Welcome to our E-Seminar:

The API-TOF Advantage – Routine Mass Accuracy and High Resolution

Agilent Technologies

Agilent LC/MSD TOF

High Resolution -Accurate Mass by LC/ESI-TOF

Accurate Mass provides

- empirical formula
  - identification and
  - Confirmation
- Added Selectivity
  - Resolution to separate isobaric compounds
  - All spectra automatically calibrated for mass peaks within 5 ppm of exact mass

How Much Accuracy in Needed?

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

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

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

165 ppm	**209**
10 ppm	**13**
5 ppm	**7**
3 ppm	**4**
2 ppm	**2**

Accurate mass reduces risk of investing effort on the wrong molecule

The Potential TOF Advantage

Routine Mass Accuracy < 3ppm

- **No user interaction required**
- **Delivered over broad dynamic range**
- **Immune to broad swings in laboratory environment**

Resolution > 4000 at low mass, > 10,000 at higher masses

Fast scanning (> 5/second) over broad mass range

Confirmation with Accurate Mass

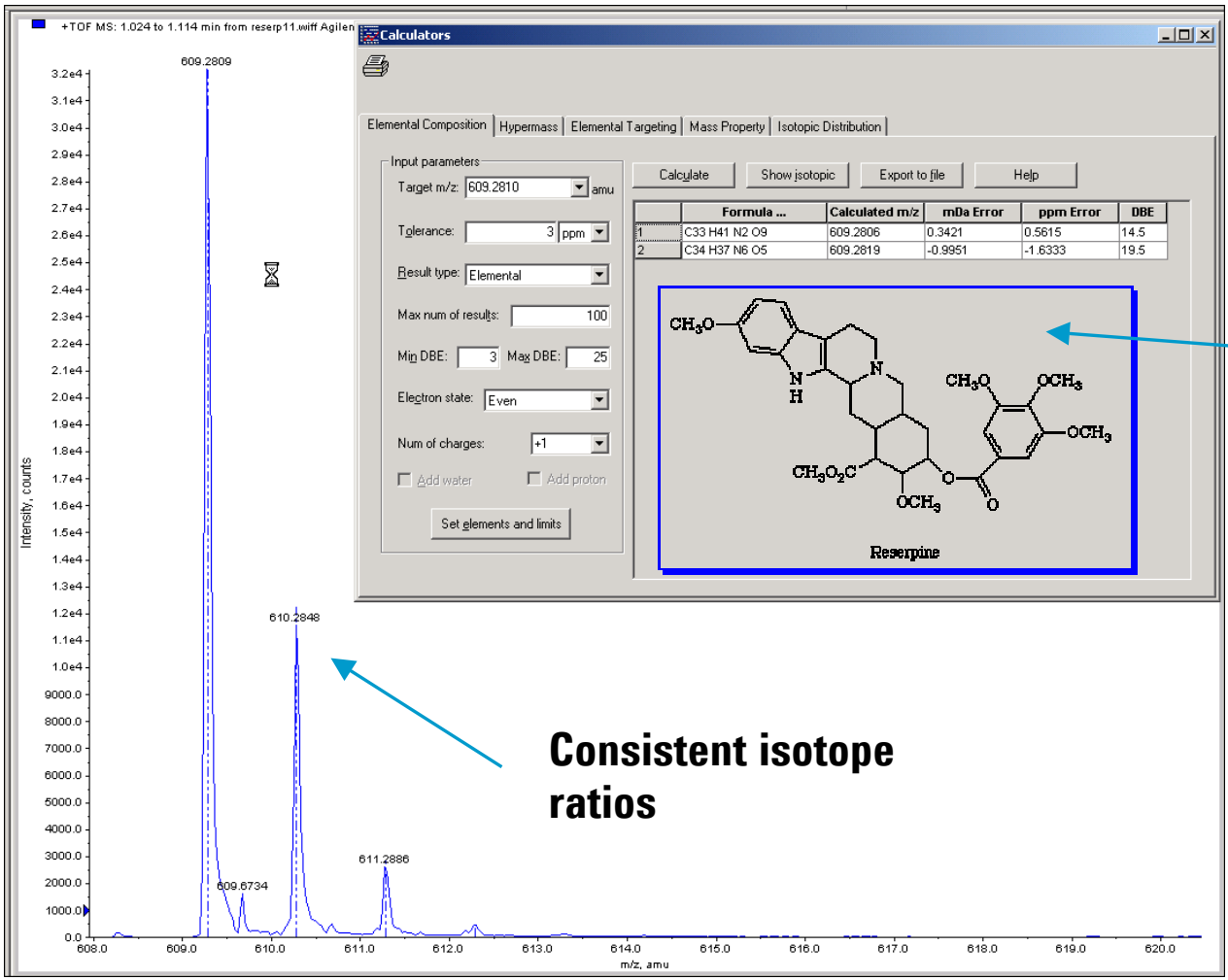
Accurate mass determination provides a level of selectivity independent of fragments from an MS/MS experiment

Determination of empirical formula

An accurate mass measurement does not guarantee a unique empirical formula at 5 ppm confidence level

Usually other information can be combined with TOF data to raise certainty

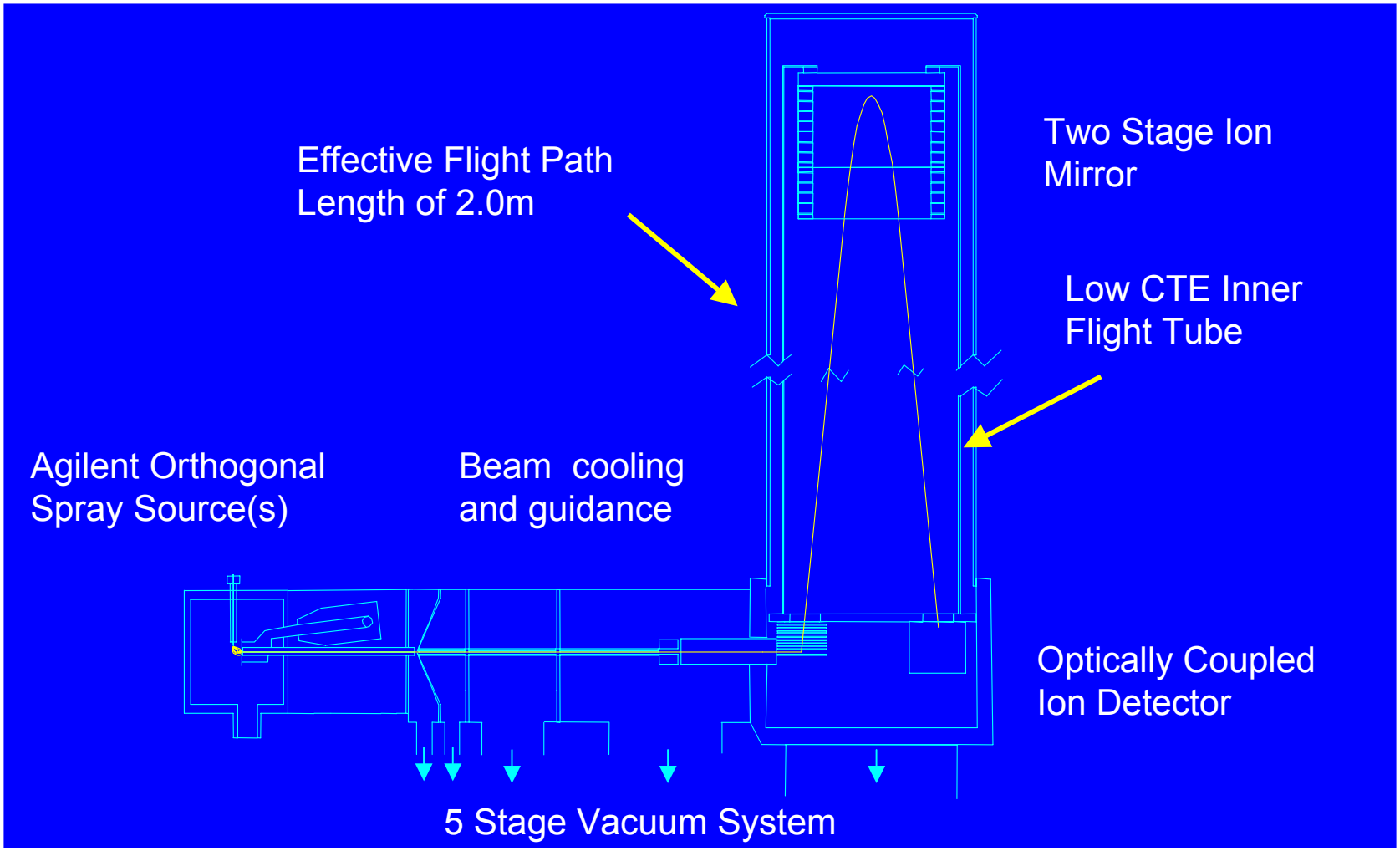
Reserpine Confirmation



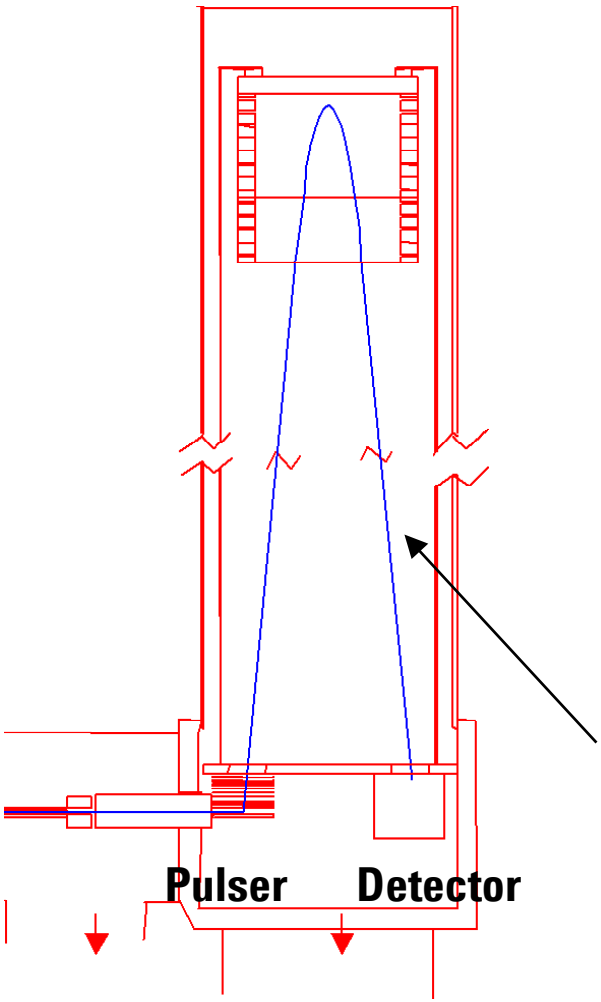
14 double bond equivalents (DBE)

Consistent isotope ratios

Agilent Design Optimizes Ion Transmission and Mass Resolving Power



A Time of Flight Scan



1. Pulse ions every 100 microseconds
2. Measure at detector each nanosecond
3. 100,000 data points in each *transient*
4. Sum 2000 – 10000 transients into one scan
5. Produces spectra with excellent ion statistics

20 µsec – *m/z* 118

46 µsec - *m/z* 622

90 µsec - *m/z* 2421

Some Key Hardware Design Features

Uses Agilent orthogonal spray sources and ion optics for superior sensitivity and flow rate range

Flight tube designed with minimal coefficient of thermal expansion

Temperature sensitive electronic components placed in temperature controlled environment

ADC digitization for maximum mass accuracy over broad dynamic range

Use CDS and dual sprayer for internal reference mass correction

Fundamentals of TOF Mass Assignment

Agilent Goal: Routine high mass accuracy

Hardware designed for highly stable mass measurement

Hardware and software for transparent internal reference mass correction

Mathematical error in mass assignment & calibration mass accuracy within ~1 ppm for peak finder & mass assignment algorithms

- **Traditional algorithms can have errors > 10 ppm with discrete data.**
- **New algorithm has intrinsic mass errors < 1 ppm with discrete data and non-symmetric peaks**

TDC versus ADC

Time to Digital Conversion (TDC) only measures time of arrival of first ion at a given m/z value

More sample means more ions means earlier arrival

Requires higher acquisition rate than ADC and peak intensity matching to accurately assign mass

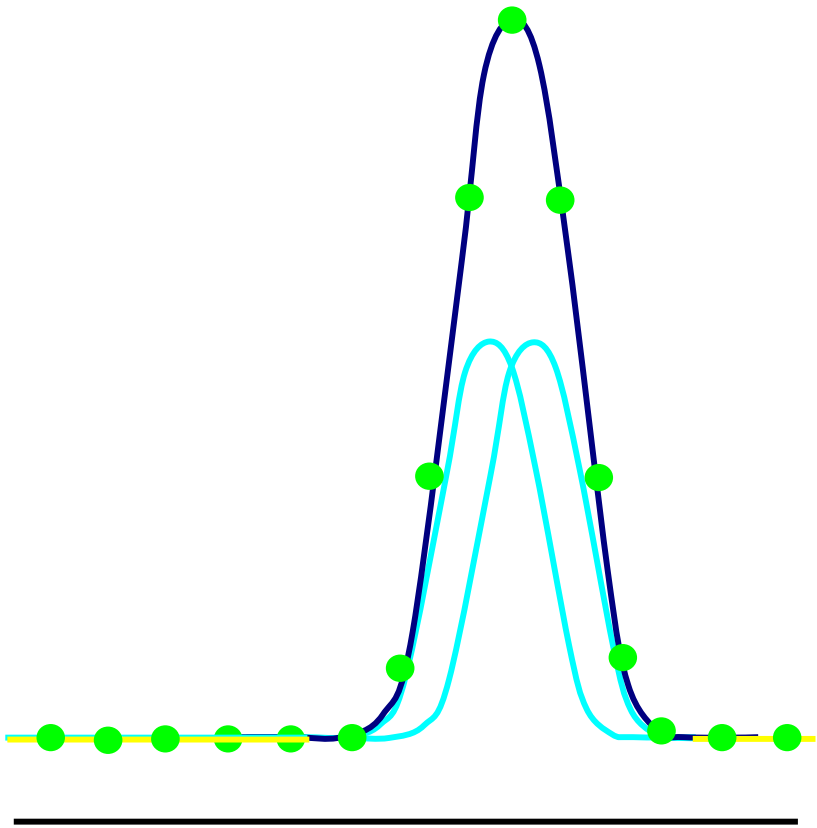
Reduced dynamic range

Analog to Digital Conversion (ADC) records time and number of ions arriving

Sample concentration does not impact (maximum) arrival time

Provides wider dynamic range

Understanding the TDC and ADC



Ion Flight Time: Is the centroid of all detected ions

1. Single ion arrives at the detector. The resulting signal crosses a preset TDC threshold and the arrival time is recorded.

