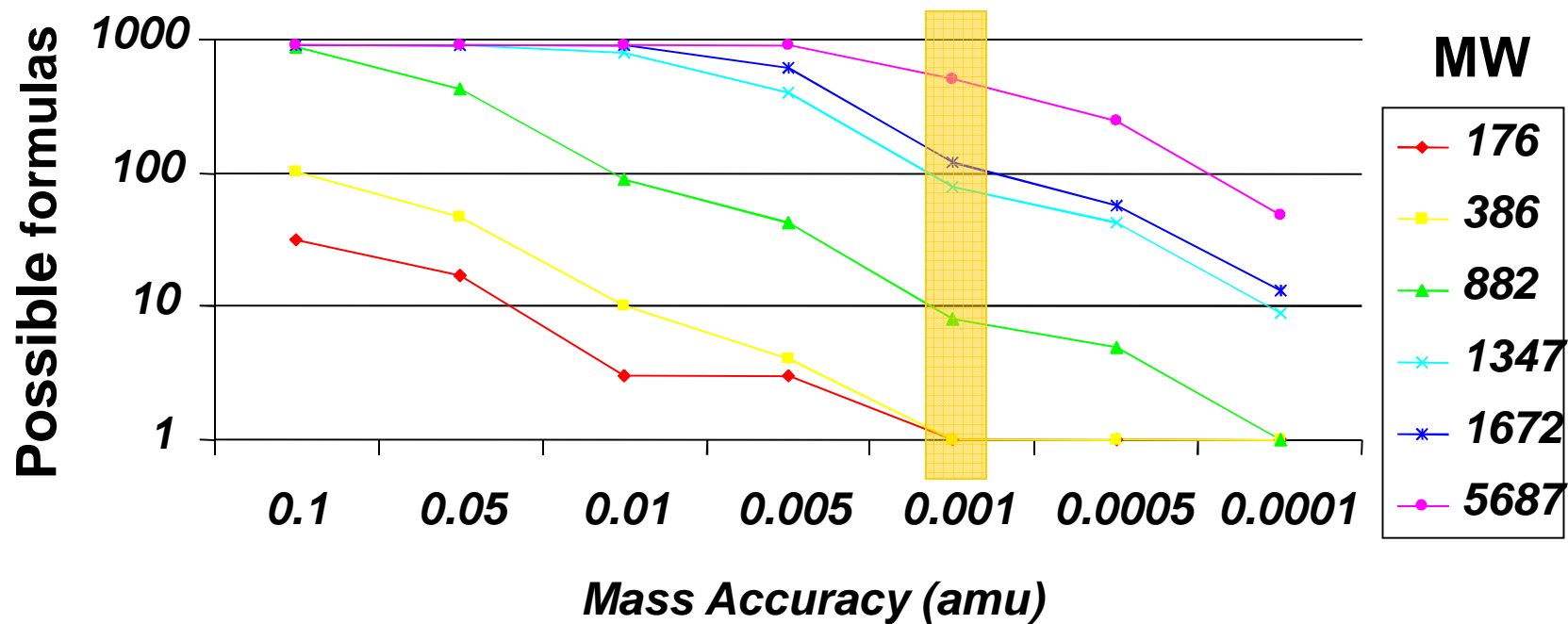
Therefore:

1 ppm @ m/z 1000 is 0.001 Da (1 mDa)

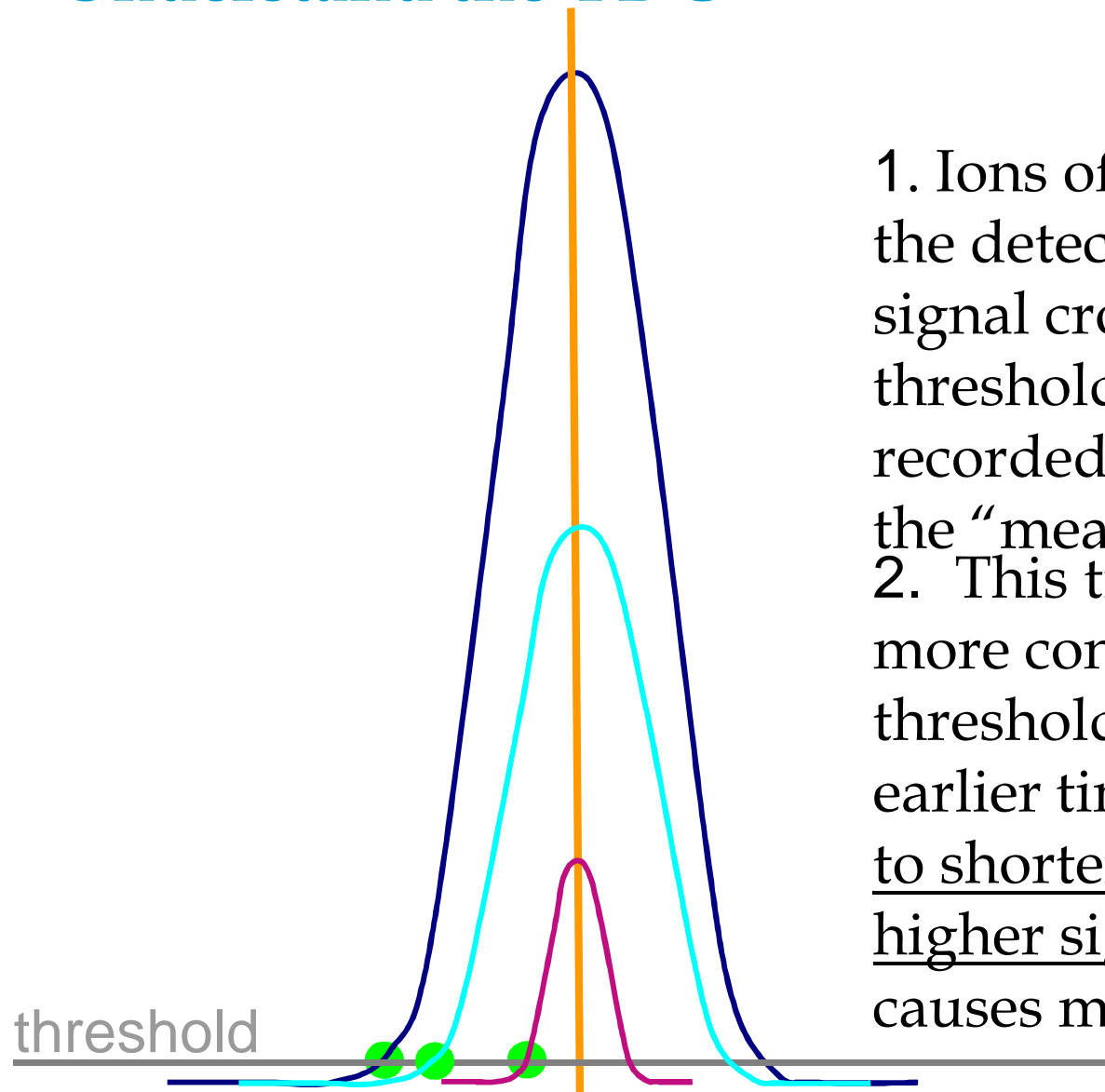


Why is Accurate Mass Useful?

Accurate masses give possible elemental compositions

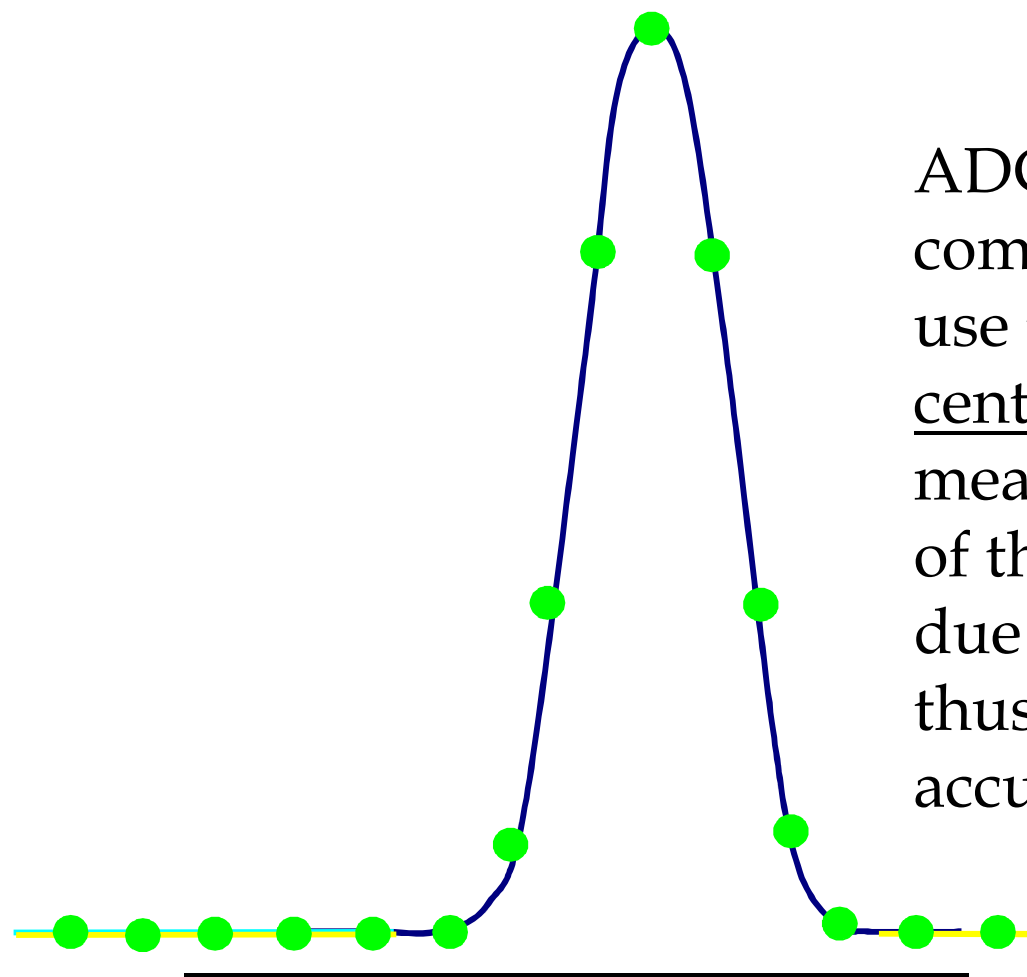


Understand the TDC



1. Ions of a compound arrive at the detector. The resulting signal crosses a preset TDC threshold and the arrival time is recorded. This time is related to the “measured mass”.
2. This time the sample is more concentrated. The TDC threshold thus triggers at an earlier time. This causes a shift to shorter arrival time for higher signal level. Therefore, causes mass assignment error.

