Conference on Small Molecule Science

COSMOS

Unknown Identification vs. Molecular Weight Confirmation – How Much Mass Accuracy is Enough?

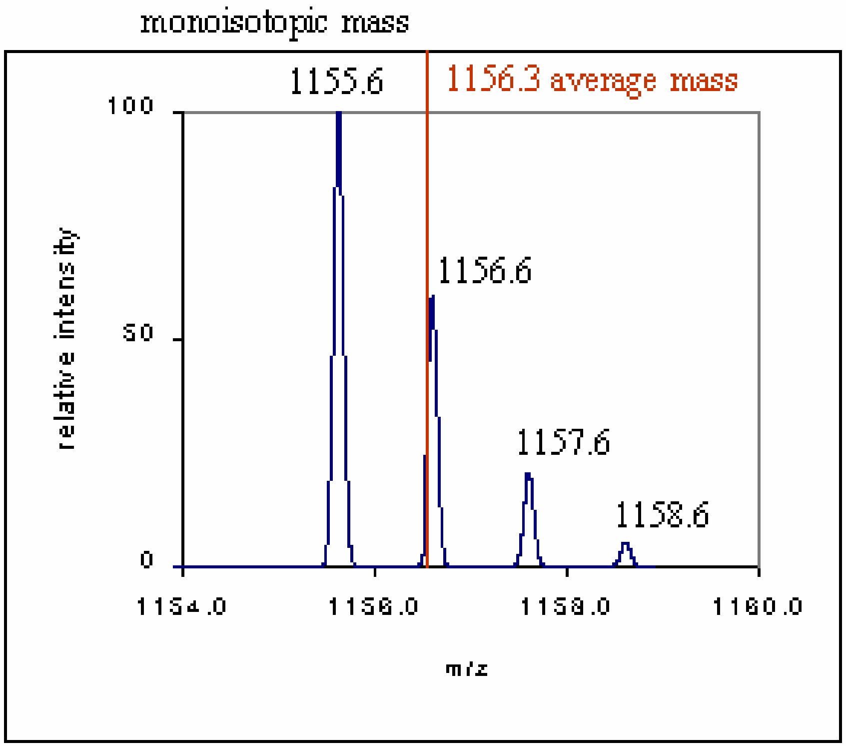
Daniel L. Norwood

Associate Director Analytical Sciences

Boehringer Ingelheim Pharmaceuticals, Inc.

Presentation Outline

- A brief historical perspective on mass measurement from the era of *Heavy Iron*
- Accurate mass measurements by Time-of-Flight
- Accurate mass measurements by Ion Cyclotron Resonance (FT-MS)



For a given compound the monoisotopic mass is the mass of the isotopic peak whose elemental composition is composed of the most abundant isotopes of those elements

The monoisotoptic mass of this compound is 1155.6

Why do we need accurate mass measurements in pharmaceutical development?

- Given a drug substance (MW 335)
- $[M+H]^+$ = 336
- Accurate monoisotopic mass 335.1897
- Molecular formula based on accurate mass $C_{19}H_{26}NO_3F$

- 0.2% photolysis product (MW 333)
- $[M+H]^+$ = 334
- Accurate monoisotopic mass 333.1940
- Molecular formula based on accurate mass $C_{19}H_{27}NO_4$

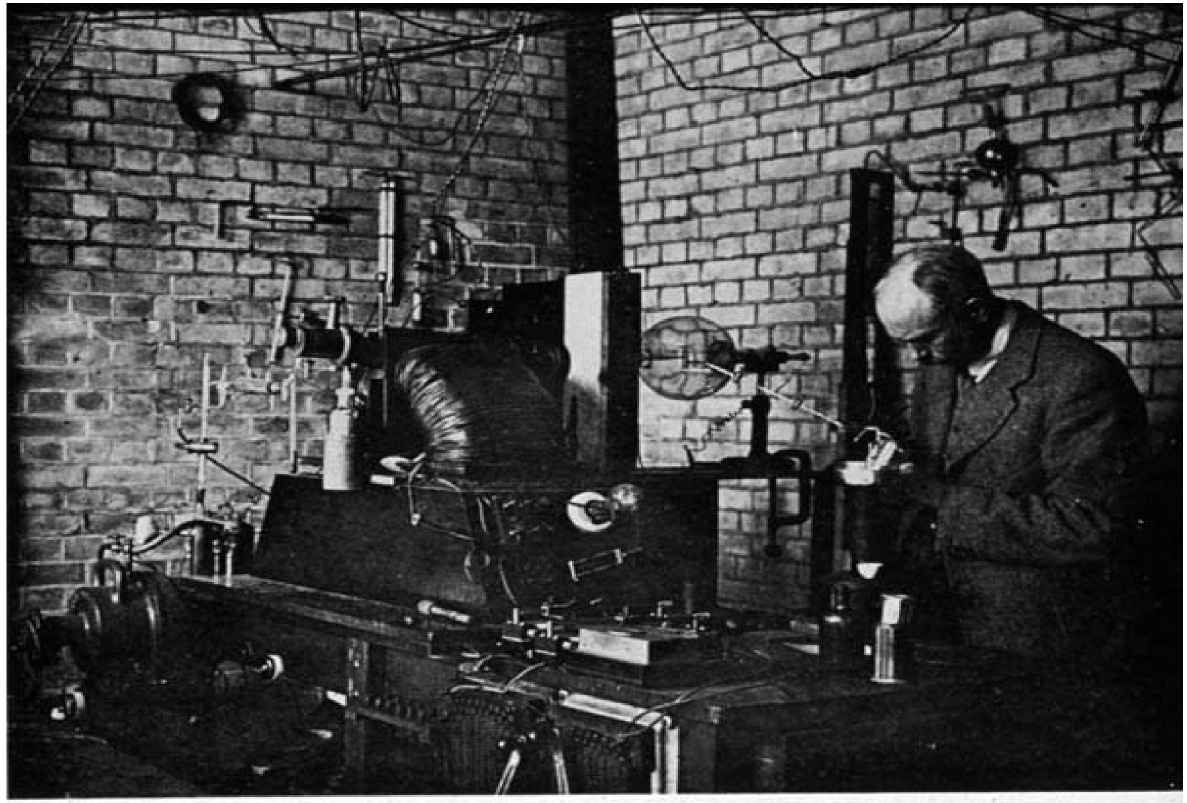


Plate 1. F. W. Aston with second mass spectrograph.

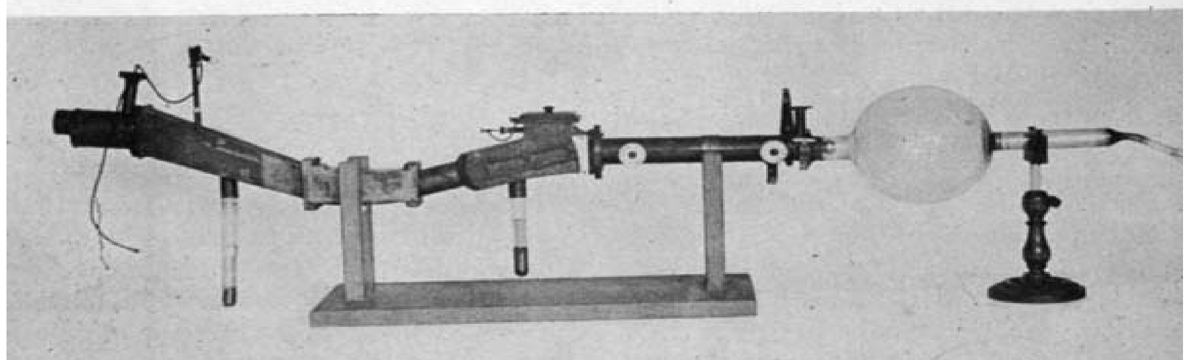
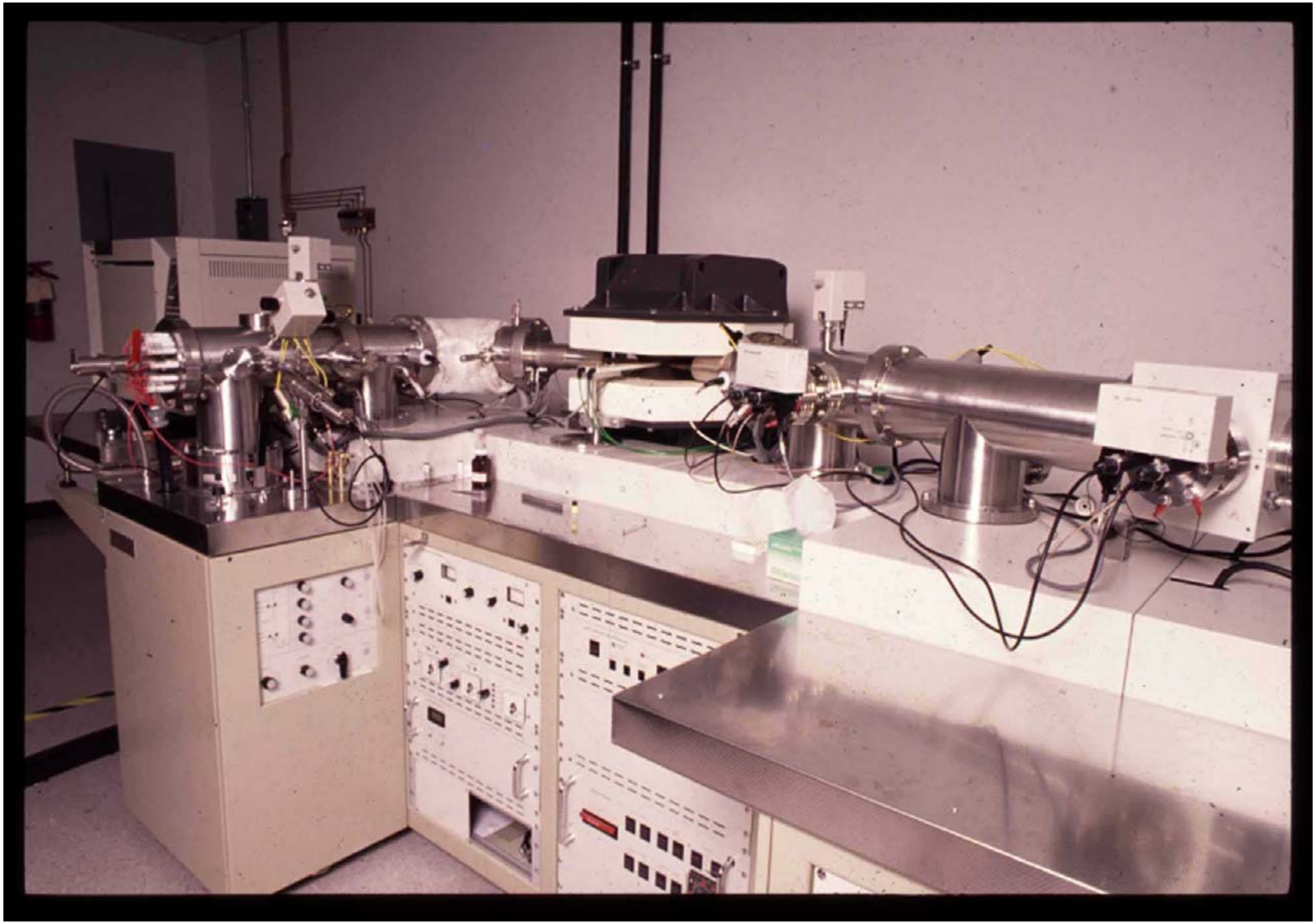