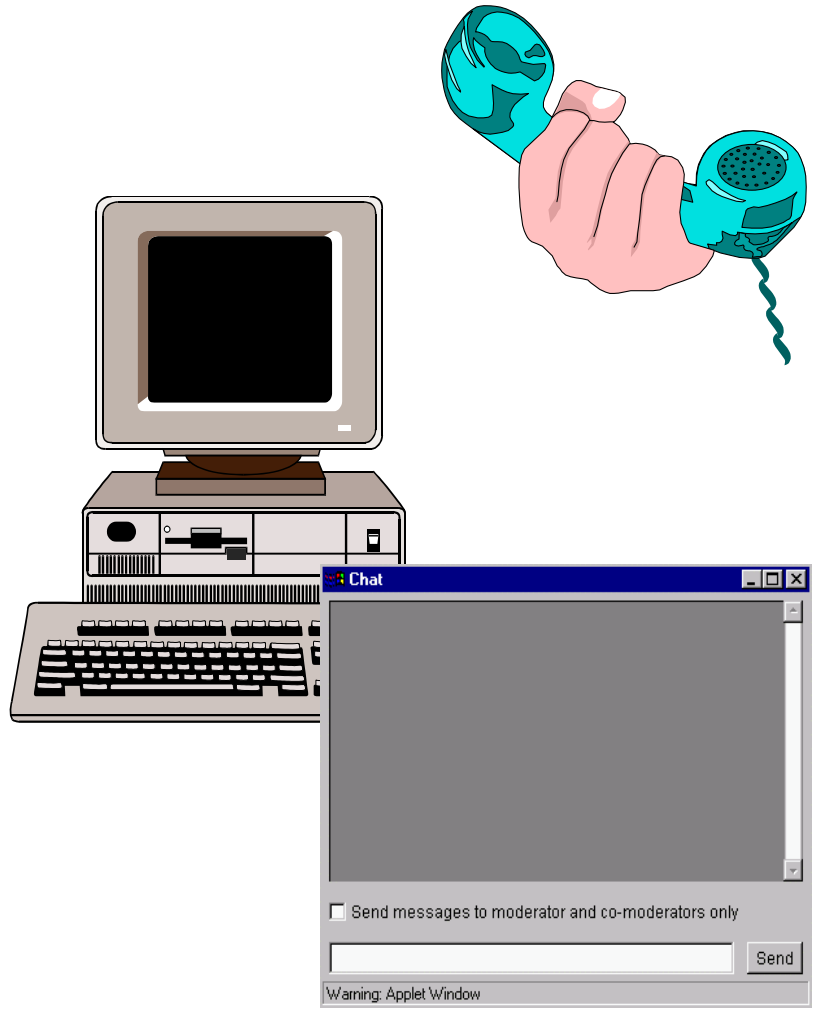
2. This time the sample is more concentrated and two ions arrive slightly spaced in time as determined by the instrument's resolving power. The yellow line is the resulting signal.

But, the TDC threshold triggers off the first ion and the arrival of the second ion is not detected. This causes as shift to shorter arrival times for higher signal levels.

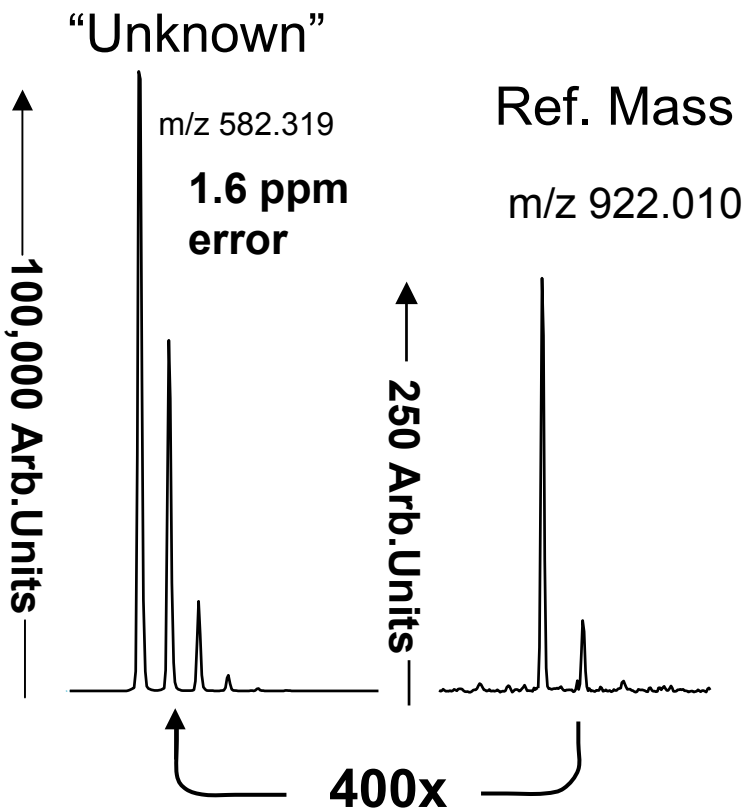
3. ADC acquisition make a conversion each 0.001usec of the total signal. ADC systems do not drop ions or suffer from "dead time" effects.

Break Number 1

For questions, at break please dial 1 on your phone, or type onto the chat screen at any time during the presentation.

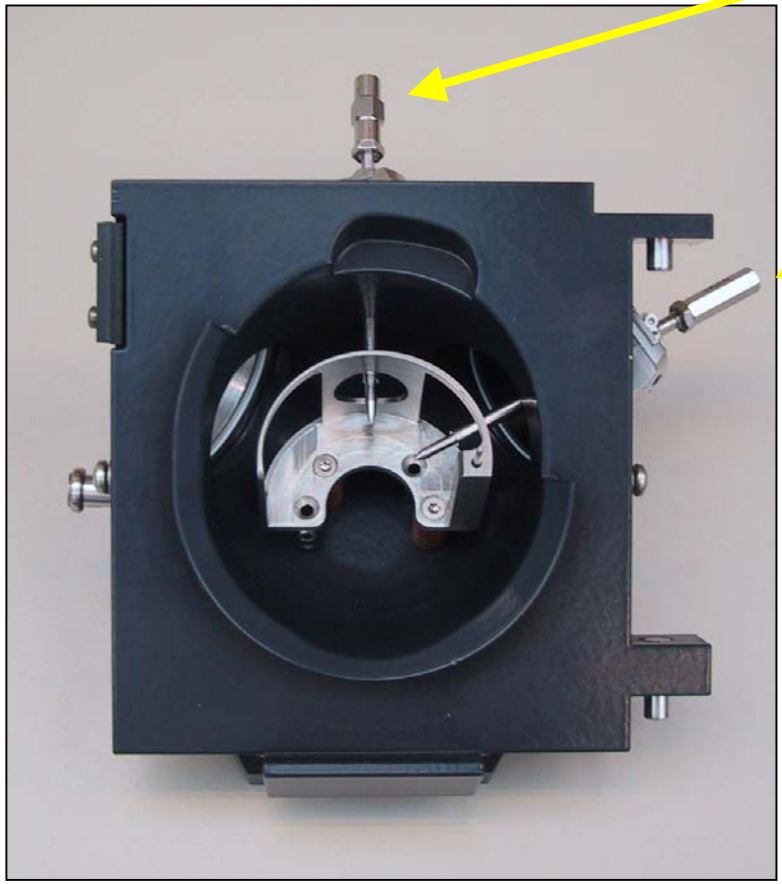


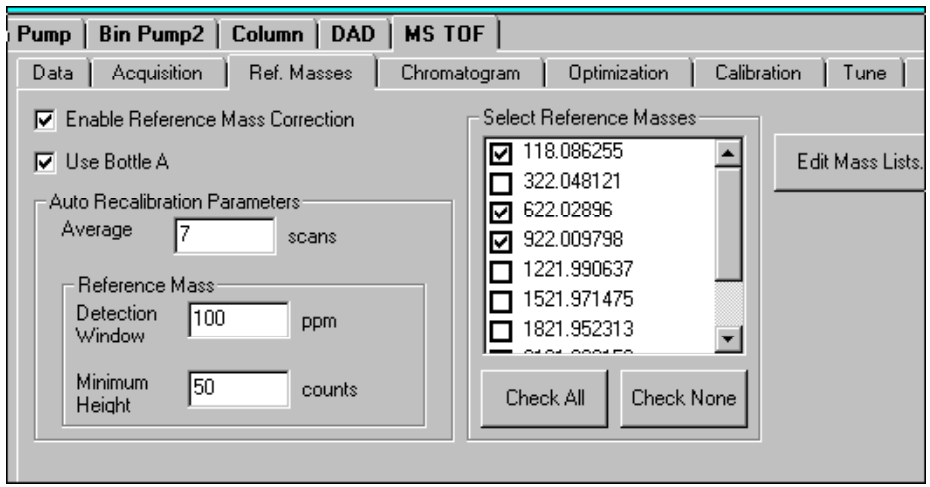
Ref. Mass Correction with *m/z* 118 and 922



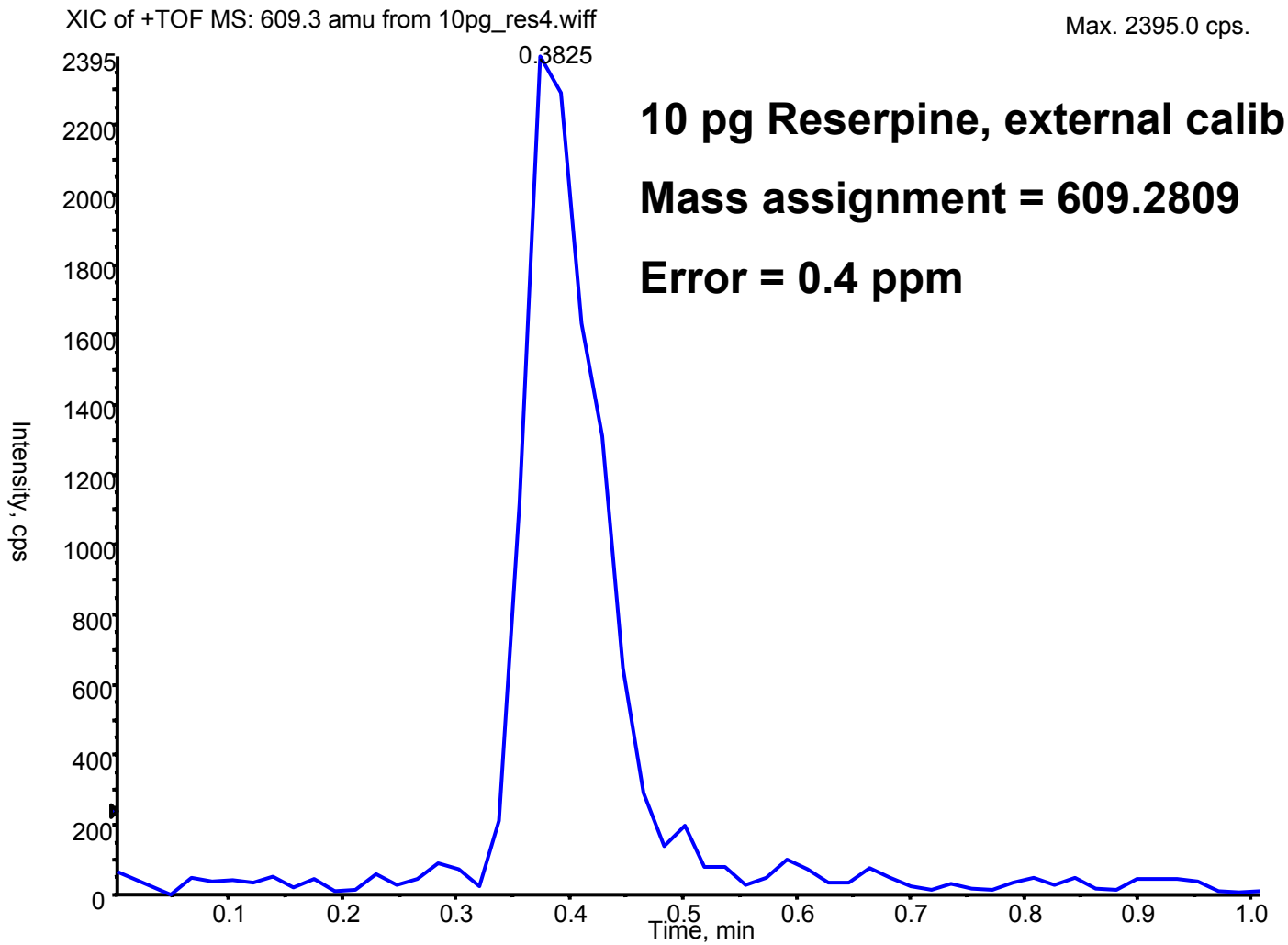
Theoretical Mass	Abundance Arb. Units	Error (ppm)
487.73253	1316	0.61
450.2364	5304	1.59
722.81666	6611	0.64
417.21191	6910	2.66
512.25457	8978	1.66
464.25036	12534	0.73
519.21717	575	0.06
653.3617	21881	1.38
435.91023	555	-0.28
501.79513	10141	-0.04
820.47251	842	0.25
526.58871	3100	0.35
674.32258	1881	-1.4
480.60877	23885	-2.52
582.31897	49027	1.57
710.84248	3863	-1.16
627.97323	13881	0.55
474.23075	24508	1.13
507.81333	11016	0.68
740.40136	9671	-0.12
784.37501	643	-3.49

