1. Nomenclature,
2. Instrument capability,
3. Sample preparation,
4. Instrument operation,
5. Acquisition method,
6. Data analysis (small molecule, protein)
7. Quantitative analysis,
8. Software,
9. Data presentation,
10. Service support.

Mass Spectrometry Workshop

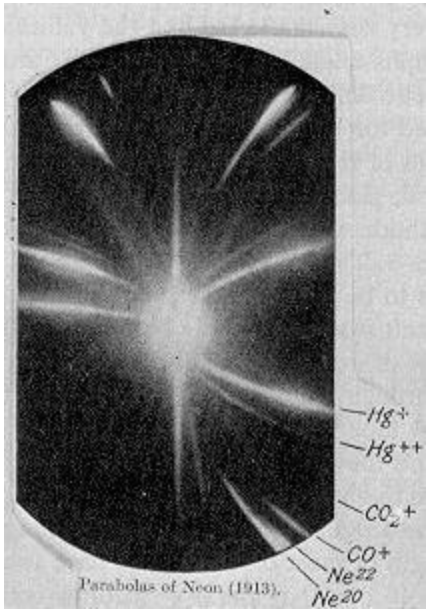
Department of Chemistry and Biochemistry,
06/25 ~ 27/2013

- Get to know the instruments,
- Exchange ideas,
- Become comfortable in data analysis

100 Years of Mass Spectrometry

light sources that generate light by sending an electrical discharge through an ionized gas, i.e. a plasma.

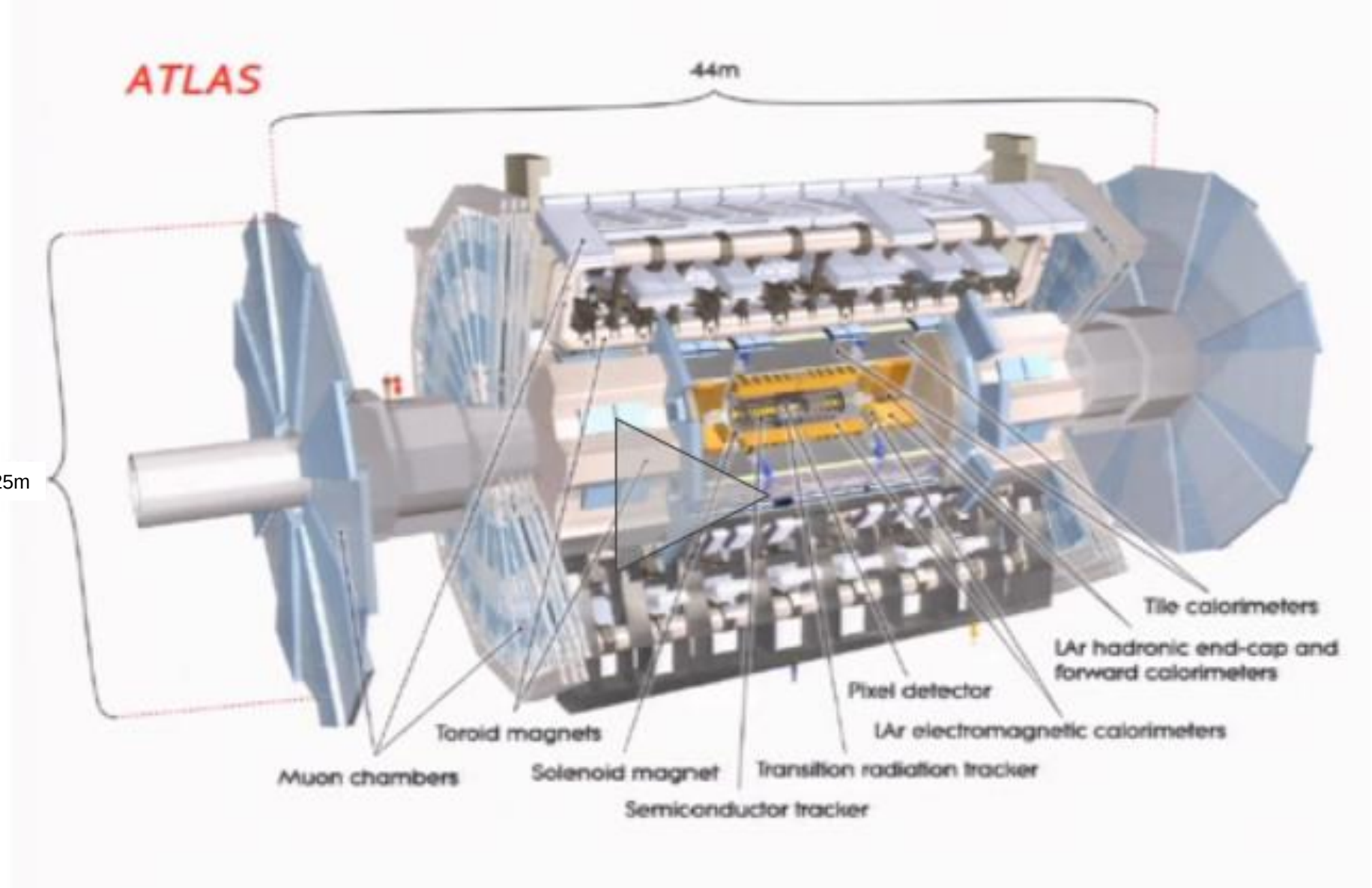
Cathode rays casting a shadow on the wall of a Crookes tube



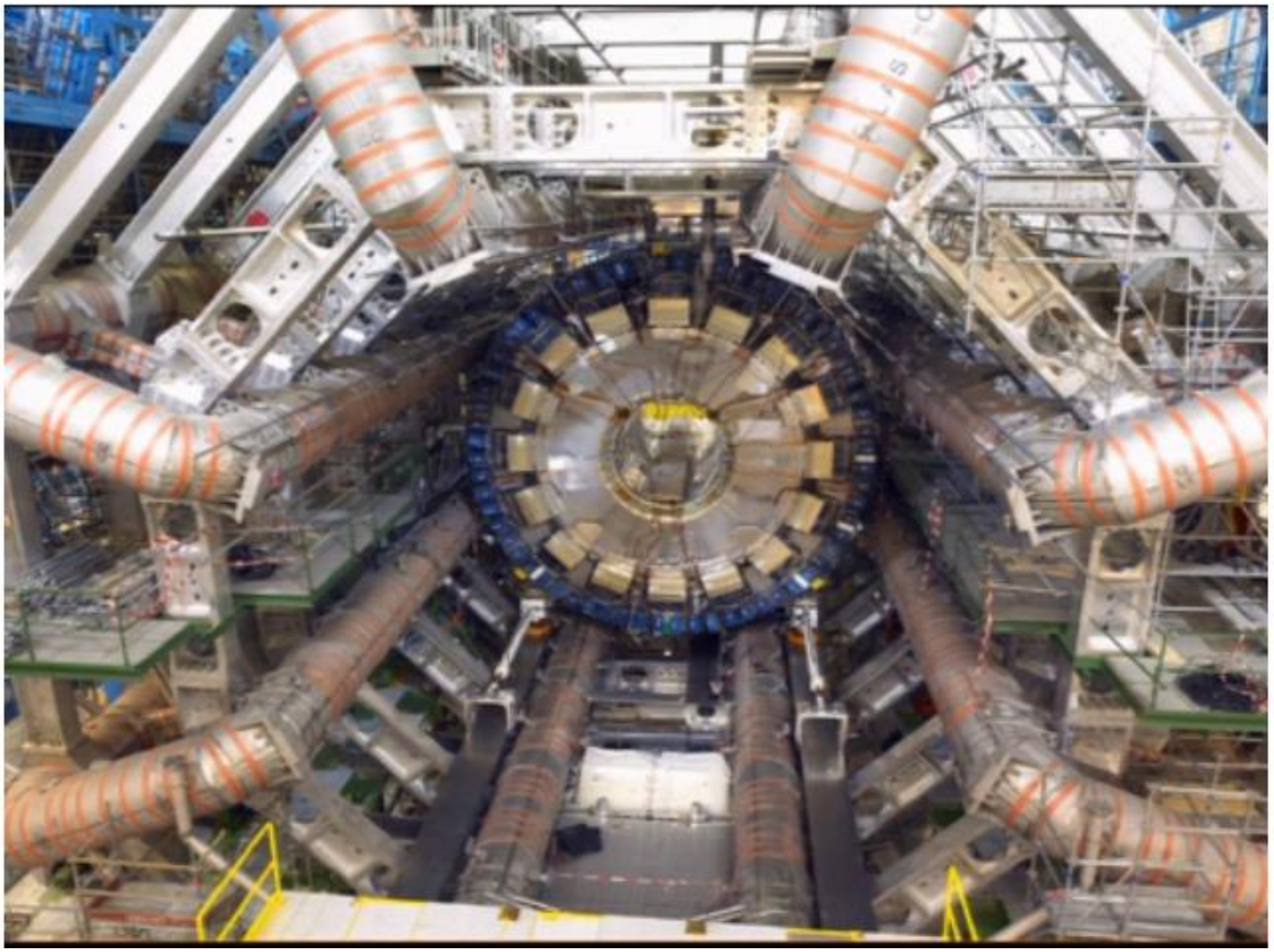
In the bottom right corner of this photographic plate are markings for the two isotopes of neon: neon-20 and neon-22.

a stream of ionized neon through a magnetic and an electric field and measured its deflection, Thomson observed two patches of light on the photographic plate, the neon gas was composed of atoms of two different atomic masses (neon-20 and neon-22).

Mass spectrometer in detecting Higgs Boson

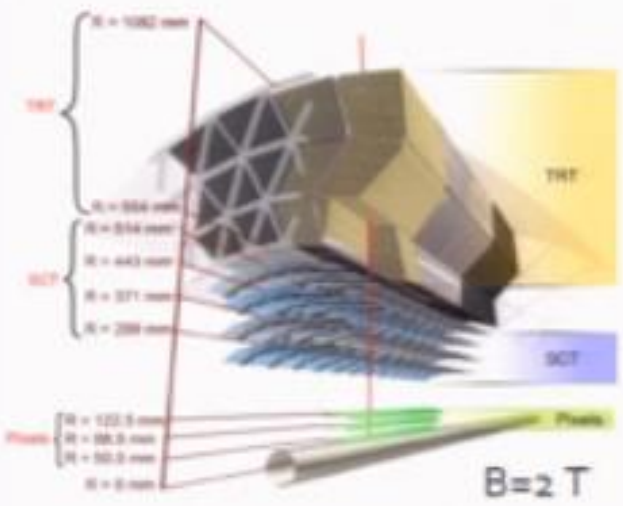


Front View of the detector



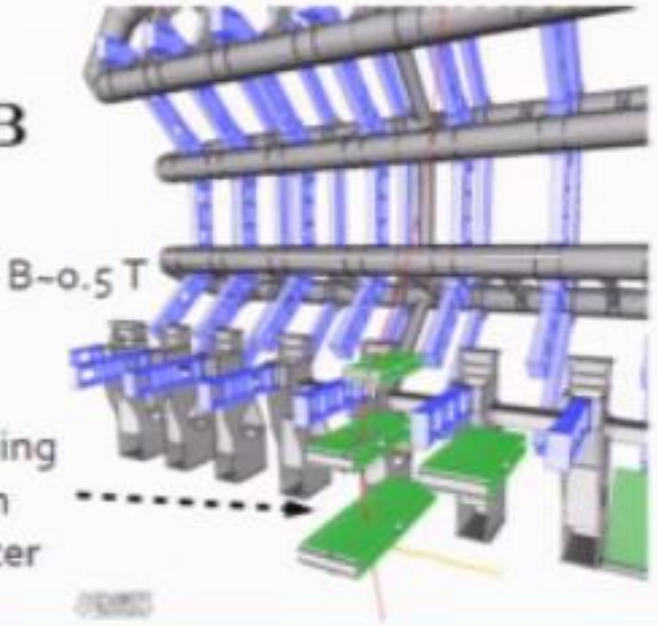
Particle Identification in ATLAS

- There are only ~ 7 kinds of particles that are detected directly (e, μ, γ, π, K, p, n) + antiparticles
- We separate these using their penetrating power
- Magnetic spectrometers used to measure (unknown) momenta
  - particles are highly relativistic: we measure p/q



Inner detector, close to collisions

$$\frac{1}{q}\frac{d\mathbf{p}}{dt} = \mathbf{v} \times \mathbf{B}$$



Muon bending in the muon spectrometer

1. Nomenclature

What is the mass by mass spec.?

Molecular weight (Dalton) = average mass: 957.0849

m/z: mass over charge

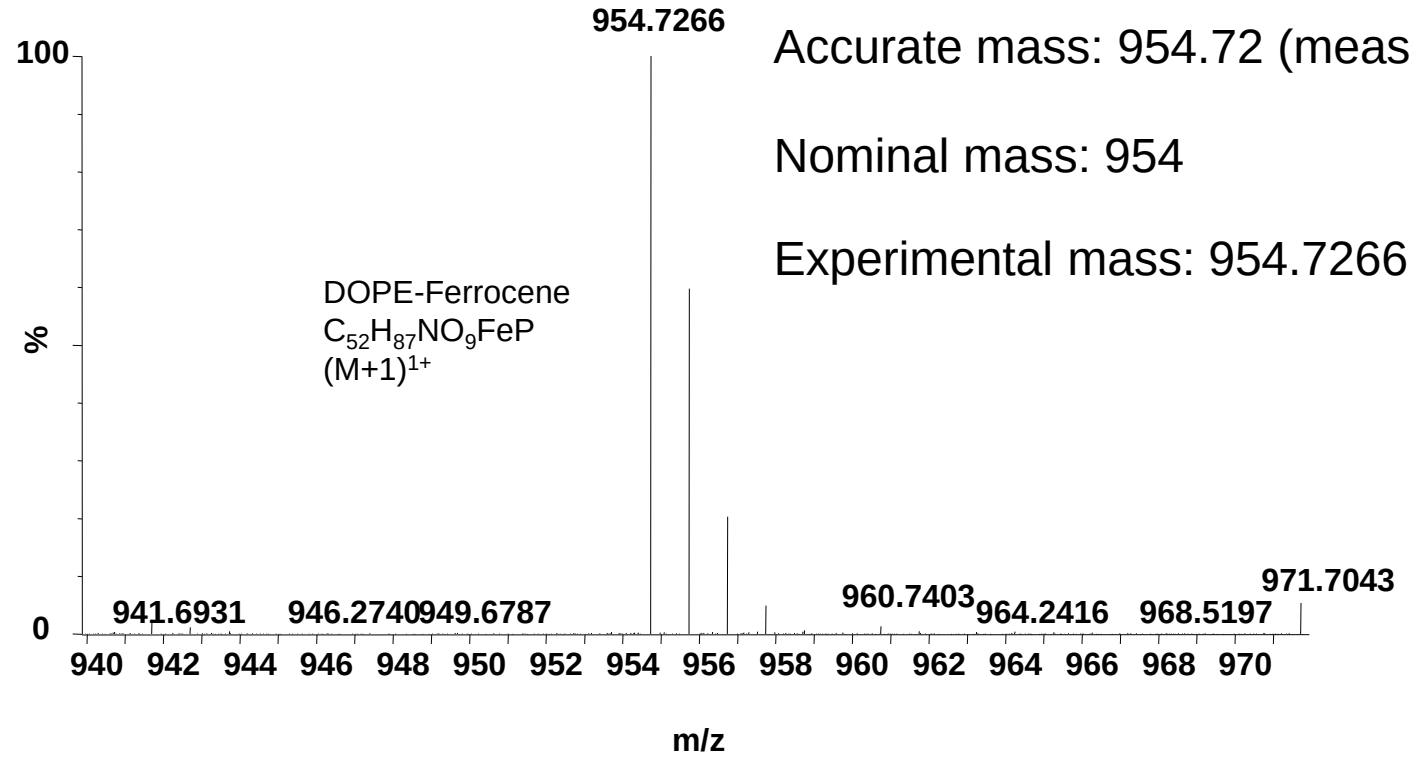
Monoisotopic mass: 954.7266

Exact mass: 954.5515 (calculated from formula)

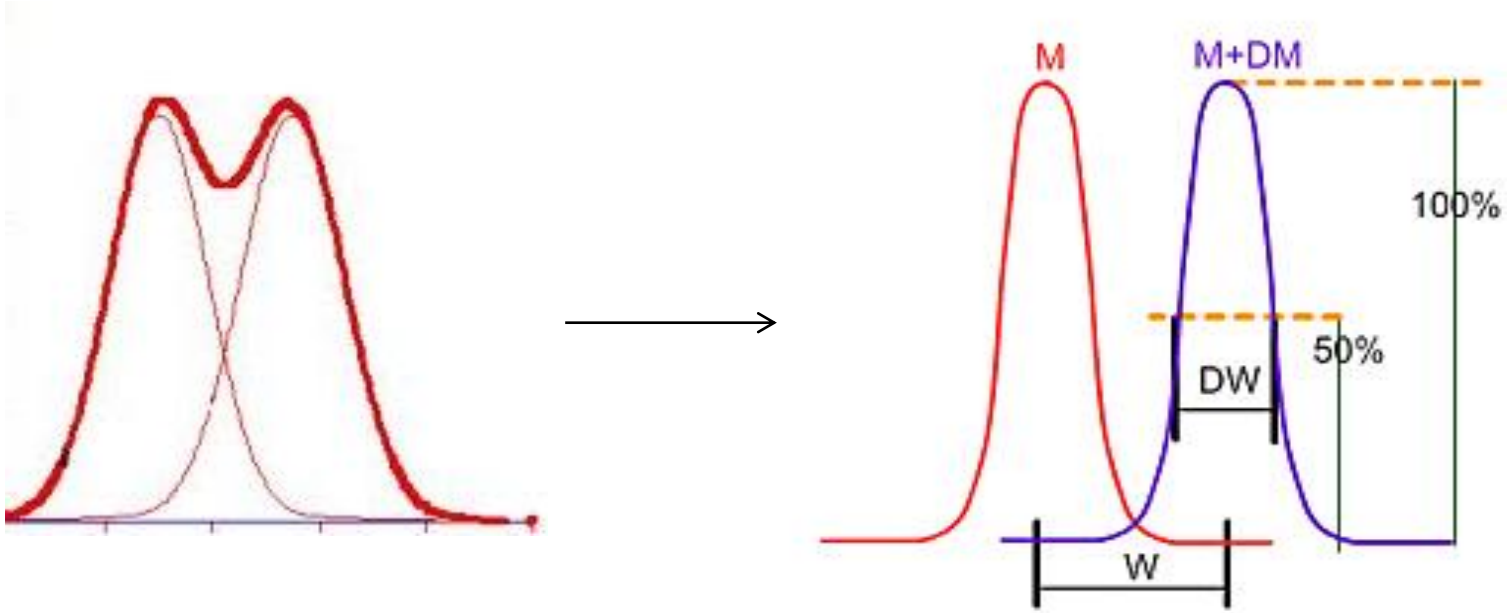
Accurate mass: 954.72 (measured)

Nominal mass: 954

Experimental mass: 954.7266

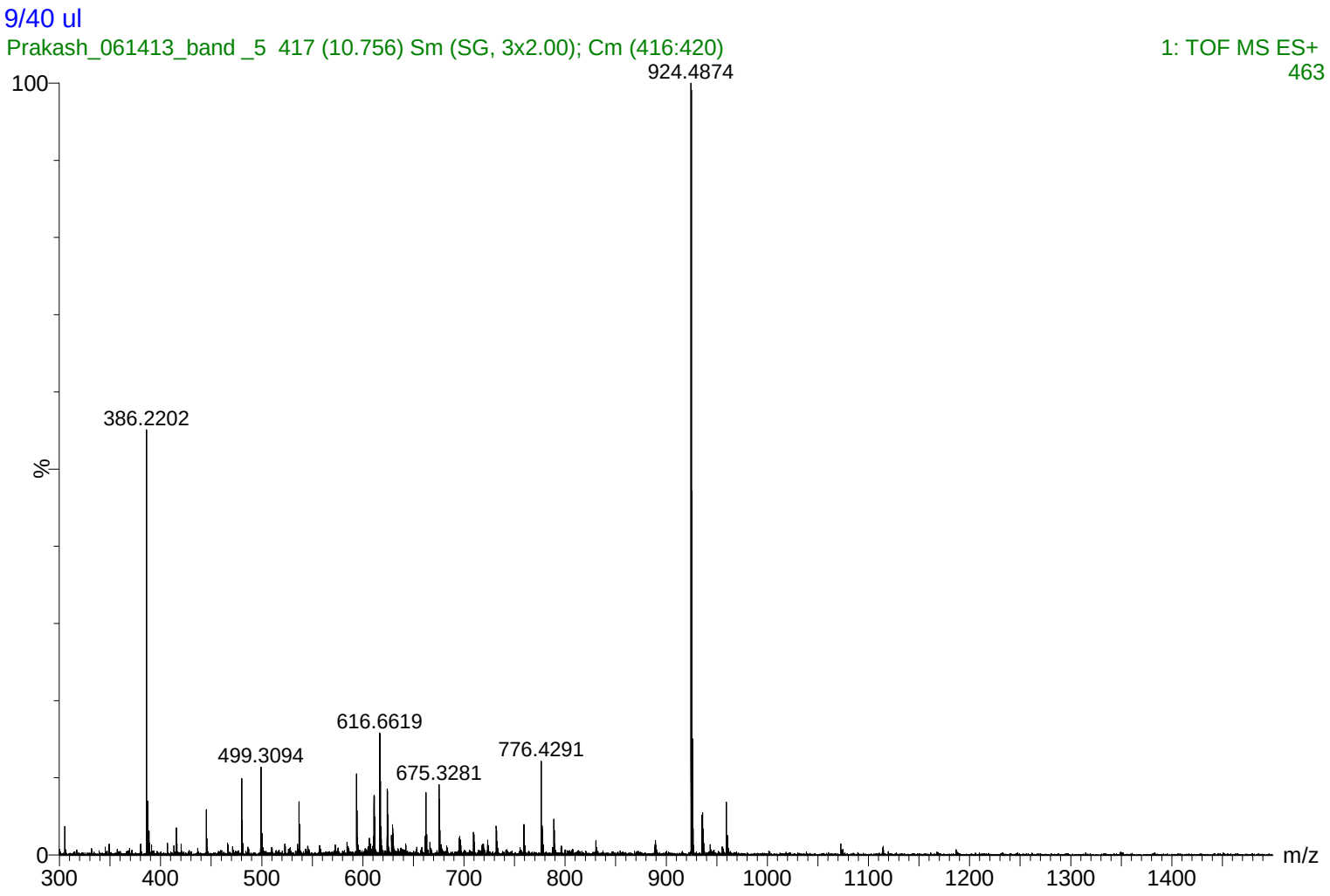


What is Mass Resolution?



$$R = M/\Delta M_{50\%}$$

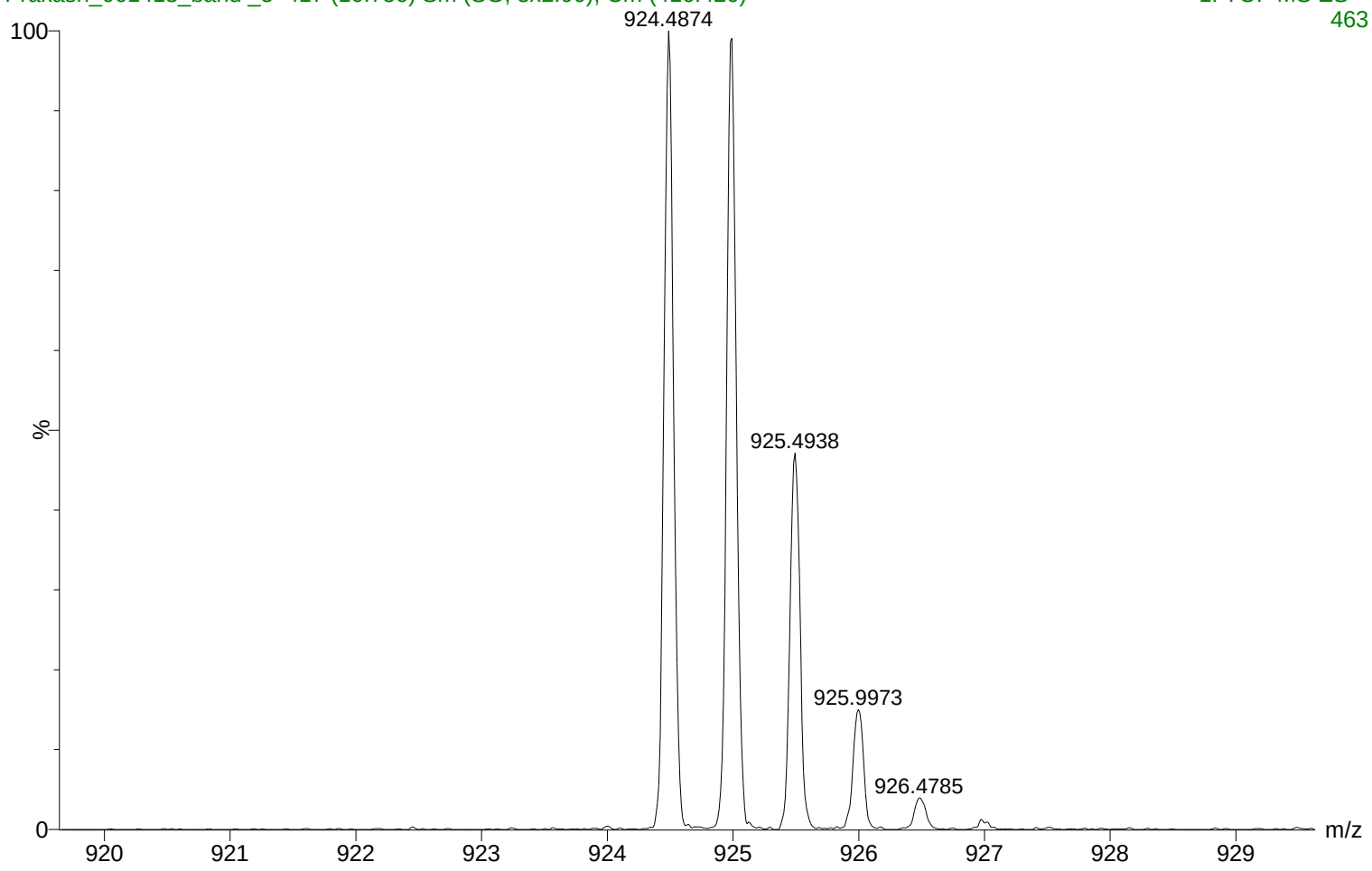
Q-TOF resolution ?



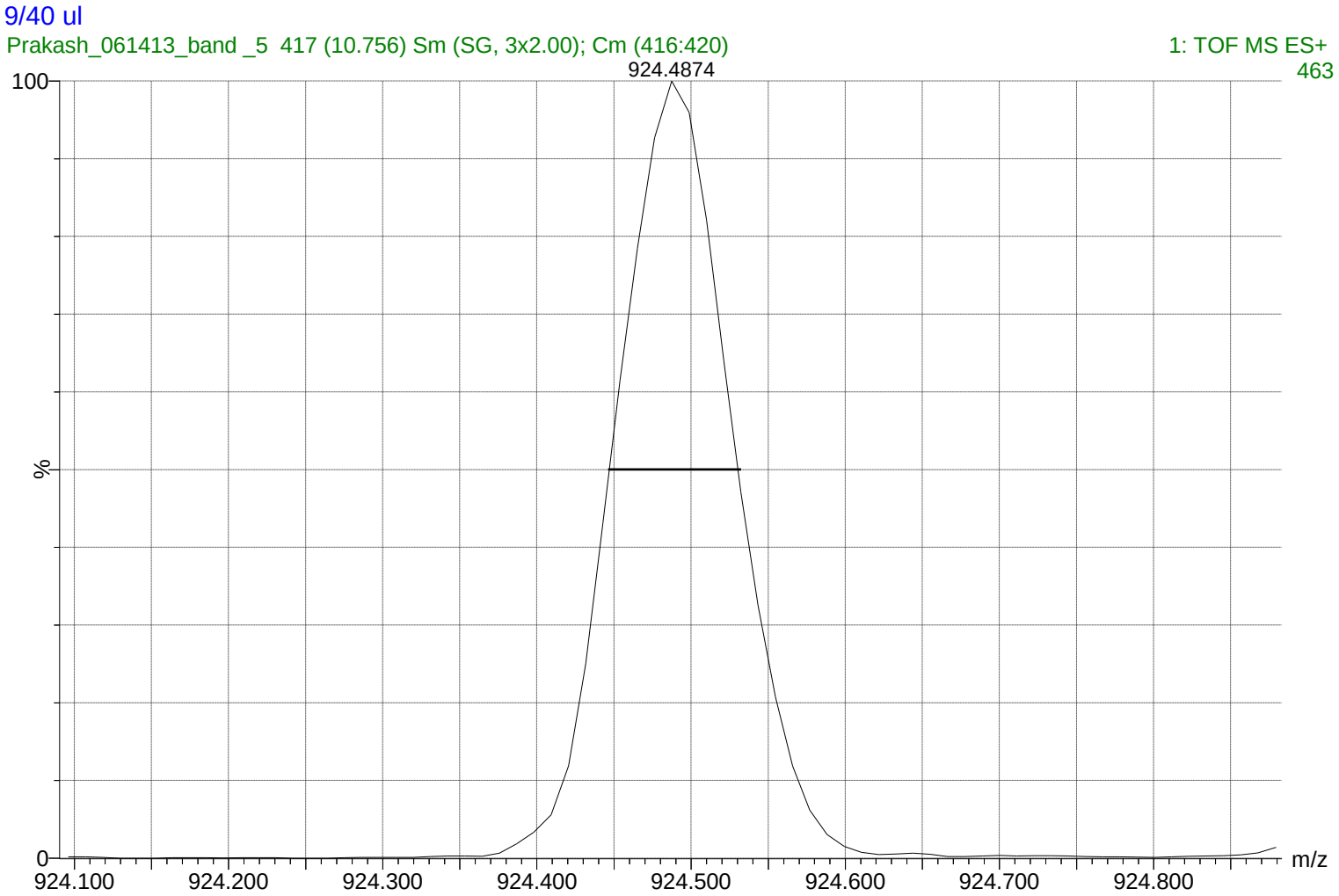
9/40 ul

Prakash_061413_band _5 417 (10.756) Sm (SG, 3x2.00); Cm (416:420)

1: TOF MS ES+
463



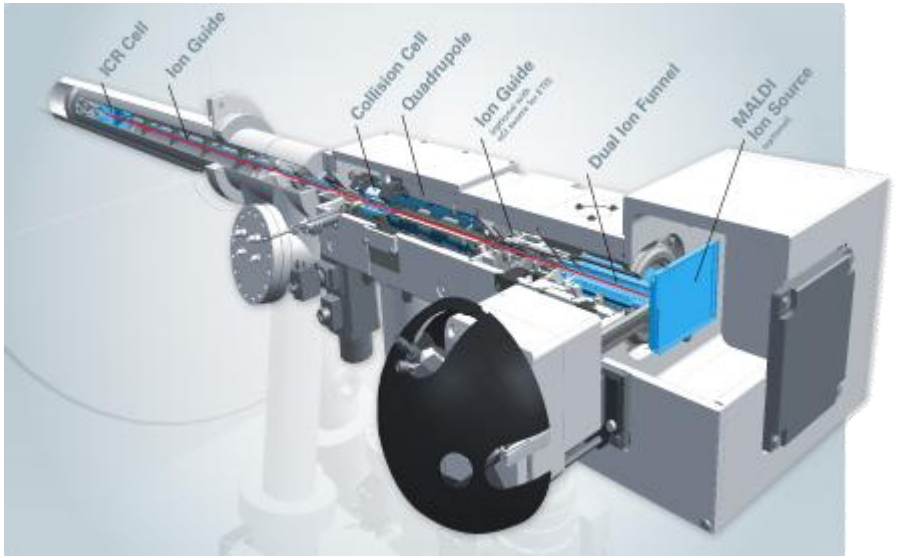
Peak width = 0.0843 924.4874/0.0843 = 10,967



HRMS (high resolution mass spectrometry)

Extreme Mass Resolution, 10 million, by solariX™ FTMS. Dynamically harmonized ParaCell™, developed by Professor Eugene Nikolaev and coworkers at the Russian Academy of Sciences in Moscow.

Reveal the fine structure in isotopic patterns that are uniquely specific to the exact molecular formulae of the detected compounds



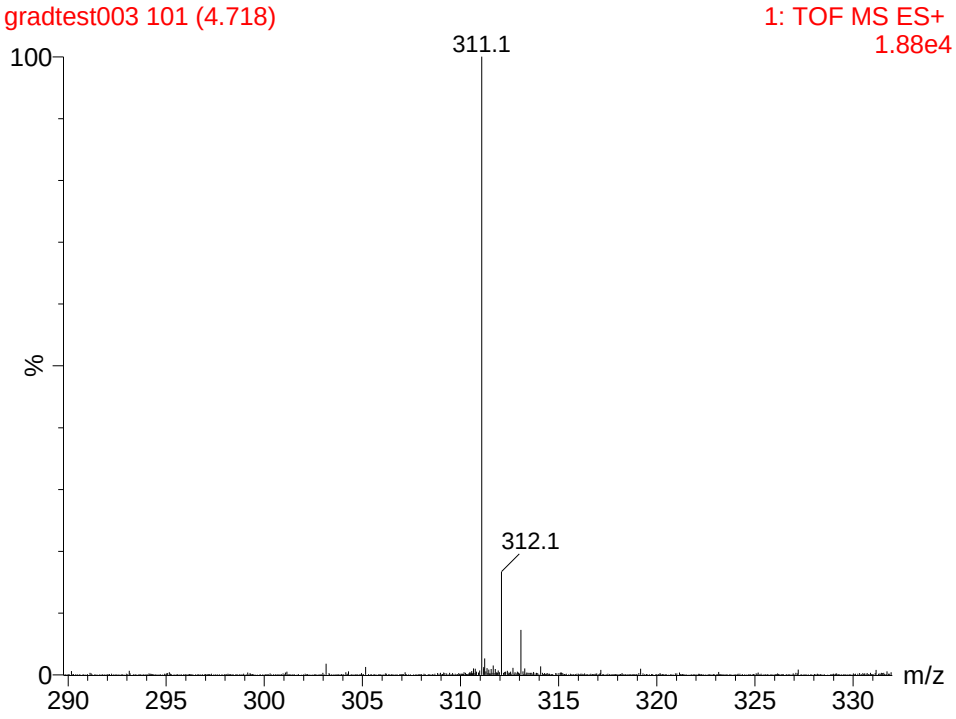
What is mass accuracy (or mass error) ?

mass accuracy (in ppm) = Δmass/calculated mass

delta mass = experimental mass – calculated mass

5 ppm, or less, is needed for the molecular formula

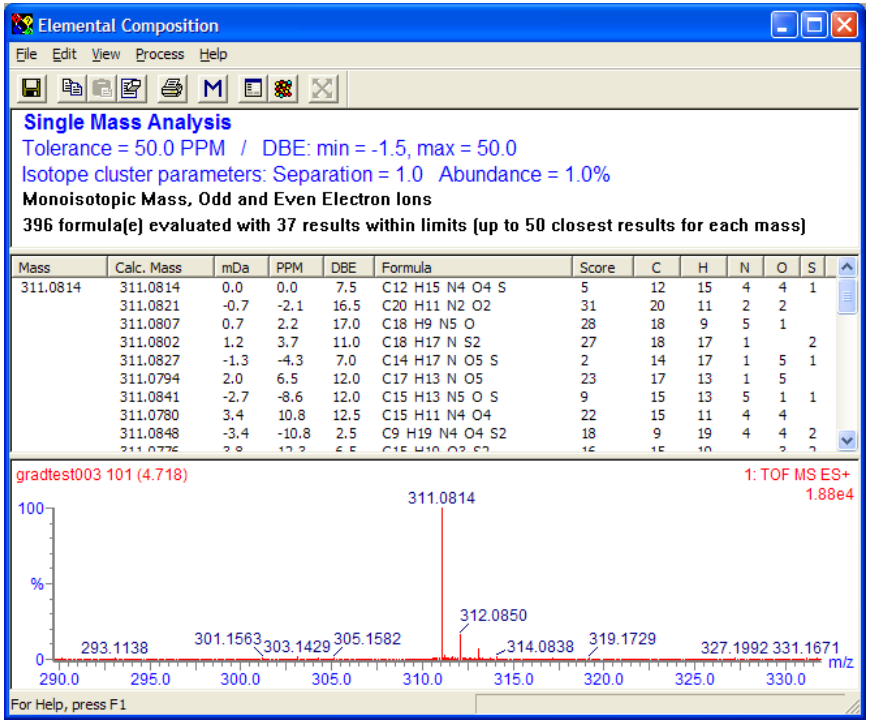
The Power of Exact Mass



Nominal mass measured spectrum

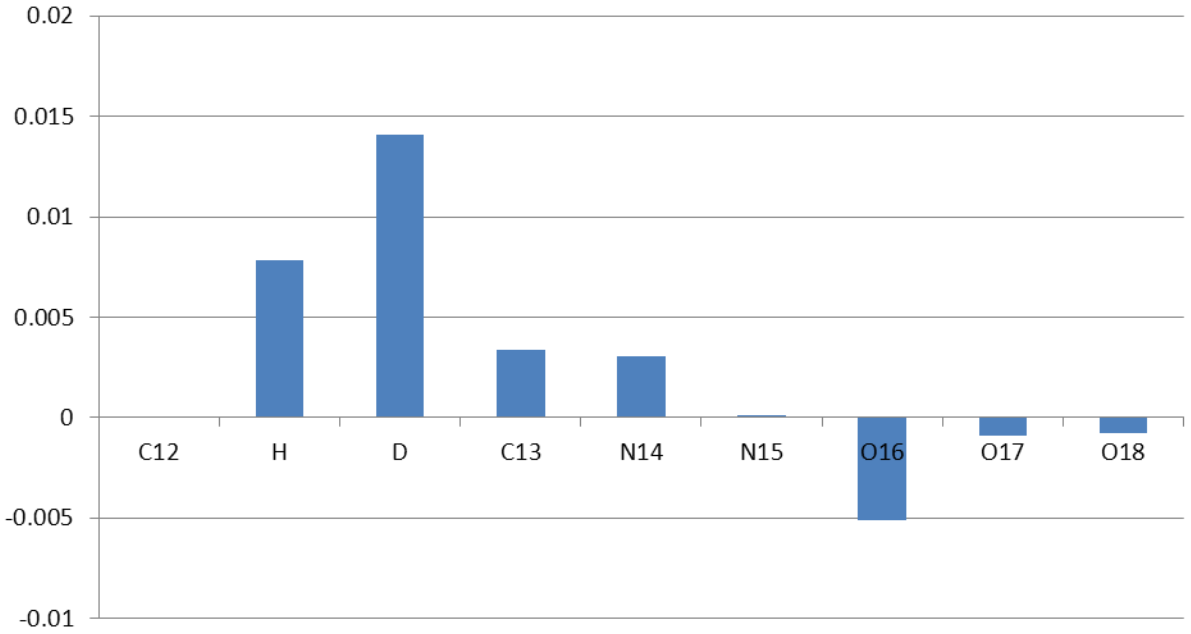
~50 ppm tolerance at *m/z* 311.0814

37 possible results



What is mass defect?