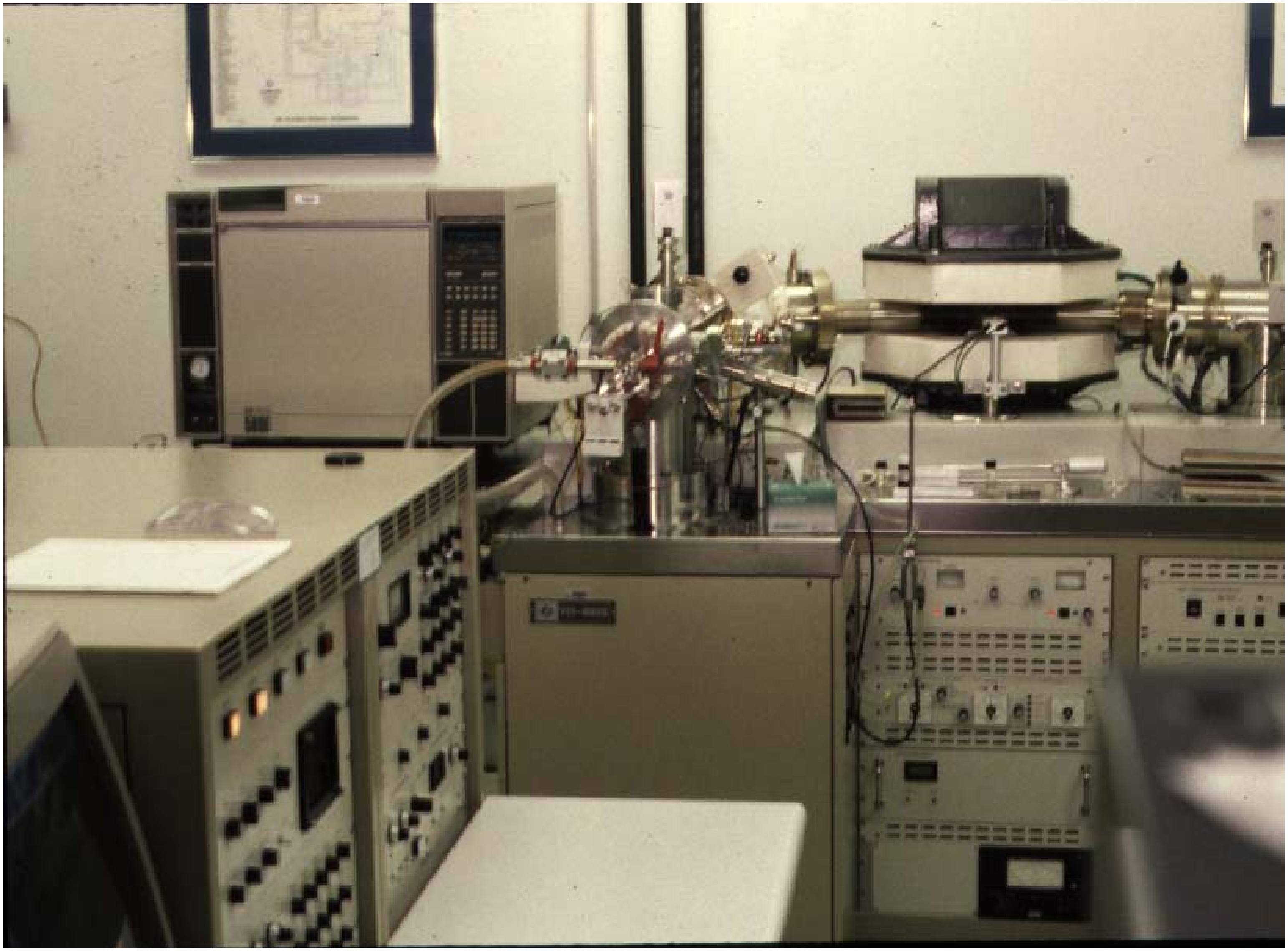
Plate 2. F. W. Aston's third mass spectrograph (1937).

Conference on Small Molecule Science
COSMOS

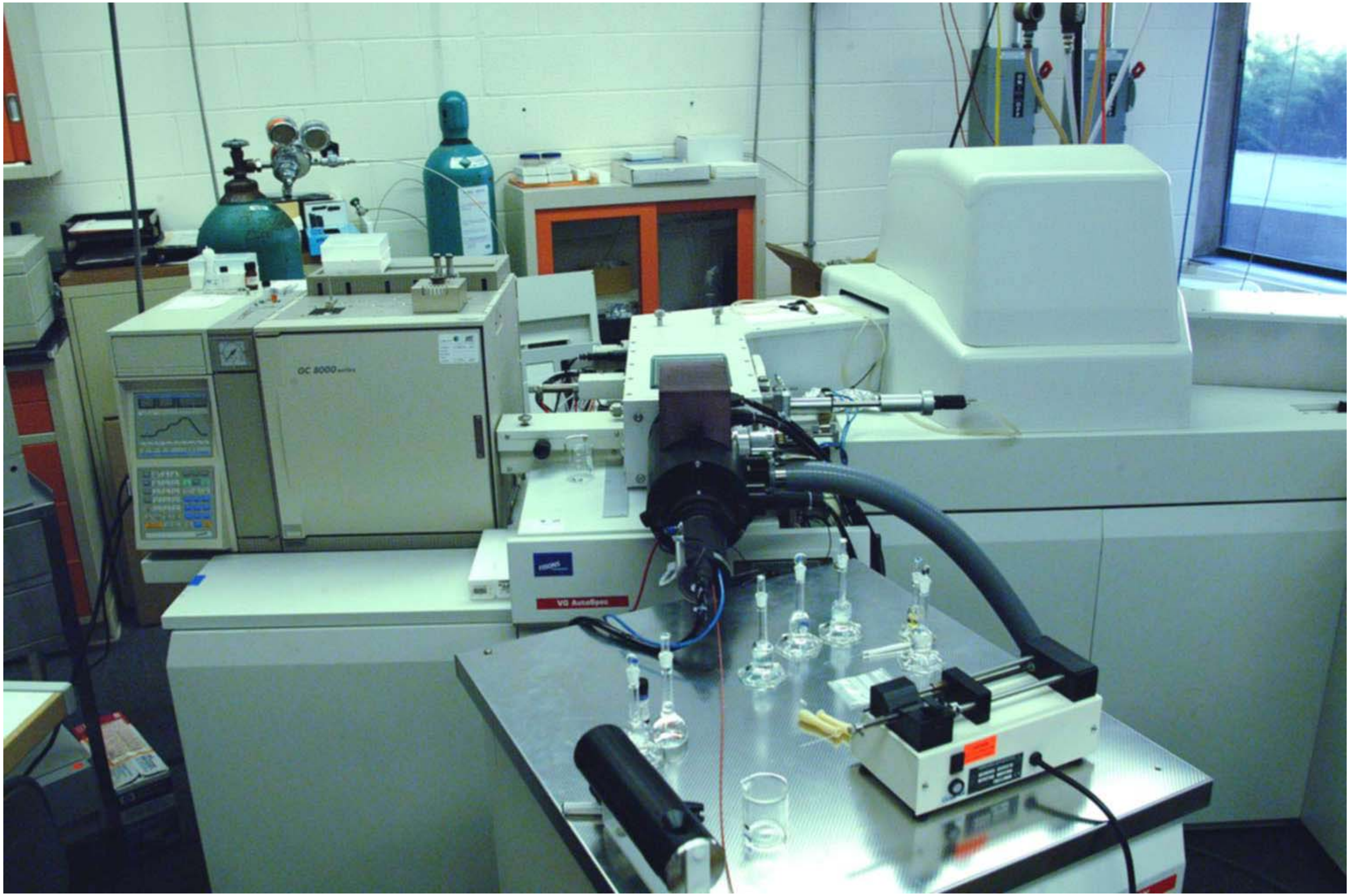


Measuring Accurate Mass with *Heavy Iron* – "Peak Matching"

- Accomplished at "High Resolution"
  - 10,000 resolving power ($m/\Delta m$; 10% valley definition)
- $m/z = B^2R^2/2V \rightarrow m_1/m_2=V_2/V_1$
- Overlay m_1 (reference ion; PFK) with m_2 (unknown) on an oscilloscope and measure V_2/V_1
- Peak shape critical for proper "match"
- "Datasystem" acquisition and control possible on later instruments

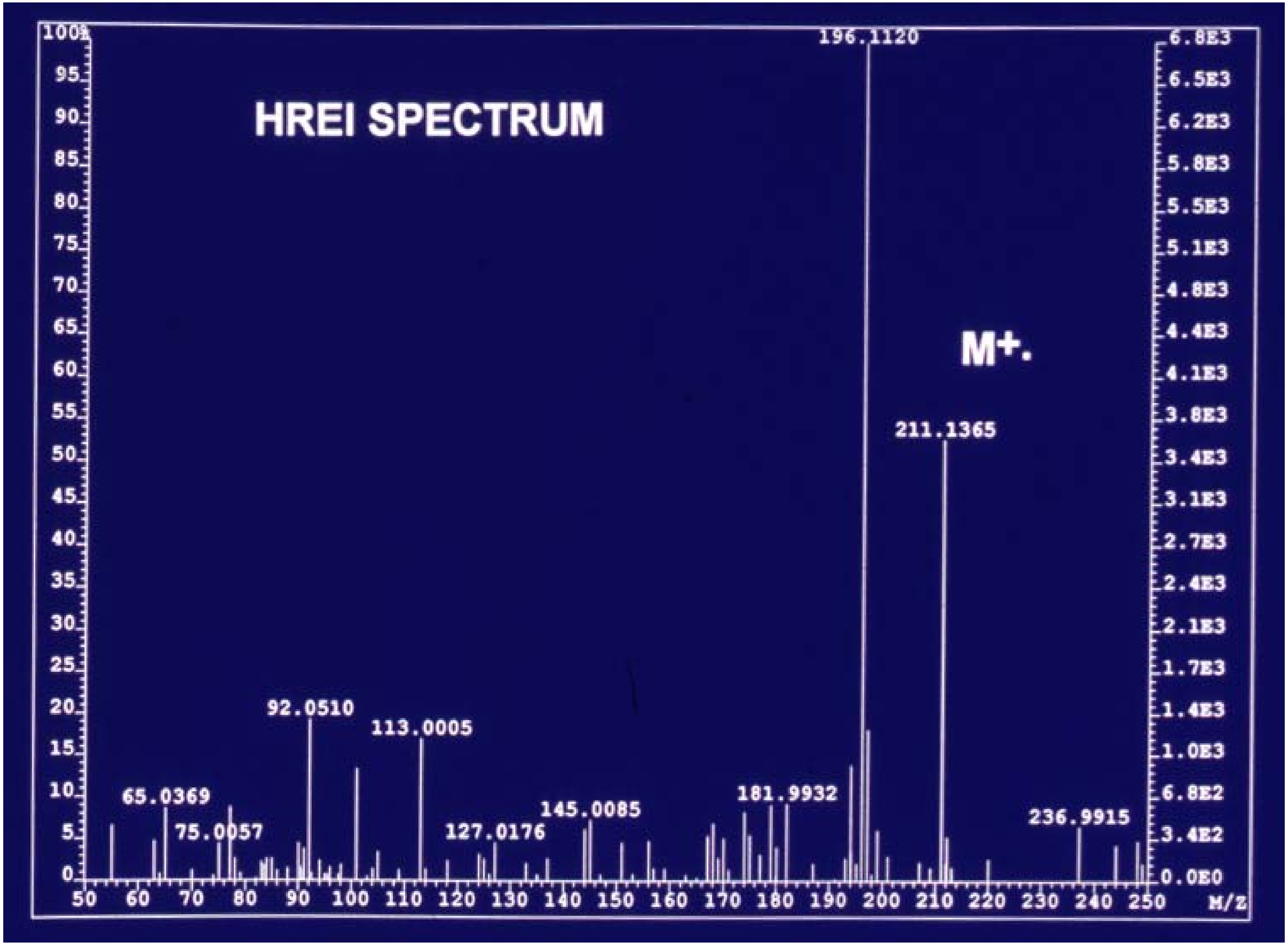


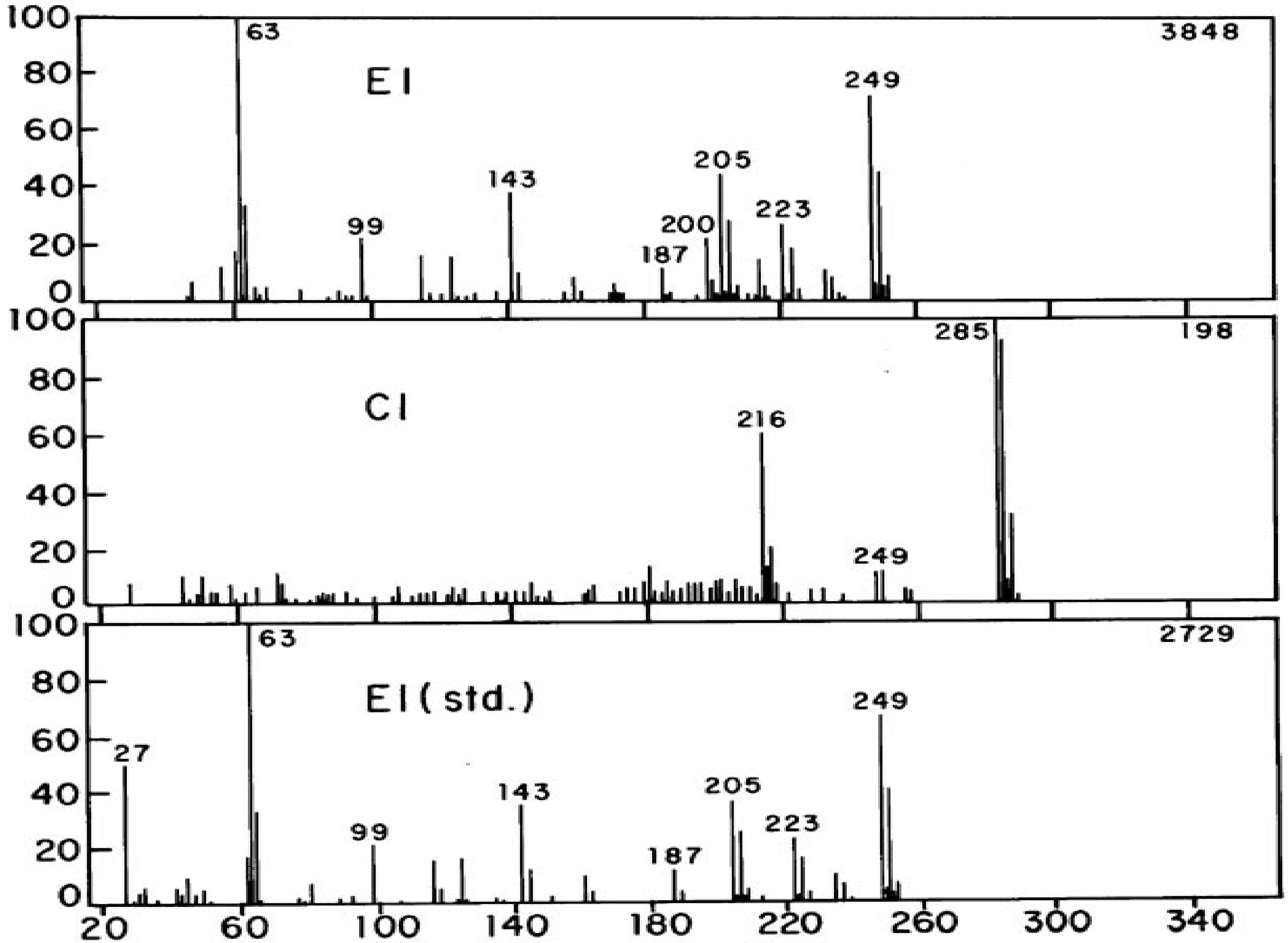
Conference on Small Molecule Science
COSMOS