Automatic Internal Referencing





Mass Accuracy and Dynamic Range

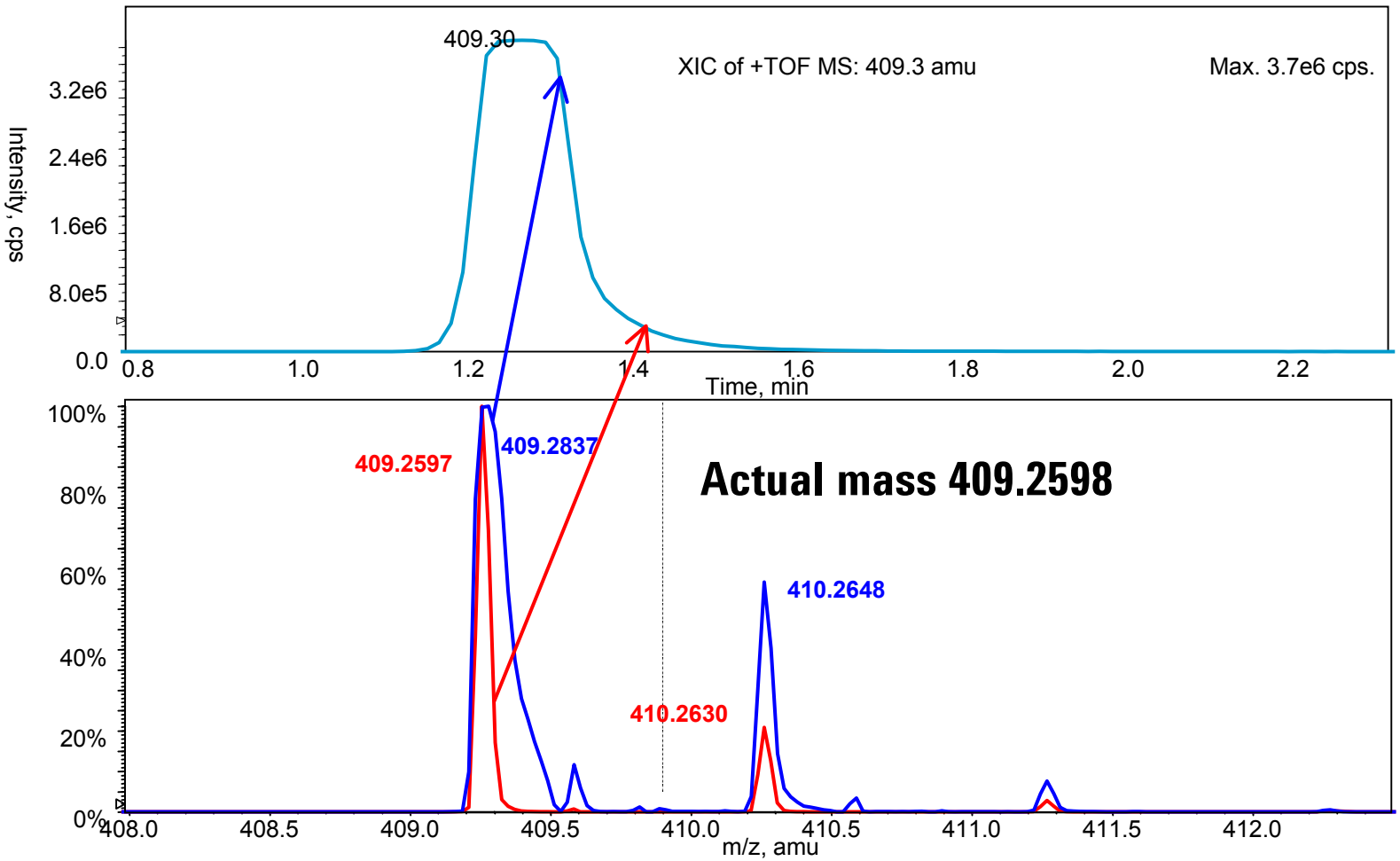


10 pg Reserpine, external calibration

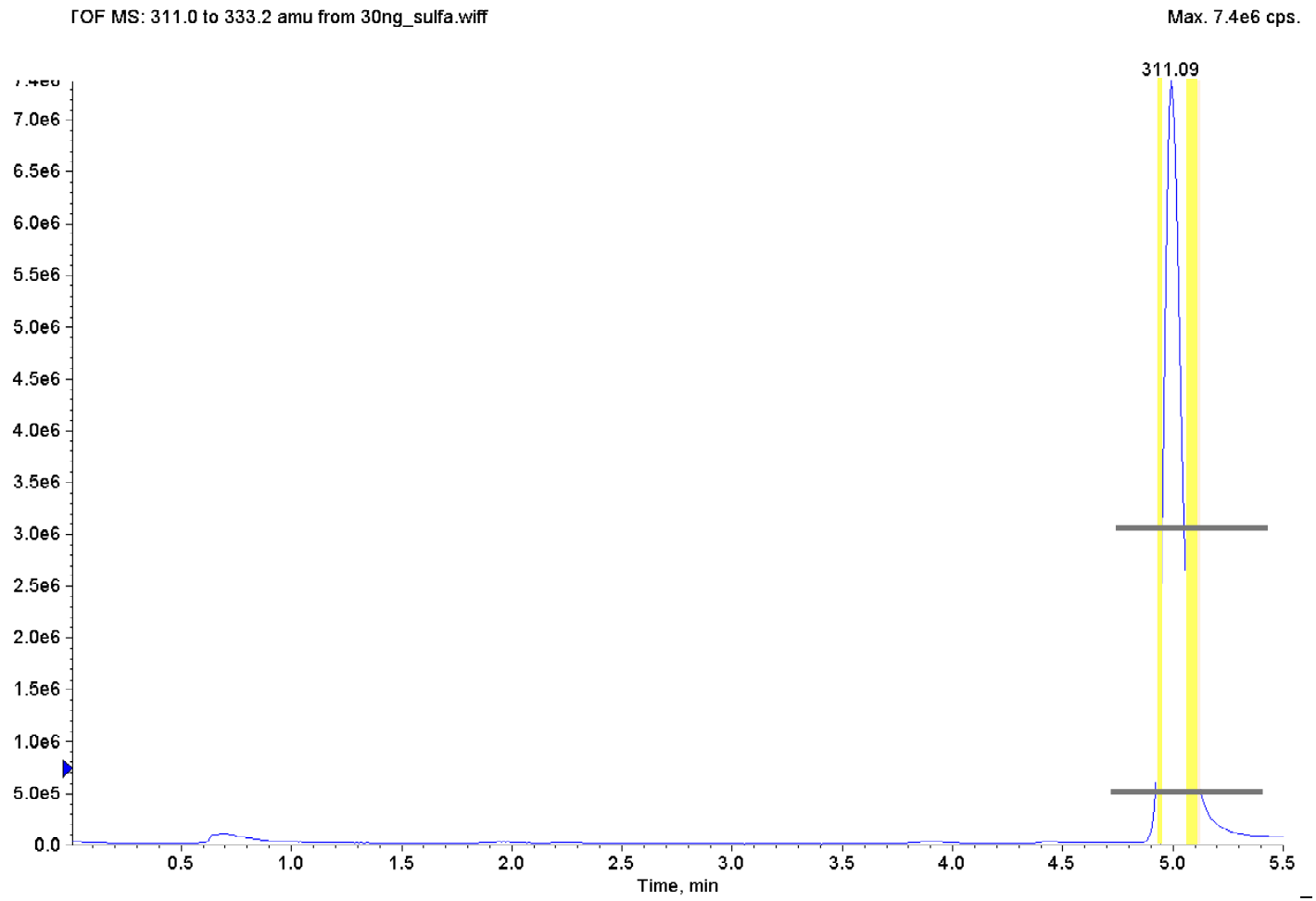
Mass assignment = 609.2809

Error = 0.4 ppm

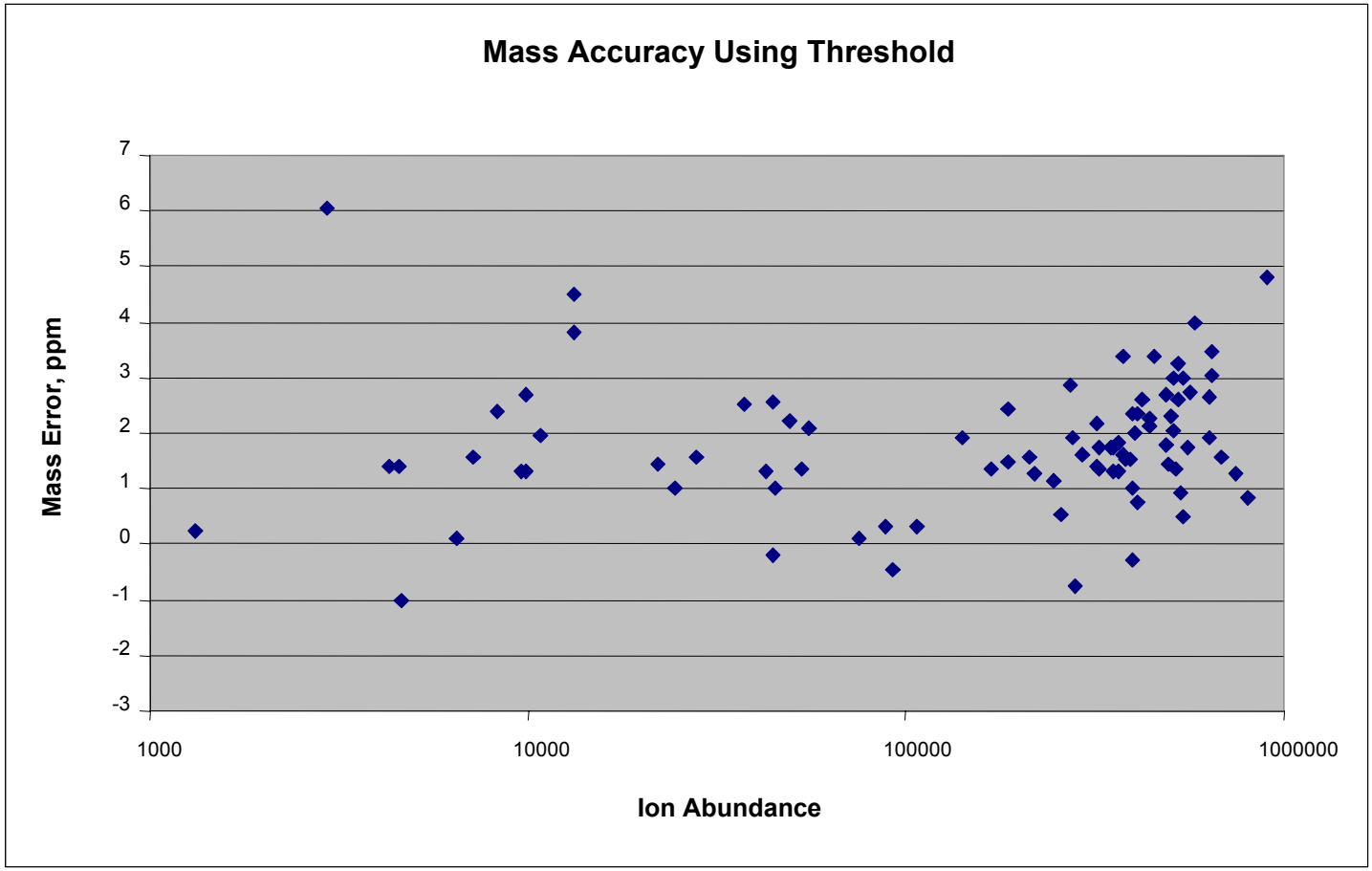
Spectral Saturation and Mass Accuracy



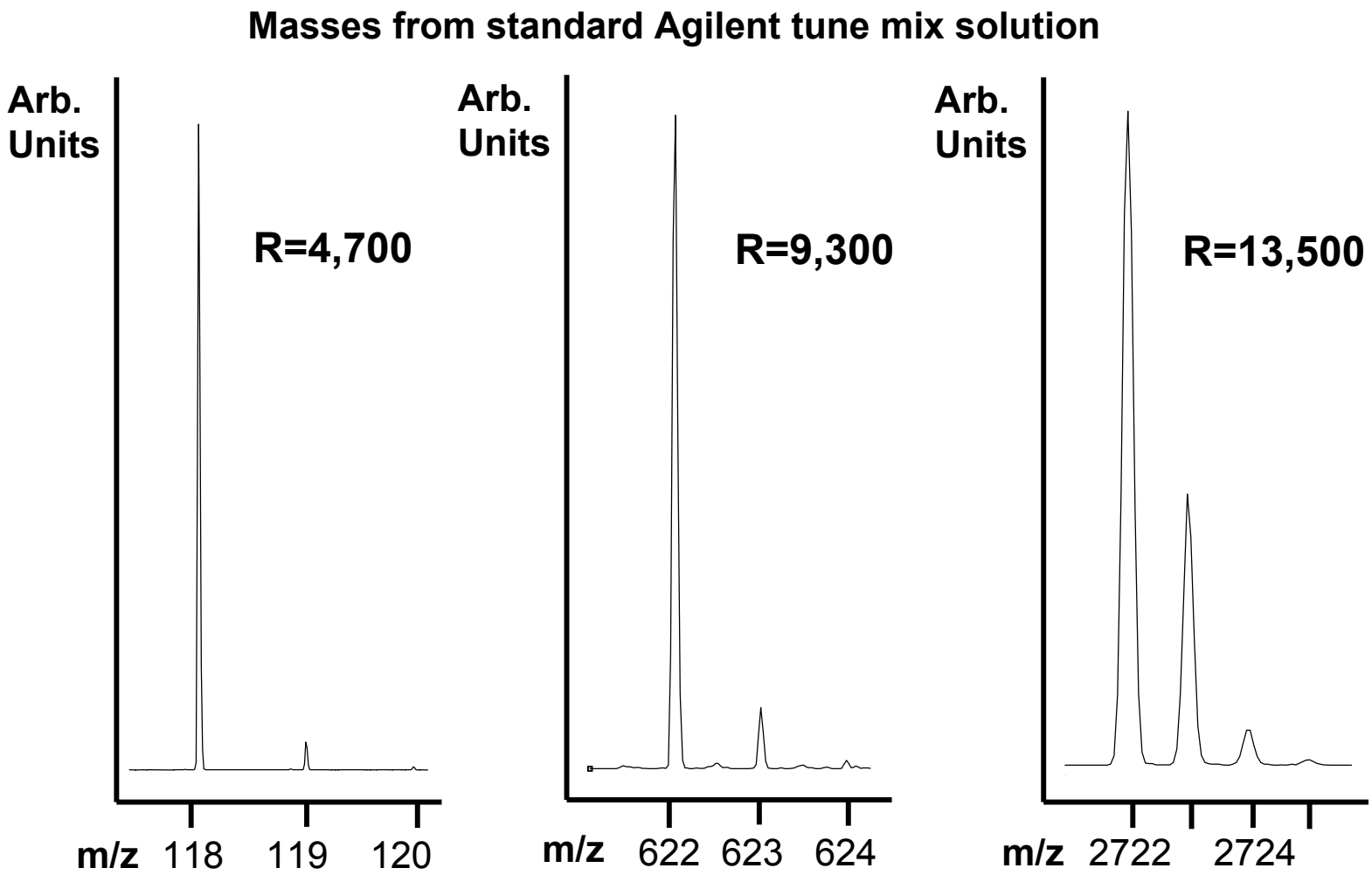
Automatic Thresholding of Spectra



Results with 50% Threshold

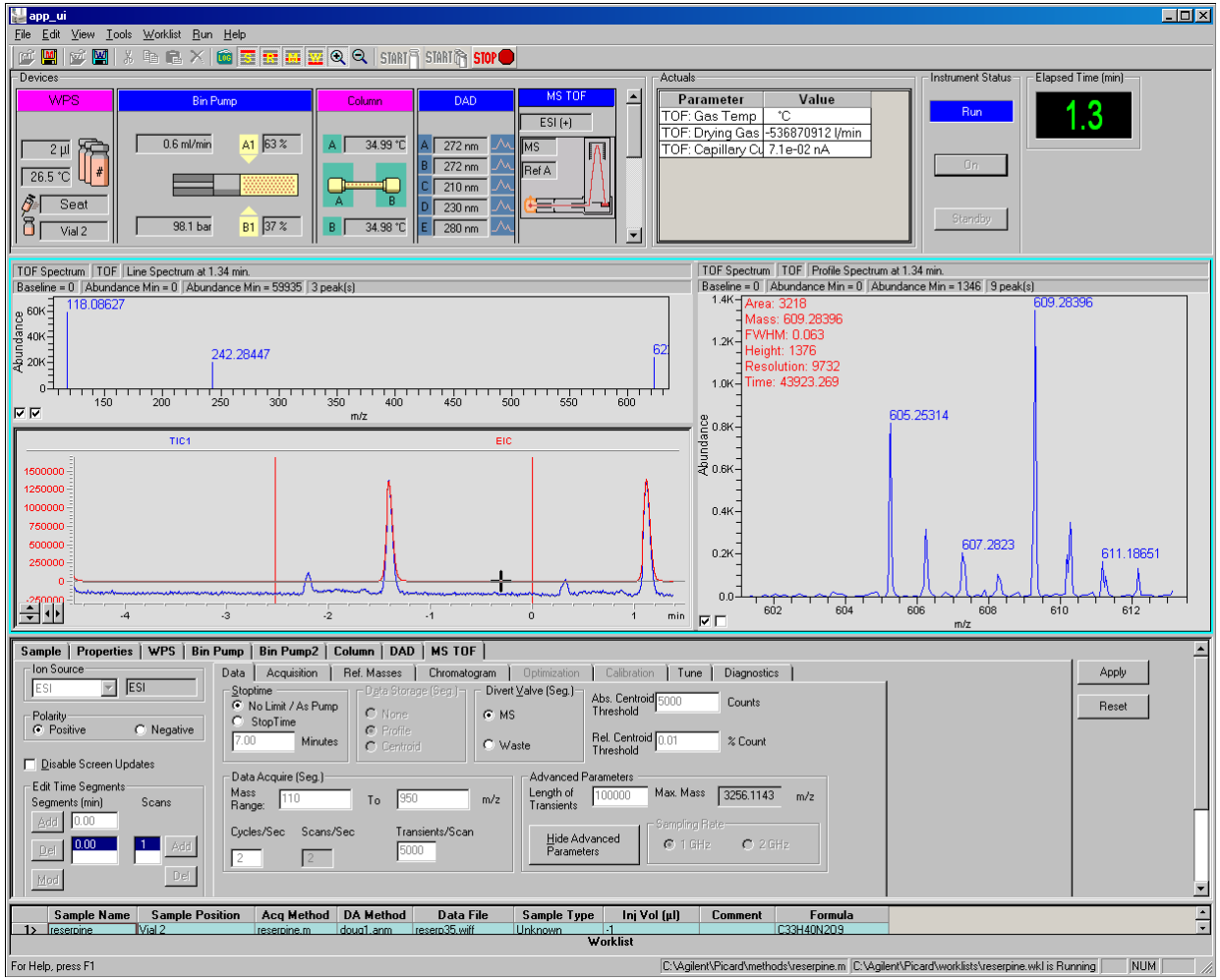


Mass Resolving Power



TOF Software User Interface

- 32-bit client server based with modular components
- Four separate panes for instrument status, real-time display, method editing and worklist management
- Each pane may be sized, displayed or hidden to meet current user needs
- Extensive use of right mouse click similar to other Windows applications



