Methods in Mass Spectrometry

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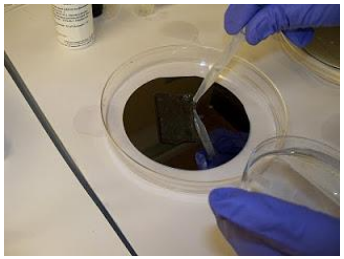
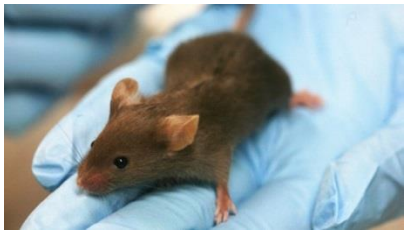
Sample

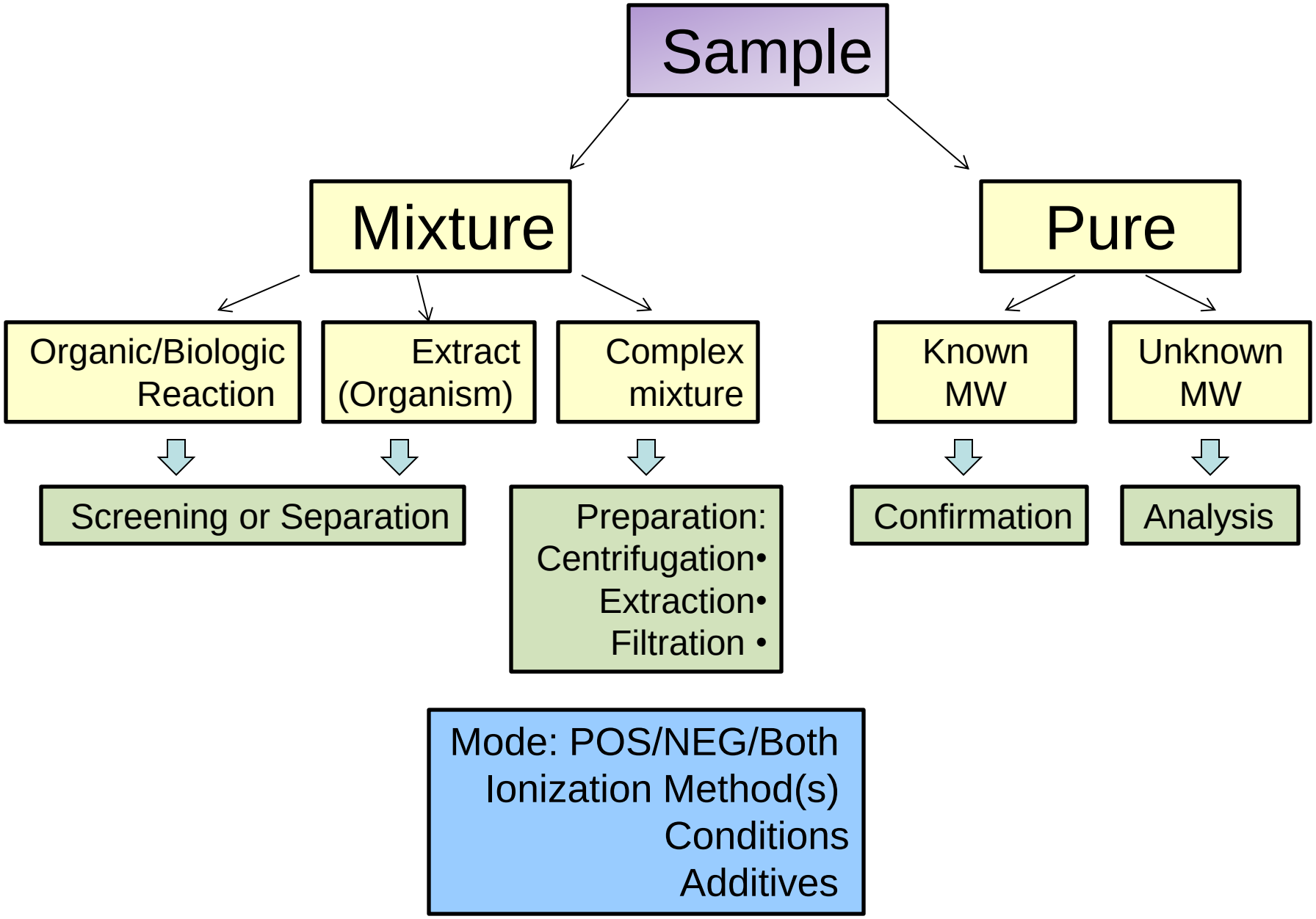
- Engineering
- Chemistry
- Biology
- Life Science
- Medicine
- Industry
- IDF / Police



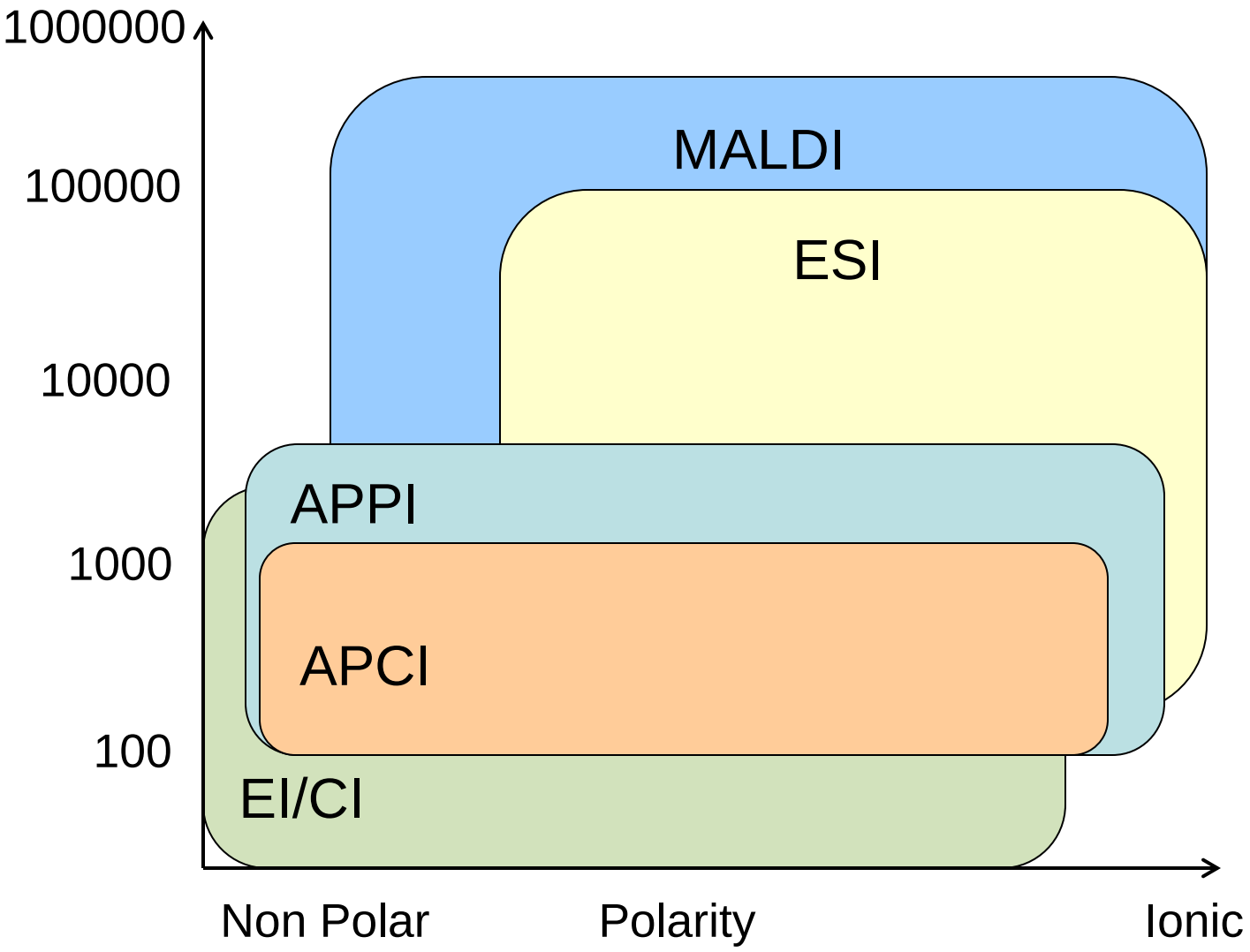






Sample
Mixture
Pure
Organic/Biologic Reaction
Extract (Organism)
Complex mixture
Known MW
Unknown MW
Screening or Separation
Preparation: Centrifugation• Extraction• Filtration •
Confirmation
Analysis
Mode: POS/NEG/Both
Ionization Method(s)
Conditions
Additives

Ionization Method



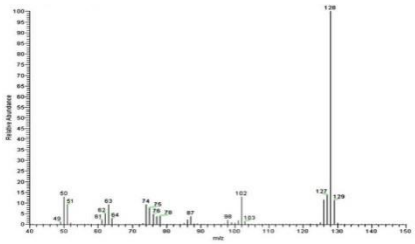
TAU MS LAB

Autospec: EI, CI, (GC), FAB

SYNAPT: ESI, APCI, APPI, (LC), MALDI

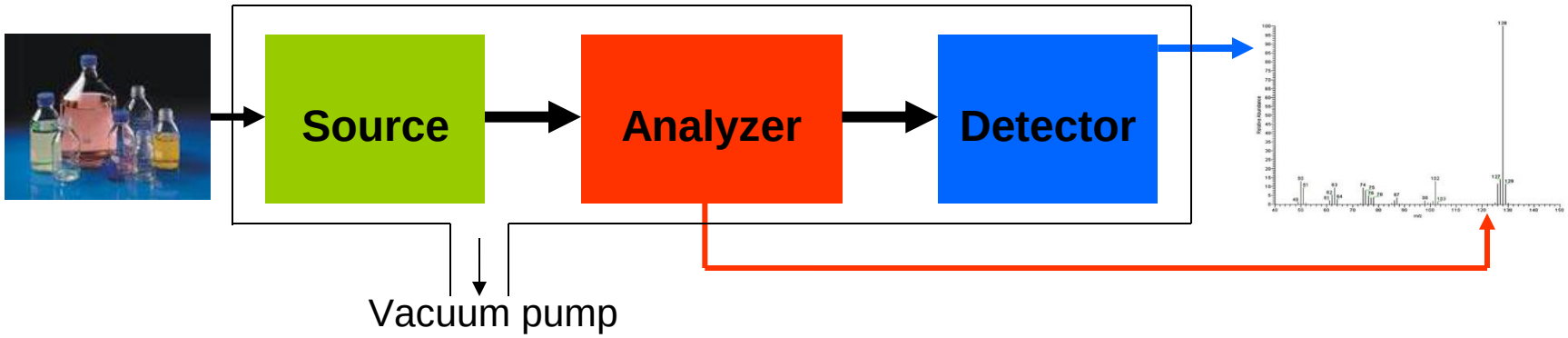
TQD: ESI, APCI, MRM (LC)

What is a Mass Spectrometer ?



- Mass spectrometer measures the ratio between mass and charge of Ions m/z
- structural Information
- Identification
- Quantification
- Very fast
- High Sensitivity (Subpicomole)
- Samples: HPLC grade chemicals - Tissue, polluted waters, Blood
- Mass range: from proton to protein
- Resolution: $M/\Delta M$ 10^3-10^6 – Elemental composition
- Very expansive….

What is a Mass Spectrometer ?



- **Source** – Ionization

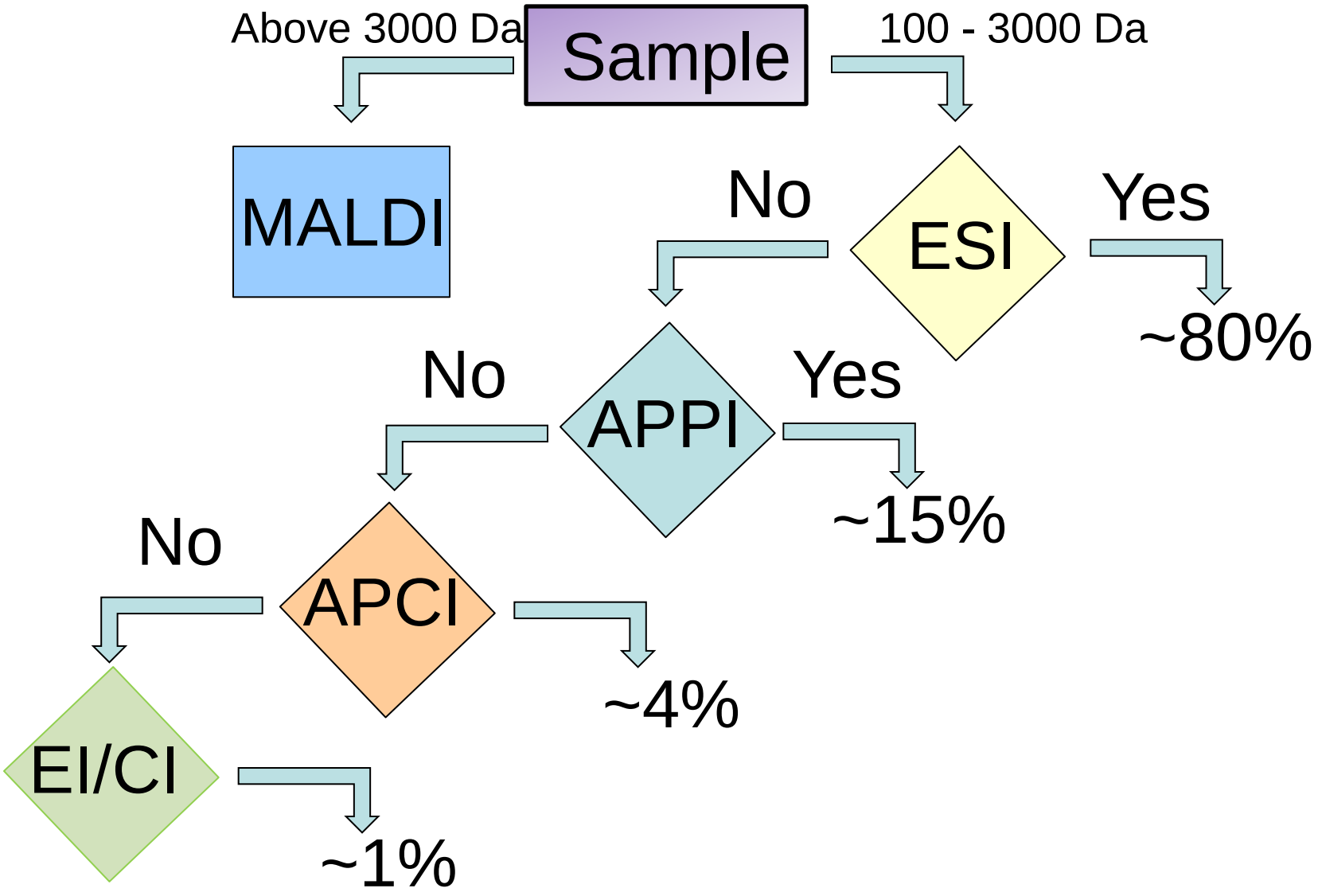
  $M^o \longrightarrow M^{+\cdot}, M^{-\cdot}, [M-H]^-, [M+H]^+$

- **Analyzer** - Mass Separation
  - Resolution
  - Mass Range

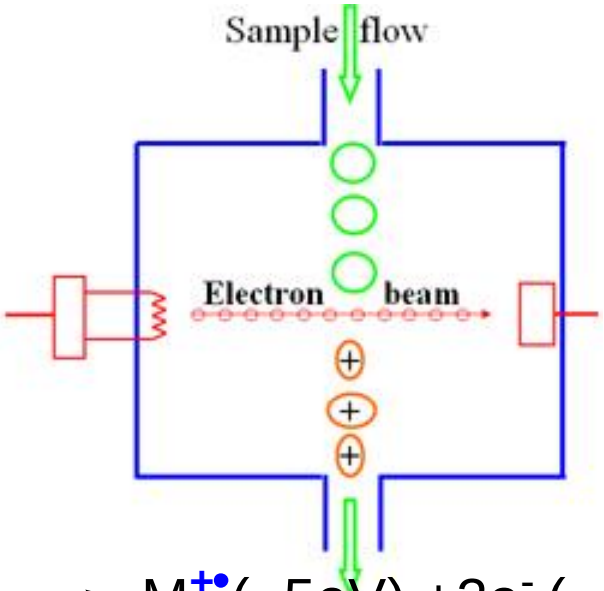
- **Detector** - Ion counter
  -SNR, -Sensitivity

Ionization Methods

- EI
- CI
- FAB
- MALDI
- ESI
- APPI
- APCI


Sample
Above 3000 Da
100 - 3000 Da
MALDI
ESI
No
Yes
~80%
APPI
No
Yes
~15%
APCI
No
~4%
EI/CI
~1%

Electron Ionization (EI)

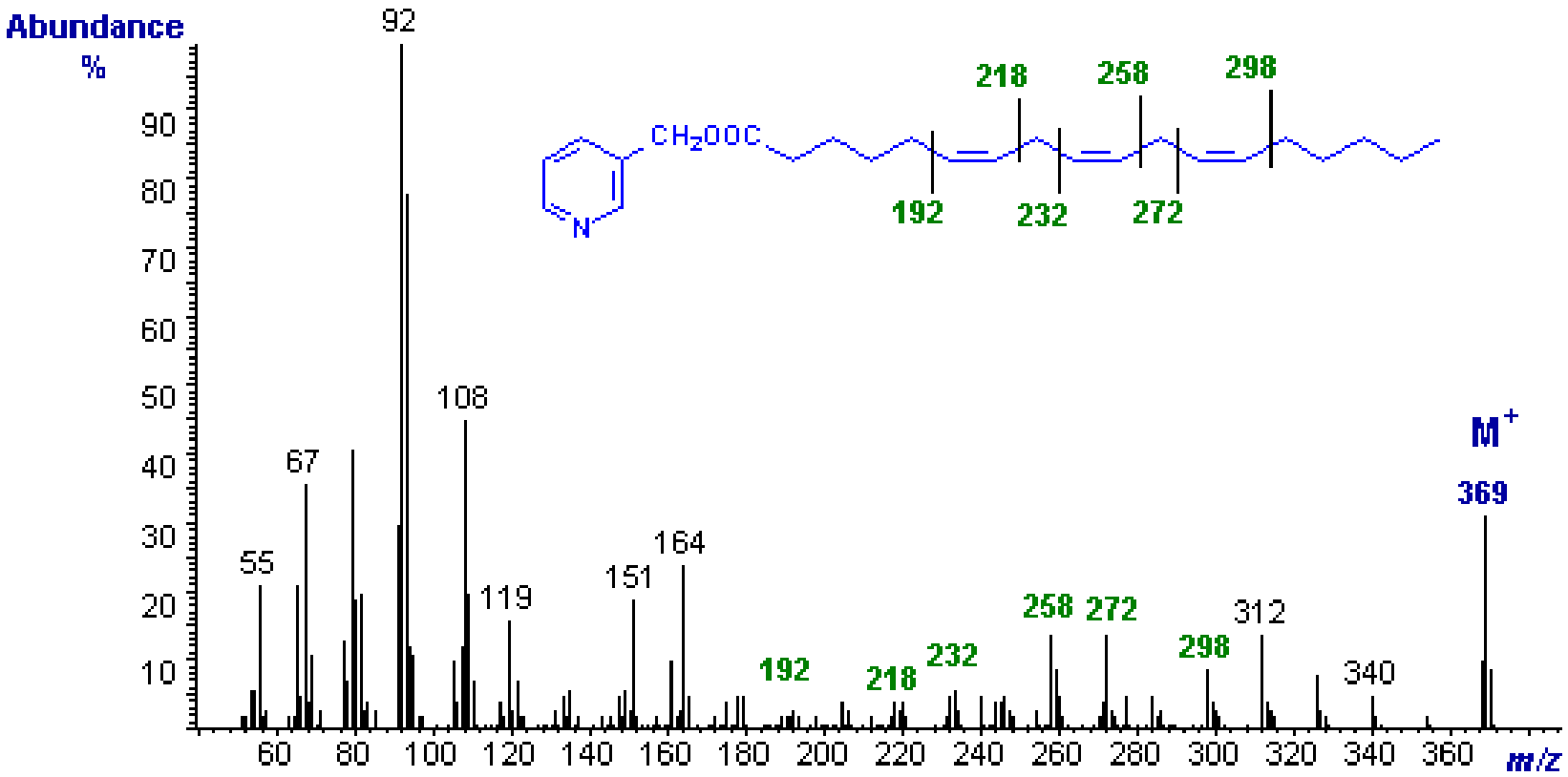


$M^{o} + e^{-}(70\ eV) \longrightarrow M^{+\bullet}(\sim 5eV) + 2e^{-}\ (\sim 65eV)$

Fragment Ions Neutral Fragments

$M^{o} + e^{-}(70\ eV) \longrightarrow M^{-}$ (only ~1%)

Electron Ionization (EI)



Electron Ionization (EI)

Advantages

- Sensitivity: Picomole
- Database: ~100,000 compounds
- Fragments: Structure + Fingerprints
- GC, Analytical chemistry

Disadvantages

- Mass range: ~1000 Da
- Fragments: Decomposition

Chemical Ionization (CI)

$$CH_4 + e^- \longrightarrow CH_4^{+\bullet} + 2e^-$$

$$CH_4^{+\bullet} \longrightarrow CH_3^{\bullet +} + H$$

$$CH_4^{+\bullet} + CH_4 \longrightarrow CH_5^+ + CH_3^{\bullet}$$

$$CH_5^+ + M \longrightarrow MH^+$$

Chemical Ionization (CI)

Advantages

- Less Fragmentation
- Suitable for fragile molecules

Disadvantages

- No library
- Mass range ~700 Da
- Less Sensitive

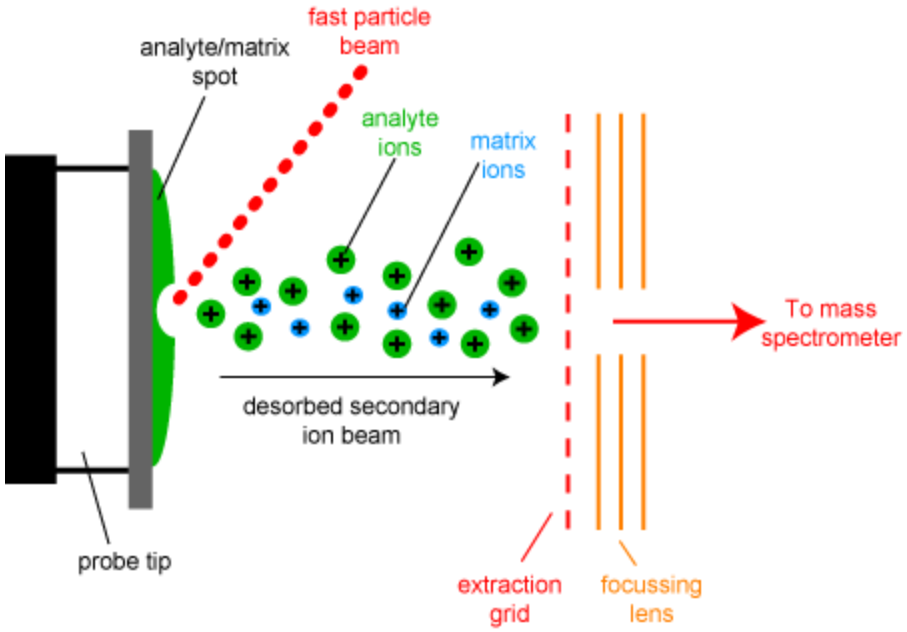
Fast Atom Bombardment (FAB)

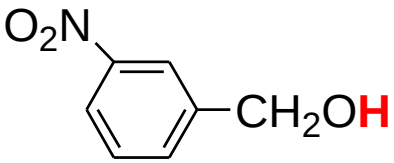
Liquid Secondary Ion Mass Spectrometry

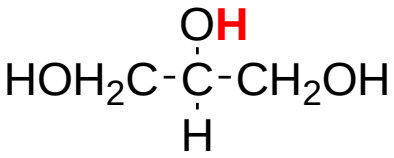
CI: $CH_5^+ + M \longrightarrow MH^+$

FAB: $Matrix + M \longrightarrow MH^+$

$Matrix + M \longrightarrow [M-H]^-$



NBA 

Glycerole 

TEA $N(CH_2CH_2OH)_3$

Fast Atom Bombardment (FAB)

Advantages

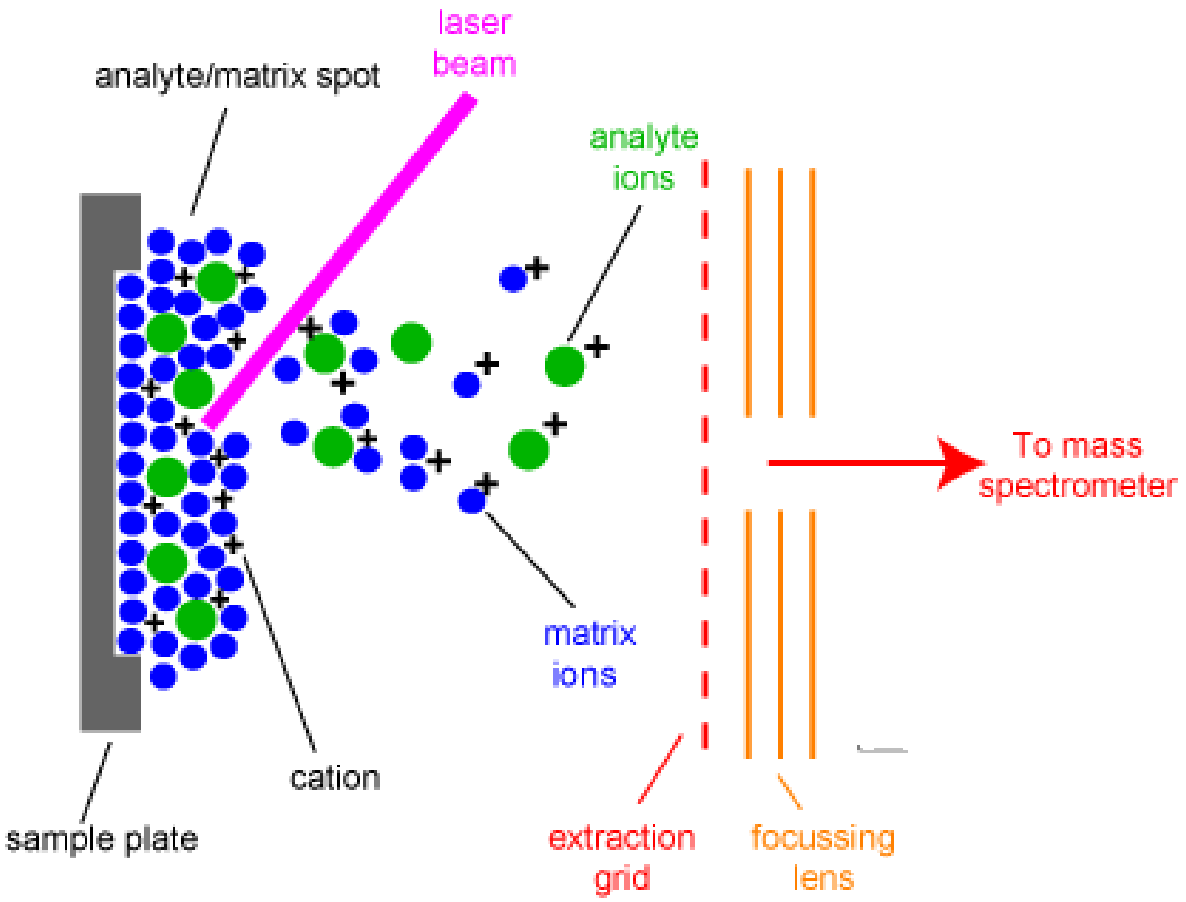
- Mass range ~7000 Da
- Soft Ionization
- Massive Cluster Impact

Disadvantages

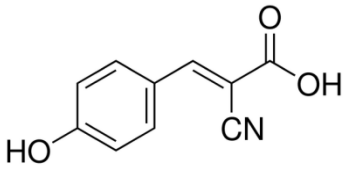
- Less Sensitive
- Only for Polar compounds

Matrix Assisted Laser Desorption Ionization (MALDI)

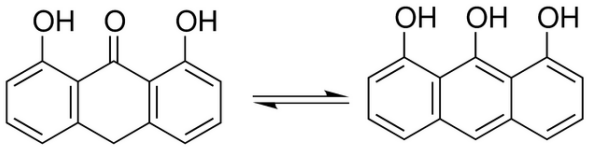
Matrix + Energy + M ⟶ $[MH]^+$, $[M\text{-}H]^-$



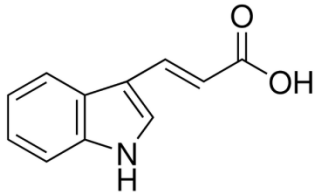
Matrix Assisted Laser Desorption Ionization (MALDI)



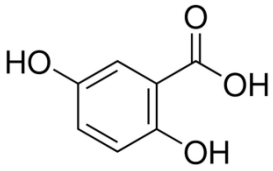
α-Cyano-4
-hydroxycinnamic acid



Dithranol

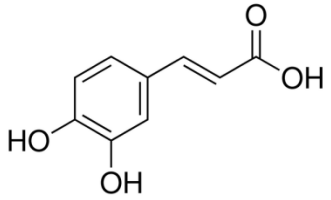


trans-3-Indoleacrylic acid

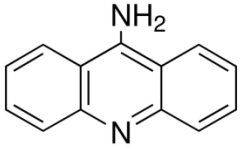


2,5-Dihydroxybenzoic acid

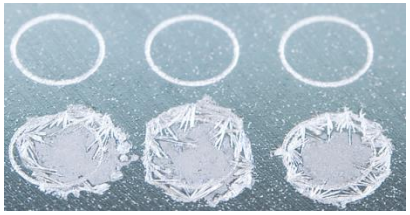
Sample plate

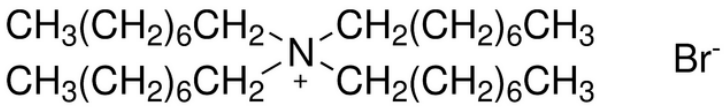


3,4-Dihydroxycinnamic acid



9-Aminoacridine



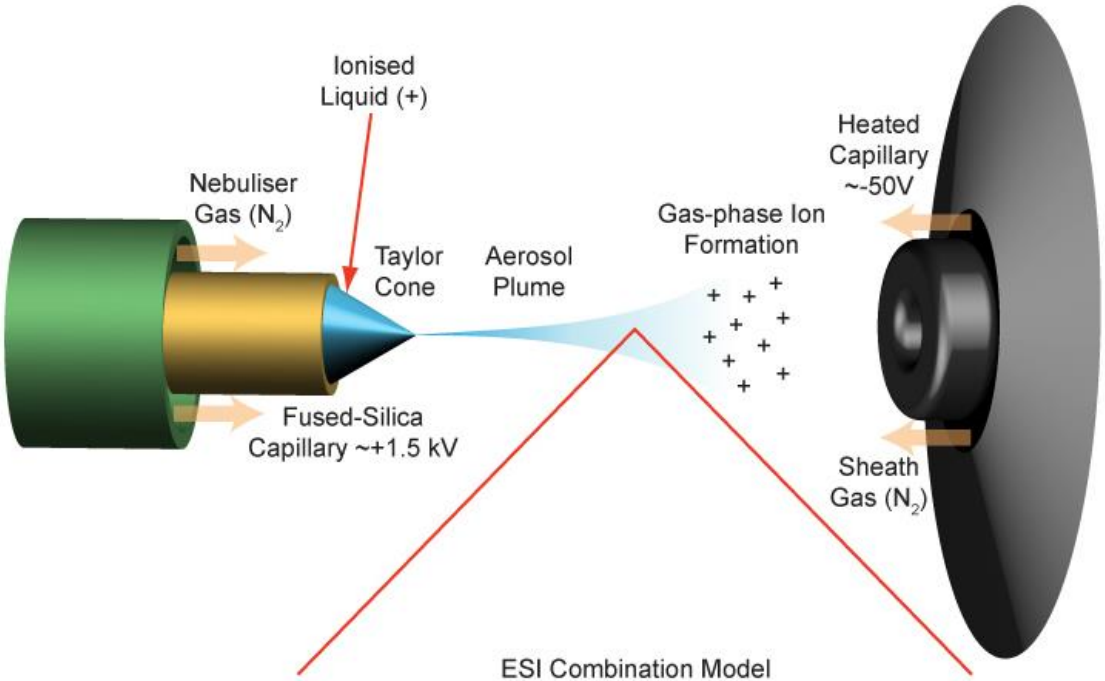


Tetraoctylammonium bromide

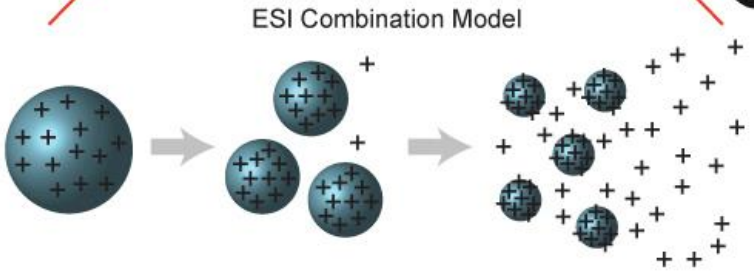
Matrix Assisted Laser Desorption Ionization (MALDI)

	FAB	MALDI
Matrix:	liquid	Solid
Energy:	Atoms/ions	LASER
Mass Range:	70,000 Da	300,000Da
Sensitivity:	Low	Femptomol
Background:	yes	<1000 Da

Electro Spray Ionization (ESI)