- deviation from unit mass



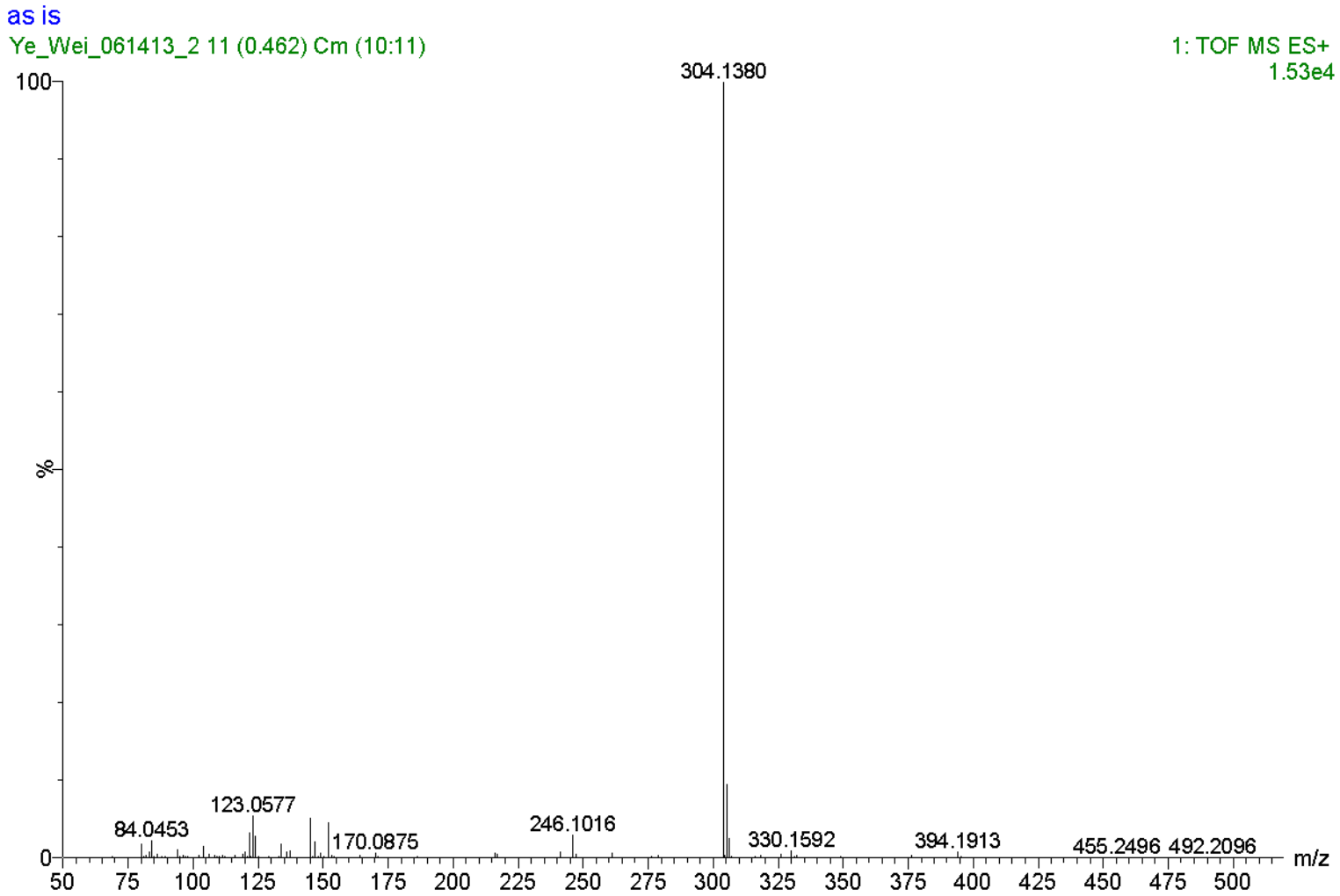
Similar or related compound have similar mass defect

Exact Mass and Isotopic Abundance of Common Elements

Element	Nuclide	Nominal Mass	Exact Mass	Mass Defect	Isotopic Abundance
Hydrogen	H	1	1.0078	0.00783	100.00%
	D	2	2.0141	0.0141	0.02%
Carbon	C^{12}	12	12.0000	0	100.00%
	C^{13}	13	13.0034	0.00336	1.10%
Nitrogen	N^{14}	14	14.0031	0.003074	100.00%
	N^{15}	15	15.0001	0.0001	0.37%
Oxygen	O^{16}	16	15.9949	-0.0051	100.00%
	O^{17}	17	16.9991	-0.0009	0.04%
	O^{18}	18	17.9992	-0.0008	0.20%
Fluorine	F^{19}	19	18.9984	-0.0016	100.00%
Phosphorus	P^{31}	31	30.9738	-0.0262	100.00%
Sulfur	S^{32}	32	31.9721	-0.0279	100.00%
	S^{33}	33	32.9725	-0.0275	0.79%
	S^{34}	34	33.9679	-0.0321	4.40%
Chlorine	Cl^{35}	35	34.9689	-0.0311	100.00%
	Cl^{37}	37	36.9659	-0.0341	32.00%
Bromine	Br^{79}	79	78.9183	-0.0817	100.00%
	Br^{81}	81	80.9163	-0.0837	97.30%

Composition Analysis

Based on mass accuracy, a composition (formula) of an unknown
Can be calculated, 5 ppm or less is needed.

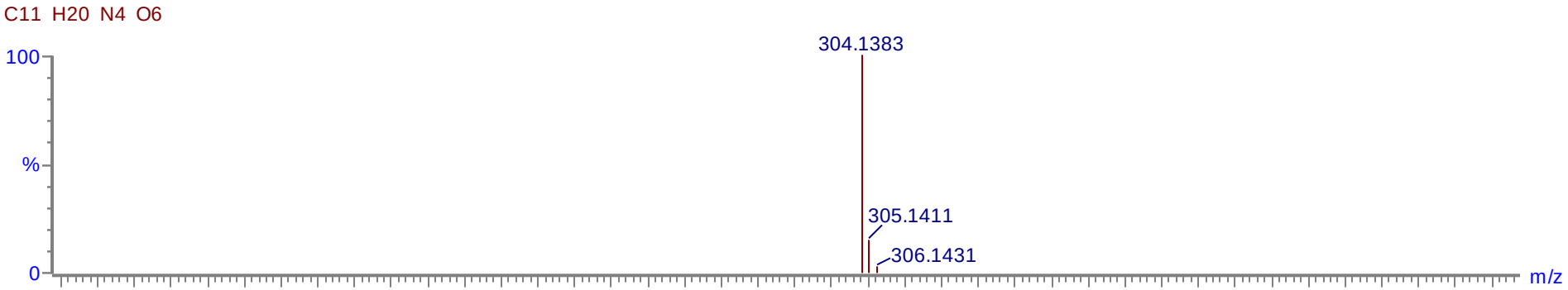


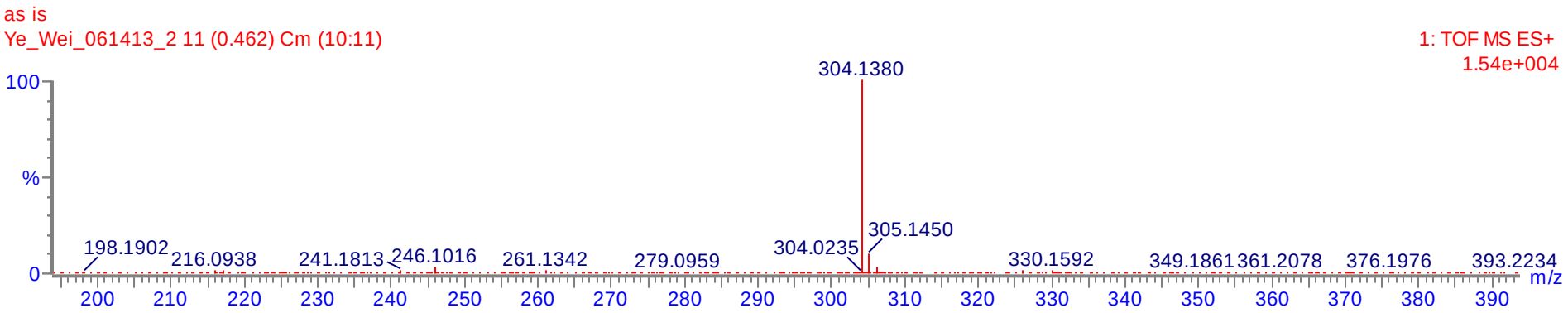
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd and Even Electron Ions
225 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-50 H: 0-60 N: 0-6 O: 0-8

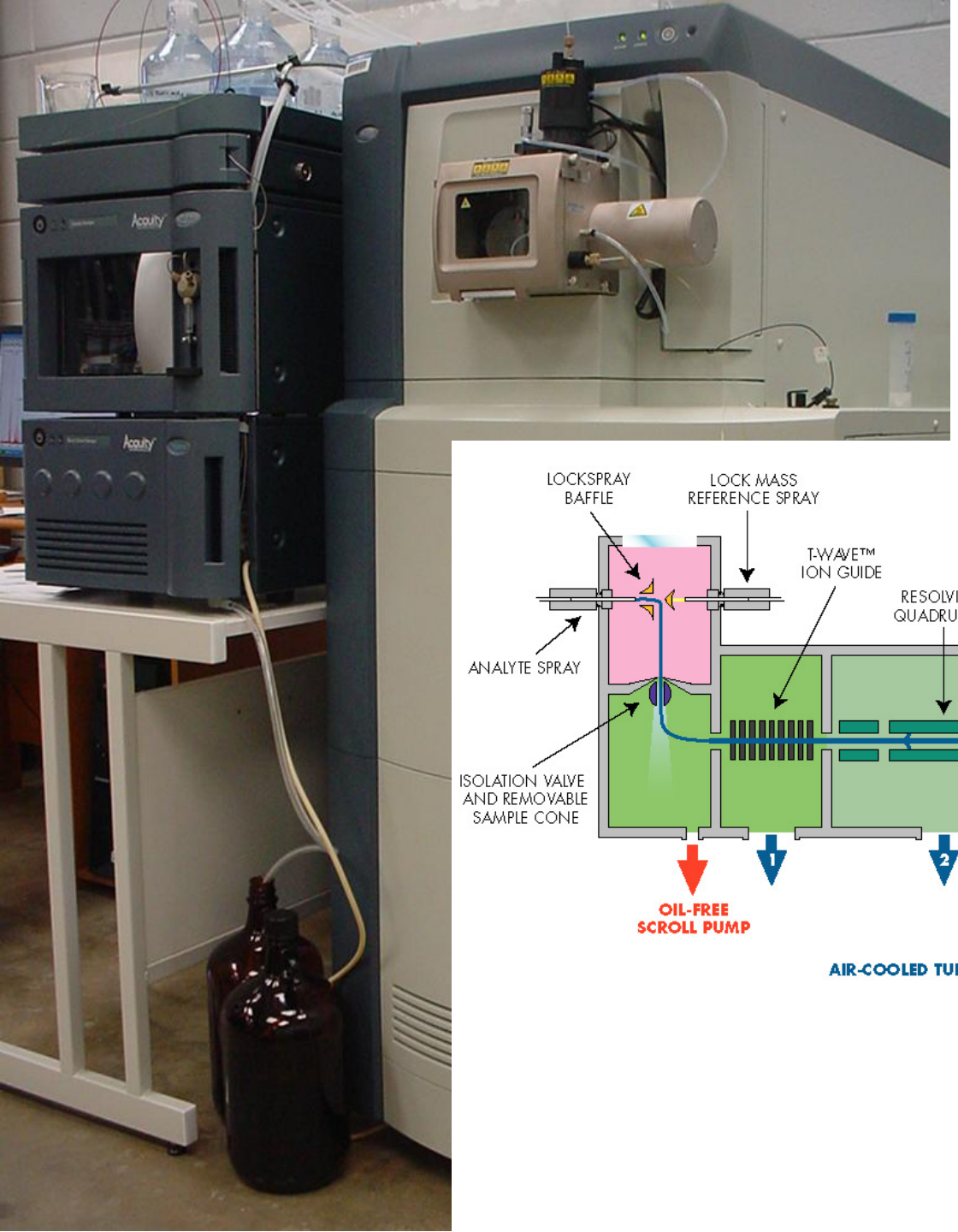
Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass		mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
304.1380	304.1383		-0.3	-1.0	4.0	86.7	0.0	C11 H20 N4 O6

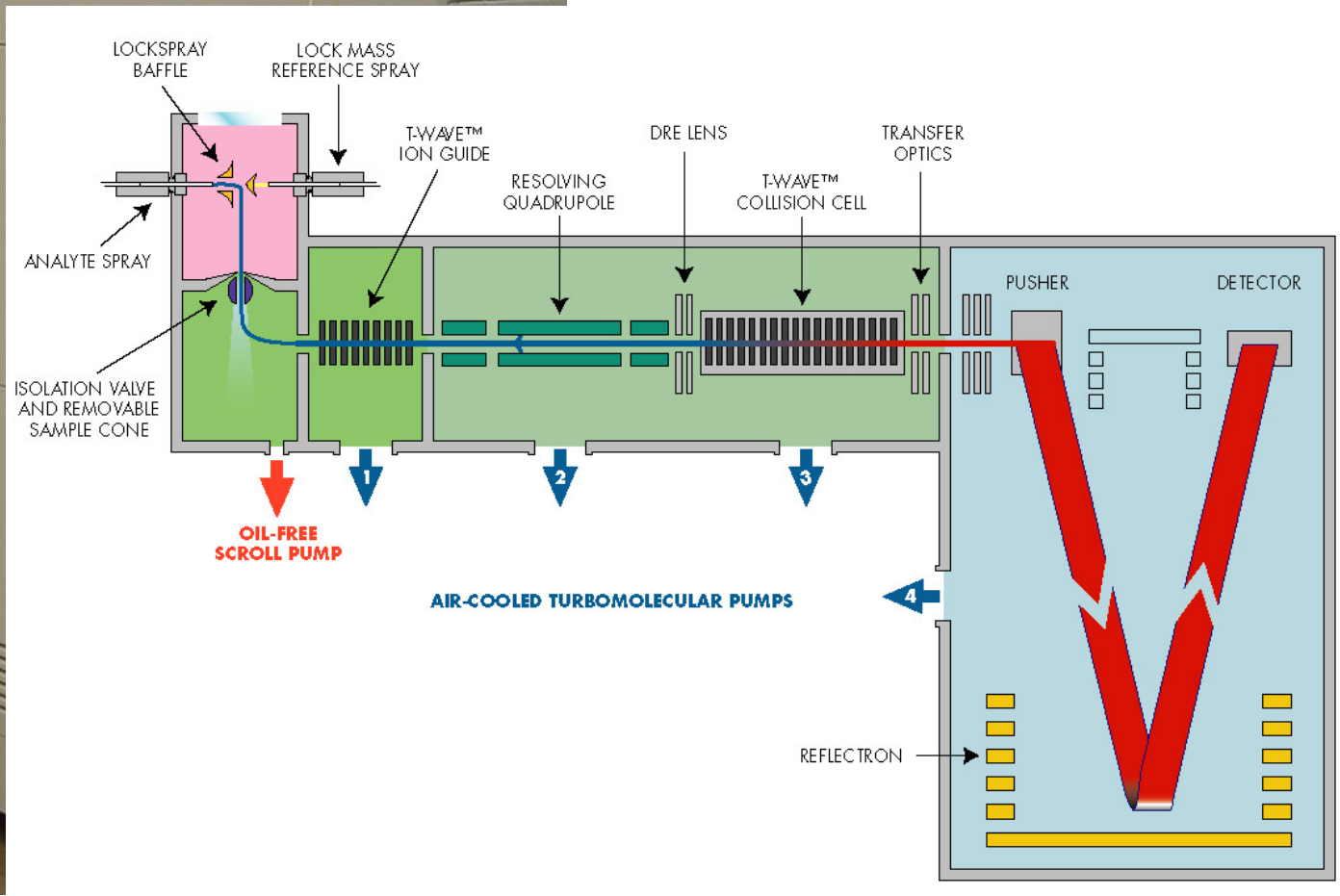




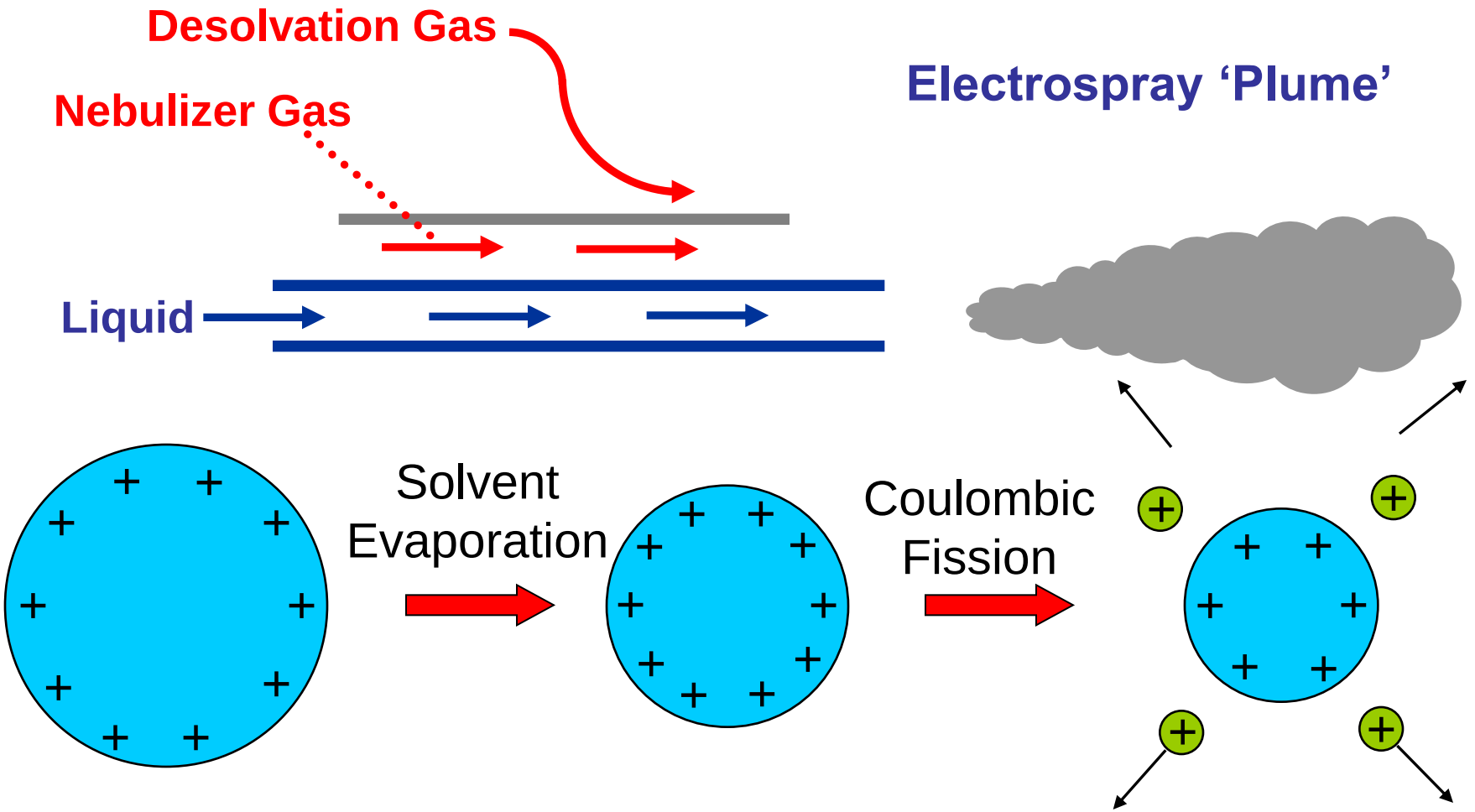
2. Instrument Capability

Q-ToF Premier
mass accuracy~ 1ppm
resolution = 17,000 (w-mode)
(Waters, $566,206)



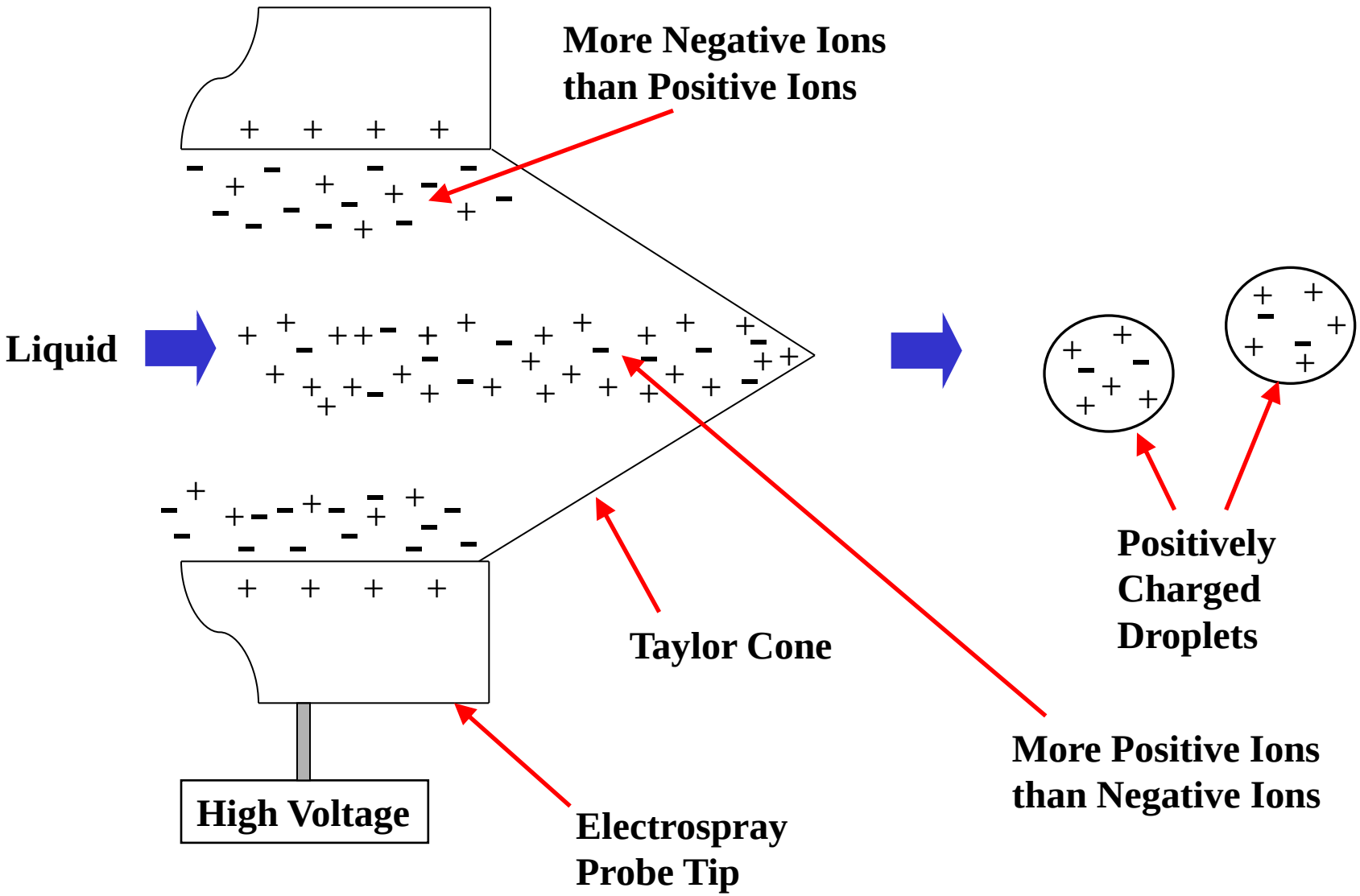


Electrospray Ionization is “soft”



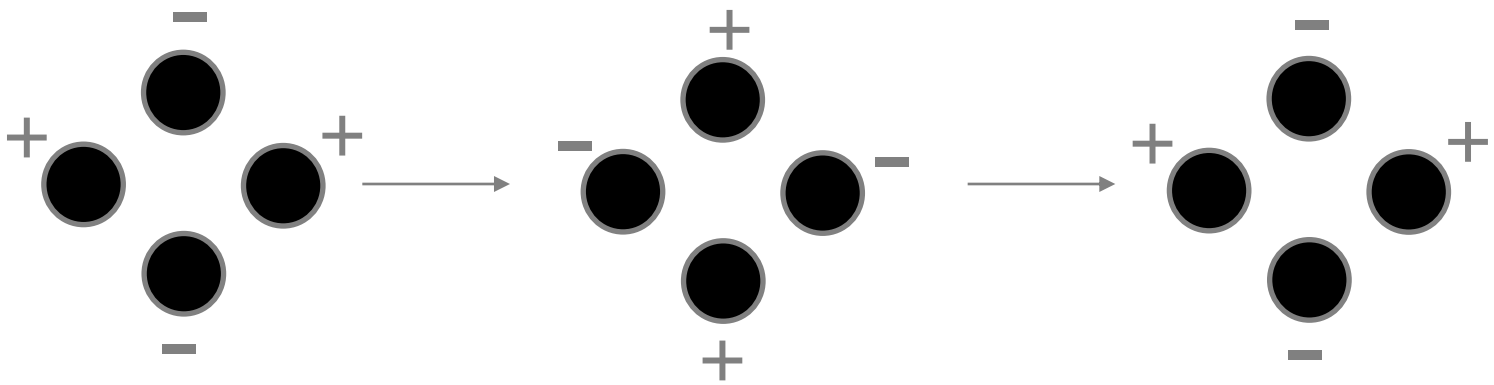
Solvent evaporates from the droplet and the droplet shrinks until the charge density on the surface reaches a point where the repulsive force between charges exceeds the liquid surface tension that holds the drop together.

Electrospray Ionization is competitive, and not that quantitative

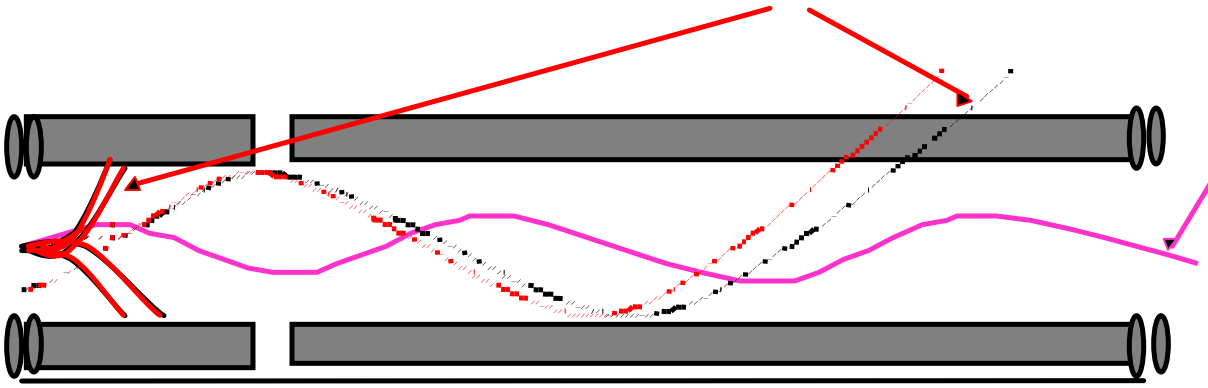


Quadruple Theory

Polarities on rods change at radio frequency.

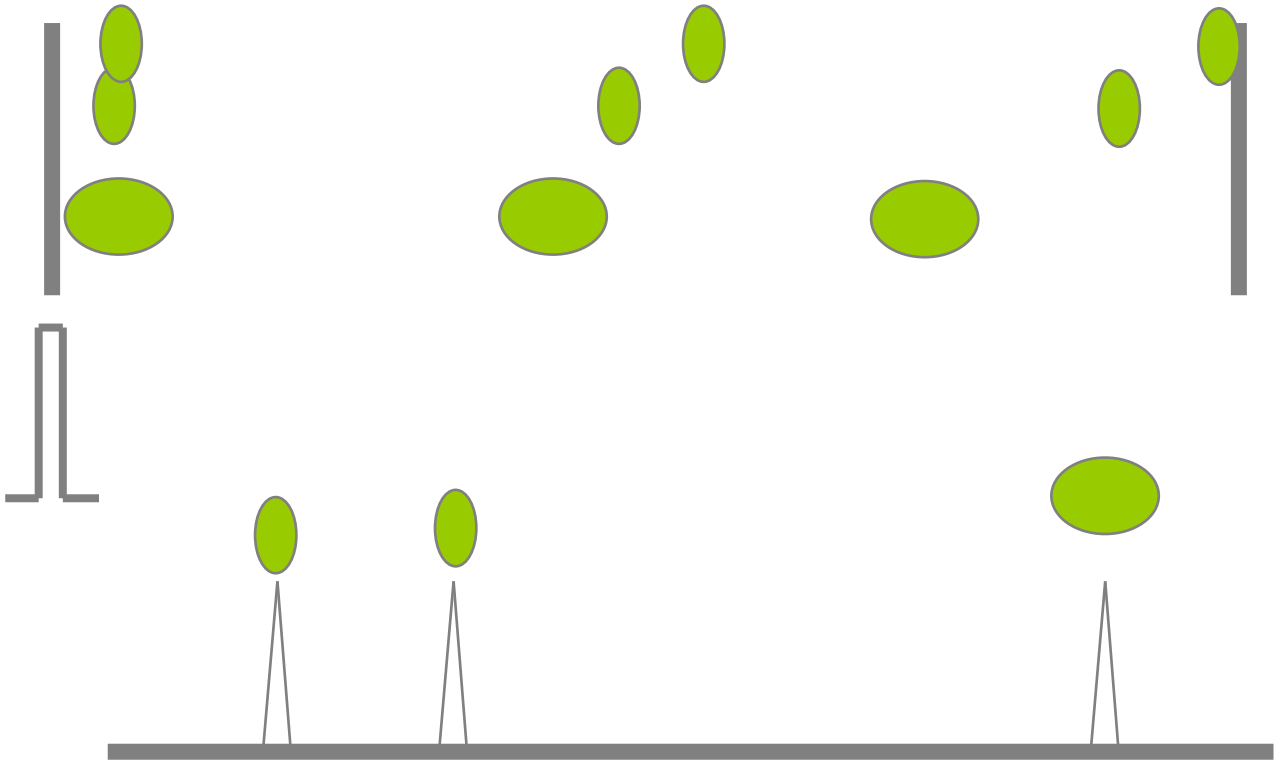


Unstable trajectories described by unbounded solutions to Mathieu equation.



Stable trajectory described by bounded solution to Mathieu equation.

Time-of-Flight MS Theory



$$\tfrac{1}{2}\, mv^2 = qE$$

Time of Flight Theory

An oa-TOF m/z measurement is initiated by a pusher pulse applied to the pusher plate, typically on the order of 800 V. Assuming that all of the energy imparted by this acceleration (a = zE, where z is the number of charges and E is the electric field strength) is converted to kinetic energy, we may relate the time it takes an ion to travel (TOF) a given distance (d) to its mass-to-charge ratio (m/z).

$KE = \frac{1}{2} mv^2 = zE$

$v = (2zE/m)^{1/2}$

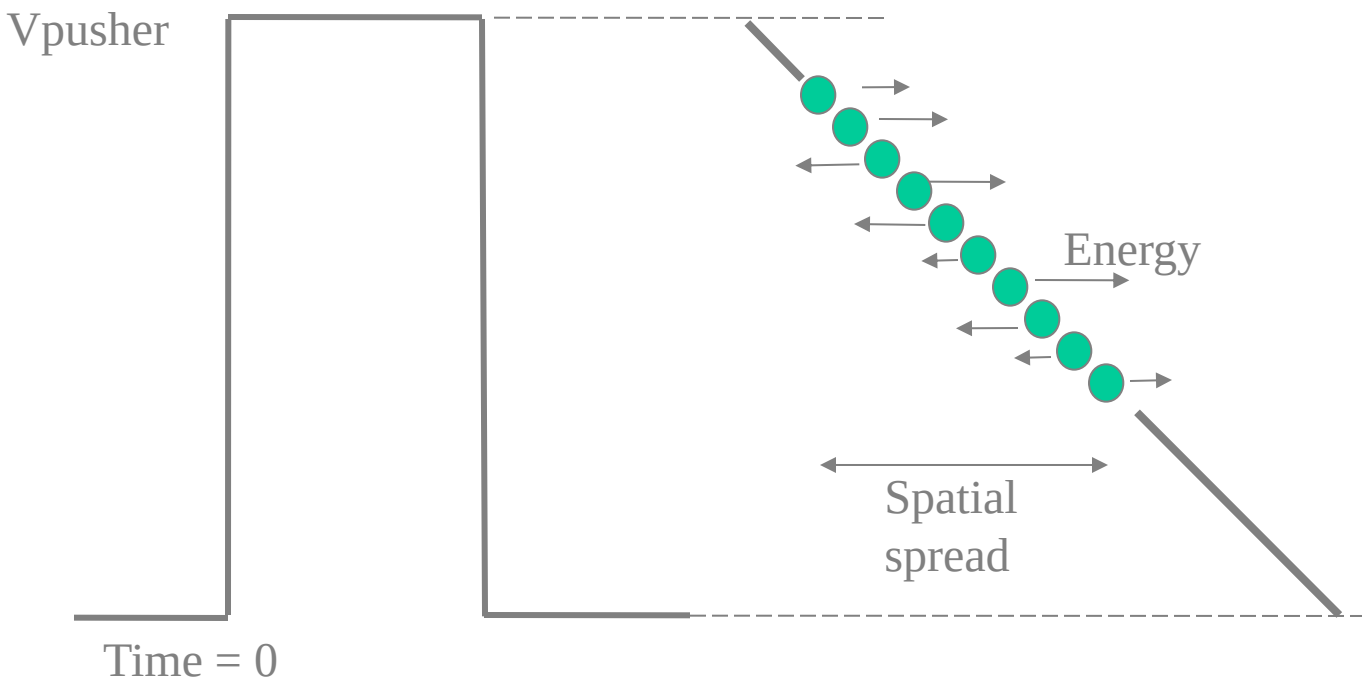
$TOF = (d/v) = d/(2zE/m)^{1/2}$

$TOF^2 = md^2/2zE = m/z\ (d^2/2E)$

So, m/z is proportional to TOF^2

Kinetic Energy Distribution

Initial energy and spatial distributions



Q-Tof Premier Quadrupole RF Settings

Q-Tof MS

Quadrupole operates as transfer lens: Only RF is applied

(Applied RF amplitude determined by MS Profile.)