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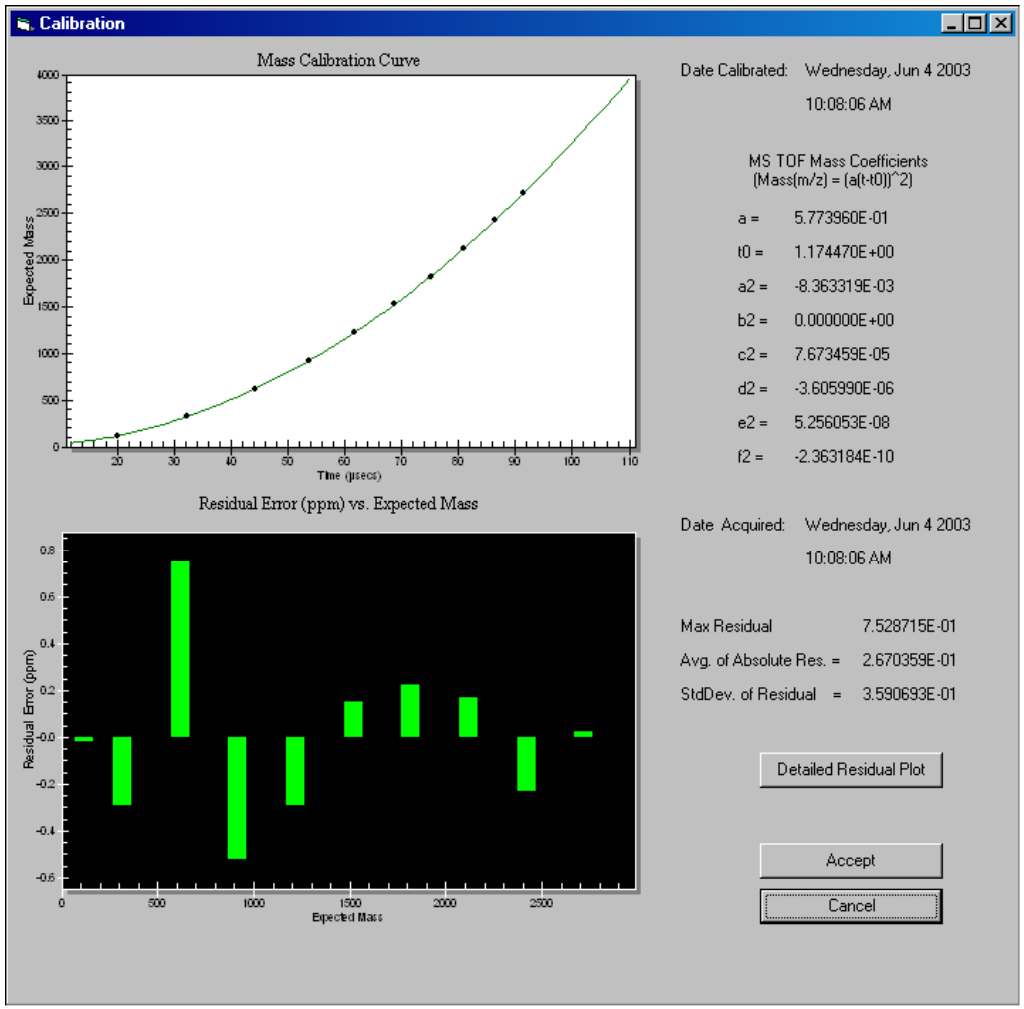
Easy Optimization and Calibration

Autotune

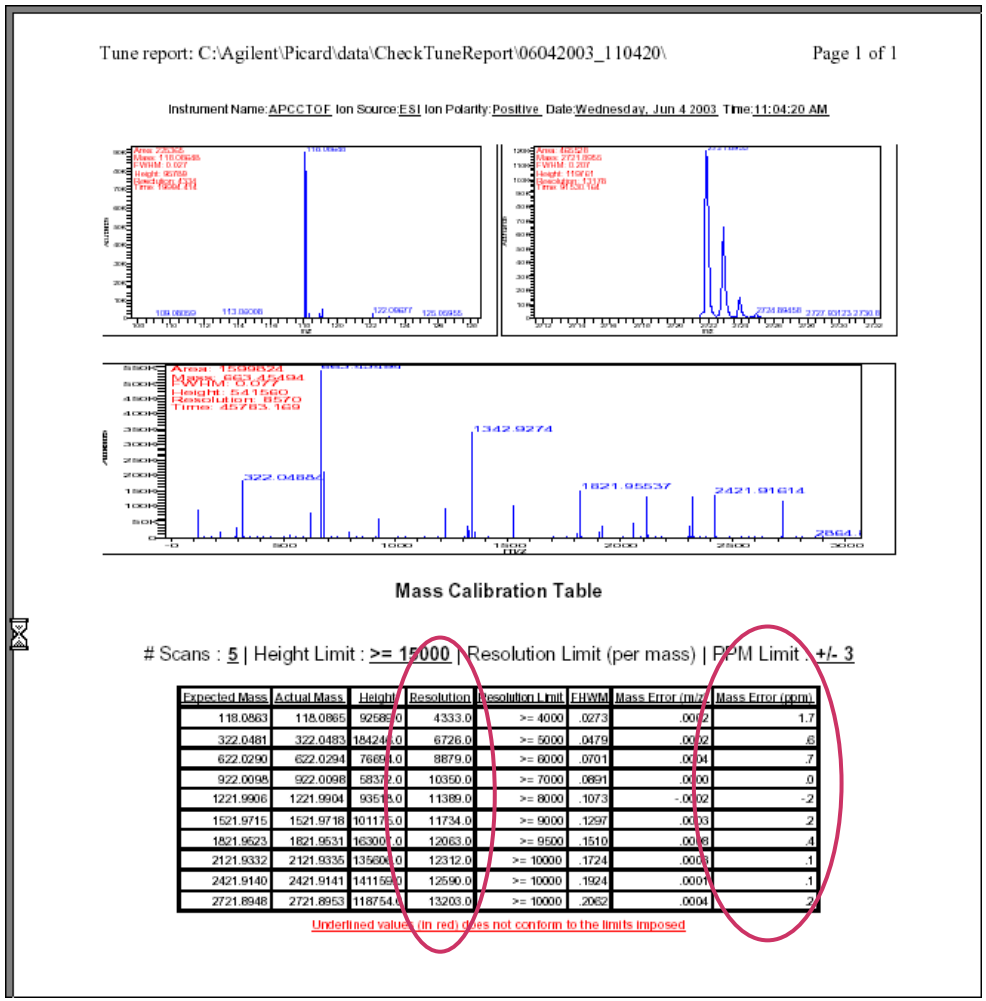
Quicktune

Checktune

Calibrate



CheckTune Report



Tune report: C:\Agilent\Picard\data\CheckTuneReport\06042003_110420\ Page 1 of 1

Instrument Name: APCCTOF Ion Source: ESI Ion Polarity: Positive Date: Wednesday, Jun 4 2003 Time: 11:04:20 AM

Mass Calibration Table

Scans : 5 | Height Limit : >= 15000 | Resolution Limit (per mass) | PPM Limit : +/- 3

Expected Mass	Actual Mass	Height	Resolution	Resolution Limit	FHWM	Mass Error (m/z)	Mass Error (ppm)
118.0863	118.0865	92585.0	4333.0	>= 4000	.0273	.0002	1.7
322.0481	322.0483	184246.0	6726.0	>= 5000	.0479	.0002	.6
622.0290	622.0294	76694.0	8879.0	>= 6000	.0701	.0004	.7
922.0098	922.0098	58372.0	10350.0	>= 7000	.0891	.0000	.0
1221.9906	1221.9904	93513.0	11389.0	>= 8000	.1073	-.0002	-.2
1521.9715	1521.9718	101175.0	11734.0	>= 9000	.1297	.0003	.2
1821.9523	1821.9531	163007.0	12063.0	>= 9500	.1510	.0008	.4
2121.9332	2121.9335	135600.0	12312.0	>= 10000	.1724	.0003	.1
2421.9140	2421.9141	141159.0	12590.0	>= 10000	.1924	.0001	.1
2721.8948	2721.8953	118754.0	13203.0	>= 10000	.2062	.0004	.2

Underlined values (in red) does not conform to the limits imposed

CheckTune Report – Expanded View

Mass Calibration Table

Scans : **5** | Height Limit : **>= 15000** | Resolution Limit (per mass) | PPM Limit : **+/- 3**

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922.0098	922.0098	58372.0	10350.0	>= 7000	.0891	.0000	.0
1221.9906	1221.9904	93518.0	11389.0	>= 8000	.1073	-.0002	-.2
1521.9715	1521.9718	101175.0	11734.0	>= 9000	.1297	.0003	.2
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Break Number 2

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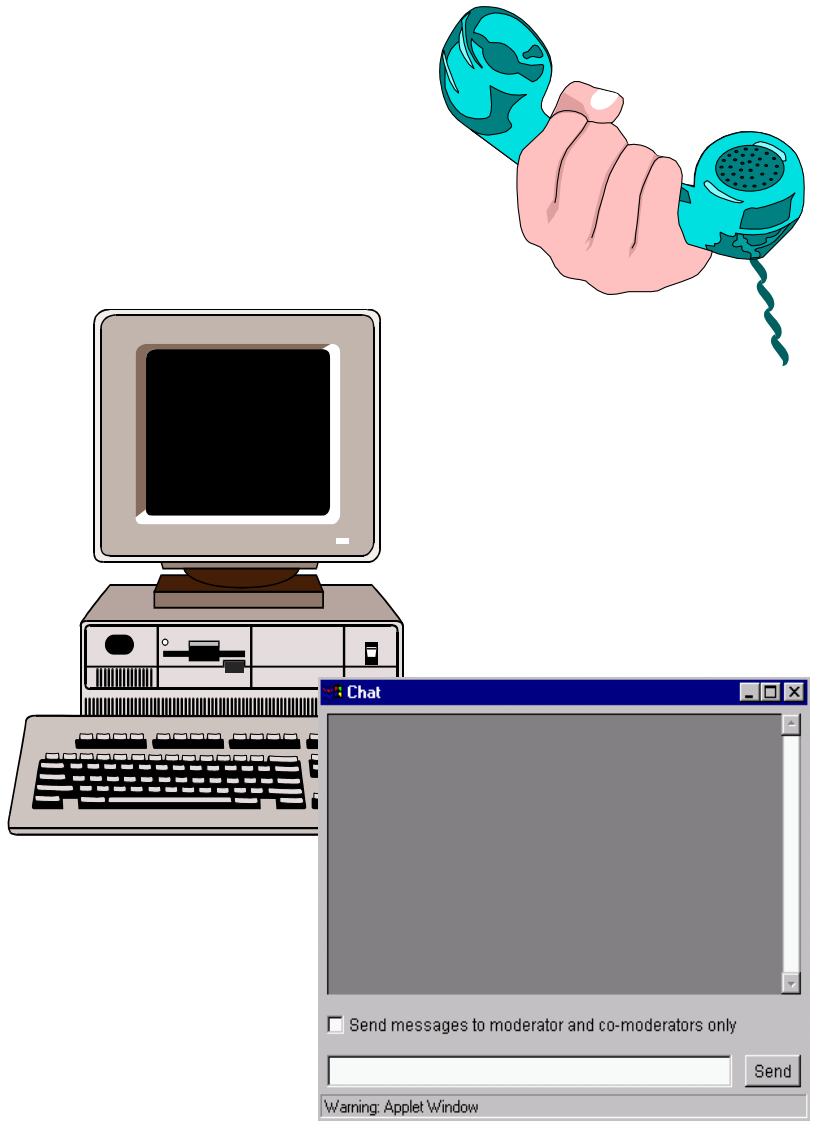
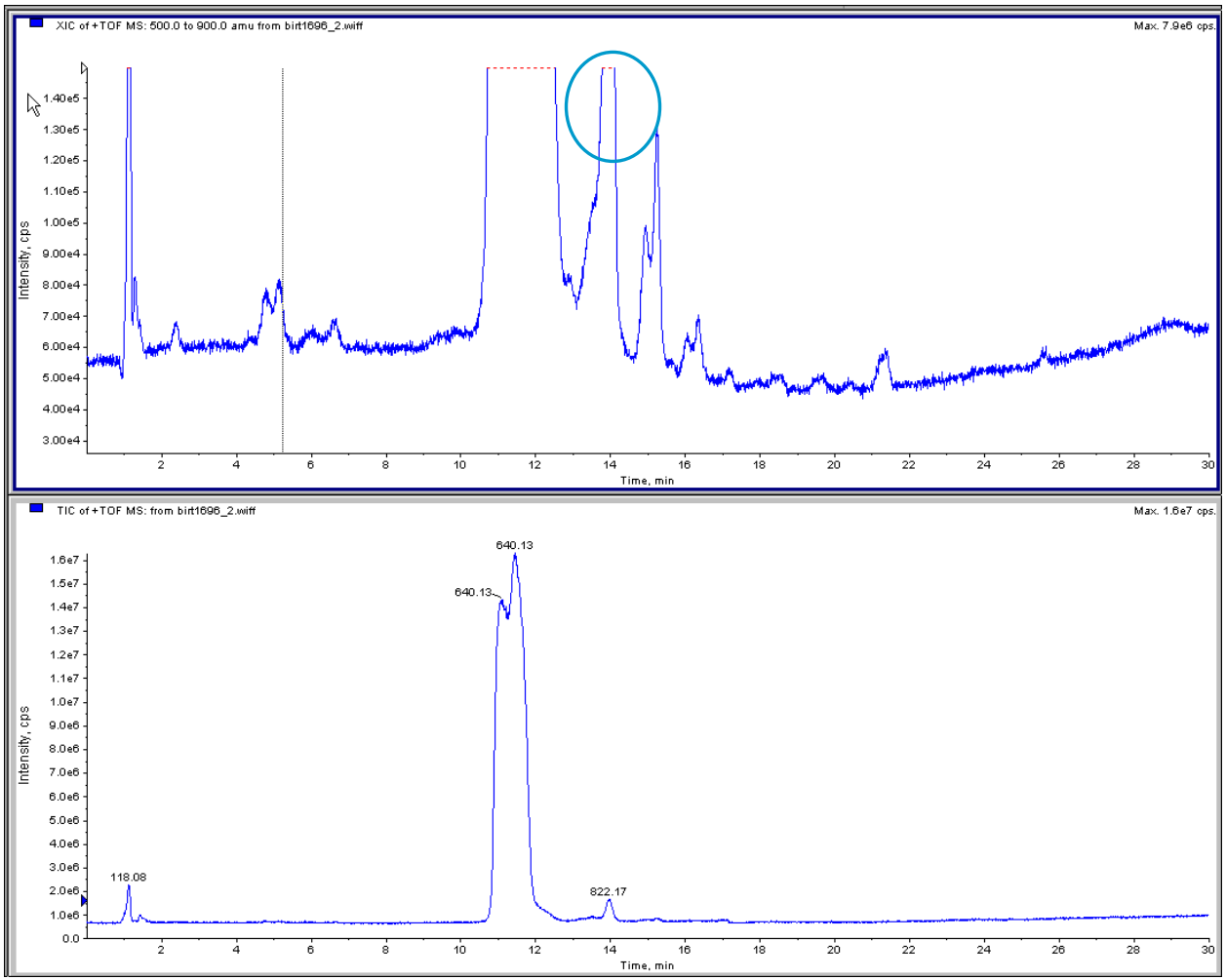


Identification of Unknowns

- Empirical formula determination can greatly assist the identification of unknowns
- The list of possibilities is smaller with mass >250
  - At 5 ppm accuracy the possibilities are few at low mass
  - The use of isotope patterns can reduce the list of possibilities even further by specifying or eliminating atoms, determining approximate carbon content etc.
- At > 250 mass units, 3 ppm accuracy reduces the possibilities