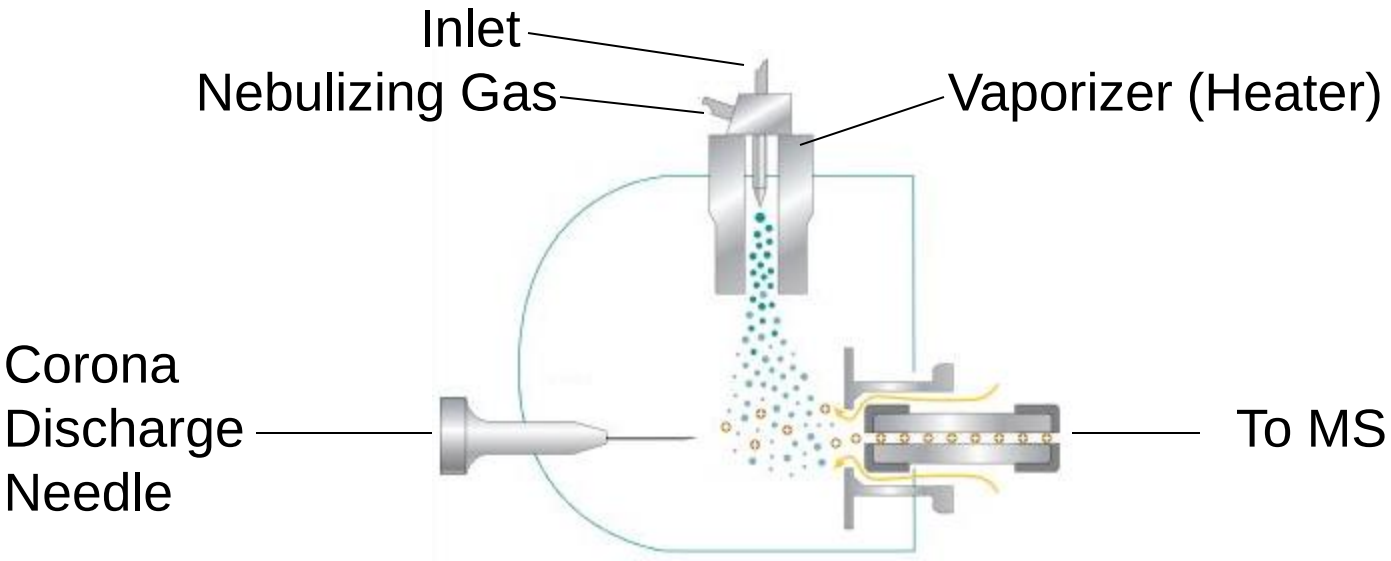
Mass Spectrometer Ion Source Region



Electro Spray Ionization (ESI)

- Polar compounds in MeOH / H_2O / MeCN
- Sensitive: Femptomole
- Multiple Charge
- Mass Range: ~100,000 Da
- Proteomics
- Atmospheric Pressure
- Interface: Direct / HPLC
- Soft Ionization: Biology, Proteomics, Non covalent compounds

Atm. Pressure Chemical Ionization (APCI)



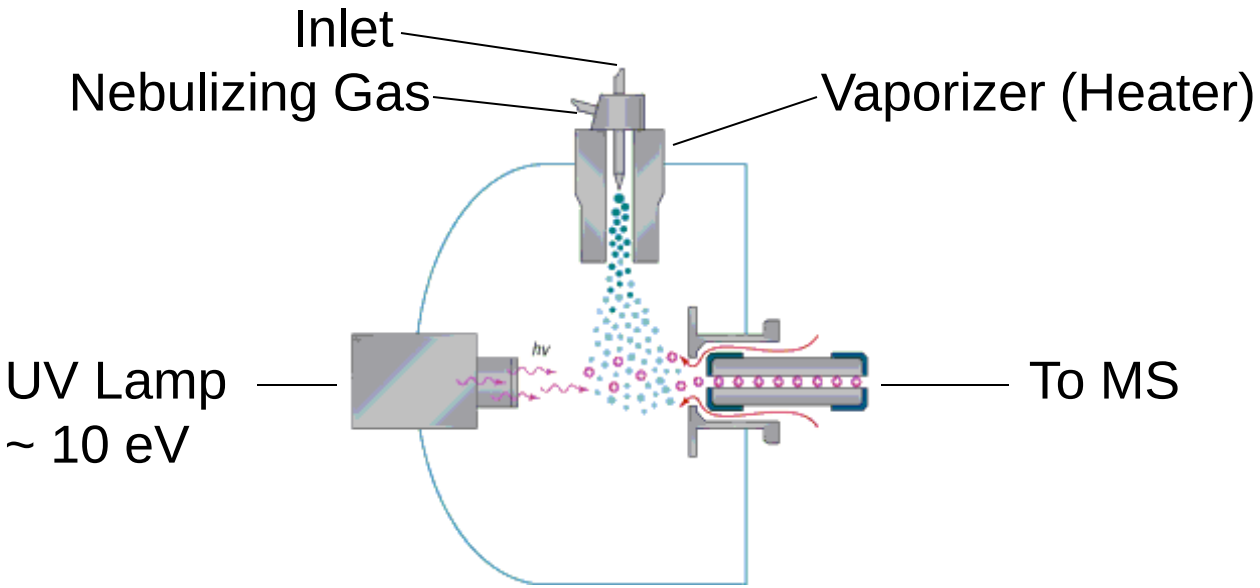
$$N_2 + e^- \longrightarrow N_2^{+\bullet} + 2e^-$$

$$N_2^{+\bullet} + M \longrightarrow M^{+\bullet} + N_2$$

Atm. Pressure Chemical Ionization (APCI)

- Less polar compounds
- Sensitivity: Pos >> Neg
- Single charge
- Volatile compounds
- Mass range: ~1500 Da
- Atmospheric Pressure
- Interface: Direct / HPLC
- Soft Ionization

Atm. Pressure Photo Ionization (APPI)



Direct APPI

$M + h\nu \longrightarrow M^{+\bullet} + e^-$

$M^{+\bullet} + \text{Solvent} \longrightarrow [MH]^+$

Dopant/Solvent assisted APPI

$D + h\nu \longrightarrow D^{+\bullet} + e^-$

$D^{+\bullet} + M + \text{Solvent} \longrightarrow [MH]^+ , [M\text{-}H]^-$

D = Photosensitizer: toluene, Acetone

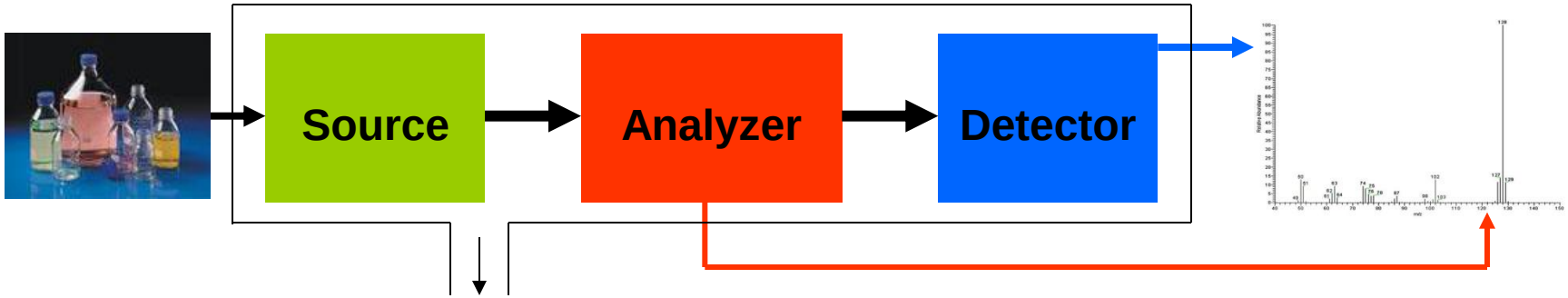
Atm. Pressure photo Ionization (APPI)

- Conjugated compounds
- Organometallic Complexes
- Conditions: Solvent. Flow rate, Temp, Dopant

	APCI	APPI
Sensitivity:	Pos >> Neg	Pos ~= Neg
Mass range	~1200 Da	~2500 Da
Aliphatic:	Yes	Limited
Conjugated:	Yes	Excellent
Organometallic:	Limited	Excellent
Conditions:	Sensitive	less sensitive

Solvent: Toluene, DCM, Hexane, MeCN, MeOH

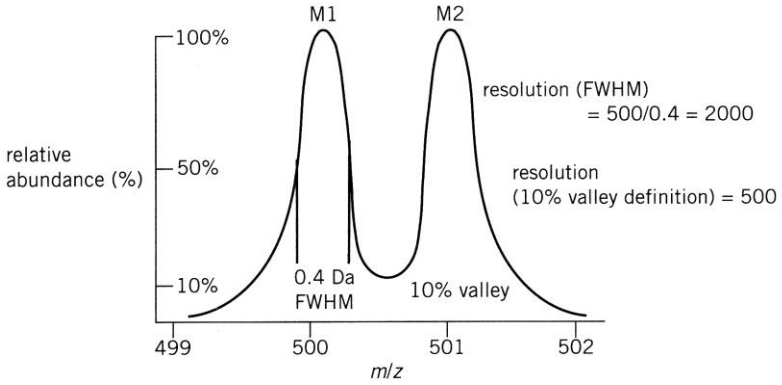
Analyzer



Analyzer: separate Ions

Mass range

Resolution: The Ability of a Mass Spectrometer to separate two masses (M1, M2) R=M/ΔM



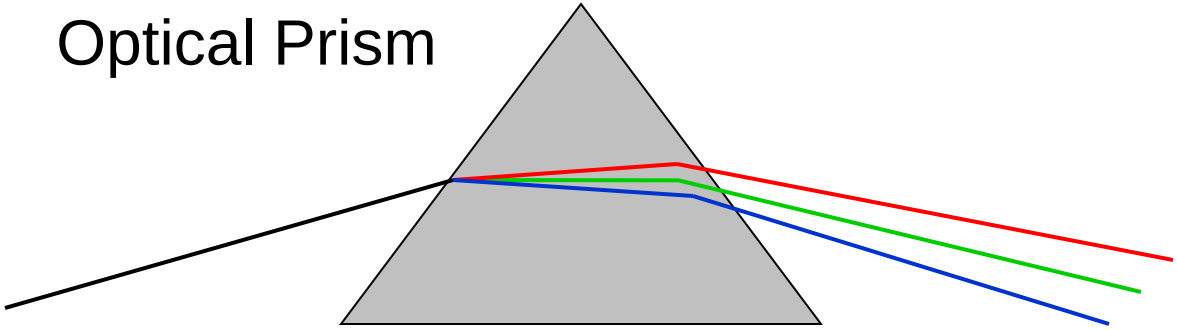
FWHM = full width at half maximum

Analyzer

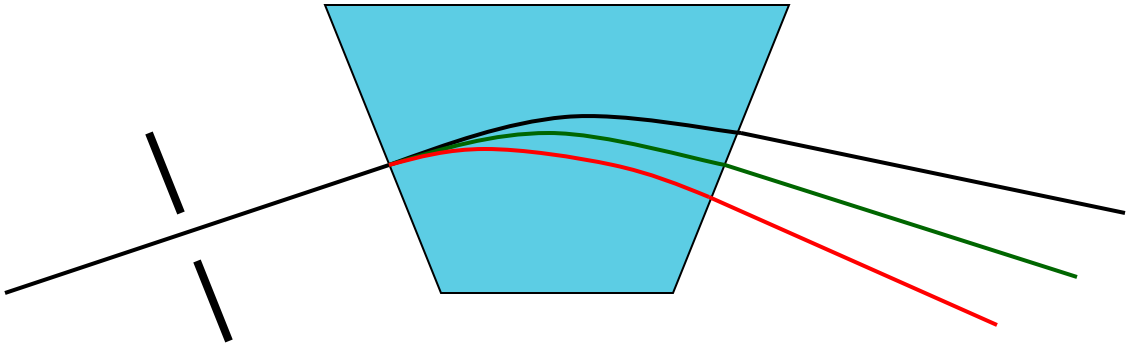
- Magnetic sector
- Quadrupole
- TOF
- Q-TOF

Magnetic Sector Analyzer

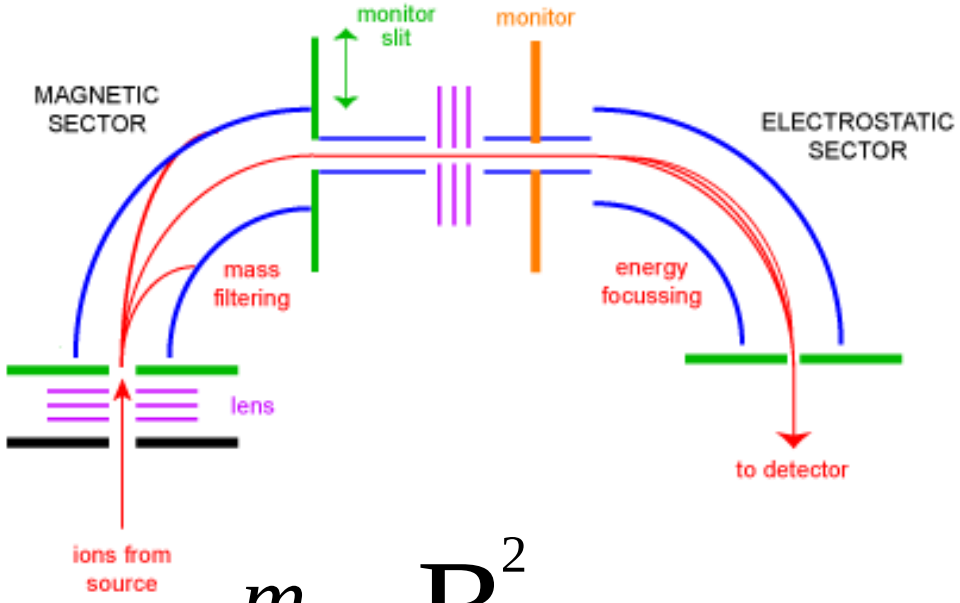
Optical Prism



Magnetic sector

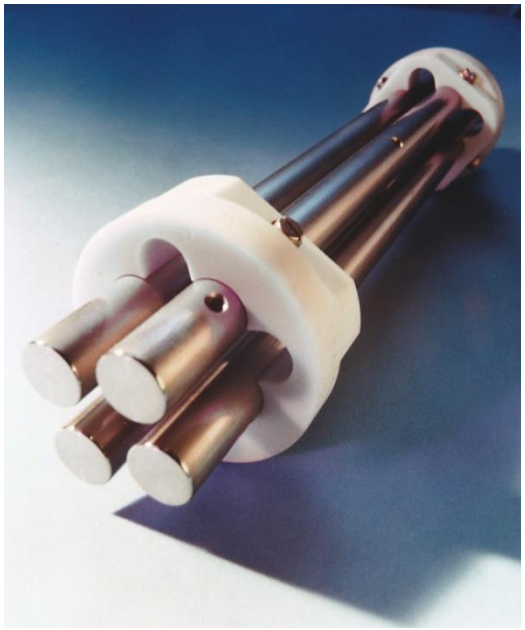


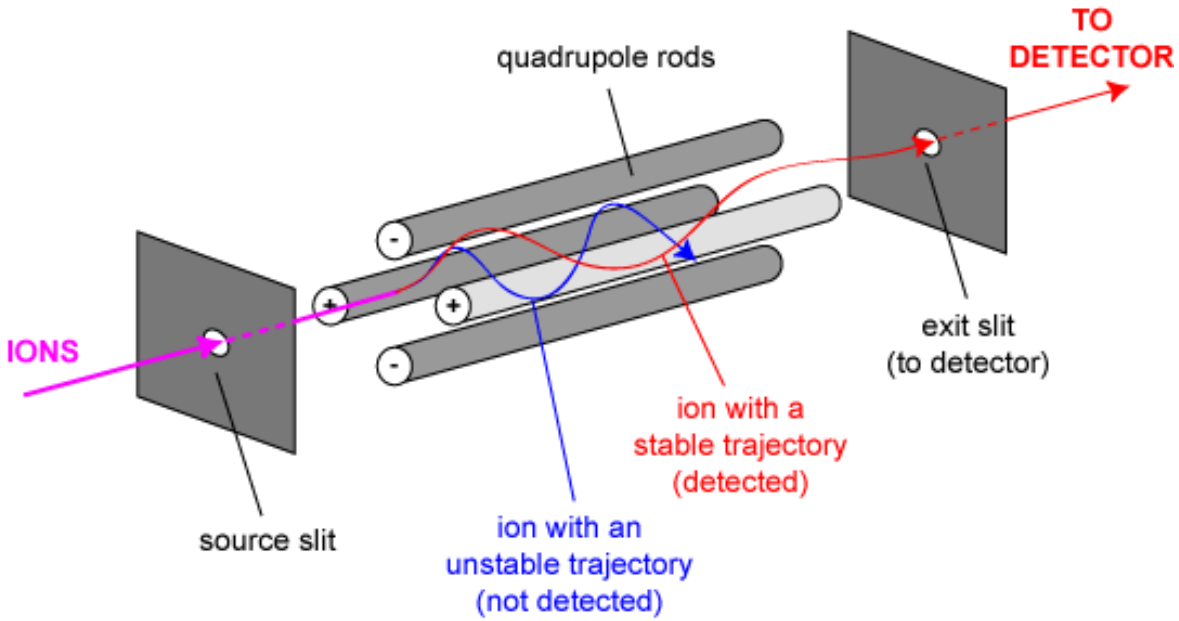
Magnetic Sector Analyzer



$$\frac{m}{z} = \frac{B^2}{V} * K$$

Quadropole





- Combination of DC and RF on the rods
- Ions moving into the Quad oscillate depending on m/z
- Only one m/z can pass
- A mass spectrum can be obtained by scanning the RF

Time Of Flight (TOF)

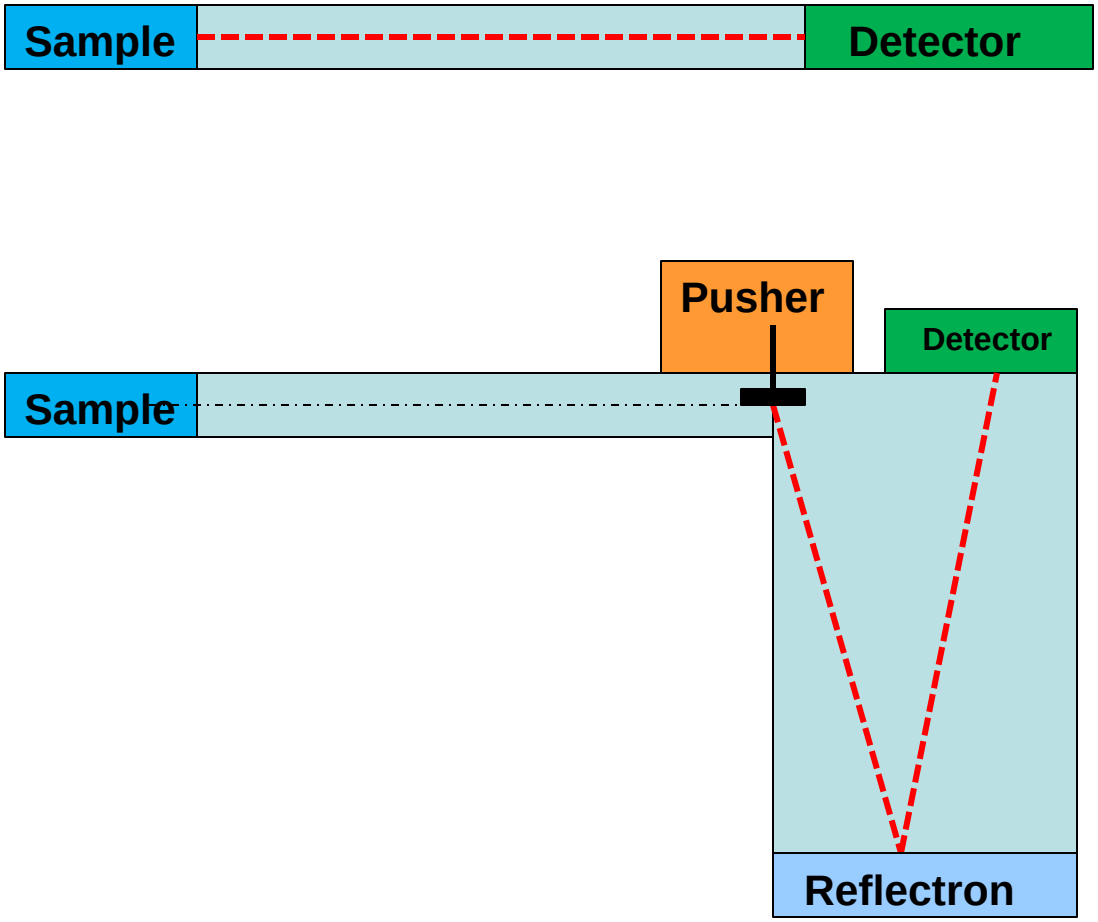
$$E_p = E_k$$

$$zeV = \frac{1}{2}mv^2$$

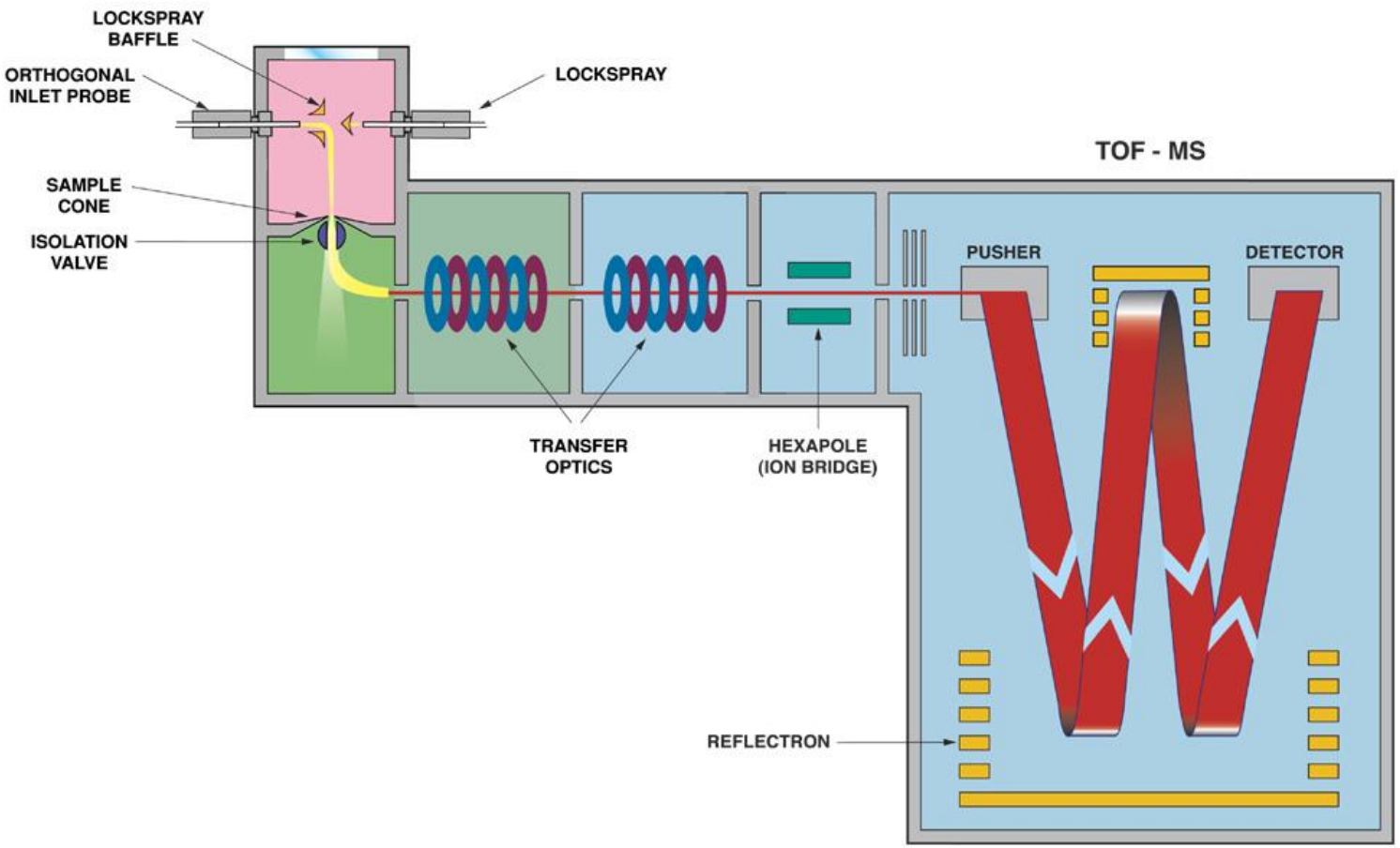
$$v = \frac{L}{t}$$

$$t = L\sqrt{\frac{m}{2zeV}}$$

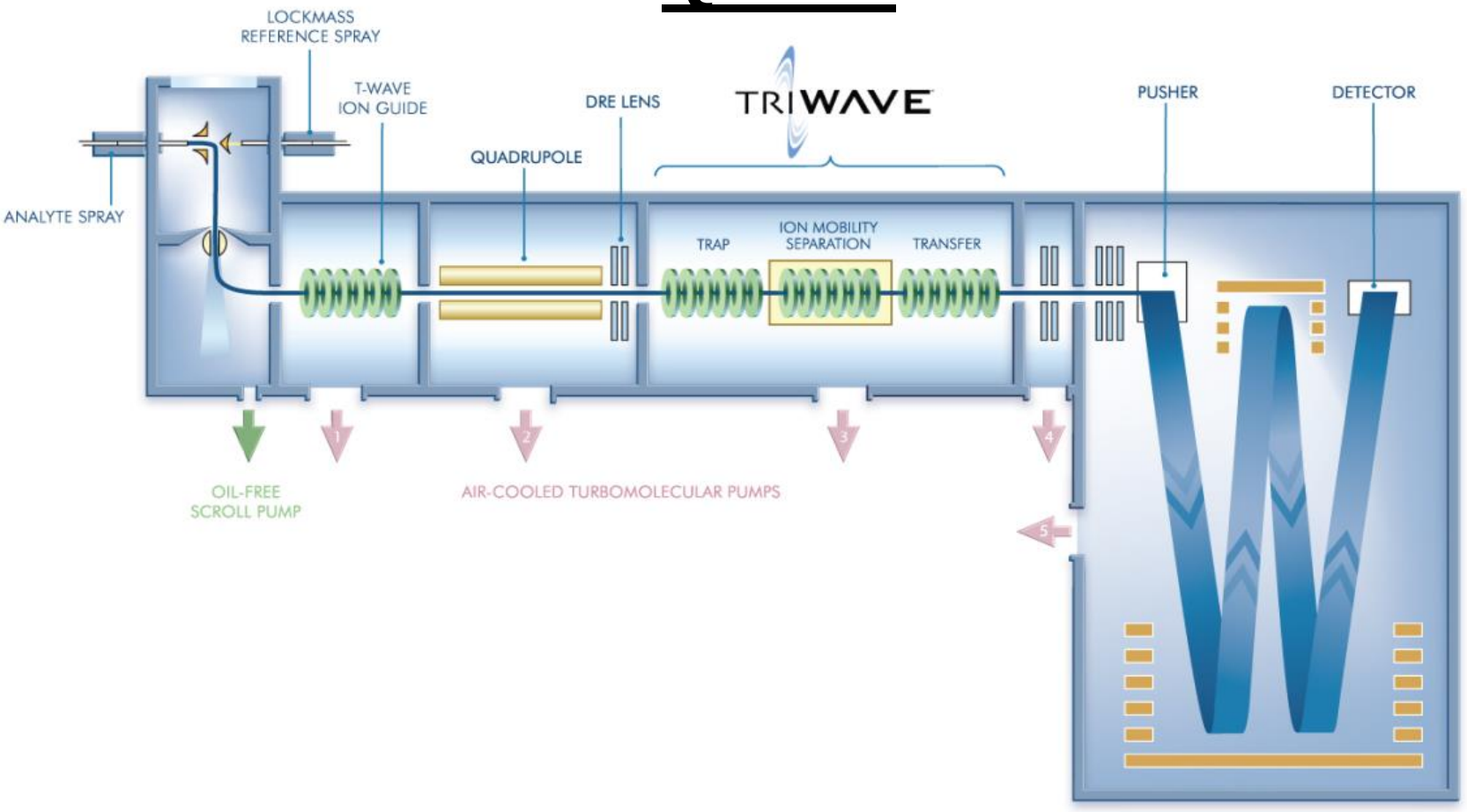
$$\frac{m}{z} = \frac{t^2}{2eVL^2}$$



Time Of Flight (TOF)



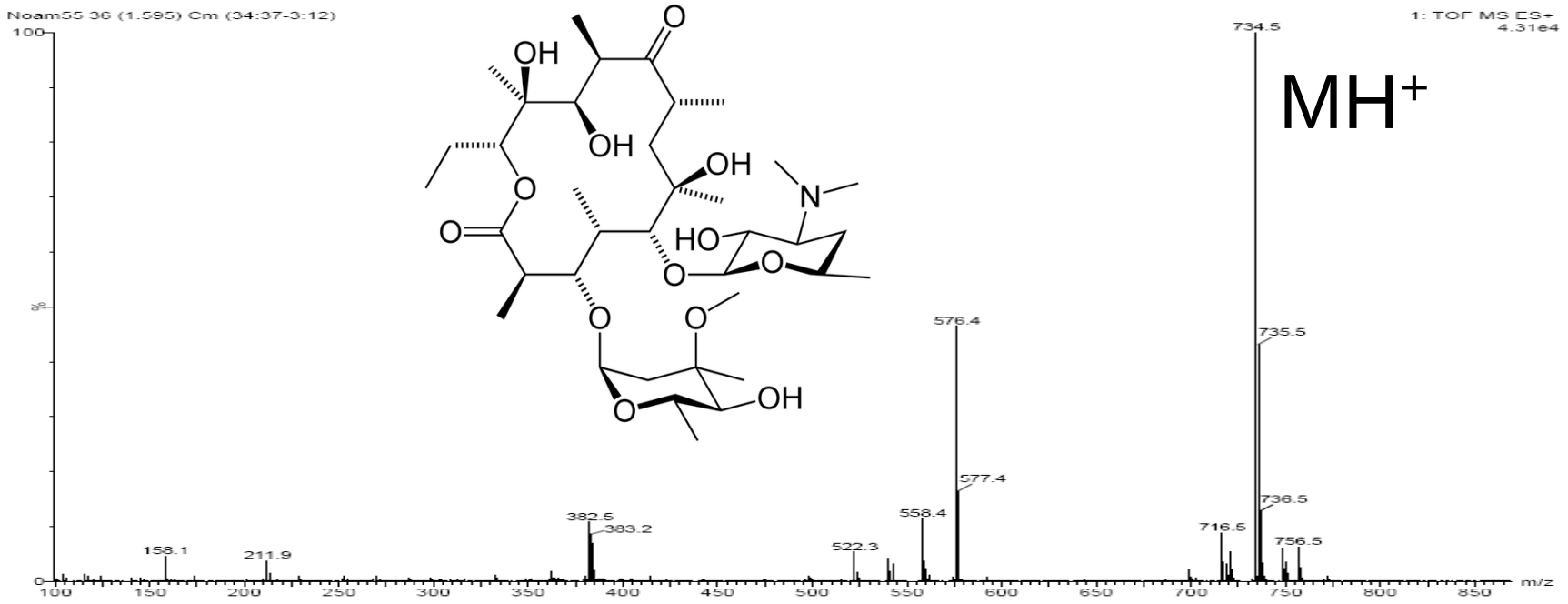
Q-TOF


LOCKMASS
REFERENCE SPRAY
T-WAVE
ION GUIDE
DRE LENS
TRIWAVE
PUSHER
DETECTOR
QUADRUPOLE
ANALYTE SPRAY
TRAP
ION MOBILITY
SEPARATION
TRANSFER
1
2
3
4
5
OIL-FREE
SCROLL PUMP
AIR-COOLED TURBOMOLECULAR PUMPS

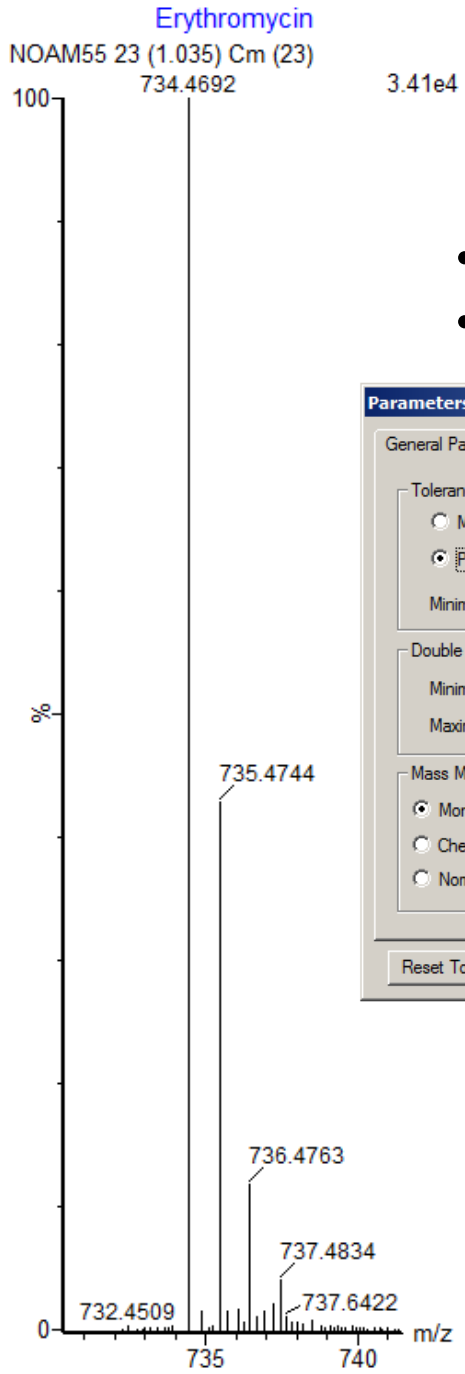
MS Analysis

- MW
- Elemental composition (HRMS)
- Charge state
- Isotope pattern
- Adducts
- POS vs NEG
- Fragmentation