Q-Tof MS/MS

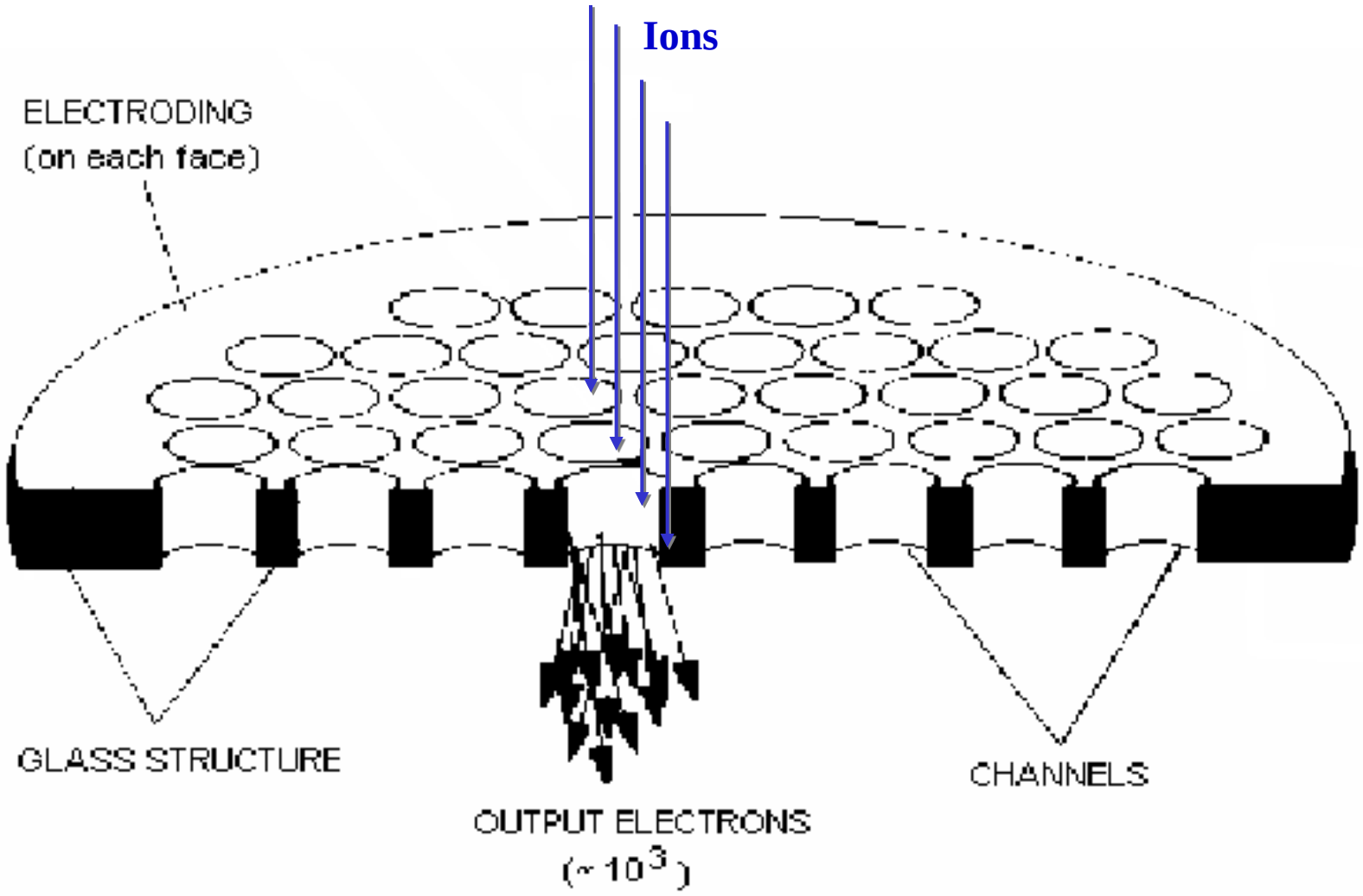
Quadrupole operated in resolving mode: RF and DC are applied

Resolution Settings during MS/MS (LM_{res}/HM_{res} on tune page):

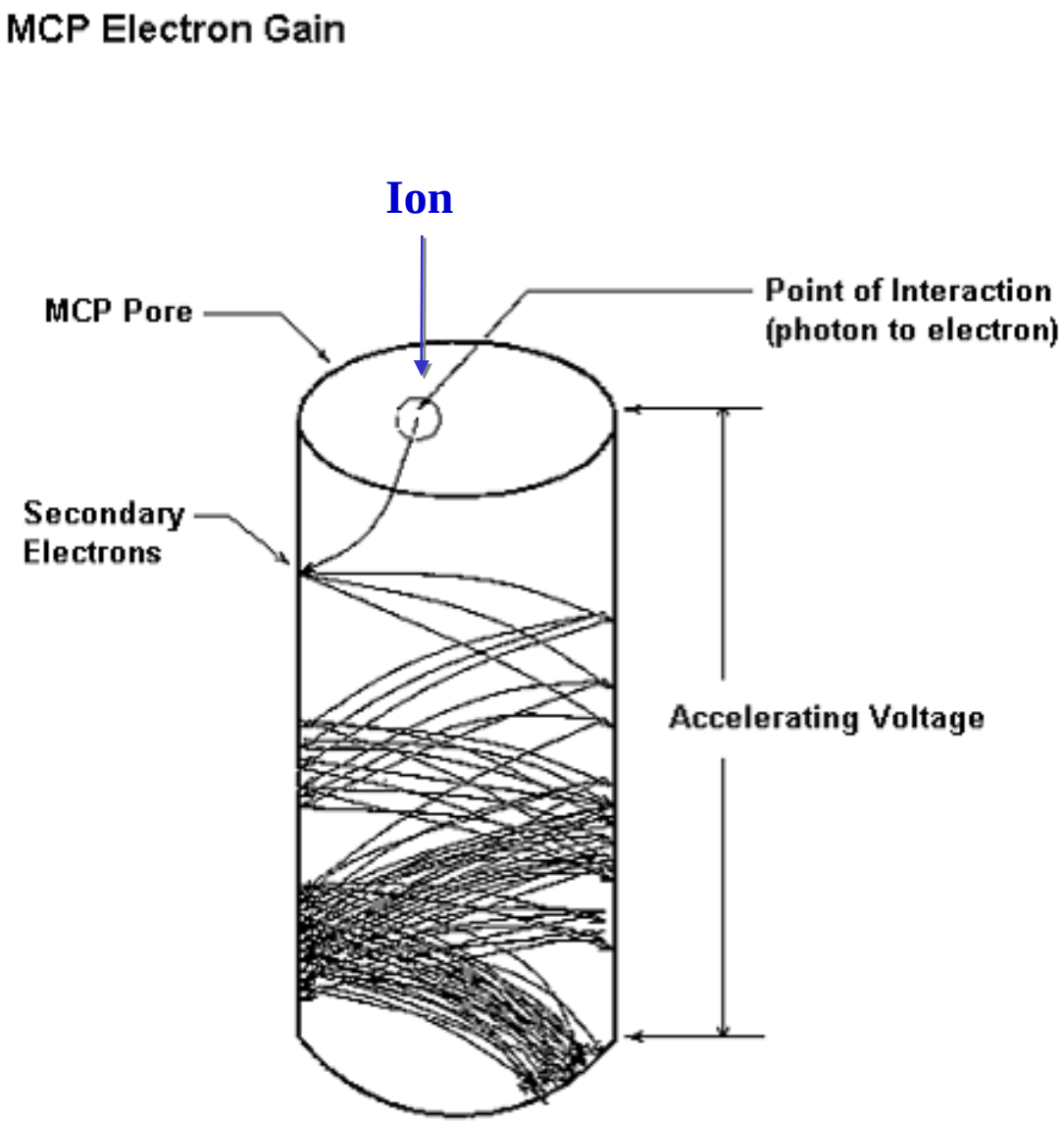
0/0	~ 20 Da window is passed
4.7/15	~ 5 Da window is passed
10/15	~ 2 Da window is passed

(note: 10/15 approximately 25% poorer transmission than 4.7/15)

Microchannel Plate (MCP)

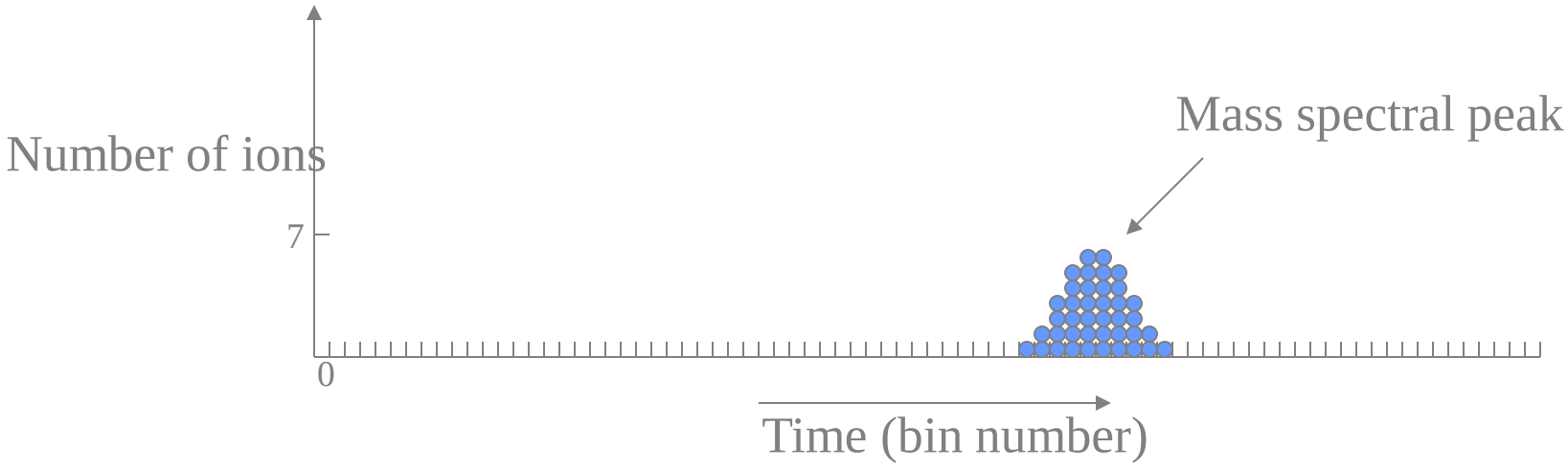


MCP Electron Gain



TDC Rapidly Sums Spectra from Individual Pulses

For a 1-sec “scan time” and 33 μsec flight time, the TDC sums ion arrivals across the 30,303 pulses on a bin-by-bin basis. The software then develops a histogram of total ion arrivals as a function of bin number (mass spectrum).

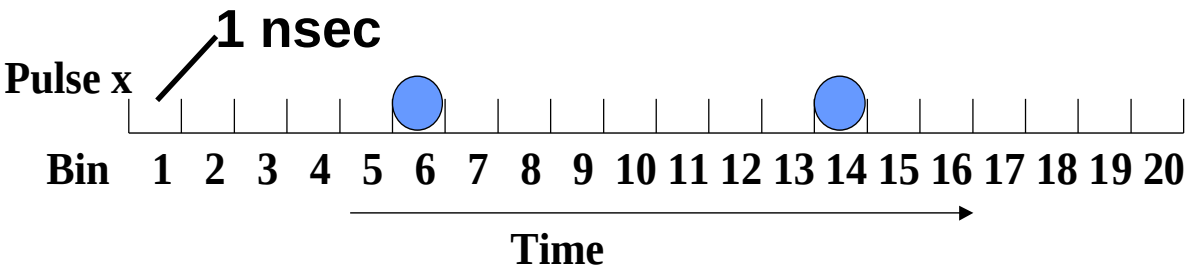


What is Dead Time?

After an ion arrival at the detector, the TDC is not ready to register another arrival during the next 4 nsec.

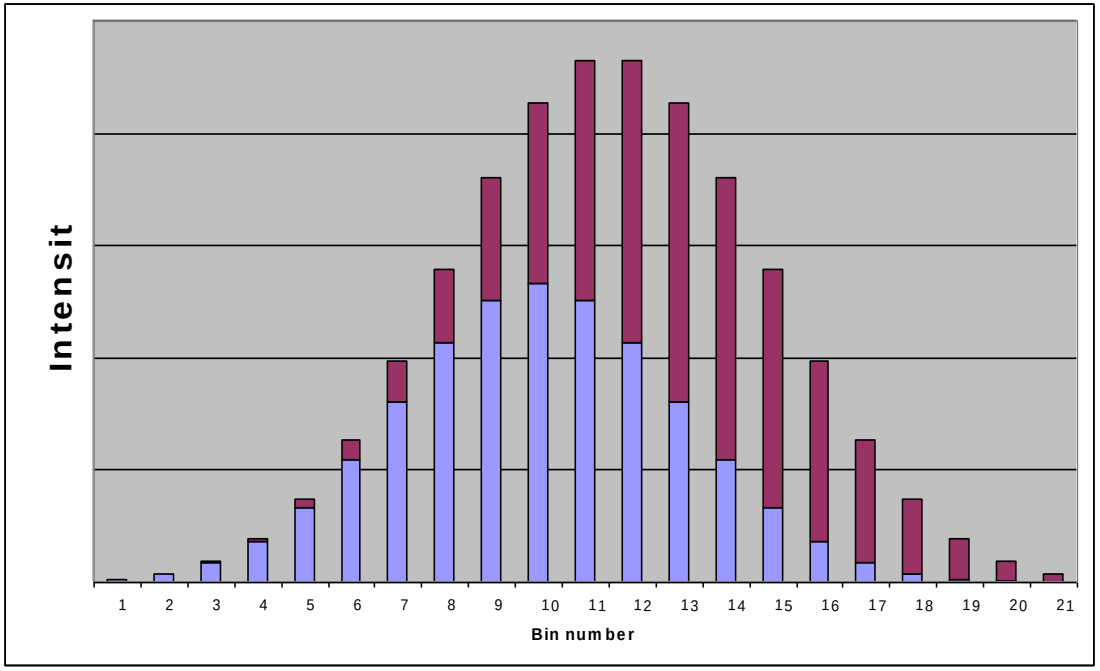
This 4 nsec inertia of the detection electronics is referred to as “dead time”.

Dead time saturation distorts peak shape and shifts the measured signal on the time (m/z) axis.



Dead Time Saturated Signal

The overall distribution (blue + red) reflects the ion arrivals at the detector. The blue distribution reflects those arrivals registered by the TDC. The red distribution reflects those arrivals in dead time.



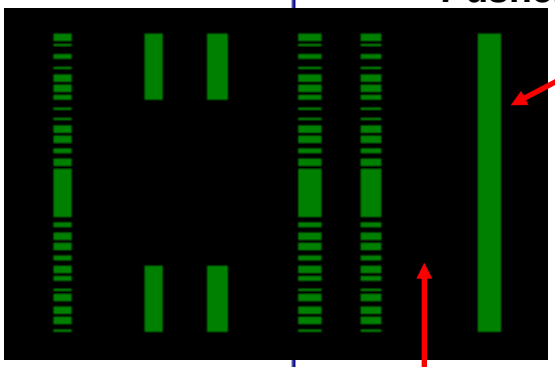
The distribution of registered arrivals is shifted to low mass relative to the distribution of total arrivals. Note that a significant fraction of arrivals go undetected, limiting the linear dynamic range.

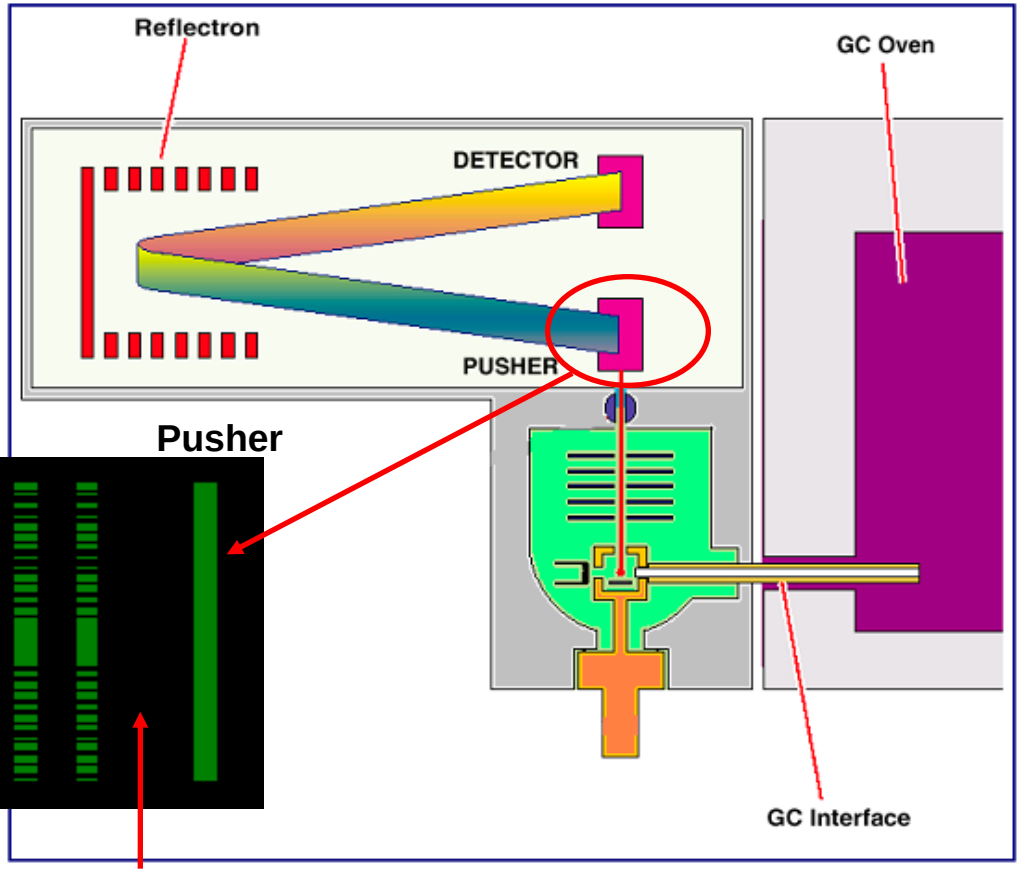
GC/MS application

GCT – Premier (Waters, $270,220)
mass accuracy< 5 ppm
resolution = 7,000

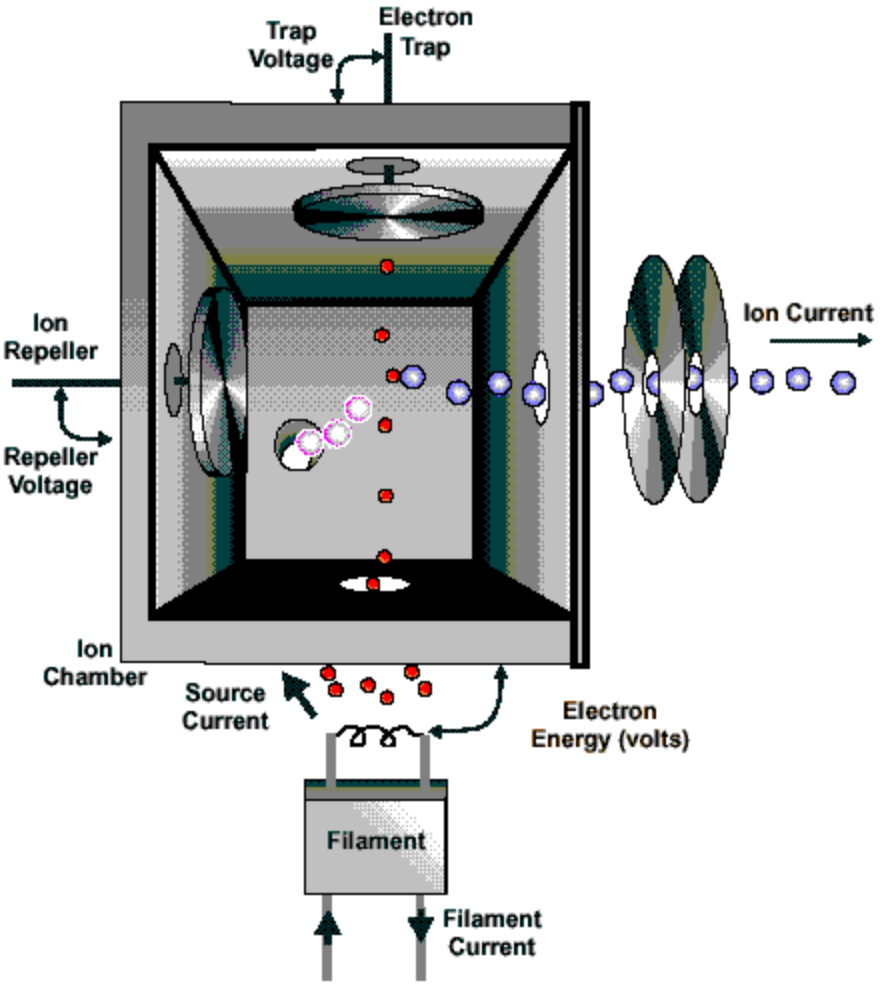
Ionization Modes:

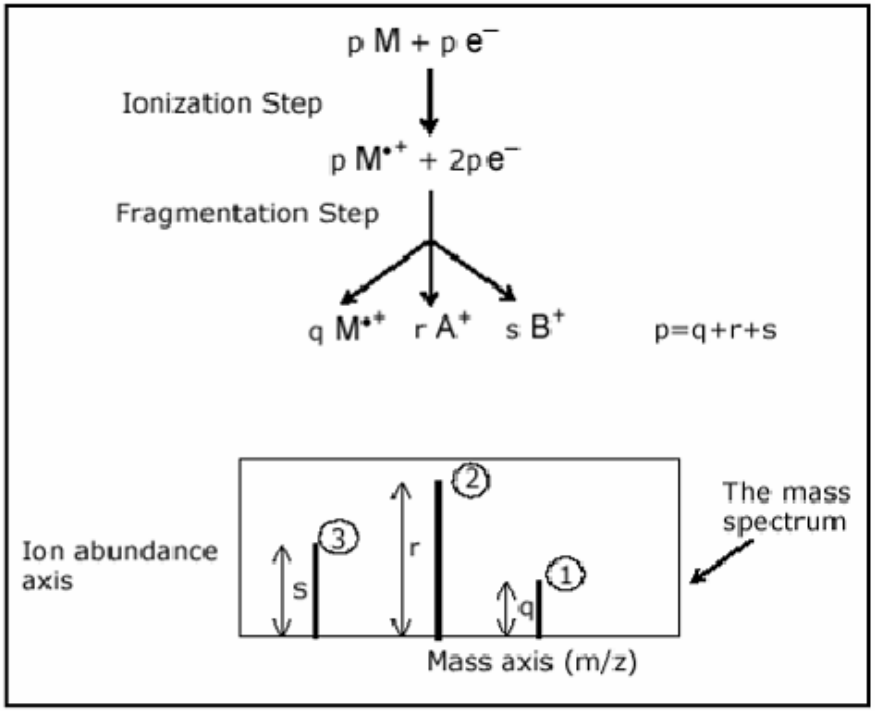
- Electron Impact,
- Chemical Ionization,
- Solids Probe





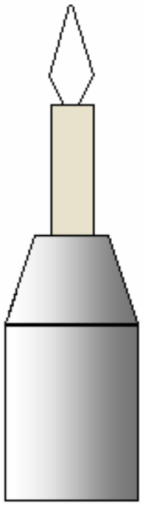
Electron Impact Ionization





The formation of a simple EI mass spectrum from a number (p) of molecules (M) interacting with electrons (e^-). Peak 1 represents $M^{\bullet+}$, the molecular ion, the ion of greatest mass (abundance q). Peaks 2, 3 represent A^+, B^+, two fragment ions (abundances r, s). Peak 2 is also the largest, and therefore the base peak.

Chemical Ionization



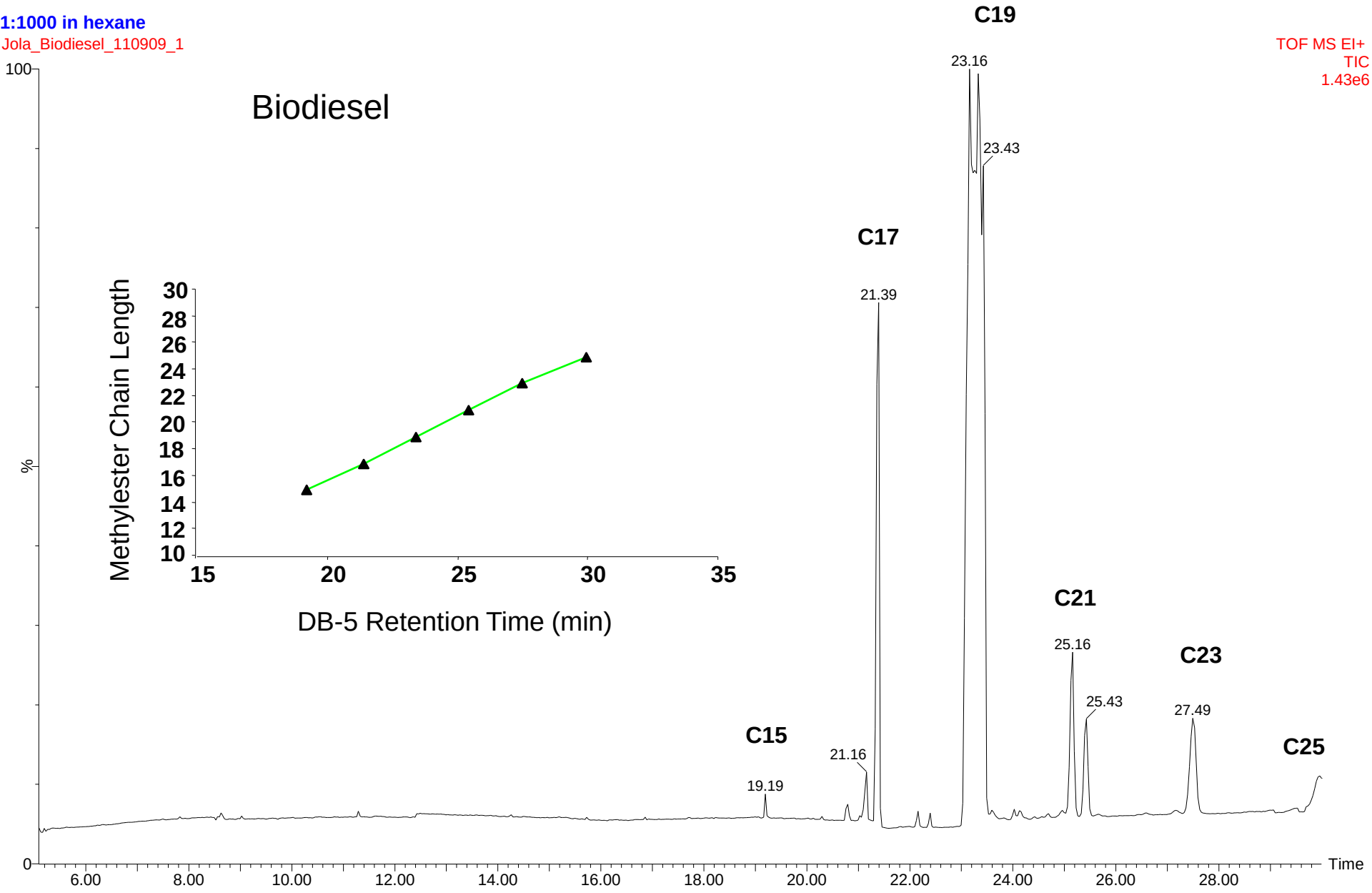
Probe

EI : M $\xrightarrow[\text{Step 1}]{\text{Ionization}}$ $M^{\bullet+}$ $\xrightarrow[\text{Step 2}]{}$ $M^{\bullet+}$, A^+, B^+ etc.
Mass Spectrum

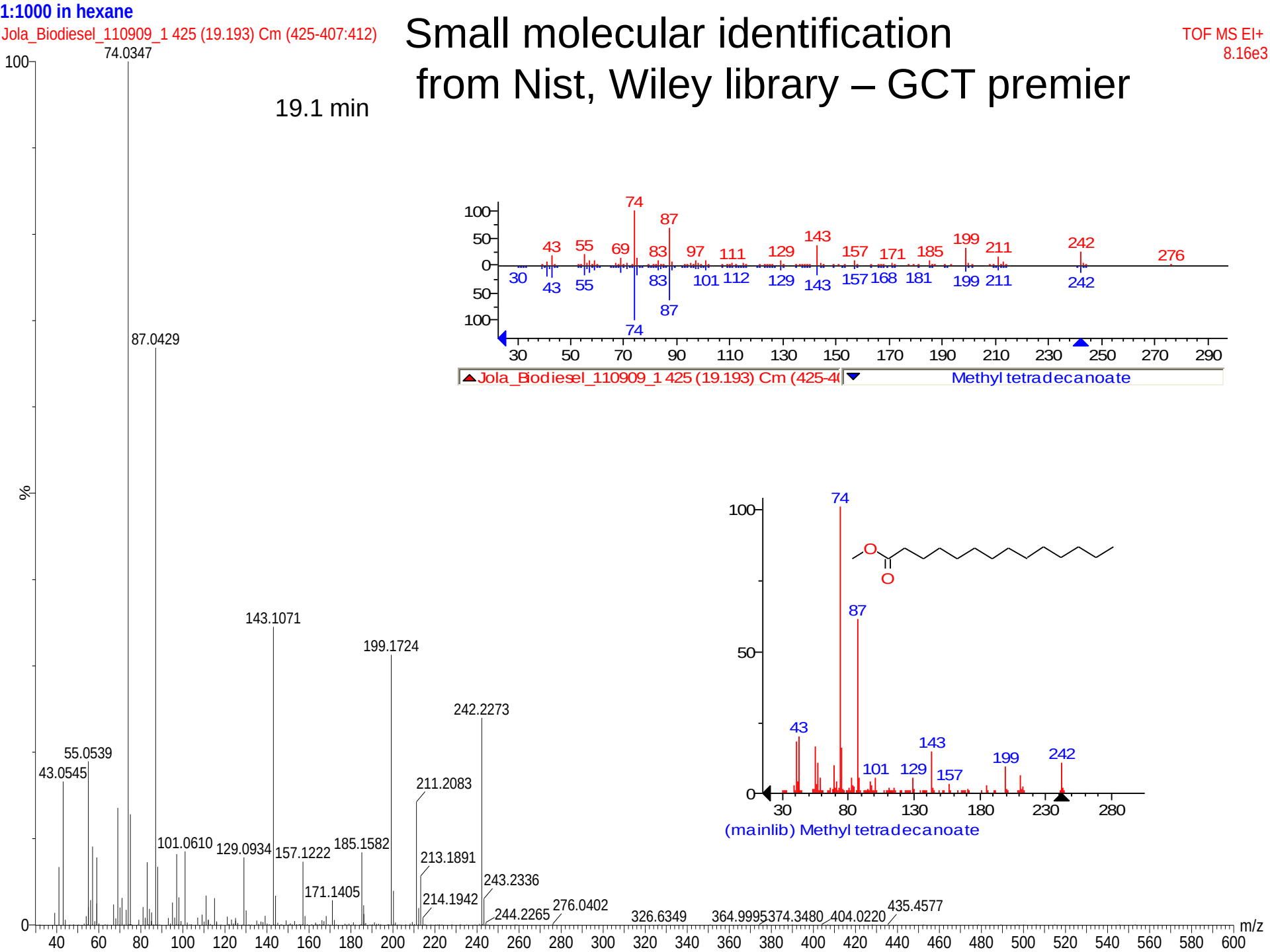
CI : R $\xrightarrow[\text{Step 1}]{\text{Ionization}}$ $R^{\bullet+}$ $\xrightarrow[\text{Step 2}]{}$ RH^+

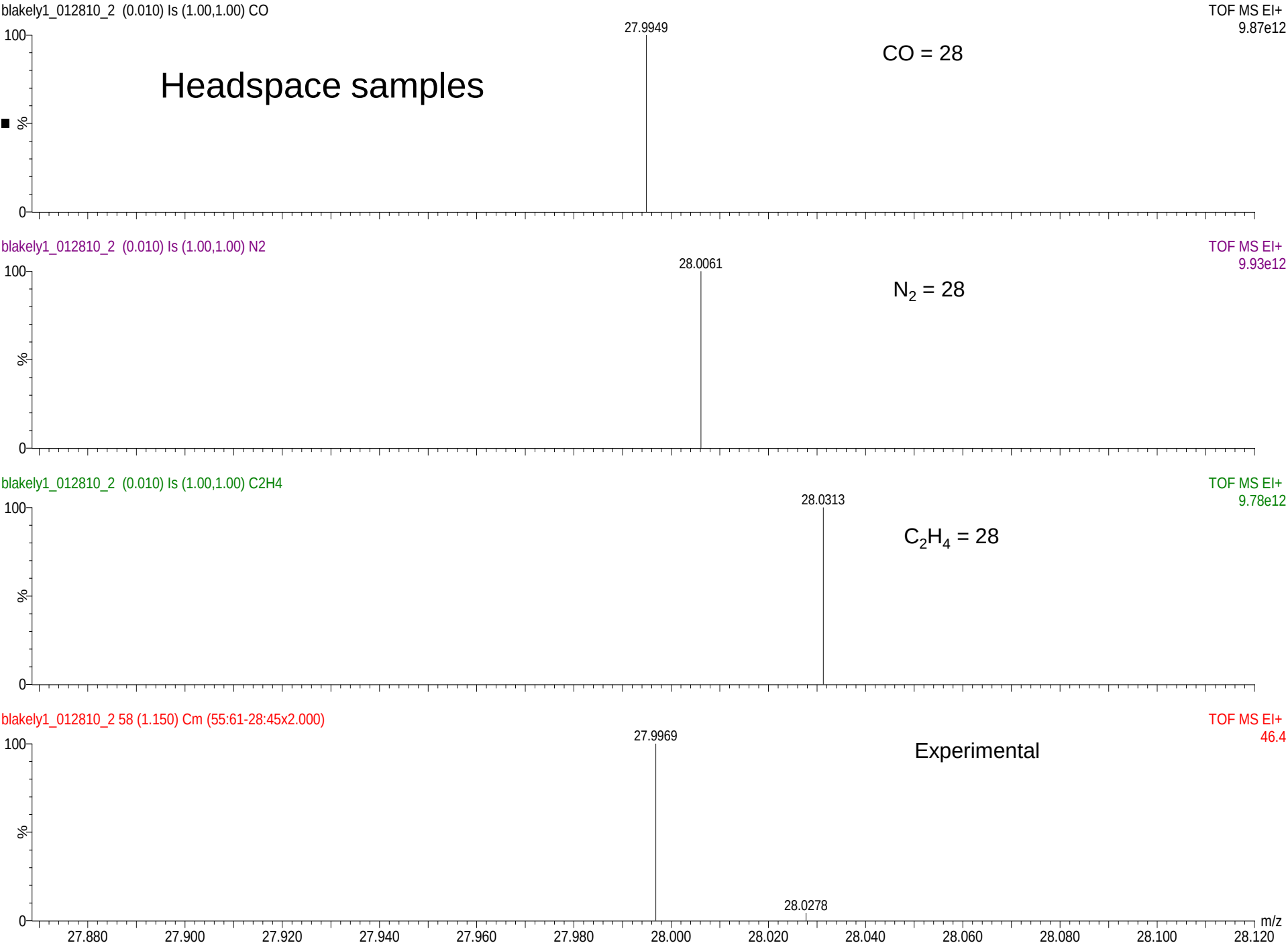
$\xrightarrow[\text{Step 3}]{M}$ $MH^+ + R$
Mass Spectrum

GC/MS is quantitative



Small molecular identification from Nist, Wiley library – GCT premier




blakely1_012810_2 (0.010) Is (1.00,1.00) CO
TOF MS EI+
9.87e12
27.9949
CO = 28
Headspace samples
100
%
0
blakely1_012810_2 (0.010) Is (1.00,1.00) N2
TOF MS EI+
9.93e12
28.0061
N_2 = 28
blakely1_012810_2 (0.010) Is (1.00,1.00) C2H4
TOF MS EI+
9.78e12
28.0313
C_2H_4 = 28
blakely1_012810_2 58 (1.150) Cm (55:61-28:45x2.000)
TOF MS EI+
46.4
27.9969
Experimental
28.0278
m/z
27.880
27.900
27.920
27.940
27.960
27.980
28.000
28.020
28.040
28.060
28.080
28.100
28.120

Selection of GC -column

ZB-5: 95% Dimethylpolysiloxane 5% phenyl

Alkaloids, Dioxins, Drugs, Essential Oils/Flavors, FAMES, Halo-hydrocarbons, PCBs/Aroclors, Pesticides/Herbicides, Phenols, Residual Solvents, Semi-volatiles.

ZB-Wax: 100% Polyethylene Glycol

Alcohols, Aldehydes, Aromatics, Basic Compounds, Essential Oils, Flavors & Fragrances, Glycols, Pharmaceuticals, Solvents/Residual Solvents, Styrene, Xylene Isomers.