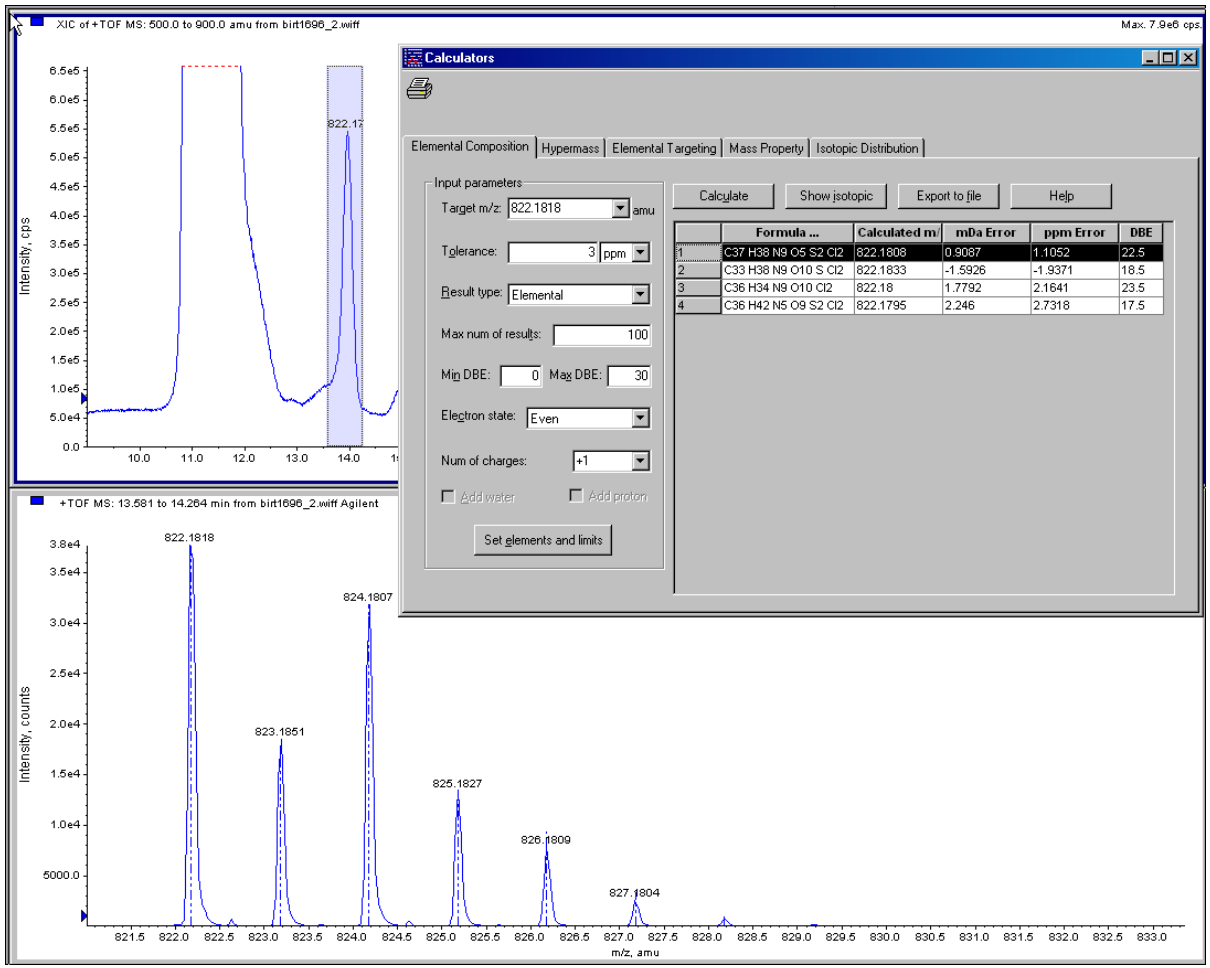
Identifying Trace Contaminants in Pharmaceuticals

100 – fold magnification

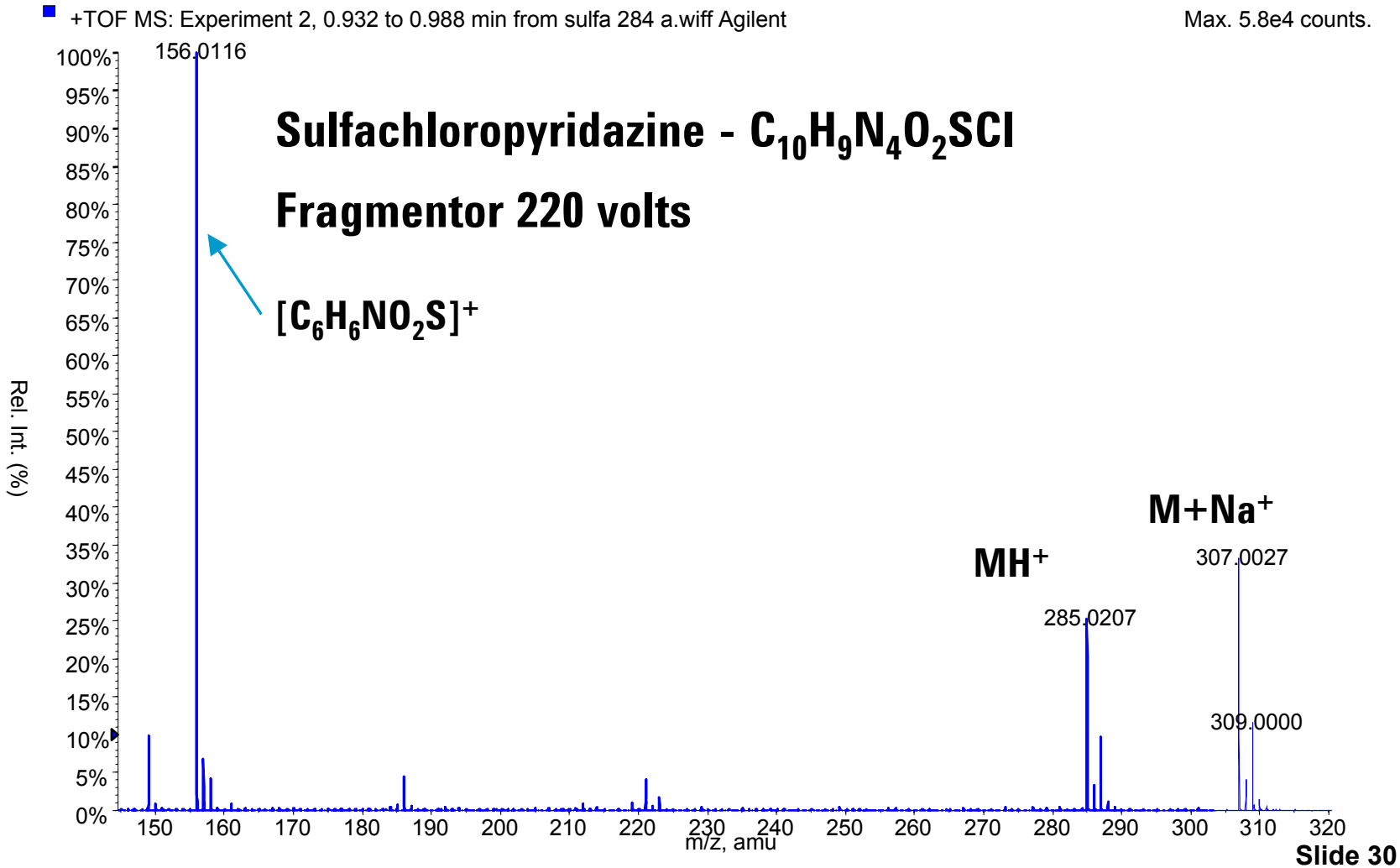


Major Contaminant

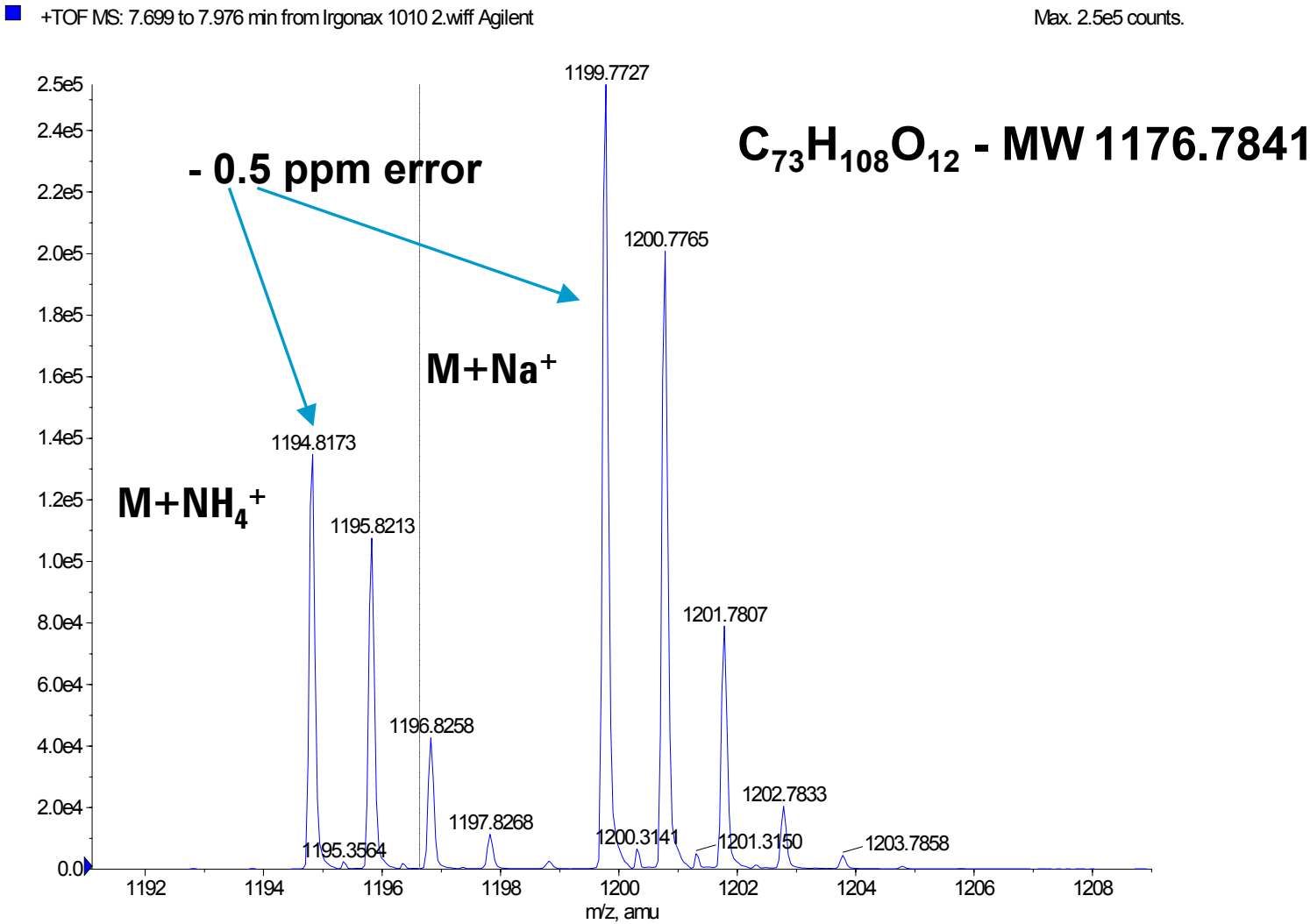
Use knowledge of major product and synthesis process to reduce possible formula



Using In-Source CID Information



Using Adduct Information



Confirmation with Accurate Mass

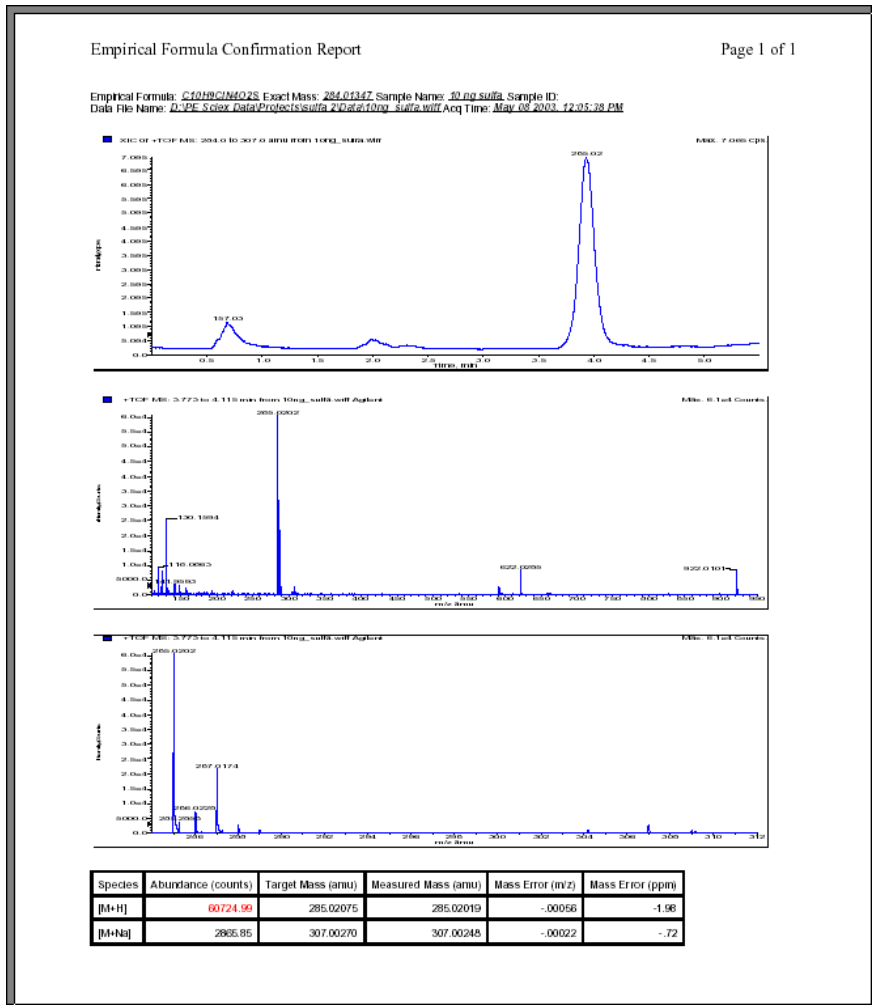
Accurate mass determination provides a level of selectivity independent of fragments from an MS/MS experiment

Determination of empirical formula

- **An accurate mass measurement is NOT exclusive of all empirical formulas at 5 ppm confidence level**
  - **A proposed compound ID or structure IS exclusive**
    - **That is there is only one empirical formula for a proposed compound- CONFIRMATION**

Empirical Formula Conformation Report

- Input one or more formulas for each sample
- Edit method to choose adducts, thresholding, report destination
- System calculates target mass, extracts ions and calculates mass error
- Reports can be printed or viewed from Internet Explorer
- Complete Worklist results output in CSV format



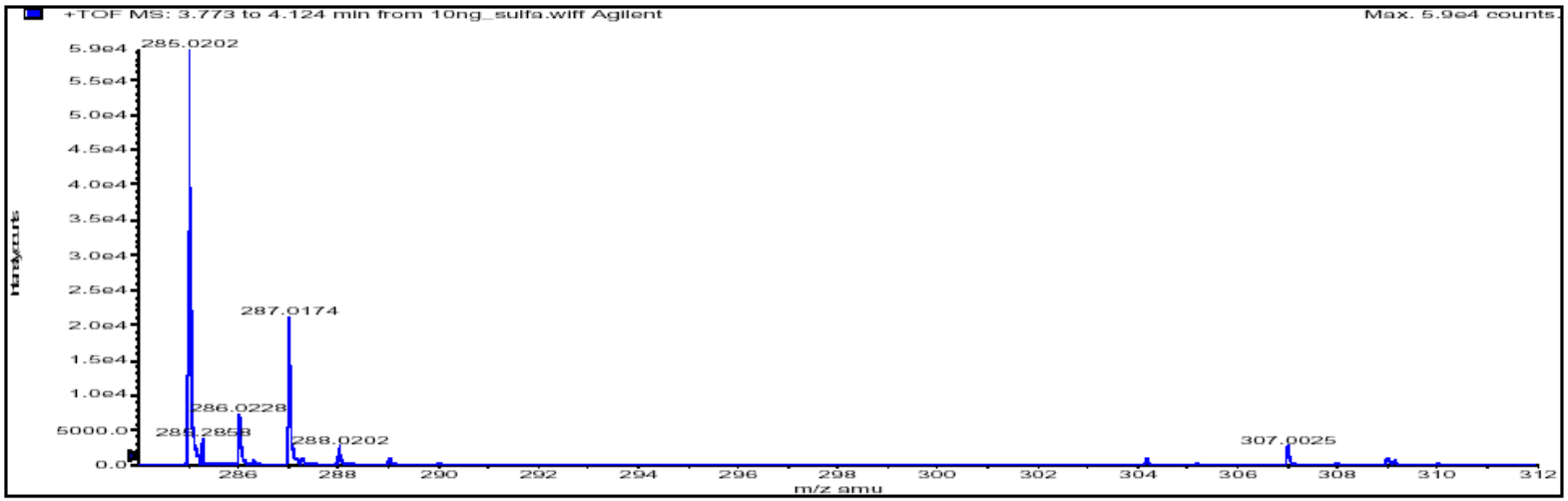
Empirical Formula Confirmation Report

Page 1 of 1

Empirical Formula: C10H9ClN4O2S Exact Mass: 284.01347 Sample Name: 10 ng sulfa Sample ID:
Data File Name: D:\PE Sciex Data\Projects\sulfa 2\Data\10ng_sulfa.wiff Acq Time: May 08 2003, 12:05:38 PM