MS

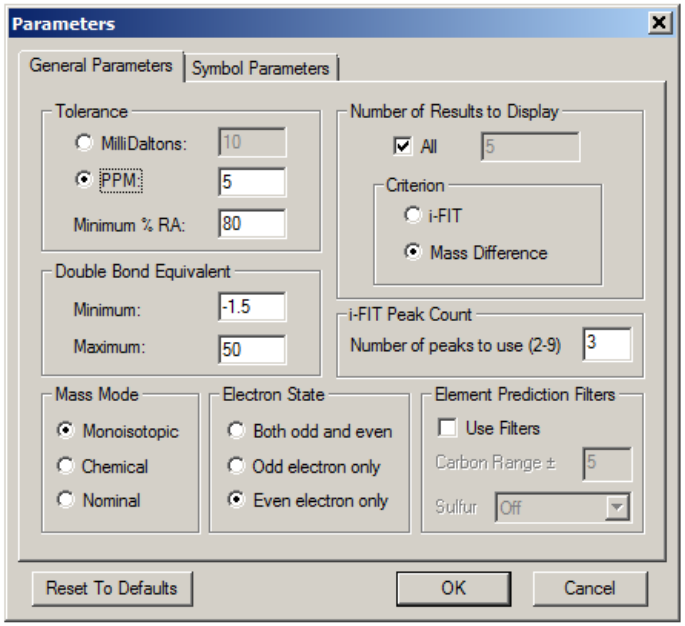


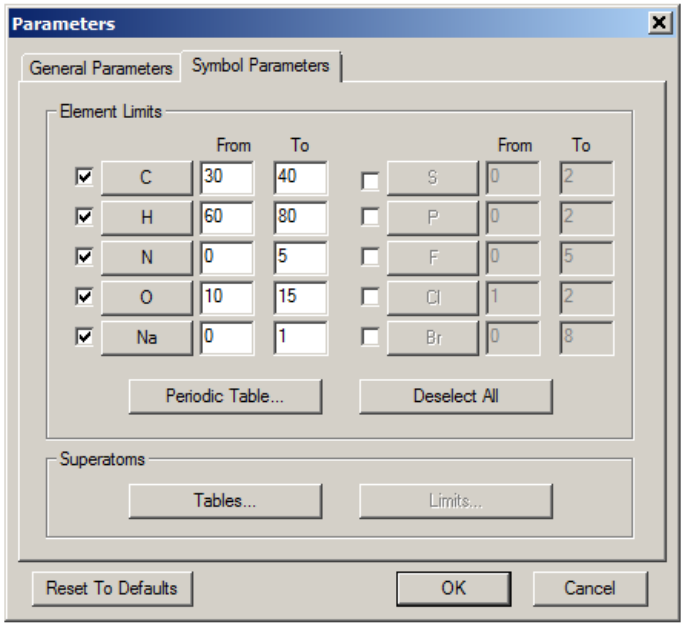
Erythromycin $C_{37}H_{67}NO_{13}$ = 733 gr/mol



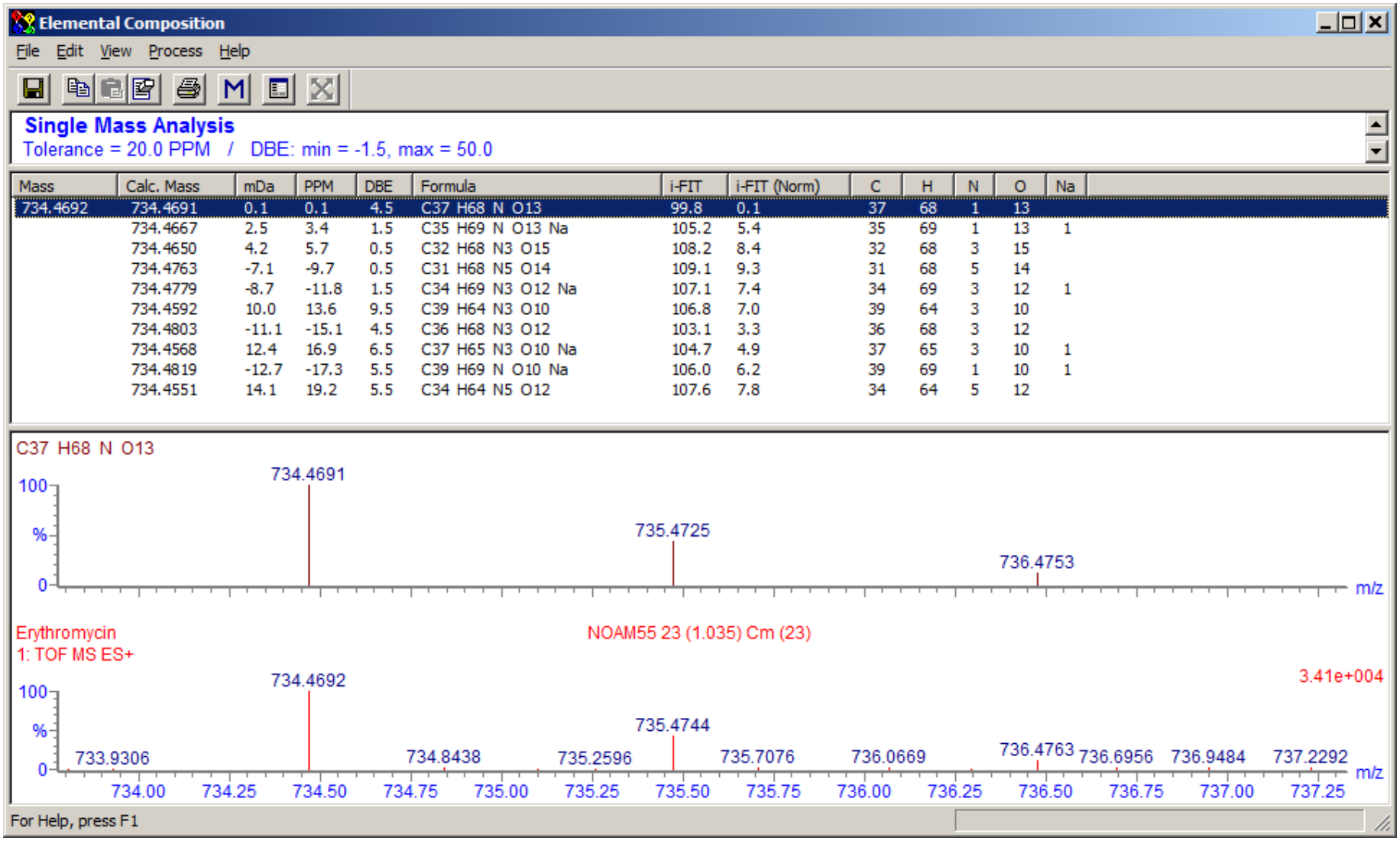
HRMS

- Resolution
- Mass Accuracy

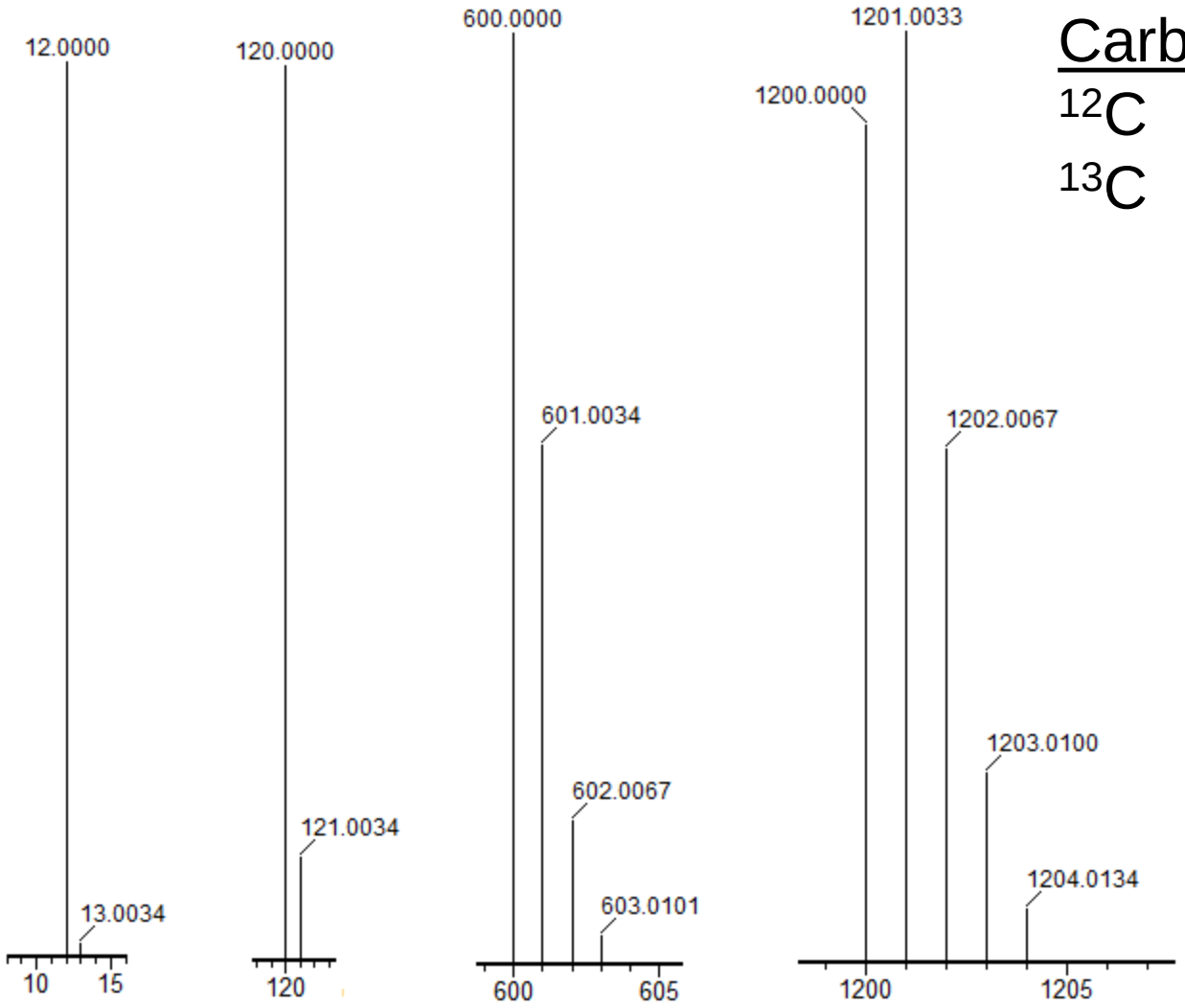




Elemental composition (HRMS)



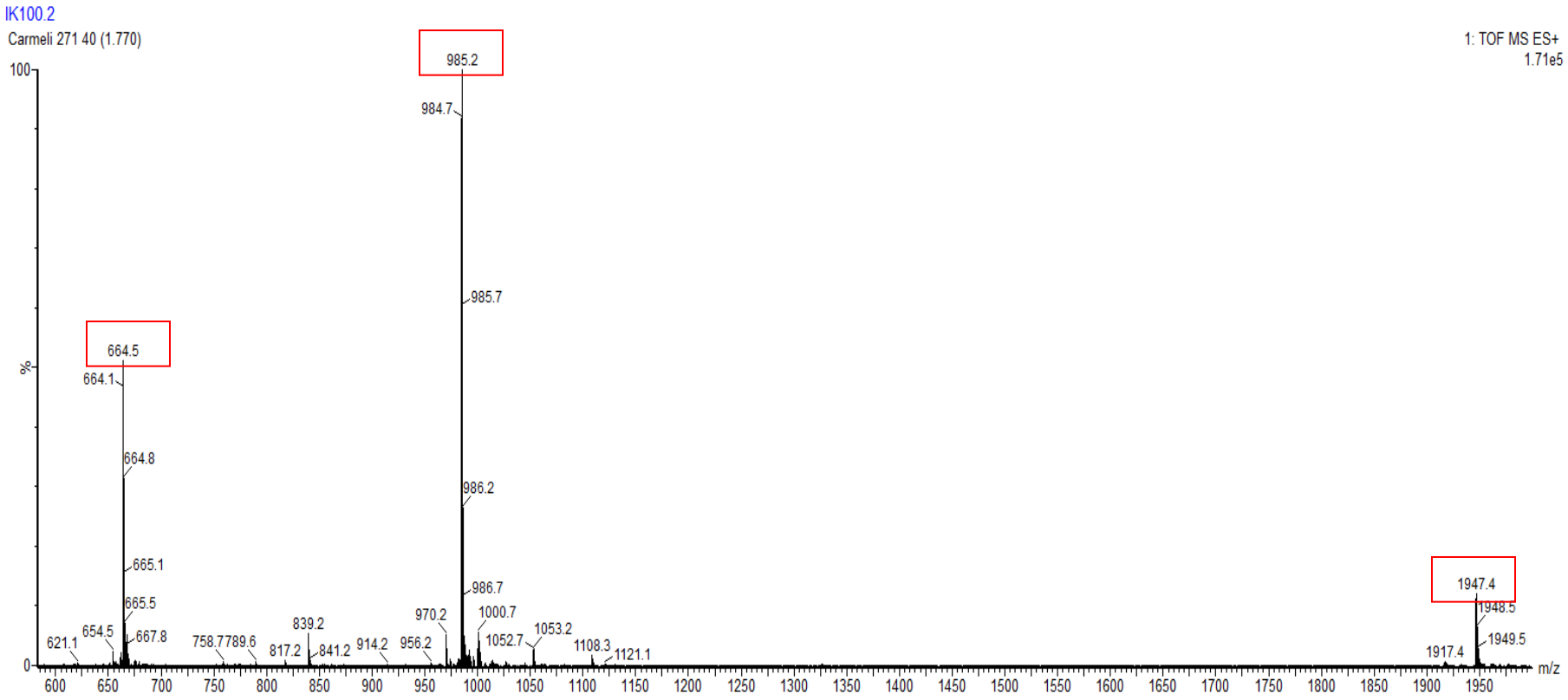
MS Analysis



Carbon Isotope

^{12}C	12.00000	98.9%
^{13}C	13.00335	1.1%

ESI


IK100.2
Carmeli 271 40 (1.770)
1: TOF MS ES+
1.71e5
100
%
0
985.2
984.7
985.7
664.5
664.1
664.8
986.2
665.1
665.5
986.7
667.8
654.5
621.1
758.7
789.6
839.2
817.2
841.2
914.2
956.2
970.2
1000.7
1052.7
1053.2
1108.3
1121.1
1947.4
1948.5
1949.5
1917.4
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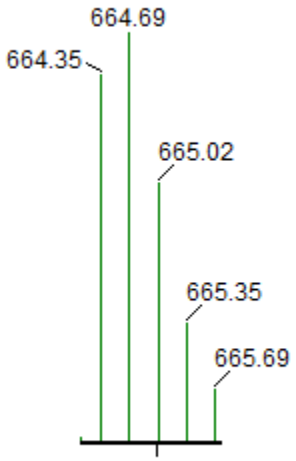
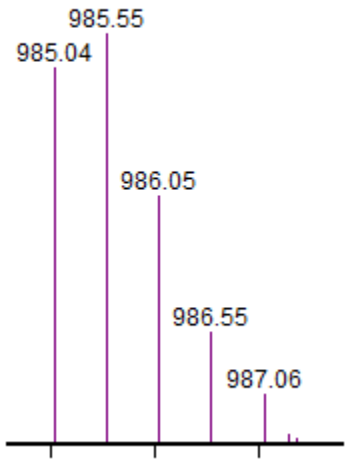
1500
1550
1600
1650
1700
1750
1800
1850
1900
1950
m/z

ESI

z=3
0.33Da

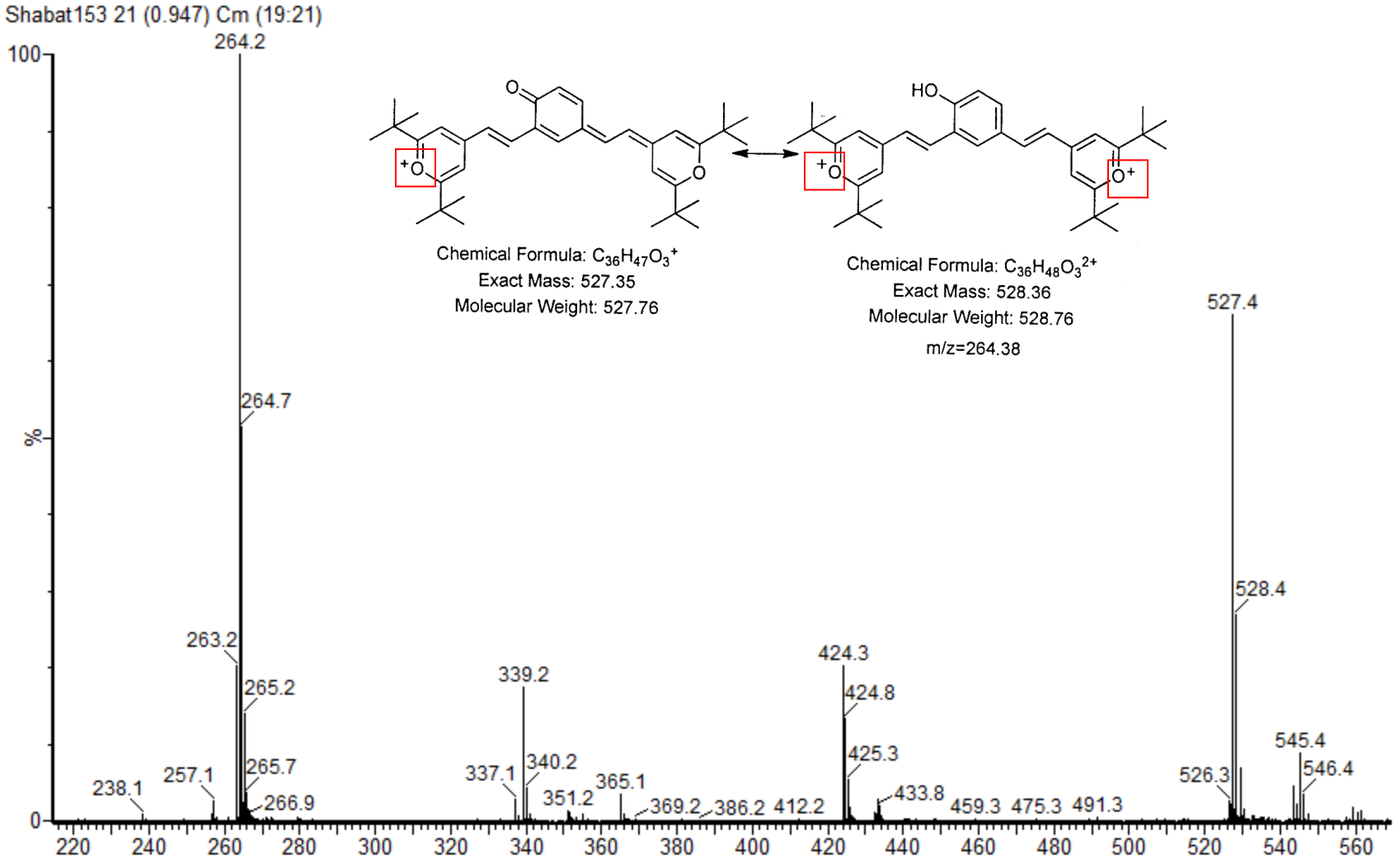


z=2
0.50Da

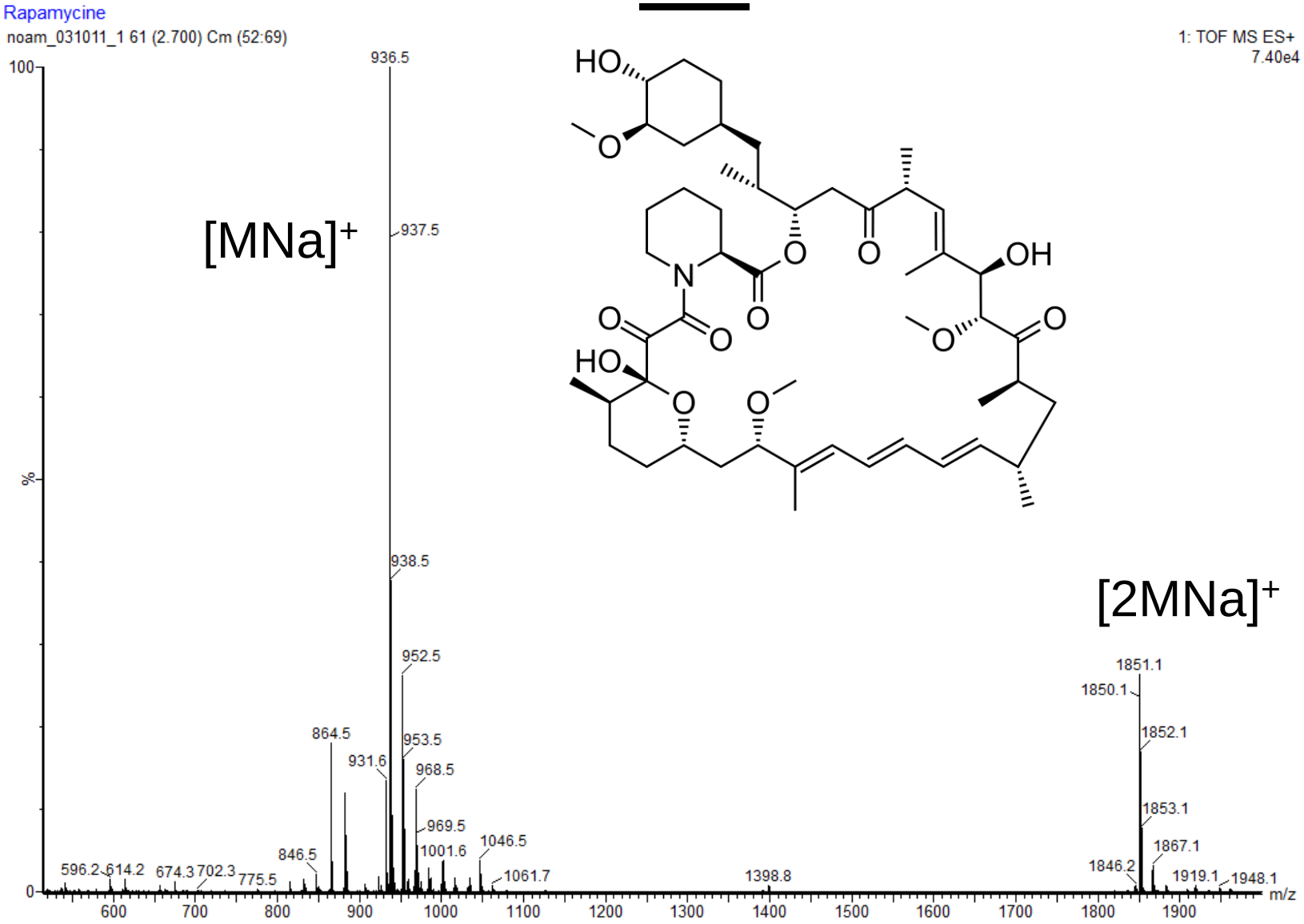


z=1
1.0 Da

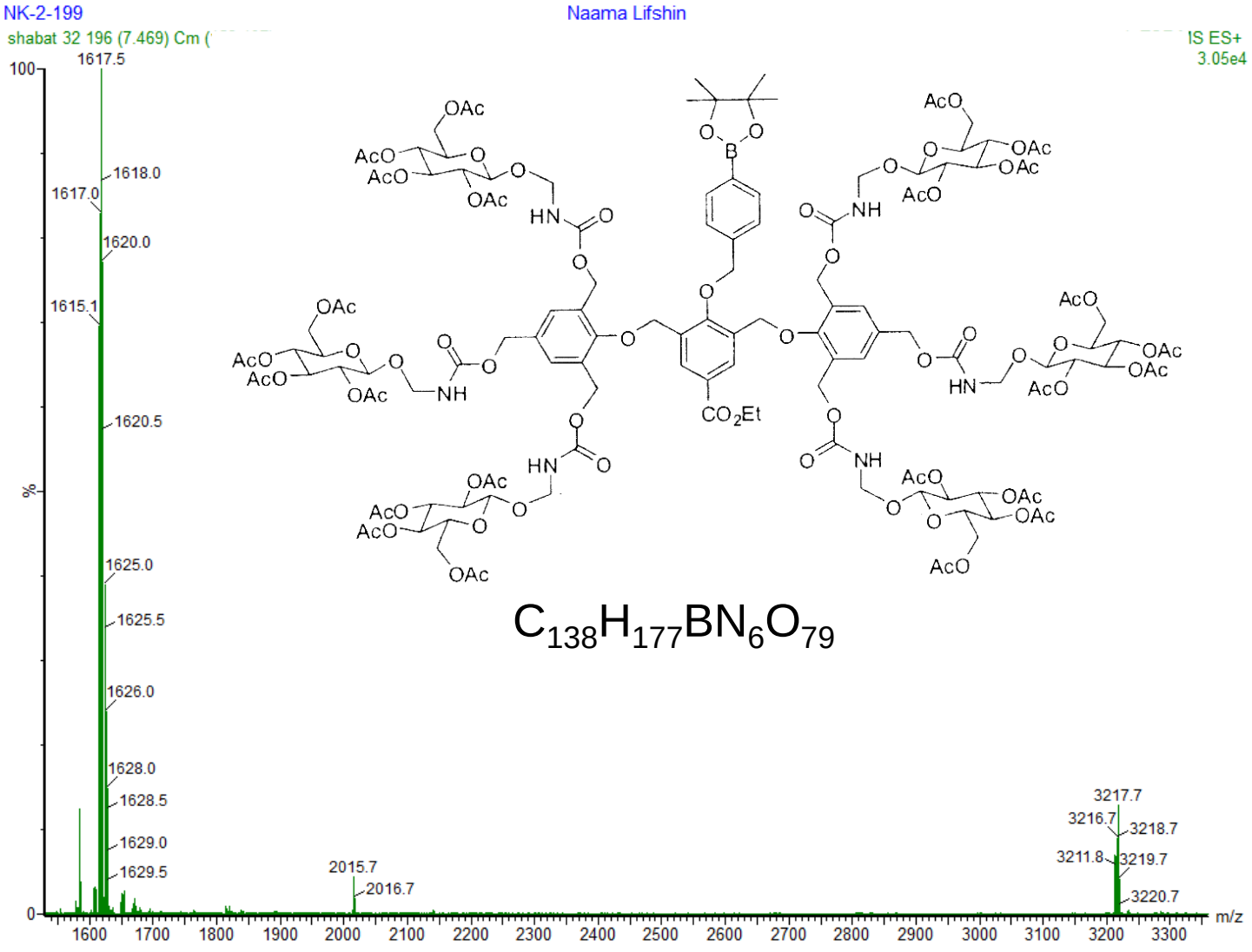
ESI


Shabat153 21 (0.947) Cm (19:21)
100
%
0
264.2
264.7
263.2
265.2
265.7
266.9
238.1
257.1
339.2
337.1
340.2
351.2
365.1
369.2
386.2
412.2
424.3
424.8
425.3
433.8
459.3
475.3
491.3
527.4
528.4
526.3
545.4
546.4
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
520
540
560
Chemical Formula: C36H47O3+
Exact Mass: 527.35
Molecular Weight: 527.76
Chemical Formula: C36H48O3 2+
Exact Mass: 528.36
Molecular Weight: 528.76
m/z=264.38

ESI


Rapamycine
noam_031011_1 61 (2.700) Cm (52:69)
1: TOF MS ES+
7.40e4
[MNa]+
[2MNa]+
100
%
0
936.5
937.5
938.5
952.5
953.5
968.5
969.5
1001.6
1046.5
1061.7
1398.8
864.5
931.6
846.5
596.2
614.2
674.3
702.3
775.5
1851.1
1850.1
1852.1
1853.1
1867.1
1846.2
1919.1
1948.1
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
m/z