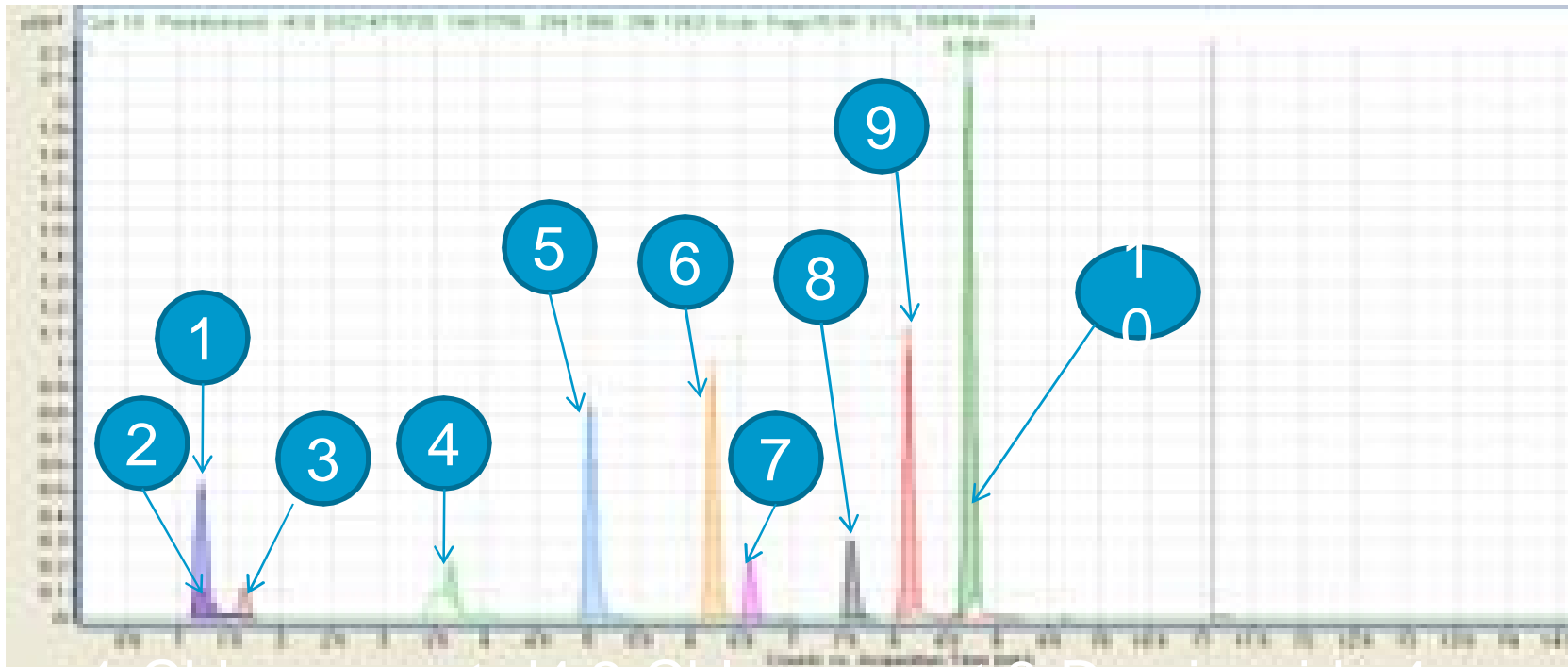
Understand the ADC



ADC systems measure the complete profile of ions and use the flight time at the centroid of the profile to assign measured mass. The location of the centroid will not change due to sample concentration, thus, maintains the mass accuracy.

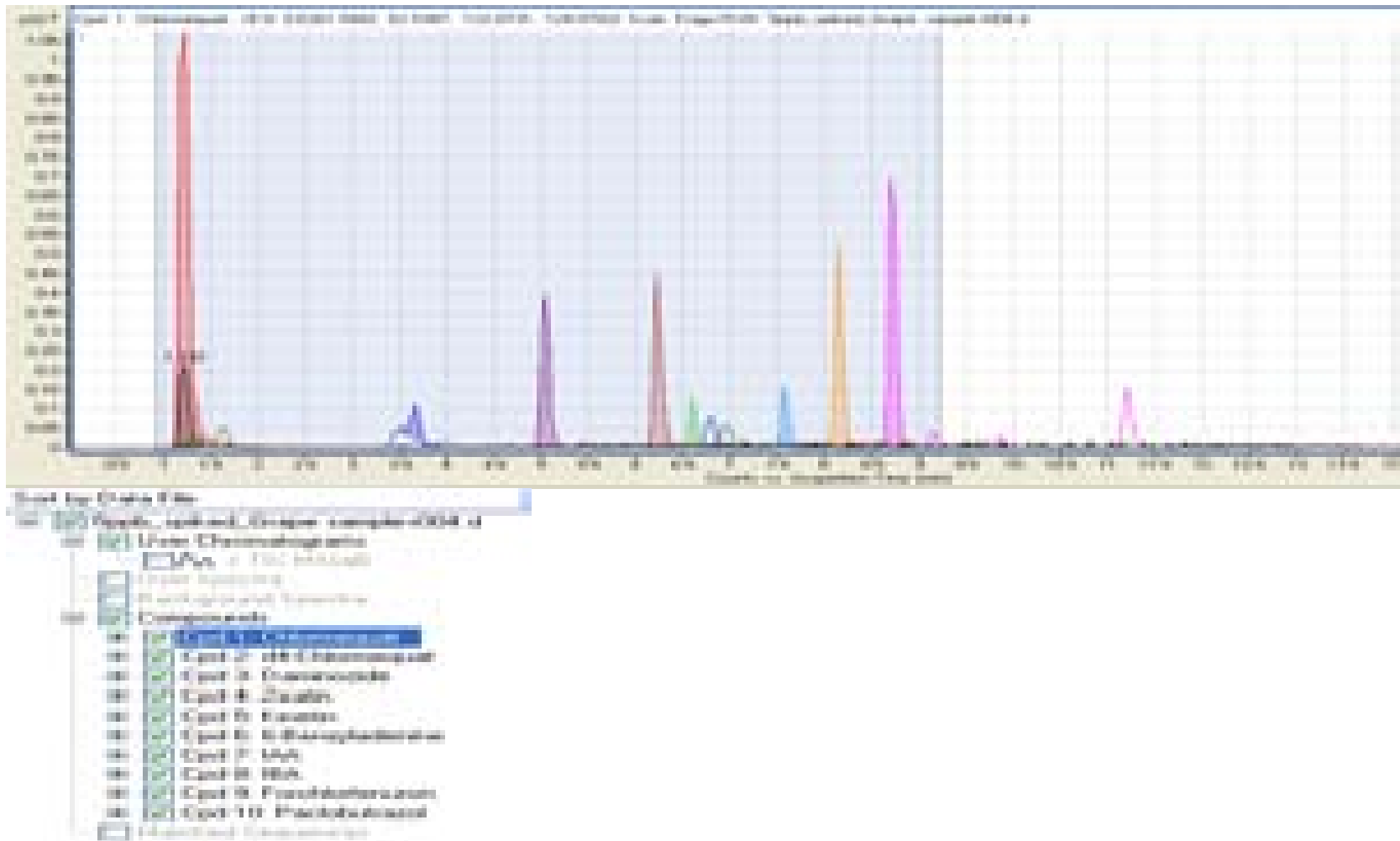
Ion Flight Time: Is the centroid of all detected ions