Species	Abundance (counts)	Target Mass (amu)	Measured Mass (amu)	Mass Error (m/z)	Mass Error (ppm)
[M+H]	60724.99	285.02075	285.02019	-.00056	-1.98
[M+Na]	2865.85	307.00270	307.00245	-.00022	-.72

Expanded View of Results



Species	Abundance (counts)	Target Mass (amu)	Measured Mass (amu)	Mass Error (m/z)	Mass Error (ppm)
[M+H]+	59329.42	285.02075	285.02019	-.00056	-1.97
[M+Na]+	2799.10	307.00270	307.00248	-.00021	-.70

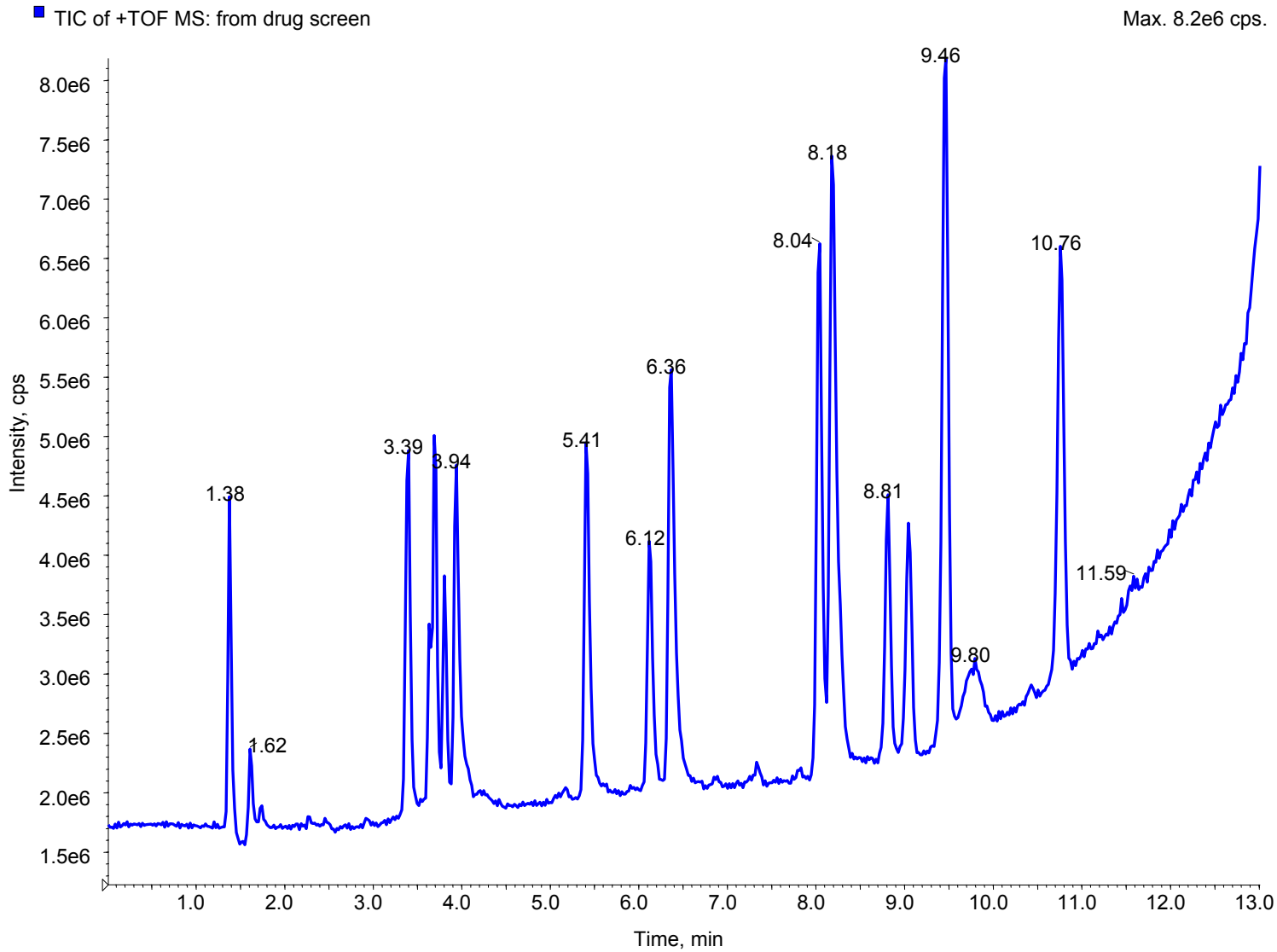
Quantification with ESI-TOF

The high resolution achieved (>5000 at *m/z*=250 and 10,000 at *m/z*= 1000) increases selectivity tremendously!

The dynamic range is greatly enhanced with the use of analog to digital conversion (ADC)!

All FULL SCAN SPECTRA are autocalibrated in the saved data file with the seamless reference mass addition

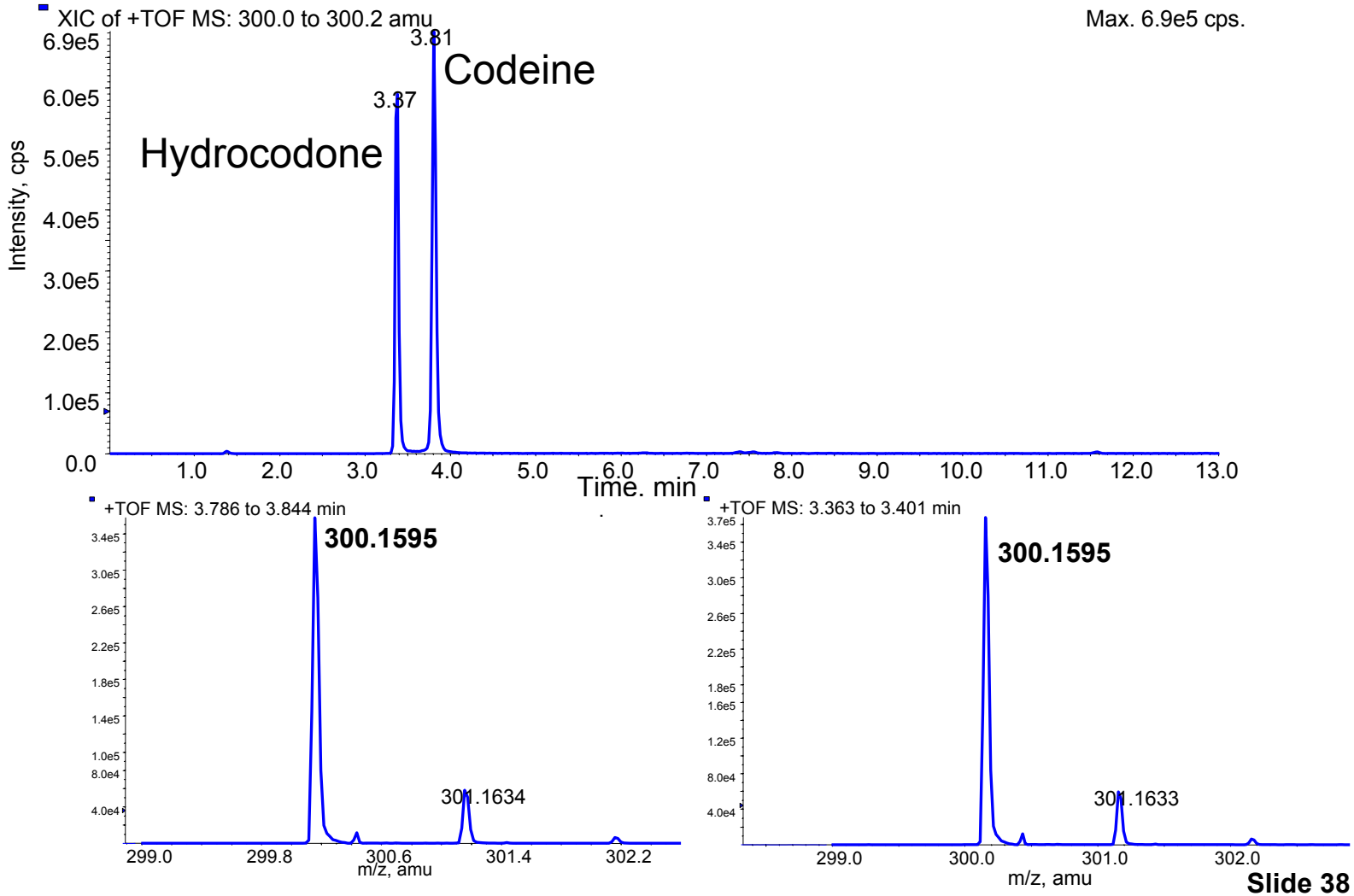
Drugs of Abuse-LC/ESI-TOF



Rt (min)	Compound
3.37	hydrocodone
3.40	unknown with M+H =166.1221
3.64	Oxycodone
3.70	Amphetamine
3.81	Codeine
3.94	Methamphetamine
5.41	Cocaine
6.12	CE
6.36	PCP
8.04	Propoxyphene
8.18	Methadone
8.81	Alprazolam
9.00	Nordiazepam
9.46	Methaqualone
10.86	Diazepam

* Special thanks to the Lucas Zarwell of the Washington D.C. Medical Examiner's Office for kindly providing the reference materials.

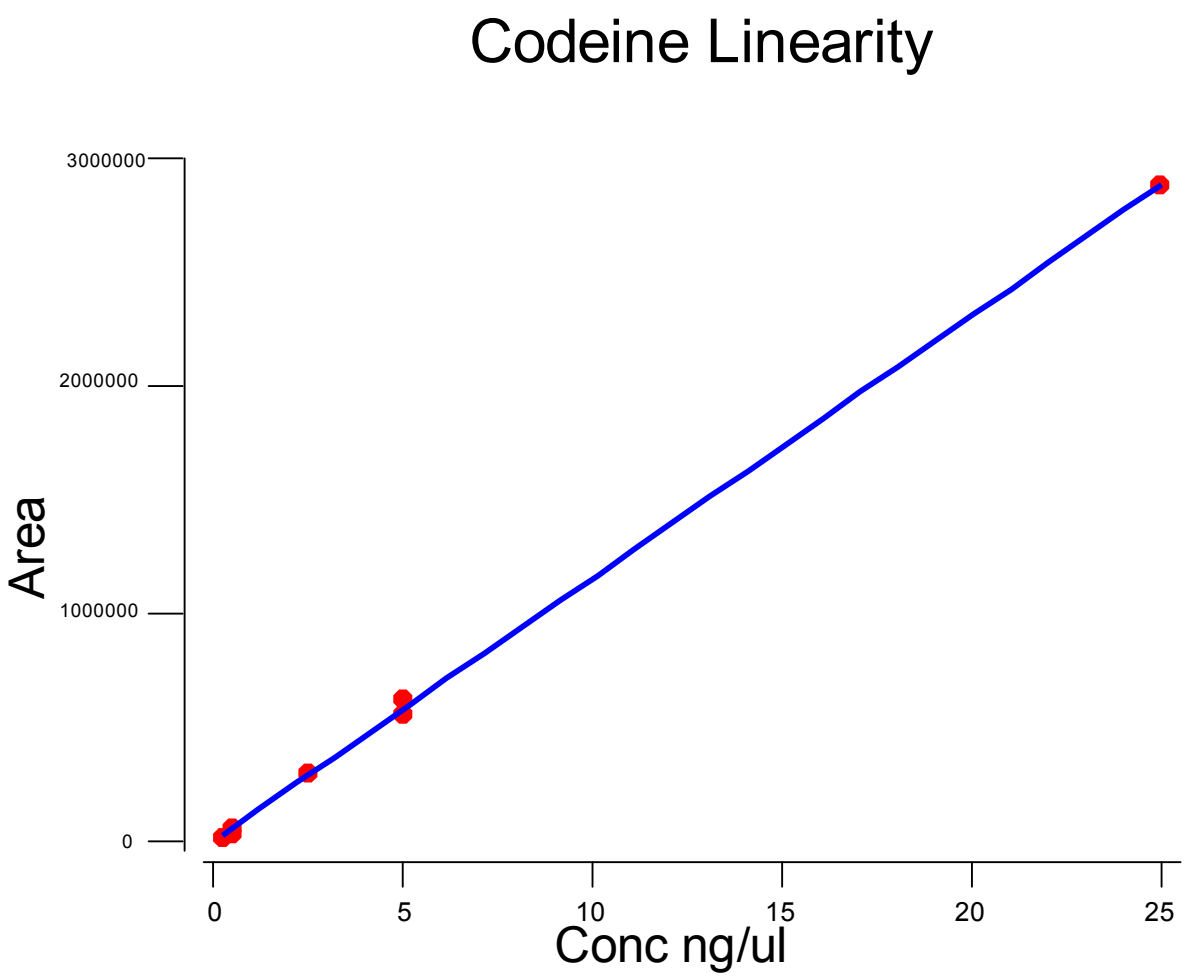
Extracted Ion Chromatogram of m/z 300 and Spectra of Two Isomers



Drugs of Abuse Mass Accuracy at 3 orders of Magnitude Concentration

Compound	Amphetamine	Methamphetamine	Hydrocodone	Codeine	Oxycodone
Nominal m/z	136.1	150.1	300.15	300.15	316.15
Conc (ng-injected)	measured error (ppm)	measured error (ppm)	measured error (ppm)	measured error (ppm)	measured error (ppm)
50	-4.71	-3.01	0.37	0.37	0.04
50	-4.53	-3.03	0.47	0.37	-0.16
50	4.53	-3.05	0.3	0.3	-0.75
25	3.53	-2.49	1.17	1.60	0.27
*2.5	-5.57	-2.88	0.60	0.27	-0.43
2.5	5	-2.78	1.6	1.6	-0.42
0.5	-4.23	-2.2	1.34	1.90	0.04
0.5	-5.01	-2.48	1.11	1.27	-0.33
0.25	-5.7	-2.69	0.95	1.27	-1.16
0.05	5.00	-5.42	3.6	2.26	0.53