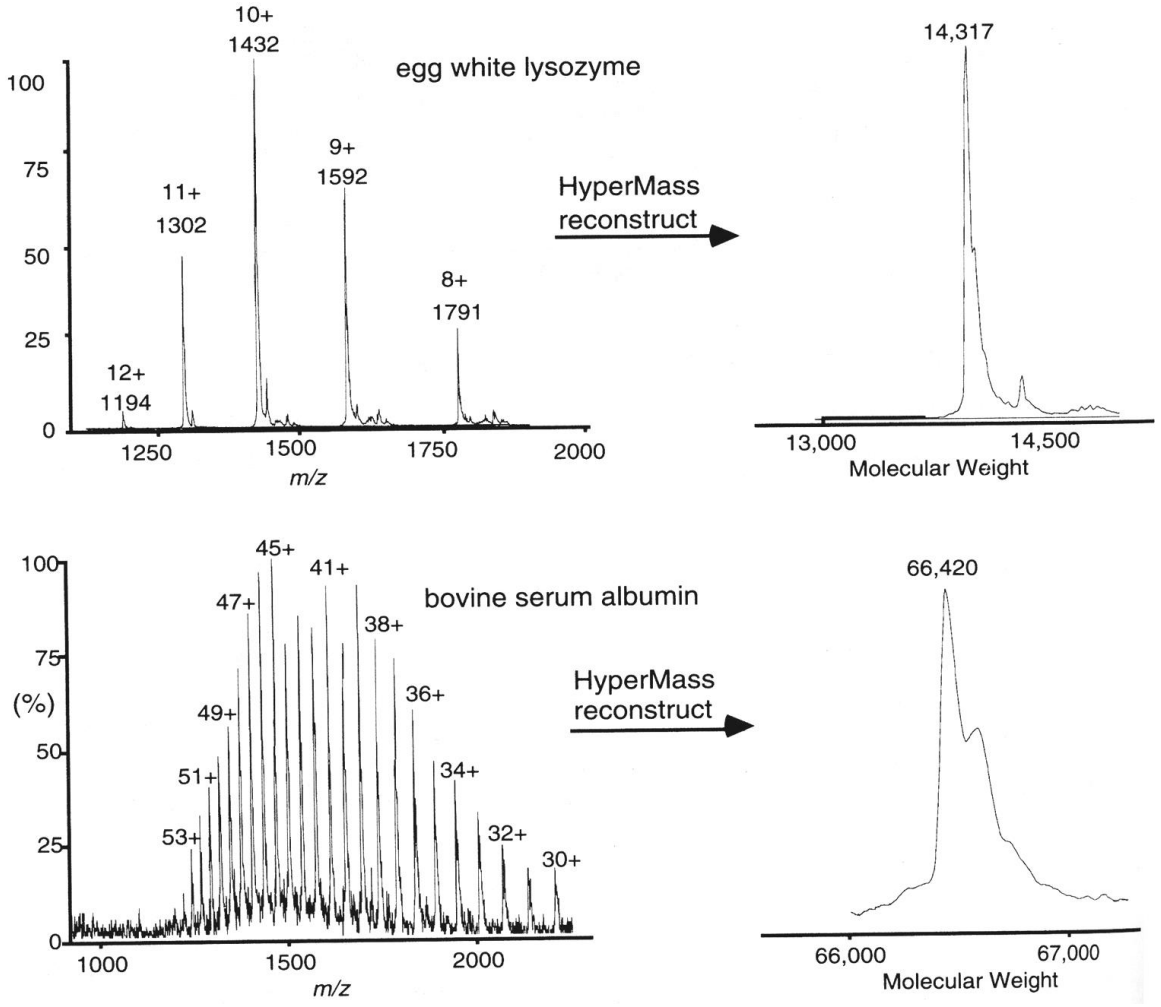
ESI



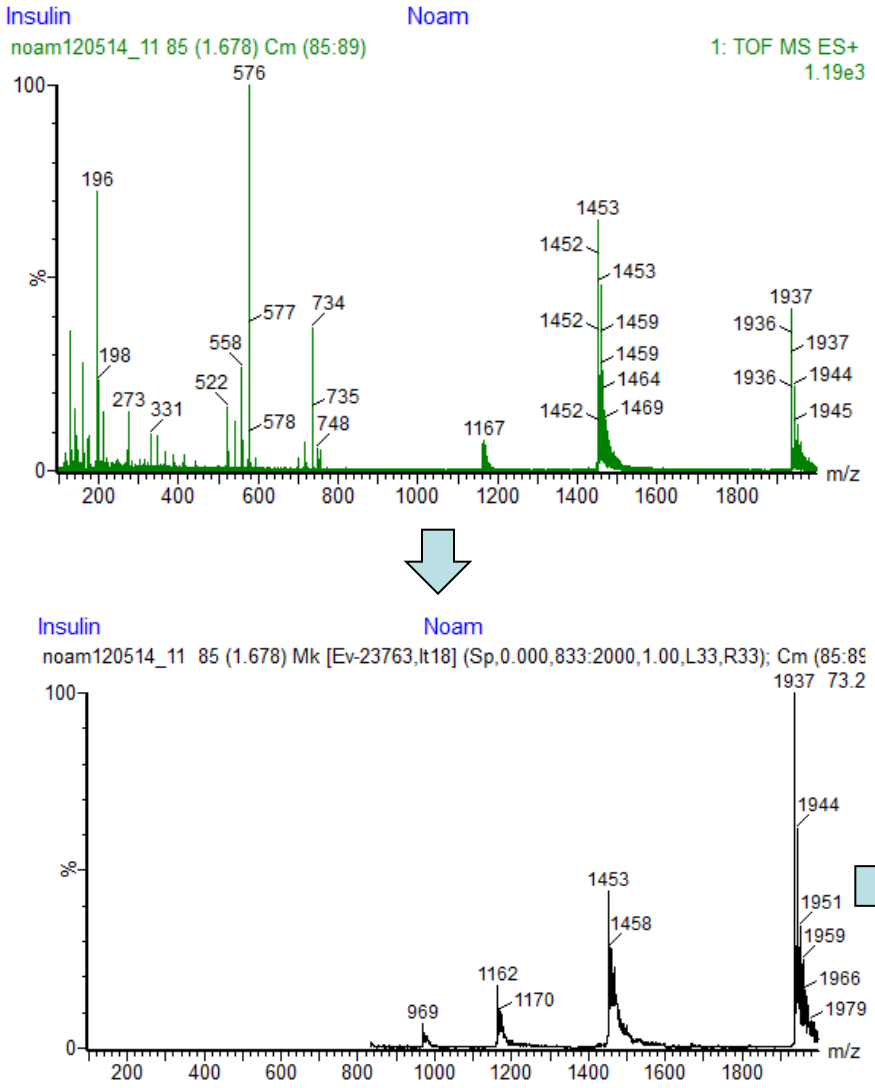
$C_{138}H_{177}BN_6O_{79}$

ESI

Protein Analysis



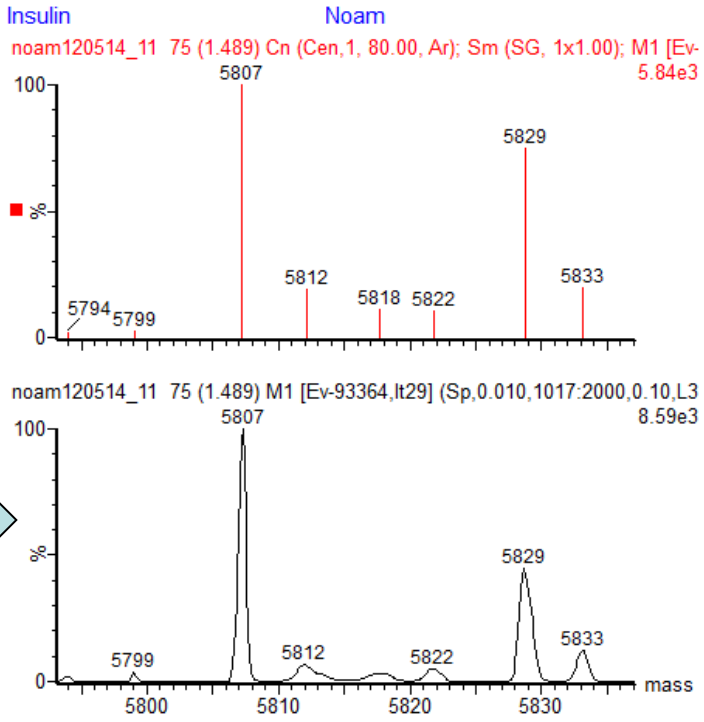
ESI



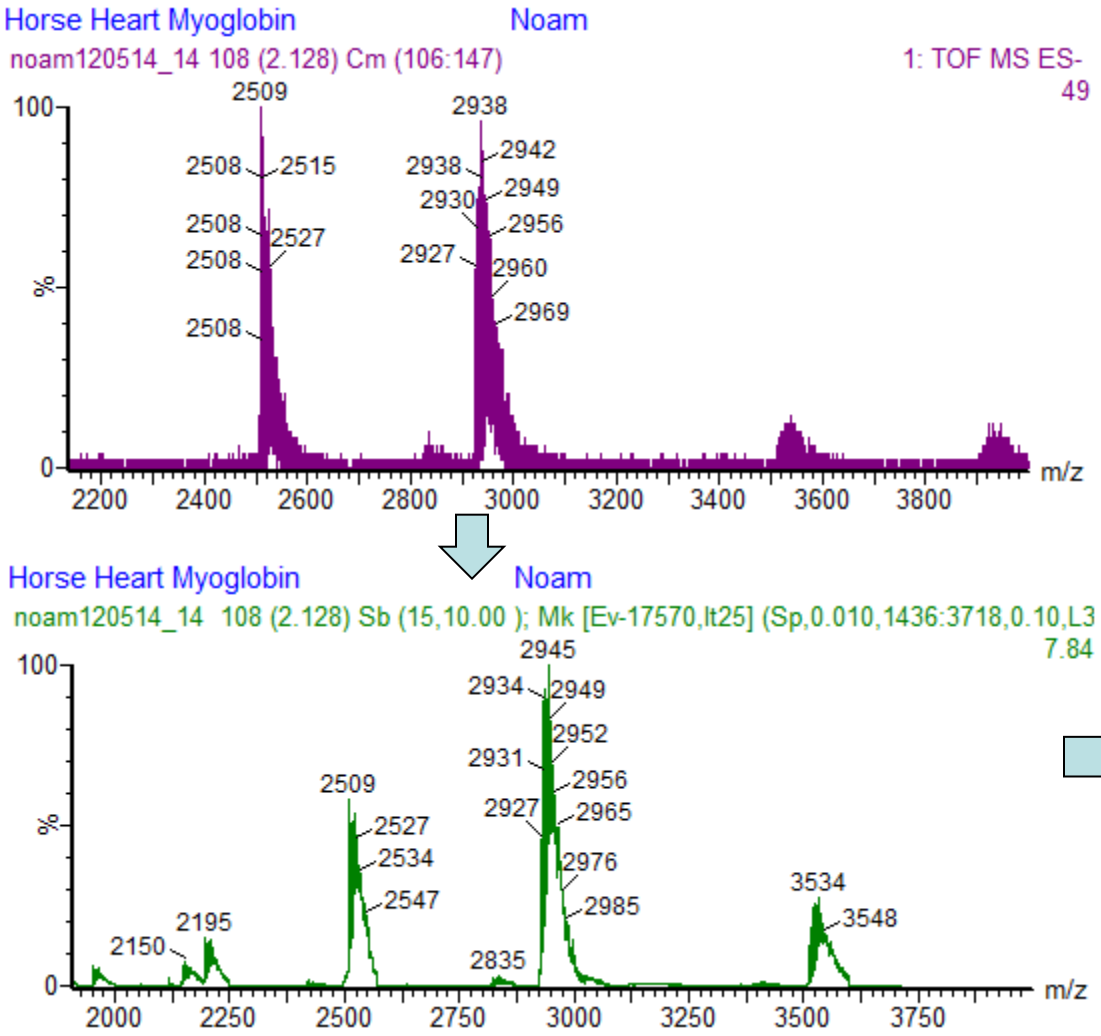
Human Insulin

$C_{257}H_{383}N_{65}O_{77}S_6$

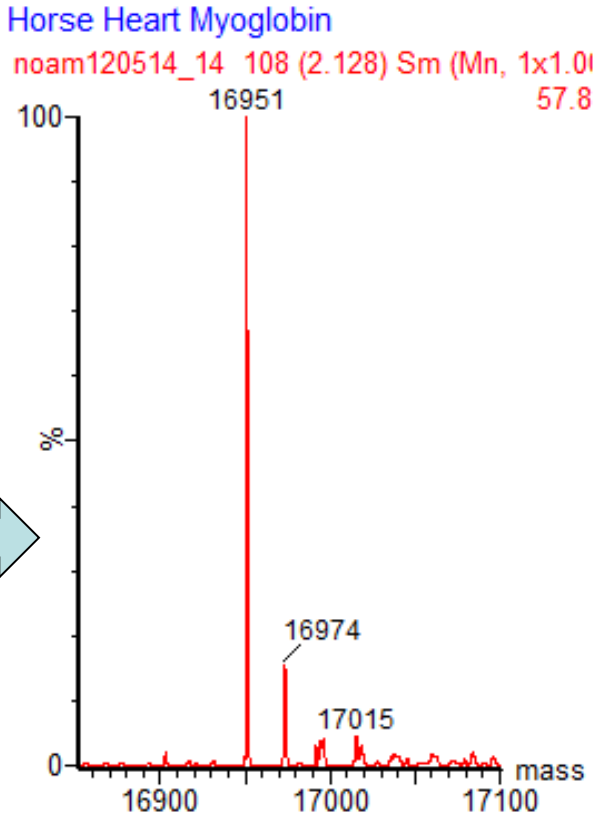
5807.57



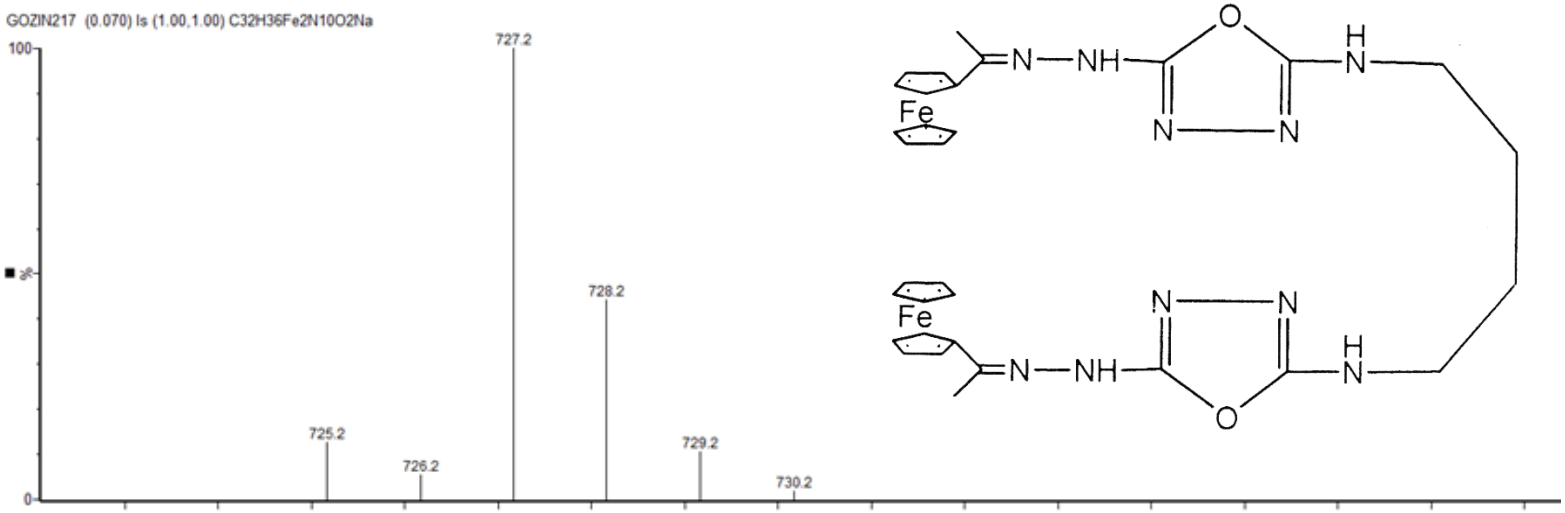
ESI

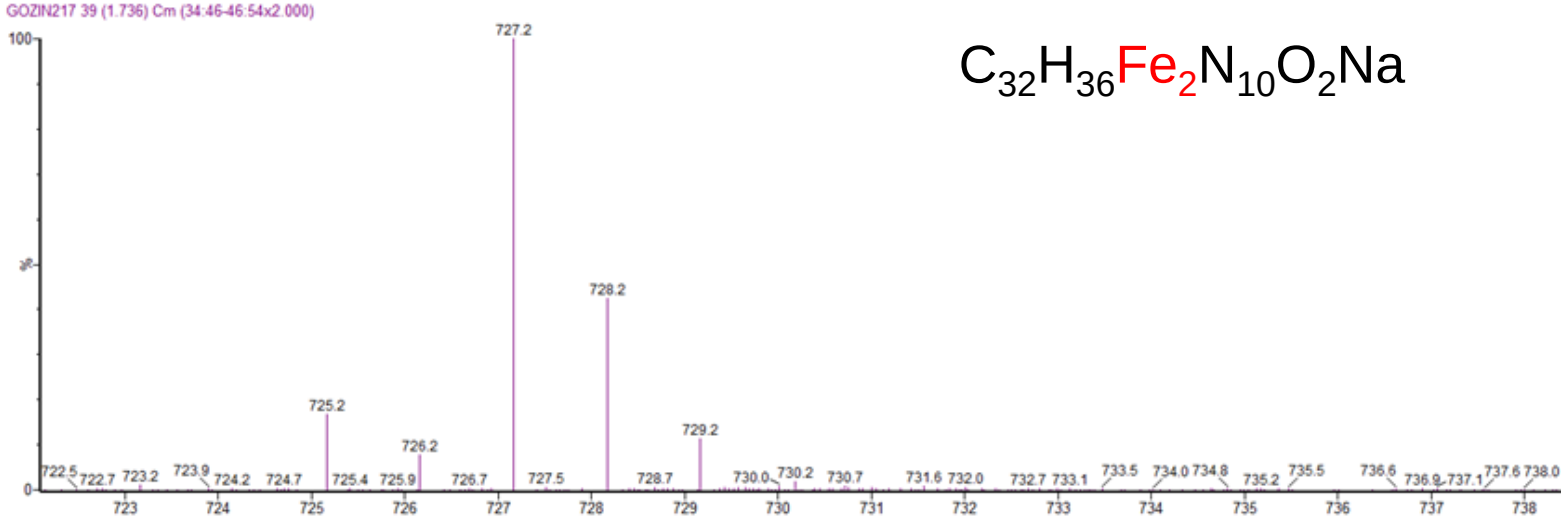


Horse myoglobin
16,951.49



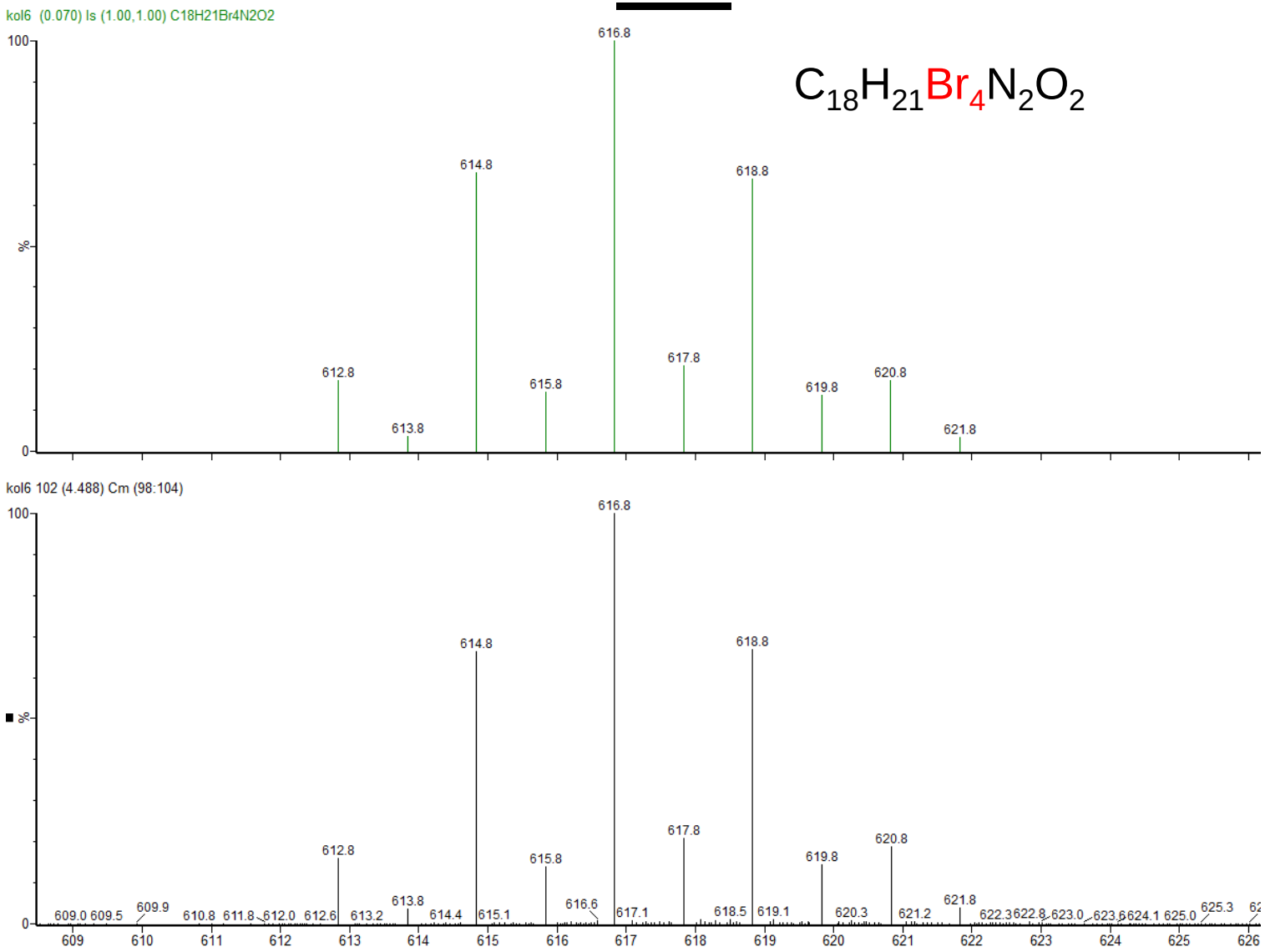
ESI





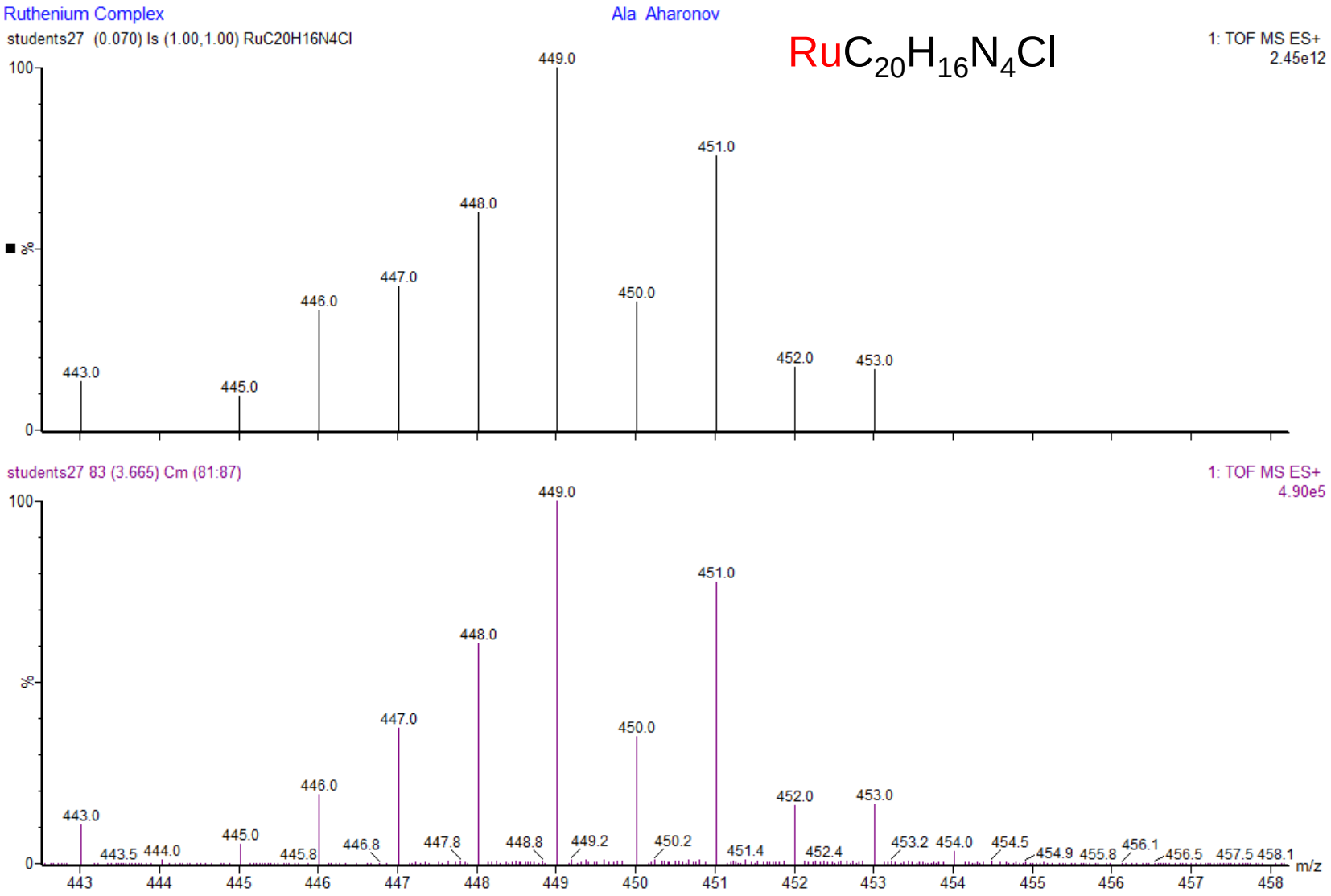
$C_{32}H_{36}Fe_2N_{10}O_2Na$

ESI



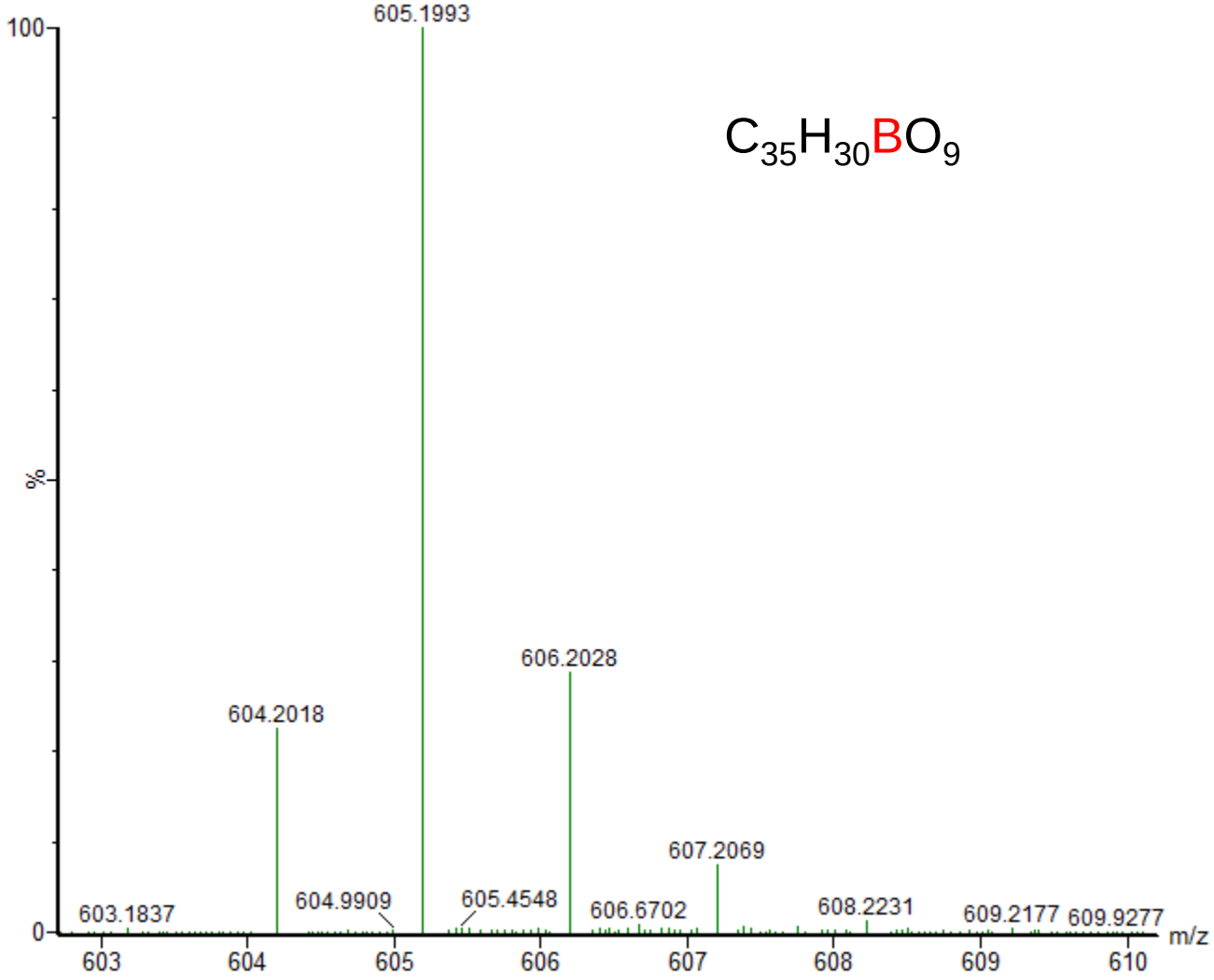
$C_{18}H_{21}Br_4N_2O_2$

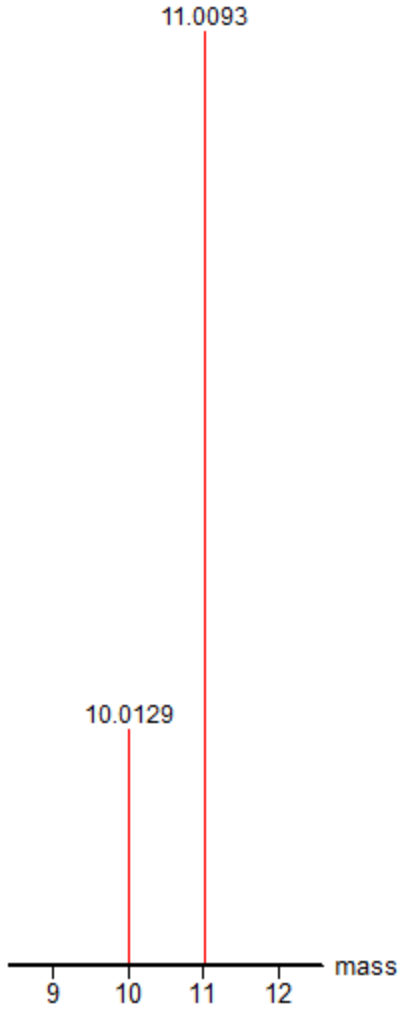
ESI


Ruthenium Complex
Ala Aharonov
$RuC_{20}H_{16}N_4Cl$
students27 (0.070) Is (1.00,1.00) RuC20H16N4Cl
1: TOF MS ES+
2.45e12
100
%
0
443.0
445.0
446.0
447.0
448.0
449.0
450.0
451.0
452.0
453.0
students27 83 (3.665) Cm (81:87)
1: TOF MS ES+
4.90e5
443.5
444.0
445.8
446.8
447.8
448.8
449.2
450.2
451.4
452.4
453.2
454.0
454.5
454.9
455.8
456.1
456.5
457.5
458.1
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
m/z

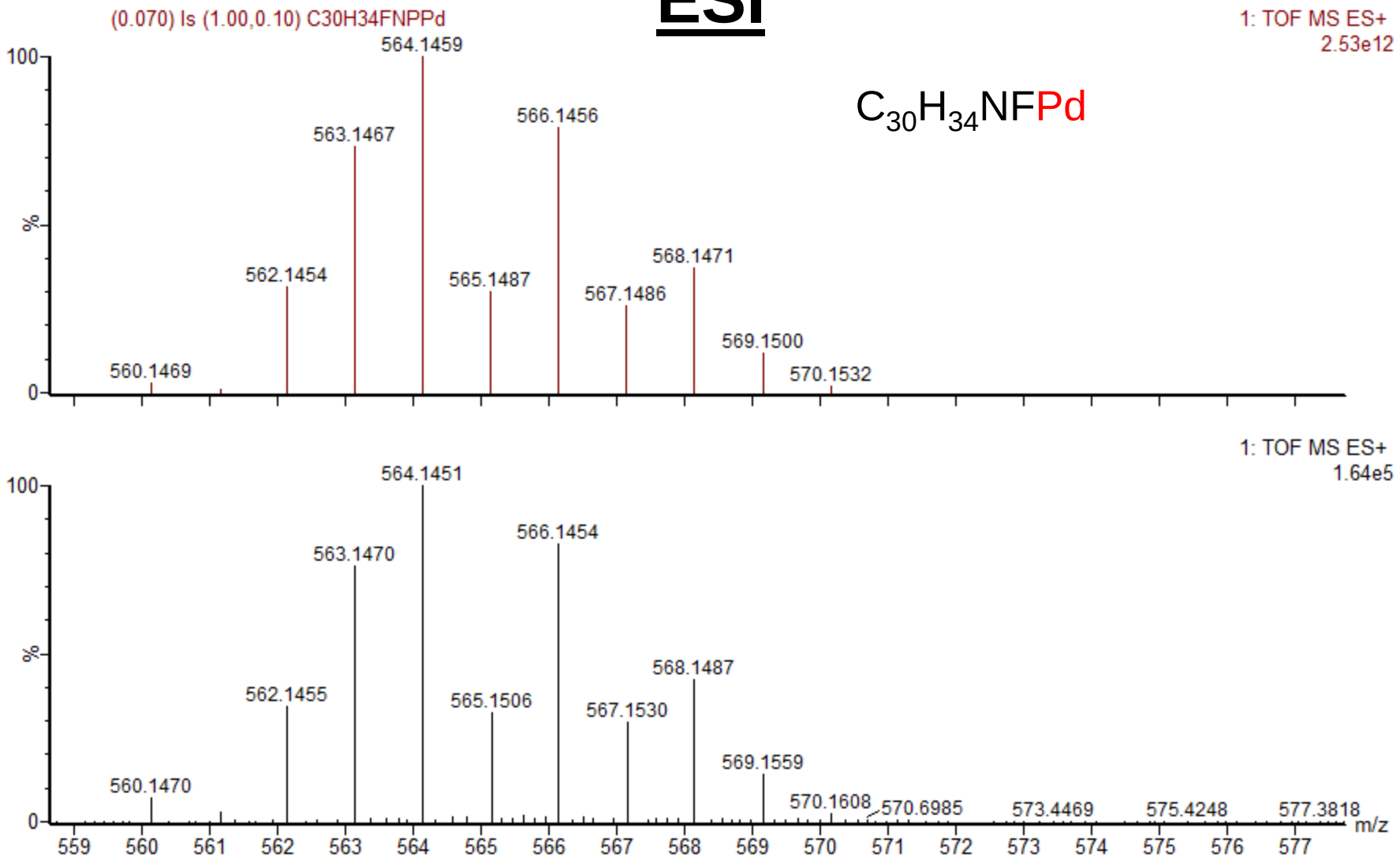
ESI

$C_{35}H_{30}BO_9$





ESI

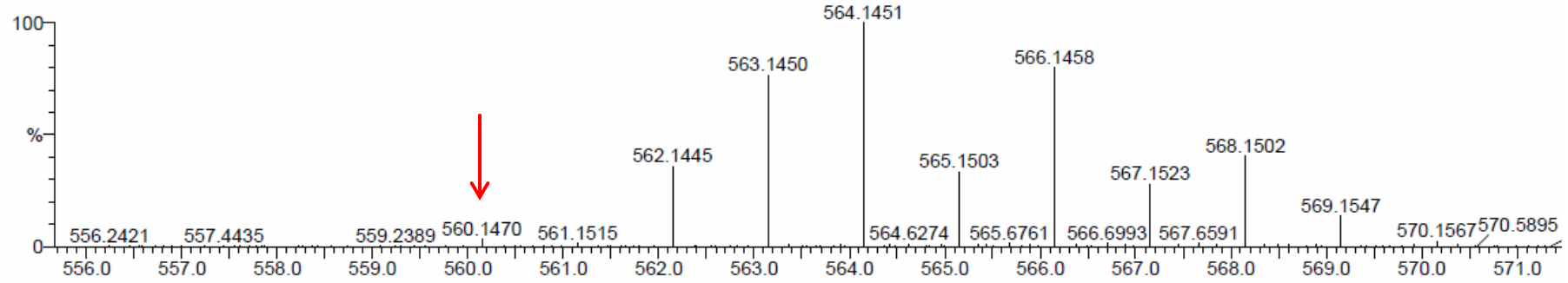


$C_{30}H_{34}NFPd$

ESI

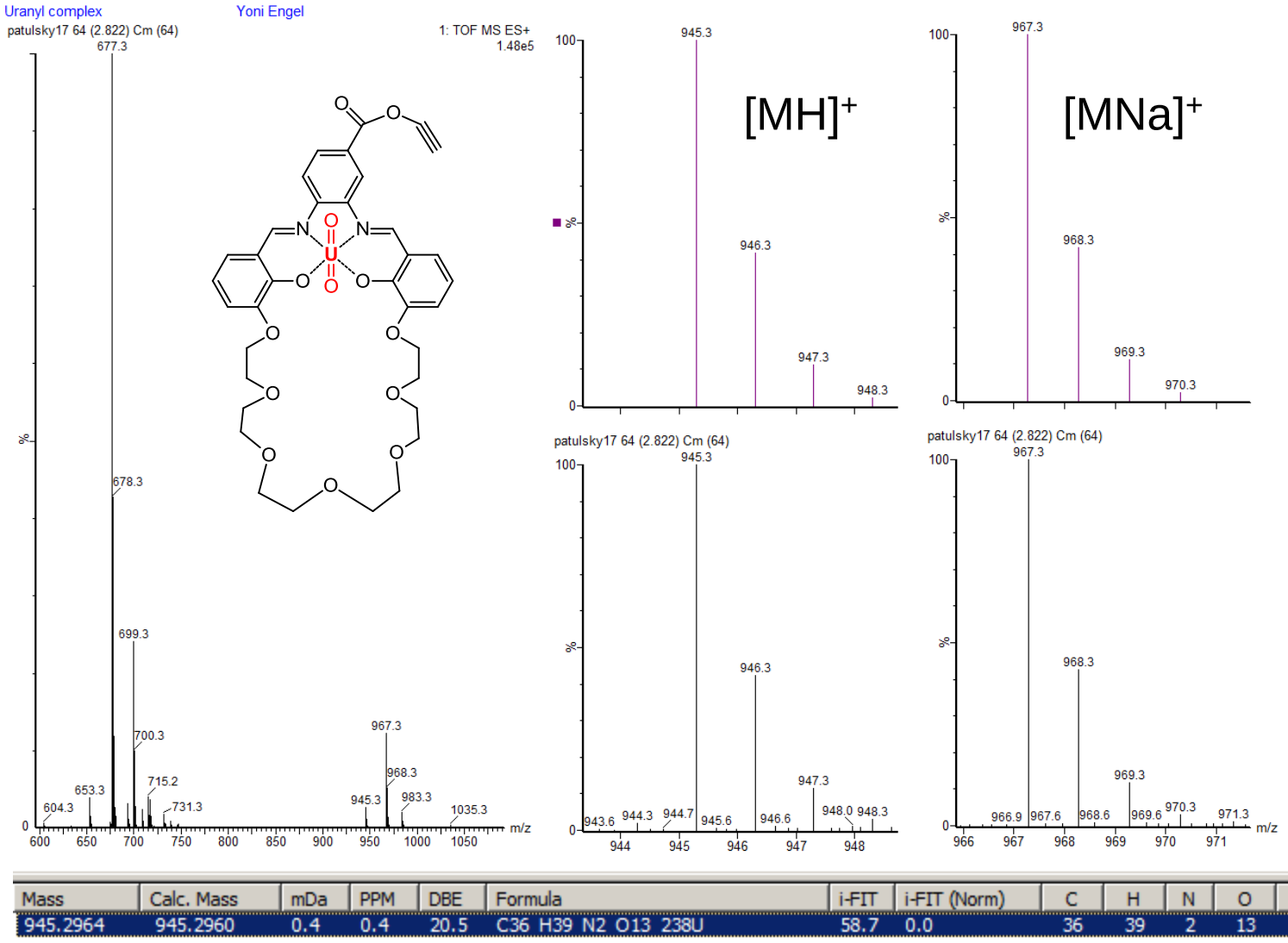
Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
1027 formula(e) evaluated with 4 results within limits (up to 10 closest results for each mass)
Elements Used:
C: 25-40 H: 30-40 N: 0-5 O: 0-5 F: 0-5 P: 0-2 102Pd: 0-1