10 PGRs standards using Q-TOF

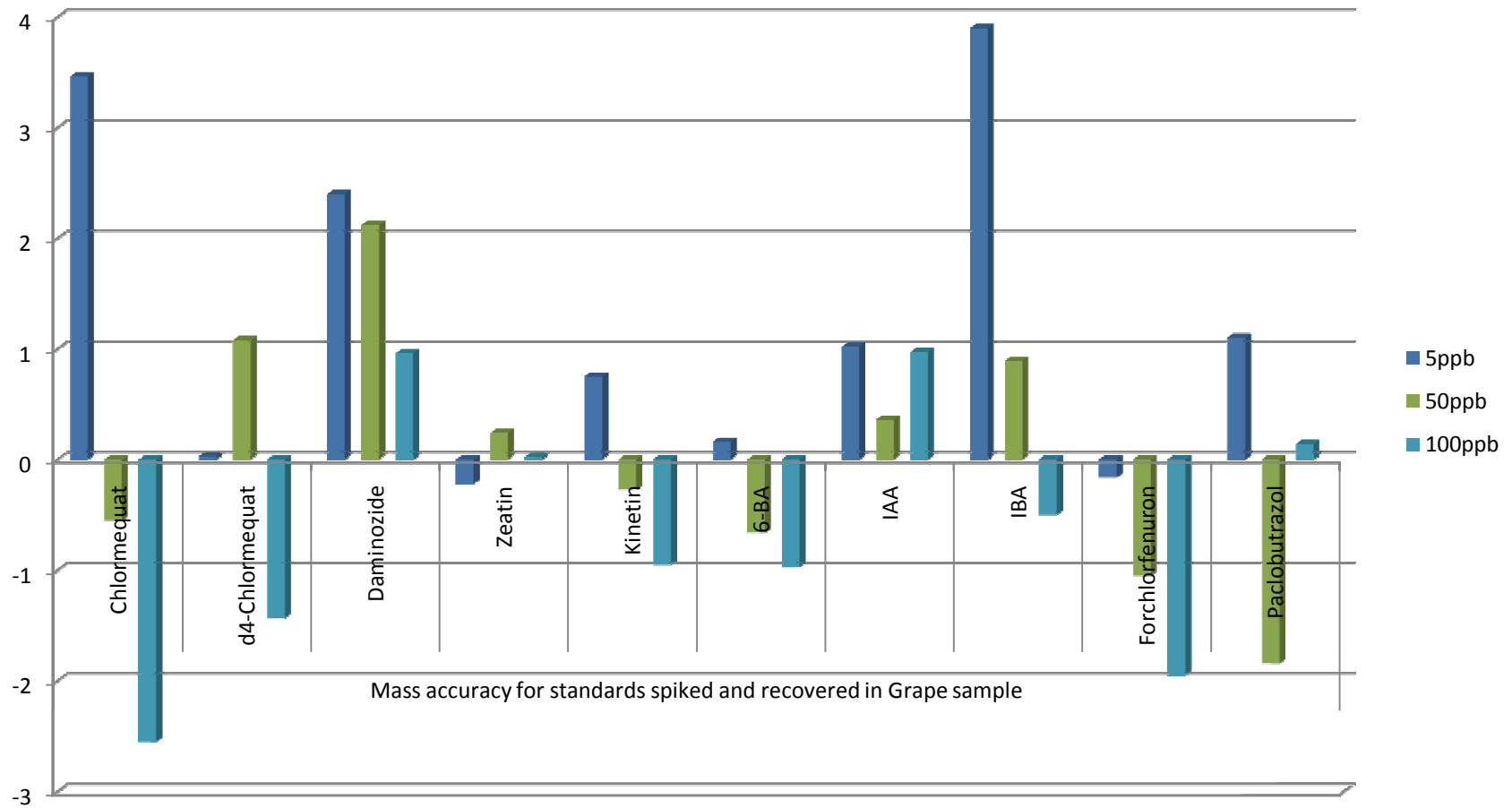


Zeatin 5-Kinetin
6-6 Benzyladenine 7-IAA 8-IBA 9-Forchlorfenuron

Screening for 10 PGRs spiked and extracted from Grape sample at 5ppb



Mass accuracy for standards spiked and extracted from Grape sample (ppm)



Resolution

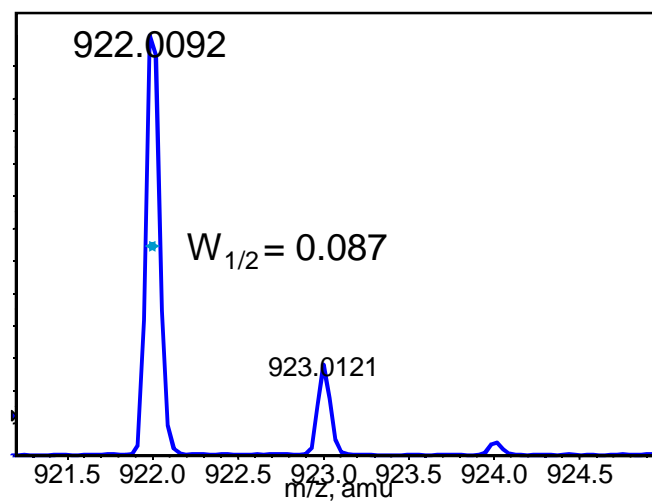


Formulae for Resolution

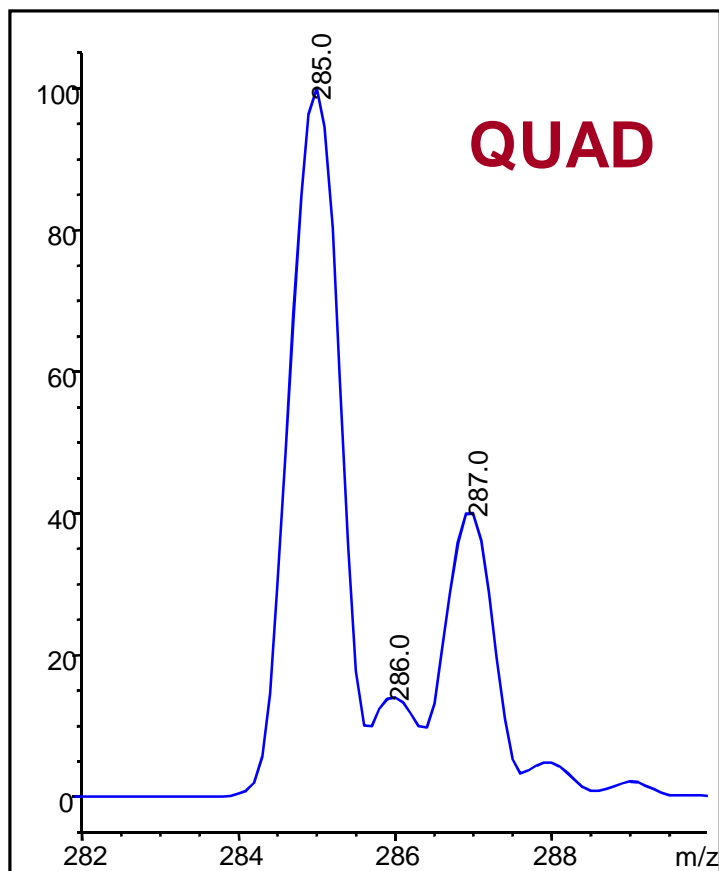
$$\text{Resolution} = (m/z)/w_{1/2}$$

$$1000/0.1 = 10,000$$

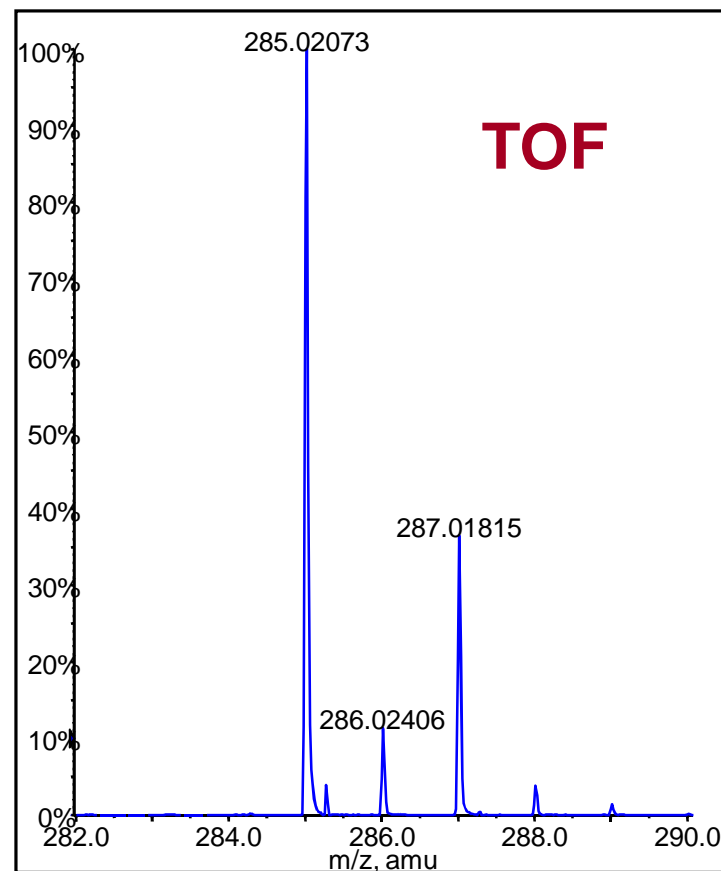
$$922/0.087 = 10,597$$



Resolution comparison of quadrupole and TOF



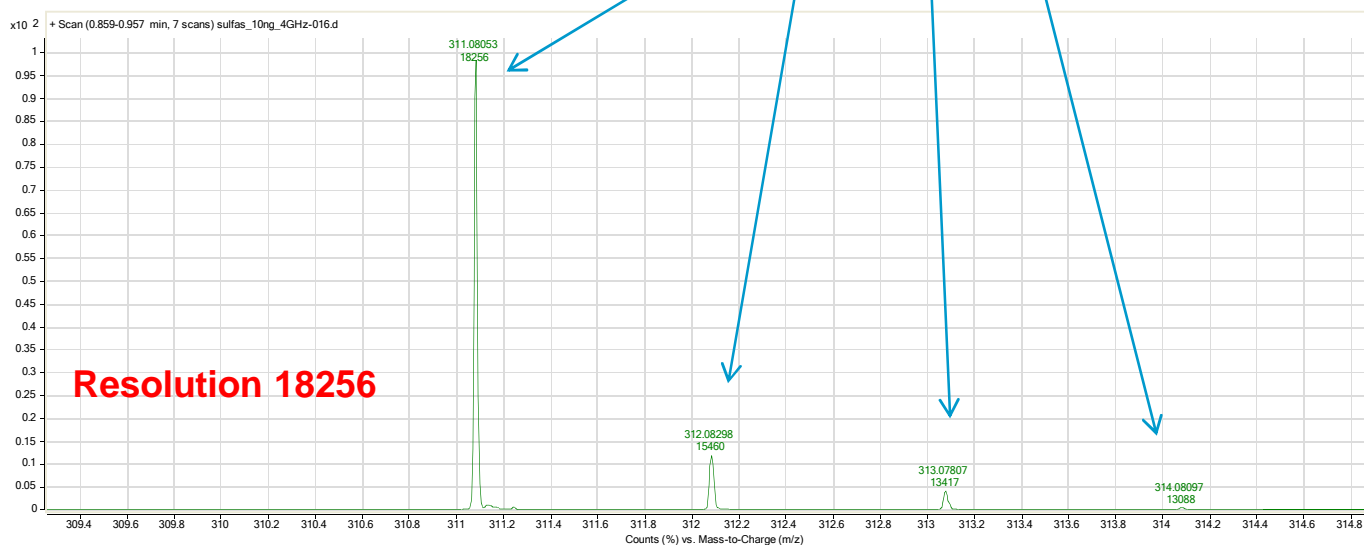
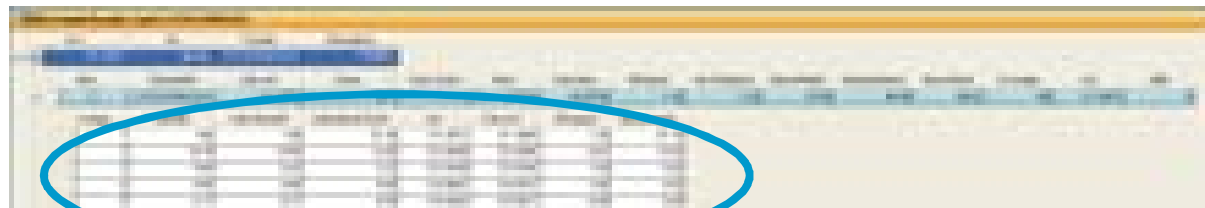
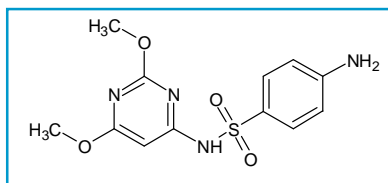
Mass axis stepped in 0.1 m/z increments
No centroid interpolation done



Mass axis stepped in 0.009 m/z increments
Advanced centroiding performed

Molecular Formula Generation from Isotopic Pattern

Sulfadimethoxine



Note: Also need dynamic range!!!