Codeine Response from 50 pg to 25 ng On-column



Food Safety Examples

Chloramphenicol (CAP) Antibiotic banned in Foods at 0.1 ppb

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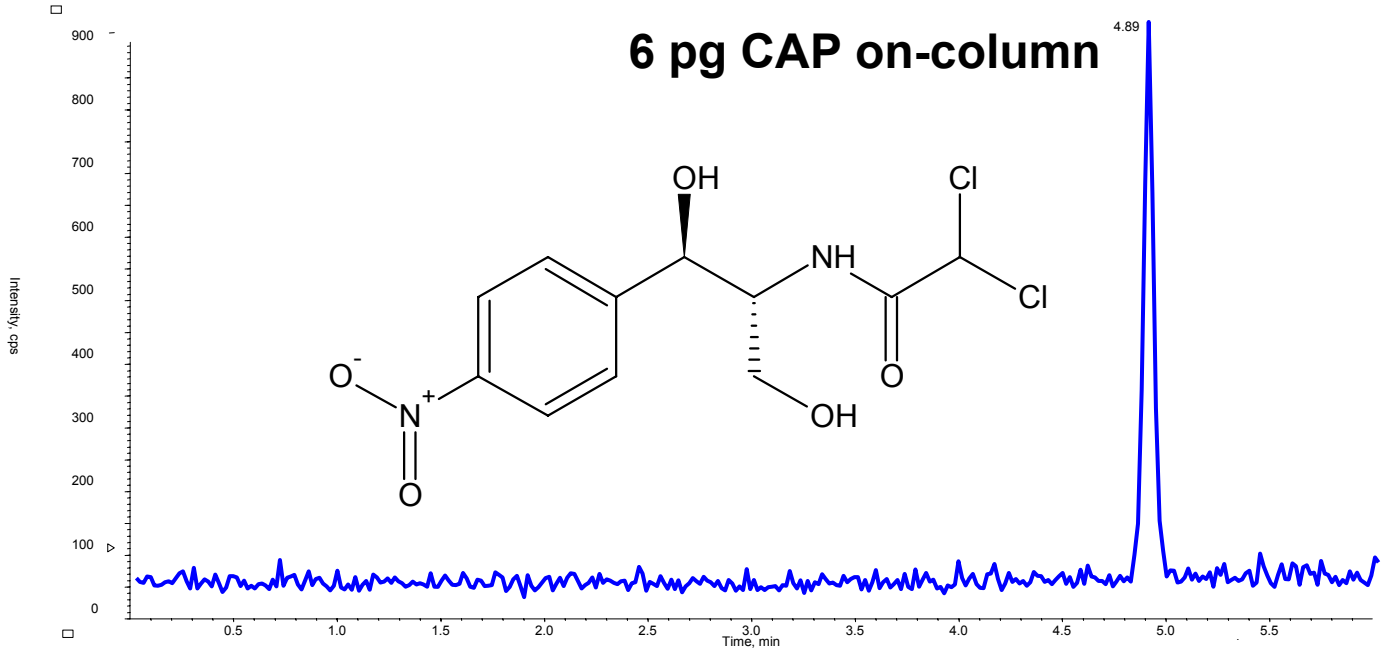
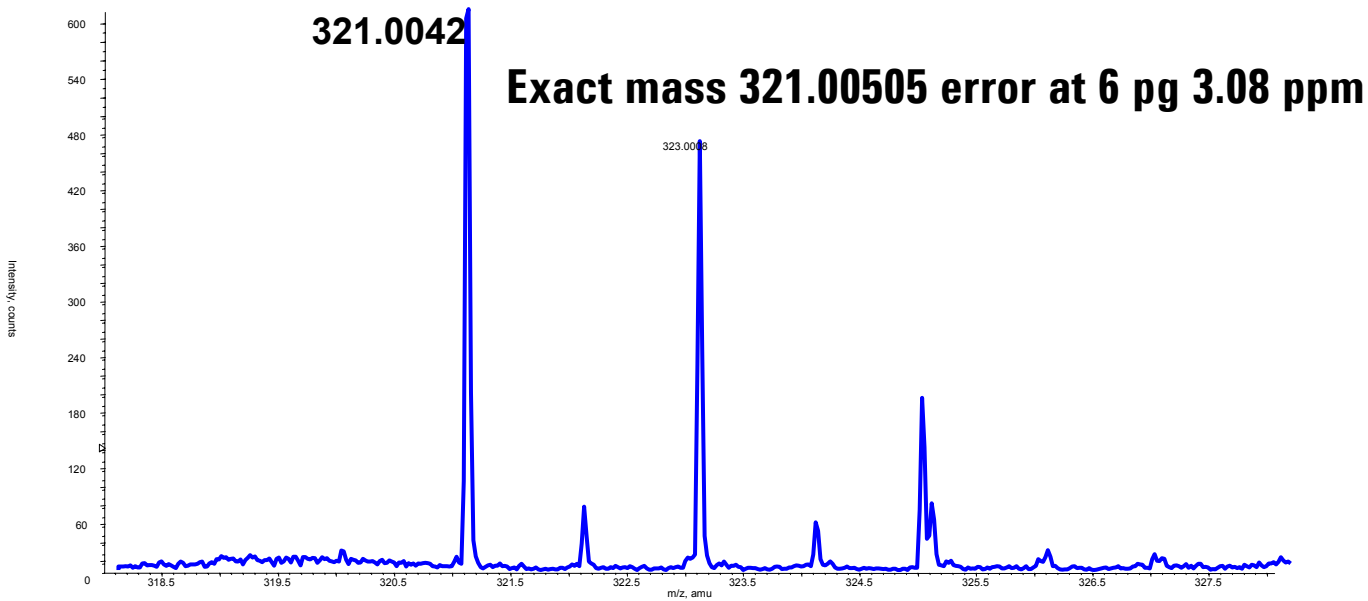
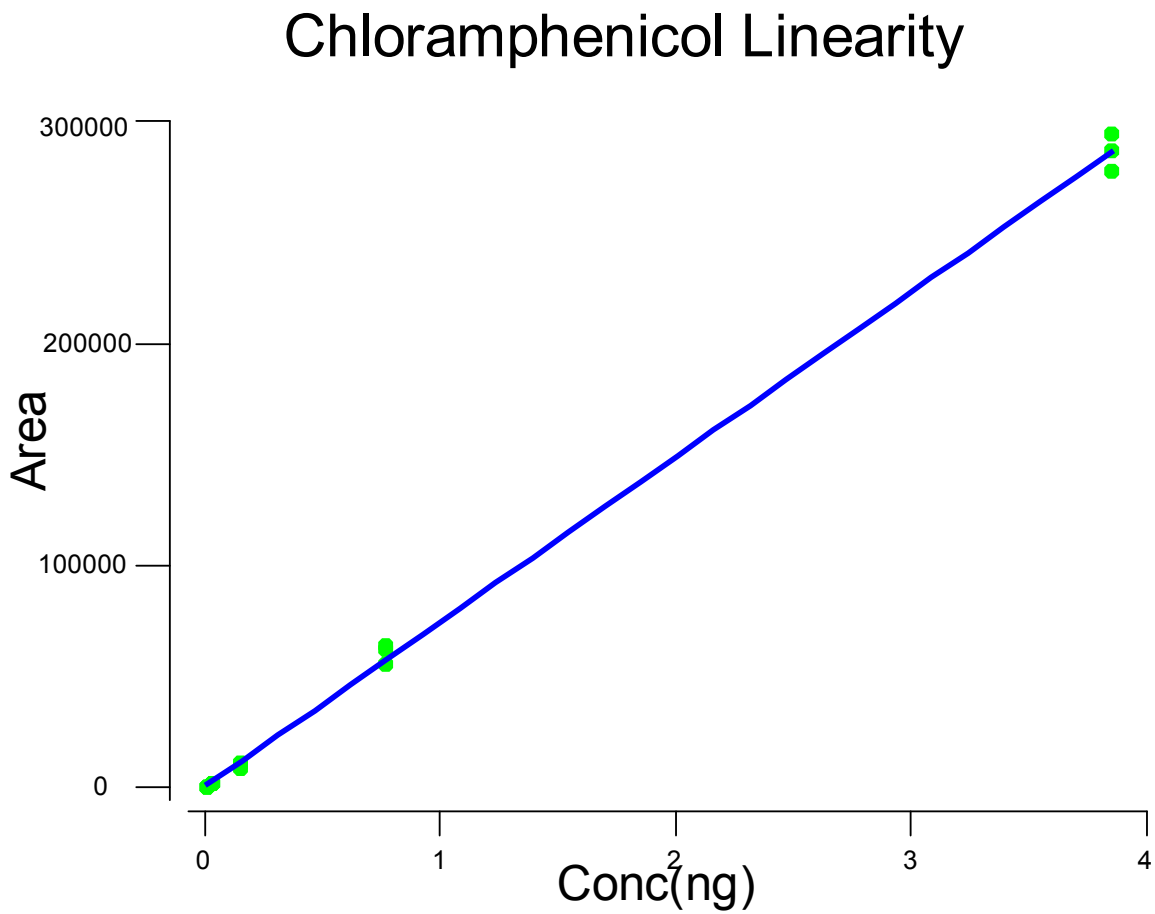




Chloramphenicol (negative ion mode) mass accuracy in ppm

pg injected	*rep 1*	*rep 2*	*rep 3*	*mean*
19000	-2.48	-2.54	-3.03	2.7
3900	-2.35	-2.94	-2.63	2.6
770	-0.84	-2.13	-1.58	1.5
154.4	-1.49	-1.66	-3.36	2.2
31	0.58	-0.03	-3.18	0.9
6.2	ND	ND	-3.08	3.1

Chloramphenicol Response from 6 pg to 4 ng On-column



Nitrofuran Metabolites in Poultry

THE SELECTIVITY OF HIGH RESOLUTION ACCURATE MASS MEASUREMENT!!

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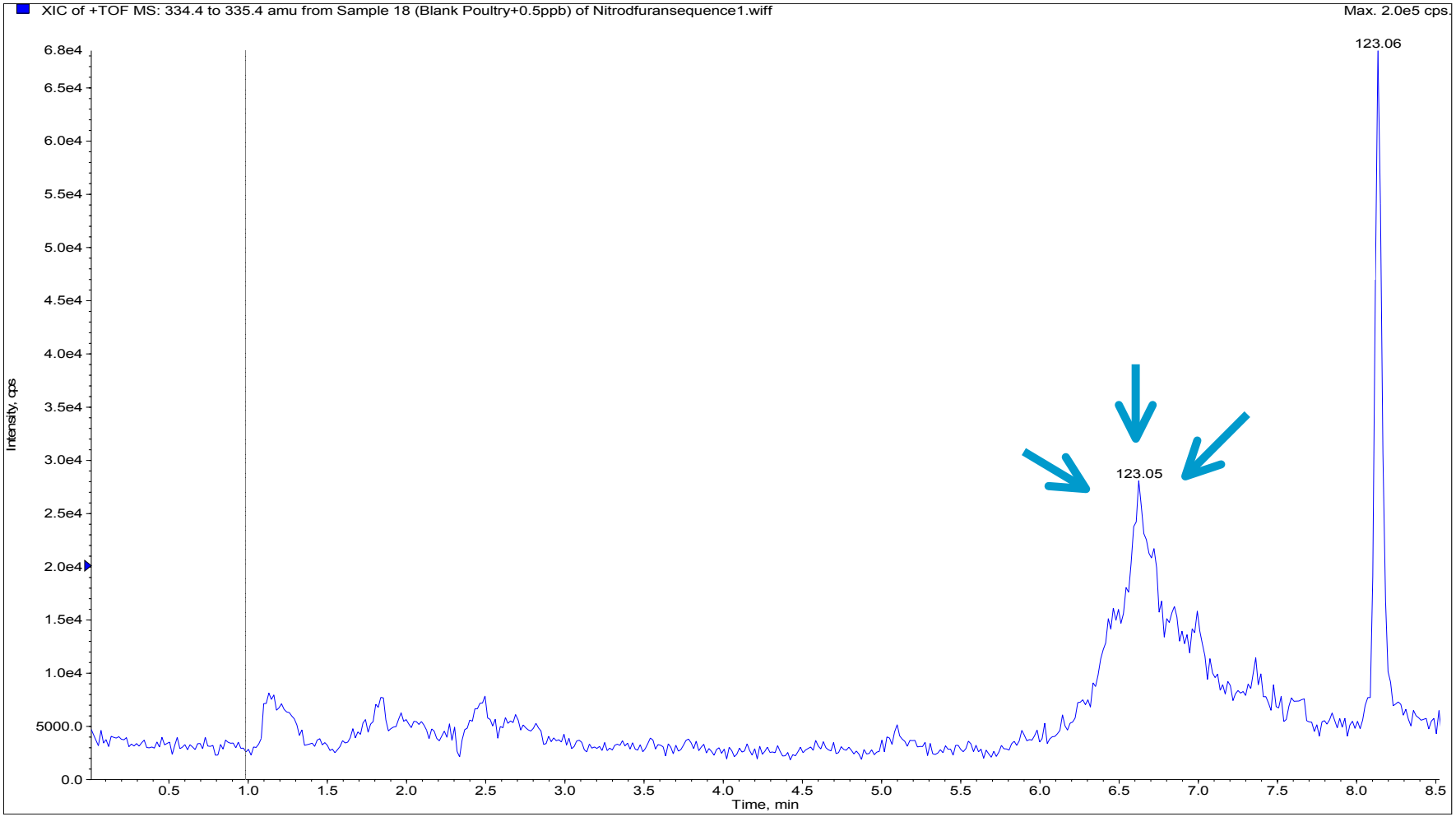
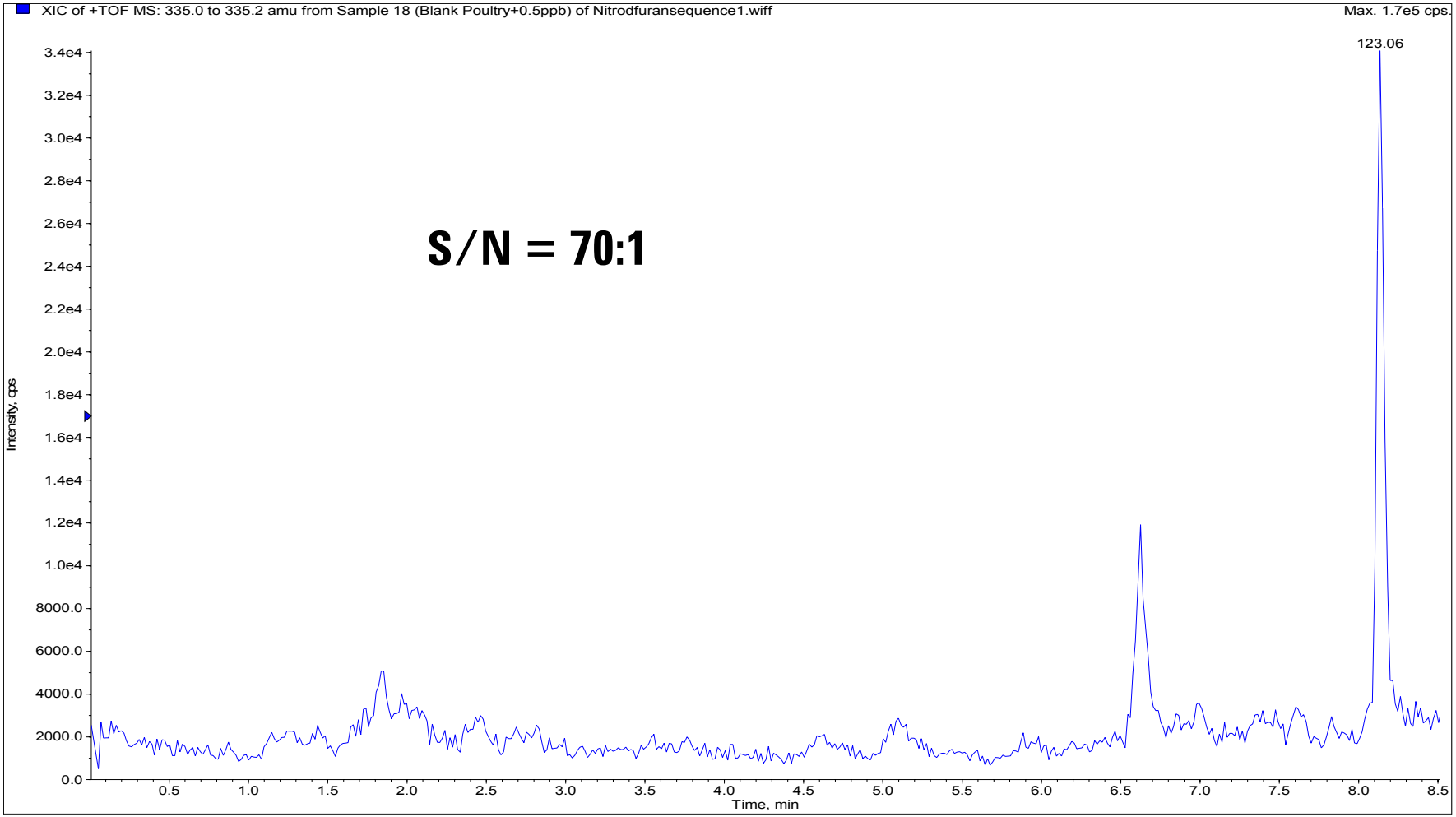
NPAMOZ at Unit Mass Resolution
335.1352 -0.7+0.3