Minimum:				-1.5			
Maximum:		10.0	2.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
560.1470	560.1469	0.1	0.2	14.5	116.5	0.6	C30 H34 N F P 102Pd
	560.1475	-0.5	-0.9	11.5	118.1	2.1	C26 H31 N3 O F3 102Pd
	560.1464	0.6	1.1	15.5	117.6	1.6	C29 H30 N3 F2 102Pd
	560.1480	-1.0	-1.8	10.5	118.1	2.1	C27 H35 N O F2 P 102Pd

ESI



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT (Norm)	C	H	N	O	238U
945.2964	945.2960	0.4	0.4	20.5	C36 H39 N2 O13 238U	58.7	0.0	36	39	2	13	1

LCMS

Liquid Chromatography - Mass Spectrometry
HPLC
UPLC
MS

Sample

UV Detector

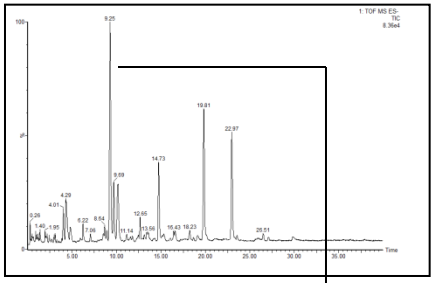
MS Detector

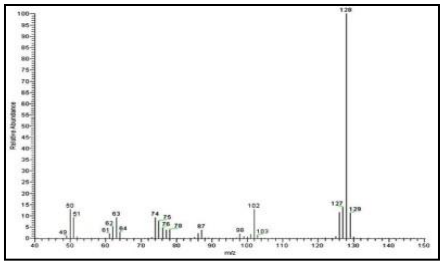
Column

Autosampler

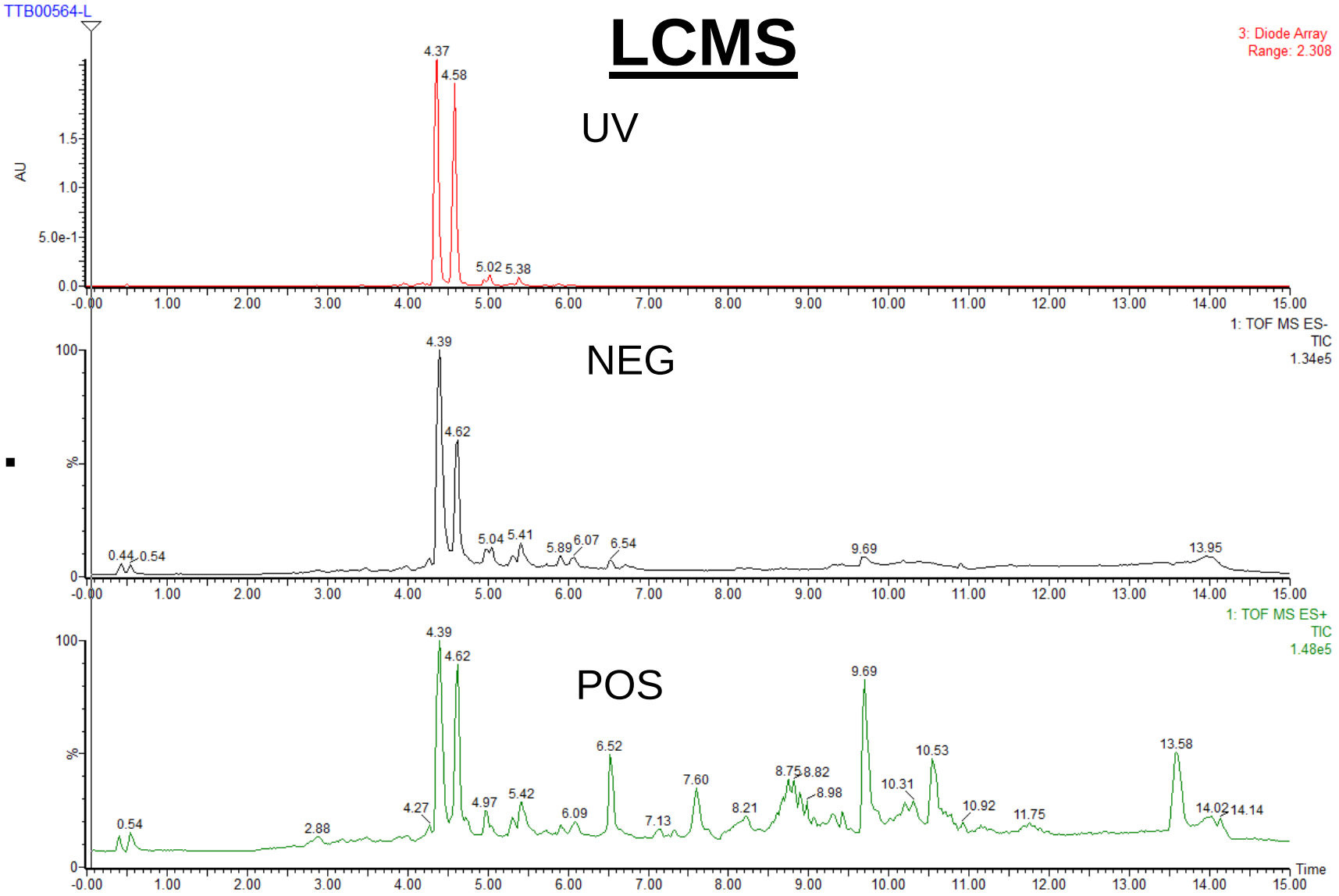
Pump

Solvents

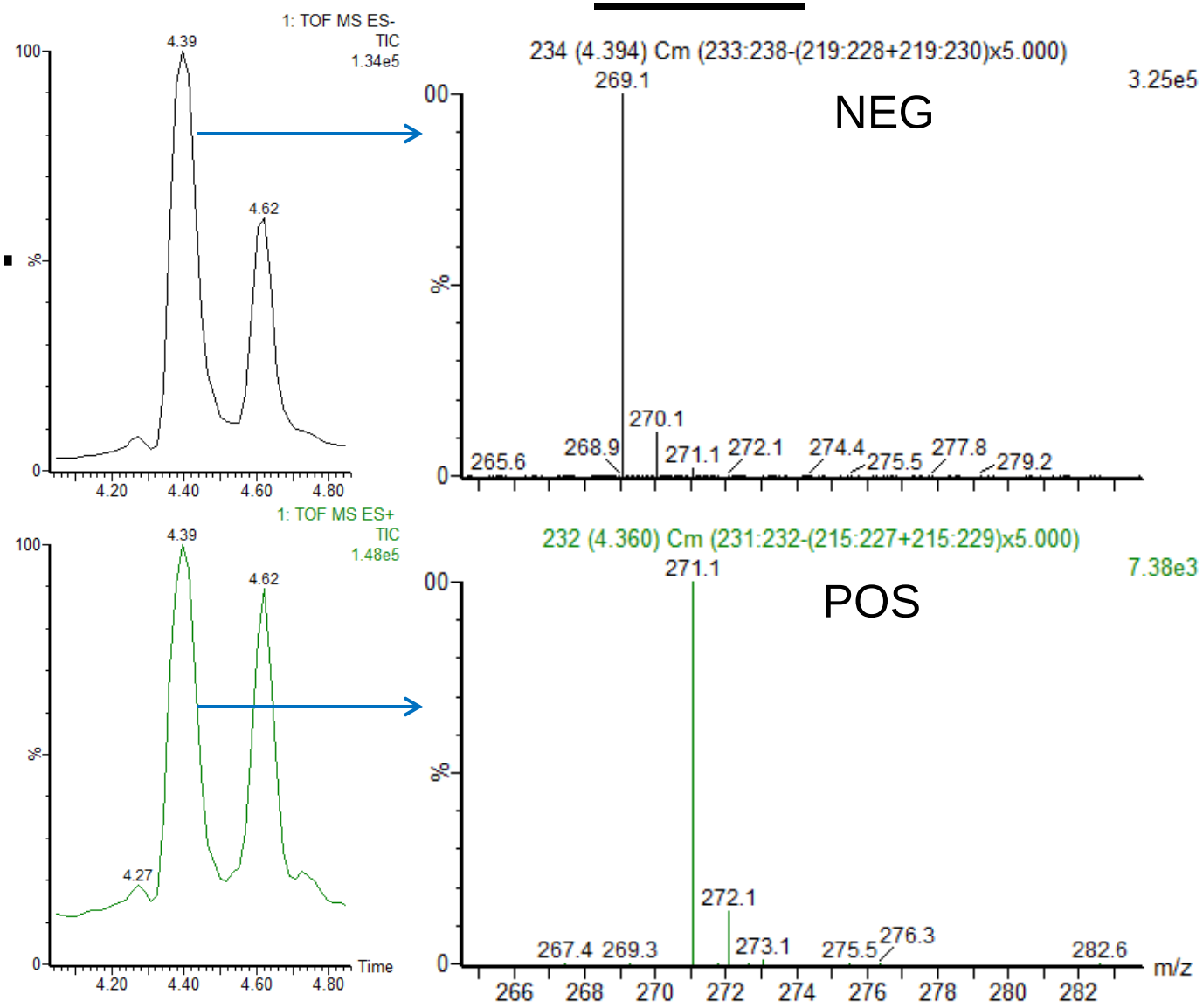




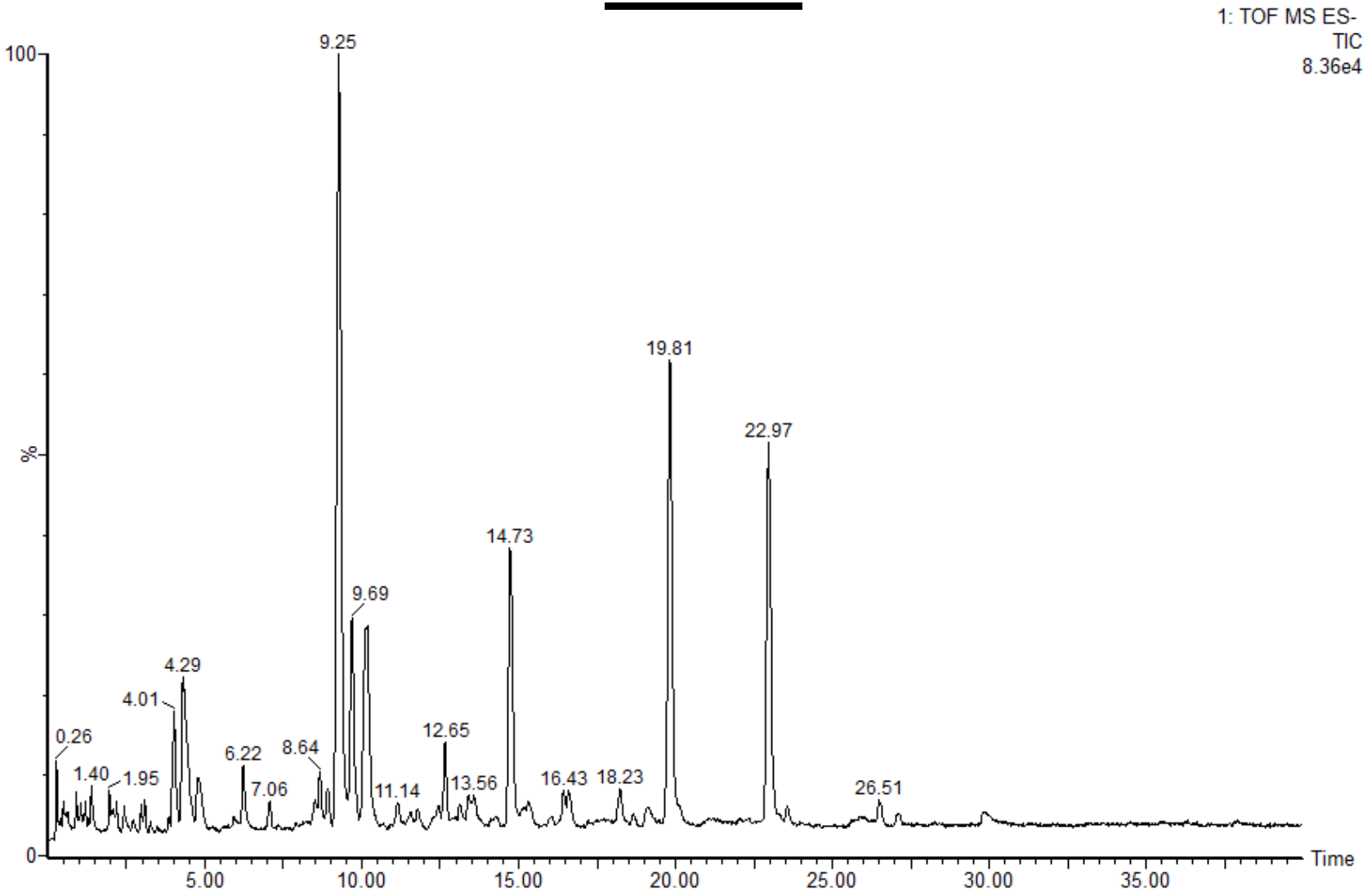
LCMS


TTB00564-L
3: Diode Array
Range: 2.308
UV
AU
4.37
4.58
5.02
5.38
1: TOF MS ES-
TIC
1.34e5
NEG
%
4.39
4.62
0.44
0.54
5.04
5.41
5.89
6.07
6.54
9.69
13.95
1: TOF MS ES+
TIC
1.48e5
POS
4.39
4.62
9.69
6.52
10.53
13.58
8.75
8.82
8.98
7.60
10.31
0.54
2.88
4.27
4.97
5.42
6.09
7.13
8.21
10.92
11.75
14.02
14.14
Time

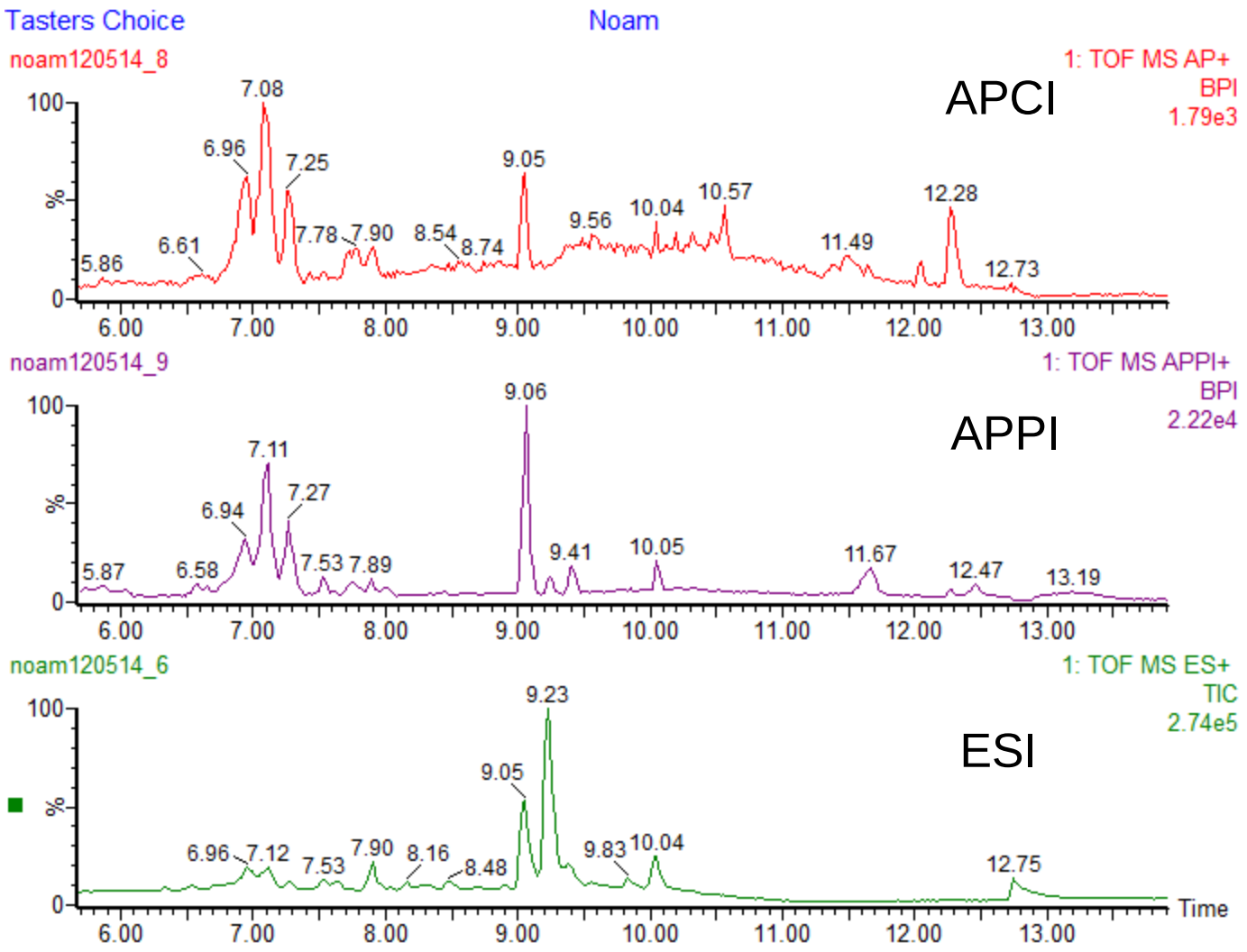
LCMS


1: TOF MS ES-
TIC
1.34e5
4.39
4.62
100
%
0
4.20
4.40
4.60
4.80
234 (4.394) Cm (233:238-(219:228+219:230)x5.000)
3.25e5
NEG
269.1
270.1
265.6
268.9
271.1
272.1
274.4
275.5
277.8
279.2
1: TOF MS ES+
TIC
1.48e5
4.27
Time
232 (4.360) Cm (231:232-(215:227+215:229)x5.000)
7.38e3
POS
271.1
272.1
267.4
269.3
273.1
275.5
276.3
282.6
m/z
266
268
270
272
274
276
278
280
282

LCMS

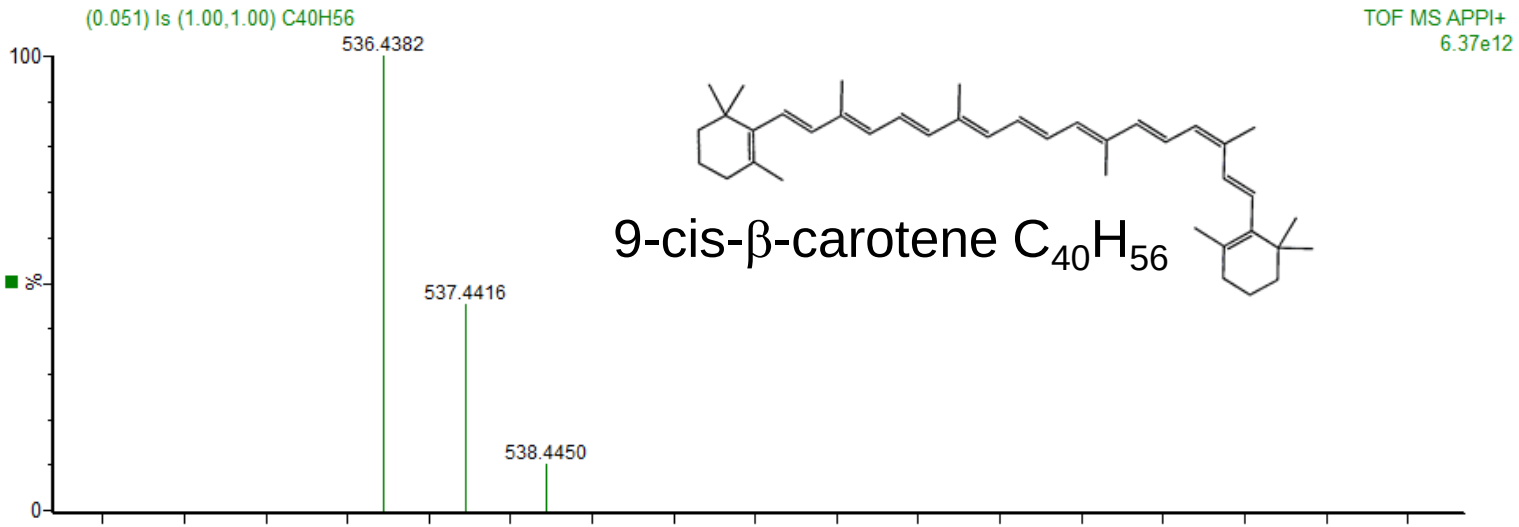

1: TOF MS ES-
TIC
8.36e4
100
%
0
9.25
19.81
22.97
14.73
9.69
4.29
4.01
0.26
12.65
6.22
8.64
1.40
1.95
7.06
11.14
13.56
16.43
18.23
26.51
5.00
10.00
15.00
20.00
25.00
30.00
35.00
Time

LCMS

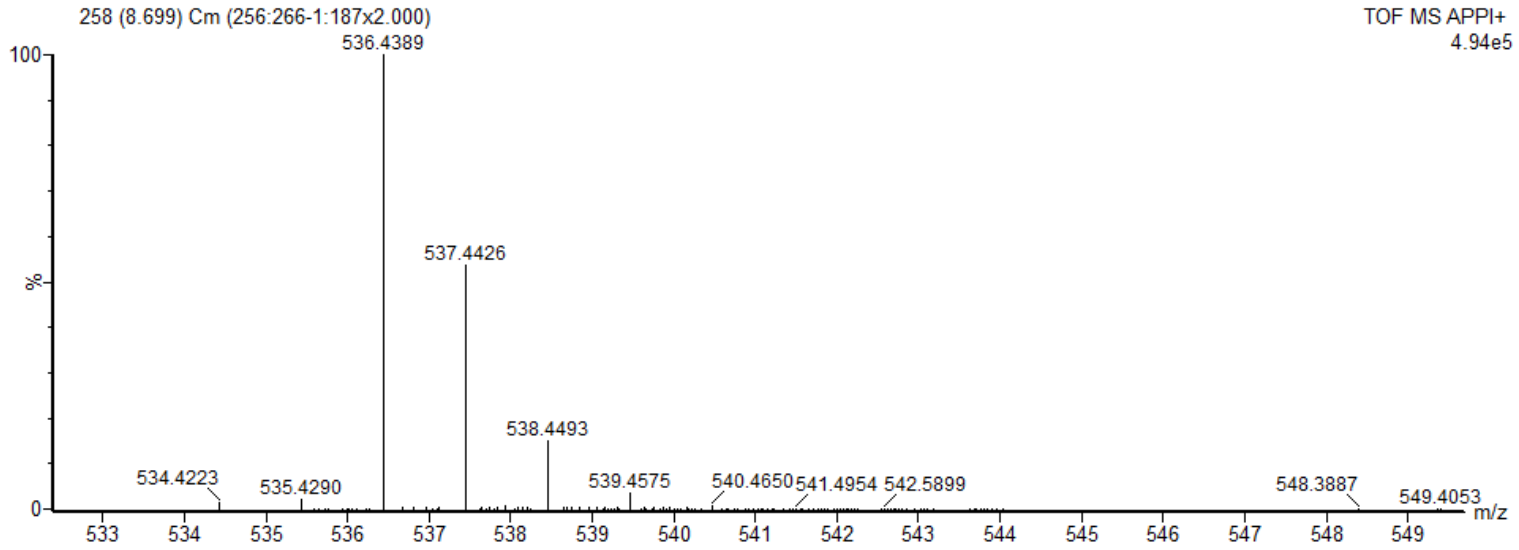


Water extract
In MeCN

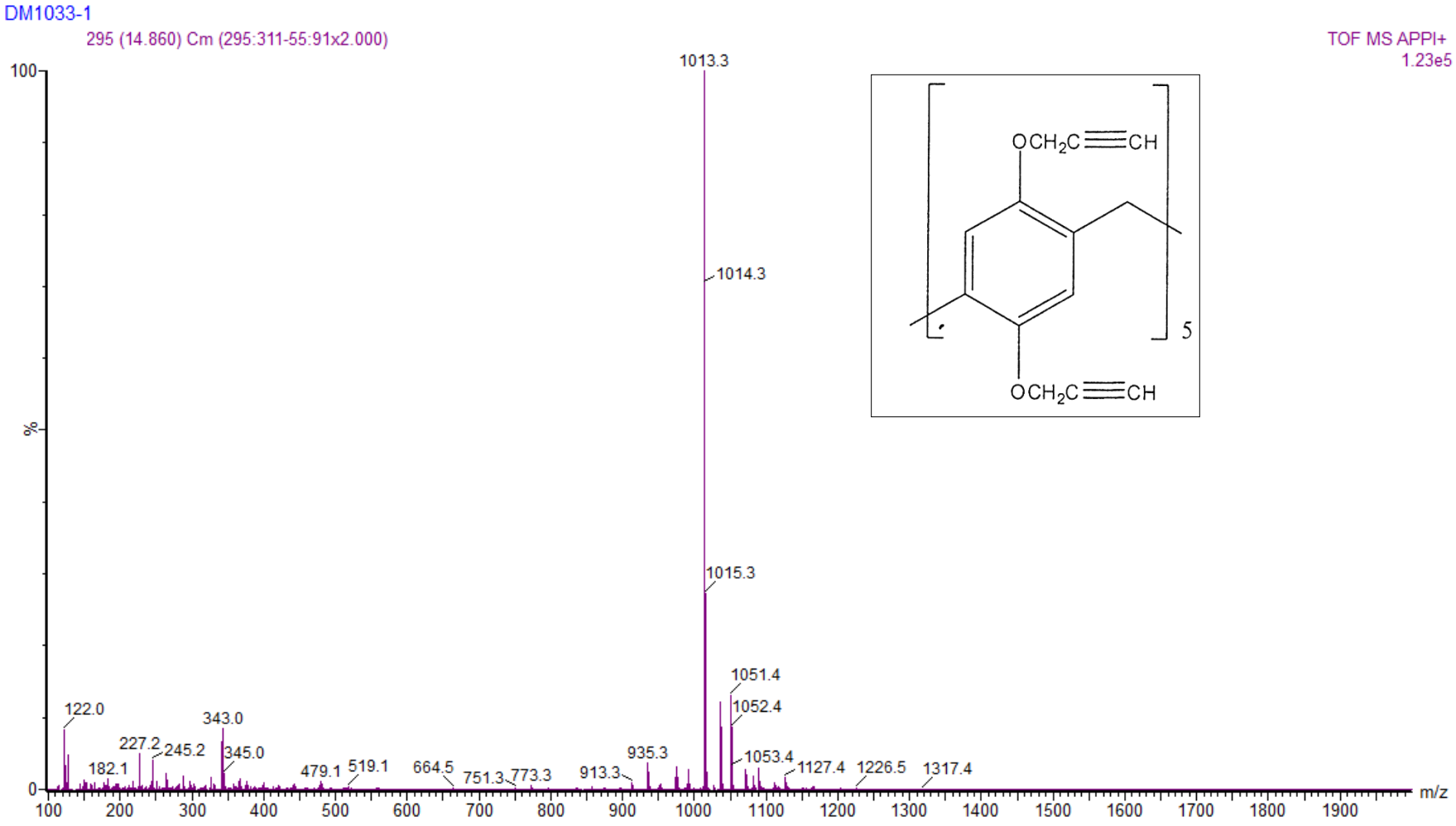
APPI



9-cis-β-carotene $C_{40}H_{56}$

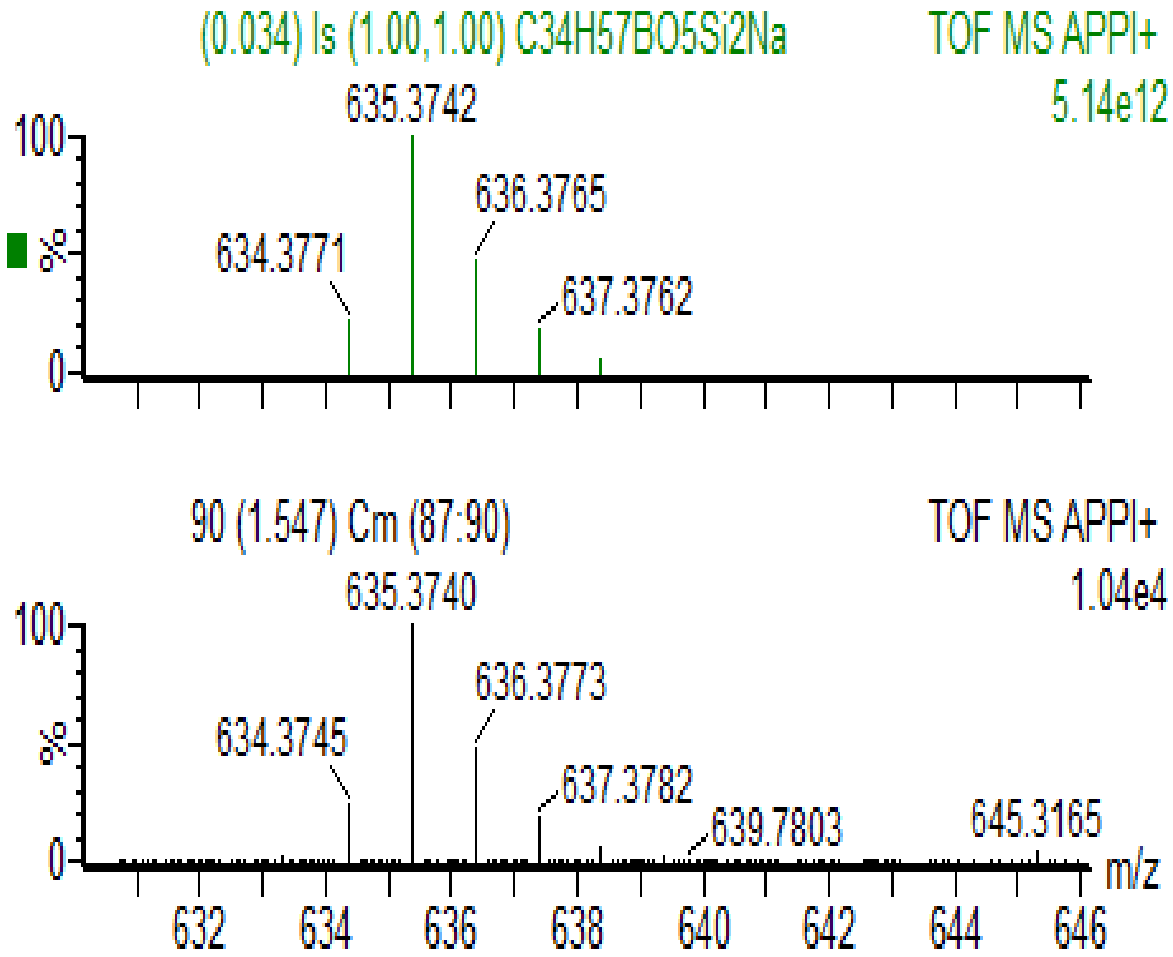


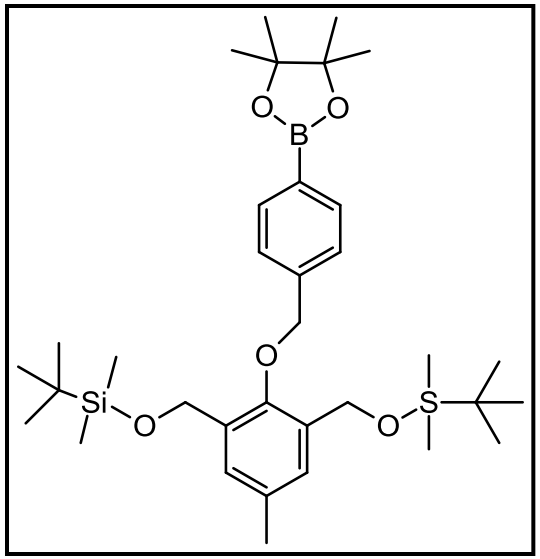
APPI


DM1033-1
295 (14.860) Cm (295:311-55:91x2.000)
TOF MS APPI+
1.23e5
100
%
0
1013.3
1014.3
1015.3
1051.4
1052.4
1053.4
122.0
343.0
227.2
245.2
345.0
182.1
479.1
519.1
664.5
751.3
773.3
913.3
935.3
1127.4
1226.5
1317.4
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
m/z
OCH2C≡CH
OCH2C≡CH
5

