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Qualitative Analysis Workshop



Stanford MS users' meeting
Theresa McLaughlin
Thursday, August 21, 2008

Qualitative Analysis Workshop



Brief introduction to ESI & APCI ionization
Qualitative Analysis



SUMS



Stanford University Mass Spectrometry Vincent Coates Foundation Mass Spectrometry Laboratory

*Core resource for Stanford community. Also serve
external academic institutions and industry
researchers.*

http://mass-spec.stanford.edu



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Last Modified 9.9.03

Welcome to the web home of the **Vincent Coates Foundation Mass Spectrometry Laboratory**. The laboratory is named in honor of a generous gift from Vincent and Stella Coates, given for the purpose of supporting the mass spectrometry facility as a core resource for researchers throughout the University and elsewhere. The laboratory is also a [Bio-X core facility](#), supported by James H. Clark and the Bio-X initiative in the spirit of interdisciplinary communication and collaboration.

At this time, we have in operation two quadrupole ion trap mass spectrometers and one hybrid quadrupole-time of flight MS which are equipped with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources.

Routine services include molecular weight determination, MS_n, LC-MS, and protein identification by proteolytic digest, LC-MS/MS and database search. Custom analyses are available; please [contact SUMS](#) to discuss.

Please check back regularly, as the website is constantly being developed and updated in response to user feedback. It is our hope that these pages will be a valuable resource to you.

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Stanford University
MASS SPECTROMETRY



ZQ Quadrupole MS

- Single Quadrupole LC-MS
- Waters Alliance HPLC and MassLynx Open Access software
 - Open Access for Stanford community
 - MW determination
 - Short column LC-MS





LCQ Classic MS

- Quadrupole Ion Trap LC-MS
- ThermoFinnigan Surveyor HPLC & LCQ "Classic" MS
 - MW determination
 - Analytical LC-MS
 - MSⁿ

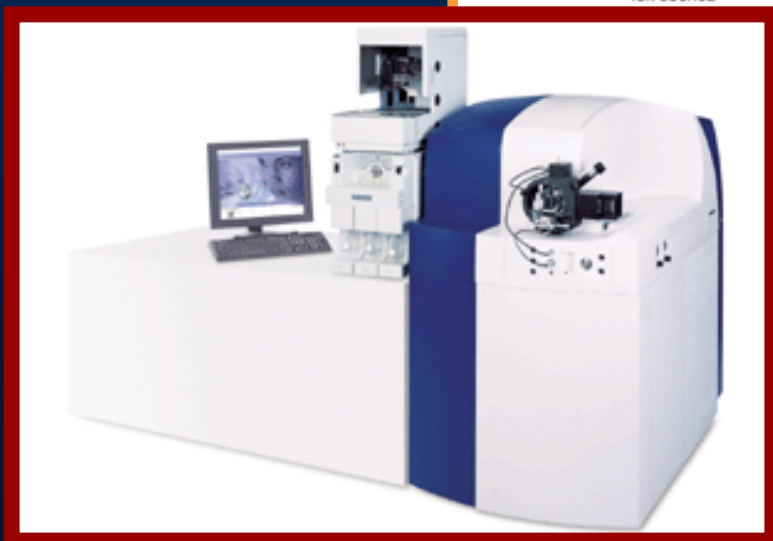
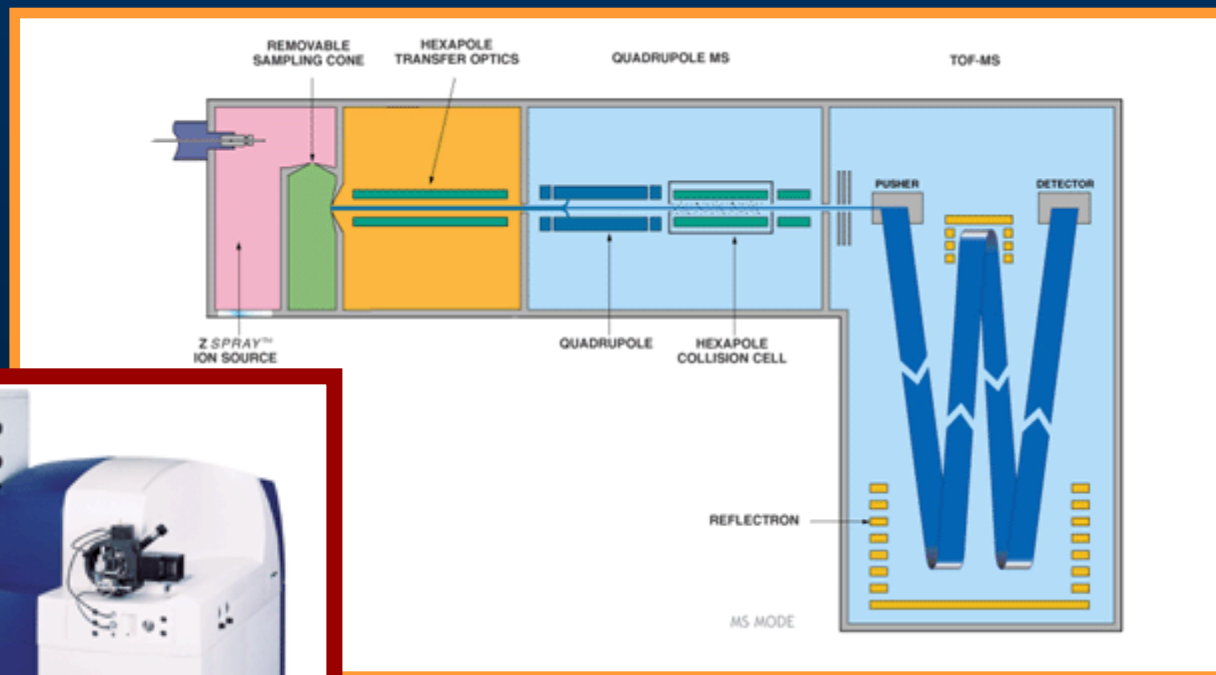