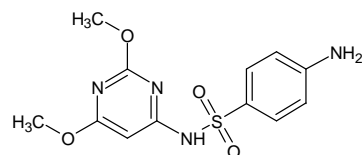
Molecular Formula Generation from Isotopic Pattern

MS Formula Results: Cpd5: C12H14N4O4S

m/z	Ion	Formula	Abundance
311.0813	(M+H) ⁺	C12H15N4O4S	175496.1

Best	Formula (M)	Calc m/z	Score	Cross Score	Mass	Calc Mass	Diff (ppm)
<input checked="" type="checkbox"/>	C12H14N4O4S	311.0809	98.7		310.074	310.0736	-1.29

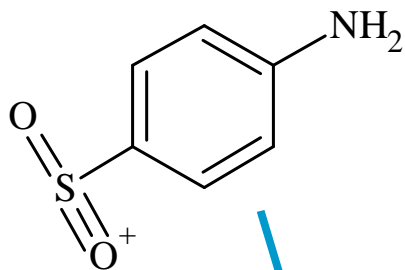
Isotope	Abund%	Calc Abund%	Calc Abund Sum%	m/z	Calc m/z	Diff (ppm)	Abund Sum%
1	100	100	81.35	311.0813	311.0809	-1.29	81.15
2	16.78	15.55	12.65	312.0835	312.0835	-0.01	13.62
3	5.69	6.43	5.23	313.0789	313.0794	1.44	4.62
4	0.65	0.84	0.68	314.0801	314.0812	3.46	0.53
5	0.11	0.11	0.09	315.0824	315.0821	-0.96	0.09



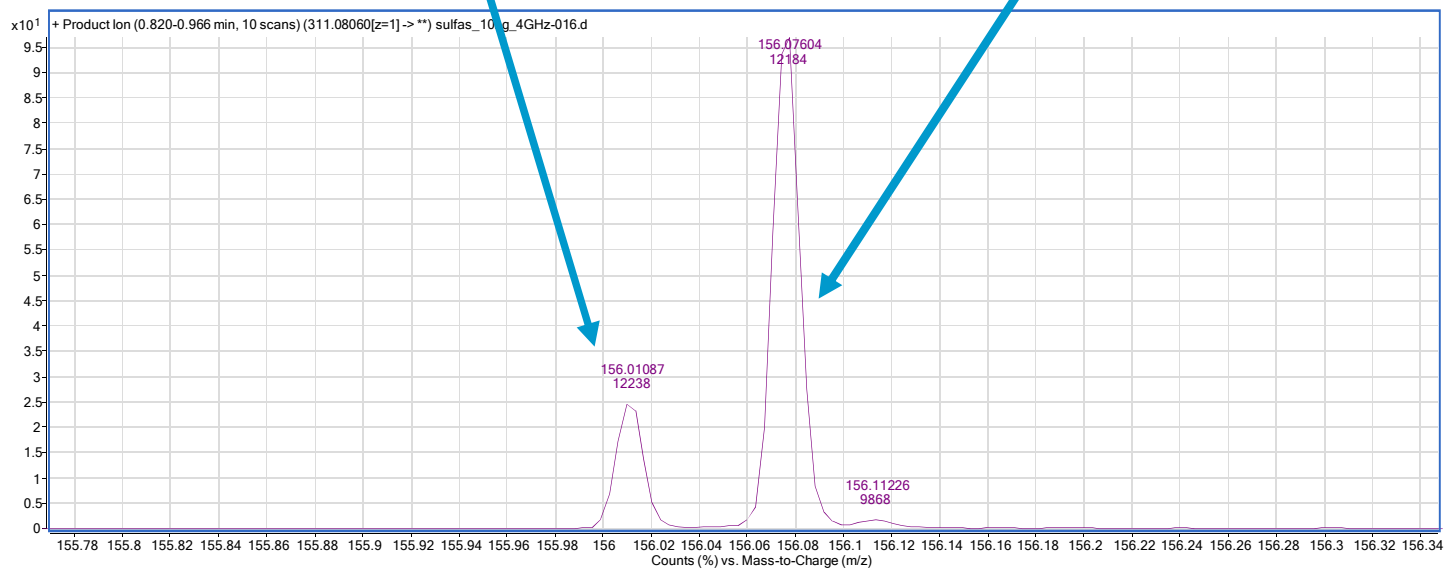
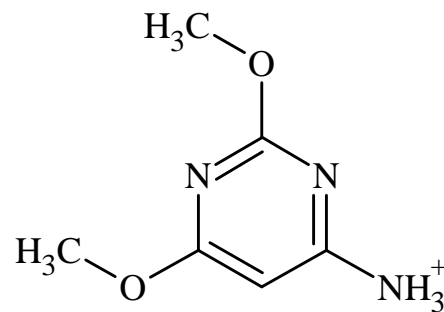
Abs Diff (ppm)	Abund Match	Spacing Match	Mass Match	Coverage	m/z	DBE
1.29	97.65	98.48	98.87	100	311.0813	8