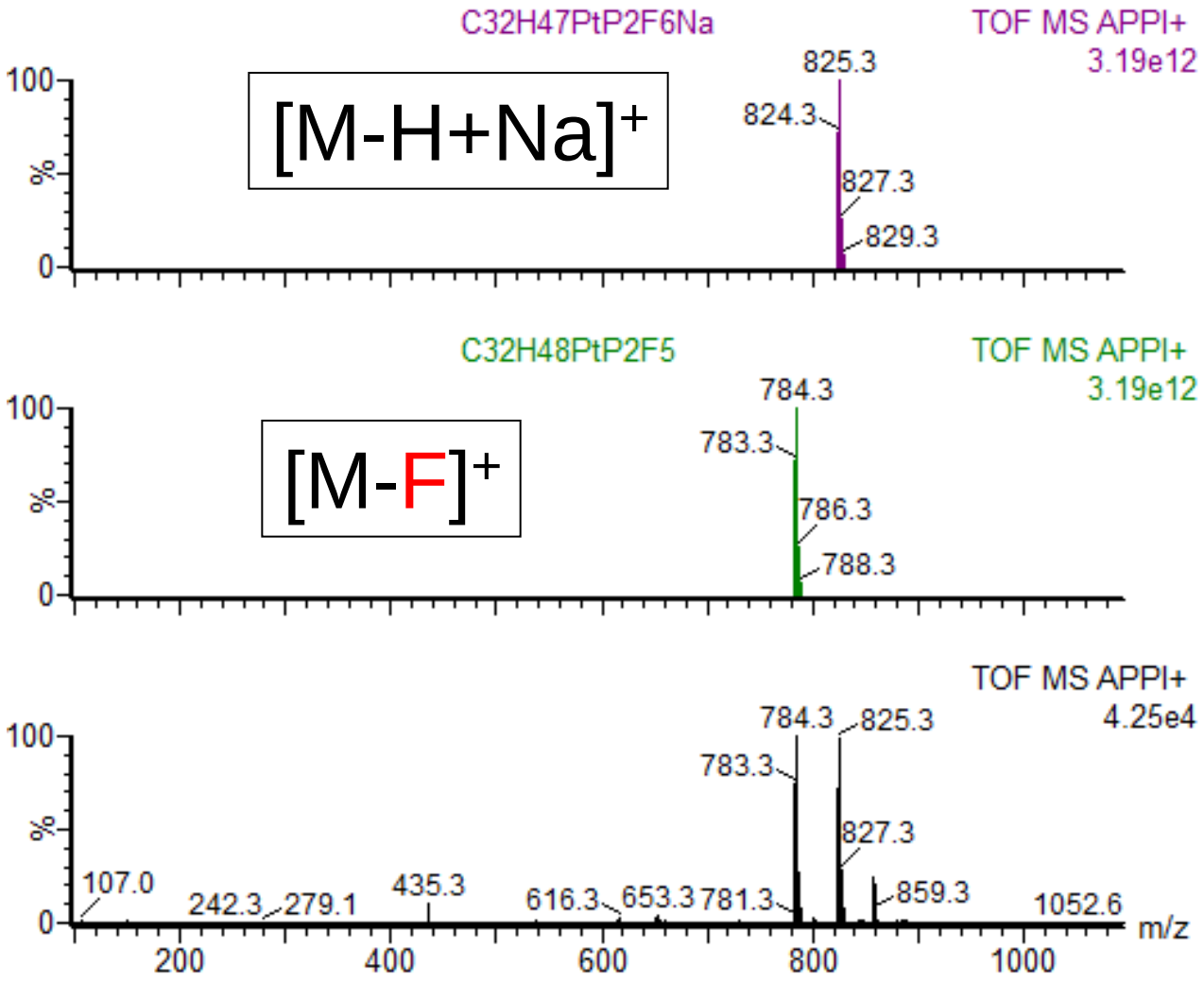
APPI

ef 2-41

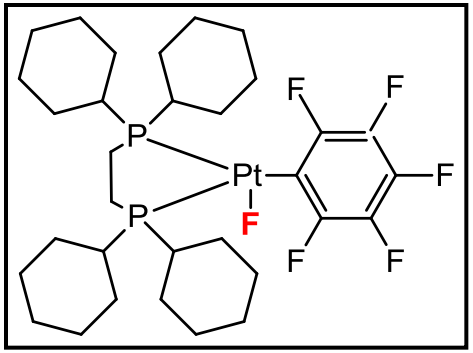

(0.034) Is (1.00,1.00) C34H57BO5Si2Na
TOF MS APPI+
5.14e12
635.3742
636.3765
634.3771
637.3762
100
%
0
90 (1.547) Cm (87:90)
TOF MS APPI+
1.04e4
635.3740
636.3773
634.3745
637.3782
639.7803
645.3165
m/z
632
634
636
638
640
642
644
646


O
B
O
O
Si
O
O
S

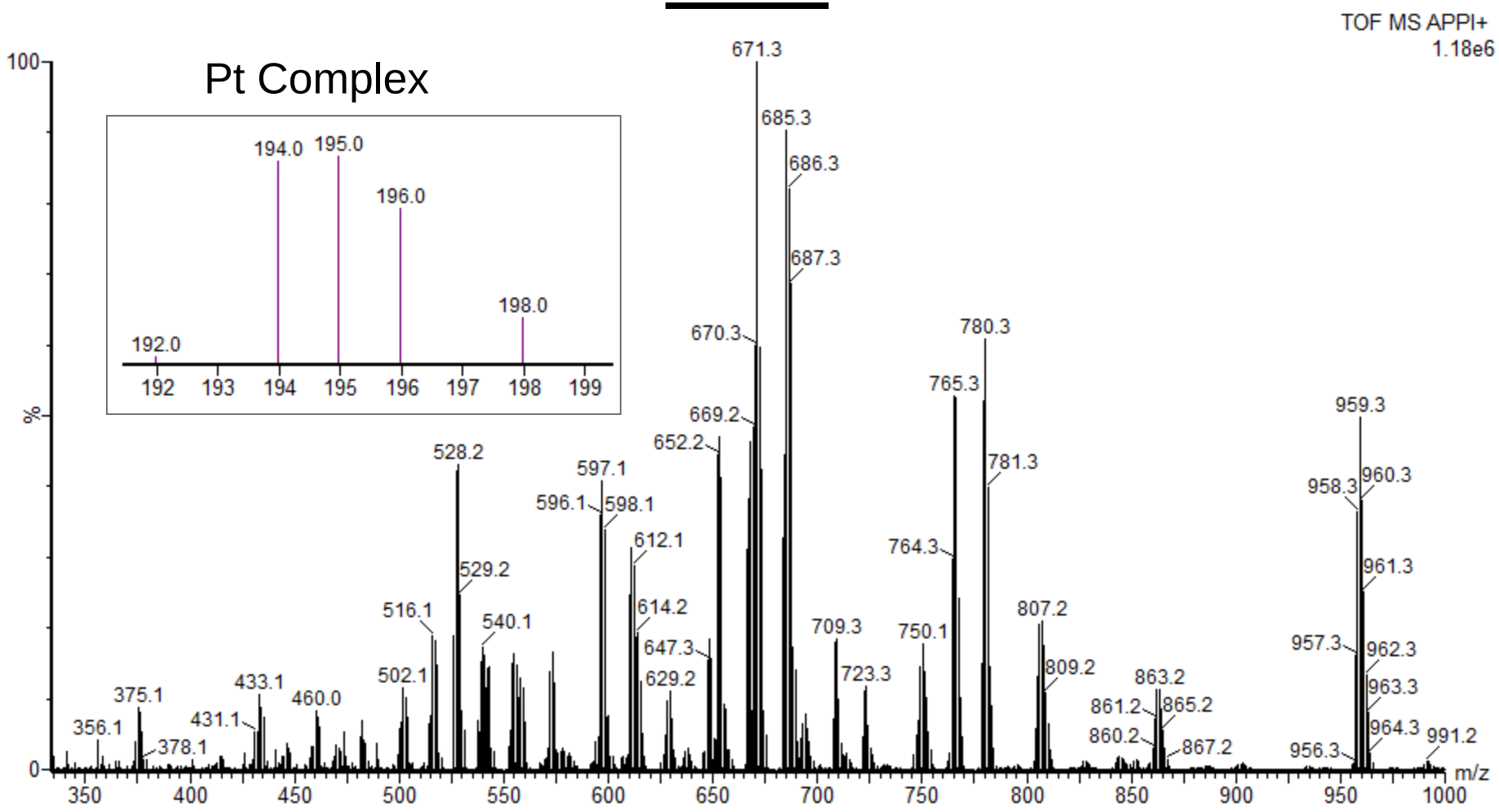
APPI



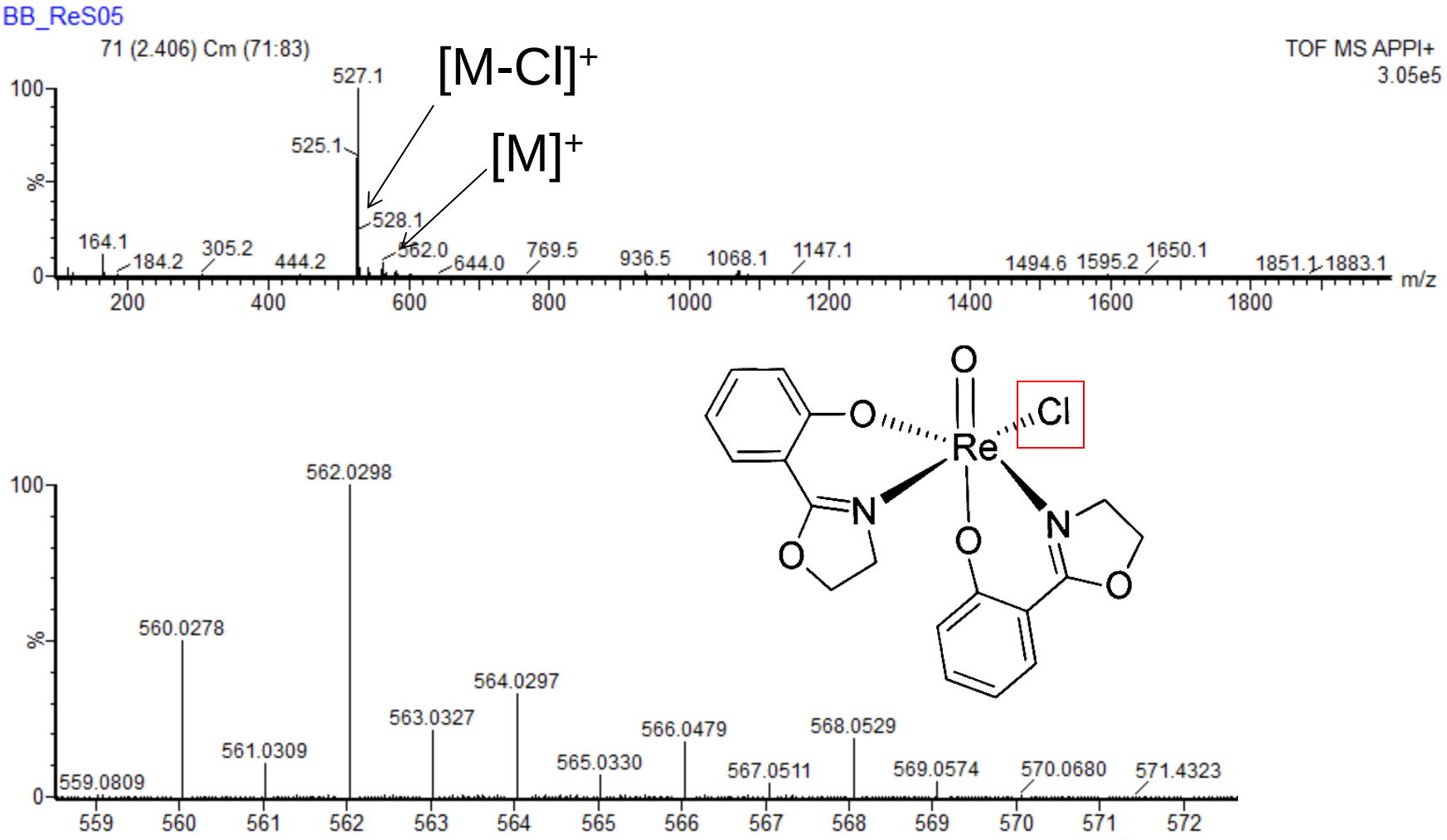
$C_{32}H_{48}PtP_2F_6$



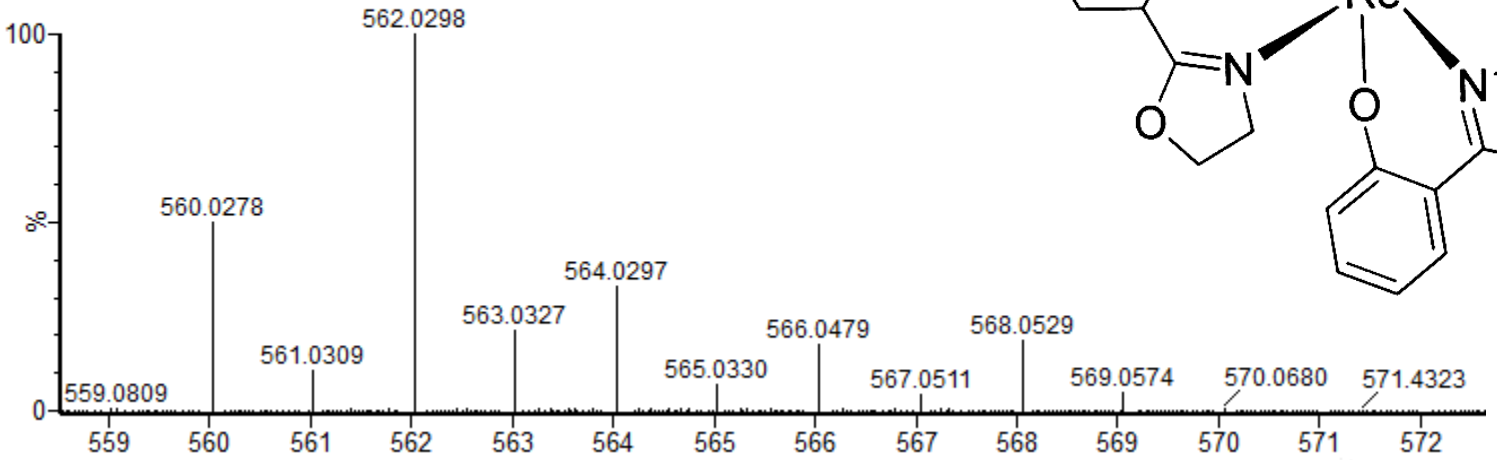
APPI


TOF MS APPI+
1.18e6
Pt Complex
194.0
195.0
196.0
198.0
192.0
192
193
194
195
196
197
198
199
100
%
0
671.3
685.3
686.3
687.3
670.3
780.3
765.3
669.2
652.2
781.3
959.3
958.3
960.3
528.2
597.1
596.1
598.1
612.1
764.3
961.3
529.2
516.1
614.2
540.1
709.3
750.1
807.2
647.3
957.3
962.3
502.1
629.2
723.3
809.2
863.2
963.3
433.1
375.1
460.0
861.2
865.2
964.3
431.1
356.1
860.2
867.2
991.2
378.1
956.3
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
m/z

APPI

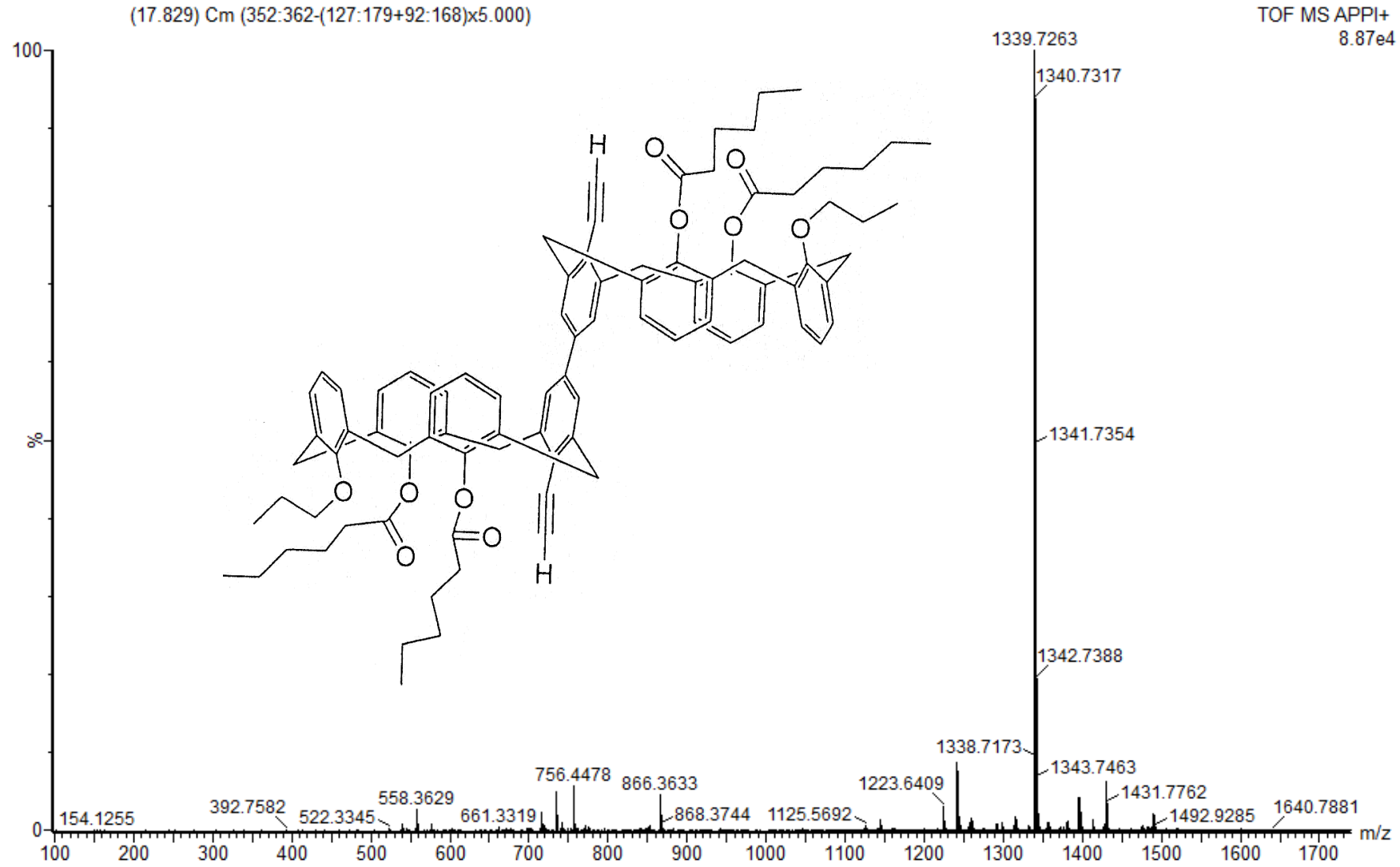

BB_ReS05
71 (2.406) Cm (71:83)
TOF MS APPI+
3.05e5
[M-Cl]+
[M]+
527.1
525.1
528.1
164.1
184.2
305.2
444.2
562.0
644.0
769.5
936.5
1068.1
1147.1
1494.6
1595.2
1650.1
1851.1
1883.1
m/z
562.0298
560.0278
564.0297
563.0327
566.0479
568.0529
561.0309
565.0330
567.0511
569.0574
570.0680
571.4323
559.0809
O
Re
Cl
N
O

APPI

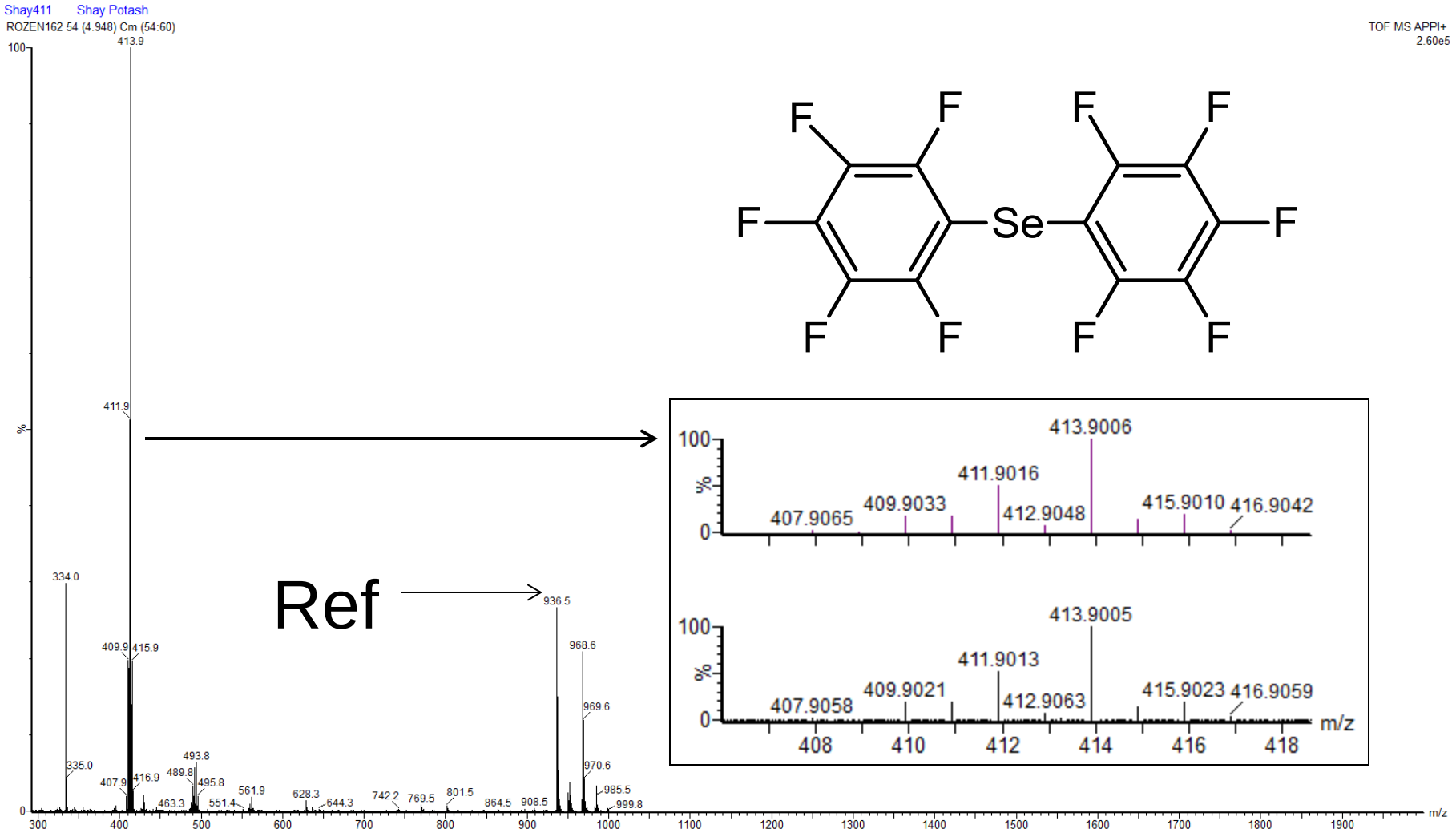


Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT (Norm)	C	H	N	O	Na	Cl	185Re	187Re
562.0298	562.0305	-0.7	-1.2	11.5	C18 H16 N2 O5 Cl 187Re	312.0	0.3	18	16	2	5		1		1
	562.0292	0.6	1.1	12.0	C16 H14 N5 O4 Cl 187Re	313.6	2.0	16	14	5	4		1		1
	562.0294	0.4	0.7	17.0	C23 H16 N4 O9 Cl2	323.2	11.6	23	16	4	9		2		
	562.0295	0.3	0.5	11.5	C15 H14 N6 O4 Cl 185Re	314.5	2.9	15	14	6	4		1	1	
	562.0297	0.1	0.2	18.5	C24 H15 N5 O6 Na Cl2	323.0	11.3	24	15	5	6	1	2		
	562.0298	0.0	0.0	7.5	C17 H19 O6 Na Cl 185Re	314.3	2.7	17	19		6	1	1	1	

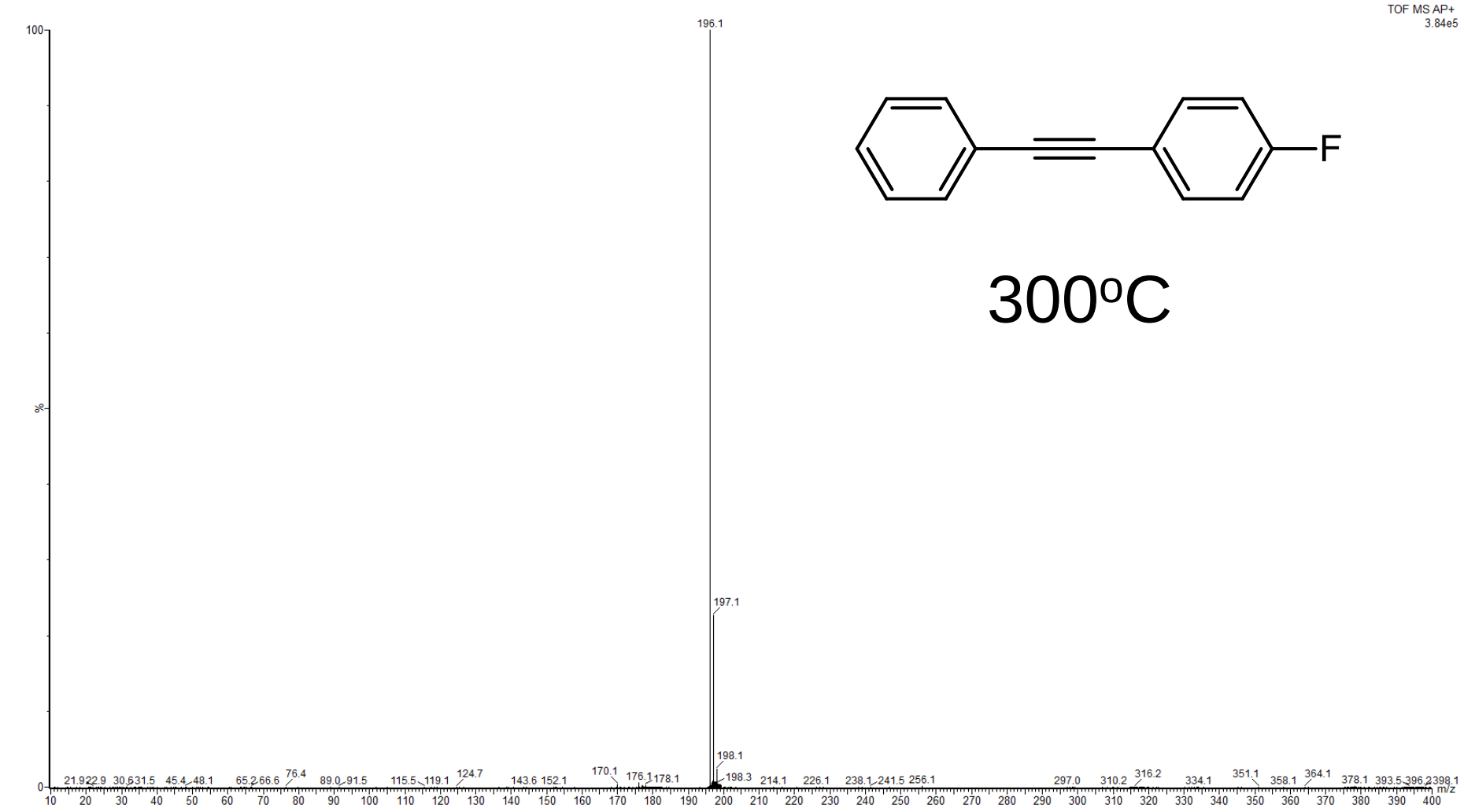
APPI


(17.829) Cm (352:362-(127:179+92:168)x5.000)
TOF MS APPI+
8.87e4
100
%
0
1339.7263
1340.7317
1341.7354
1342.7388
1338.7173
1343.7463
1431.7762
1492.9285
1640.7881
1223.6409
756.4478
866.3633
868.3744
1125.5692
558.3629
661.3319
392.7582
522.3345
154.1255
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
m/z

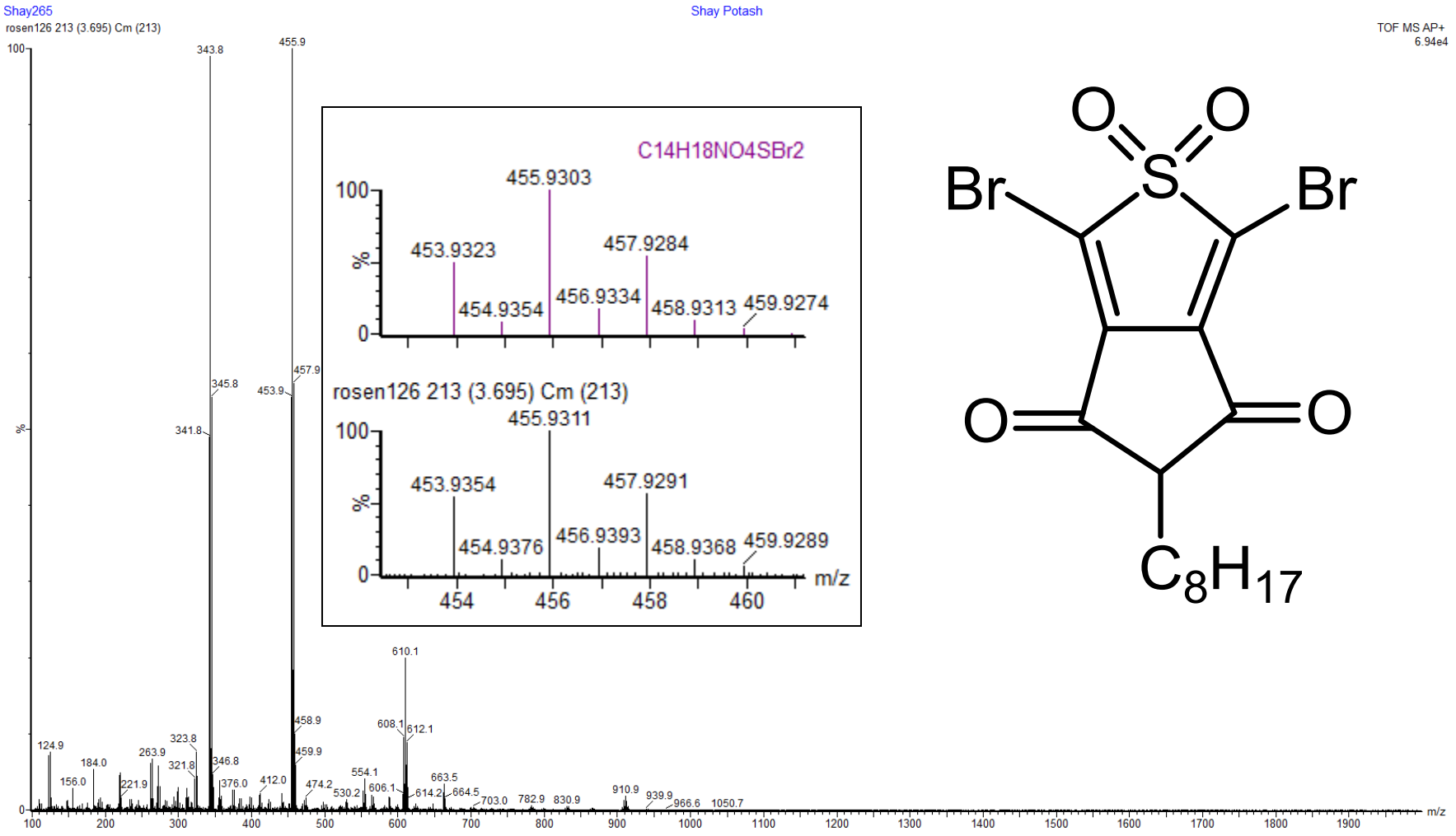
APPI


Shay411 Shay Potash
ROZEN162 54 (4.948) Cm (54:60)
TOF MS APPI+
2.60e5
Ref
413.9006
411.9016
407.9065
409.9033
412.9048
415.9010
416.9042
413.9005
411.9013
407.9058
409.9021
412.9063
415.9023
416.9059
m/z