Maintenance

- GC/MS has been over injected, need to dilute your samples to µM, nM or less,

- Over injection increases burden on filament ($250/piece, and last a couple of month),
  Inner and outer ion source clean up,
  over $50,000 spent on the maintenance,

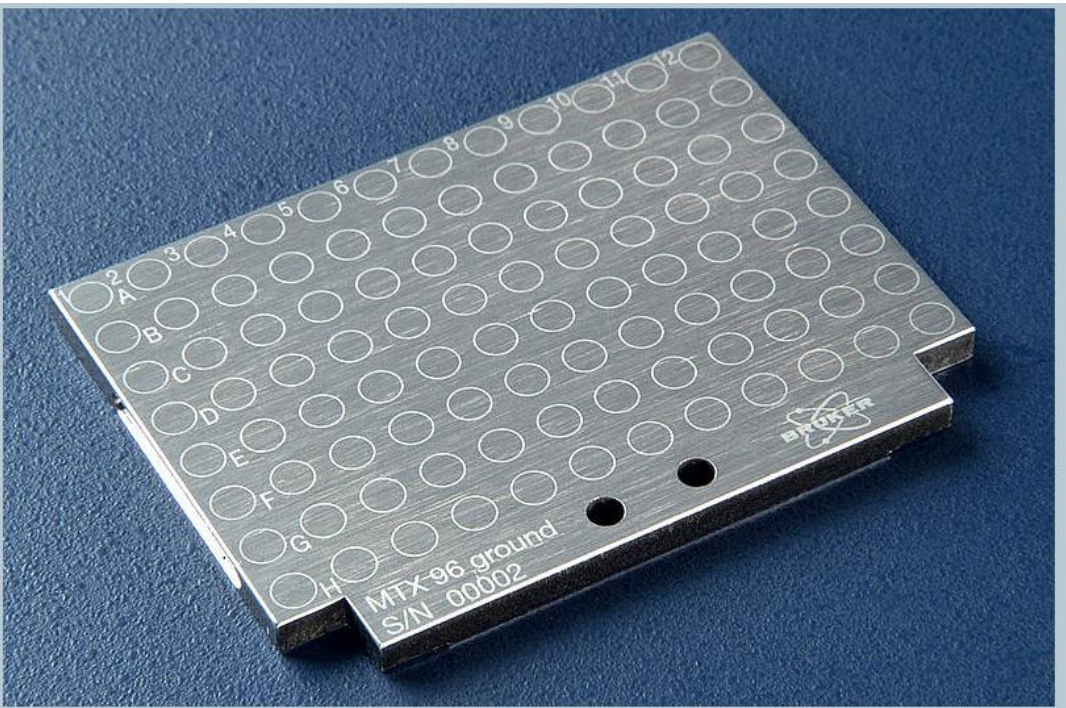
- available Probes: EI/CI probe, Direct insertion probe (DI), Direct chemical ionization probe (DCI),

MALDI/TOF (matrix-assisted laser desorption ionization/time of flight)

Microflex (Bruker)

This technology provides homogeneous, exactly-positioned samples on the MALDI target for robust and rapid automated data collection, as well as up to two orders of magnitude increase in sensitivity.

detection of even a broad mass range of small molecule, polymer, protein, and peptide analysts.



flexControl - tof - microflex - [Negative_insulin.par]

File Display View Tools Help

Sample: IN | Select Method

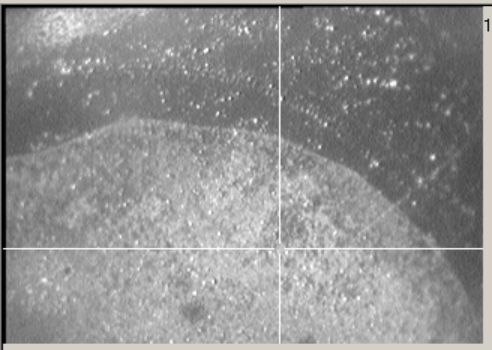
System: READY | Negative_insulin.par

Auto: AVAILABLE | Start Automatic Run

Carrier: G_FE721B00_3339_4E0F_BE15D850E6604A9F

AutoX: (Please select a sequence file) | Browse

BRUKER



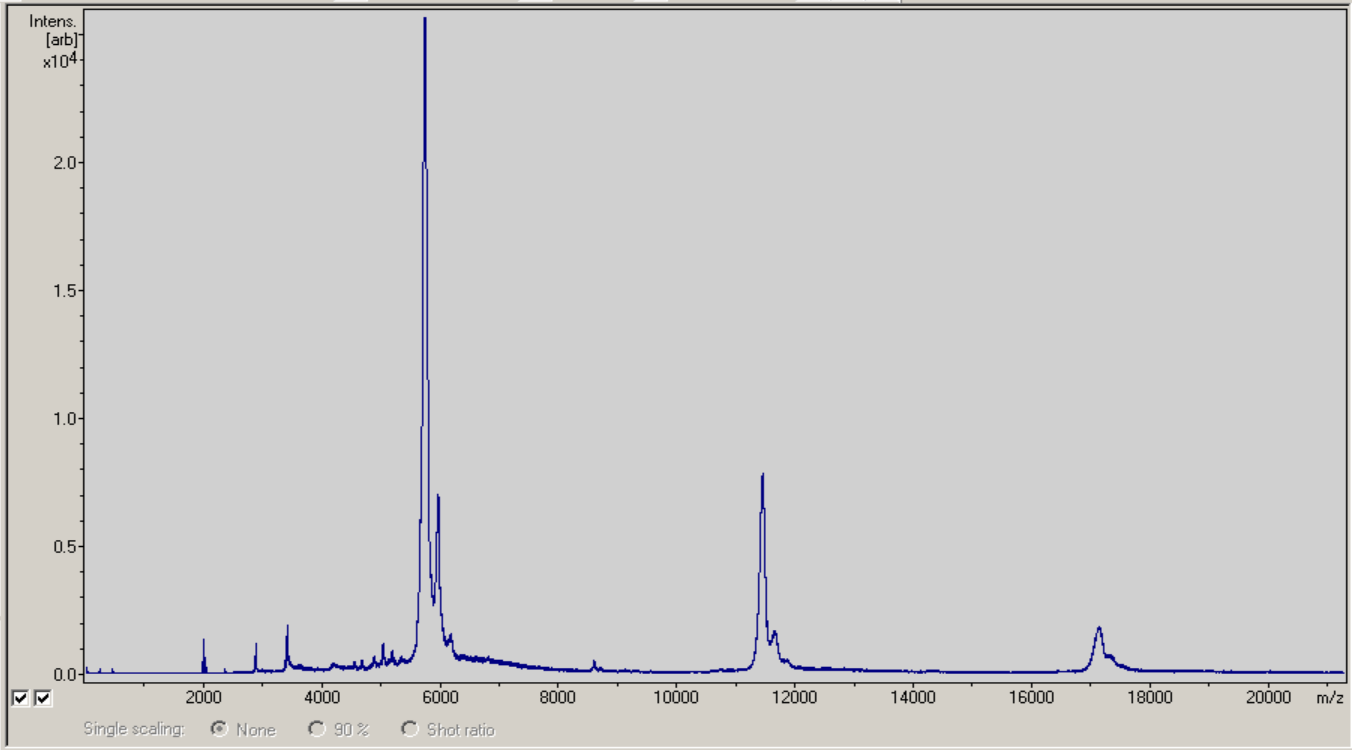
Start | Add | Undo

Save | Save As... | Clear Sum

Shots: 100 | MSP 96

Current: 100 | Frequency: 5.0 Hz | Spot: C9

Added: 0 | Laser Power: 3 %



Sample Carrier | Spectrometer | Detection | AutoXecute | Setup | Calibration | Status

Sample Selection

	1	2	3	4	5	6	7	8	9	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	○	○	○	○	○	○	○	○	○	○	○
C	○	○	○	○	○	○	○	○	●	○	○	○
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○

C9

First | Previous | Next | Last

Geometry: MSP 96

Teaching File:

Advanced >>>

Default Teaching

For Help, press F1

Linear measurement mode | Maximum cursor

Start | flexControl - tof - microfle... | flexAnalysis - tof - (*) | 10:23 AM

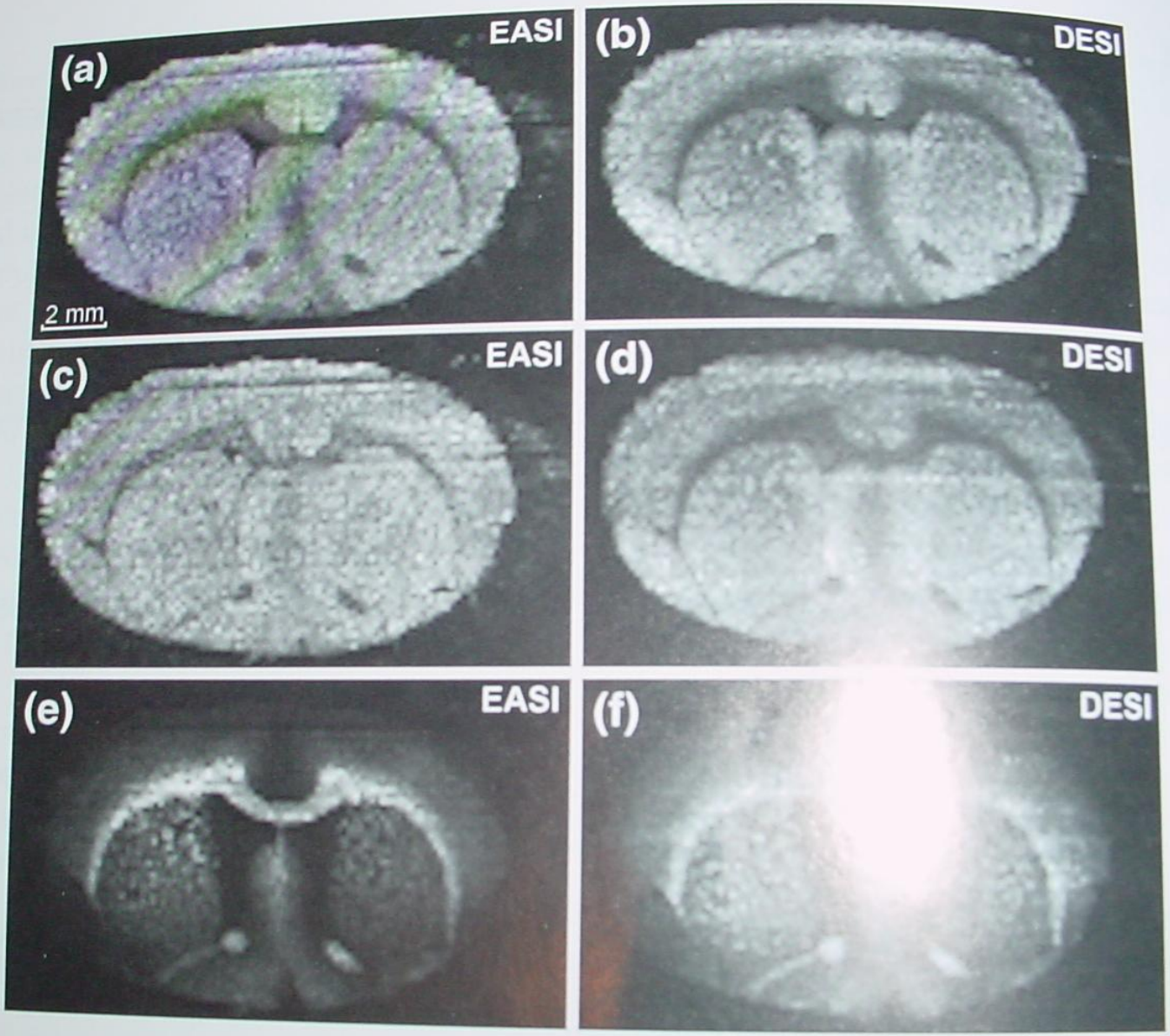
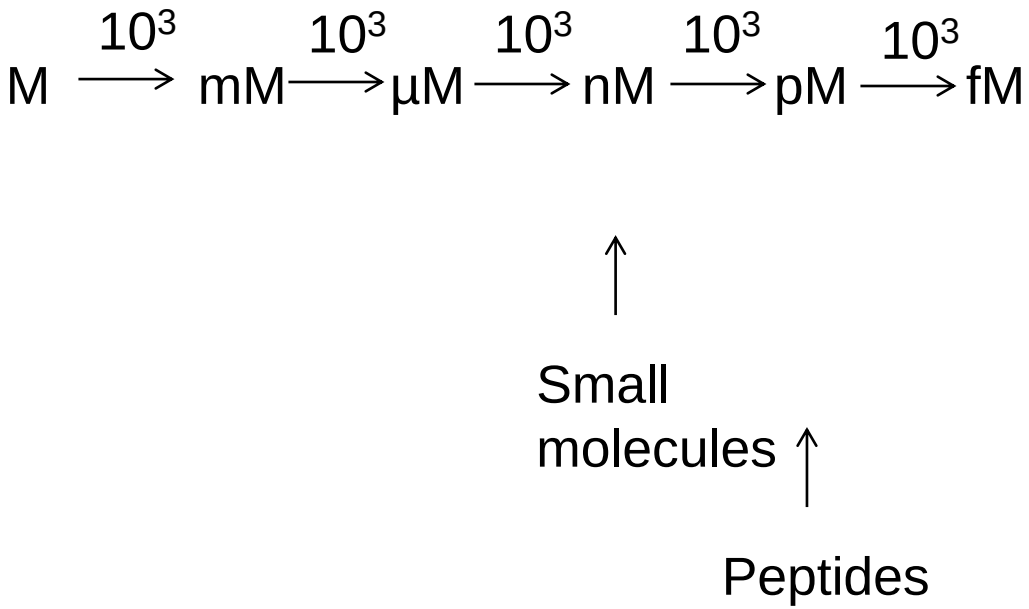


Figure 2. Desorption ionization images of rat brain, recorded with 150 µm spatial resolution in negative ion
column, (**a**), (**c**), (**e**) are recorded with EASI, and the right column, (**b**), (**d**), (**f**) are recorded with DESI. (**a**), (**b**): PS(4
(**c**), (**d**): PI(18:0/20:4) (*m/z* 885.5); (**e**), (**f**): ST(24:2) (*m/z* 888.6)

3. Sample Preparation

- Sample must be soluble in solvent/H_2O, no particulate,
- Typically inject 1µL of µM, or less material of 50 µL in 2 ml Vial,
- Most samples were over injected; dilute 10~100 x,
- Place small molecules in glass vial, proteins in plastic vial,
- Add acid or base to protonate or deprotonate in + or – ion mode.

Q-Tof sensitivity



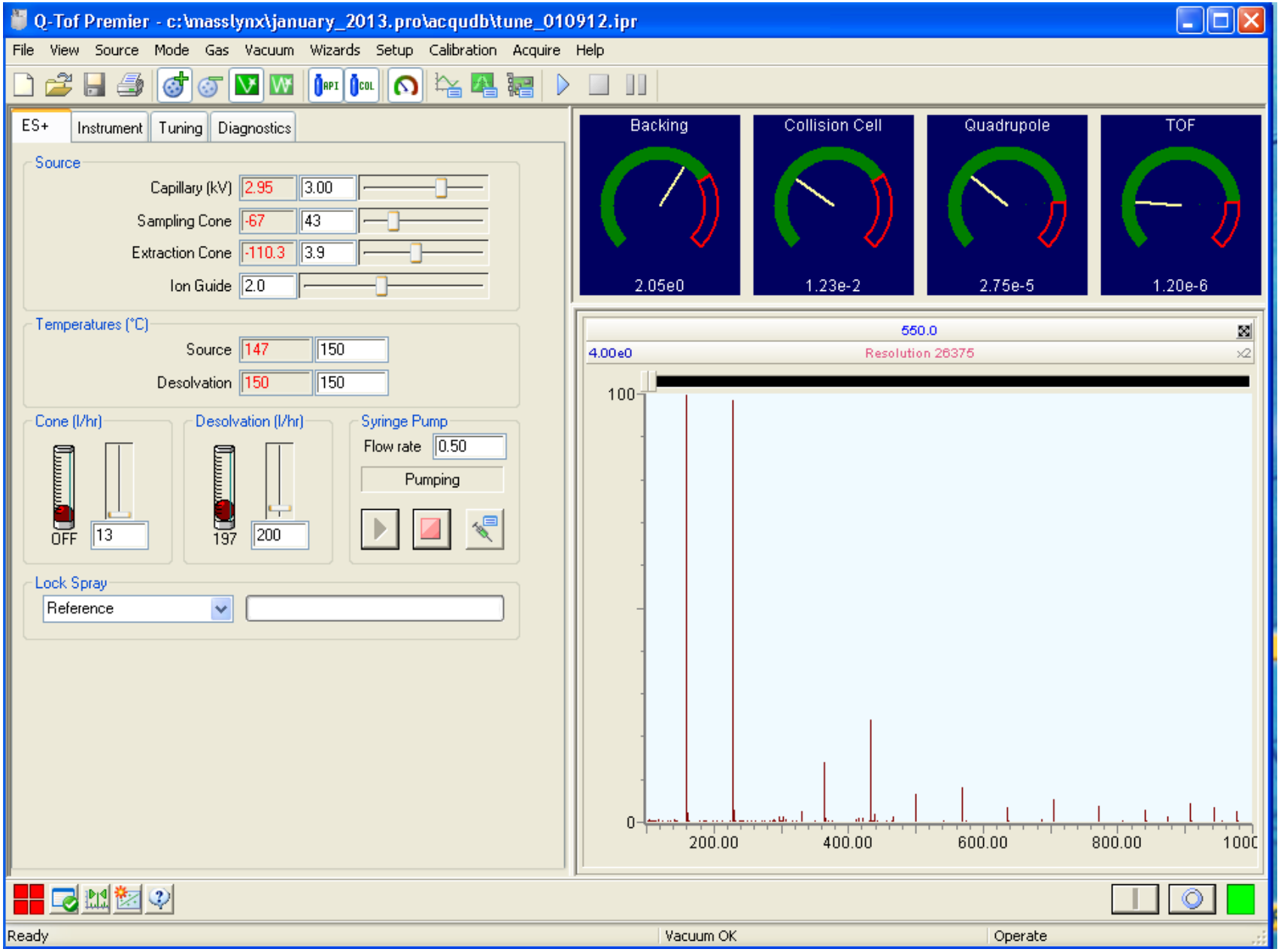
Instrument maintenance

New

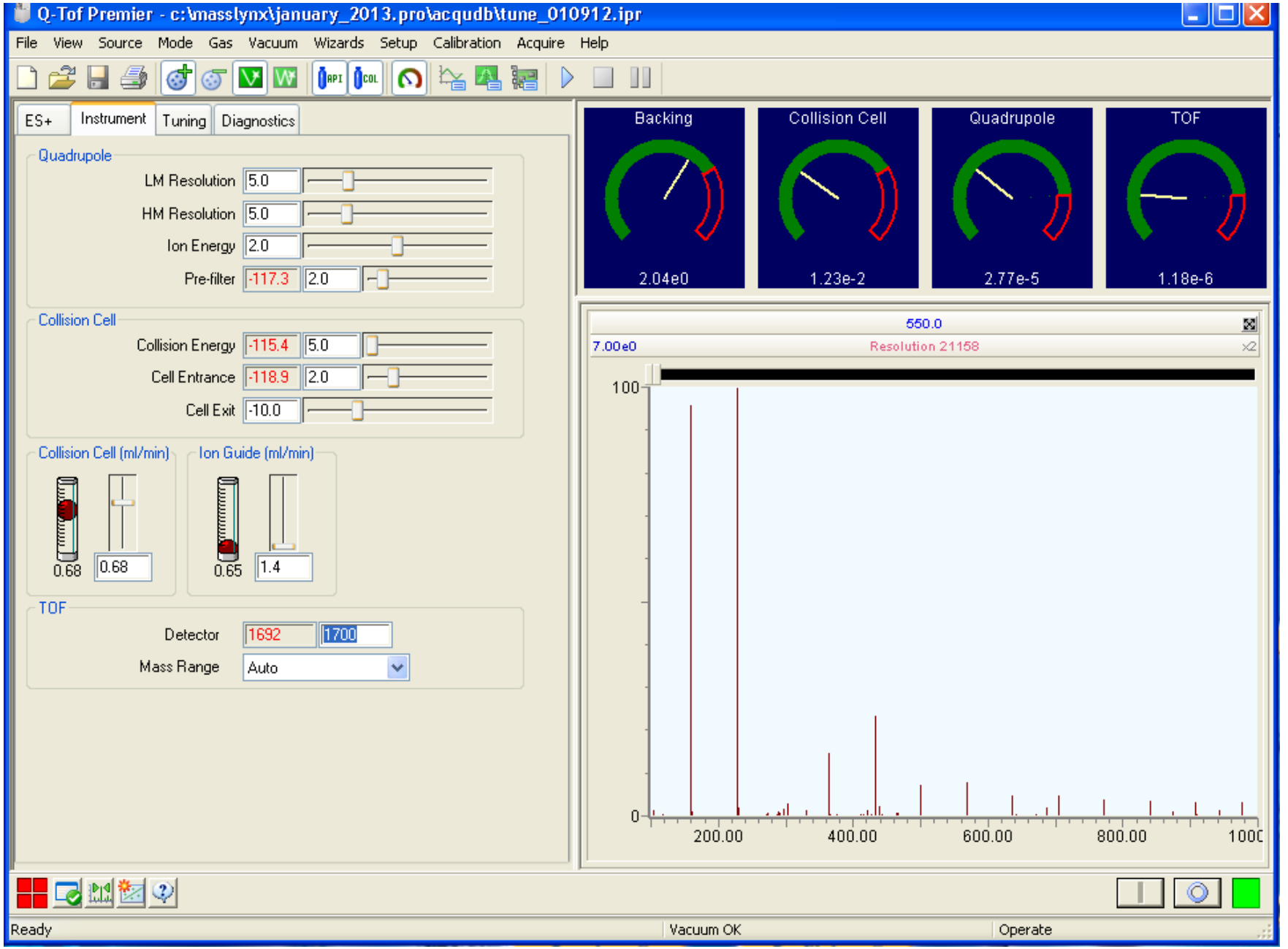
Damaged

Sample Cone

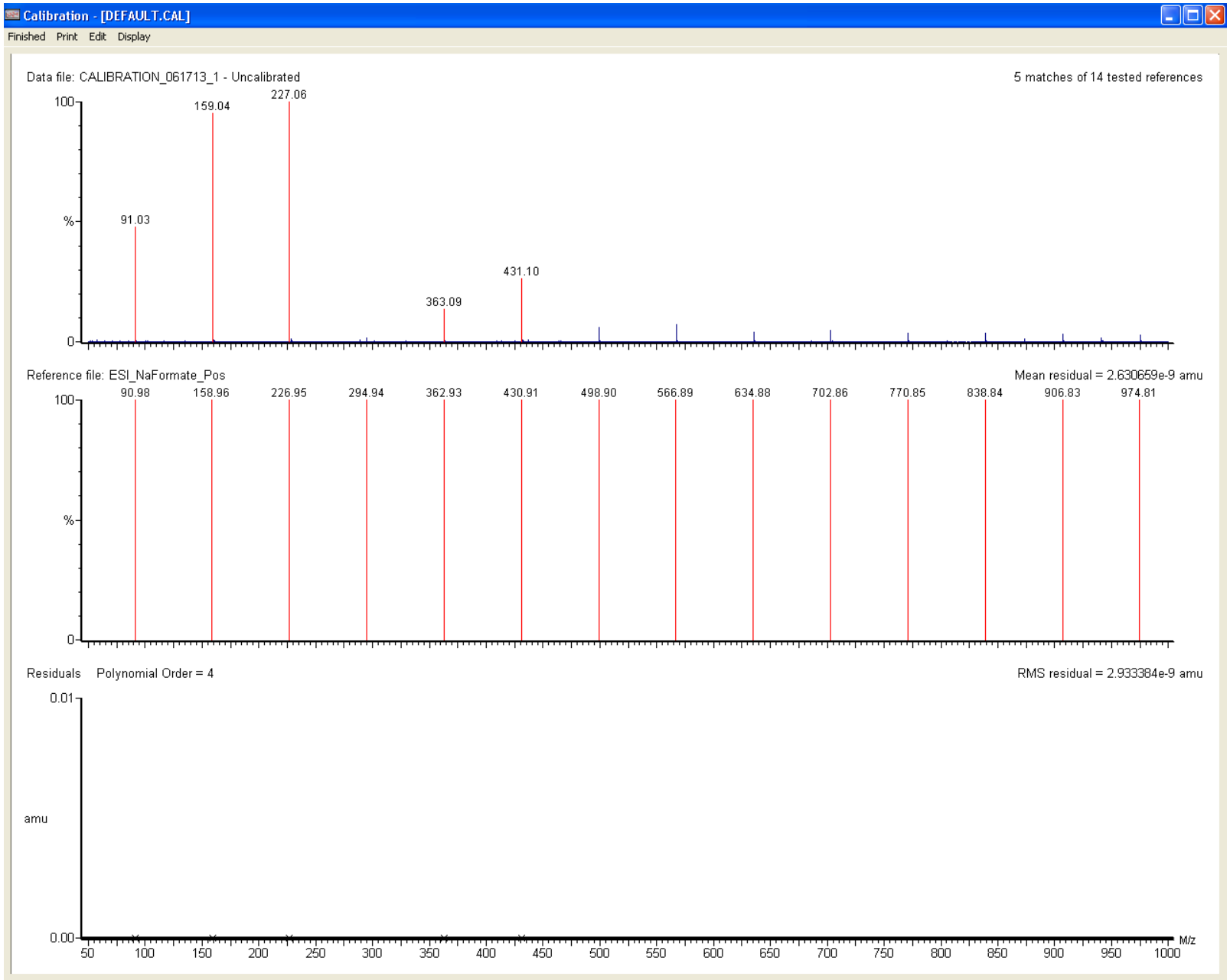
4. Instrument Operation, Tune Page



Tune Page -continue



Calibration with NaFormate



Change Ion mode

- Set the instrument in Stand By (bottom right on the cycle button),
- Select ion mode (first tab on the up left on the Tune page),
- Bring in the corresponding tune page,
- Wait for 1 min,
- Set the instrument in Operation,
- Check detector voltage (1,700) and lockmass intensity,
- Change back when finish your work.

Sample loss (2% efficiency)

Only a small proportion of ions entering the pusher are detected by the MCPs. There are three main factors limiting the overall sensitivity of the orthogonal TOF:

Sampling Efficiency

This is the proportion of ions entering the pusher that are pushed out orthogonally. A Q-TOF has a sampling efficiency of 22%.

Transmission of Grids

The ions have to pass through the three pusher grids as well as the reflectron grid. At each grid a proportion of the ions are lost. For a Q-TOF, the overall transmission of the grids is 12%.

Detection Efficiency

Not all ions that hit the MCP are detected. The detection efficiency of the MCP is 65%.

Multiplying together these factors we find that the overall sensitivity of the orthogonal-TOF is 2%. This is true for ions whose flight times match the pusher frequency. For ions of lower masses there is a further reduction in sensitivity, given by the factor T/T_m where T is the flight time of the ion, and T_m is the maximum flight time for the pusher frequency in use.

This sensitivity may not sound a lot, but it is 100 times greater that that achieved by a quadrupole scanning over a range of 1000 Da, at unit resolution.

1 ion count from1 billion molecules

Tips for Accurate Mass Work

- Use separate calibrations for positive and negative ion work,
- Save the instrument tuning files and note which calibrations apply (general housekeeping),
- Leave the instrument in operate at all times,
  - Stabilization is greater than two hours,
- Familiarize yourself with the limits of deadtime correction.

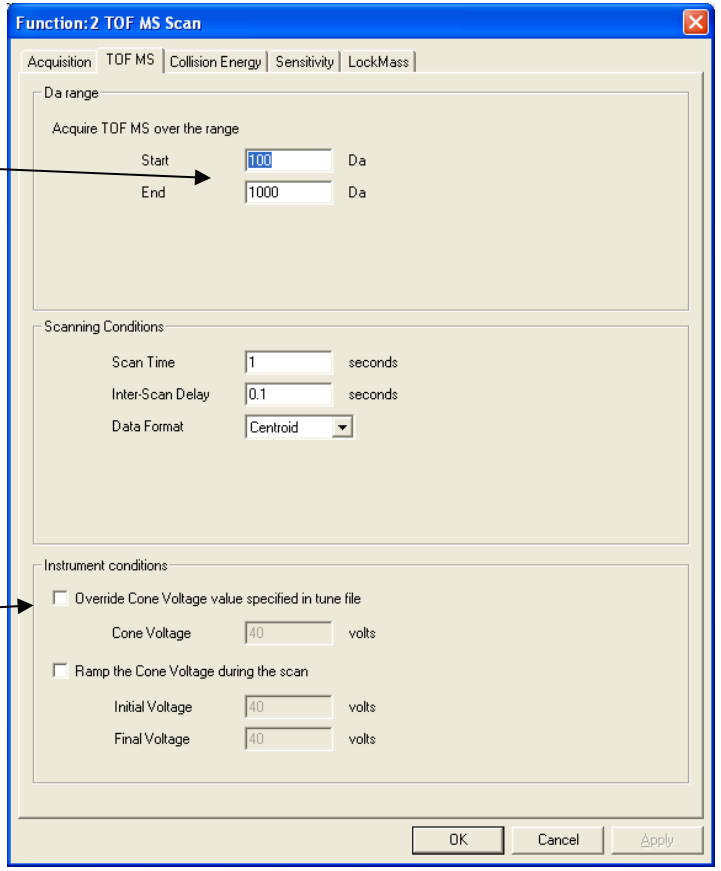
5. Acquisition method for Q-ToF

- **MS Methods** (small molecule, polar compounds)
- **MS/MS Methods** (structural analysis)
- **Data Dependent Analysis:** Survey Methods (peptide sequencing)
- **Data Dependent Analysis**: Parent Ion Discovery via Product Ions (structural analysis)

MS scan

- **Remember to use a mass range that is consistent with your calibration.**

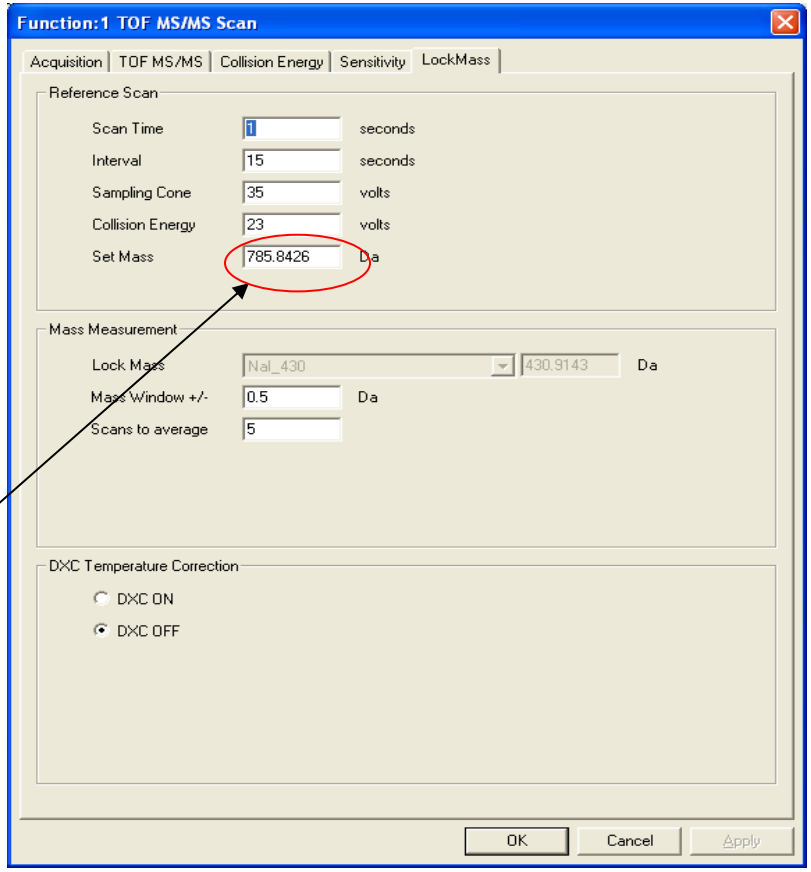
- **You can use higher cone voltage for in source fragmentation**



MS/MS Methods

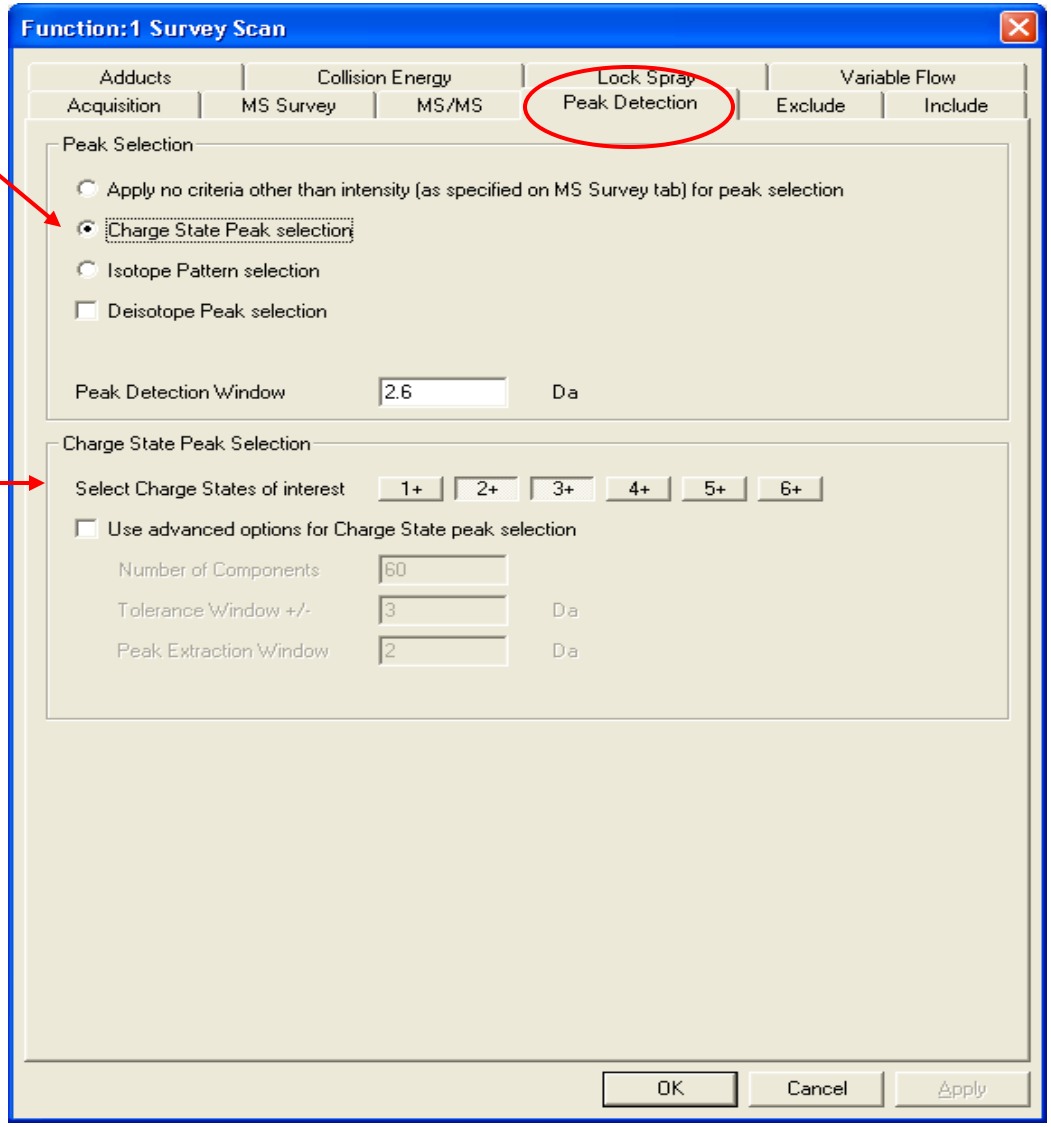
- **With glufibrinopeptide, an abundant m/z 785.8426^{2+} and 684.3469^{+} are generated to use as lockmasses for multiply charged peptides (2+, 3+, 4+) or singly charged peptides respectively**

- **Set mass to parent ion of 785.8426**



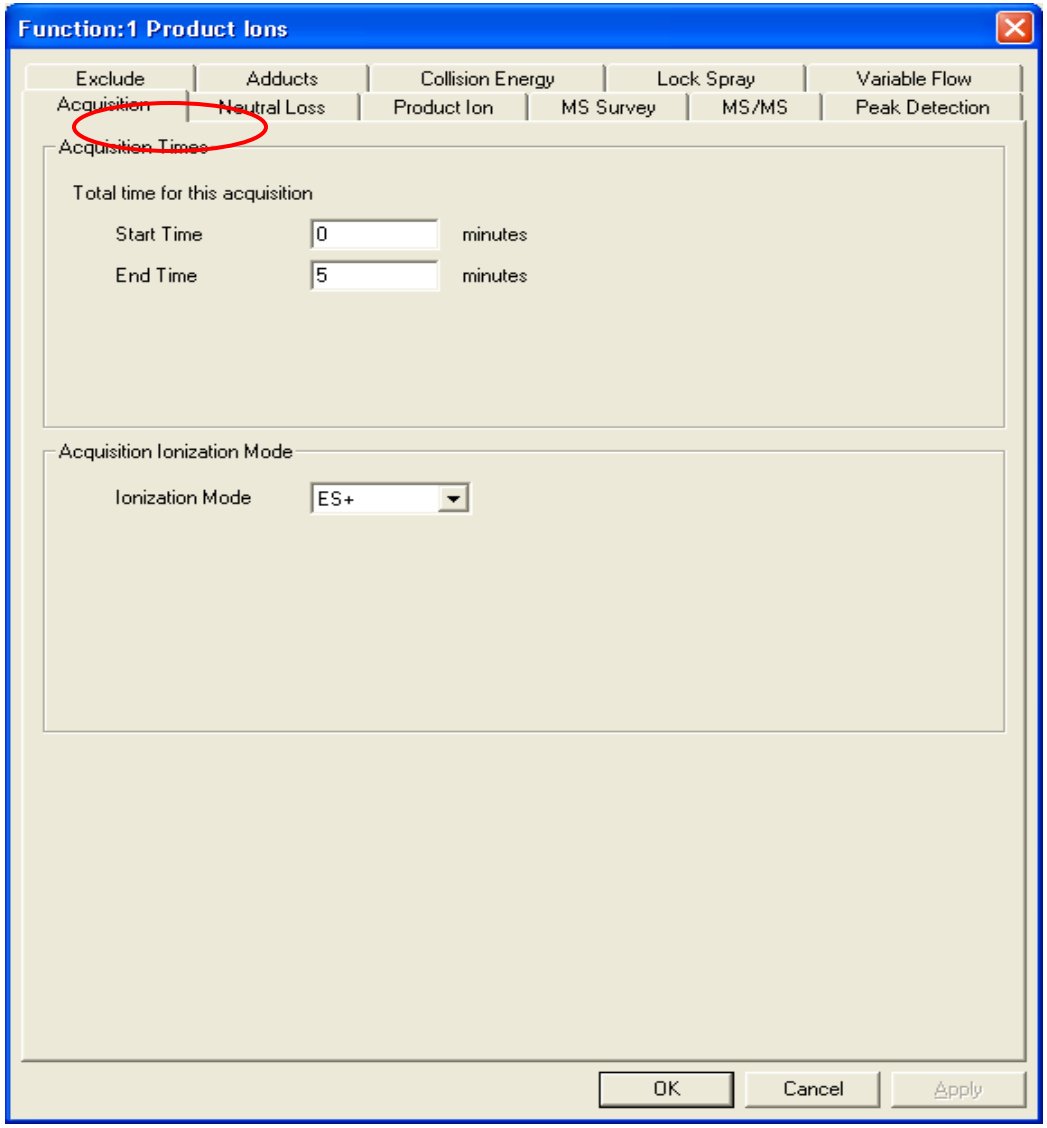
DDA Methods *Collision Energy Profiling*

- **By selecting Charge State Peak selection, the charge state species preferentially desired in an experiment may be switched on for MS/MS while all others are excluded from consideration. In tryptic digests, the majority of peptides are 2^+ and 3^+.**



Parent Ion Discovery
Product Ion

- **Same as with DDA.**



Typical ESI Positive Samples:

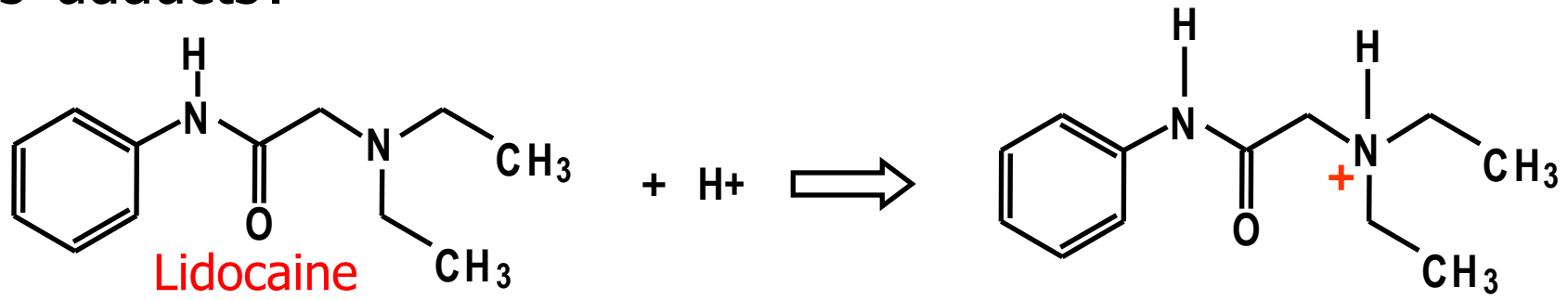
Peptides and proteins
Small polar molecules
Drugs and their metabolites
Environmental contaminants
Dye compounds
Some organometallics
Small saccharides

Typical ESI Negative Samples:

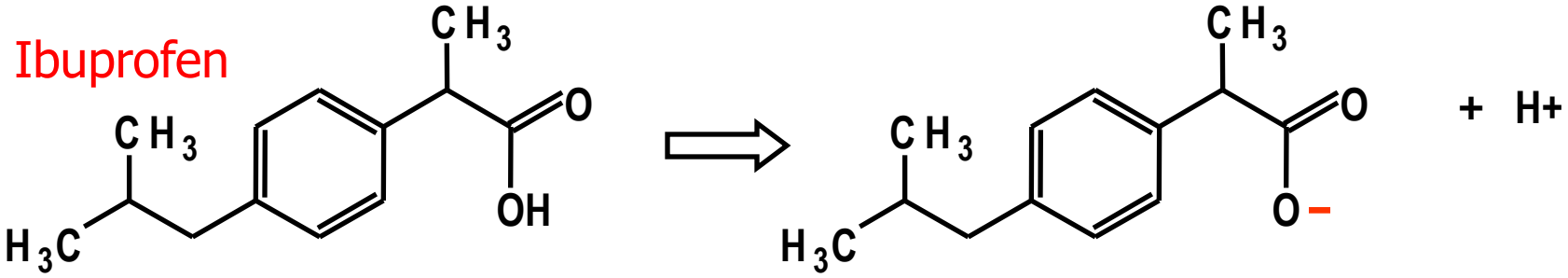
Some proteins
Some drug metabolites (e.g. conjugates)
Oligonucleotides
Some saccharides and polysaccharides

Production of positive and negative ions

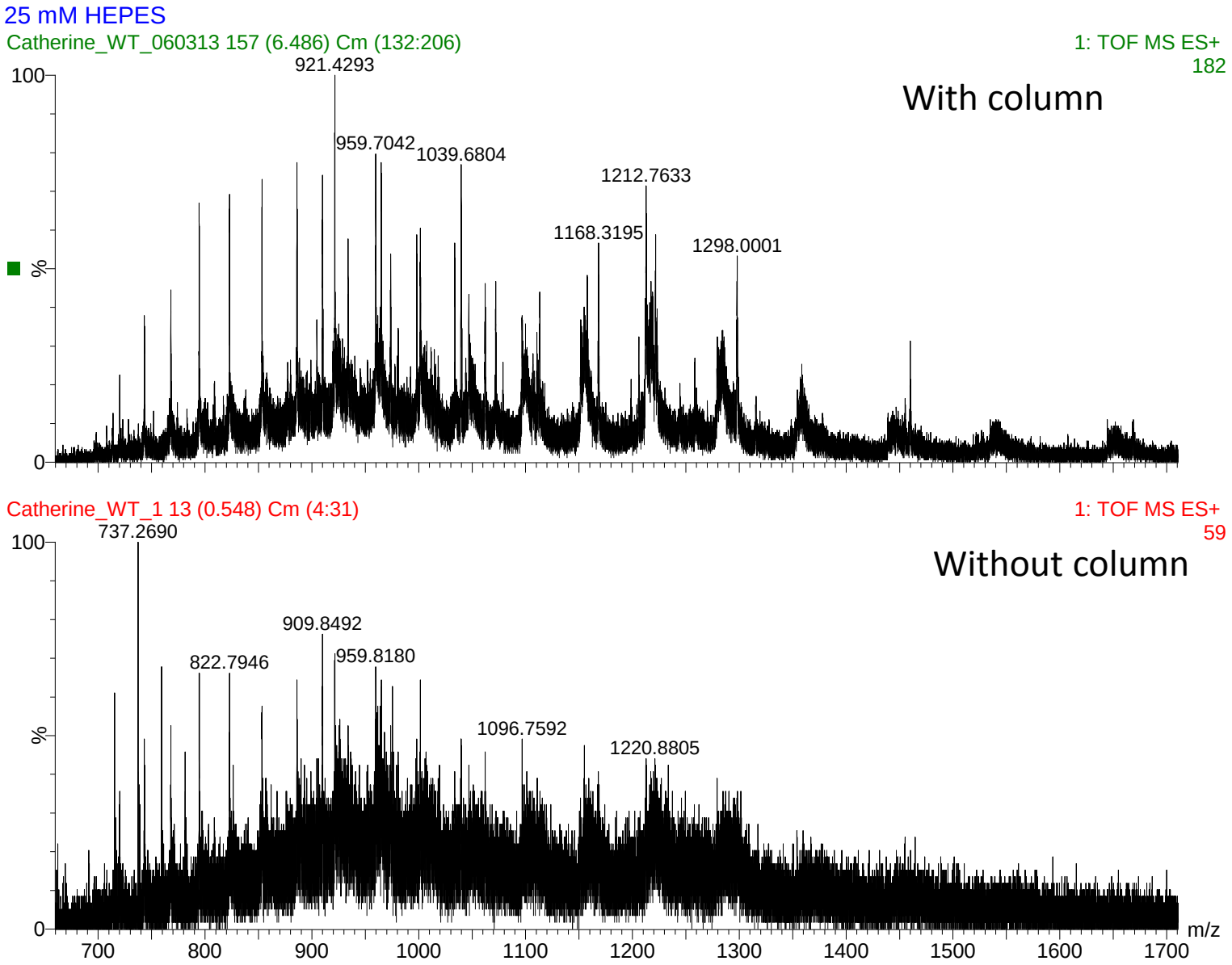
Positive Electrospray Ions are produced by the addition to a molecule of a positively ion (e.g H+, NH4+, Na+). These positively charged ions that are added are often referred to as 'adducts'.