Measuring Accurate Mass with *Heavy Iron* – "Scanning"

- Accomplished at "High Resolution"
  - 10,000 resolving power (m/Δm; 10% valley definition) for short range linear voltage scans
  - 5000 resolving power for GC/MS with exponential magnet scans
- Datasystem acquisition and calibration with reference ions (PFK internal reference)
- Peak shape very critical for proper ion beam centroiding by datasystem


HREI SPECTRUM
196.1120
M+.
211.1365
92.0510
113.0005
65.0369
75.0057
127.0176
145.0085
181.9932
236.9915
M/Z



M/E	C	H	O 16	CL 35	P	MMU	OBS. MASS
249	7	5	10	0	0	0.8	248.9874
	13	7	1	2	0	-0.0	
	18	2	0	0	1	2.0	
	12	7	2	1	1	-0.2	
	6	12	4	2	1	-2.4	
205	6	2	6	1	0	-2.0	204.9559
	5	2	7	0	1	-2.1	
	4	8	3	2	1	2.9	

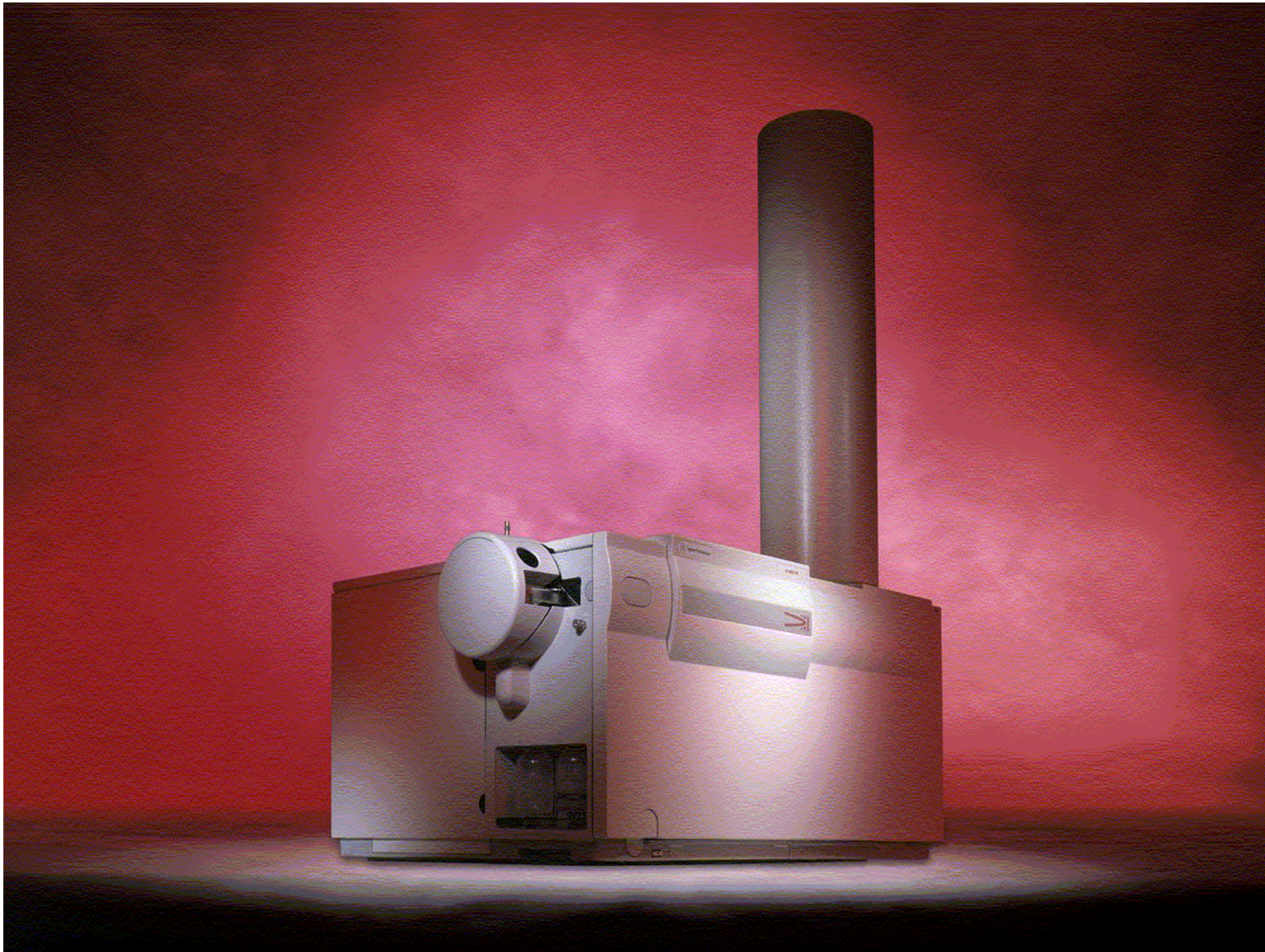
$(C_2H_4ClO)_3P{=}O$

Measuring Accurate Mass with *Time-of-Flight*

- Instrumentation in use in 1950s
- $T=L/v$ (T=flight time (sec); L=flight distance (m); v=velocity)
- Substituting into $mv^2/2=zV$ gives

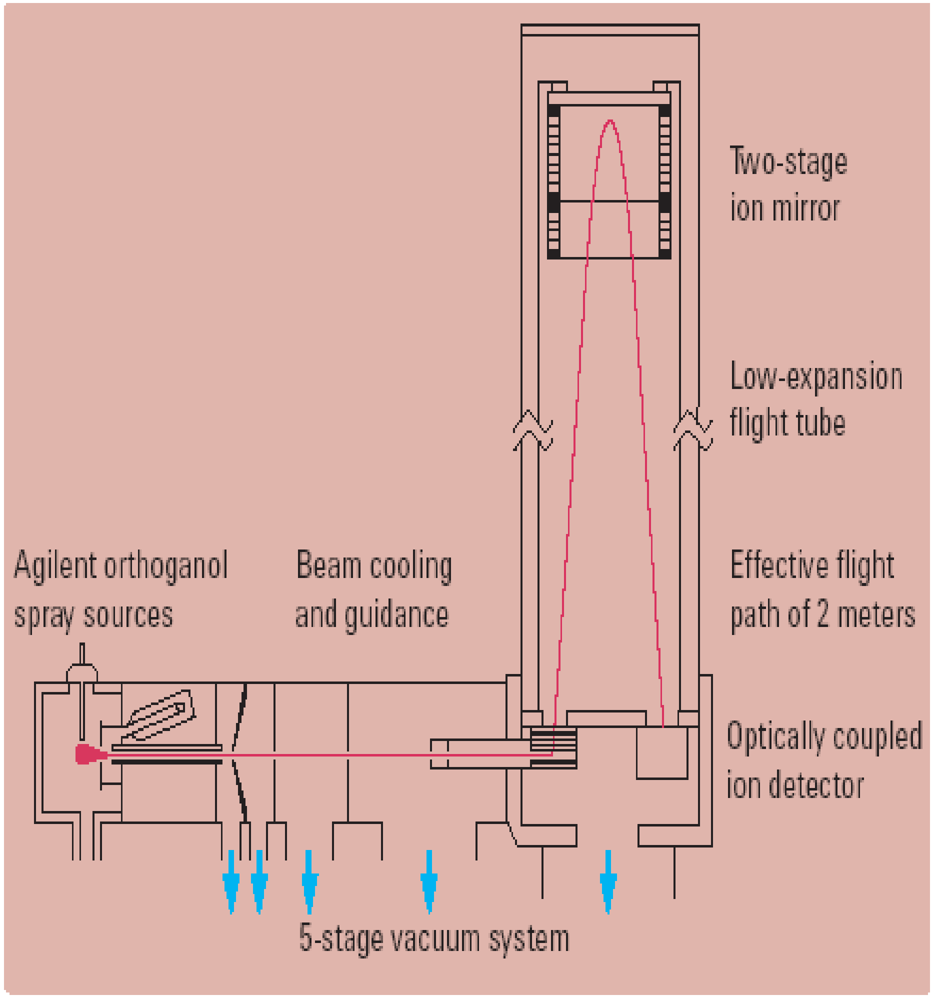
$$t=Lm^{1/2}/(2zV)^{1/2}$$

- For high mass accuracy L and V must be carefully controlled or monitored/compensated
- Adequate mass accuracy requires higher resolution (~5000, 50% valley definition), and internal reference ion(s)
- Modern fast computers and electronics have made accurate mass TOF possible and routine