APCI

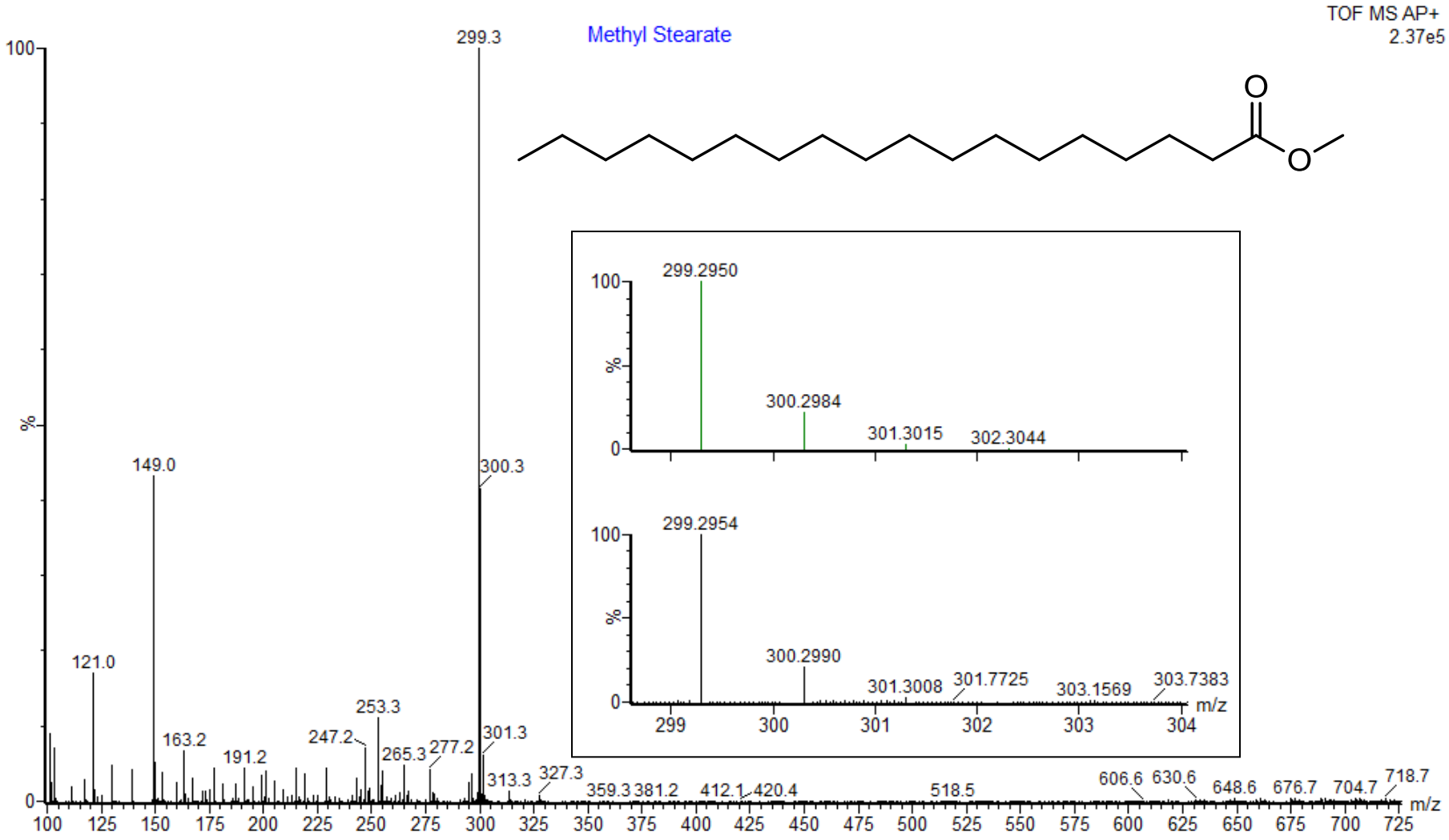


APCI

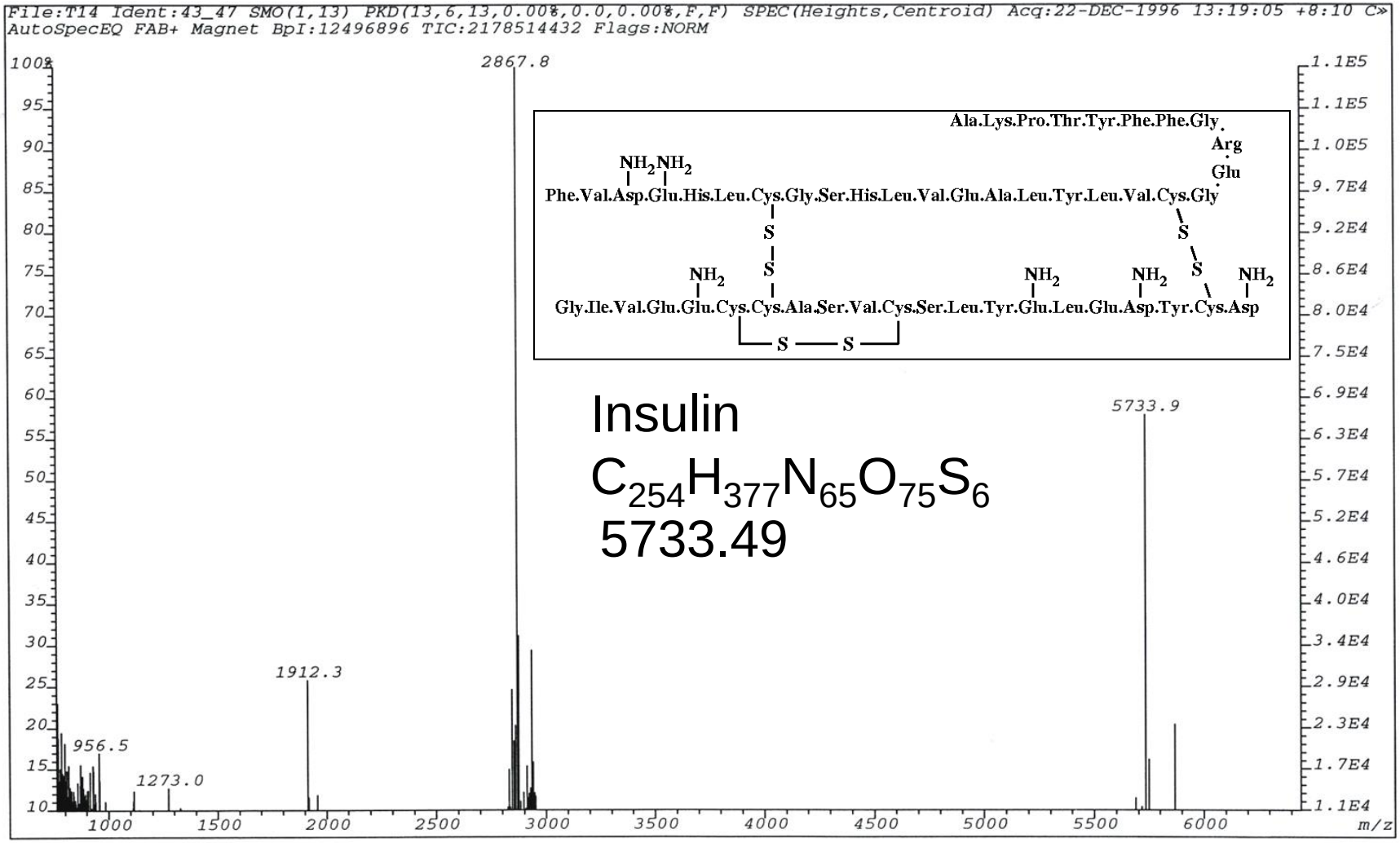

Shay265
rosen126 213 (3.695) Cm (213)
Shay Potash
TOF MS AP+
6.94e4
C14H18NO4SBr2
455.9303
453.9323
457.9284
454.9354
456.9334
458.9313
459.9274
rosen126 213 (3.695) Cm (213)
455.9311
453.9354
457.9291
454.9376
456.9393
458.9368
459.9289
m/z
454
456
458
460
343.8
455.9
345.8
457.9
453.9
341.8
610.1
458.9
608.1
612.1
459.9
124.9
323.8
263.9
184.0
321.8
346.8
156.0
221.9
376.0
412.0
474.2
554.1
530.2
606.1
614.2
663.5
664.5
703.0
782.9
830.9
910.9
939.9
966.6
1050.7
m/z

O
O
S
Br
Br
O
O
C8H17

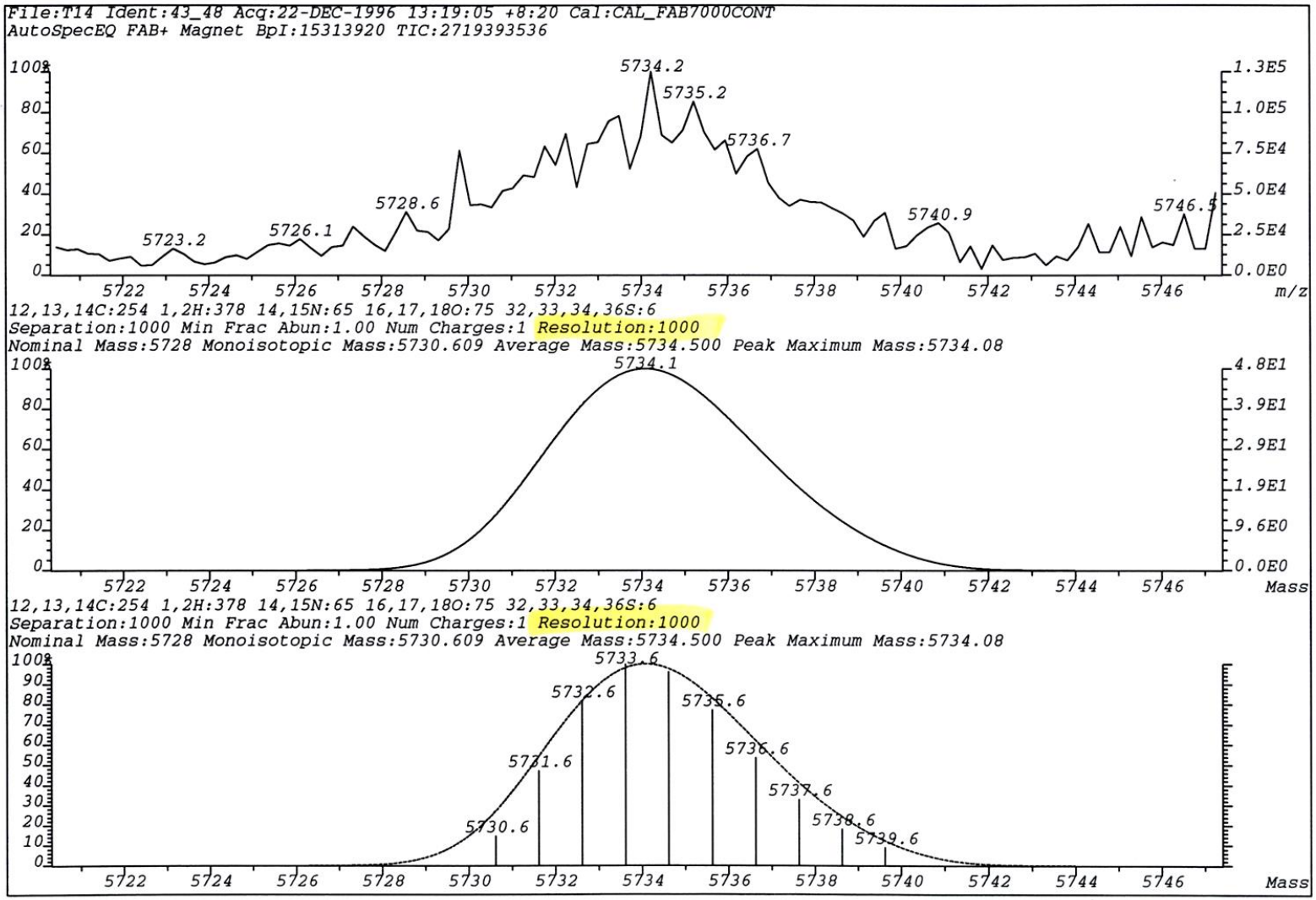
APCI


TOF MS AP+
2.37e5
Methyl Stearate
O
O
299.3
100
%
0
149.0
300.3
121.0
253.3
163.2
247.2
191.2
265.3
277.2
301.3
313.3
327.3
359.3
381.2
412.1
420.4
518.5
606.6
630.6
648.6
676.7
704.7
718.7
m/z
100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550 575 600 625 650 675 700 725
299.2950
300.2984
301.3015
302.3044
299.2954
300.2990
301.3008
301.7725
303.1569
303.7383
299 300 301 302 303 304

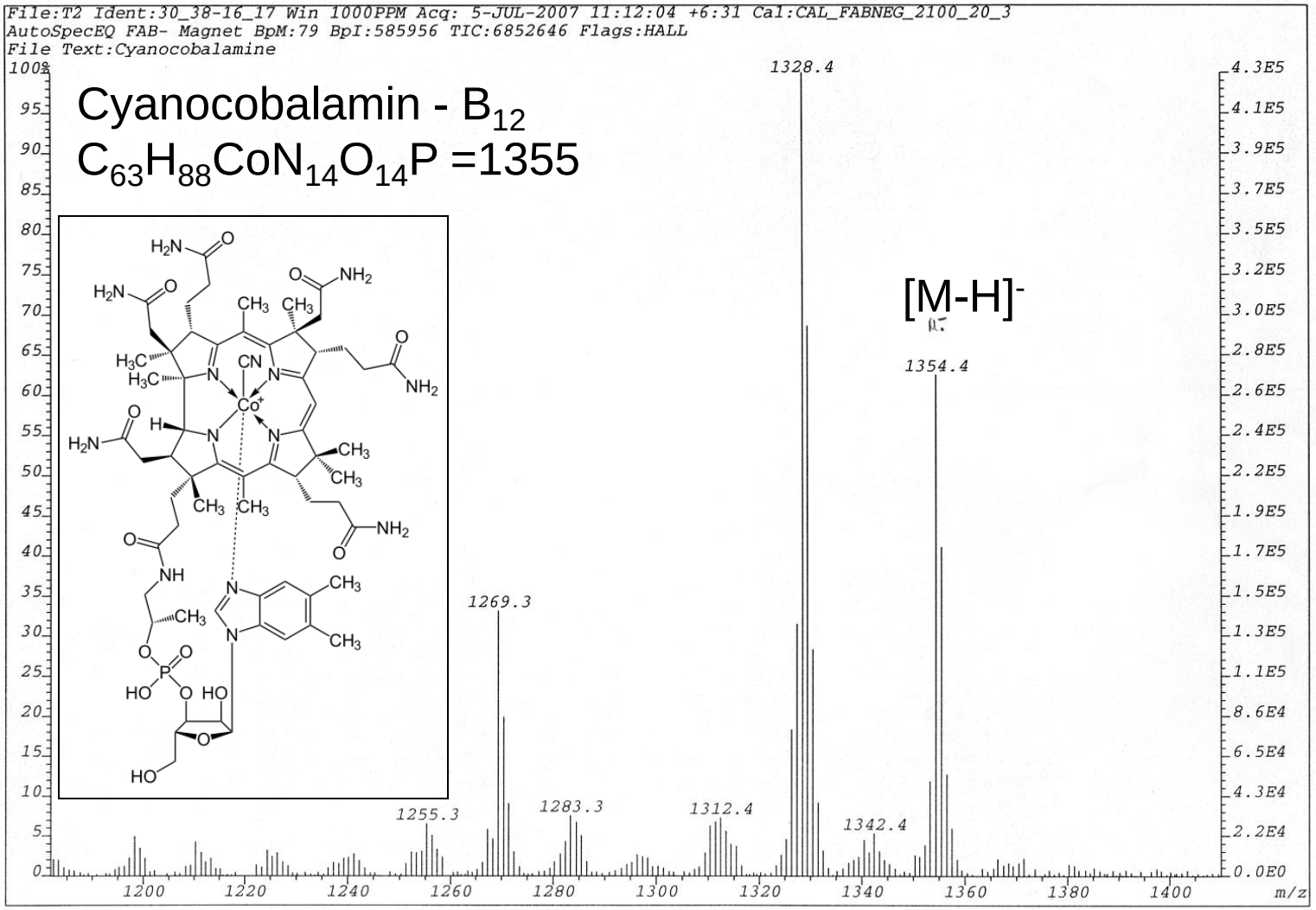
FAB


File:T14 Ident:43_47 SMO(1,13) PKD(13,6,13,0.00%,0.0,0.00%,F,F) SPEC(Heights,Centroid) Acq:22-DEC-1996 13:19:05 +8:10 C»
AutoSpecEQ FAB+ Magnet BpI:12496896 TIC:2178514432 Flags:NORM
2867.8
5733.9
1912.3
956.5
1273.0
m/z
Ala.Lys.Pro.Thr.Tyr.Phe.Phe.Gly
Arg
Glu
Phe.Val.Asp.Glu.His.Leu.Cys.Gly.Ser.His.Leu.Val.Glu.Ala.Leu.Tyr.Leu.Val.Cys.Gly
Gly.Ile.Val.Glu.Glu.Cys.Cys.Ala.Ser.Val.Cys.Ser.Leu.Tyr.Glu.Leu.Glu.Asp.Tyr.Cys.Asp
Insulin
C254H377N65O75S6
5733.49

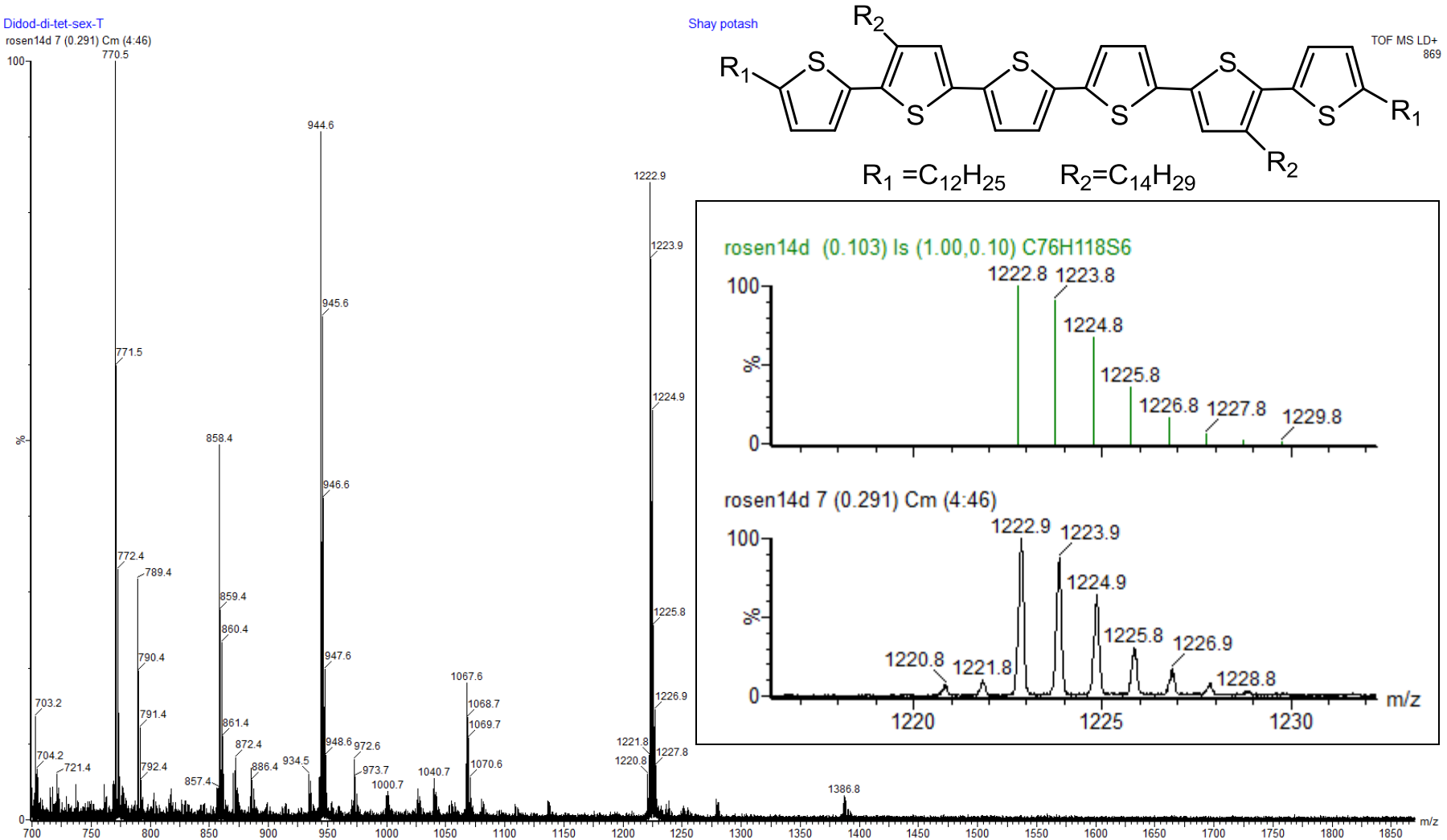
FAB


File:T14 Ident:43_48 Acq:22-DEC-1996 13:19:05 +8:20 Cal:CAL_FAB7000CONT
AutoSpecEQ FAB+ Magnet BpI:15313920 TIC:2719393536
5734.2
5735.2
5736.7
5728.6
5723.2
5726.1
5740.9
5746.5
m/z
12,13,14C:254 1,2H:378 14,15N:65 16,17,18O:75 32,33,34,36S:6
Separation:1000 Min Frac Abun:1.00 Num Charges:1 Resolution:1000
Nominal Mass:5728 Monoisotopic Mass:5730.609 Average Mass:5734.500 Peak Maximum Mass:5734.08
5734.1
Mass
12,13,14C:254 1,2H:378 14,15N:65 16,17,18O:75 32,33,34,36S:6
Separation:1000 Min Frac Abun:1.00 Num Charges:1 Resolution:1000
Nominal Mass:5728 Monoisotopic Mass:5730.609 Average Mass:5734.500 Peak Maximum Mass:5734.08
5733.6
5732.6
5735.6
5731.6
5736.6
5737.6
5730.6
5738.6
5739.6
Mass

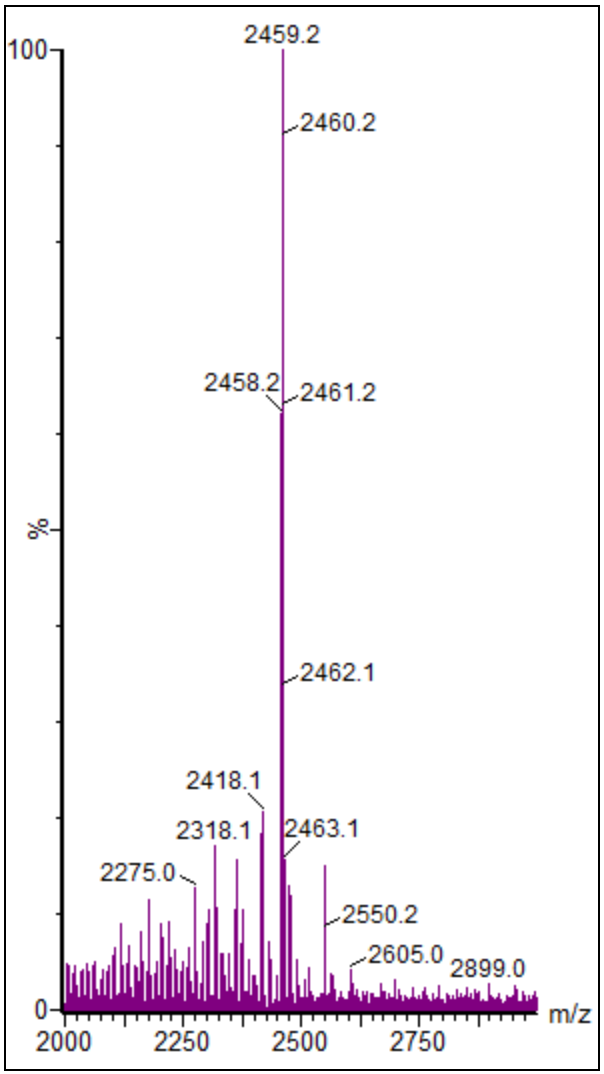
FAB

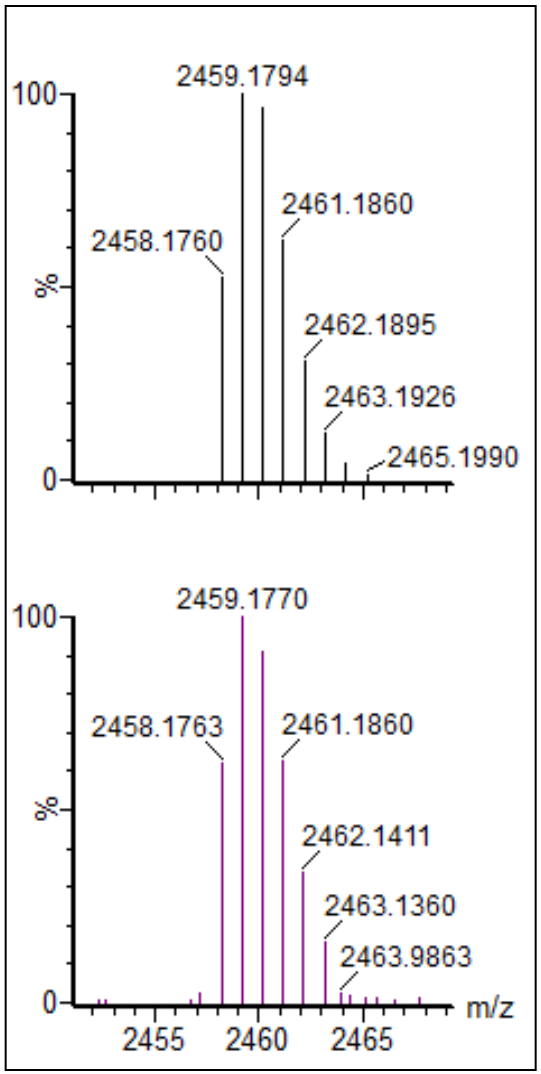

File:T2 Ident:30_38-16_17 Win 1000PPM Acq: 5-JUL-2007 11:12:04 +6:31 Cal:CAL_FABNEG_2100_20_3
AutoSpecEQ FAB- Magnet BpM:79 BpI:585956 TIC:6852646 Flags:HALL
File Text:Cyanocobalamine
Cyanocobalamin - B12
C63H88CoN14O14P =1355
[M-H]-
1328.4
1354.4
1269.3
1255.3
1283.3
1312.4
1342.4
m/z

MALDI

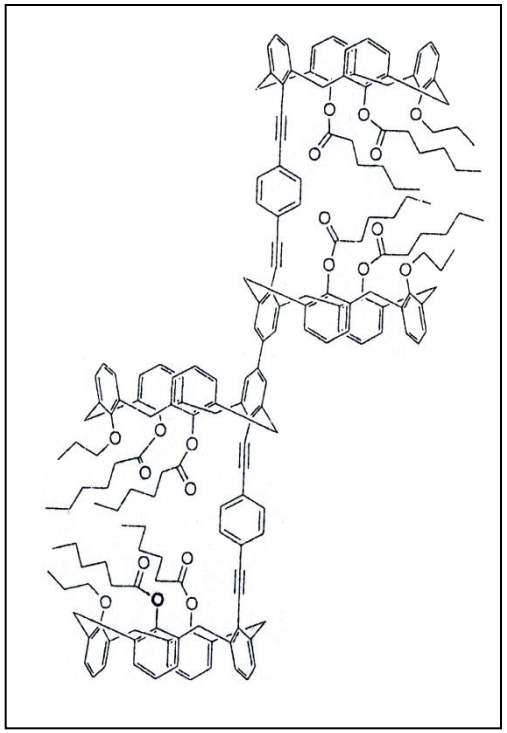

Didod-di-tet-sex-T
rosen14d 7 (0.291) Cm (4:46)
Shay potash
TOF MS LD+
869
R1 =C12H25
R2=C14H29
rosen14d (0.103) Is (1.00,0.10) C76H118S6
1222.8
1223.8
1224.8
1225.8
1226.8
1227.8
1229.8
rosen14d 7 (0.291) Cm (4:46)
1222.9
1223.9
1224.9
1225.8
1226.9
1220.8
1221.8
1228.8
m/z
%

MALDI

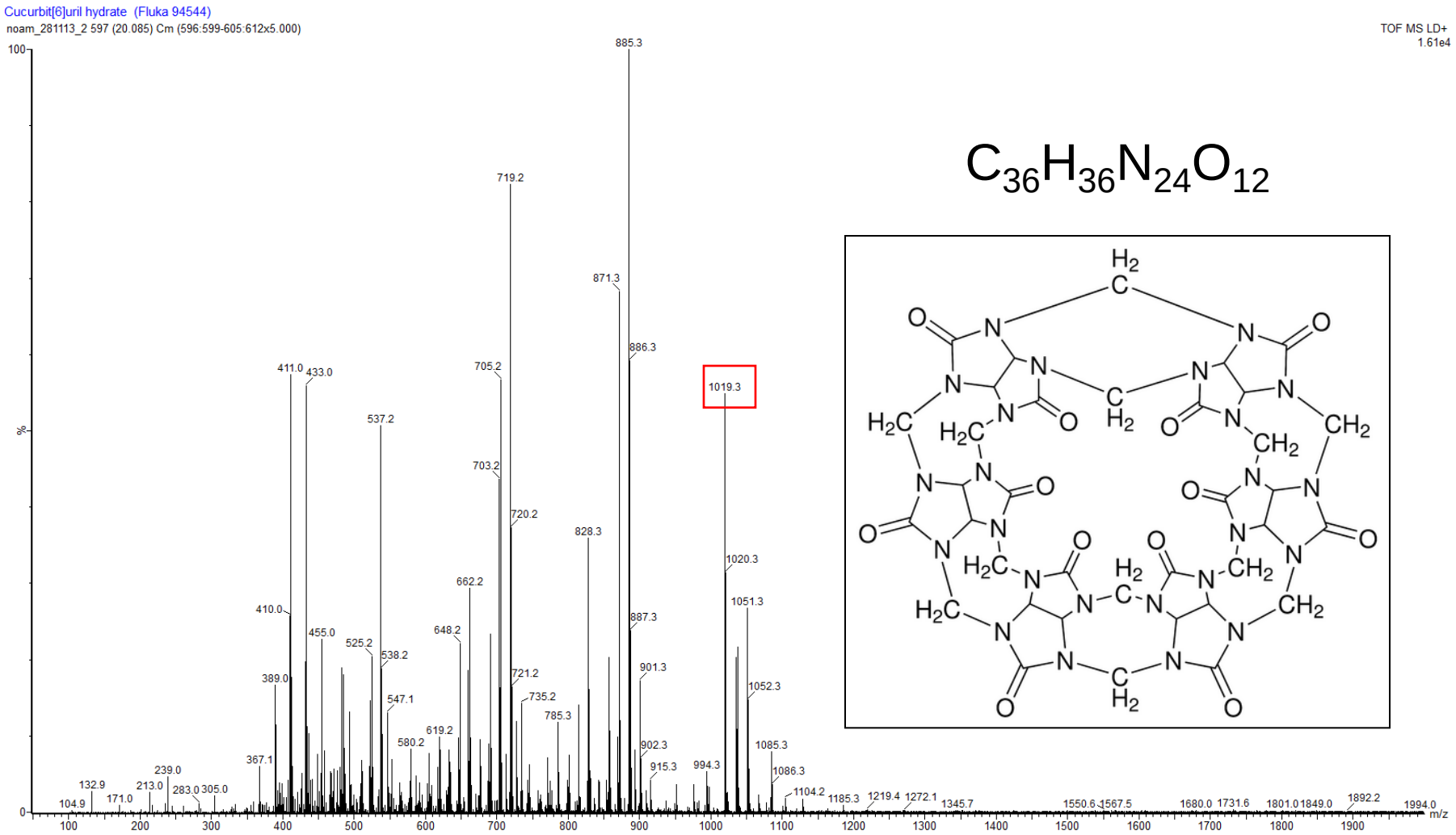




$C_{168}H_{162}O_{16}Na$



MALDI



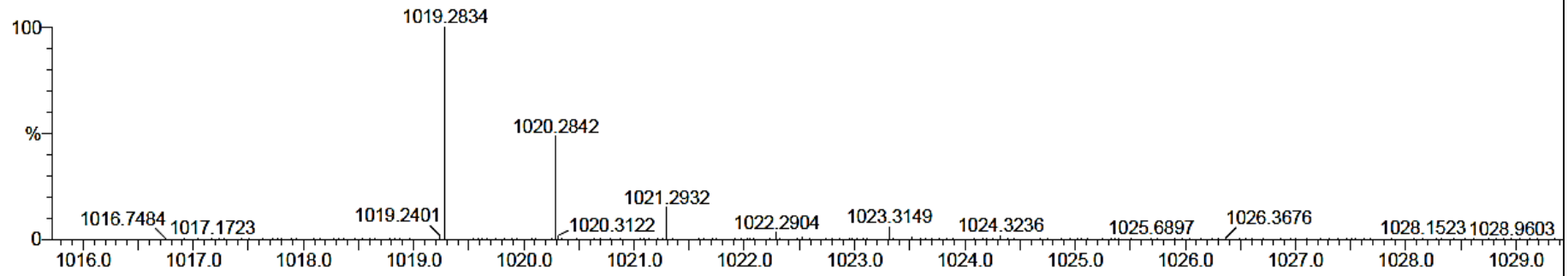
$C_{36}H_{36}N_{24}O_{12}$

MALDI

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 = 2

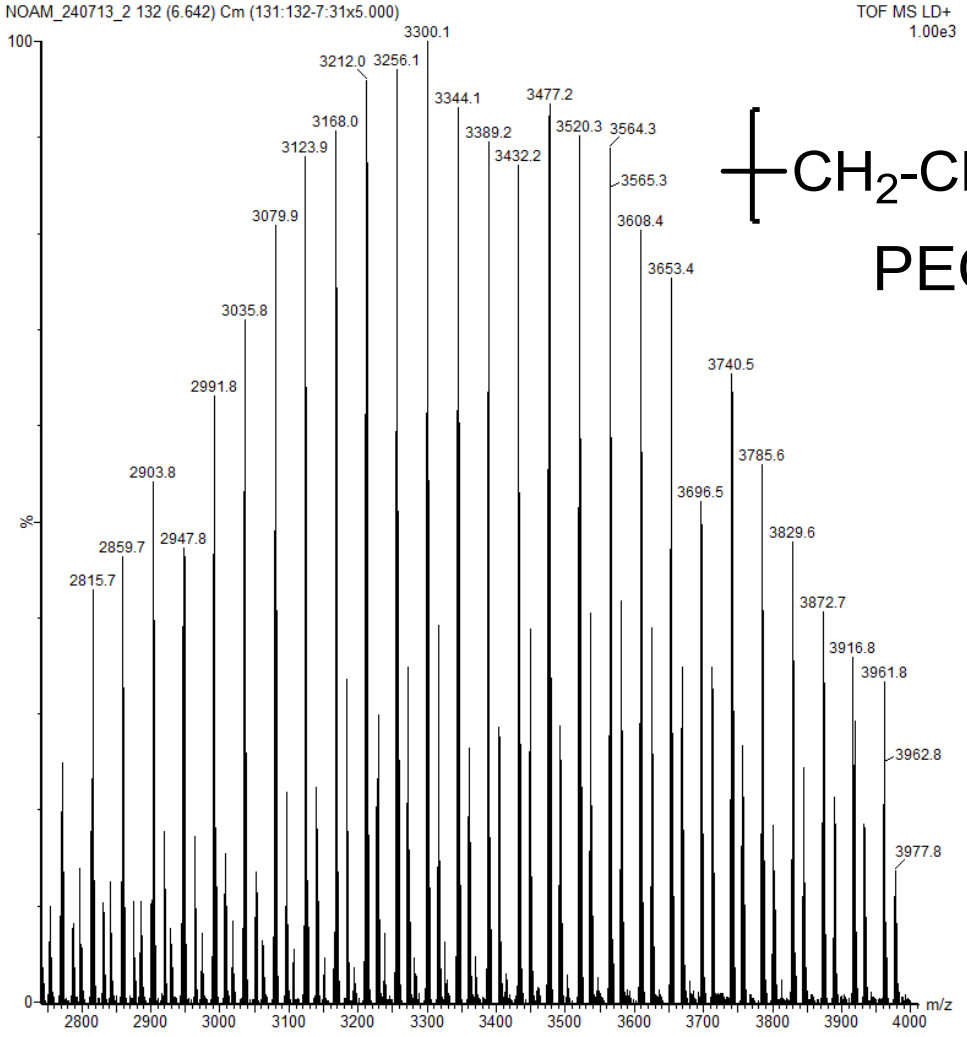
Monoisotopic Mass, Odd and Even Electron Ions
231 formula(e) evaluated with 22 results within limits (up to 10 closest results for each mass)
Elements Used:
C: 30-40 H: 30-40 N: 20-30 O: 5-20 Na: 0-1