Q-ToF API

- Hybrid Tandem Quadrupole – Time of Flight MS

Micromass
Q-Tof



- High resolution MS
- Protein identification & characterization
- De novo* peptide sequencing
- Post-translational modification ID



The Mass Spectrometer: Components

1. Ion source
2. Mass analyzer, including:
 - a. Mass filter (quadrupole, ion trap, TOF, *etc.*)
 - b. Vacuum system
 - c. Some electronics
3. Detector (photomultiplier or electron multiplier)
4. Data storage, (processing), and output device (usually a computer)

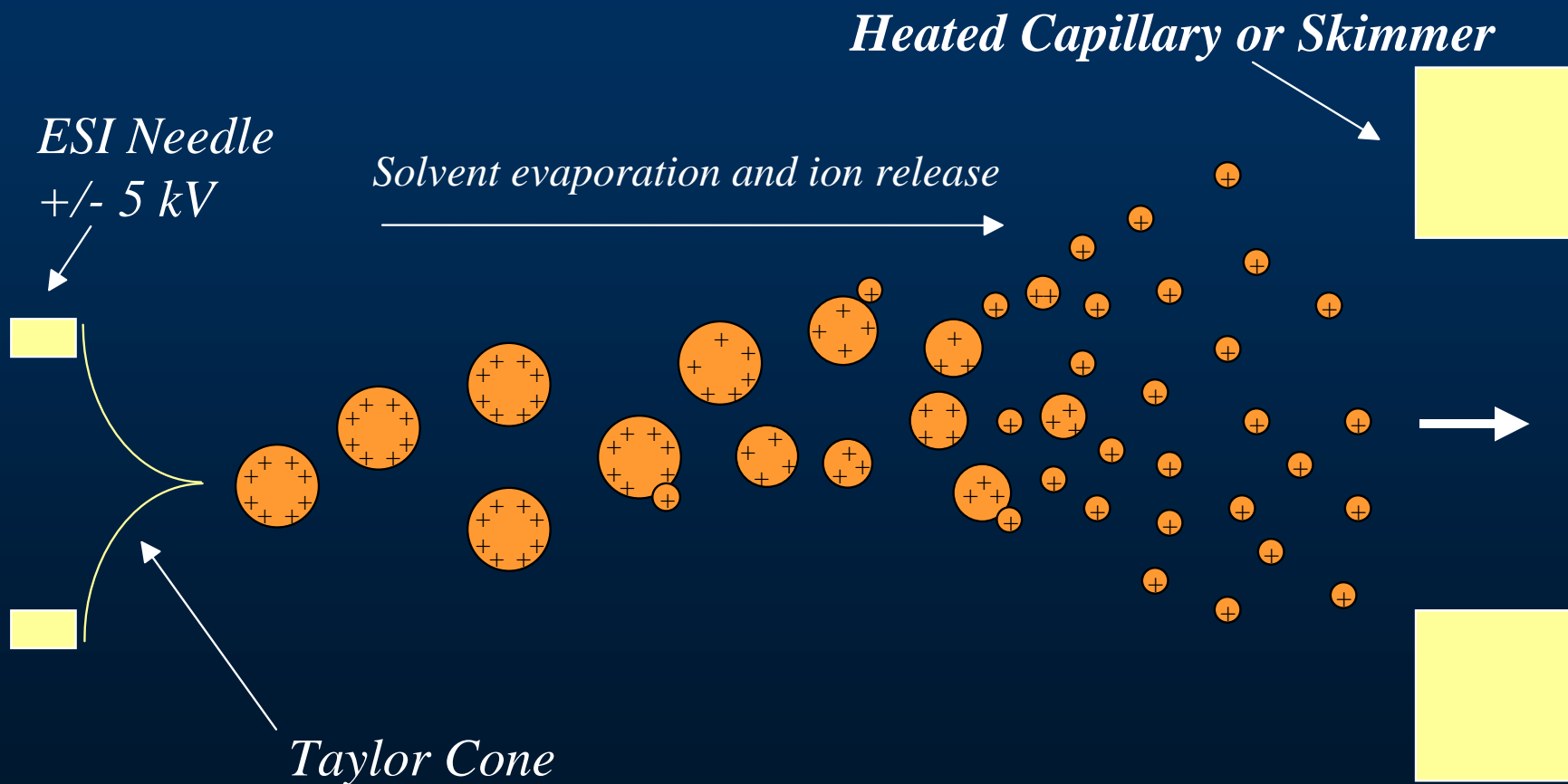


What is API?

- Atmospheric Pressure Ionization
 - ESI – Electrospray Ionization
 - Soft ionization technique
 - Solution-phase process (for the most part)
 - APCI – Atmospheric Pressure Chemical Ionization
 - Gas-phase process
- An interface between HPLC and Mass Detection
 - Designed to separate and ionize analytes from HPLC solvents