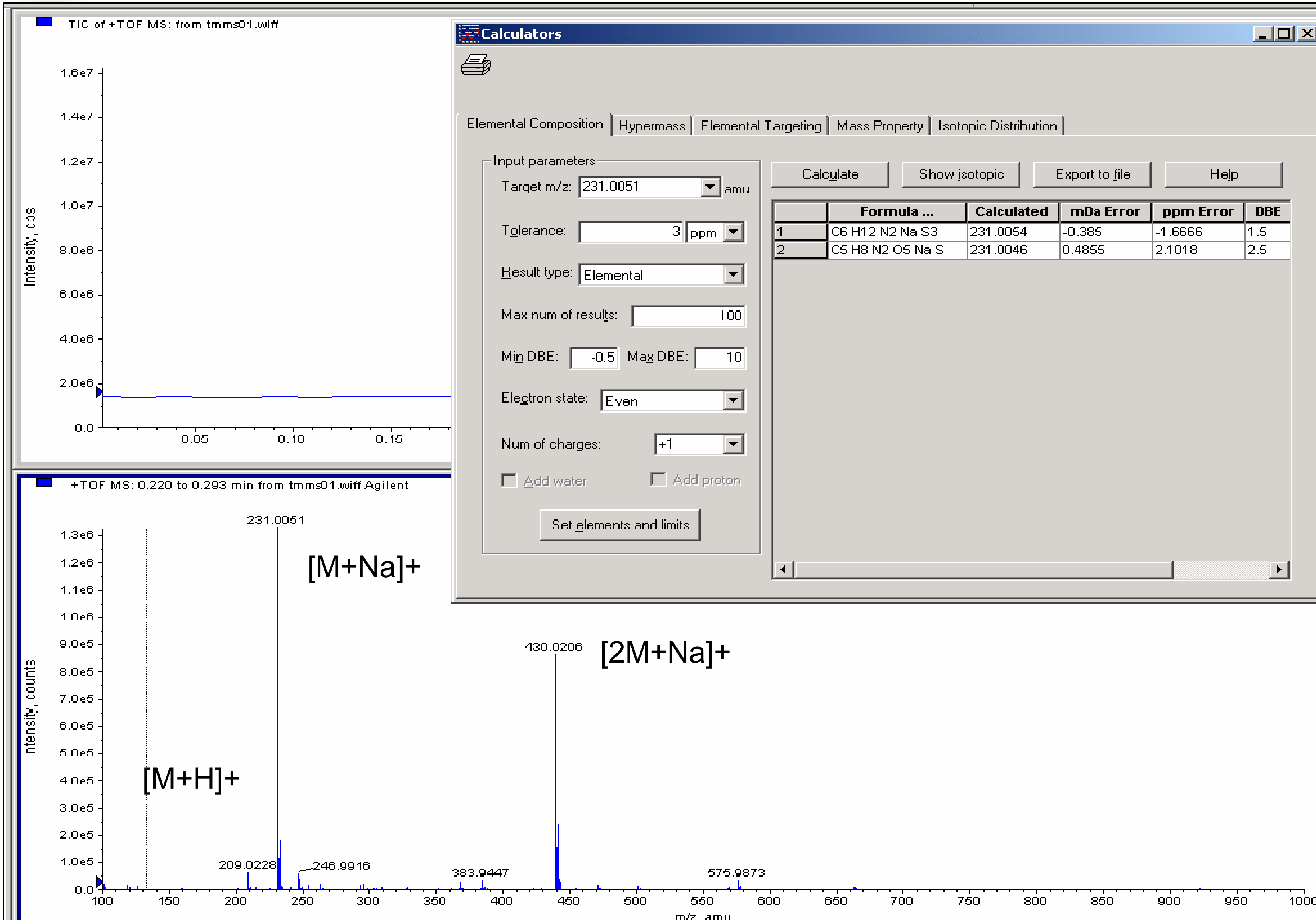


Functioning of LC/MSD TOF

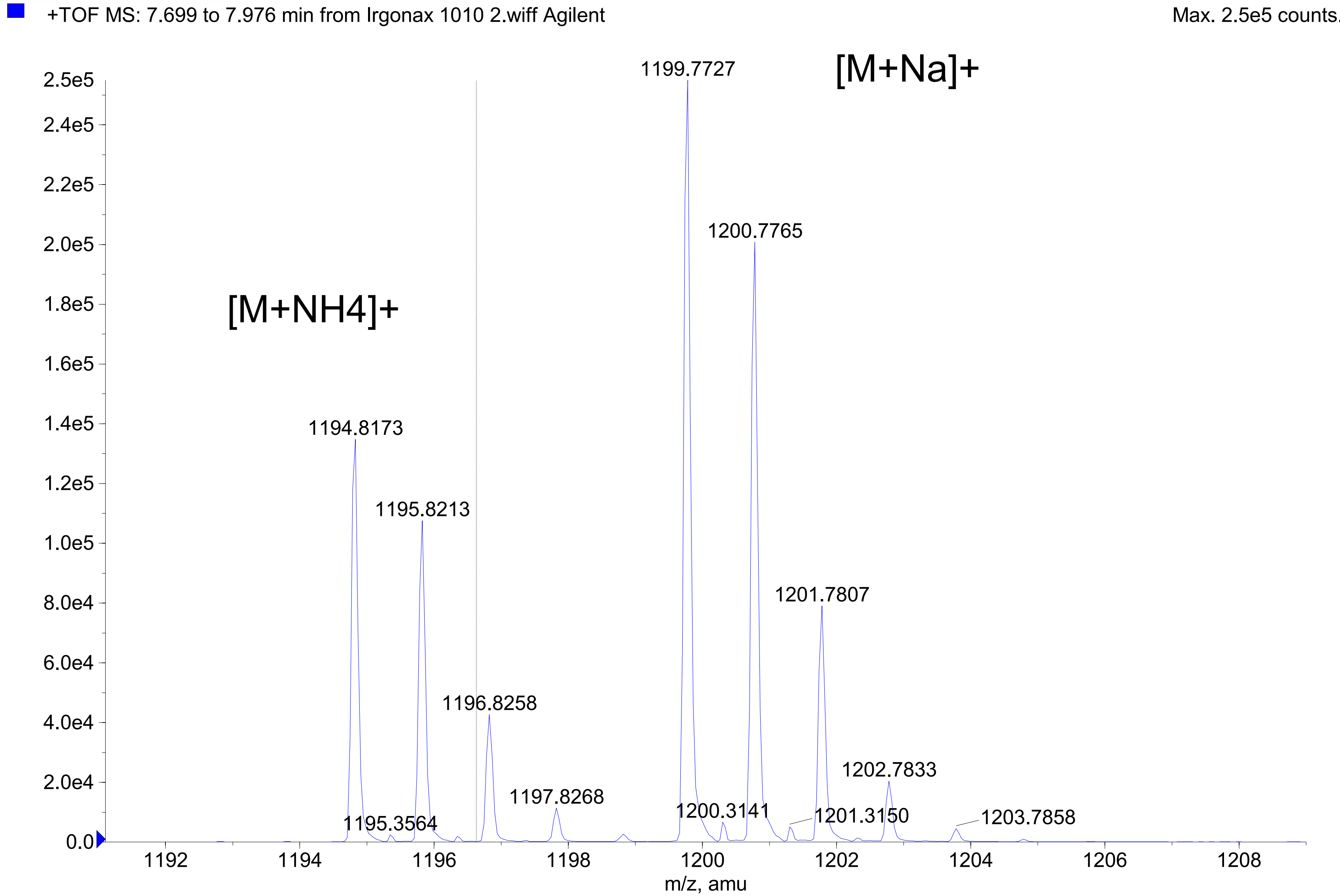
- An internal reference solution is added post column.
- Several ionization methods available, including ESI, APCI, APPI.
- Ion beam is pulsed sending the ions down the flight tube to spatially separate according the m/z.
- The ions are reflected off an ion mirror and further separated until they arrive at the front of the detector (at different flight times depending on there mass-to-charge ratio).
- The signal is passes to a microchannel plate/photomultiplier detector were it is amplified and recorded by the data system.



ESI+ TOF Mass Spectrum of TMTMS



Expanded ESI+ TOF Mass Spectrum of Irganox 1010



Elemental Composition Report for Irganox 1010 from ESI+ TOF-MS

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -0.5, max = 40.0

Monoisotopic Mass, Odd and Even Electron Ions
289500 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)

Mass	Calc. Mass	mDa	PPM	DBE	Formula
1199.7727	1199.7738	-1.1	-1.0	19.5	12C73 1H108 16O12 23Na
	1199.7763	-3.6	-3.0	22.5	12C75 1H107 16O12
	1199.7680	4.7	3.9	28.5	12C80 1H104 16O7 23Na

Calculators

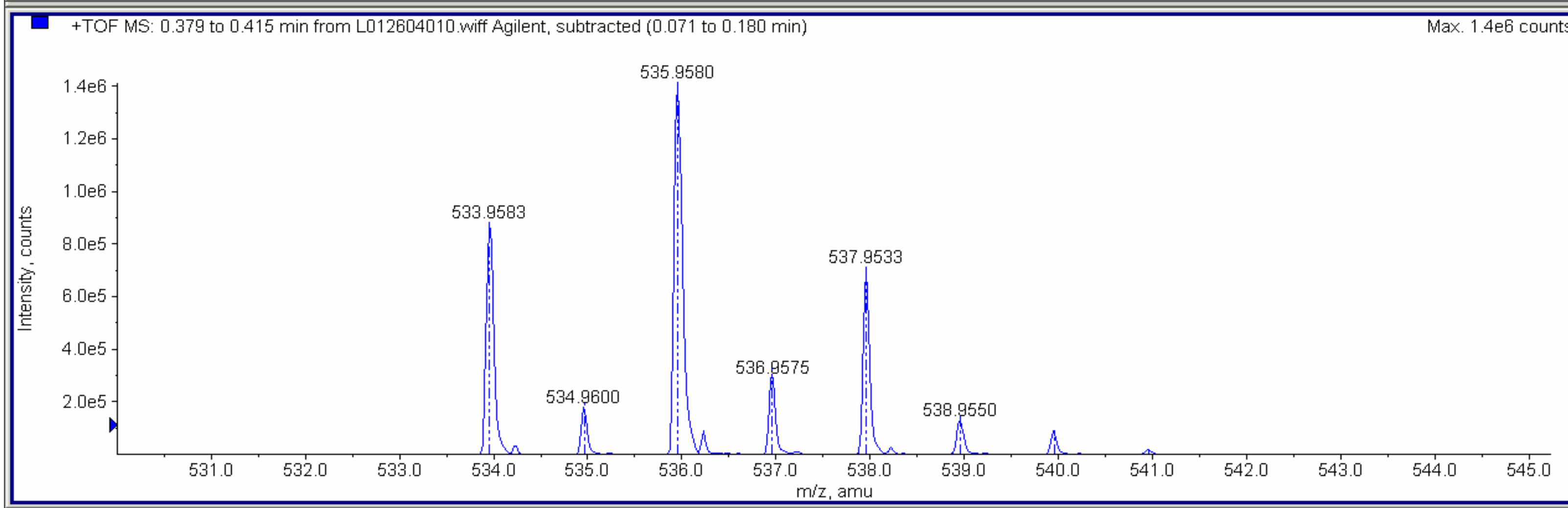
Elemental Composition | Hypermass | Elemental Targeting | Mass Property | Isotopic Distribution

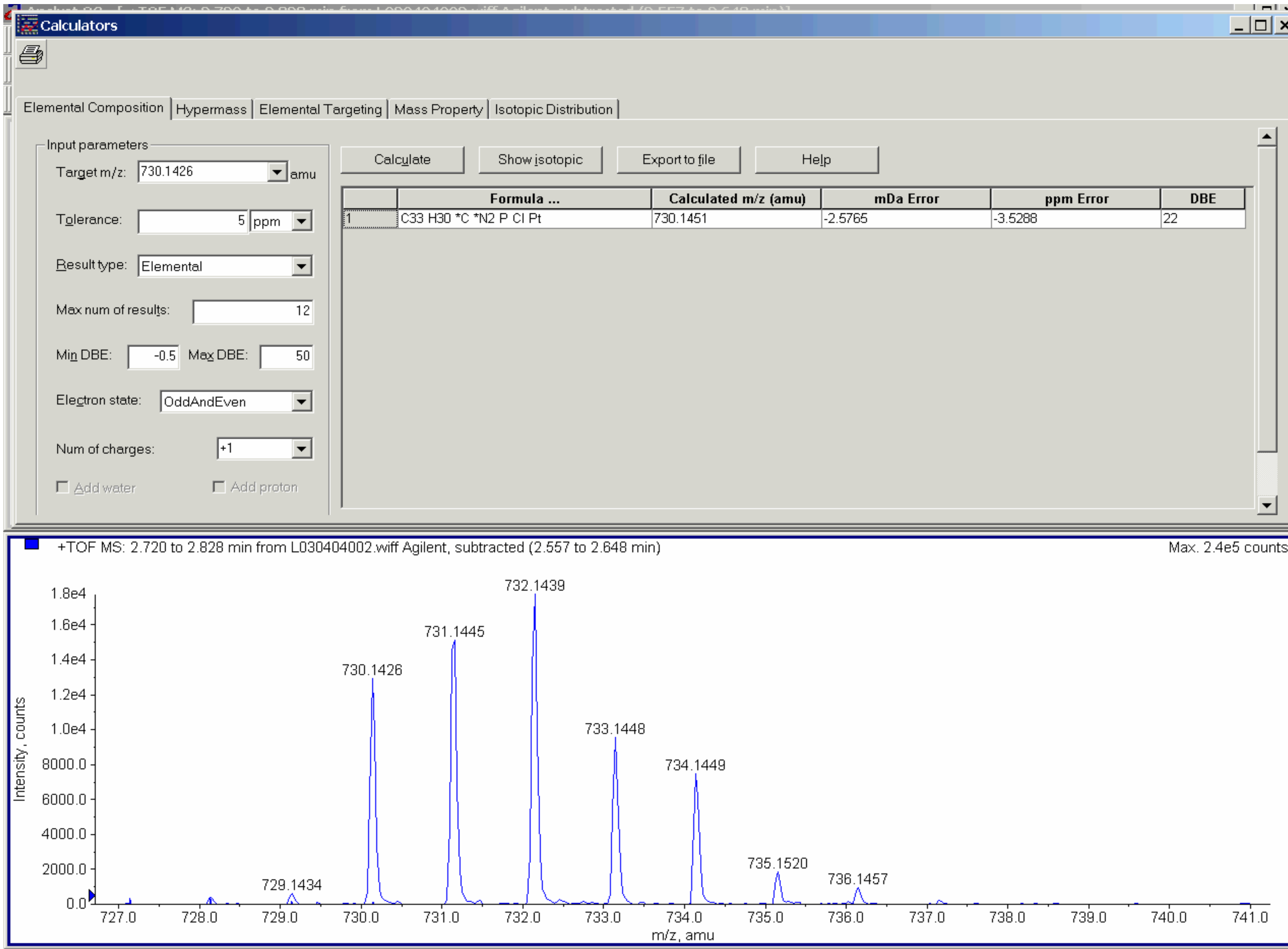
Input parameters

- Target m/z: 533.9580 amu
- Tolerance: 5 ppm
- Result type: Elemental
- Max num of results: 20
- Min DBE: -0.5 Max DBE: 50
- Electron state: OddAndEven
- Num of charges: +1
- Add water
- Add proton

Calculate | Show isotopic | Export to file | Help

	Formula ...	Calculated m/z (amu)	mDa Error	ppm Error	DBE
1	C23 H13 N3 O F2 Cl2 Br	533.9581	-0.1616	-0.3027	16.5
2	C23 H11 N2 O3 F3 Cl Br	533.9588	-0.8173	-1.5307	17
3	C14 H19 N3 O4 F3 Cl3 Br	533.9571	0.8881	1.6634	3.5
4	C19 H20 N3 O2 F Cl Br2	533.9589	-0.9317	-1.7448	9.5
5	C13 H25 N3 O4 F Cl2 Br2	533.9567	1.261	2.3617	0.5
6	C20 H14 N3 O2 F3 Cl2 Br	533.9593	-1.3045	-2.4432	12.5
7	C22 H18 N O F2 Cl3 Br	533.9599	-1.9915	-3.7298	11.5
8	C17 H18 N3 O3 F2 Cl3 Br	533.9559	2.0311	3.8039	7.5
9	C16 H21 N3 O3 F2 Cl Br2	533.96	-2.0746	-3.8853	5.5
10	C20 H15 N2 O4 F2 Cl2 Br	533.9554	2.5183	4.7163	12