Sulfadimethoxine - MS/MS Fragmentation

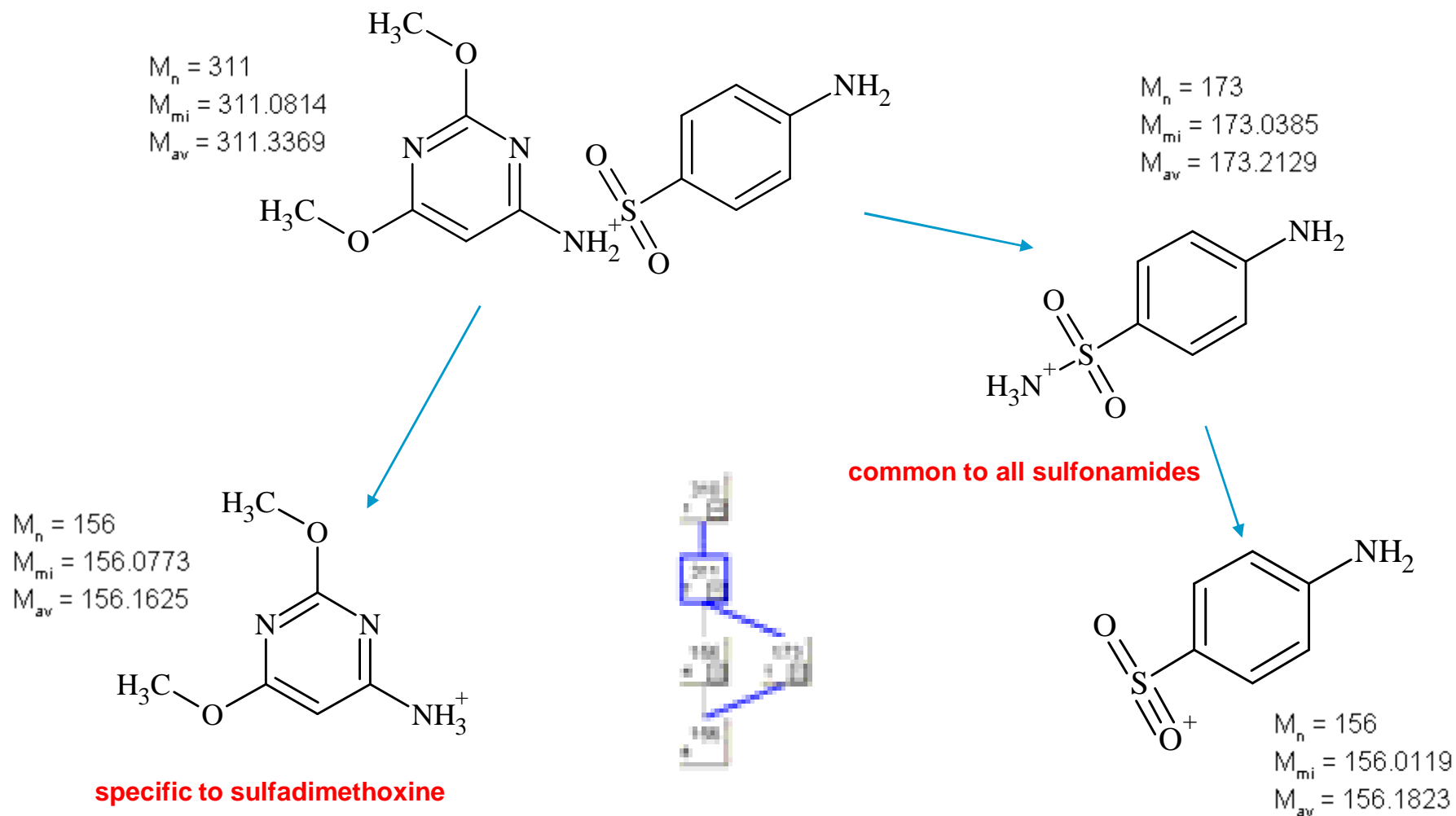
m/z 156.0119



m/z 156.0773



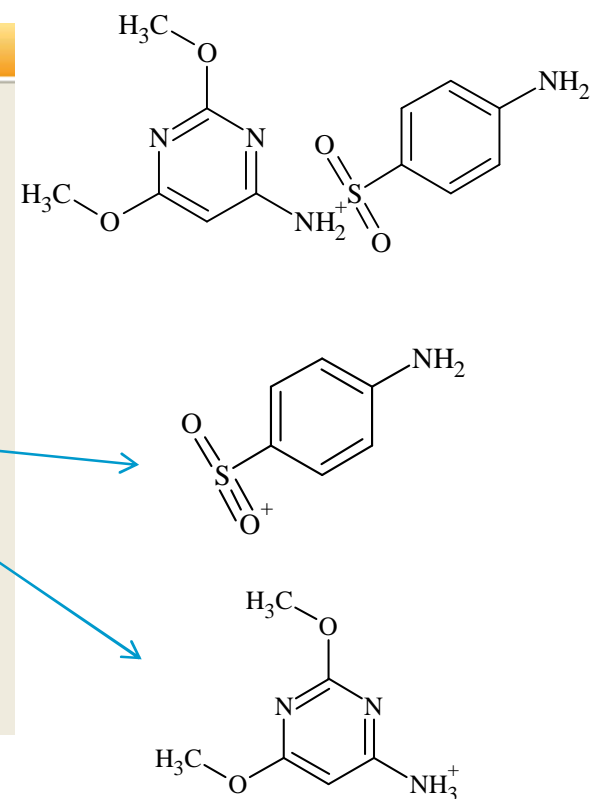
MS/MS Fragmentation Pathways



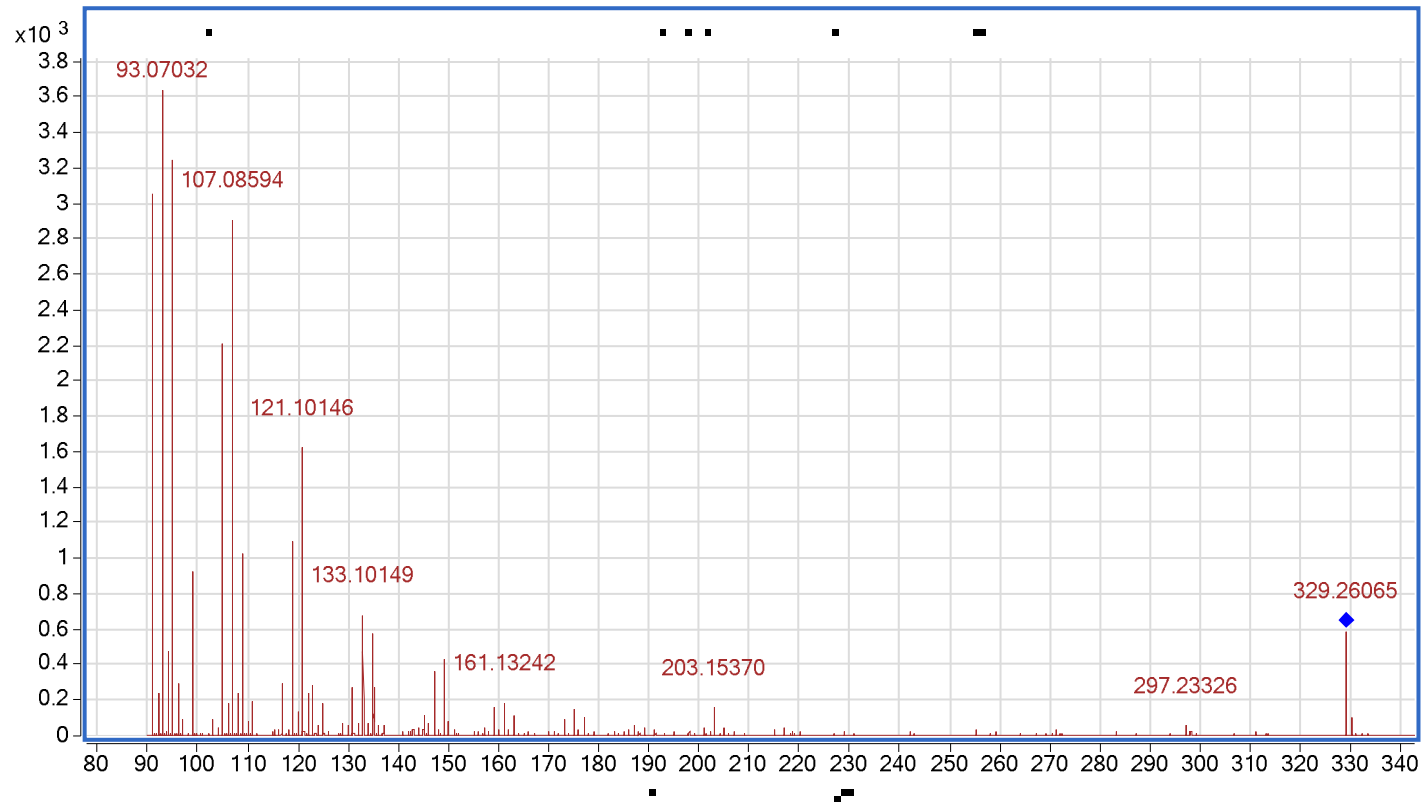
Elemental Compositions of Observed Signals

MS/MS Formula Details: Cpd5: C12H14N4O4S C12H14N4O4S

m/z	Δ	Formula	Abund%	Diff (ppm)	Loss Mass	Loss Formula
92.0497		C6H6N	10.4	-2.22	219.0314	C6H9N3O4S
92.0497		C3H10NS	10.4	34.4	219.028	C9H5N3O4
108.0442		C6H6NO	12.36	1.84	203.0365	C6H9N3O3S
108.0442		C3H10NOS	12.36	33.04	203.0331	C9H5N3O3
108.0442		CH6N3O3	12.36	-35.39	203.0405	C11H9NO3S
155.0683		C8H11O3	2.05	12.89	156.0106	C4H4N4O3S
155.0683		C7H11N2S	2.05	-29.19	156.0171	C5H4N2O4
156.0108		C6H6NO2S	25.04	3.94	155.0695	C6H9N3O2
156.0108		C9H2NO2	25.04	-17.67	155.0728	C3H13N3O2S
156.076		C6H10N3O2	37.91	4.96	155.0041	C6H5NO2S
218.0222		C6H8N3O4S	5.32	3.71	93.0578	C6H7N
218.0222		C9H4N3O4	5.32	-11.75	93.0612	C3H11NS
245.1027		C12H13N4O2	6.92	2.32	65.9776	H2O2S

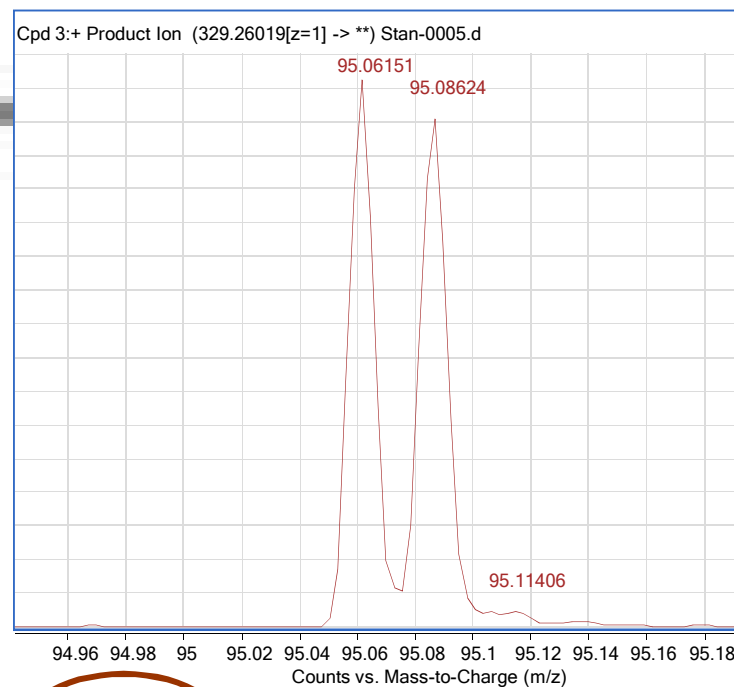
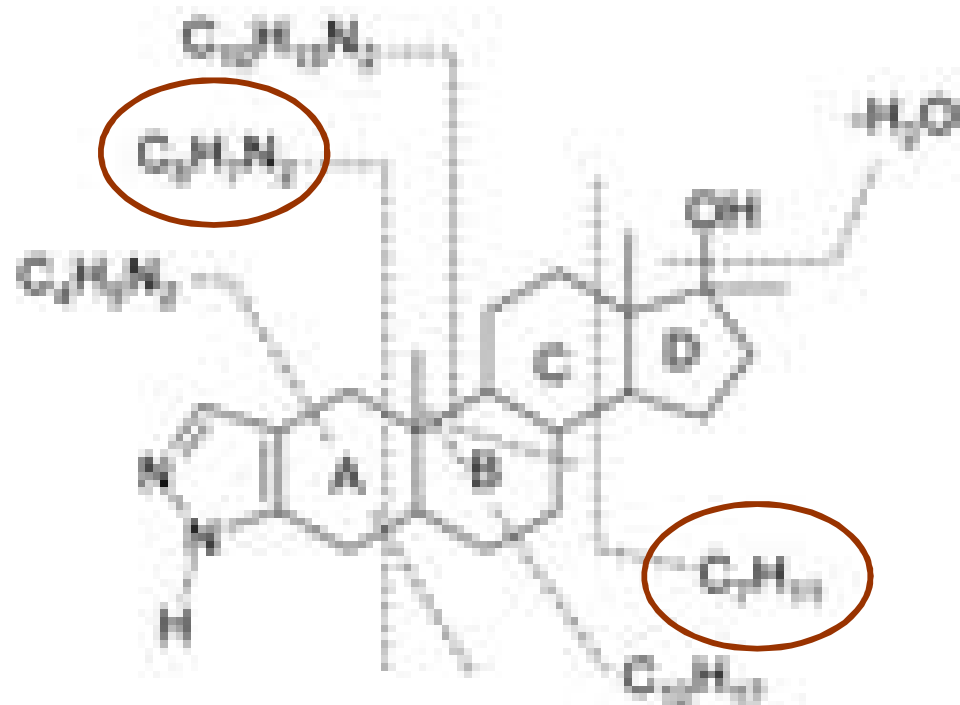