Cucurbit[6]uril hydrate (Fluka 94544) NOAM_281113_2 26 (0.882) Cm (25:70)
TOF MS LD+

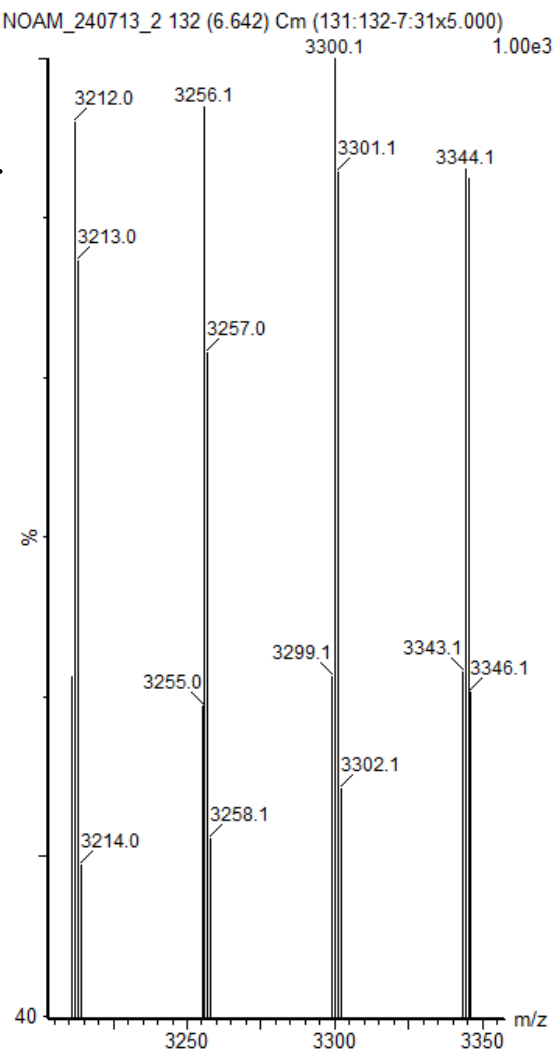


Minimum:				-1.5							
Maximum:		10.0	5.0	50.0							
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula				
1019.2834	1019.2839	-0.5	-0.5	29.0	70.0	2.2	C35	H37	N23	O15	
	1019.2839	-0.5	-0.5	34.5	70.0	2.2	C34	H31	N30	O10	
	1019.2829	0.5	0.5	31.0	69.8	2.0	C34	H34	N27	O11	Na
	1019.2829	0.5	0.5	25.5	69.9	2.1	C35	H40	N20	O16	Na
	1019.2842	-0.8	-0.8	30.5	70.2	2.4	C36	H36	N24	O12	Na
	1019.2826	0.8	0.8	29.5	69.9	2.1	C33	H35	N26	O14	
	1019.2815	1.9	1.9	31.5	70.1	2.2	C32	H32	N30	O10	Na
	1019.2853	-1.9	-1.9	34.0	70.9	3.0	C36	H33	N27	O11	
	1019.2853	-1.9	-1.9	28.5	70.7	2.8	C37	H39	N20	O16	
	1019.2815	1.9	1.9	26.0	70.4	2.5	C33	H38	N23	O15	Na

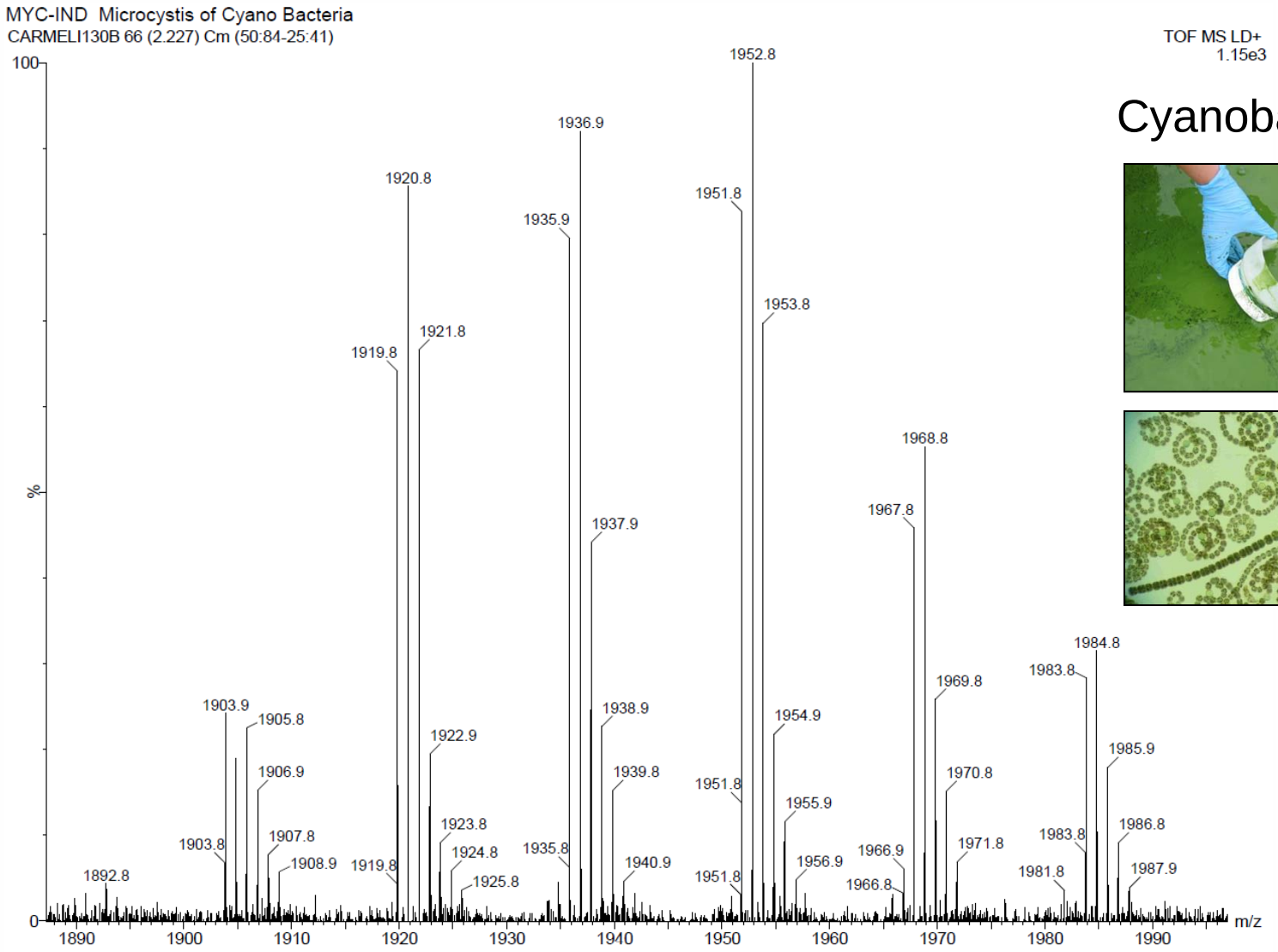
MALDI


NOAM_240713_2 132 (6.642) Cm (131:132-7:31x5.000)
TOF MS LD+
1.00e3
100
%
0
2815.7
2859.7
2903.8
2947.8
2991.8
3035.8
3079.9
3123.9
3168.0
3212.0
3256.1
3300.1
3344.1
3389.2
3432.2
3477.2
3520.3
3564.3
3565.3
3608.4
3653.4
3696.5
3740.5
3785.6
3829.6
3872.7
3916.8
3961.8
3962.8
3977.8
2800
2900
3000
3100
3200
3300
3400
3500
3600
3700
3800
3900
4000
m/z

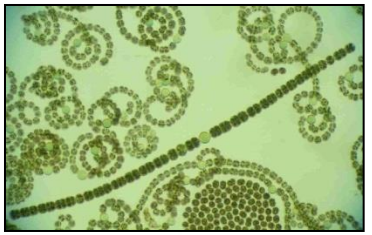
CH2-CH2-O
n
PEG


NOAM_240713_2 132 (6.642) Cm (131:132-7:31x5.000)
1.00e3
%
40
3212.0
3213.0
3214.0
3255.0
3256.1
3257.0
3258.1
3299.1
3300.1
3301.1
3302.1
3343.1
3344.1
3346.1
3250
3300
3350
m/z

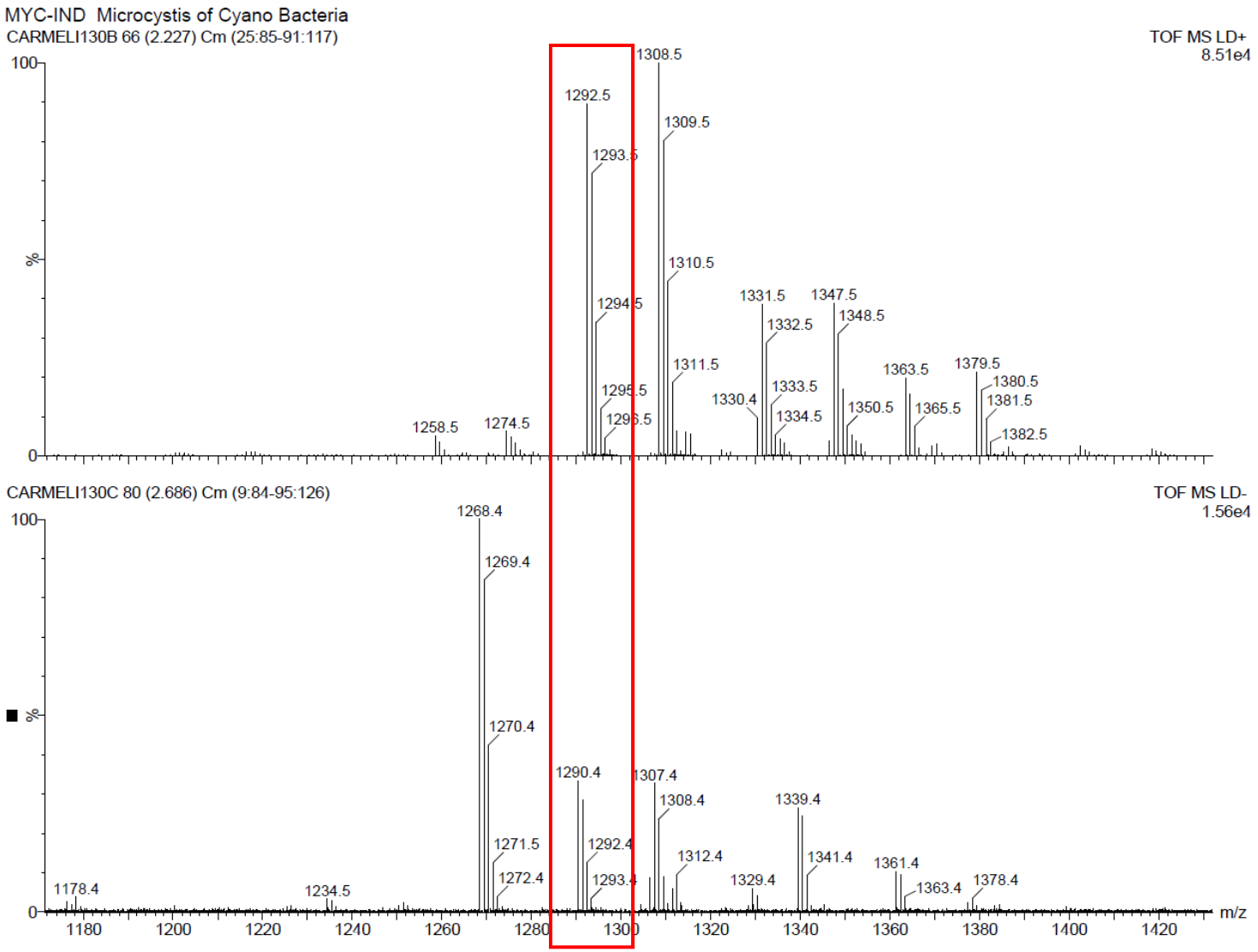
MALDI



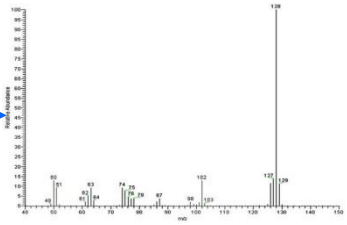
Cyanobacteria

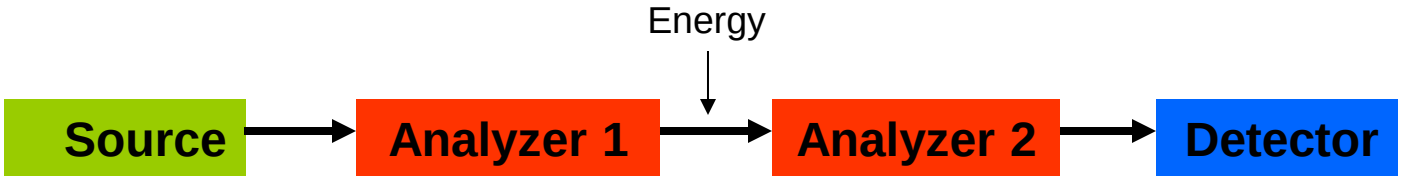


MALDI


MYC-IND Microcystis of Cyano Bacteria
CARMELI130B 66 (2.227) Cm (25:85-91:117)
TOF MS LD+
8.51e4
100
%
0
1308.5
1292.5
1309.5
1293.5
1310.5
1294.5
1331.5
1347.5
1332.5
1348.5
1311.5
1363.5
1379.5
1380.5
1295.5
1333.5
1330.4
1381.5
1334.5
1350.5
1365.5
1296.5
1258.5
1274.5
1382.5
CARMELI130C 80 (2.686) Cm (9:84-95:126)
TOF MS LD-
1.56e4
100
%
0
1268.4
1269.4
1270.4
1290.4
1307.4
1308.4
1339.4
1271.5
1292.4
1312.4
1341.4
1361.4
1272.4
1293.4
1329.4
1363.4
1378.4
1178.4
1234.5
m/z
1180
1200
1220
1240
1260
1280
1300
1320
1340
1360
1380
1400
1420

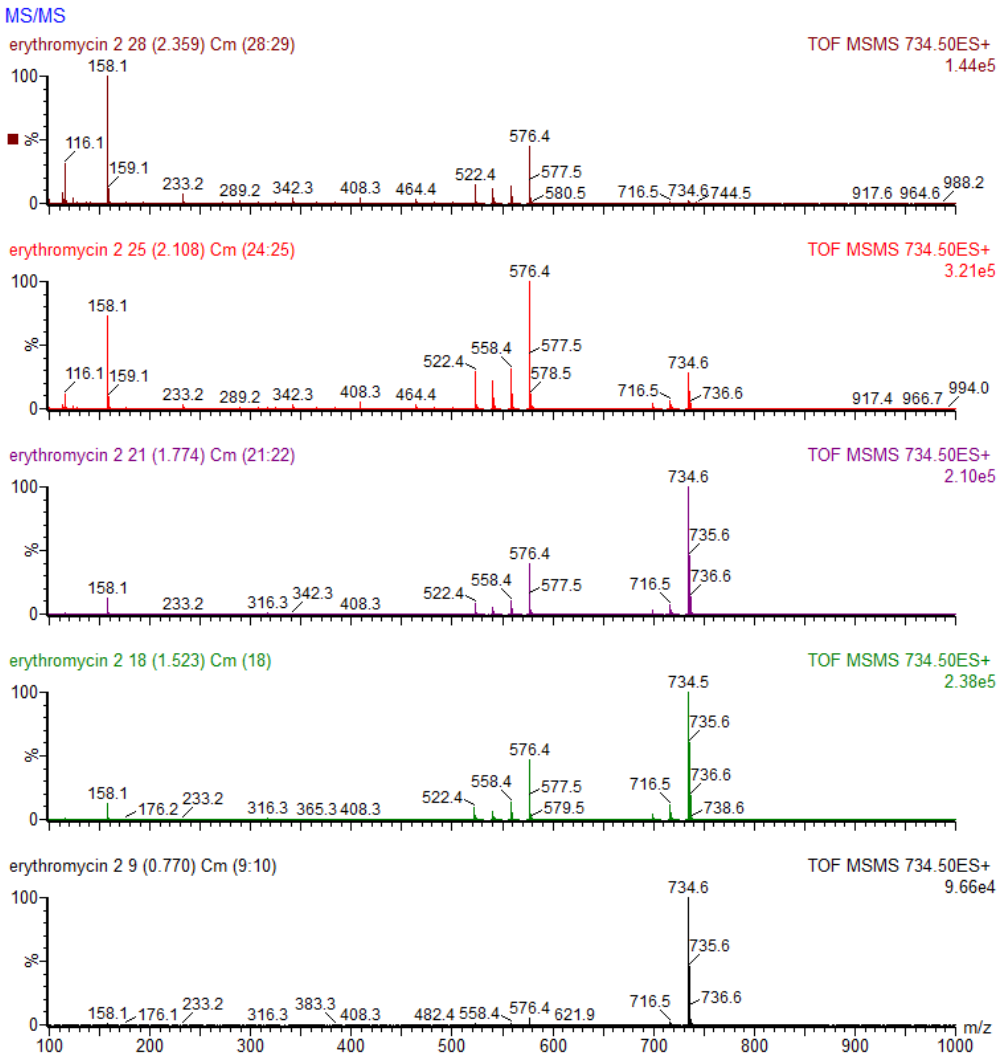
Tandem Mass Spectrometry



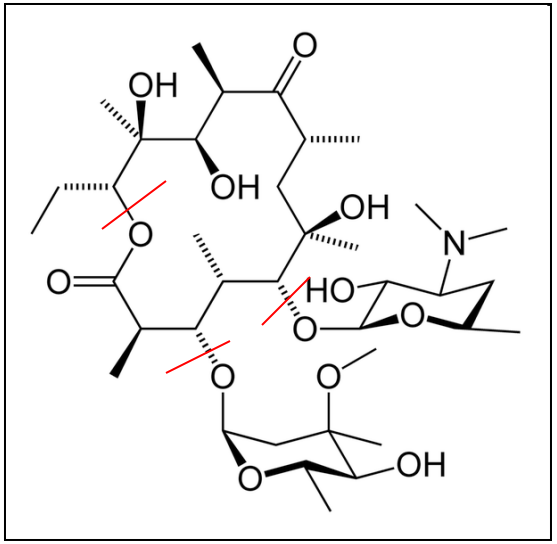


- Gentle ionization do not produce a significant amount of fragment ion
- Fragments are Important for identification and structure study.
- Tandem MS Induce fragmentation by collision with gas molecules (Argon)

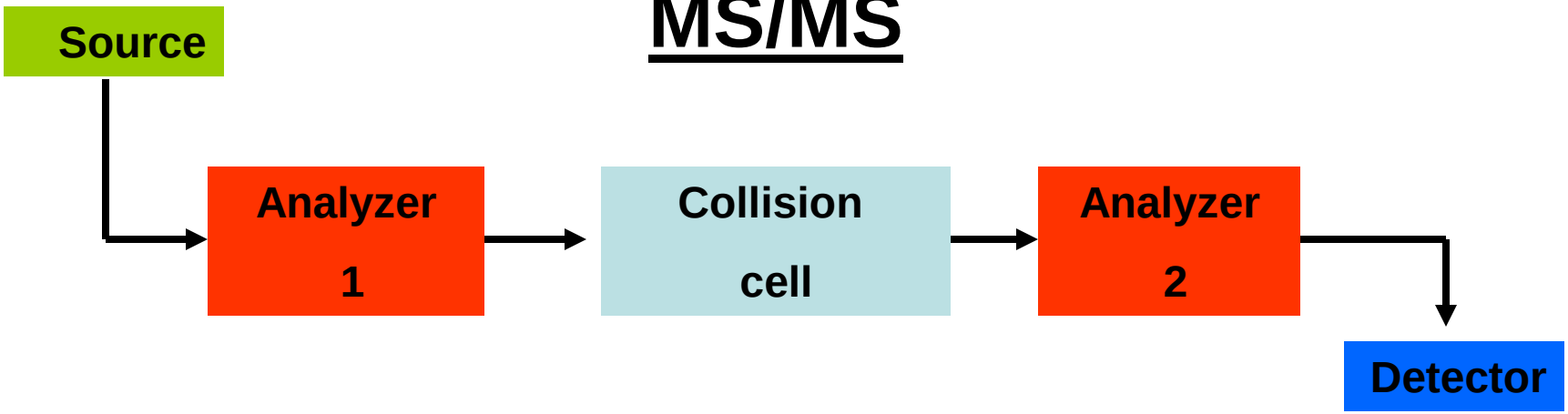
MS/MS



Collision Energy



MS/MS

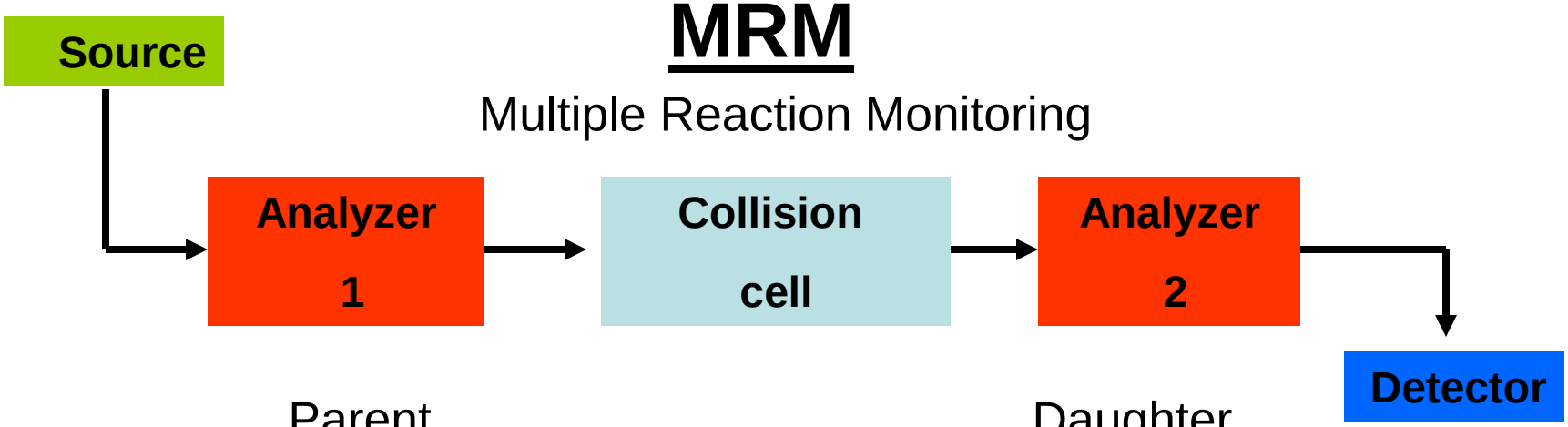


	Filter	Fragmentation	Scanner or Filter
M1			
M2	**M2**	F1, F2, F3…Fn	**F3**
M3			

Compound ⟶ **Fragments**

MRM

Multiple Reaction Monitoring



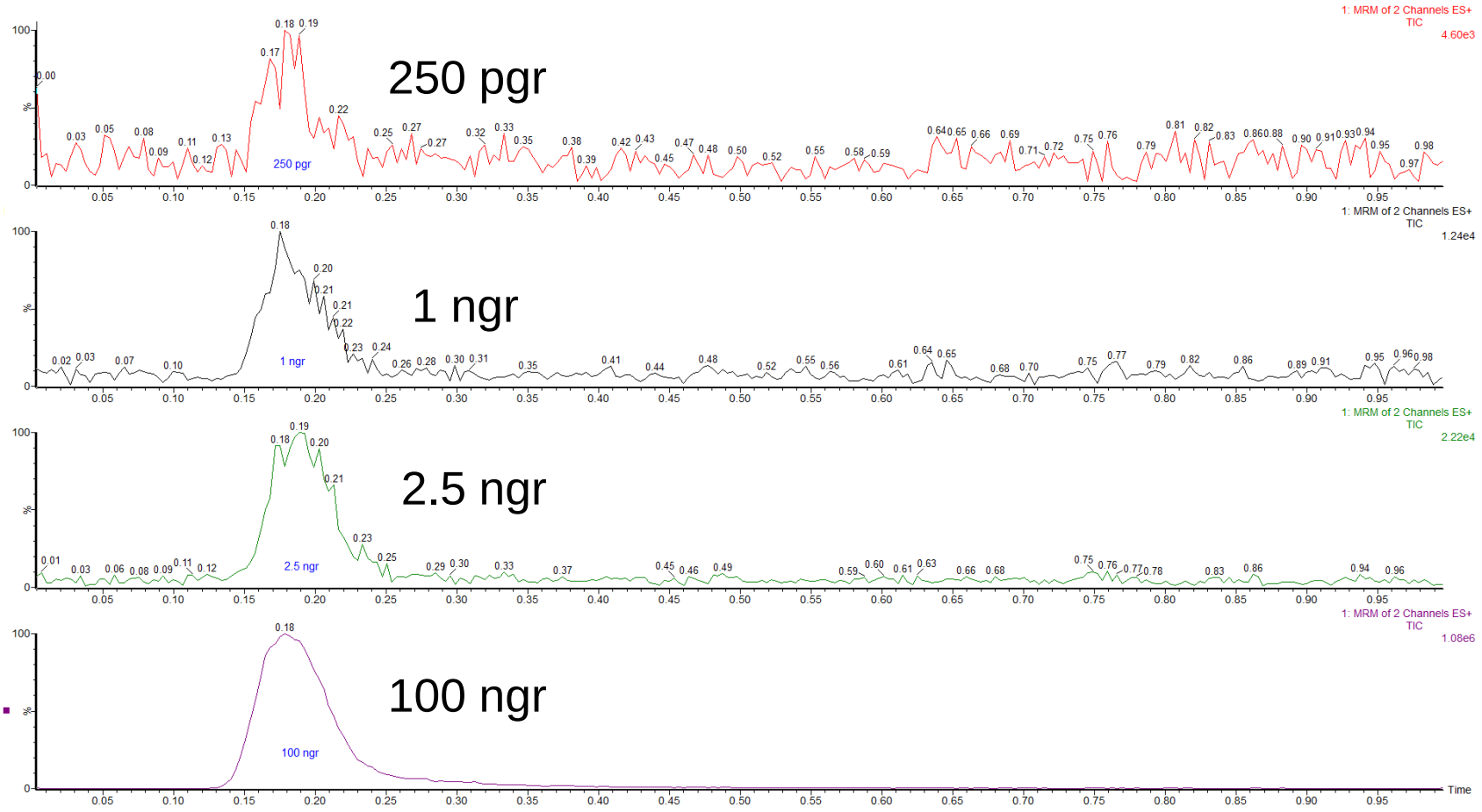
Parent	Daughter
500 (**A**)	406 (20) 322 (100) 158 (56)
500 (**B**)	478 (100) 188 (37)

Parent (500) → Fragments

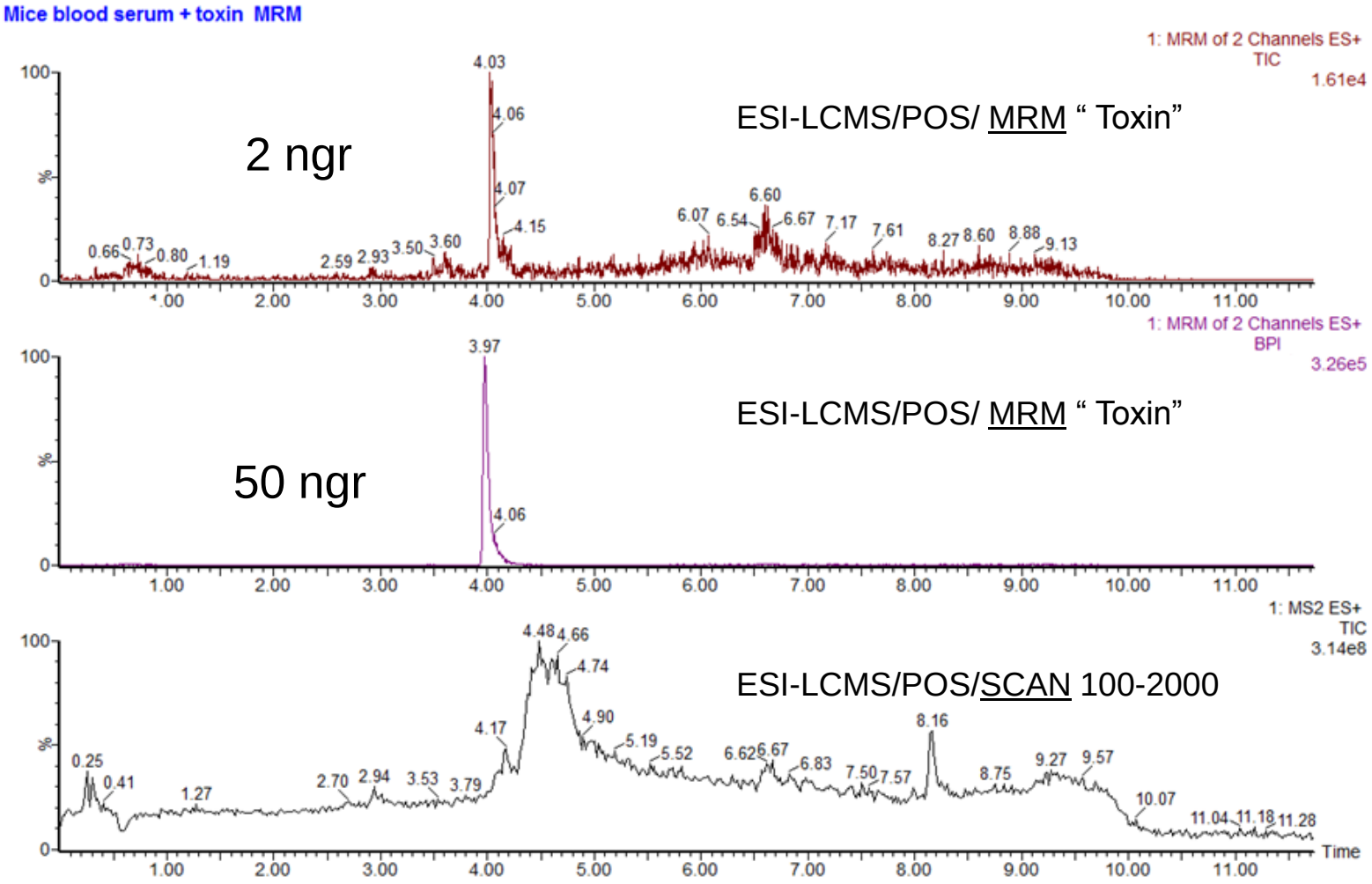
(A) or **(B)**

MRM

MRM of “Toxin”



MRM


Mice blood serum + toxin MRM
1: MRM of 2 Channels ES+
TIC
1.61e4
2 ngr
ESI-LCMS/POS/ MRM " Toxin"
1: MRM of 2 Channels ES+
BPI
3.26e5
50 ngr
ESI-LCMS/POS/ MRM " Toxin"
1: MS2 ES+
TIC
3.14e8
ESI-LCMS/POS/SCAN 100-2000
Time

Summary

- Ionization Method
- MS/ HRMS
- Isotope pattern
- Adducts
- Fragmentation
- MRM
- **Conditions** Solvent, additives, temp, flow, potential etc’.