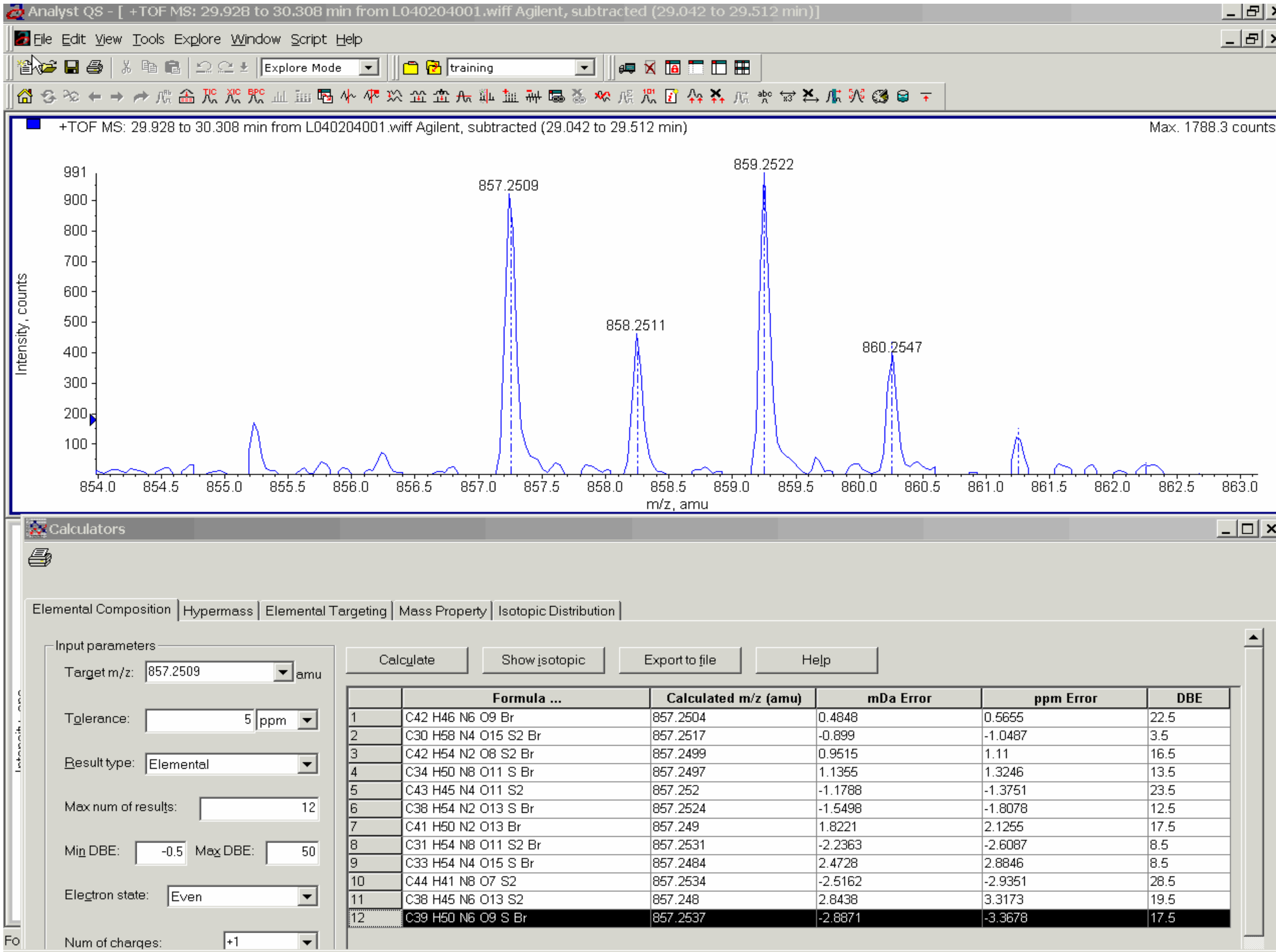



Calculators
Elemental Composition
Hypermass
Elemental Targeting
Mass Property
Isotopic Distribution
Input parameters
Target m/z: 730.1426 amu
Tolerance: 5 ppm
Result type: Elemental
Max num of results: 12
Min DBE: -0.5
Max DBE: 50
Electron state: OddAndEven
Num of charges: +1
Add water
Add proton
Calculate
Show isotopic
Export to file
Help
Formula ...
Calculated m/z (amu)
mDa Error
ppm Error
DBE
1
C33 H30 *C *N2 P Cl Pt
730.1451
-2.5765
-3.5288
22
+TOF MS: 2.720 to 2.828 min from L030404002.wiff Agilent, subtracted (2.557 to 2.648 min)
Max. 2.4e5 counts.
Intensity, counts
m/z, amu
729.1434
730.1426
731.1445
732.1439
733.1448
734.1449
735.1520
736.1457

Analyst QS - [TIC of +TOF MS: from L040204001.wiff]
File Edit View Tools Explore Window Script Help
Explore Mode
training
TIC of +TOF MS: from L040204001.wiff
Max. 6.6e7 cps.
136.05
120.06
922.02
358.12
466.26
438.03
452.05
480.07
492.07
871.25
352.33
803.53
915.66
Intensity, cps
Time, min
XIC of +TOF MS: 857.3 to 857.3 amu from L040204001.wiff
Max. 1.1e4 cps.
922.02
922.02
131.97
131.97
Intensity, cps
Time, min
+TOF MS: 29.928 to 30.308 min from L040204001.wiff Agilent, subtracted (29.042 to 29.512 min)
Max. 1788.3 counts.
857.2509
859.2522
858.2511
860.2547
Intensity, counts
m/z, amu

Conference on Small Molecule Science

COSMOS



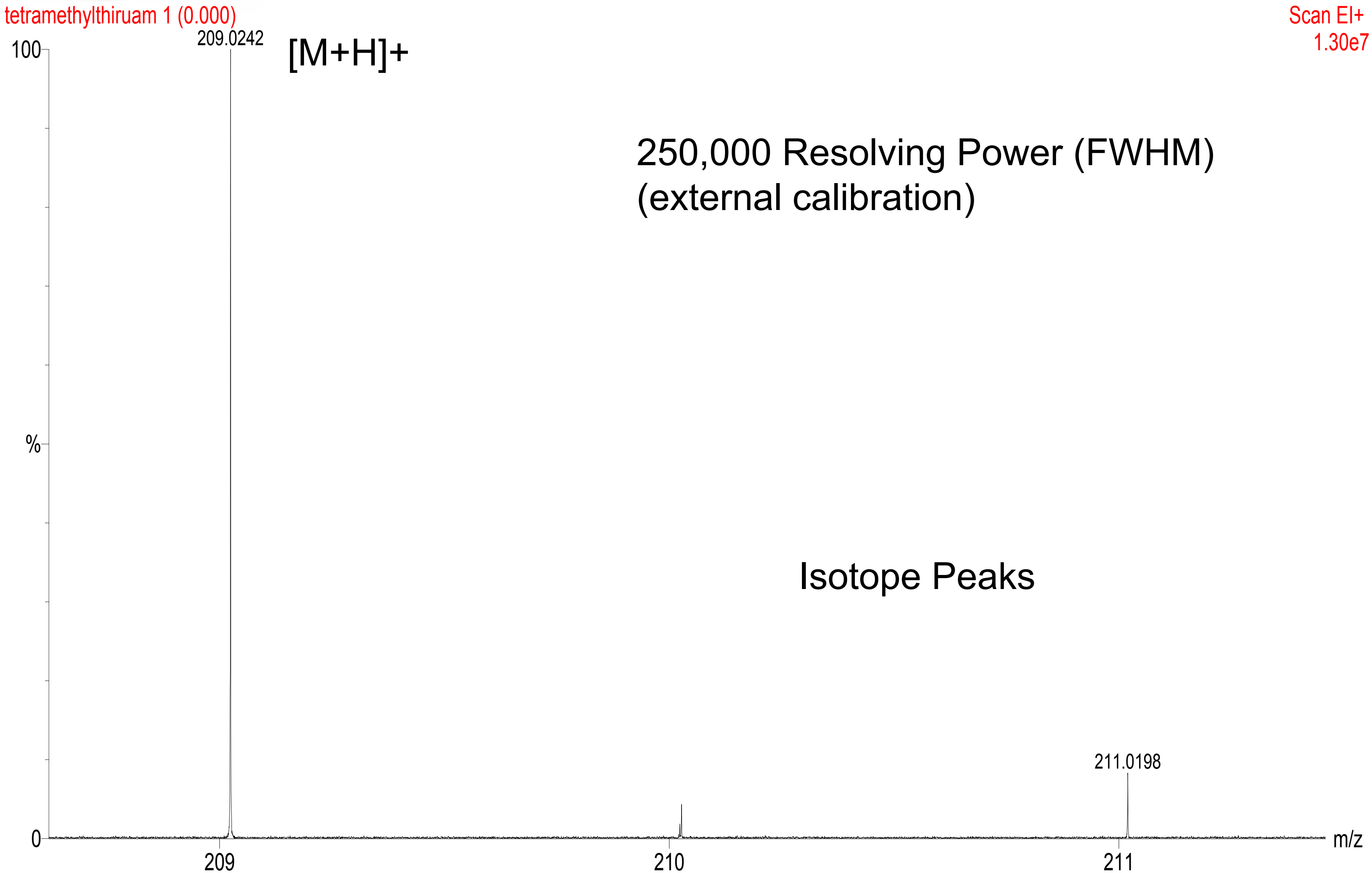
	Formula ...	Calculated m/z (amu)	mDa Error	ppm Error	DBE
1	C42 H46 N6 O9 Br	857.2504	0.4848	0.5655	22.5
2	C30 H58 N4 O15 S2 Br	857.2517	-0.899	-1.0487	3.5
3	C42 H54 N2 O8 S2 Br	857.2499	0.9515	1.11	16.5
4	C34 H50 N8 O11 S Br	857.2497	1.1355	1.3246	13.5
5	C43 H45 N4 O11 S2	857.252	-1.1788	-1.3751	23.5
6	C38 H54 N2 O13 S Br	857.2524	-1.5498	-1.8078	12.5
7	C41 H50 N2 O13 Br	857.249	1.8221	2.1255	17.5
8	C31 H54 N8 O11 S2 Br	857.2531	-2.2363	-2.6087	8.5
9	C33 H54 N4 O15 S Br	857.2484	2.4728	2.8846	8.5
10	C44 H41 N8 O7 S2	857.2534	-2.5162	-2.9351	28.5
11	C38 H45 N6 O13 S2	857.248	2.8438	3.3173	19.5
12	C39 H50 N6 O9 S Br	857.2537	-2.8871	-3.3678	17.5

Measuring Accurate Mass with *Ion Cyclotron Resonance (FT-MS)*

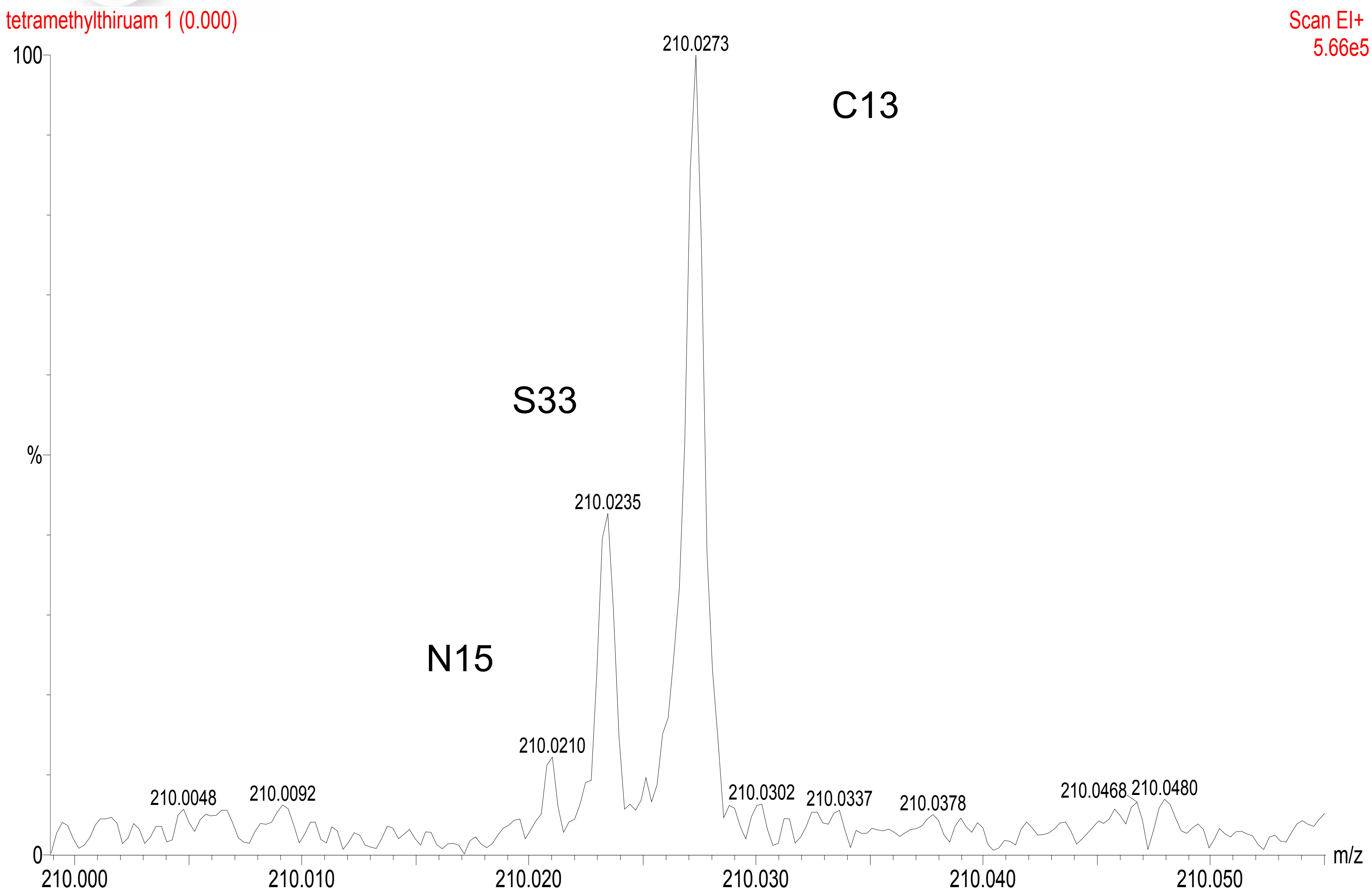
- Instrumentation in use in 1950s
- $m/z=B/2f_c$
- B created by a superconducting magnet
- All f_c measured simultaneously producing an FID (interfering sine wave pattern)
- Fourier Transform converts data from time domain to frequency domain
- Accomplished at "Ultra-High Resolution"
  - ≥100,000 resolving power typical
- Datasystem acquisition and calibration with or without reference ions (internal reference)
- Modern fast computers and electronics have made accurate mass FT-MS possible and routine