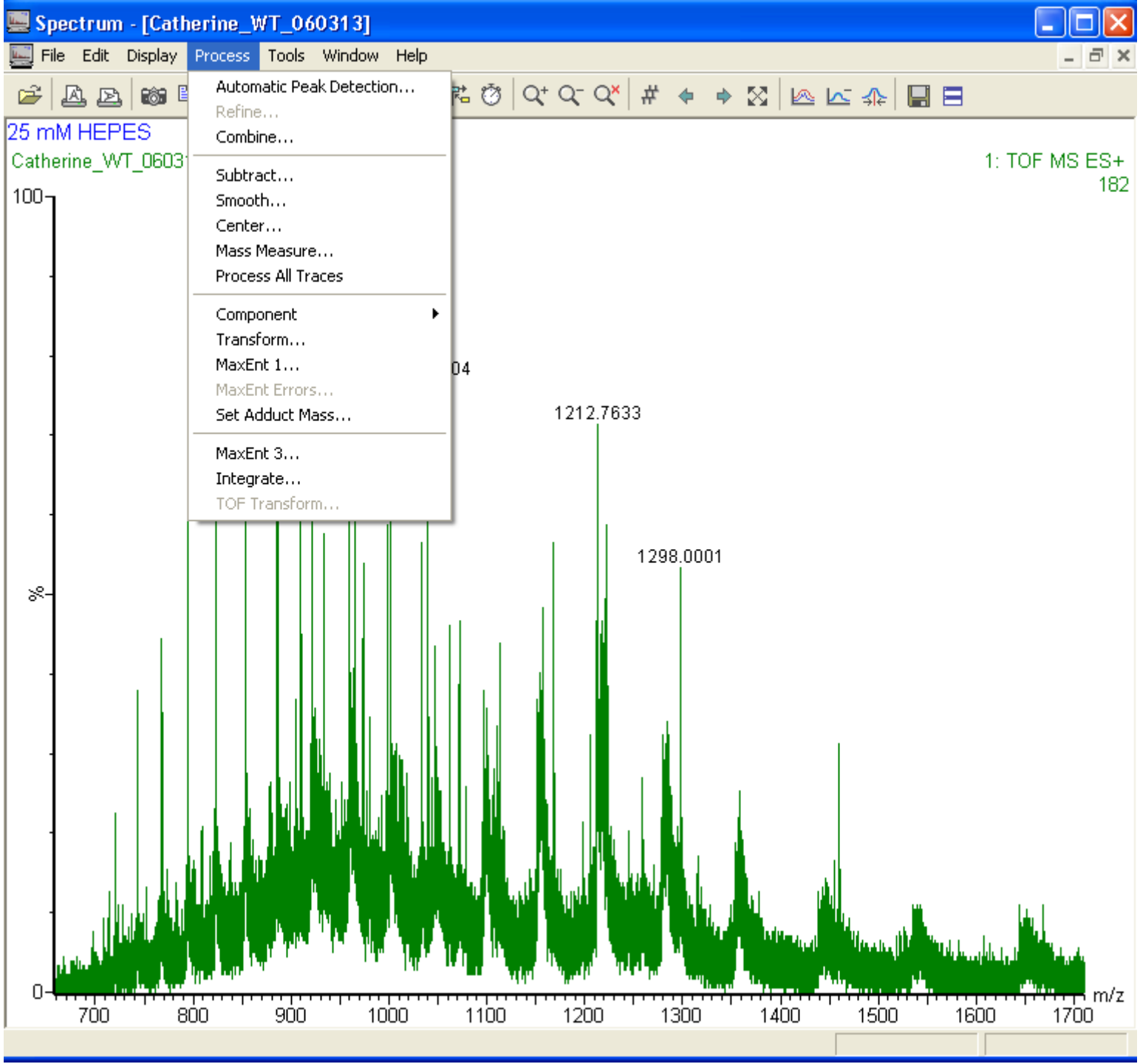


Negative Electrospray Ions are most often produced by the removal of a proton (hydrogen ion) from a molecule.

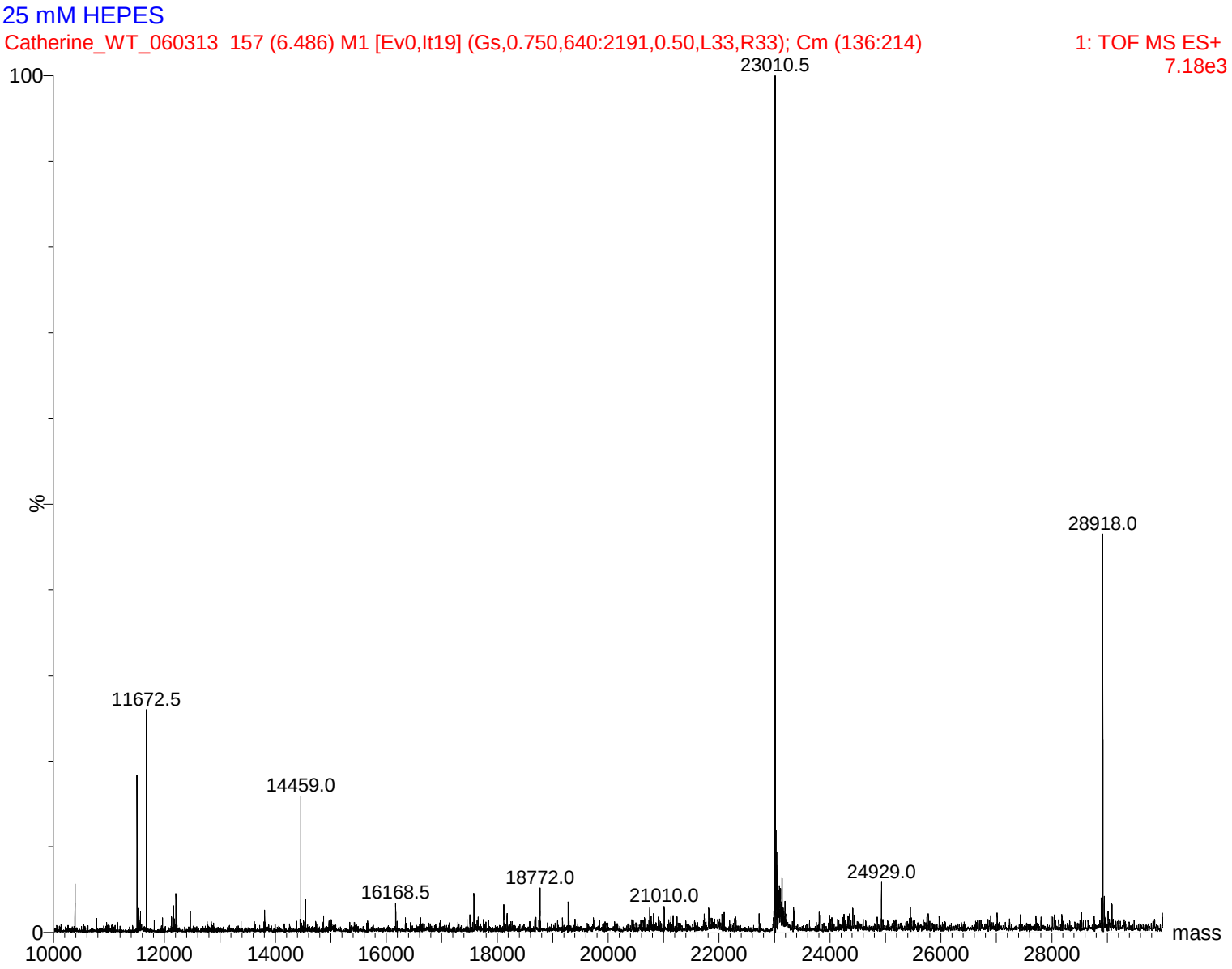


Protein, loop injection vs. C18 column

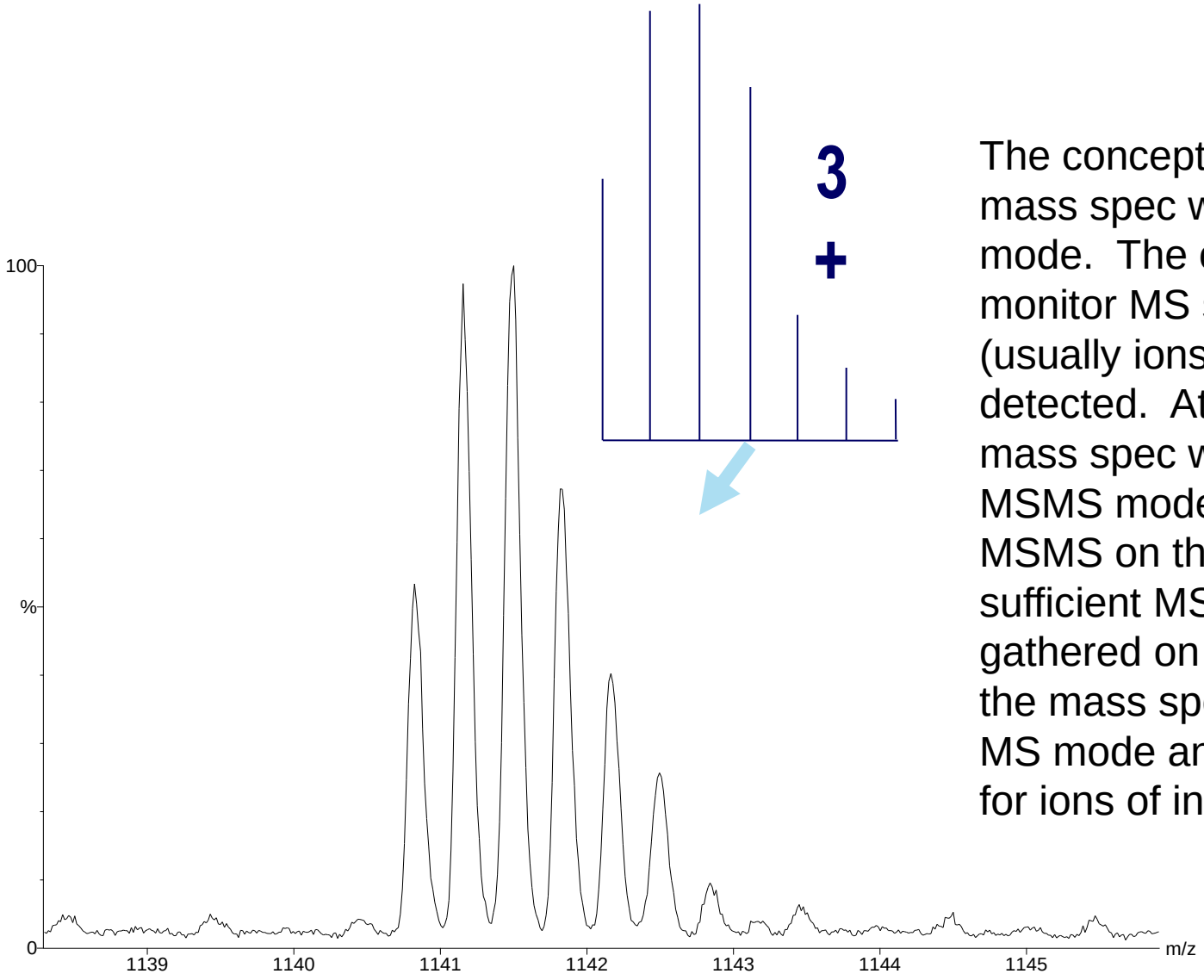



Spectrum - [Catherine_WT_060313]
File
Edit
Display
Process
Tools
Window
Help
Automatic Peak Detection...
Refine...
Combine...
Subtract...
Smooth...
Center...
Mass Measure...
Process All Traces
Component
Transform...
MaxEnt 1...
MaxEnt Errors...
Set Adduct Mass...
MaxEnt 3...
Integrate...
TOF Transform...
25 mM HEPES
Catherine_WT_0603
1: TOF MS ES+
182
1212.7633
1298.0001
100
%
0
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
m/z

Deconvolution by MaxEnt 1

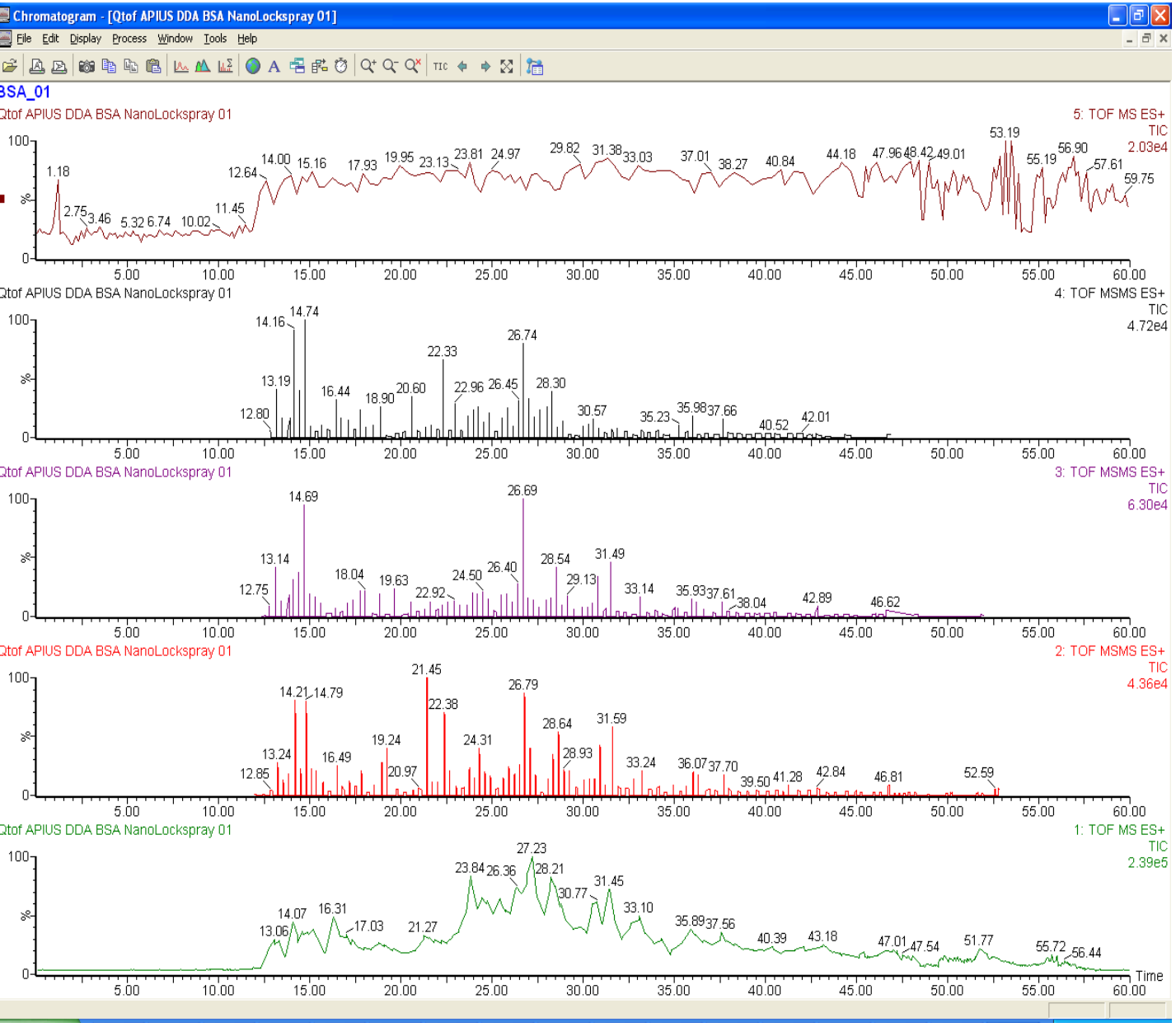


Peak Detection, DDA method



The concept of DDA is that the mass spec will run in MS mode. The computer will monitor MS scans until an ion (usually ions) of interest is detected. At that point the mass spec will switch into MSMS mode and perform MSMS on the ion/s. When sufficient MSMS data is gathered on the selected ions the mass spec will go back to MS mode and resume looking for ions of interest.

A typical DDA result is composed of 5 functions (there can be more)



Optional
Function 5
Lockmass function

Function 4
3rd MSMS function

Function 3
2nd MSMS function

Function 2
1st MSMS function

Function 1
MS function

6. Data Analysis

I. Check lockmass to examine the mass accuracy,
Look for ion of interest,

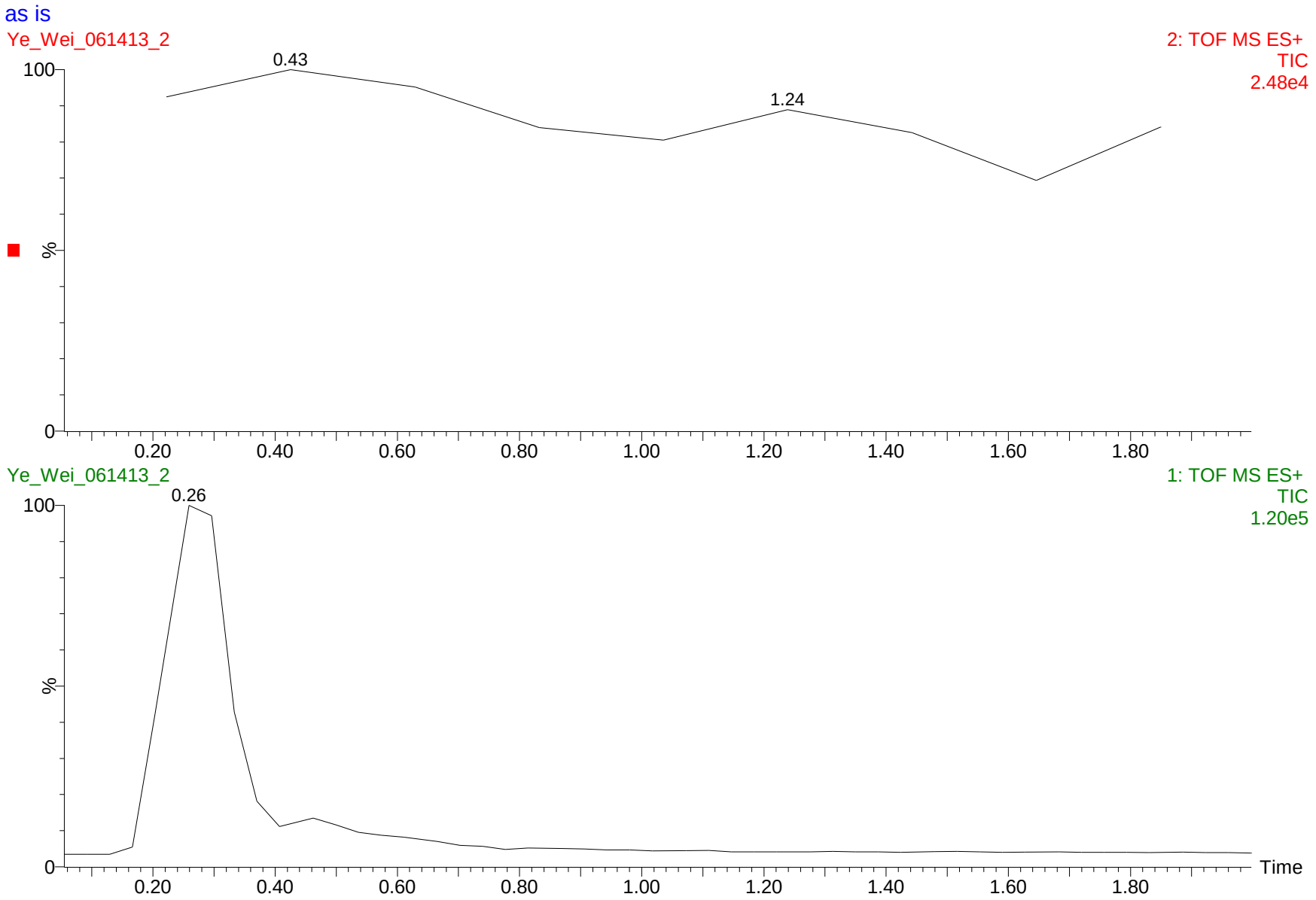
II. For continuum spectrum: do smooth, centering (and subtraction) to get the accurate mass,

III. Pay attention to mass defect of other ions,

IV. Deconvolution:
MaxEnt I (for protein molecular weight)
MaxEnt III (for peptide mapping),

V. Do composition analysis and isotope modeling

Loop Injection method

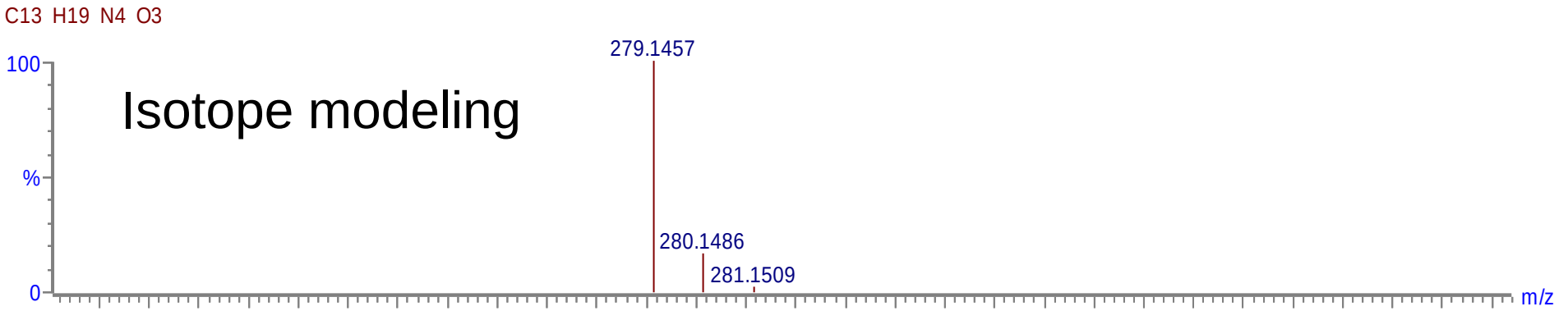


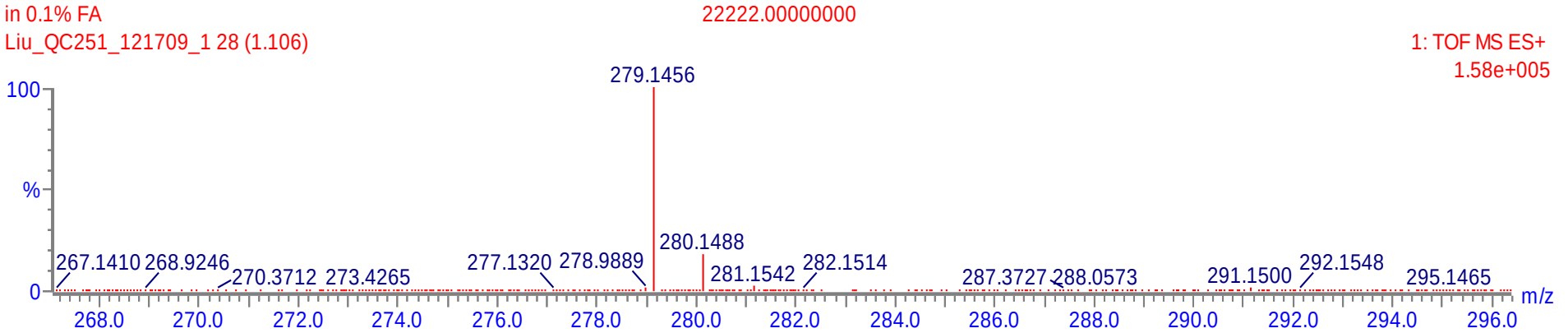
Elemental Composition Report

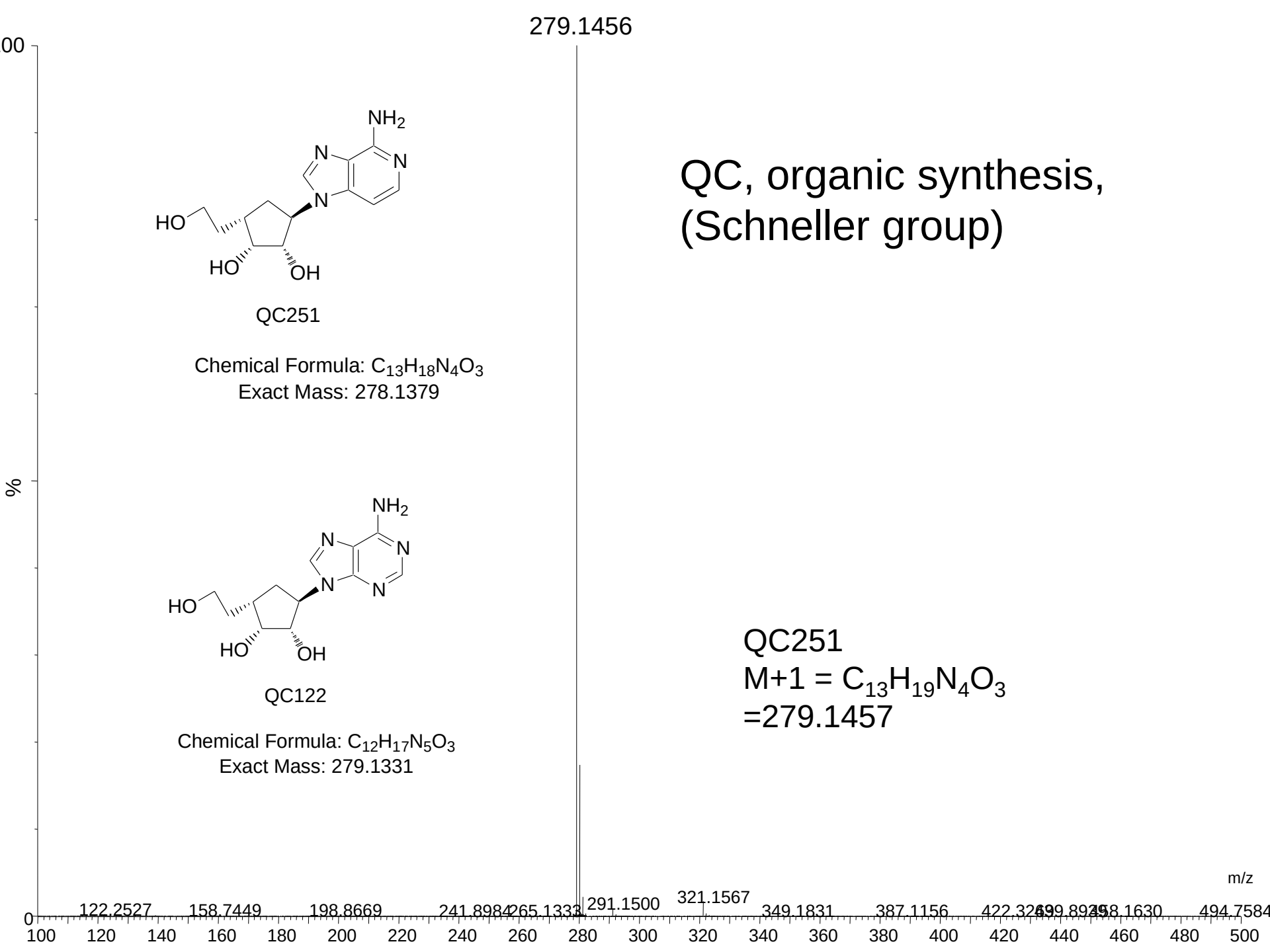
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
302 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-50 H: 0-100 N: 2-10 O: 0-20

Minimum:				-1.5				
Maximum:		20.0	5.0	50.0				
Mass	**Calc. Mass**		mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
279.1456	**279.1457**		-0.1	-0.4	6.5	396.5	0.0	C13 H19 N4 O3







QC251

Chemical Formula: $C_{13}H_{18}N_4O_3$
Exact Mass: 278.1379

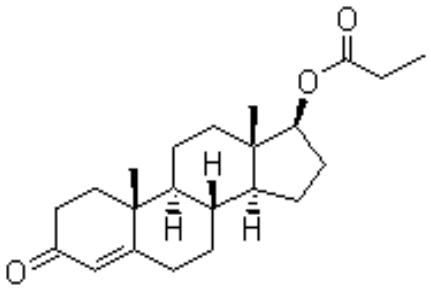
QC122

Chemical Formula: $C_{12}H_{17}N_5O_3$
Exact Mass: 279.1331

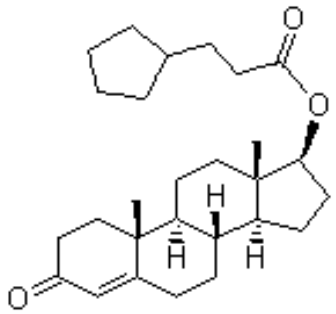
QC, organic synthesis, (Schneller group)