Resolution in MS/MS - Stanozolol



Resolution in MS/MS – Stanozolol

Fragmentation – 25 mDaltons



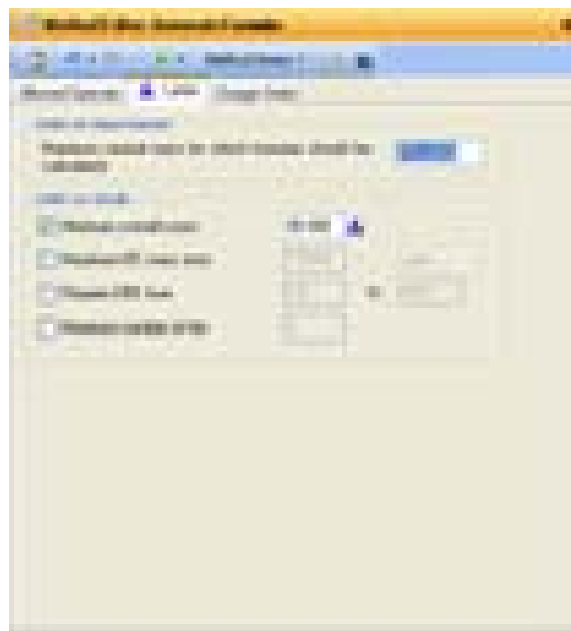
				Formula	Loss Mass	Loss Formula	Abund
95.06151	95.06037	-1.13	-11.93	$C_5H_7N_2$	234.19837	$C_{16}H_{26}O$	3495
95.08624	95.08553	-0.72	-7.53	C_7H_{11}	234.17321	$C_{14}H_{22}N_2O$	3337
119.0604	119.06037	-0.02	-0.18	$C_7H_7N_2$	210.19837	$C_{14}H_{26}O$	357
119.08578	119.08553	-0.26	-2.15	C_9H_{11}	210.17321	$C_{12}H_{22}N_2O$	1990
135.09166	135.09167	0.02	0.11	$C_8H_{11}N_2$	194.16707	$C_{13}H_{22}O$	628
135.11642	135.11683	0.41	3.05	$C_{10}H_{15}$	194.14191	$C_{11}H_{18}N_2O$	389
147.09201	147.09167	-0.33	-2.28	$C_9H_{11}N_2$	182.16707	$C_{12}H_{22}O$	376
147.11785	147.11683	-1.02	-6.96	$C_{11}H_{15}$	182.14191	$C_{10}H_{18}N_2O$	301
161.10823	161.10732	-0.9	-5.6	$C_{10}H_{13}N_2$	168.15142	$C_{11}H_{20}O$	112
161.13242	161.13248	0.05	0.32	$C_{12}H_{17}$	168.12626	$C_9H_{16}N_2O$	190

Strict Database search parameters to minimize False Positives

Mass Accuracy



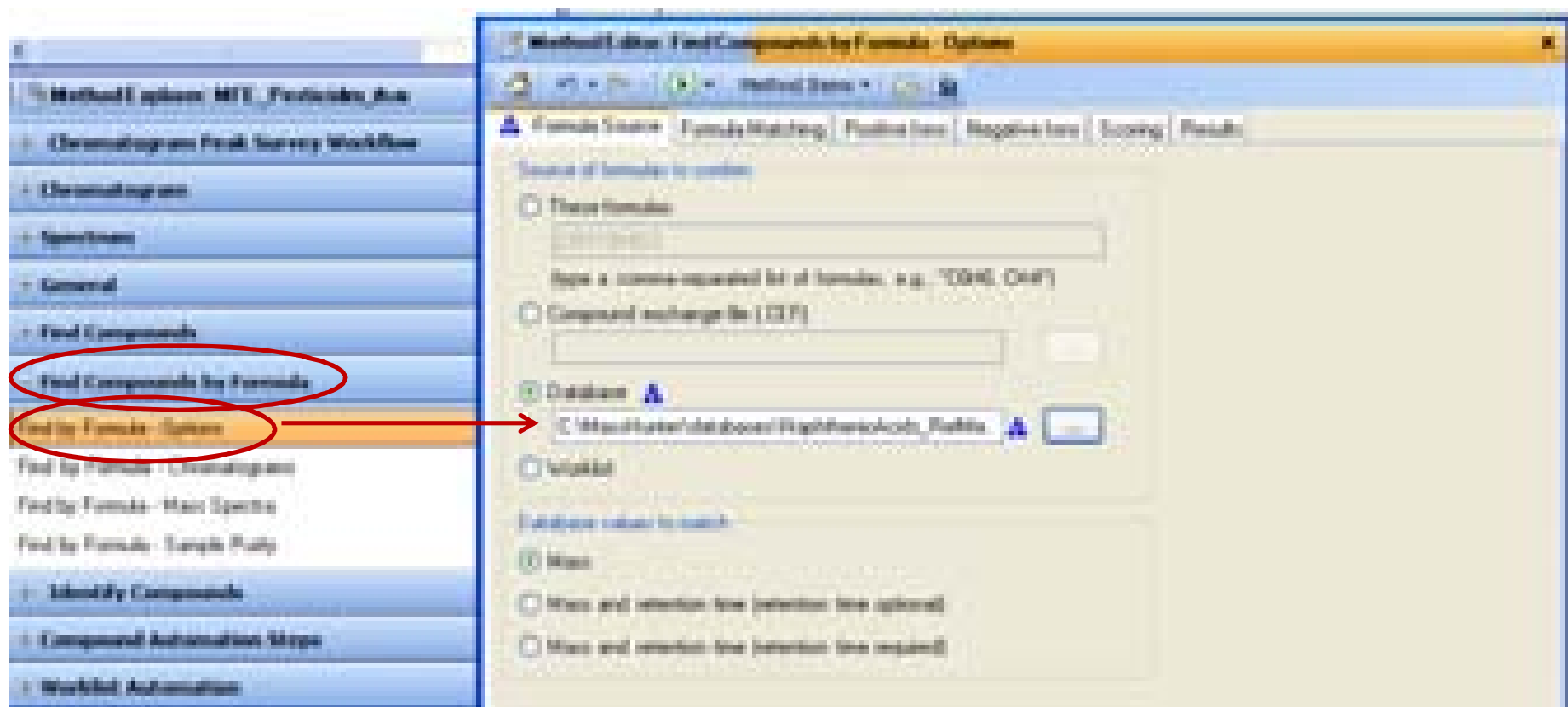
Isotopic pattern score



Retention times can be added to database for stricter matches



Search Your Own Database - Excel



Sensitivity



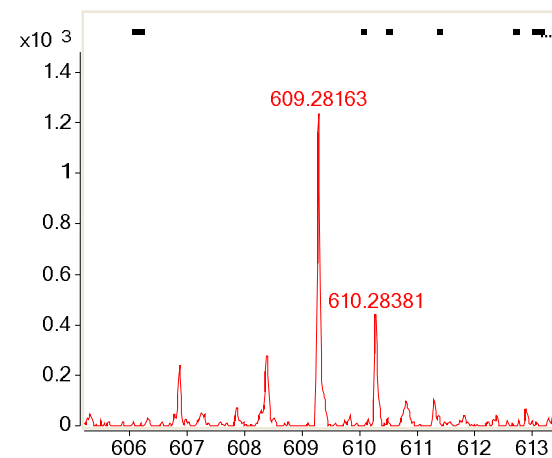
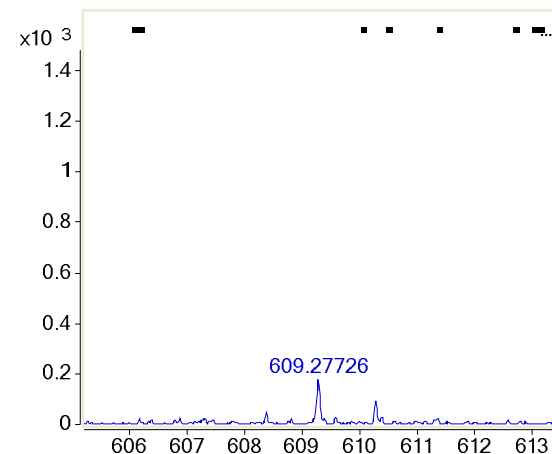
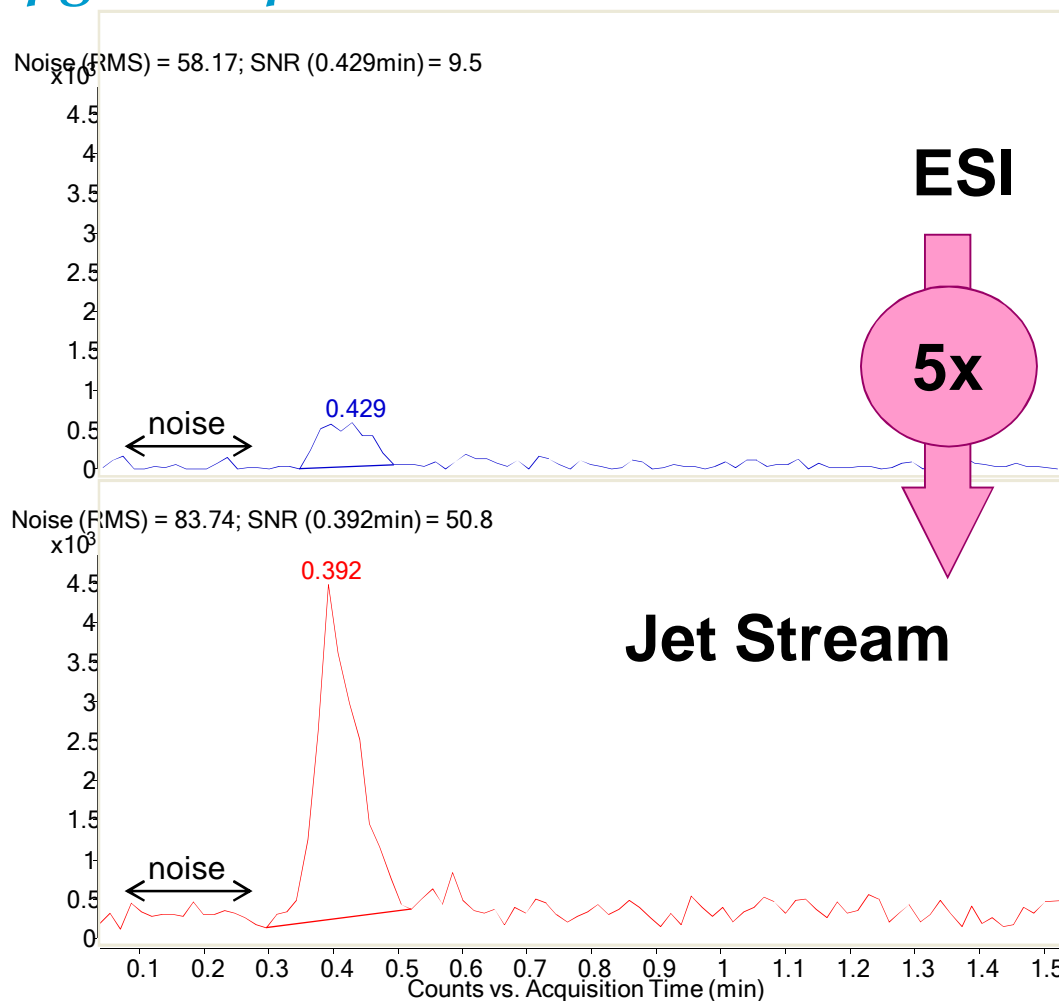
Sensitivity