BRUKER
DALTONICS
BRUKER

ESI-FT/MS Mass Spectrum of Tetramethylthiuram Monosulfide (TMTMS)



Resolution of [M+H+1]+ Isotope Peak

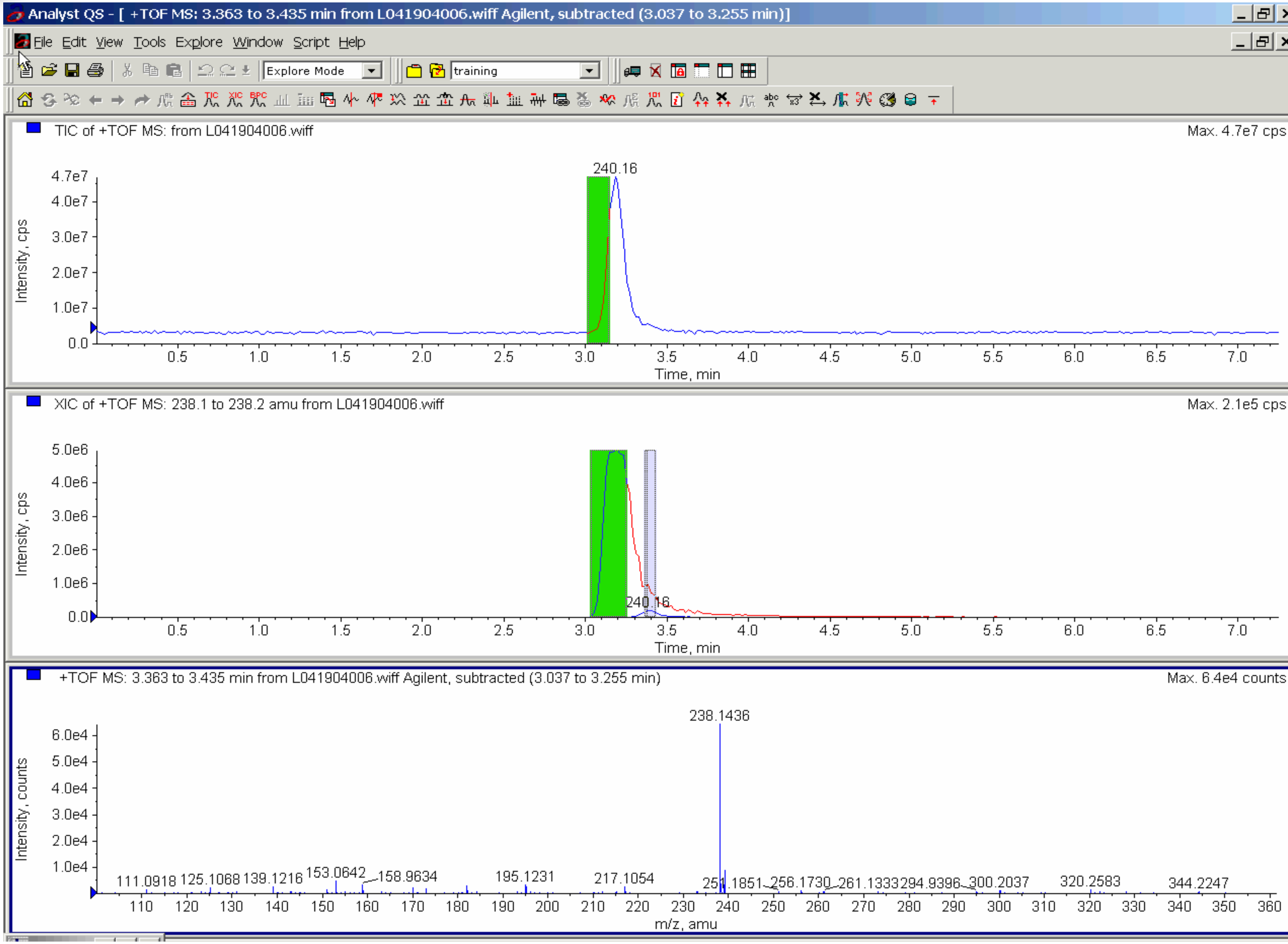


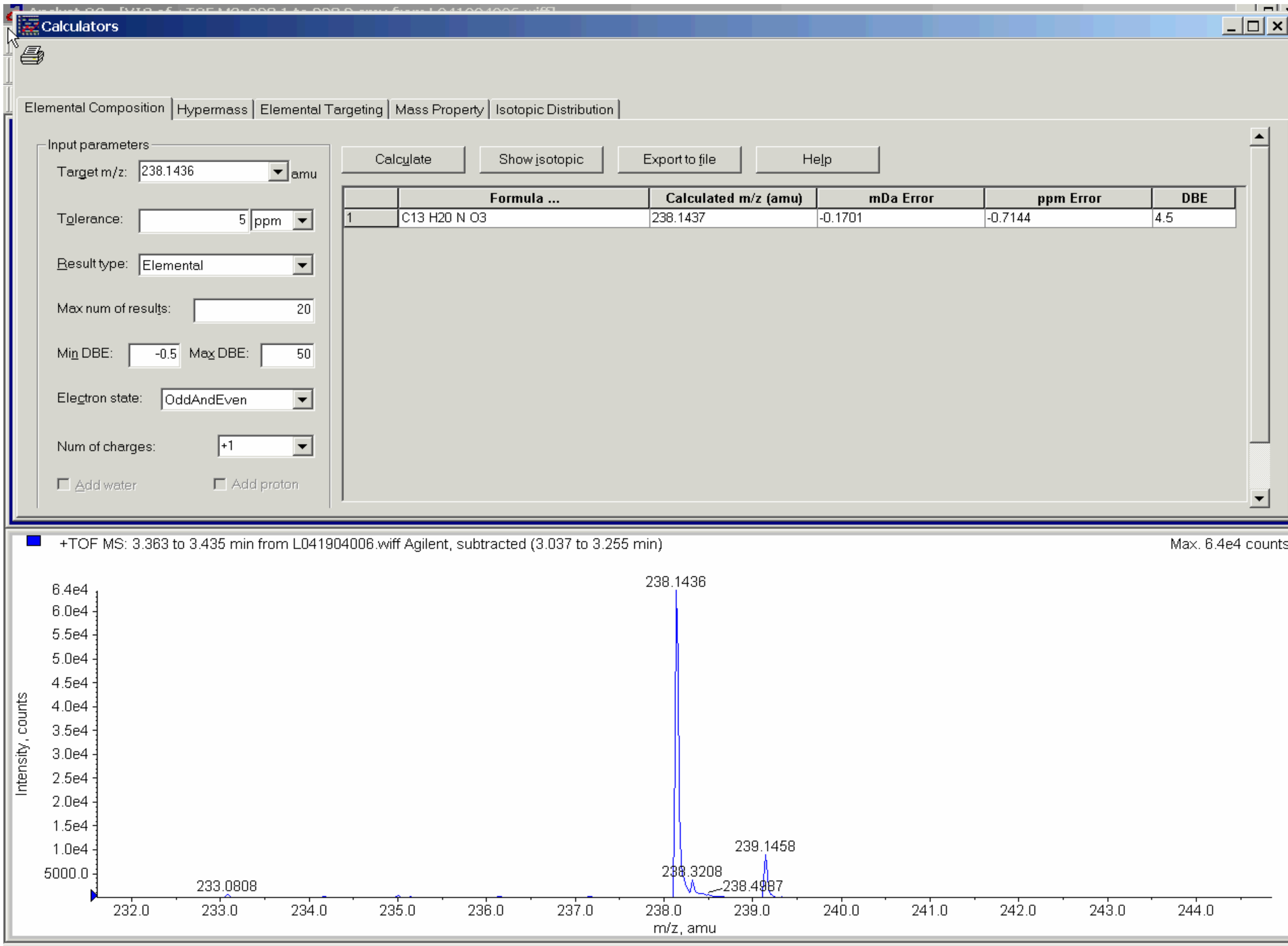
Elemental Composition Report for TMTMS from ESI+ FT-MS

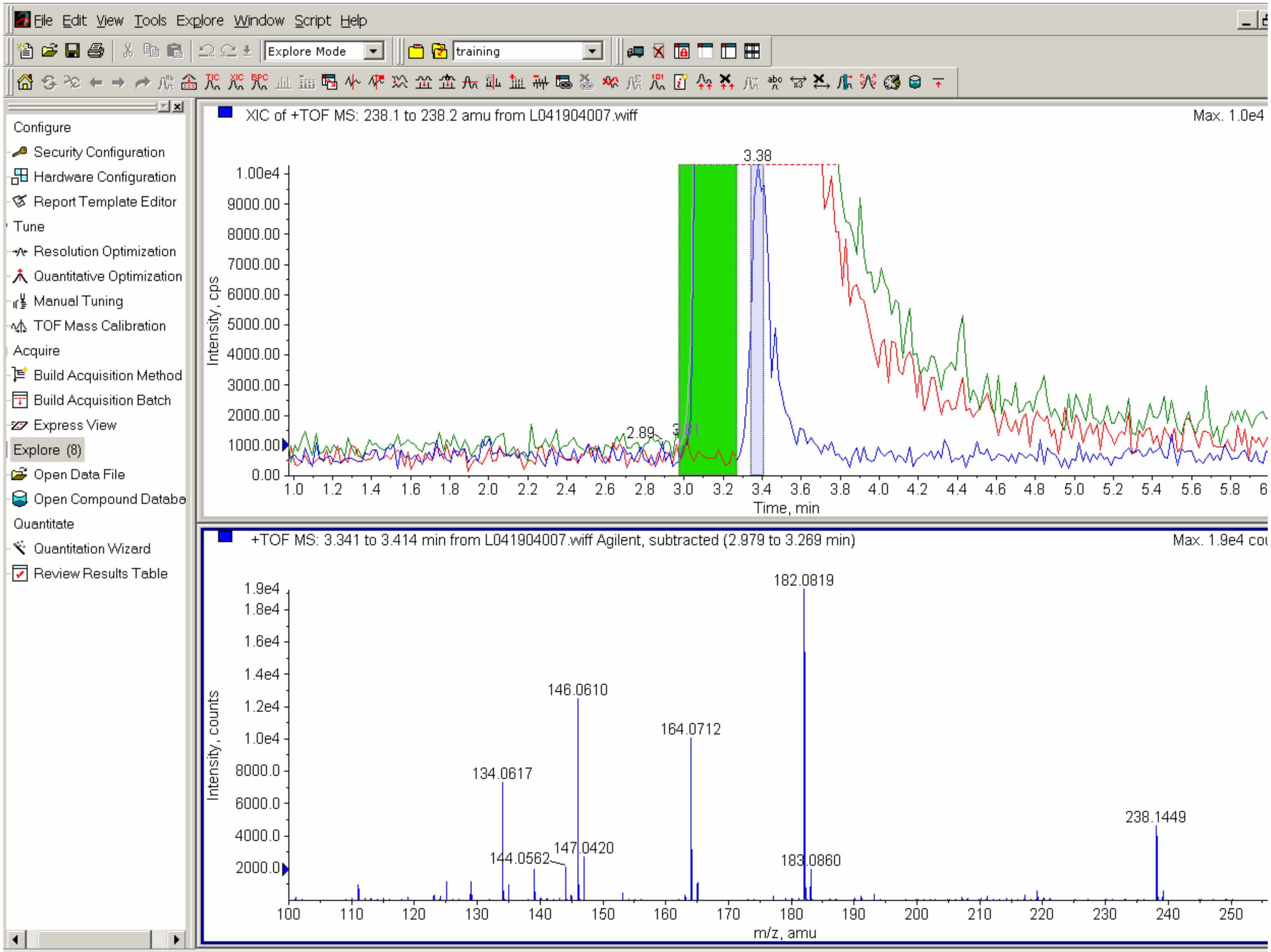
Single Mass Analysis (displaying only valid results)
Tolerance = 5.0 PPM / DBE: min = -0.5, max = 20.0

Monoisotopic Mass, Odd and Even Electron Ions
556 formula(e) evaluated with 5 results within limits (all results (up to 1000) for each mass)