QC251
M+1 = $C_{13}H_{19}N_4O_3$
=279.1457

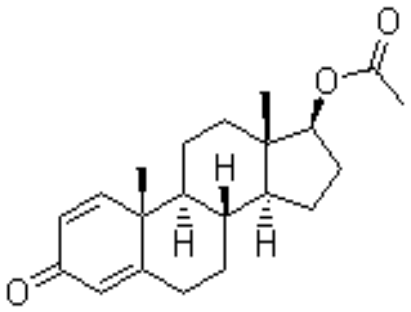
Steroids by ESI^+



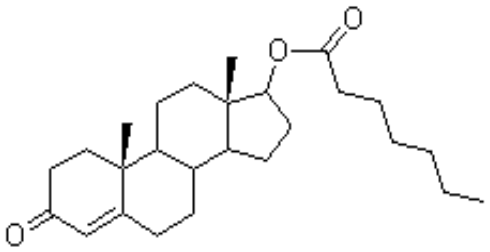
Testosterone propionate



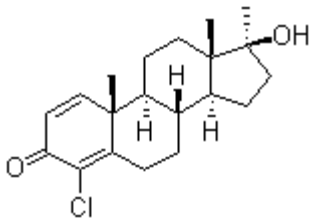
Testosterone cypionate



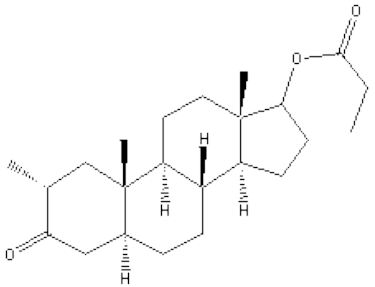
Boldenone 17-acetate



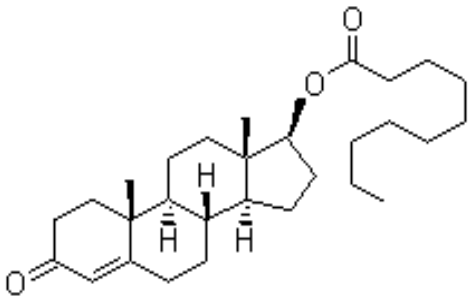
Testosterone enanthate



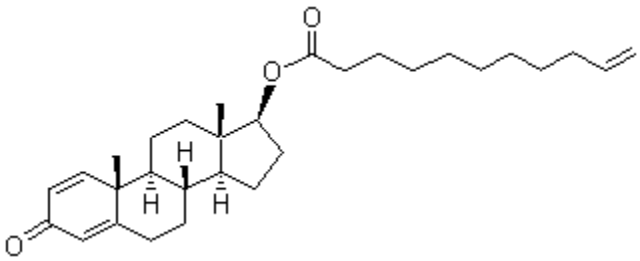
4-Chlorodehydromethyltestosterone



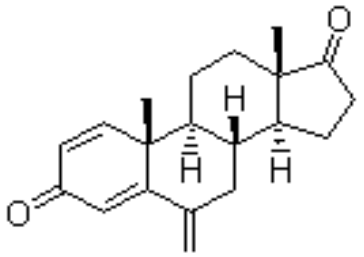
Dromostanolone propionate



Testosterone decanoate

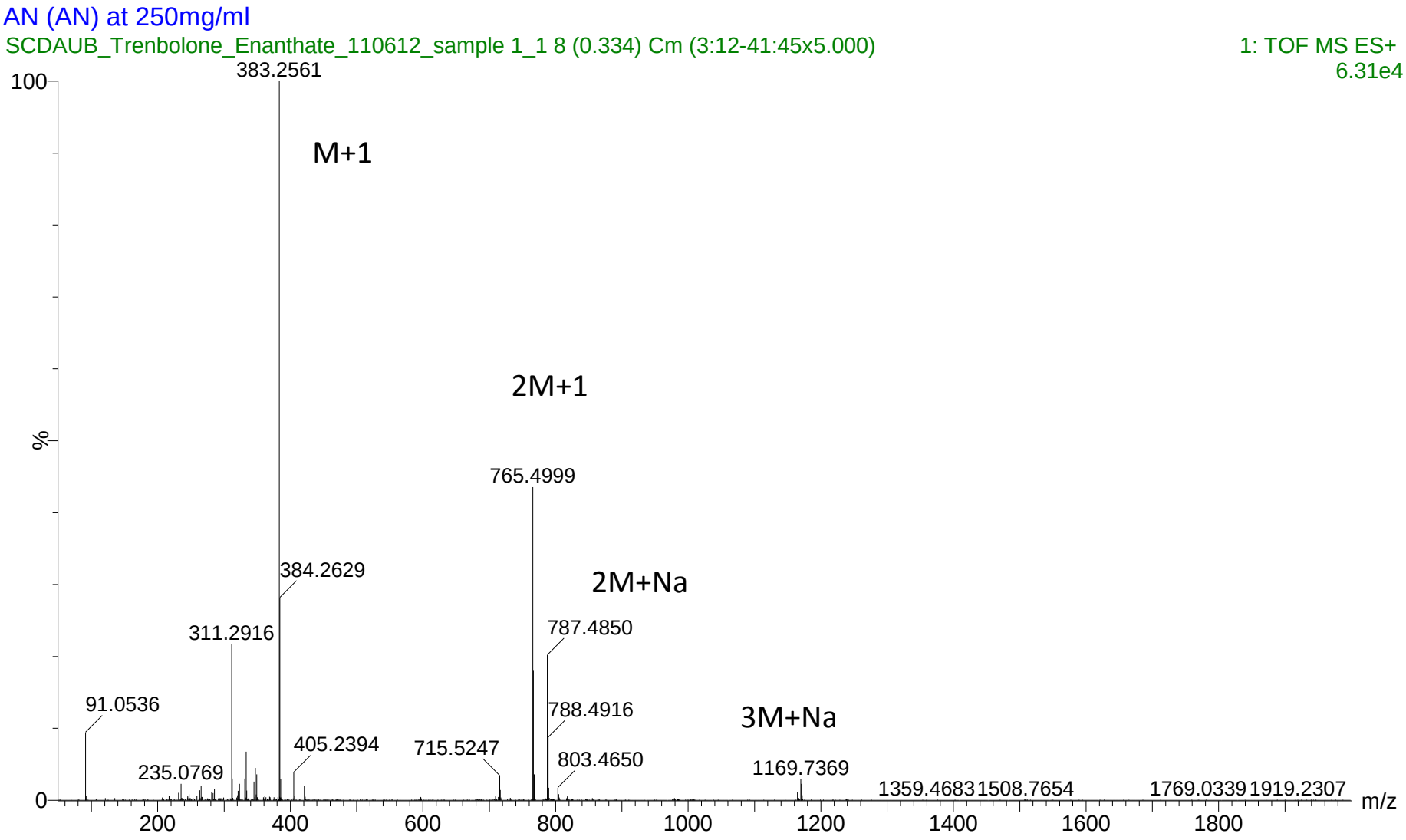


Boldenone undecylenate

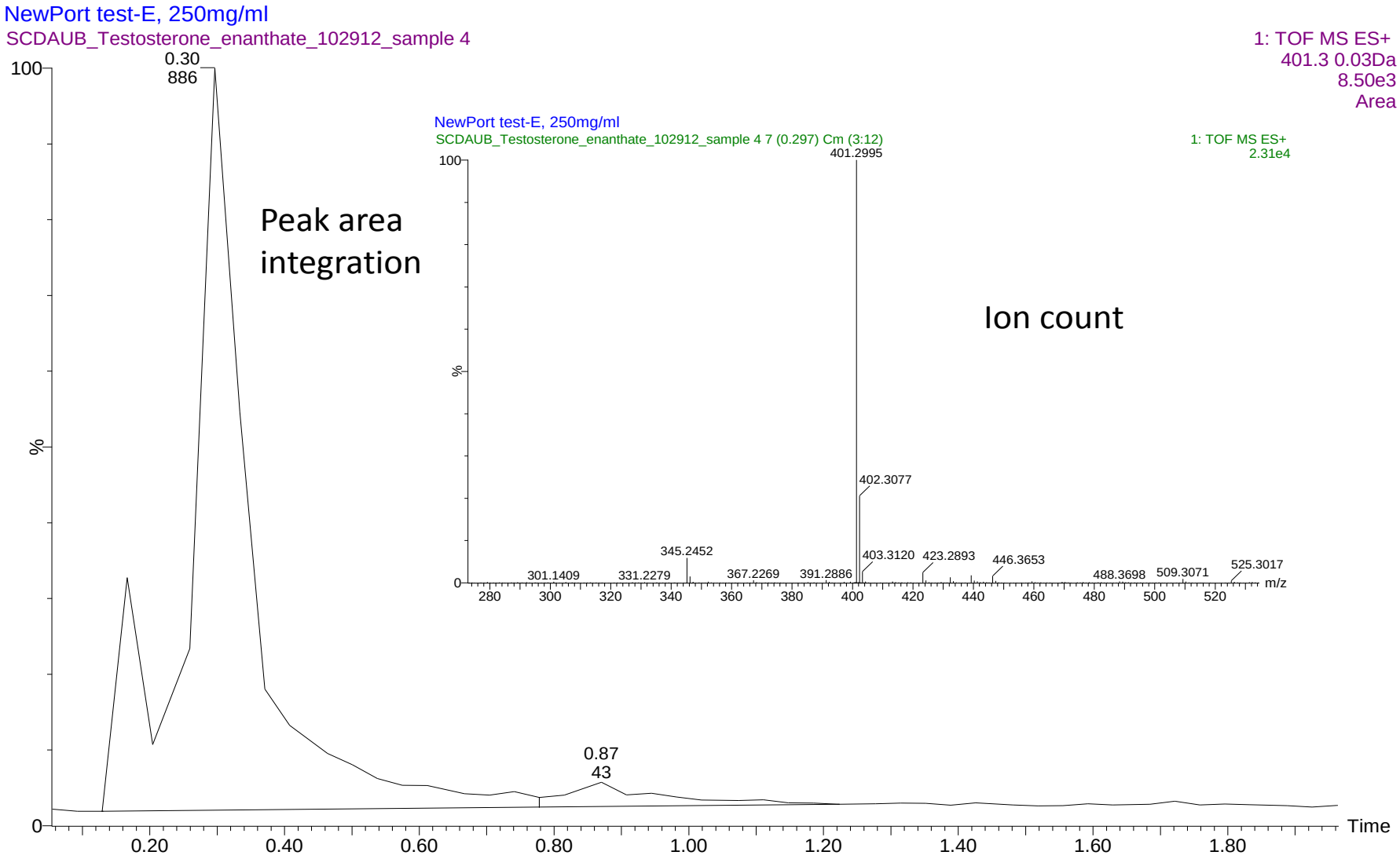


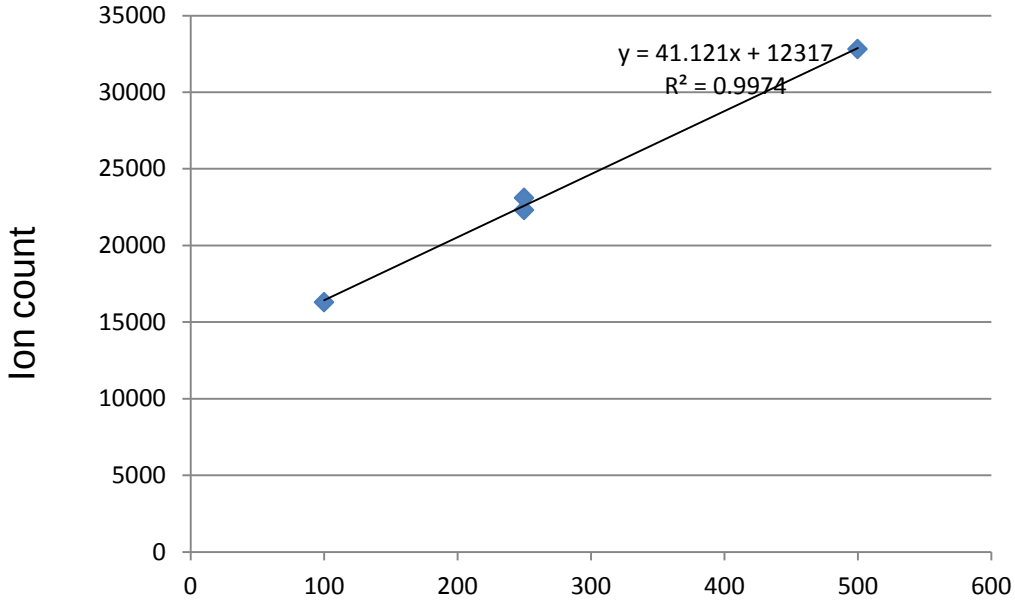
Exemestane

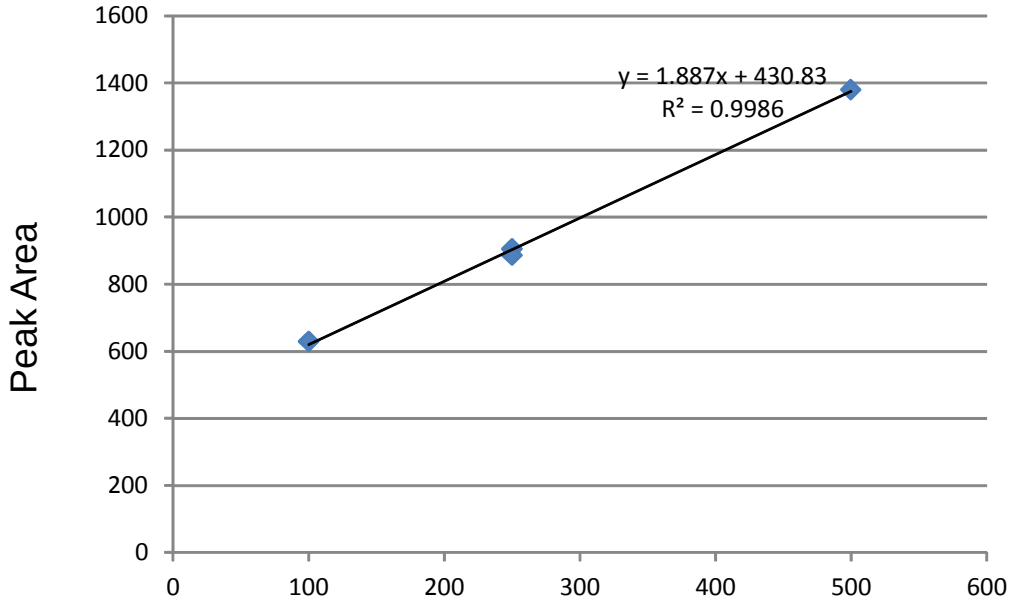
Spectrum

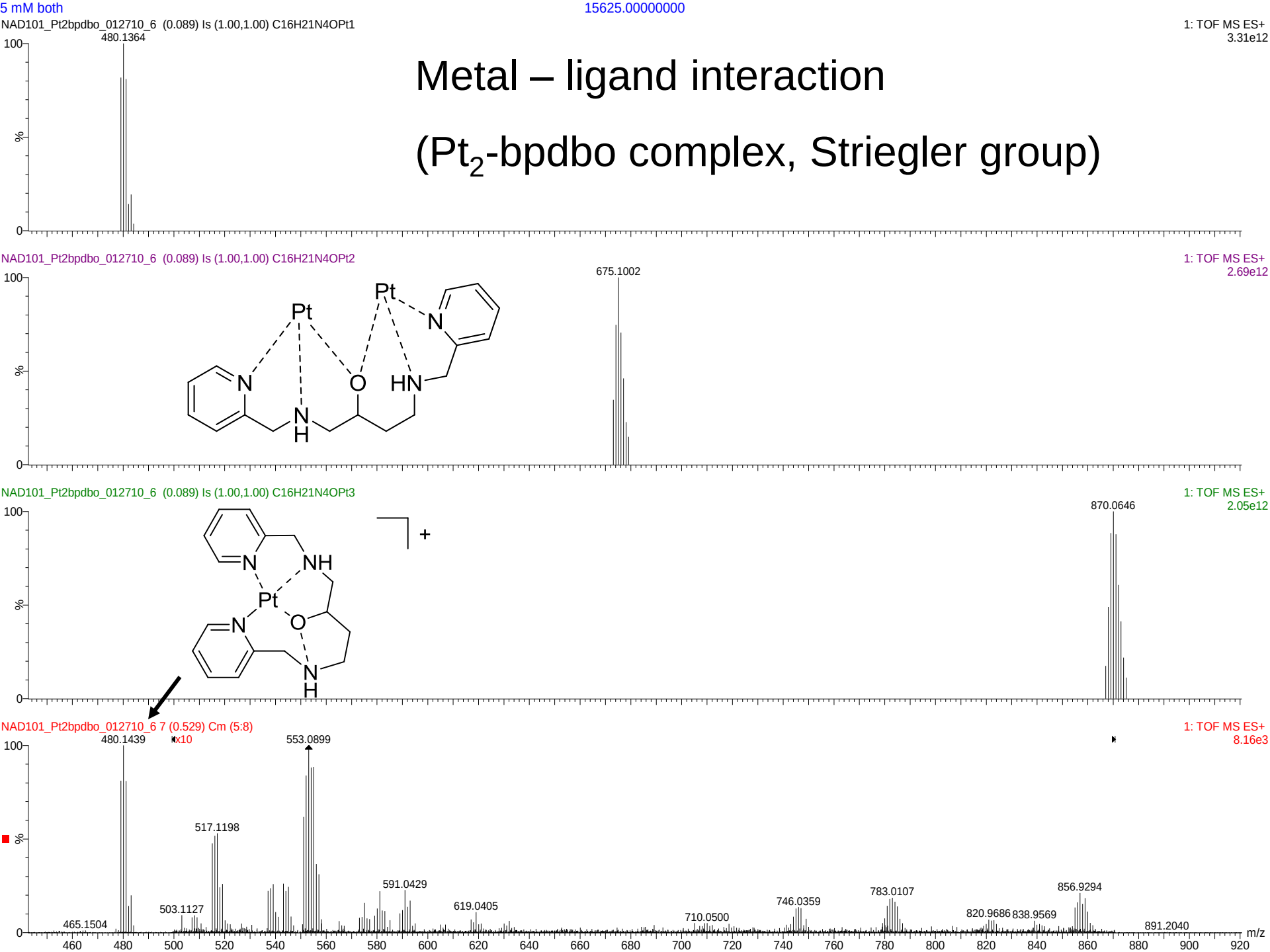

AN (AN) at 250mg/ml
SCDAUB_Trenbolone_Enanthate_110612_sample 1_1 8 (0.334) Cm (3:12-41:45x5.000)
1: TOF MS ES+
6.31e4
100
%
0
383.2561
M+1
2M+1
765.4999
384.2629
2M+Na
311.2916
787.4850
91.0536
788.4916
3M+Na
405.2394
715.5247
803.4650
235.0769
1169.7369
1359.4683
1508.7654
1769.0339
1919.2307
m/z
200
400
600
800
1000
1200
1400
1600
1800

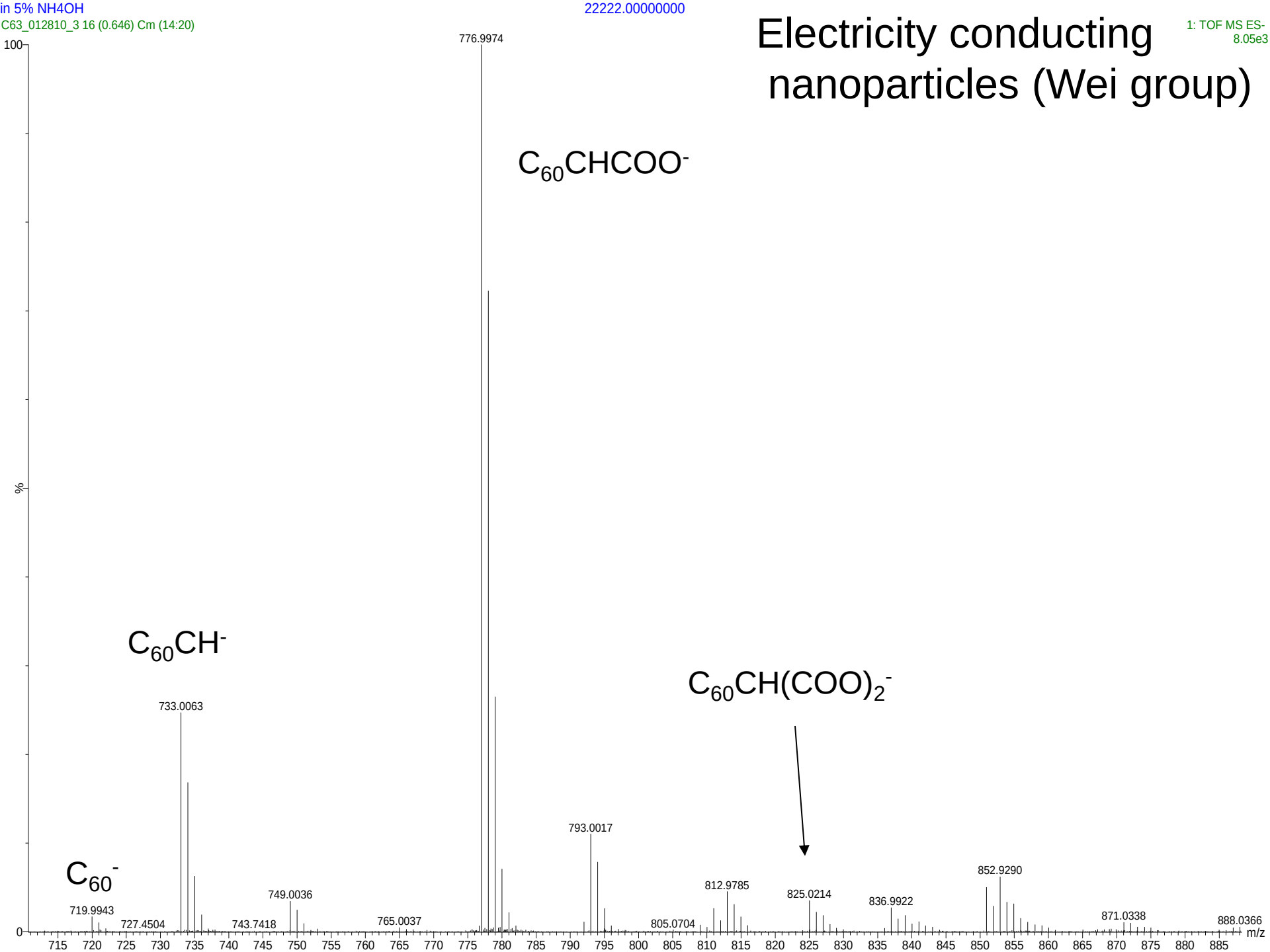
Purity assessment without column chromatography



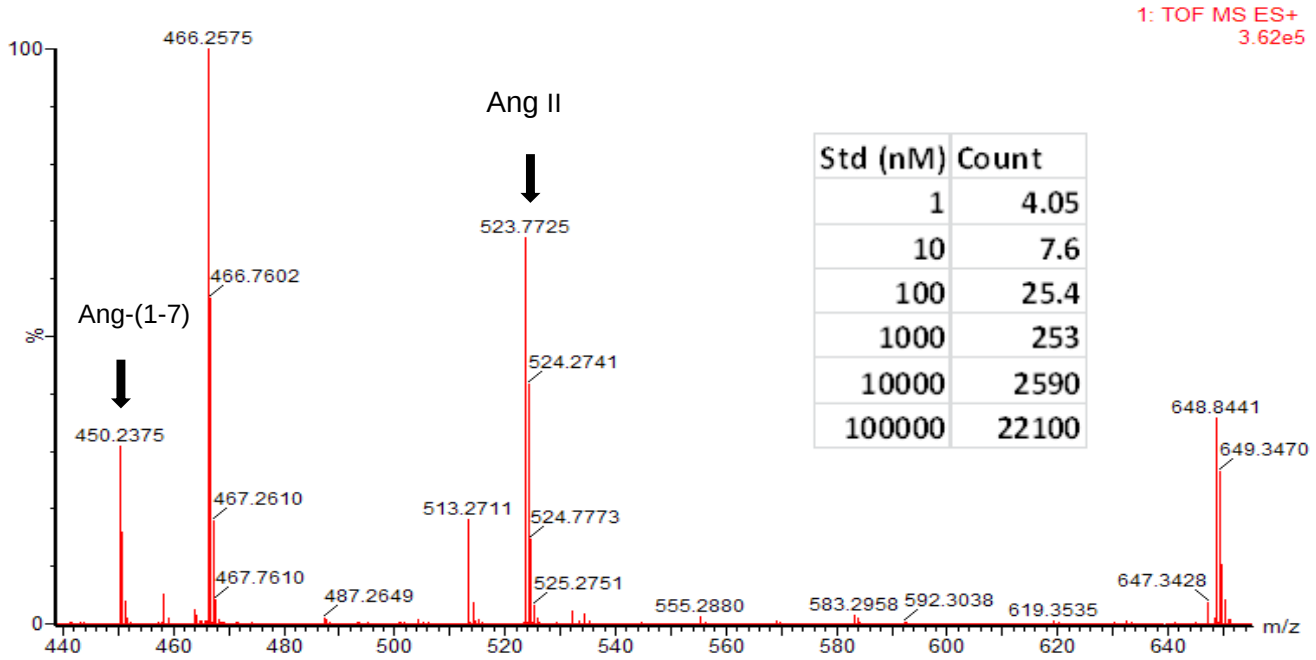

Ion count
35000
30000
25000
20000
15000
10000
5000
0
0
100
200
300
400
500
600
y = 41.121x + 12317
R² = 0.9974


Peak Area
1600
1400
1200
1000
800
600
400
200
0
0
100
200
300
400
500
600
y = 1.887x + 430.83
R² = 0.9986
Testosterone Enanthate (mg/ml)

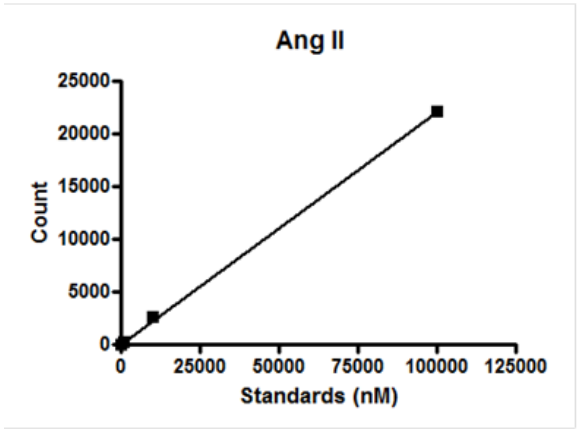

5 mM both
15625.00000000
NAD101_Pt2bpdbo_012710_6 (0.089) Is (1.00,1.00) C16H21N4OPt1
1: TOF MS ES+
3.31e12
480.1364
100
%
0
Metal – ligand interaction
(Pt2-bpdbo complex, Striegler group)
NAD101_Pt2bpdbo_012710_6 (0.089) Is (1.00,1.00) C16H21N4OPt2
1: TOF MS ES+
2.69e12
675.1002
Pt
Pt
N
N
O
HN
N
H
NAD101_Pt2bpdbo_012710_6 (0.089) Is (1.00,1.00) C16H21N4OPt3
1: TOF MS ES+
2.05e12
870.0646
N
NH
Pt
N
O
N
H
+
NAD101_Pt2bpdbo_012710_6 7 (0.529) Cm (5:8)
1: TOF MS ES+
8.16e3
480.1439
x10
553.0899
517.1198
591.0429
503.1127
619.0405
465.1504
710.0500
746.0359
783.0107
856.9294
820.9686
838.9569
891.2040
460
480
500
520
540
560
580
600
620
640
660
680
700
720
740
760
780
800
820
840
860
880
900
920
m/z

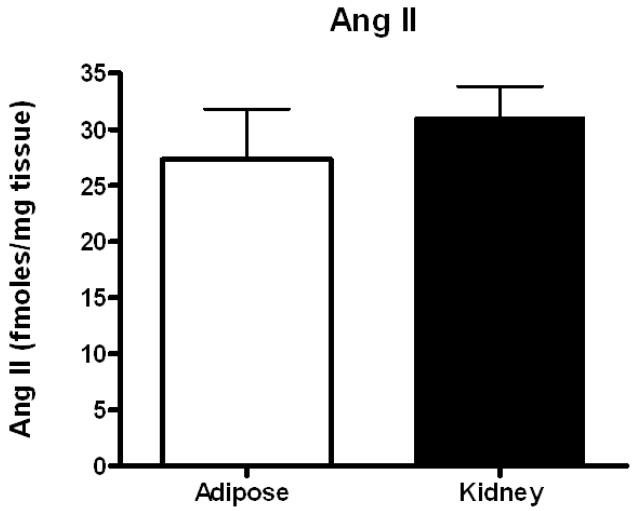

in 5% NH4OH
C63_012810_3 16 (0.646) Cm (14:20)
22222.00000000
1: TOF MS ES-
8.05e3
Electricity conducting nanoparticles (Wei group)
100
%
0
776.9974
C60CHCOO-
C60CH-
733.0063
C60CH(COO)2-
C60-
719.9943
727.4504
743.7418
749.0036
765.0037
793.0017
805.0704
812.9785
825.0214
836.9922
852.9290
871.0338
888.0366
715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885
m/z

Angiotensin (Hussain group)



Std (nM)	Count
1	4.05
10	7.6
100	25.4
1000	253
10000	2590
100000	22100

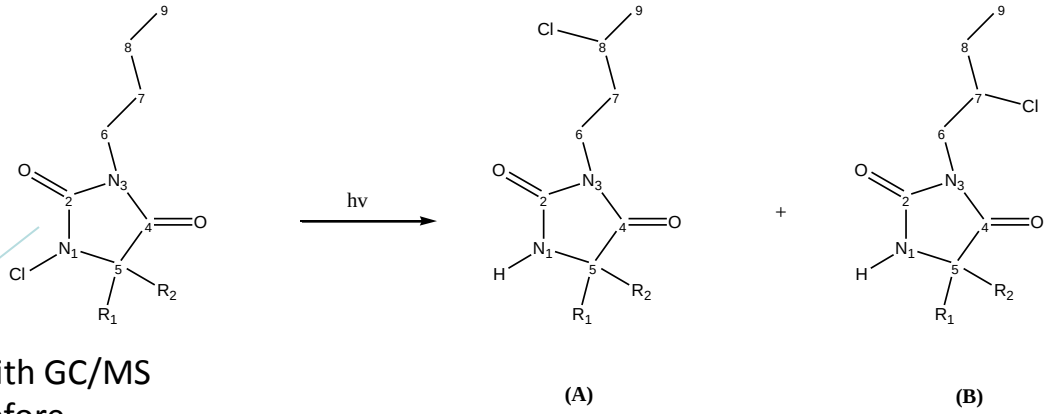




BP-gulf oil spill, fish and sediment PAH analysis by GC/MS

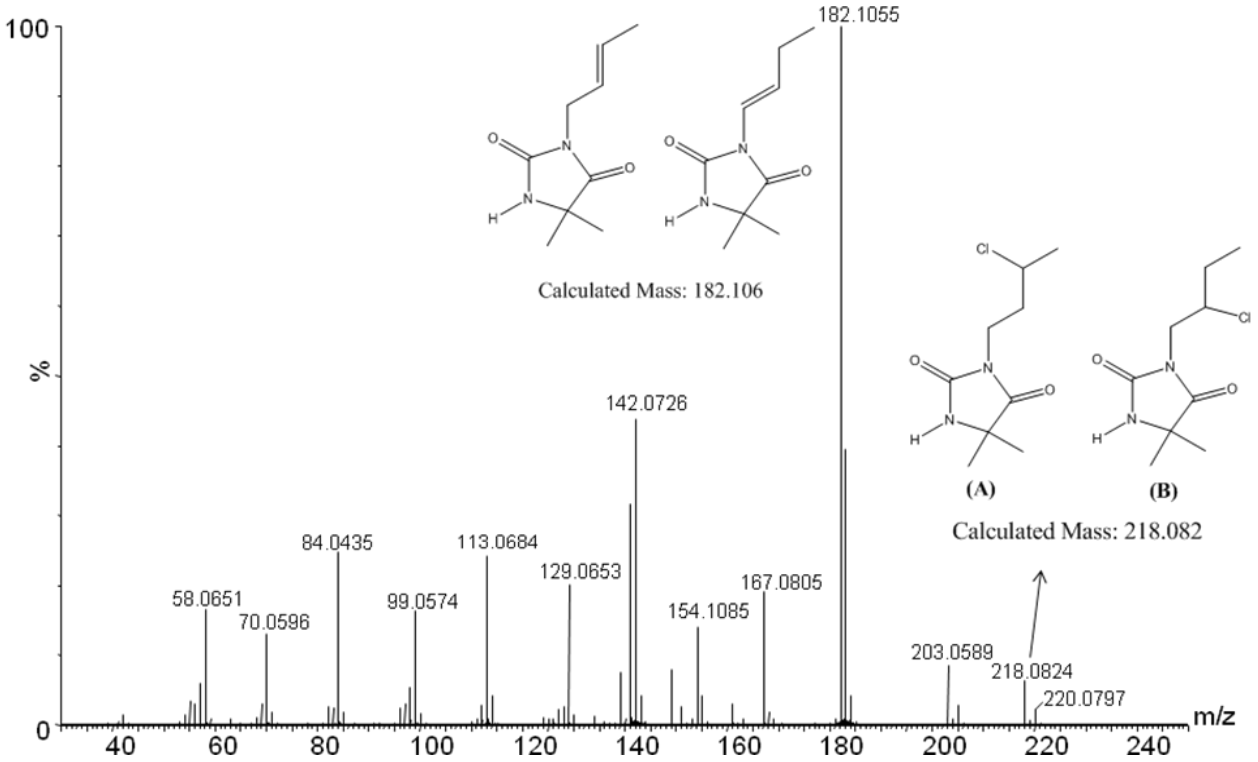
BP_fish_5878_4-19-2012_121312_1							
Alpha#	**PAH name**	**RT**	**Parent ion**	**Formula**	**Area**	**ng**	**ppb**
		(min)	mass		(peak)		
		ZB-5MS					
14	Naphthalene	8.13	128.0626	C10H8	470.4	3	1.24
2	Acenaphthylene	9.86	152.0626	C12H8	10.4	0	0.05
1	Acenaphthene	10.86	154.0783	C12H10	4.1	0	0.03
12	Fluorene	12.06	166.0783	C13H10	17.1	0	0.12
15	Phenanthrene	14.93	178.0783	C14H10	86.4	1	0.31
3	Anthracene	15.09	178.0783	C14H10	8.5	0	0.04
11	Fluoranthene	19.93	202.0783	C16H10	22.5	0	0.08
16	Pyrene	20.96	202.0783	C16H10	34.2	0	0.11
					total	5.2	2.0
					Ave	0.7	0.247

Mechanism Study by EI and ESI (Worley Group)

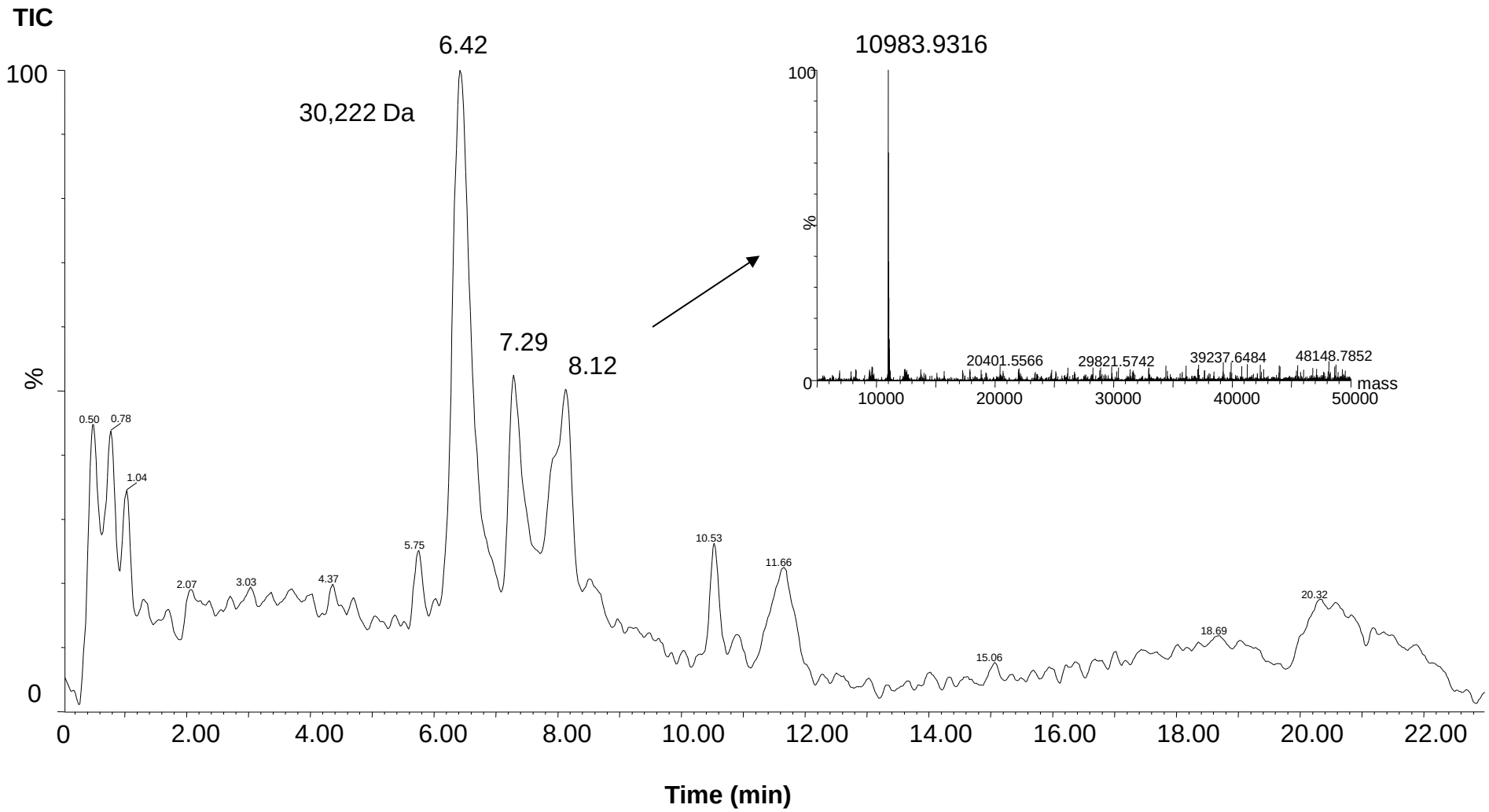


could not be detected with GC/MS since N-Cl bond broke before the volatilization temperature.

Proposed mechanism was proved with GC/MS.

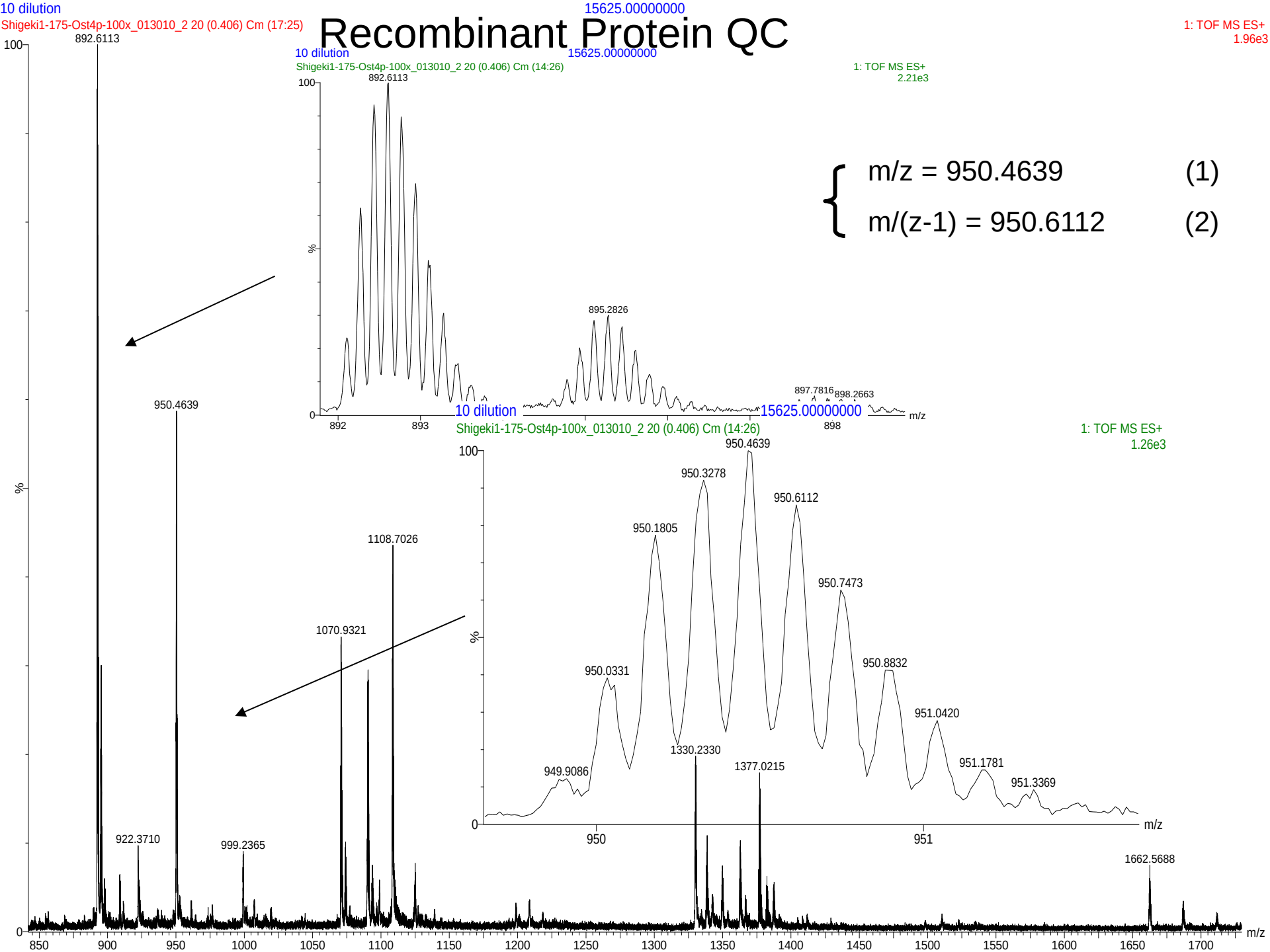


Protein analysis (Ellis group)



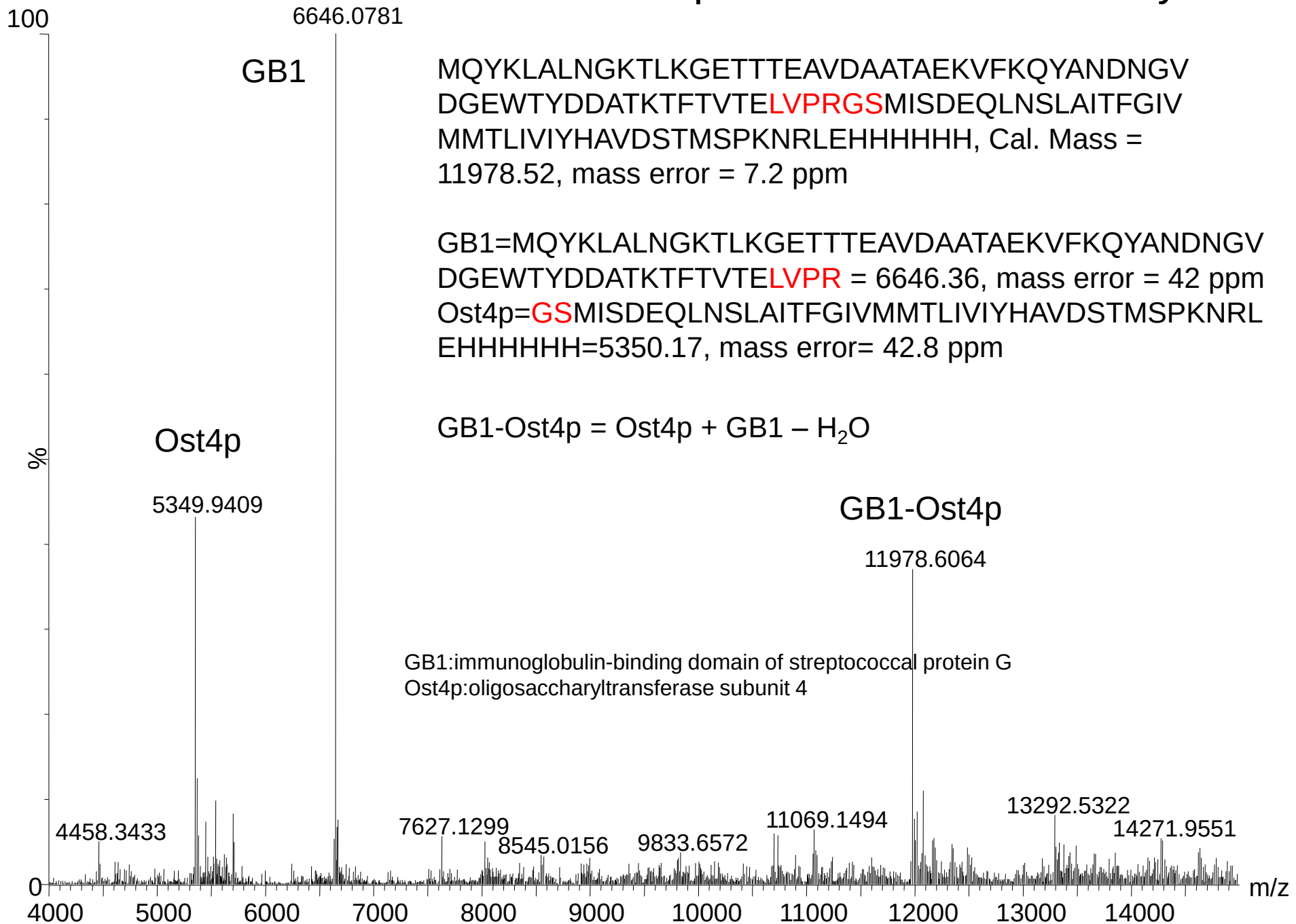
Deconvolution: iteration until converge, Calibration: to accurate mass