NPAMOZ 335.1352 ± 0.1



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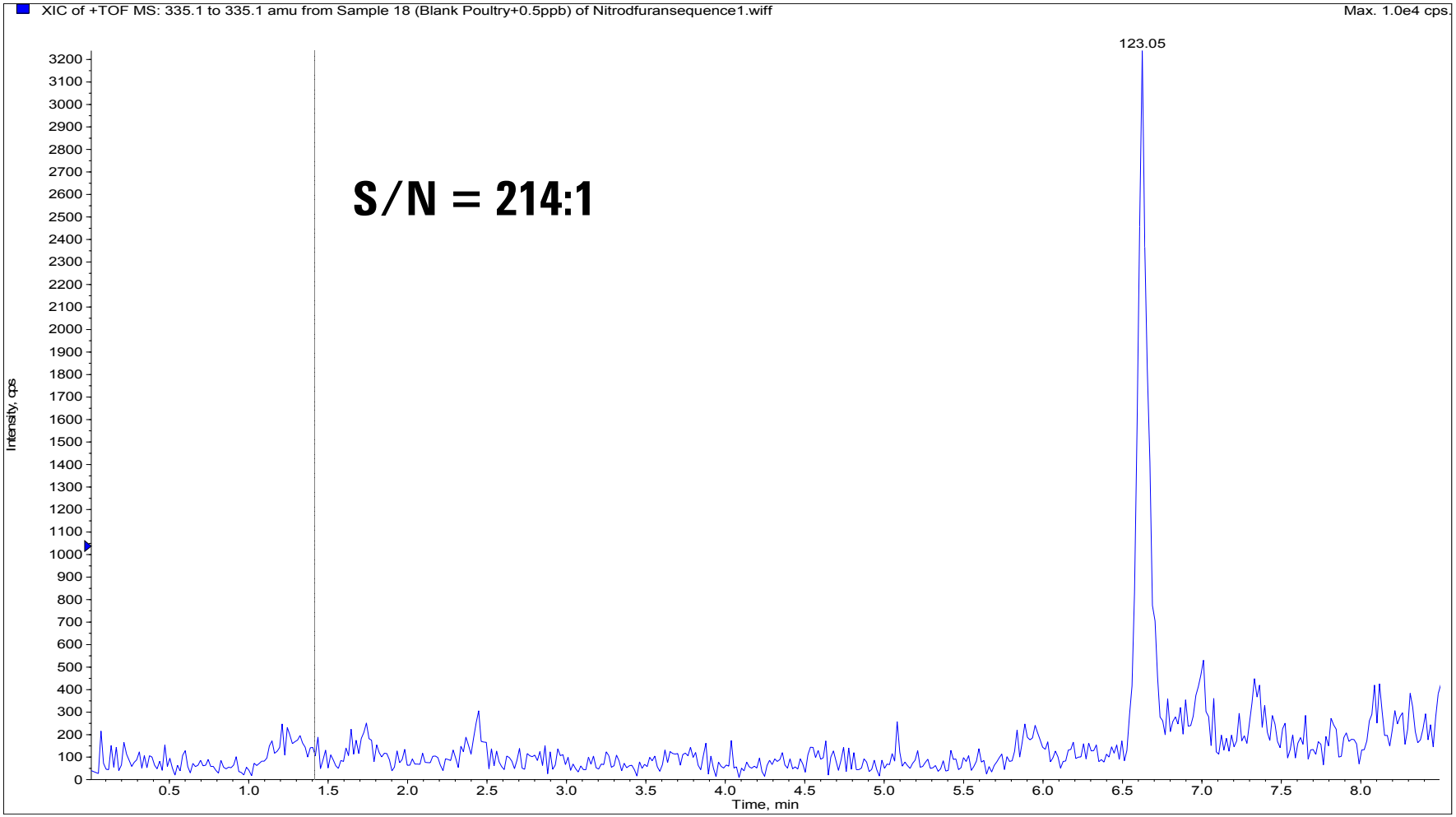
NPAMOZ 335.1352
+- 20ppm(335.1262-335.1396)



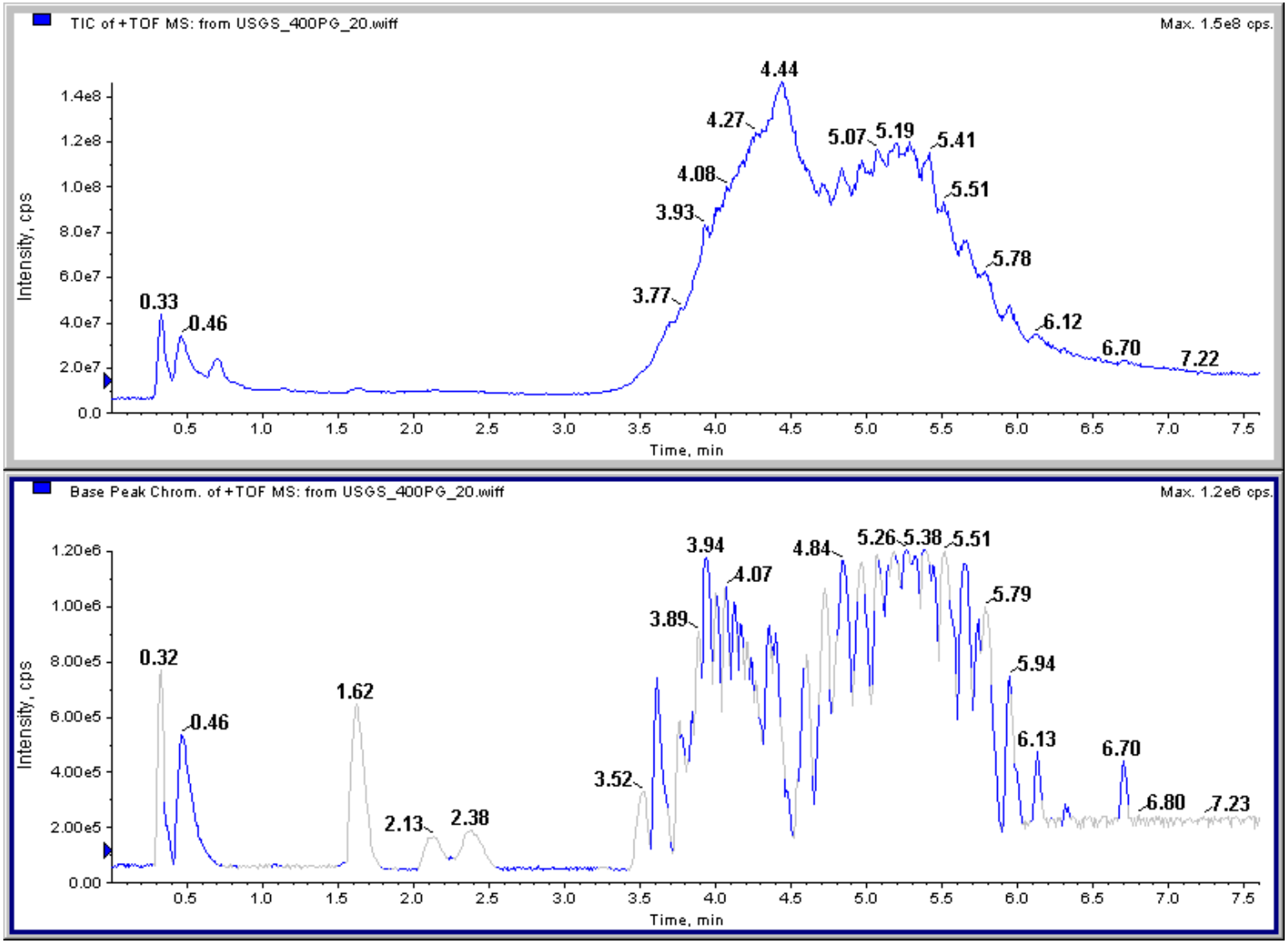
Chromatographic and Spectral Data

ESI TOF Positive ion

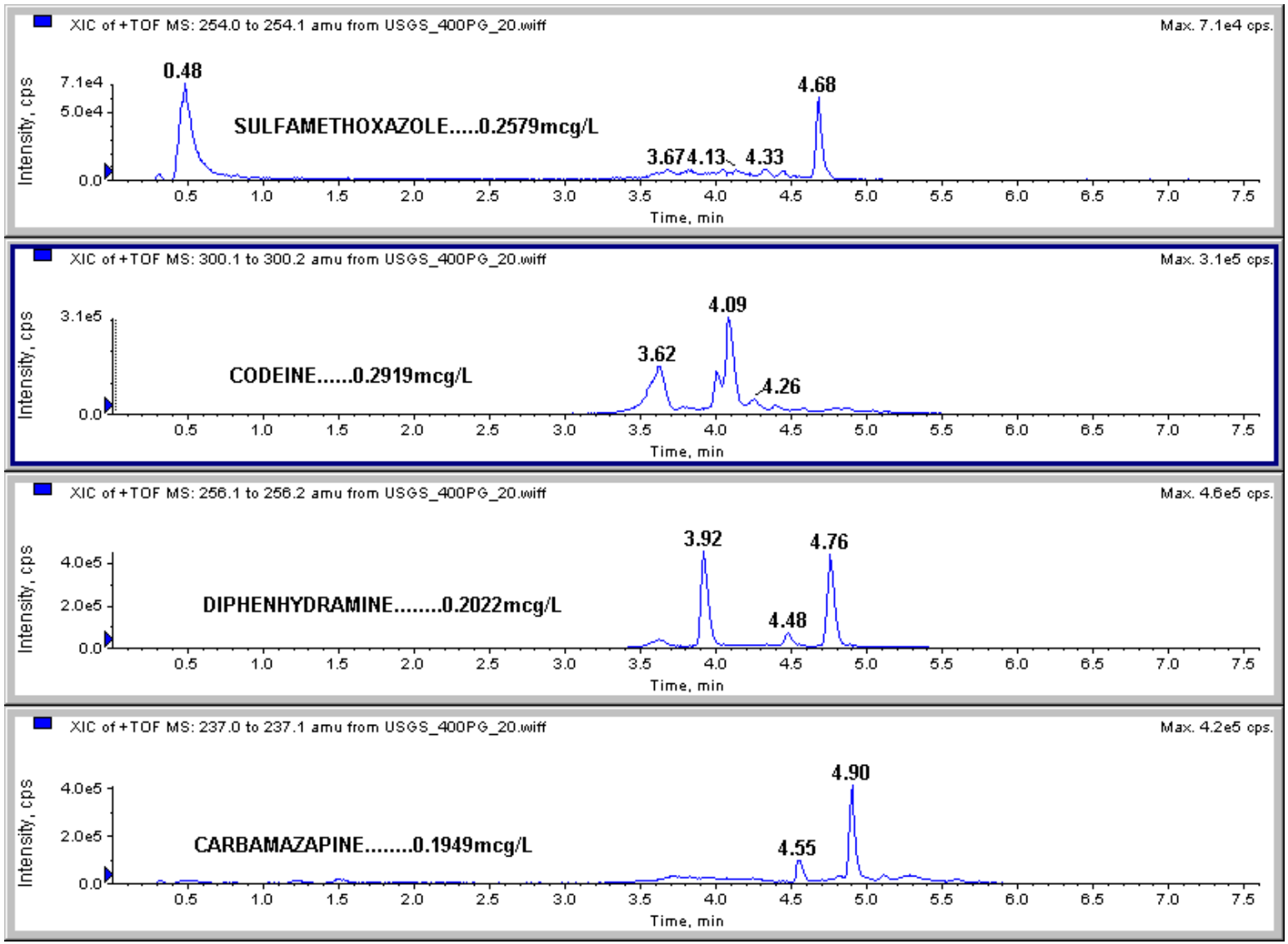
20 MICROLITER INJECTION OF
WATER SAMPLE 119

Agilent Technologies

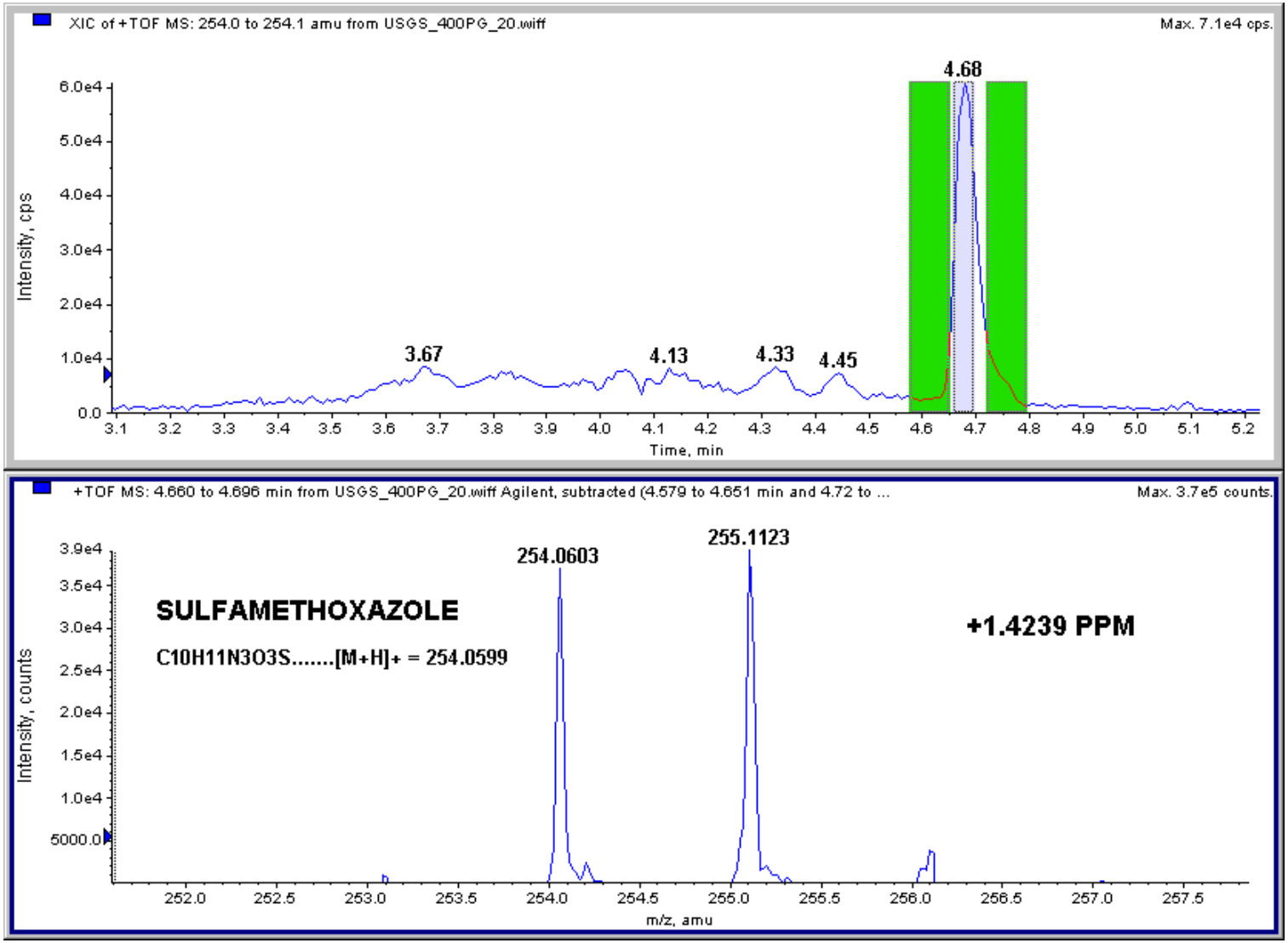
TIC and BPC OF WATER SAMPLE 119



EICs OF WATER SAMPLE 119



EICs OF WATER SAMPLE 119



Conclusions

- Agilent LC/MSD ESI-TOF offers routinized accurate mass measurement
  - ✓Seamless addition of lock-mass
  - ✓Auto-calibration of every stored scan
  - ✓Better than 5 ppm at low mass and 3 ppm at higher mass
- Confirmation and identification of unknowns
- Quantitative response
  - ✓ up to 3 orders of magnitude linearity (within range of ESI)
  - ✓Greater selectivity
    - ➢From higher mass resolution (>4000 for low mass and 10000 for high mass)
    - ➢And accurate mass automatically stored for every scan