	Mass	Calc. Mass	mDa	PPM	DBE	Formula
Minimum:					-0.5	
Maximum:			2.0	5.0	20.0	
	209.0241	209.0241	0.0	0.1	1.5	C6 H13 N2 S3
		209.0239	0.2	1.1	11.5	C13 H5 O3
		209.0246	-0.5	-2.2	7.5	C6 H5 N6 O S
		209.0232	0.9	4.2	2.5	C5 H9 N2 O5 S
		209.0232	0.9	4.2	8.0	C4 H3 N9 S


Analyst QS - [+TOF MS: 3.363 to 3.435 min from L041904006.wiff Agilent, subtracted (3.037 to 3.255 min)]
File Edit View Tools Explore Window Script Help
Explore Mode
training
TIC of +TOF MS: from L041904006.wiff
Max. 4.7e7 cps.
240.16
Intensity, cps
Time, min
XIC of +TOF MS: 238.1 to 238.2 amu from L041904006.wiff
Max. 2.1e5 cps.
240.16
Intensity, cps
Time, min
+TOF MS: 3.363 to 3.435 min from L041904006.wiff Agilent, subtracted (3.037 to 3.255 min)
Max. 6.4e4 counts.
238.1436
111.0918 125.1068 139.1216 153.0642 158.9634 195.1231 217.1054 256.1730 261.1333 294.9396 300.2037 320.2583 344.2247
Intensity, counts
m/z, amu

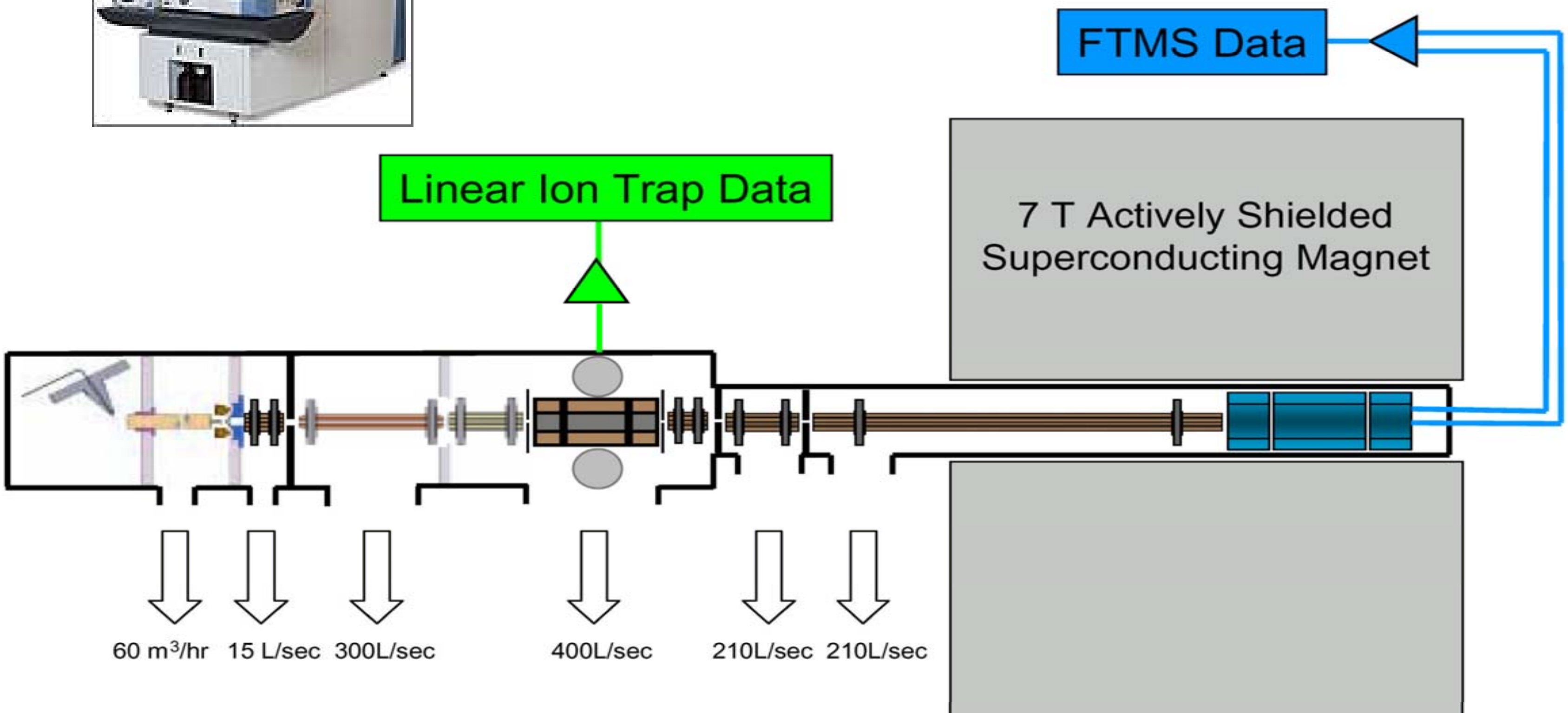

Calculators
Elemental Composition
Hypermass
Elemental Targeting
Mass Property
Isotopic Distribution
Input parameters
Target m/z: 238.1436 amu
Tolerance: 5 ppm
Result type: Elemental
Max num of results: 20
Min DBE: -0.5
Max DBE: 50
Electron state: OddAndEven
Num of charges: +1
Add water
Add proton
Calculate
Show isotopic
Export to file
Help
Formula ...
Calculated m/z (amu)
mDa Error
ppm Error
DBE
1
C13 H20 N O3
238.1437
-0.1701
-0.7144
4.5
+TOF MS: 3.363 to 3.435 min from L041904006.wiff Agilent, subtracted (3.037 to 3.255 min)
Max. 6.4e4 counts.
Intensity, counts
m/z, amu
238.1436
239.1458
238.3208
238.4987
233.0808
6.4e4
6.0e4
5.5e4
5.0e4
4.5e4
4.0e4
3.5e4
3.0e4
2.5e4
2.0e4
1.5e4
1.0e4
5000.0
232.0
233.0
234.0
235.0
236.0
237.0
238.0
239.0
240.0
241.0
242.0
243.0
244.0


File Edit View Tools Explore Window Script Help
Explore Mode
training
Configure
Security Configuration
Hardware Configuration
Report Template Editor
Tune
Resolution Optimization
Quantitative Optimization
Manual Tuning
TOF Mass Calibration
Acquire
Build Acquisition Method
Build Acquisition Batch
Express View
Explore (8)
Open Data File
Open Compound Databa
Quantitate
Quantitation Wizard
Review Results Table
XIC of +TOF MS: 238.1 to 238.2 amu from L041904007.wiff
Max. 1.0e4
Intensity, cps
Time, min
3.38
2.89
+TOF MS: 3.341 to 3.414 min from L041904007.wiff Agilent, subtracted (2.979 to 3.269 min)
Max. 1.9e4 cou
Intensity, counts
m/z, amu
182.0819
146.0610
164.0712
134.0617
238.1449
144.0562
147.0420
183.0860

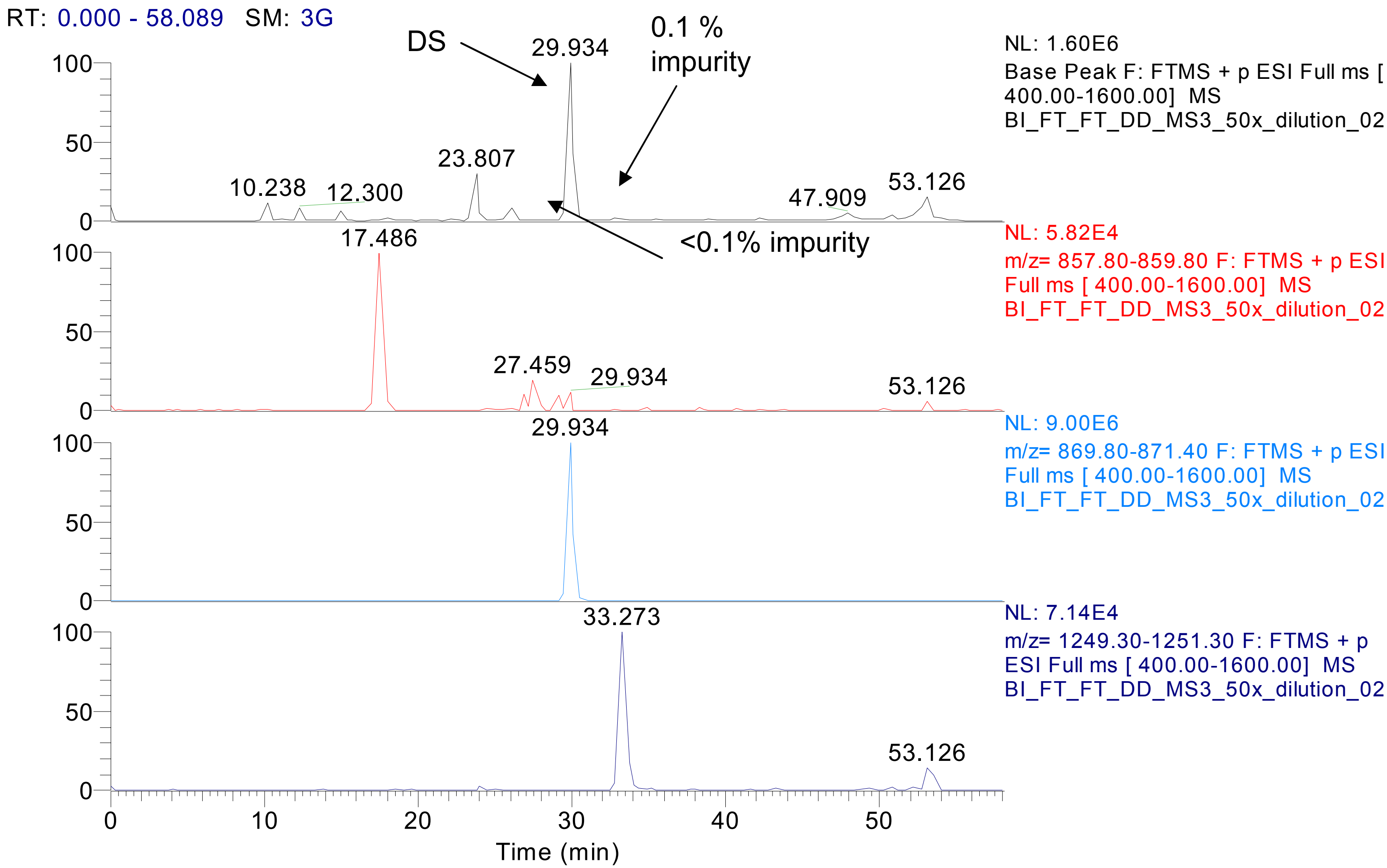
Elemental Formulae of Albuterol Ketone Fragment ions from LC-MSD TOF

Formula	Theoretical	Acquired	mDa	ppm	DBE
$C_9H_{12}NO_3$	182.0811	182.0819	0.7301	4.0101	4.5
$C_9H_{10}NO_2$	164.0706	164.0712	0.5948	3.6258	5.5
C_9H_8NO	146.0600	146.0610	0.9596	6.5699	6.5

Recent development in Hybrid FT/MS: LTQ-FT/MS



Base Peak Plot of Full Scan MS and Extracted Ion Chromatograms for the Three Ions of Interest