Recombinant Protein QC



$$\begin{cases} m/z = 950.4639 & (1) \\ m/(z-1) = 950.6112 & (2) \end{cases}$$

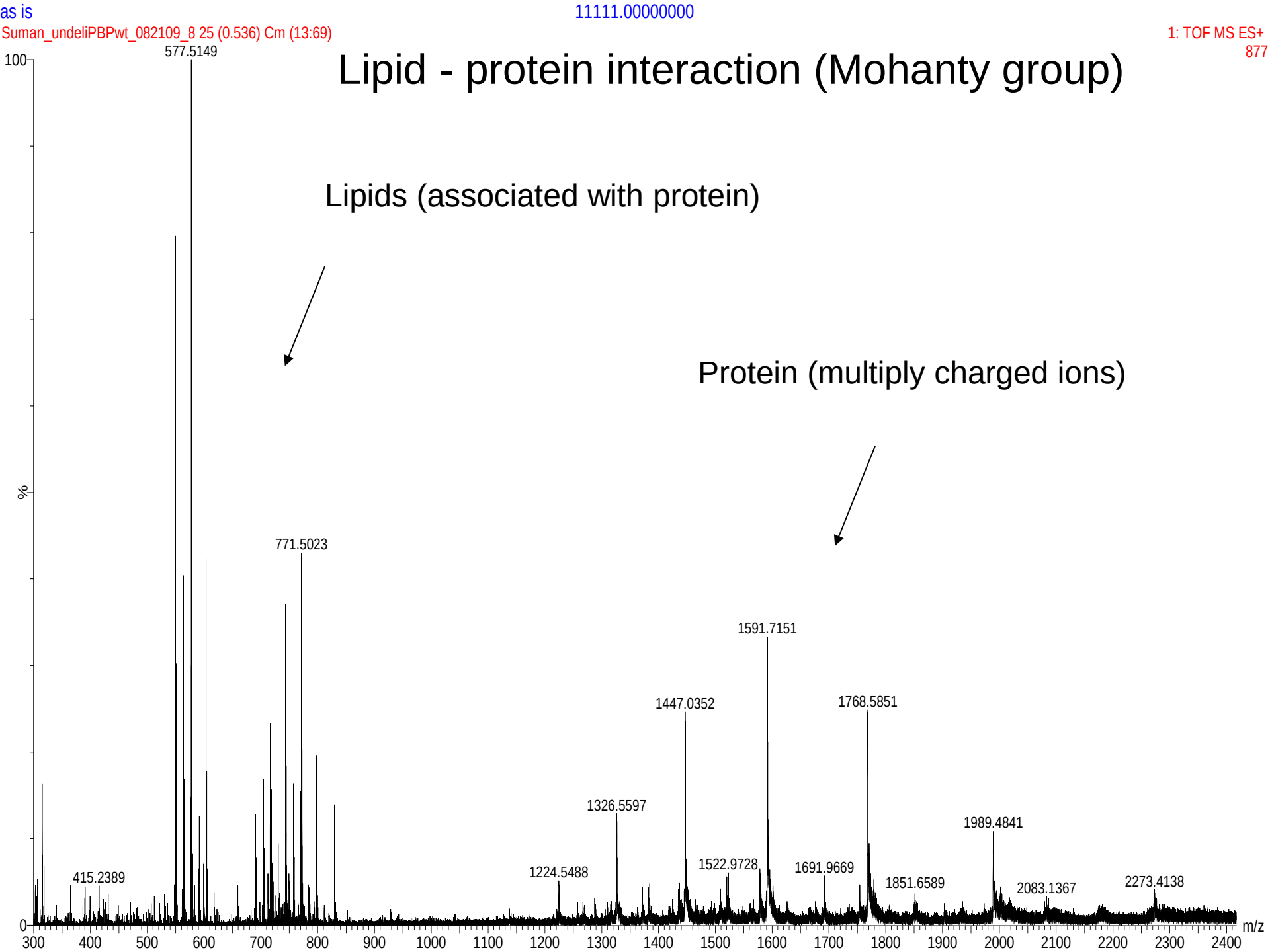
Deconvoluted protein accurate mass by ESI



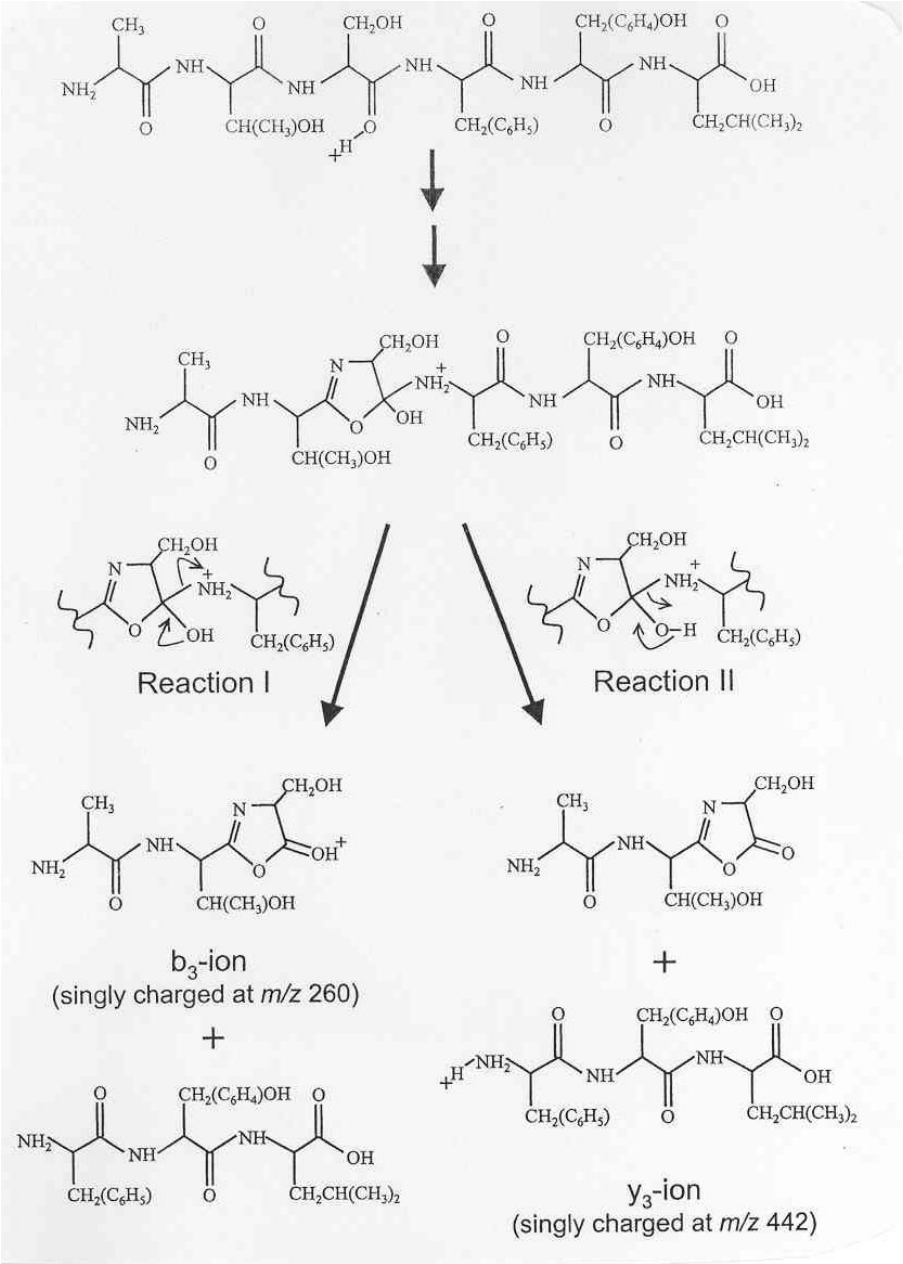
MQYKLALNGKTLKGETTTEAVDAATAEKVFKQYANDNGV
DGEWTYDDATKTFTVTELVPRGSMISDEQLNSLAITFGIV
MMTLIVIYHAVDSTMSPKNRLEHHHHHH, Cal. Mass =
11978.52, mass error = 7.2 ppm

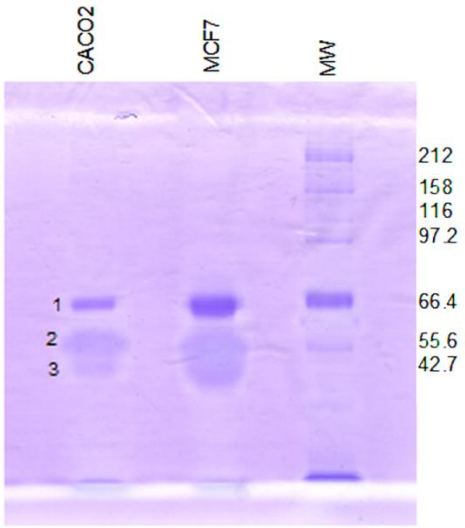
GB1=MQYKLALNGKTLKGETTTEAVDAATAEKVFKQYANDNGV
DGEWTYDDATKTFTVTELVPR = 6646.36, mass error = 42 ppm
Ost4p=GSMISDEQLNSLAITFGIVMMTLIVIYHAVDSTMSPKNRL
EHHHHHH=5350.17, mass error= 42.8 ppm

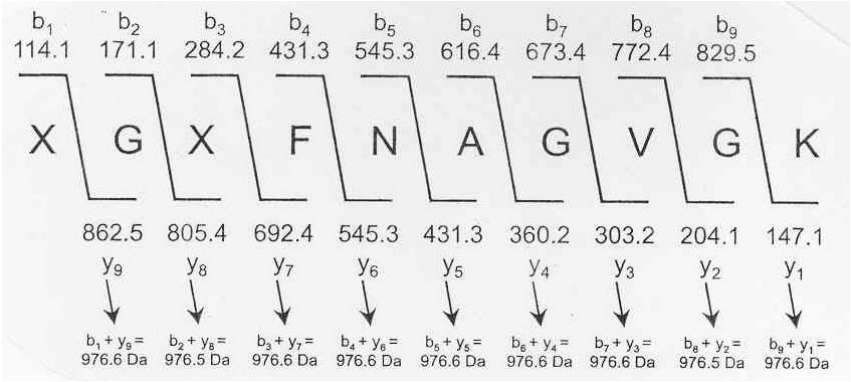
GB1-Ost4p = Ost4p + GB1 – H_2O


as is
11111.00000000
Suman_undeliPBPwt_082109_8 25 (0.536) Cm (13:69)
1: TOF MS ES+
877
Lipid - protein interaction (Mohanty group)
Lipids (associated with protein)
Protein (multiply charged ions)
577.5149
771.5023
1591.7151
1447.0352
1768.5851
1326.5597
1989.4841
415.2389
1224.5488
1522.9728
1691.9669
1851.6589
2083.1367
2273.4138
100
%
0
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
2100
2200
2300
2400
m/z

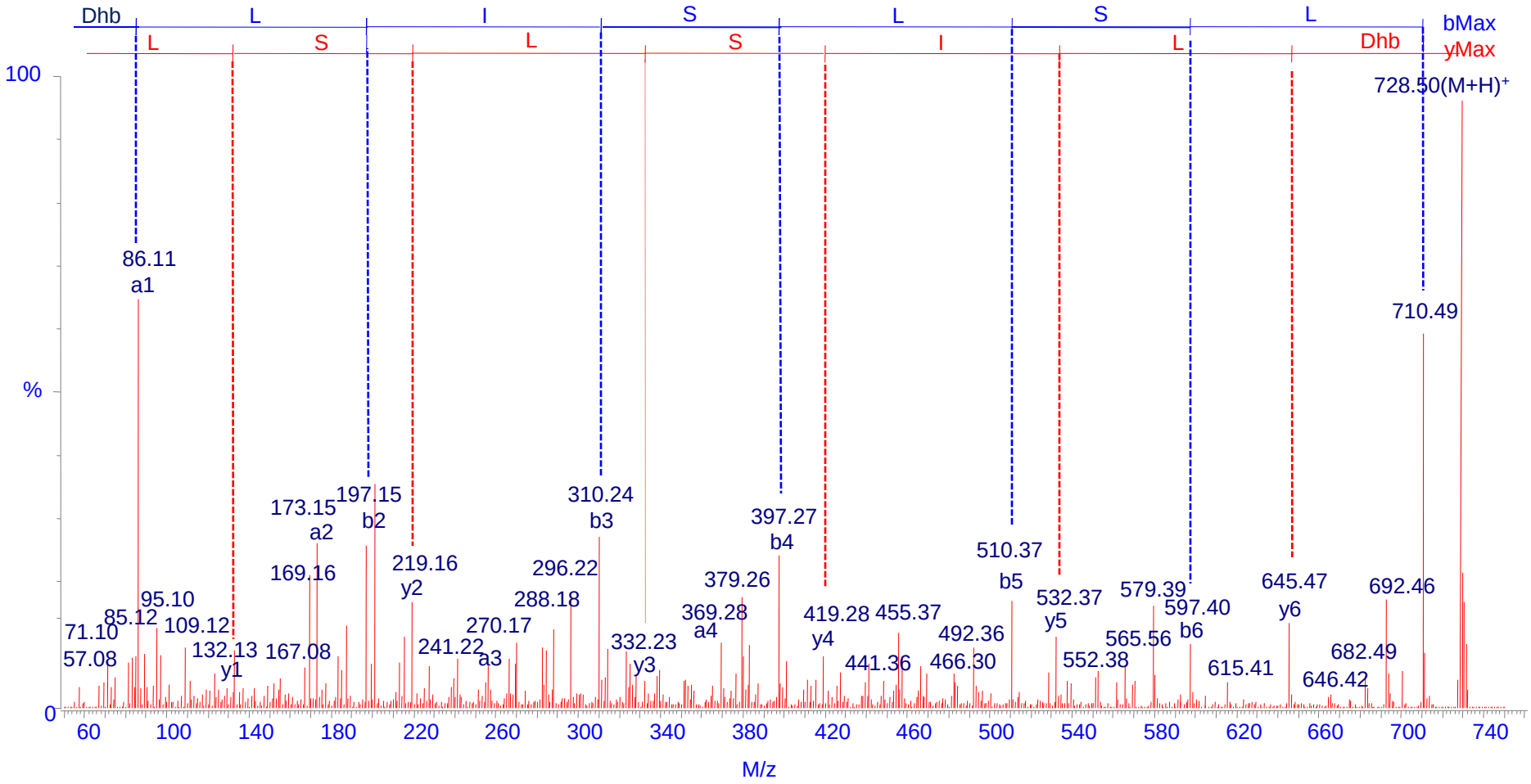
Proteomics, micro sequencing



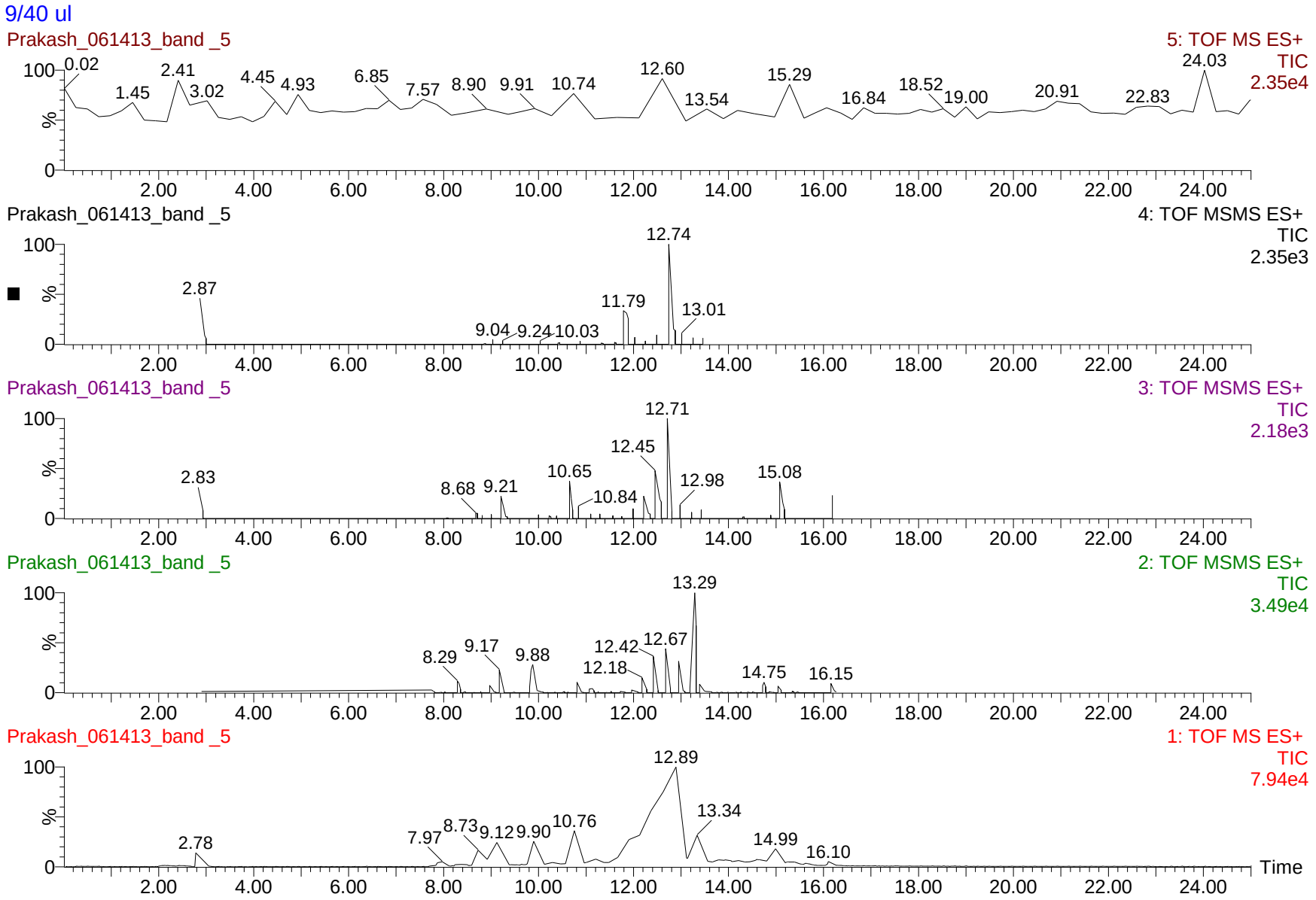


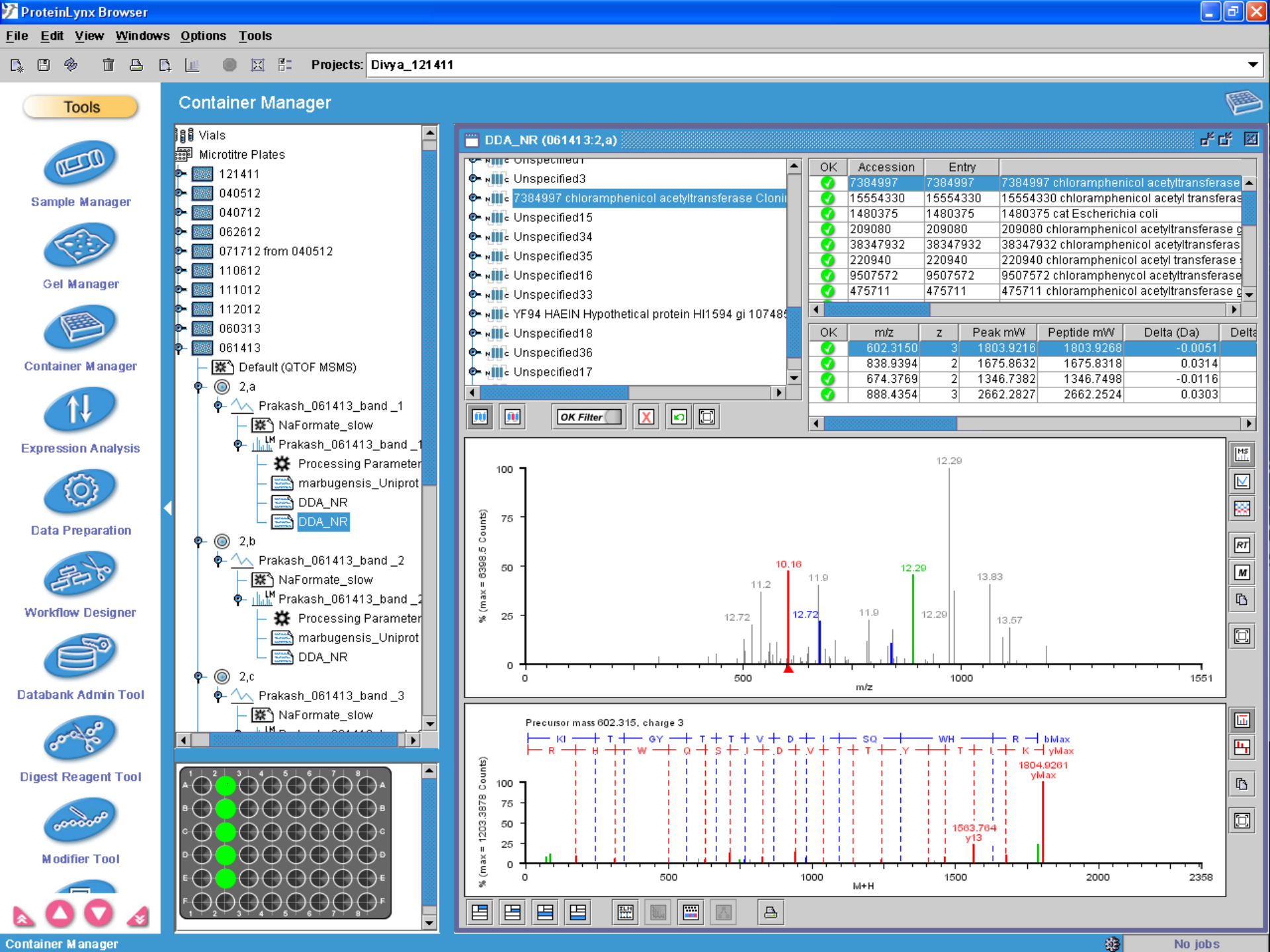


MS/MS spectrum of 728.50 in positive ESI.

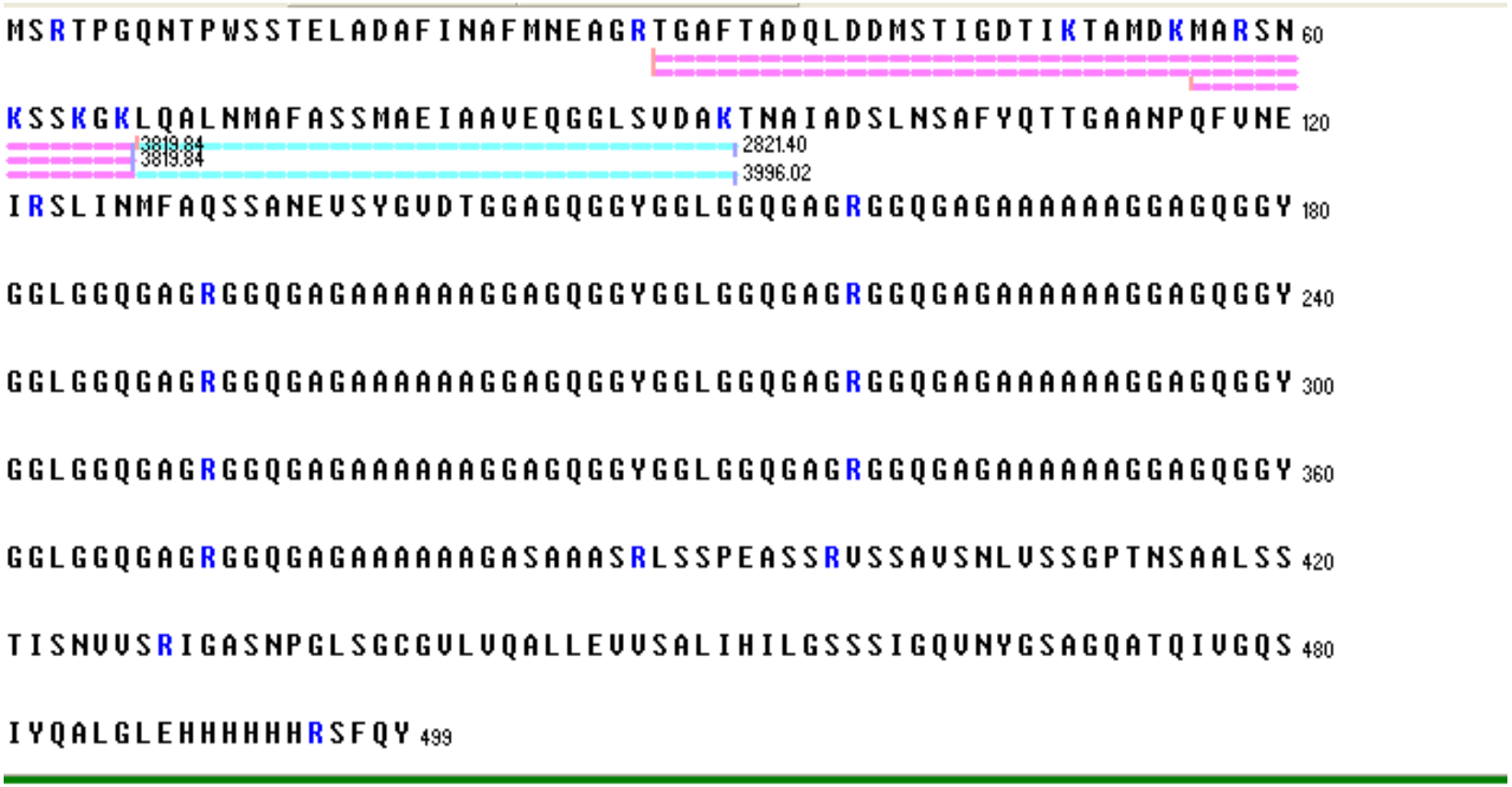


Slow flow rate, shallow gradient, and right CE




ProteinLynx Browser
File Edit View Windows Options Tools
Projects: Divya_121411
Tools
Sample Manager
Gel Manager
Container Manager
Expression Analysis
Data Preparation
Workflow Designer
Databank Admin Tool
Digest Reagent Tool
Modifier Tool
Container Manager
Vials
Microtitre Plates
121411
040512
040712
062612
071712 from 040512
110612
111012
112012
060313
061413
Default (QTOF MSMS)
2,a
Prakash_061413_band _1
NaFormate_slow
Processing Parameter
marbugensis_Uniprot
DDA_NR
DDA_NR
2,b
Prakash_061413_band _2
NaFormate_slow
Processing Parameter
marbugensis_Uniprot
DDA_NR
2,c
Prakash_061413_band _3
NaFormate_slow
DDA_NR (061413:2,a)
Unspecified3
7384997 chloramphenicol acetyltransferase Cloni
Unspecified15
Unspecified34
Unspecified35
Unspecified16
Unspecified33
YF94 HAEIN Hypothetical protein HI1594 gi 10748
Unspecified18
Unspecified36
Unspecified17
OK Filter
OK Accession Entry
7384997 7384997 7384997 chloramphenicol acetyltransferase
15554330 15554330 15554330 chloramphenicol acetyl transferas
1480375 1480375 1480375 cat Escherichia coli
209080 209080 209080 chloramphenicol acetyltransferase
38347932 38347932 38347932 chloramphenicol acetyltransferas
220940 220940 220940 chloramphenicol acetyl transferase
9507572 9507572 9507572 chloramphenycol acetyltransferase
475711 475711 475711 chloramphenicol acetyltransferase
OK m/z z Peak mW Peptide mW Delta (Da)
602.3150 3 1803.9216 1803.9268 -0.0051
838.9394 2 1675.8632 1675.8318 0.0314
674.3769 2 1346.7382 1346.7498 -0.0116
888.4354 3 2662.2827 2662.2524 0.0303
% (max = 6398.5 Counts)
m/z
Precursor mass 602.315, charge 3
% (max = 1203.3878 Counts)
M+H
1804.9261
yMax
1563.764
y13
Container Manager
No jobs

Peptide Mapping



Residue coverage: 12% [64 of 499]
Peptide hits: 3 Modified: 1 Not identified: 149

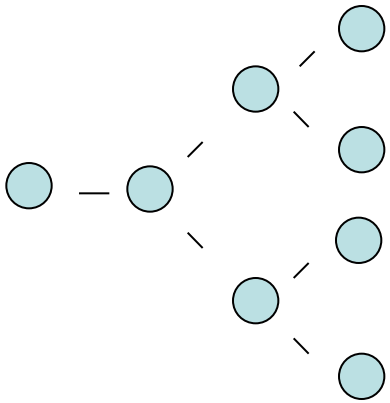
- **Peptides identified without modifications:**

input	found	dev.	mc	from-to	sequence
3820.167 /	3819.844	0.684	5	31- 66	TGAFTADQLDDM....KMARSNKSSKGK metOx present Missed cleavage
3820.864 /	3819.844	-0.012	5	31- 66	TGAFTADQLDDM....KMARSNKSSKGK metOx present Missed cleavage
3997.257 /	3996.023	-0.226	4	56- 94	MARSNKSSKGKL....AVEQGGLSVDAK metOx missing Missed cleavage

- **Peptides identified with modifications:**

3993.016 /	2821.399	-0.261	0	67- 94	mannose-8 LQALNMAFASSM....AVEQGGLSVDAK

Glycomics of silk protein (Marcot group)

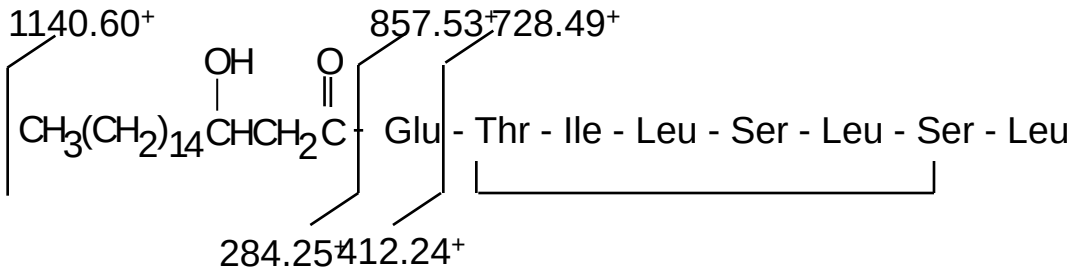


Glycan with 8 mannose, net formula to N- glycan
= $C_{48}O_{33}H_{66}$, = 1170.3486

Find attach to residue 67-94, LQALNMA…

Mannose-8

Also searched with Mannose-9, 1349.3964, did not find the modification



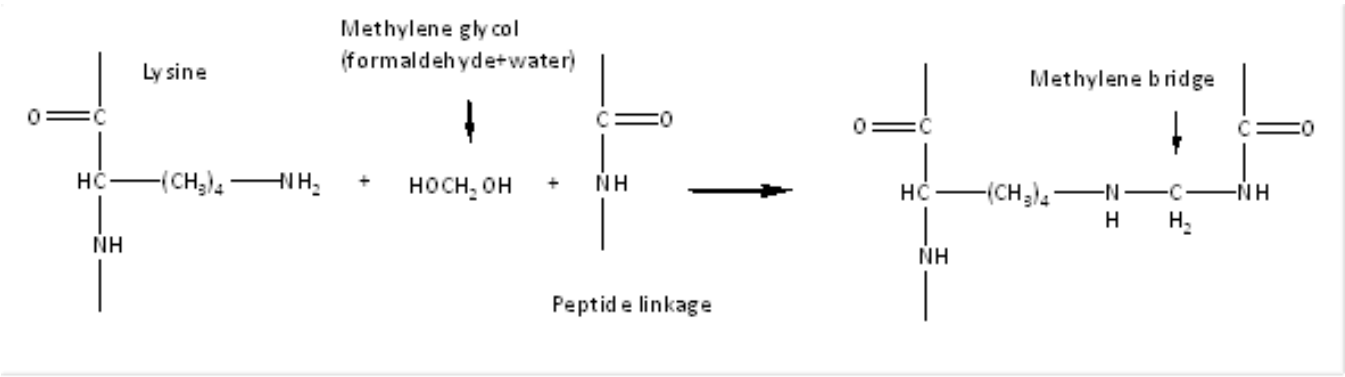
3-hydroxy Octadecanoic acid $(M+1)^+$ - OH

$C_{18}H_{37}O_3^+ - OH = 284.25^+$

Deduced BG33R surfactant structure

BSA crosslinking by formaldehyde - peptide mapping and de novo sequencing to check for linkage

m/z	Amount (ratio*)	Peptide Mapping (MS)	de novo Sequencing (MS/MS)	Position	Modification (mass, type)
343.648^{+}	3.8	A233LK or L372AK**	VPK	521-523	A, 12
582.3198^{2+}	4.5	no match	TEPGALHPV**DK**	196-197	A, or B
649.142^{2+}	1.9	no match	EPHPLGPY**TMR**	207-209	A, or B
659.3618^{2+}	3.2	no match	TPVGHGG**PQNLLK**	490-492, 407-412	A, or B
727.284^{2+}	4.3	no match	TMGLVPAHNM**LNR**	480-482	A, or B
747.295^{2+}	1.9	**LGEYGFEDALLVR****	**LG$_{422}$EYGFEDALLVR**	424-433	A, 12
784.3785^{2+}	3.1	**DAFLGSFLYEYSR**	**DAFLGSFLYEYSR**	347-359	Unmodified
1163.6398^{+}	6.6	**LVNELTEFAK**	**LVNELTEFAK**	66-75	Unmodified
1175.6296^{+}	3.1	**LVNELTEFAK****	**LV$_{67}$NELTEFAK**	66-75	A, 12



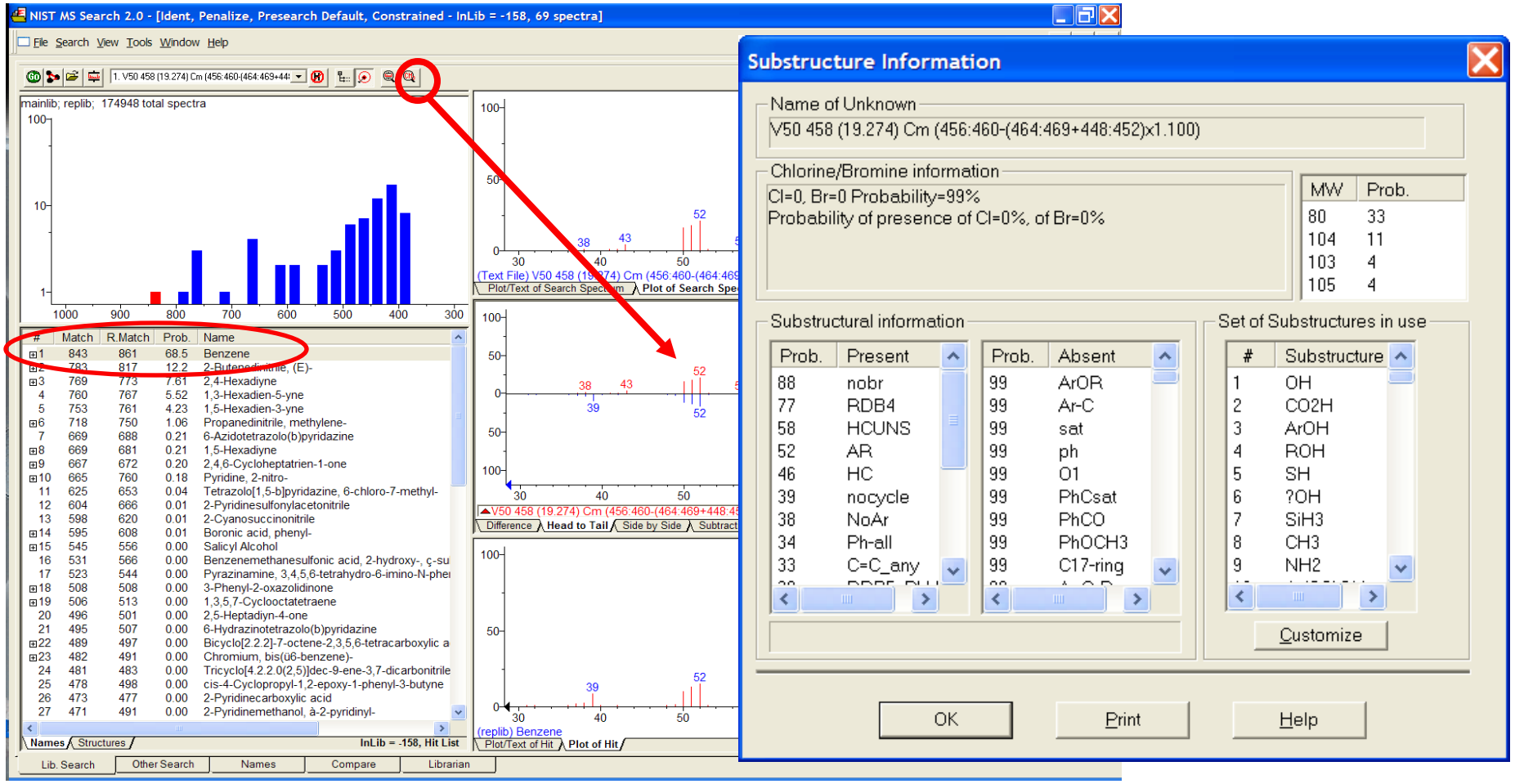
BSA fiber from protein aggregation

TABLE 2. Mechanical properties of individual single BSA fibers in comparison of cotton and silk

Specimen	Yield Strength (MPa)	Young's Modulus (GPa)	Elongation (%)
BSA fiber at pH 6, crosslinked with formaldehyde	61±16	2.71±0.6	3.6±1.4
BSA fiber at pH 4.7, crosslinked with formaldehyde	132±30	5.31±2.0	3.9±1.6
BSA fiber at pH 4.7, crosslinked with glutaraldehyde	148±4	5.72±0.3	>30[a]
BSA fiber at 4.7, crosslinked with EDC	214±97	8.26±4.3	>30[a]
Cotton single fiber	77±7	2.12±0.4	13.8±3.3
Silk single fiber	233±88	4.78±2.0	>30[a]

[a]Data beyond test limitation

Library Search and Substructure Identification



Indexing a User Library

7. Quantitation Methods by ESI

A: Loop injection: extract ion,

(1) peak height,

(2) peak area,

(3) ion count (percent of total ions)

2 min run, quick result,
high throughput,
ion suppression,
less sensitive.