All of it matters!!!

- 1: Ion generation
- 2: Heated drying gas and dielectric capillary
- 3: Octopole ion guides
- 4: RF ion lenses
- 5: Hyperbolic quadrupole
- 6: Three-stage ion detection
- 7: ADC

QTOF Sensitivity with Jet Stream

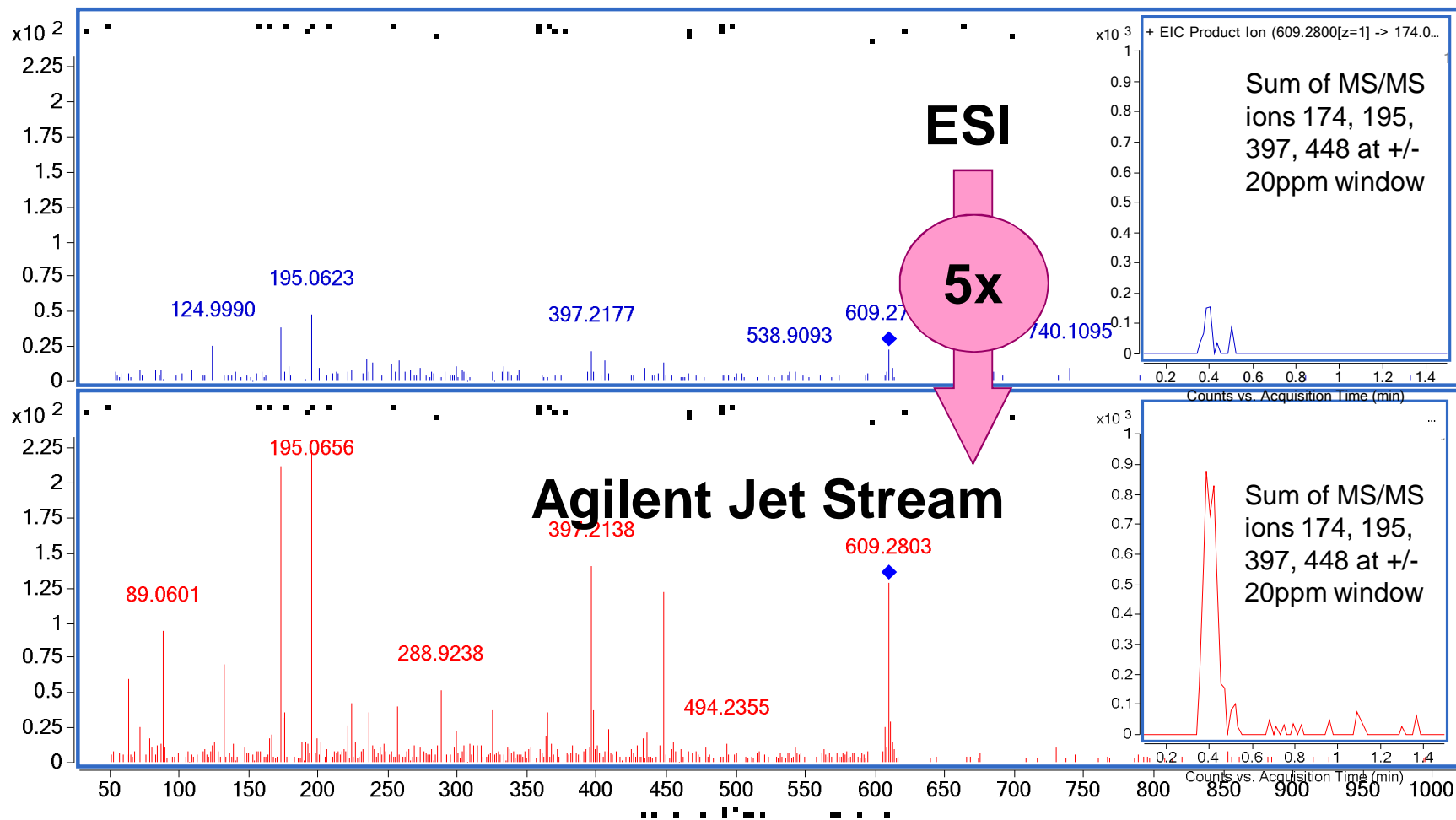
1 pg Reserpine, MS S/N 10:1



These data were acquired with 75/25 MeOH/water on a 2.1x30mm 3.5um column. Final specs will use ACN/water and a 1.8um column and be common with the 6460 QQQ checkout

QTOF Sensitivity with Jet Stream

1 pg Reserpine, MS/MS



These data were acquired with 75/25 MeOH/water on a 2.1x30mm 3.5um column. Final specs will use ACN/water and a 1.8um column and be common with the 6460 QQQ checkout