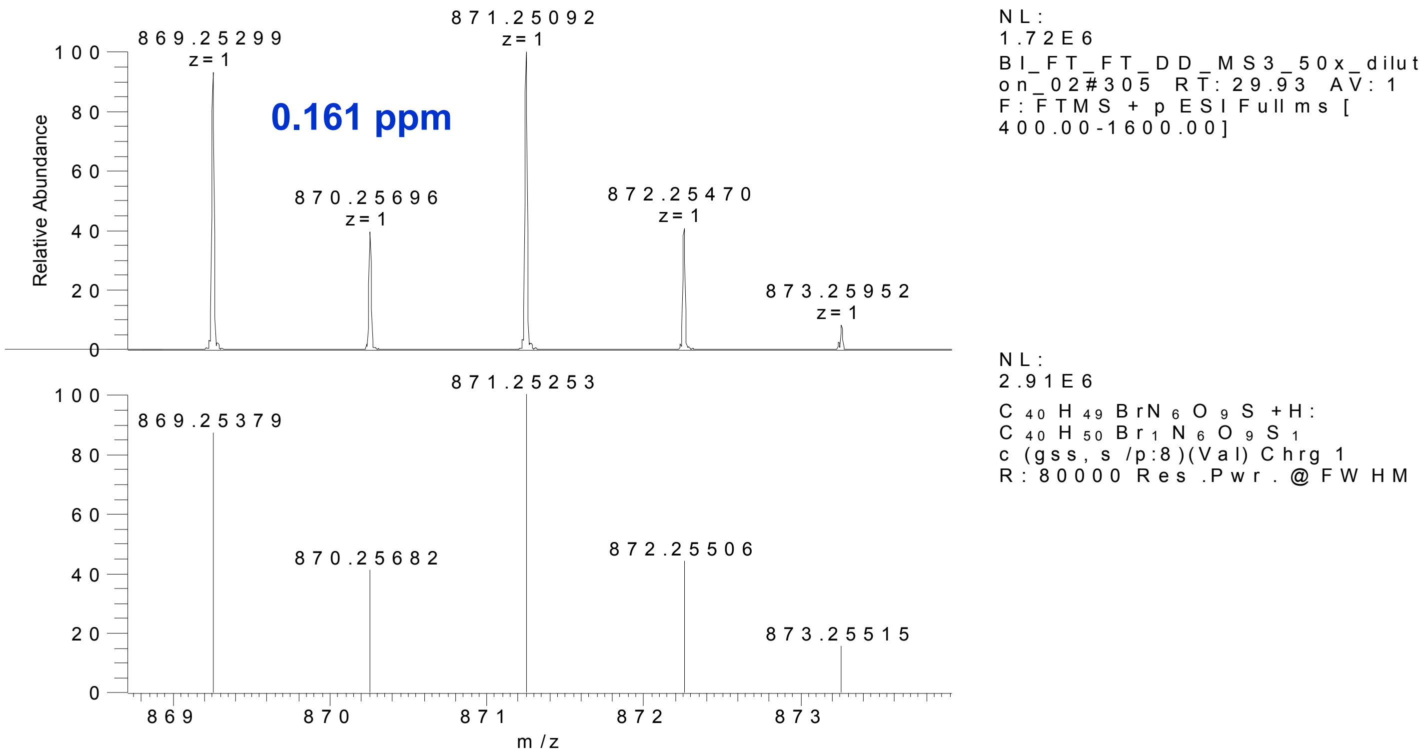


Conference on Small Molecule Science
COSMOS

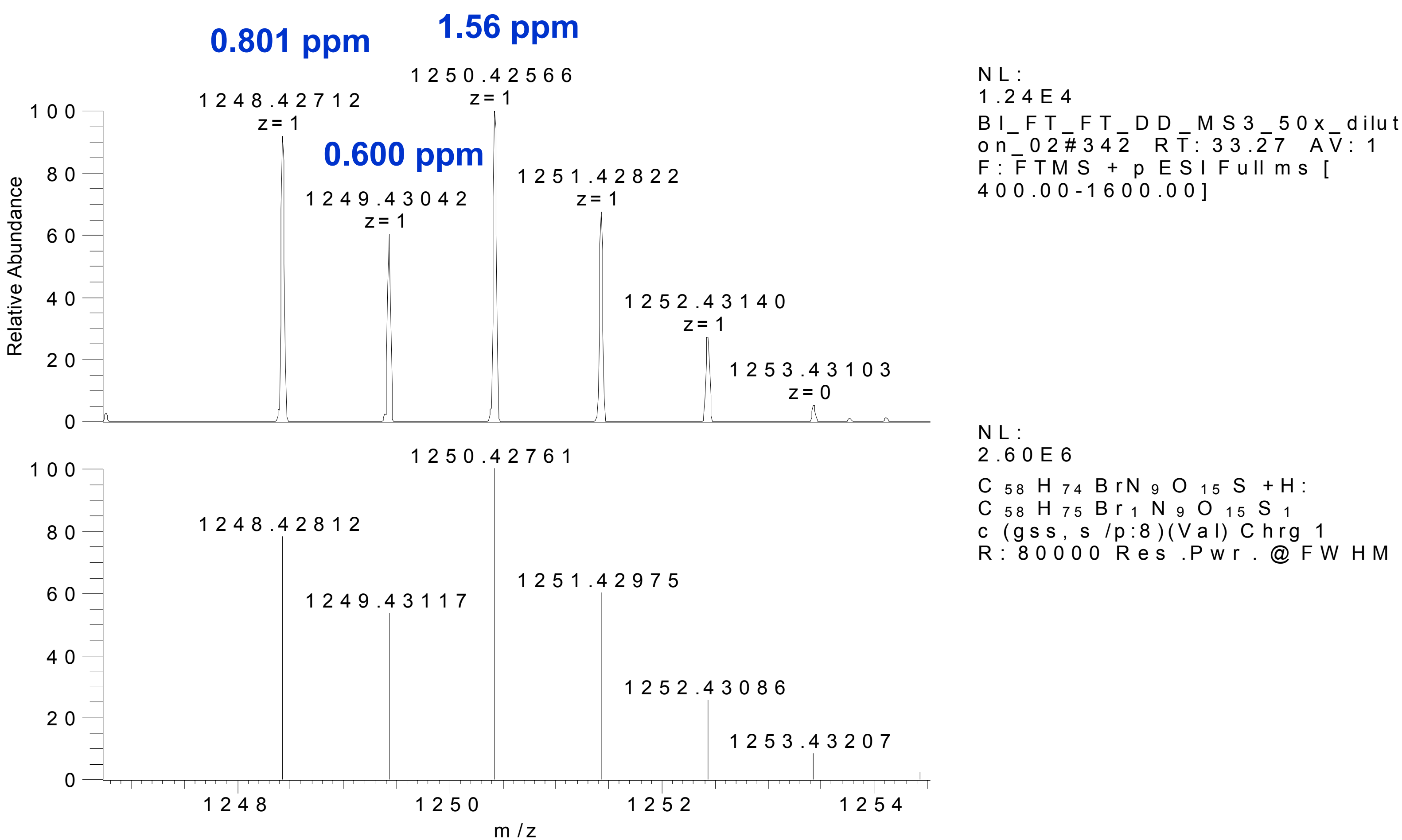
Accurate Mass Comparison and Isotopic Profile for the Elution Peak at 29.9 Minutes

0.920 ppm **1.85 ppm**

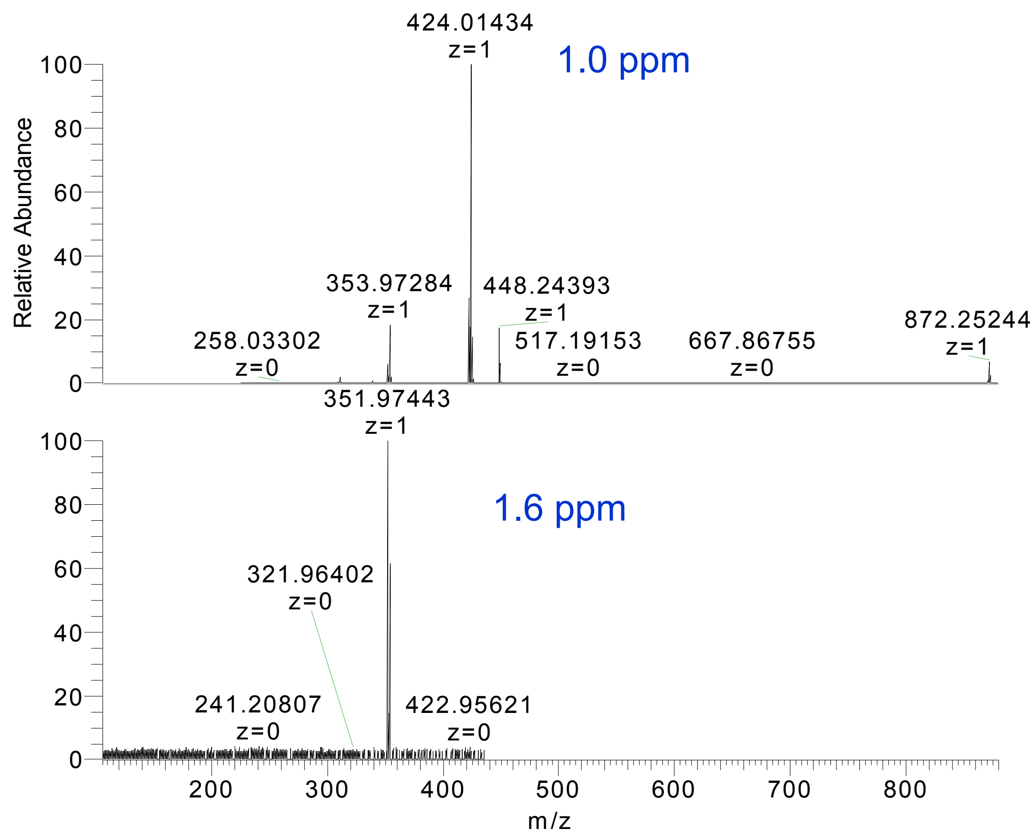
0.161 ppm



Accurate Mass Comparison and Isotopic Profile for the Elution Peak at 33.3 Minutes



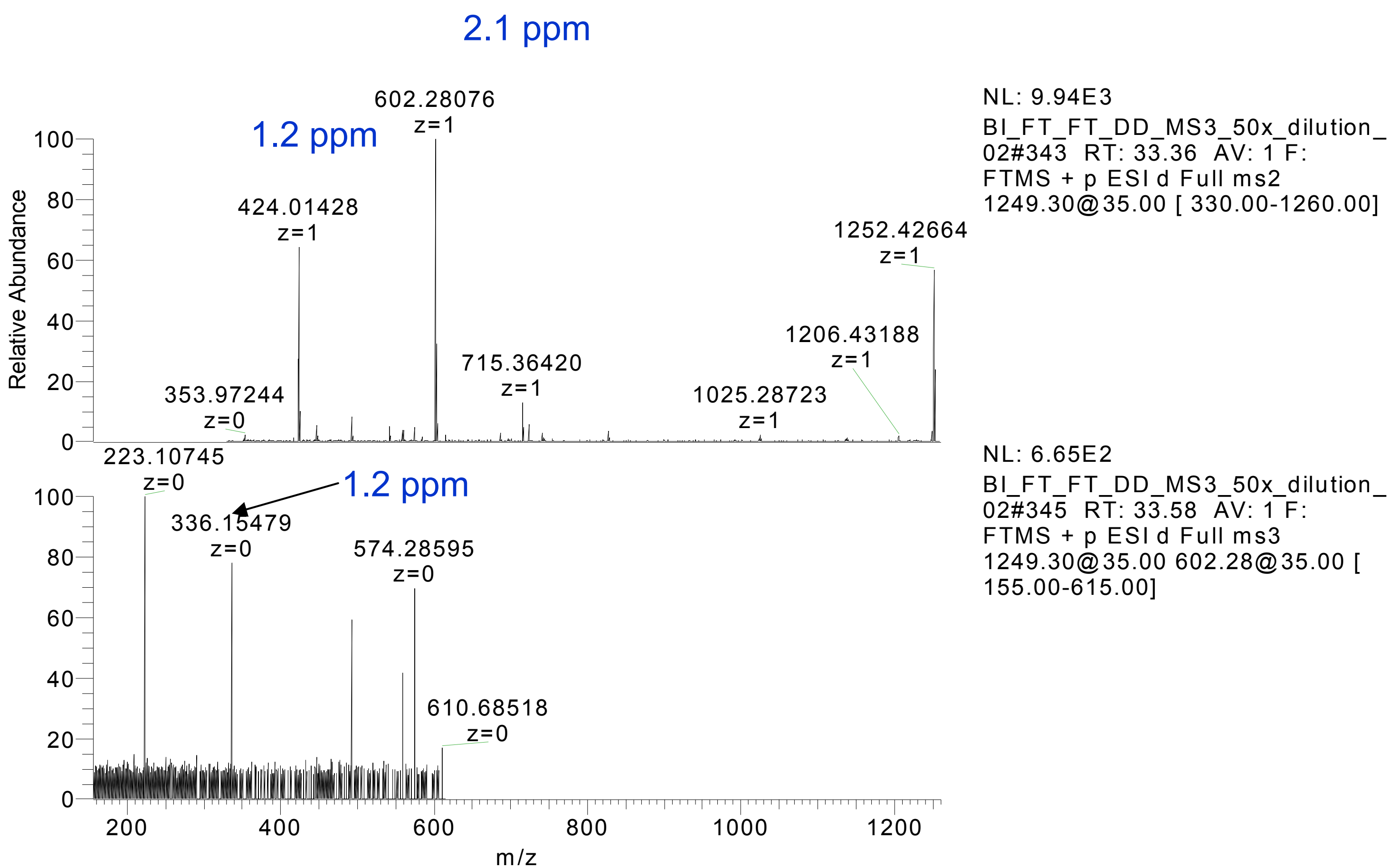
Full Scan MS/MS and MS³ Spectra for the 29.9 Minute Elution Peak



NL: 7.90E5
BI_FT_FT_DD_MS3_50x_dilution_02#306 RT: 29.96 AV: 1 F: FTMS + p ESI d Full ms2 869.84@35.00 [225.00-880.00]

NL: 1.74E3
BI_FT_FT_DD_MS3_50x_dilution_02#318 RT: 30.86 AV: 1 F: FTMS + p ESI d Full ms3 869.84@35.00 422.02@35.00 [105.00-435.00]

Full Scan MS/MS and MS3 Spectra for the 33.4 Minute Elution Peak



Concluding Statement

As a result of the availability of LC-TOF (and FT-MS) technology, accurate mass measurement will again become routine for small molecule structure elucidation.

Conference on Small Molecule Science

COSMOS

Acknowledgments

- Analytical Sciences Department, Boehringer-Ingelheim Pharmaceuticals, Inc.
- Dr. Fenghe Qiu (BIPI Analytical Sciences)
- Walter Davidson (BIPI Biology)
- Bryan Lavery and Doug McIntyre (Aglient Technologies)
- Dr. Scott Peterman (Thermo/Finnigan)