B: Column chromatography: extract ion,

(1) peak height,

(2) peak area,

(3) ion count (percent of total ions)

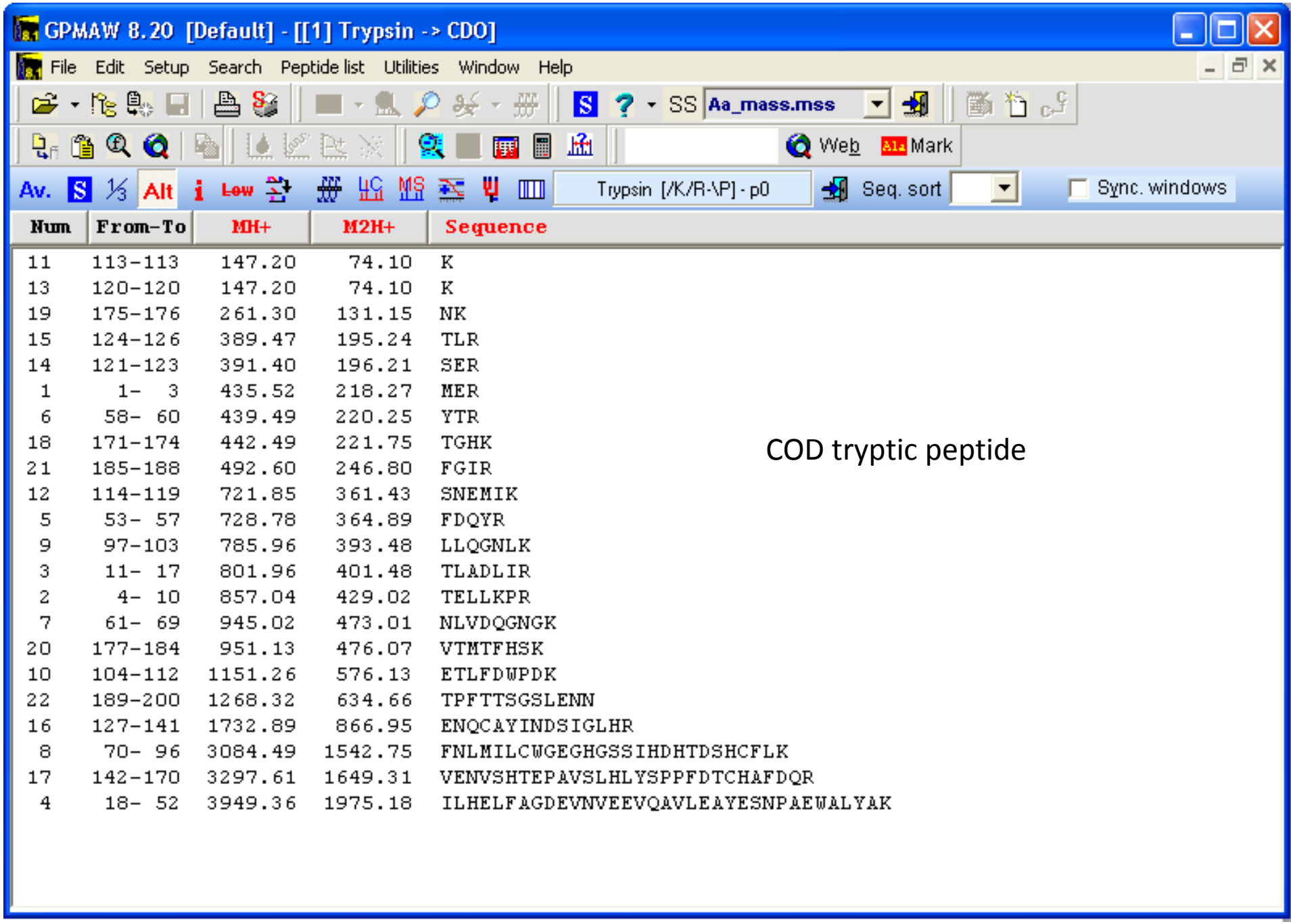
10 min run,
desalt, less ion suppression,
more sample load,
more sensitive,
more quantitative.

QC in quantitative analysis (for both GC/MS and LC/MS)

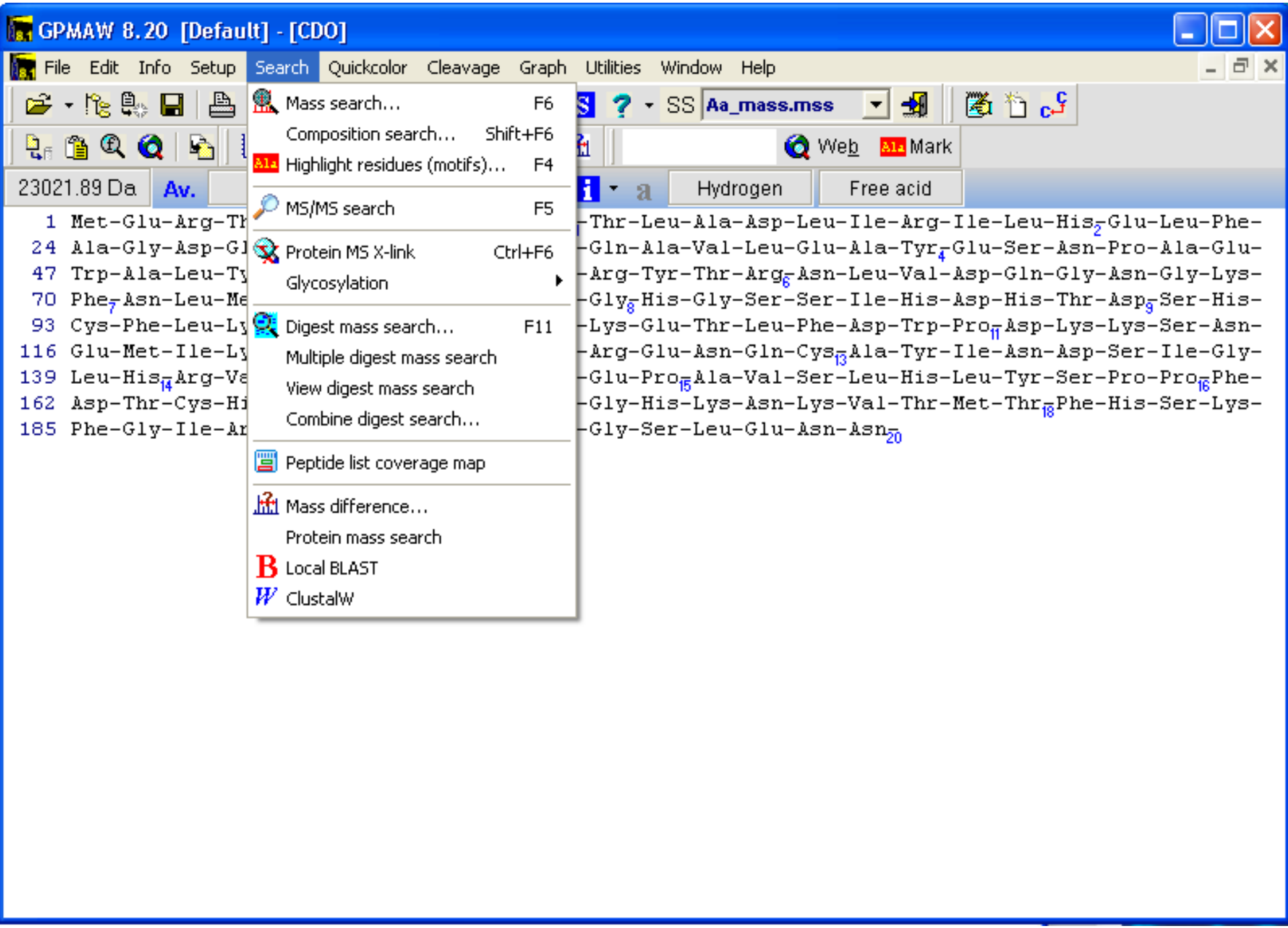
- Calibration curve,
- Dynamic range,
- Detection Limit,
- Quantitation limit,
- Mean and standard deviation, statistics,
- Precision (error analysis, reproducibility).

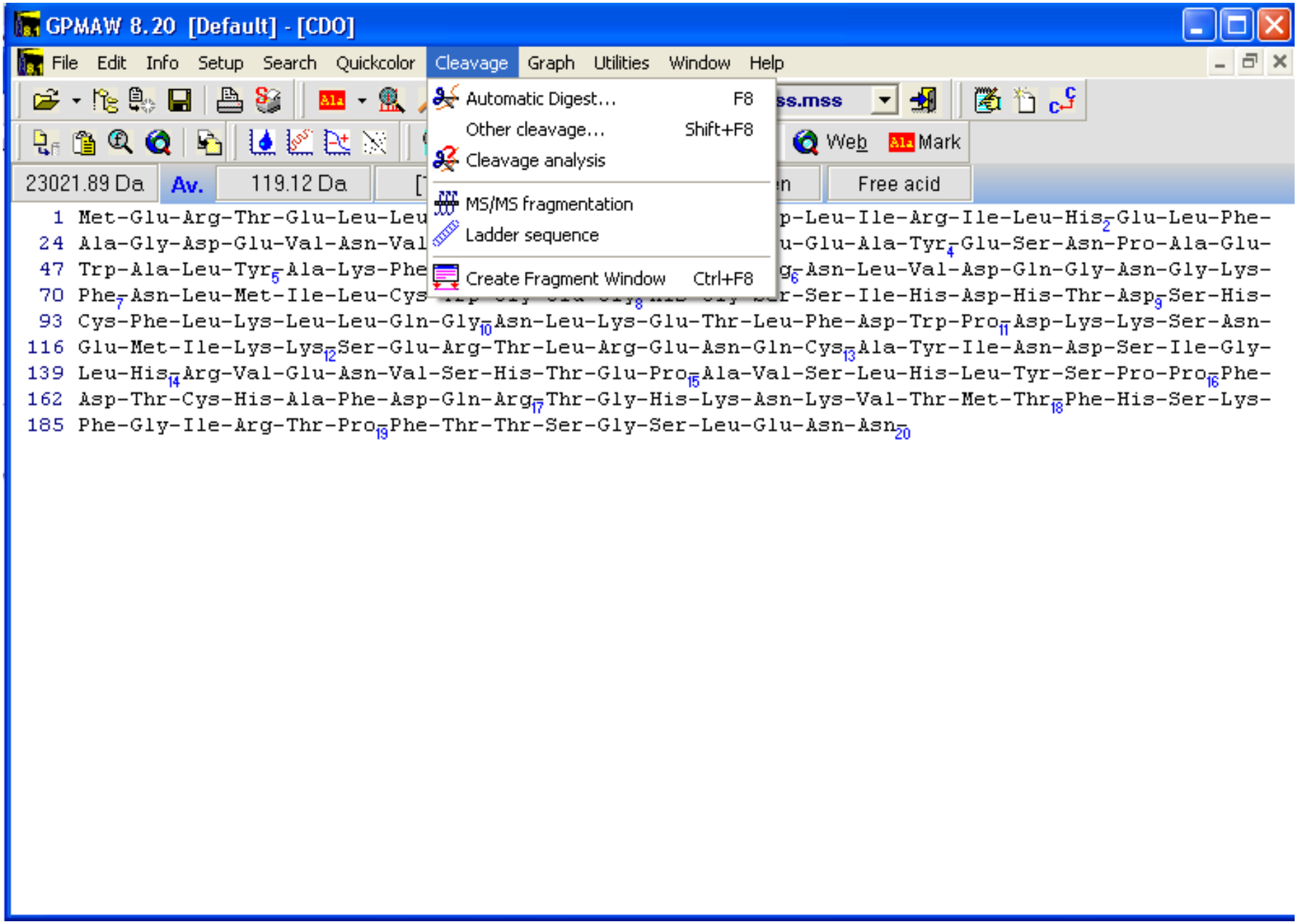
8. Software/database

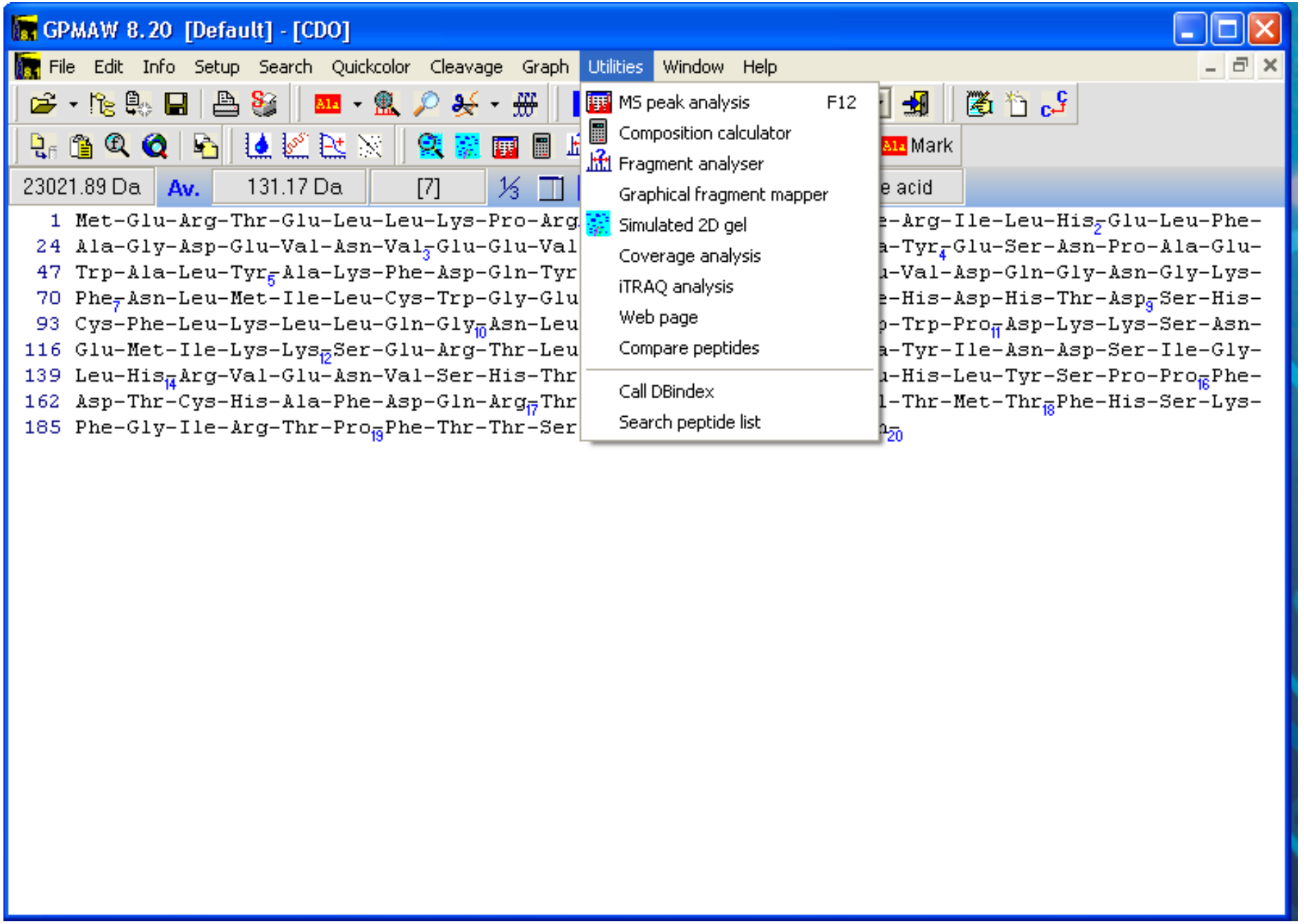
- Masslynx (run both Q-TOF and GCT),
- Nist Library (EI compound library, 250,000 spectra)
- Metabolite library (LipidMap, Human metabolome, Chemspider, Chemical entities of Biological interest)
- Proteinlynx, (SwissProt, NR, and user protein database)
- GPMAW (general protein molecular weight analysis for windows)
- flexcontrol (run MALDI), flexAnalysis (data analysis)



COD tryptic peptide


GPMAW 8.20 [Default] - [CDO]
File Edit Info Setup Search Quickcolor Cleavage Graph Utilities Window Help
Mass search... F6
Composition search... Shift+F6
Highlight residues (motifs)... F4
MS/MS search F5
Protein MS X-link Ctrl+F6
Glycosylation
Digest mass search... F11
Multiple digest mass search
View digest mass search
Combine digest search...
Peptide list coverage map
Mass difference...
Protein mass search
Local BLAST
ClustalW
Aa_mass.mss
Web
Mark
23021.89 Da
Av.
Hydrogen
Free acid
1 Met-Glu-Arg-Th
24 Ala-Gly-Asp-Gl
47 Trp-Ala-Leu-Ty
70 Phe-Asn-Leu-Me
93 Cys-Phe-Leu-Ly
116 Glu-Met-Ile-Ly
139 Leu-His-Arg-Va
162 Asp-Thr-Cys-Hi
185 Phe-Gly-Ile-Ar
-Thr-Leu-Ala-Asp-Leu-Ile-Arg-Ile-Leu-His-Glu-Leu-Phe-
-Gln-Ala-Val-Leu-Glu-Ala-Tyr-Glu-Ser-Asn-Pro-Ala-Glu-
-Arg-Tyr-Thr-Arg-Asn-Leu-Val-Asp-Gln-Gly-Asn-Gly-Lys-
-Gly-His-Gly-Ser-Ser-Ile-His-Asp-His-Thr-Asp-Ser-His-
-Lys-Glu-Thr-Leu-Phe-Asp-Trp-Pro-Asp-Lys-Lys-Ser-Asn-
-Arg-Glu-Asn-Gln-Cys-Ala-Tyr-Ile-Asn-Asp-Ser-Ile-Gly-
-Glu-Pro-Ala-Val-Ser-Leu-His-Leu-Tyr-Ser-Pro-Pro-Phe-
-Gly-His-Lys-Asn-Lys-Val-Thr-Met-Thr-Phe-His-Ser-Lys-
-Gly-Ser-Leu-Glu-Asn-Asn-


GPMAW 8.20 [Default] - [CDO]
File Edit Info Setup Search Quickcolor Cleavage Graph Utilities Window Help
Automatic Digest... F8
Other cleavage... Shift+F8
Cleavage analysis
MS/MS fragmentation
Ladder sequence
Create Fragment Window Ctrl+F8
Web Mark
23021.89 Da Av. 119.12 Da Free acid
1 Met-Glu-Arg-Thr-Glu-Leu-Leu
24 Ala-Gly-Asp-Glu-Val-Asn-Val
47 Trp-Ala-Leu-Tyr5-Ala-Lys-Phe
70 Phe7-Asn-Leu-Met-Ile-Leu-Cys
93 Cys-Phe-Leu-Lys-Leu-Leu-Gln-Gly10-Asn-Leu-Lys-Glu-Thr-Leu-Phe-Asp-Trp-Pro11-Asp-Lys-Lys-Ser-Asn-
116 Glu-Met-Ile-Lys-Lys12-Ser-Glu-Arg-Thr-Leu-Arg-Glu-Asn-Gln-Cys13-Ala-Tyr-Ile-Asn-Asp-Ser-Ile-Gly-
139 Leu-His14-Arg-Val-Glu-Asn-Val-Ser-His-Thr-Glu-Pro15-Ala-Val-Ser-Leu-His-Leu-Tyr-Ser-Pro-Pro16-Phe-
162 Asp-Thr-Cys-His-Ala-Phe-Asp-Gln-Arg17-Thr-Gly-His-Lys-Asn-Lys-Val-Thr-Met-Thr18-Phe-His-Ser-Lys-
185 Phe-Gly-Ile-Arg-Thr-Pro19-Phe-Thr-Thr-Ser-Gly-Ser-Leu-Glu-Asn-Asn20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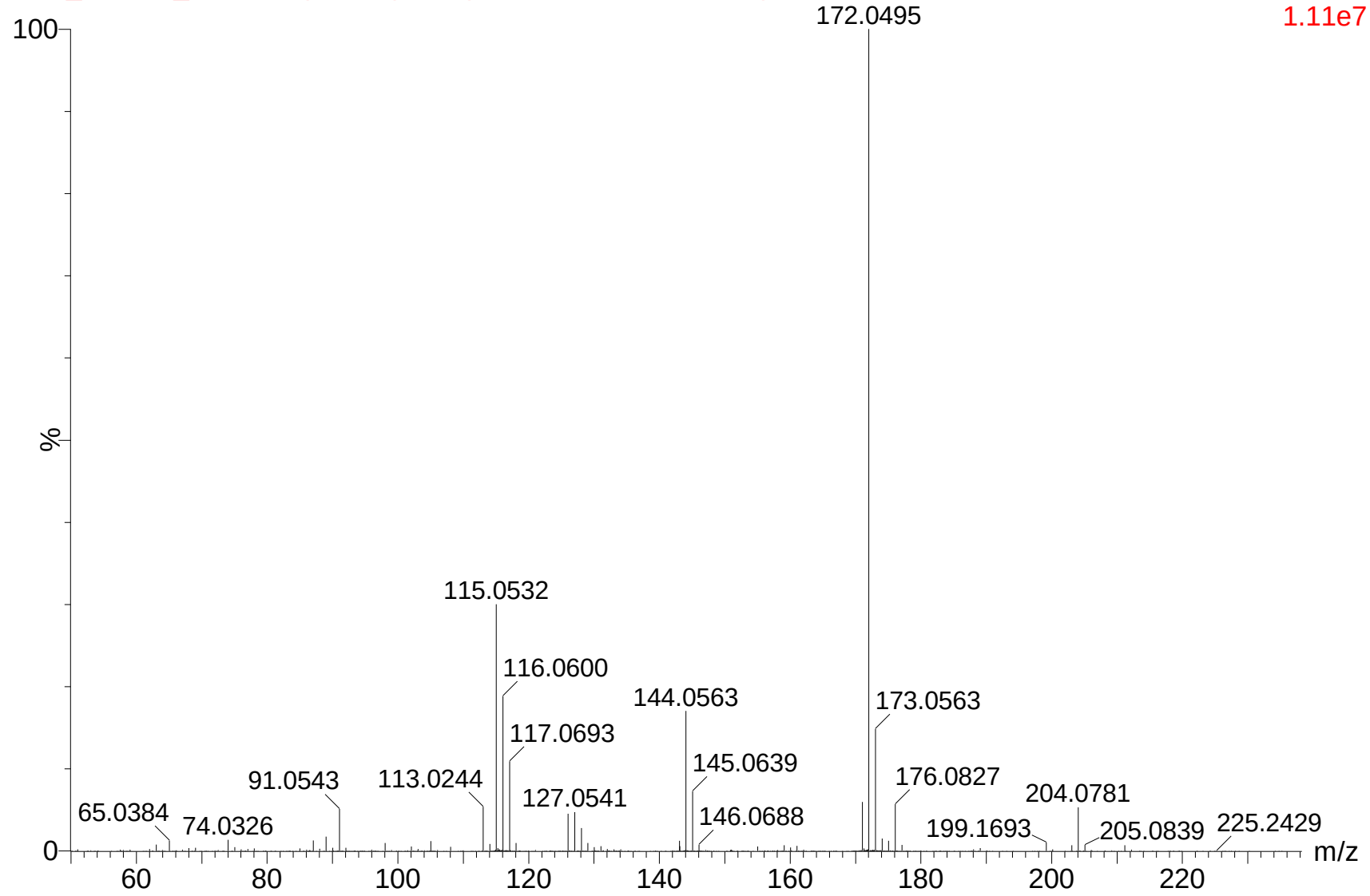
MS peak analysis F12
Composition calculator
Fragment analyser
Graphical fragment mapper
Simulated 2D gel
Coverage analysis
iTRAQ analysis
Web page
Compare peptides
Call DBindex
Search peptide list
23021.89 Da
Av.
131.17 Da
[7]
Mark
e acid
1 Met-Glu-Arg-Thr-Glu-Leu-Leu-Lys-Pro-Arg
24 Ala-Gly-Asp-Glu-Val-Asn-Val-Glu-Glu-Val
47 Trp-Ala-Leu-Tyr-Ala-Lys-Phe-Asp-Gln-Tyr
70 Phe-Asn-Leu-Met-Ile-Leu-Cys-Trp-Gly-Glu
93 Cys-Phe-Leu-Lys-Leu-Leu-Gln-Gly-Asn-Leu
116 Glu-Met-Ile-Lys-Lys-Ser-Glu-Arg-Thr-Leu
139 Leu-His-Arg-Val-Glu-Asn-Val-Ser-His-Thr
162 Asp-Thr-Cys-His-Ala-Phe-Asp-Gln-Arg-Thr
185 Phe-Gly-Ile-Arg-Thr-Pro-Phe-Thr-Thr-Ser
-Arg-Ile-Leu-His-Glu-Leu-Phe-
-Tyr-Glu-Ser-Asn-Pro-Ala-Glu-
-Val-Asp-Gln-Gly-Asn-Gly-Lys-
-His-Asp-His-Thr-Asp-Ser-His-
-Trp-Pro-Asp-Lys-Lys-Ser-Asn-
-Tyr-Ile-Asn-Asp-Ser-Ile-Gly-
-His-Leu-Tyr-Ser-Pro-Pro-Phe-
-Thr-Met-Thr-Phe-His-Ser-Lys-

9. Data Storage, Retrieval, and Presentation

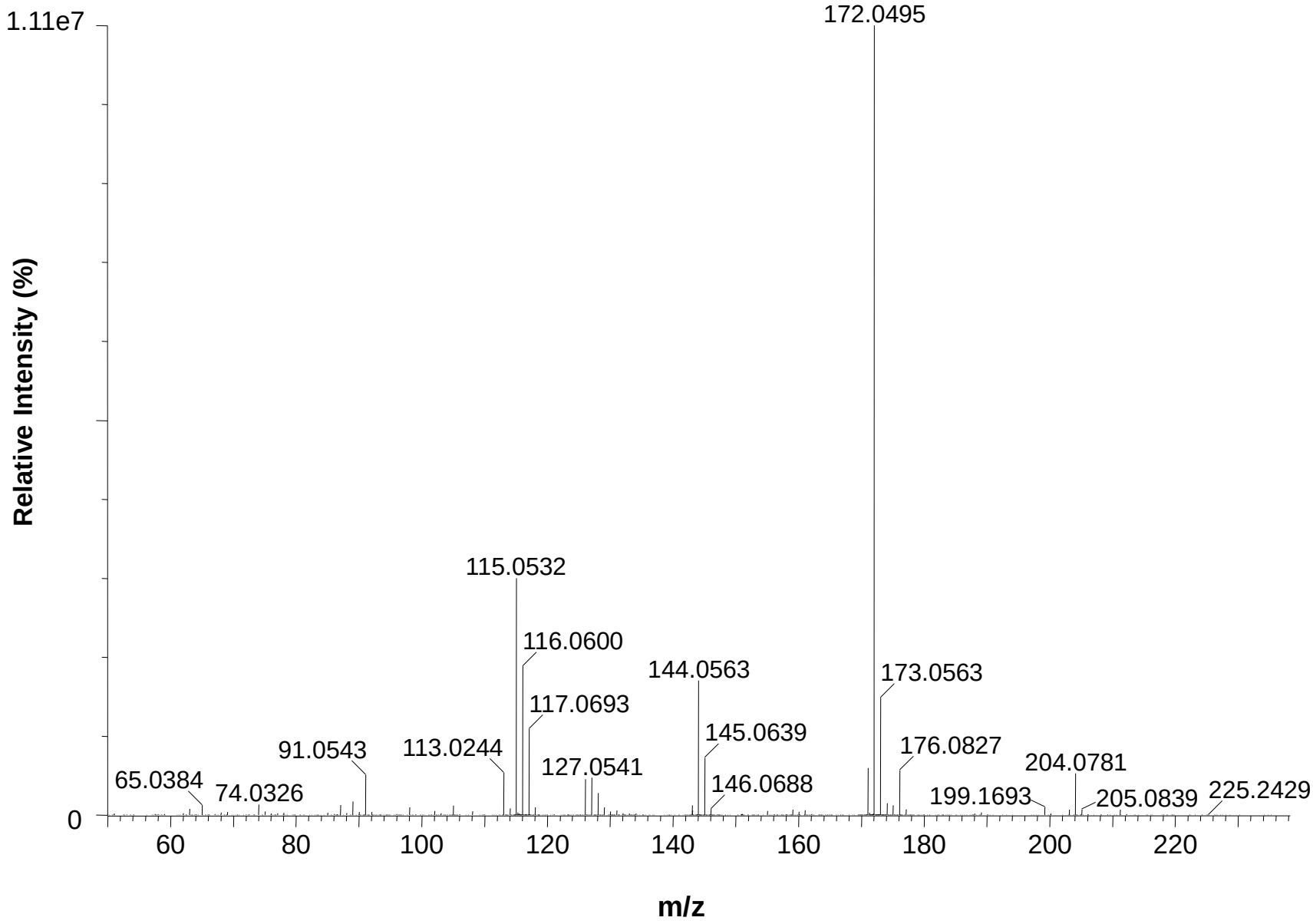
- All data are stored in C drive and backed up in two other locations, they can be retrieved at any time.
- Data can be downloaded into other formate (Excel, PPT…)
- Chromatogram and spectrum can be reformatted .

Example of data presentation, original spectrum

as is



Modified spectrum



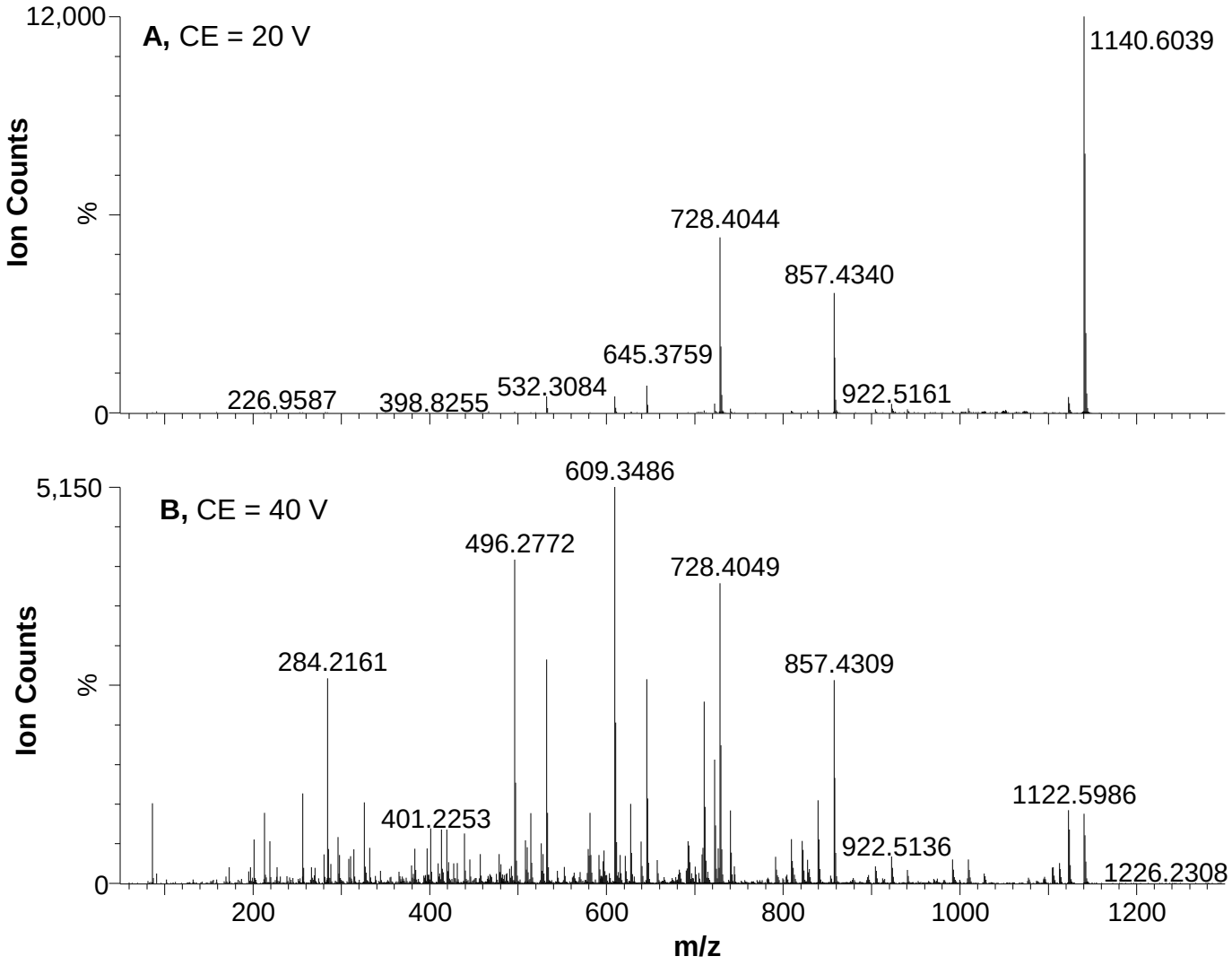


Fig. 2, MSMS scan of ion 1140 with low and high collision energy

10. Service Support

- Experiment design
- Data acquisition (same day turnaround)
- Data analysis
- Technical write-up
- Consultation and brain-storming (free)

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http://www.auburn.edu/cosam/departments/chemistry/Mass_Spec%20Usage.html

SOP for analyzing LHRH (luteinizing hormone releasing hormone)

1. Turn on power supplies for Waters Sample Manager and Binary Solvent Manager if they are not already on.
2. Open Masslynx (V.4.1, Waters) software on the desktop if it is not already open.
3. Ensure the communication establishes between computer, embedded PC, UPLC and QToF instrument.
4. If communication failed, check connection in the router: it can be reset by unplug the power supply, turn off the PC and EPC, restart the PC and login, but not open the Masslynx, turn on the EPC and wait for 5 min, then open the Masslynx.
5. In the instrument menu, click on MS Tune, examine the read-back voltages, they should agree with the set values.
6. Make sure the collision gas is on all the time, click API gas, desolvation flow rate should be 200 liter/hour, if not, check the high pressure nitrogen gas tank to see if it is getting close to empty and ready to be refilled.
7. Set the detector voltage to 1700 by increasing 500 volts stepwise.
8. Check the solvent bottles for solution A (95% Water, 0.1% Formic acid 5% Acetonitrile) and solution B (95% Acetonitrile, 5% water, 0.1% Formic acid). Prepare more solutions if they are low.
9. Degas solvent by placing the solvent bottle in sonication water bath and sonicate for 5 minutes, or by bubbling solvent bottle under nitrogen gas for 5 minutes in the hood.
10. Open the Inlet Method; launch Acquity UPLC Console, in the binary Solvent Manager, Control menu, solvent A/B prime, seal wash can be performed.
11. In the Sample Manager, Control tab, prime syringes, wash needle can be performed.
12. Prime pumps when change solvents or when system pressure is unstable.
13. Equilibrate the system with Direct method in 50:50= A:B followed by 95:5 = A:B for at least 5 minutes each.

Note: if the C18 column (or normal phase column) and system is already clean, 50:50=A:B step can be skipped.

14. Monitor the analyte ion source chromatogram to the baseline and system pressure is stable (around 60 psi for direct injection and 4000 psi with C18 column indicating no leaks) before inject any sample.

Mass Calibration

15. Turn on the syringe pump and LC pump to make the lockmass ion and analyte ions flow, the detector voltage and API gas.
16. Check the sensitivity of the instrument: in the analyte ion source with the Direct LC method on, the chemical noise should be less than 1% of the total ion count.
17. In the reference ion source, the lockmass ion count per scan should be over 1,000 for the highest one.
18. Clean the ion source if the sensitivity is poor. Depend on the usage, the ion source should be cleaned every two to three weeks.
19. Check the resolution of the reference ions, it should be around 8,000.
20. Acquire the lockmass chromatogram for 1 min by clicking on the blue triangle button on the top menu bar in the tune page; give the file name with Calibration-date-number format.
21. Click the “Clock” icon in the chromatogram to update the run real time.
22. Combine the scan to have most lockmass over 1,000 counts.
23. Do smooth (2 channel 3 times by Savitzky Golay method) and center (4 min peak width, create added area) to lockmass ions in the spectrum , under the “Process” menu bar.
24. Save the spectrum under the “File” menu, click OK.
25. Stop the acquisition, Click on the “Calibration” Calibrate TOF, in the popup menu, click on Calibrate, select “create calibration…”
26. Find the Calibration file just acquired, click at the bottom of the window on “History” select “AccMass”, click OK,
27. Accept calibration if residue errors are less than 0.01 Dalton, and all data points are tight around zero.
28. Save tune page with newly calibrated TOF.

Preparation of Sample

III. 29. Add formic acid to the sample for a final concentration of 0.1% FA for ion paring in the hood if needed. Avoid acid if samples are unstable in the acidic condition.

30. Centrifuge sample in the tabletop centrifuge at 13,000 PRM for 5 minutes to precipitate particulates that could clog the system.

31. Place the sample in the glass insert (hold 200 µL) with spring at the bottom in the injection vial for samples with less volume.

32. Use glass vial for small molecules and plastic vials for protein/peptide samples.

33. Check for air bubbles in the sample vials.

34. Open the Sample Manager carrier door underneath the Waters Autosampler, place the sample in the slot in the tray and record the position. Each tray has 48 wells, Tray in the left is the number 1 tray and the one in the right is the number 2 tray.

35. Enter sample name, separation method, injecting volume and vial position in the “sample queue” field. Position format uses: 1:24 stands for tray 1 at the end of row 4.

Note: the maximum volume is 10 µL with current installed sample loop.

36. Enter the list of samples in the sample queue field to run the batch operation.

37. Use the MS_584 method for MS method, Direct for Inlet File.

Data Acquisition:

VI. 38. Arrange samples from low to high concentration, Place water wash run in between samples to prevent the carryover if needed.
39. Observe real time lockmass ions are plentiful and ready for calibration.
40. Make injection by selecting either single injection of batch injection, and run chromatogram.
41. Estimate the LHRHa content (and fragment 1 and 2 from pepsin digestion by combining scans at the injection peak area.
42. Estimate the concentration of unknown peptide by comparing to known amount of peptides.
43. Estimate the concentration by running a standard curve with at least 4 concentrations spanning two orders of magnitude.
44. Report the result, save and copy the chromatogram into Windows PowerPoint and record in the notebook.
45. Finish the operation by leaving the system in 50:50=A:B for 5 minutes.

Set the instrument in idle

46. When instrument is not in use, turn off the solvent flow by clicking on the “water fountain”
47. Set the detector voltage to zero.
48. Turn off the API gas by clicking on the icon, leave COL gas on.
49. Turn off the syringe pump of the lockmass, by clicking on the pump arrow; it changes red color to blue color at the center of the “instrument” tab.

Materials and Reagents

Kimwipe
Glove
Pipetmans: 5 mL, 1 mL, 200 µL, 100 µL and 1~10µL
Pipet tips: 1-10 µL, 200 µL, 1 mL and 5 mL
1.5 mL eppendorf tubes
1 mL glass vials and insert
Tube Blocks
MilliQ water
HPLC grade Acetonitrile
Formic acid (very volatile, work in hood)
Beakers
Bottles
Sonicator
Tabletop Centrifuge (Argosflexifuge)