Dynamic Range

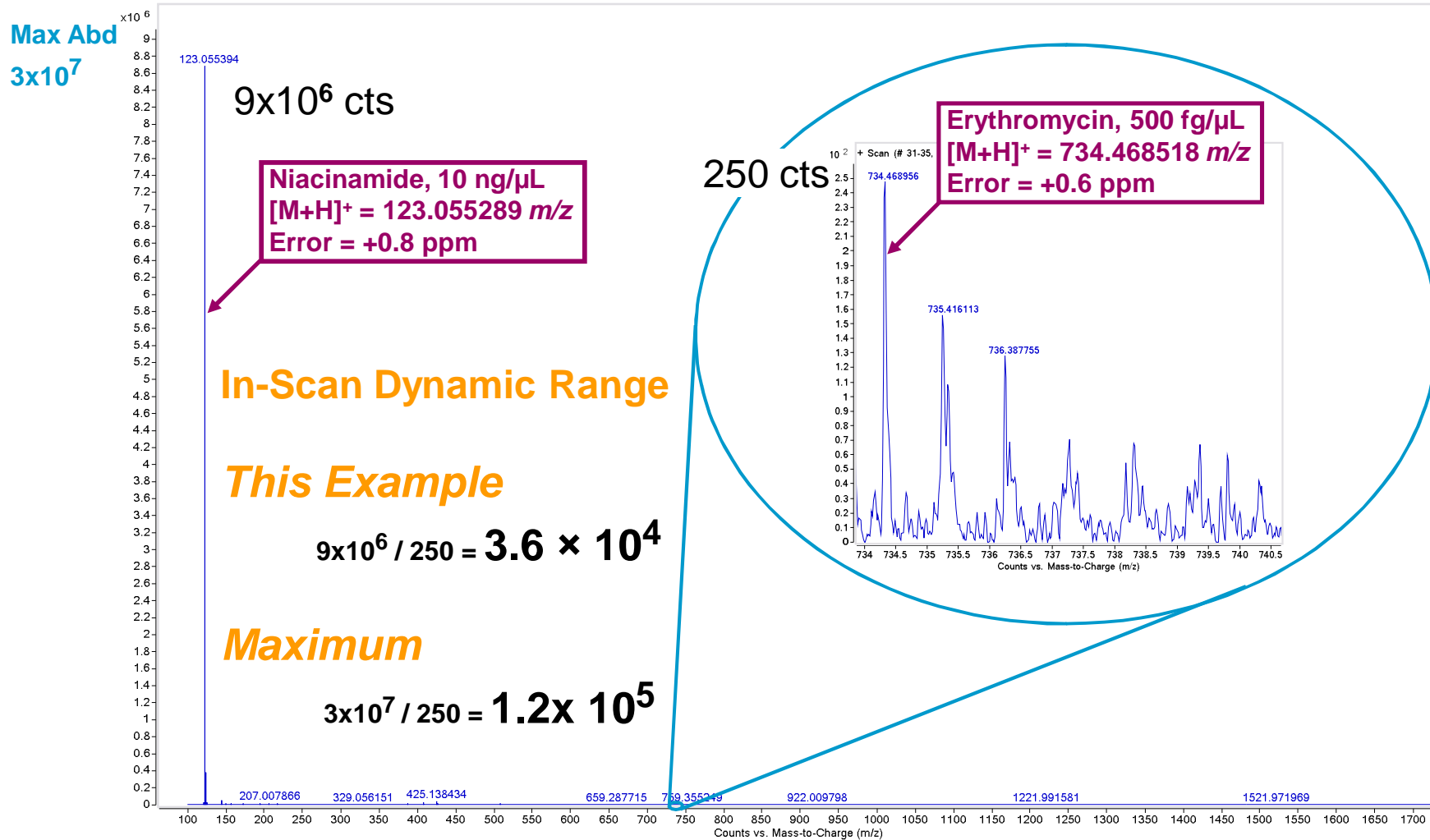


Sensitivity
+
**ADC/TDC
technology**
+
High speed electronics
=
Dynamic Range



New 4GHz Acquisition System

up to 5 Decades of In-Spectrum Dynamic Range



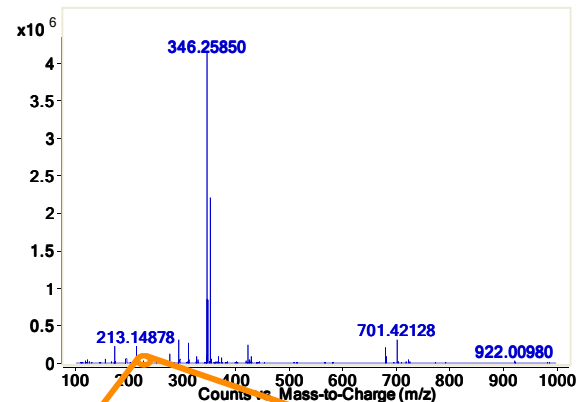
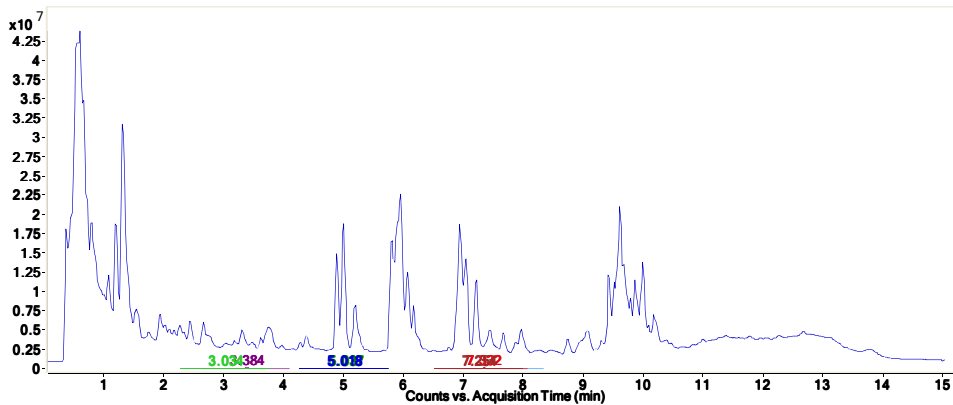
Triazines Spiked Into Tomato Matrix, 500 ppt

6530 QTOF, MS Mode, Extended Dynamic Range

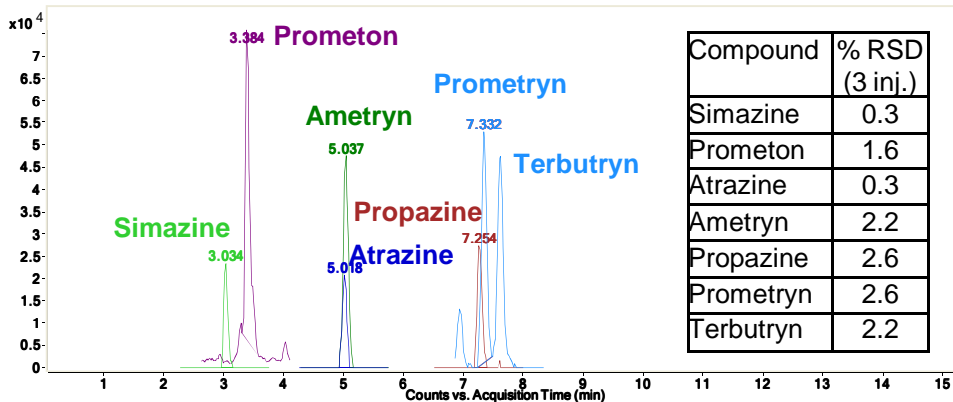
Notice 4×10^6 signal

Spectrum at 5.018 min

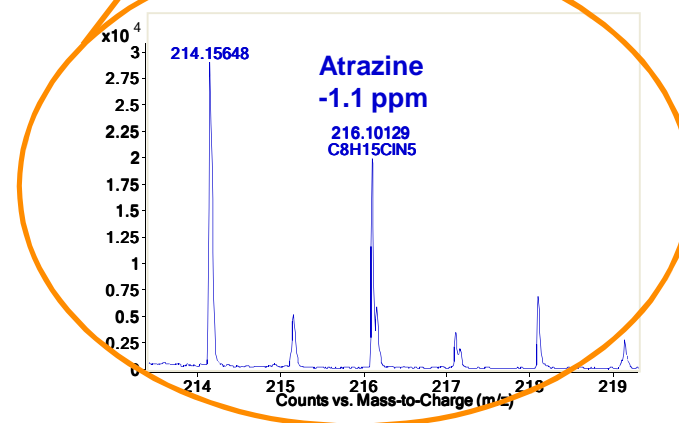
TIC



EICs



Zoom view



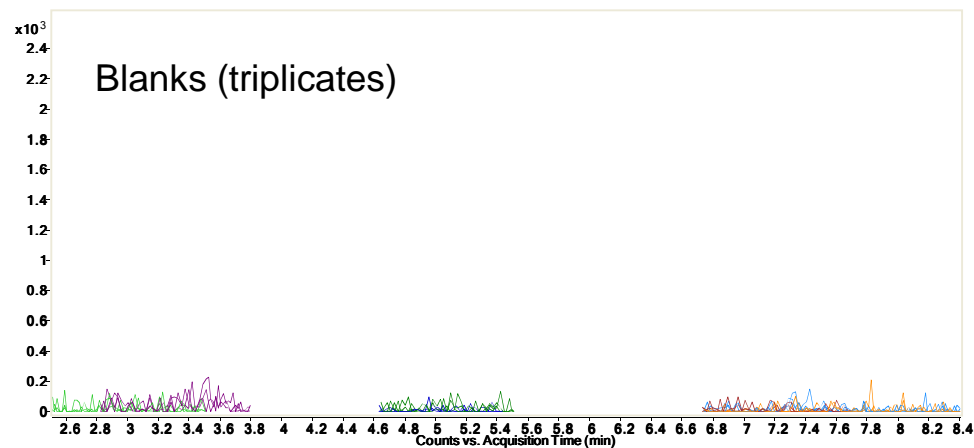
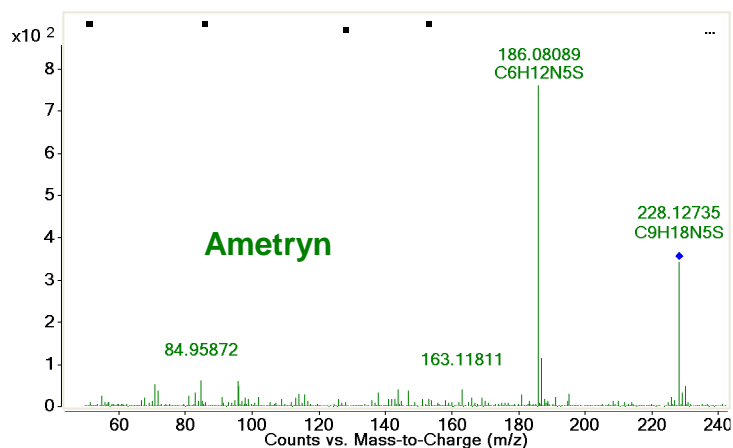
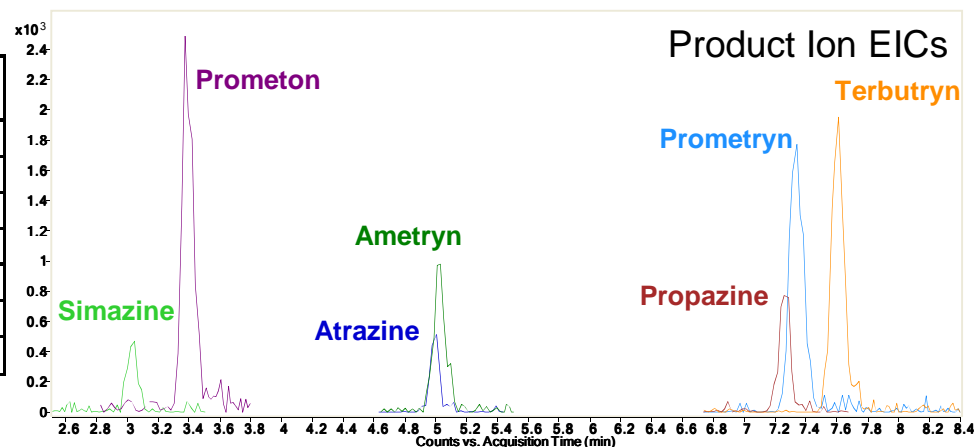
Note: on-column amount is 5 pg (10 μ L x 500 ppt).
Identification done by database searching

20,000 cts

Triazines Spiked Into Tomato Matrix, 100 ppt 6530 QTOF, Targeted MS/MS Mode

On-column amount is 1 pg (10 μ L x 100 ppt)

Compound	Precursor	Product Ion(s)	Collision energy, V	% RSD (3 inj.)
Simazine	202.0849	124.0868+132.0317	20	13.2
Prometon	226.1662	142.0717+184.1189	20	1.5
Atrazine	216.1010	174.0539	20	16.0
Ametryn	228.1277	186.0803	20	10.1
Propazine	242.1435	158.0487+200.0962	20	17.2
Prometryn	230.1177	146.0226+188.0692	25	14.2
Terbutryn	242.1435	186.0803	20	12.6



Questions??????????