Electrospray – Basic Layout





Leading Theories

Ion evaporation - **Dole Model (1968)**

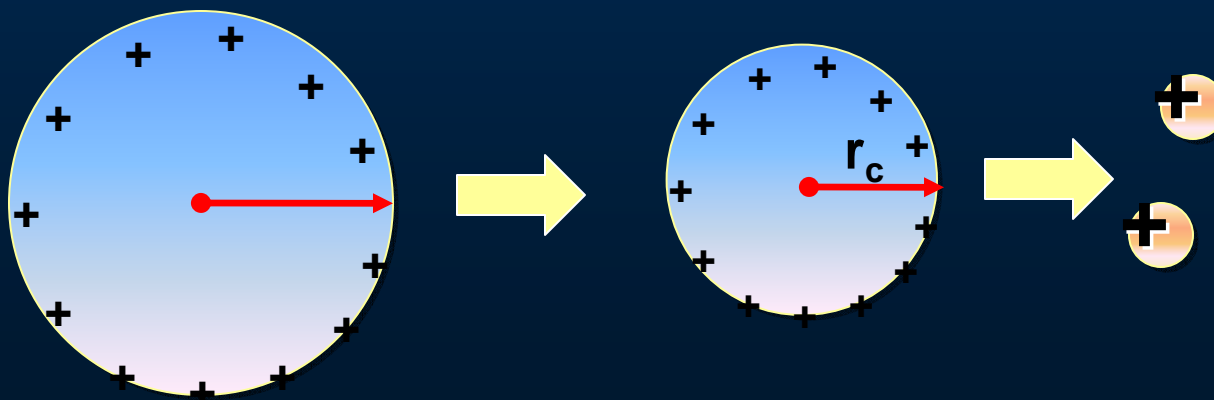
- Studied/Supported by Röllgen et al. 1989
- Requires formation of extremely small droplets ($r \sim 1 \text{ nm}$) containing only one ion.
- Solvent evaporation leaves formation of a gas phase ion
- Also known as Single Ion in Droplet Theory (SIDT)



Leading theories

Ion ejection - Iribarne and Thompson Model (1976)

- Ion emission from highly charged droplets
- Requires critical onset size and charge ($r=8\sim 10\text{nm}$ & $n\sim 70+$ charges)
- Does not require formation of very small droplets ($r\sim 1\text{nm}$) that contain only one charge





APCI: Atmospheric Pressure Chemical Ionization

Mechanism for positive ion formation

Primary ion formation:



Secondary ion formation:

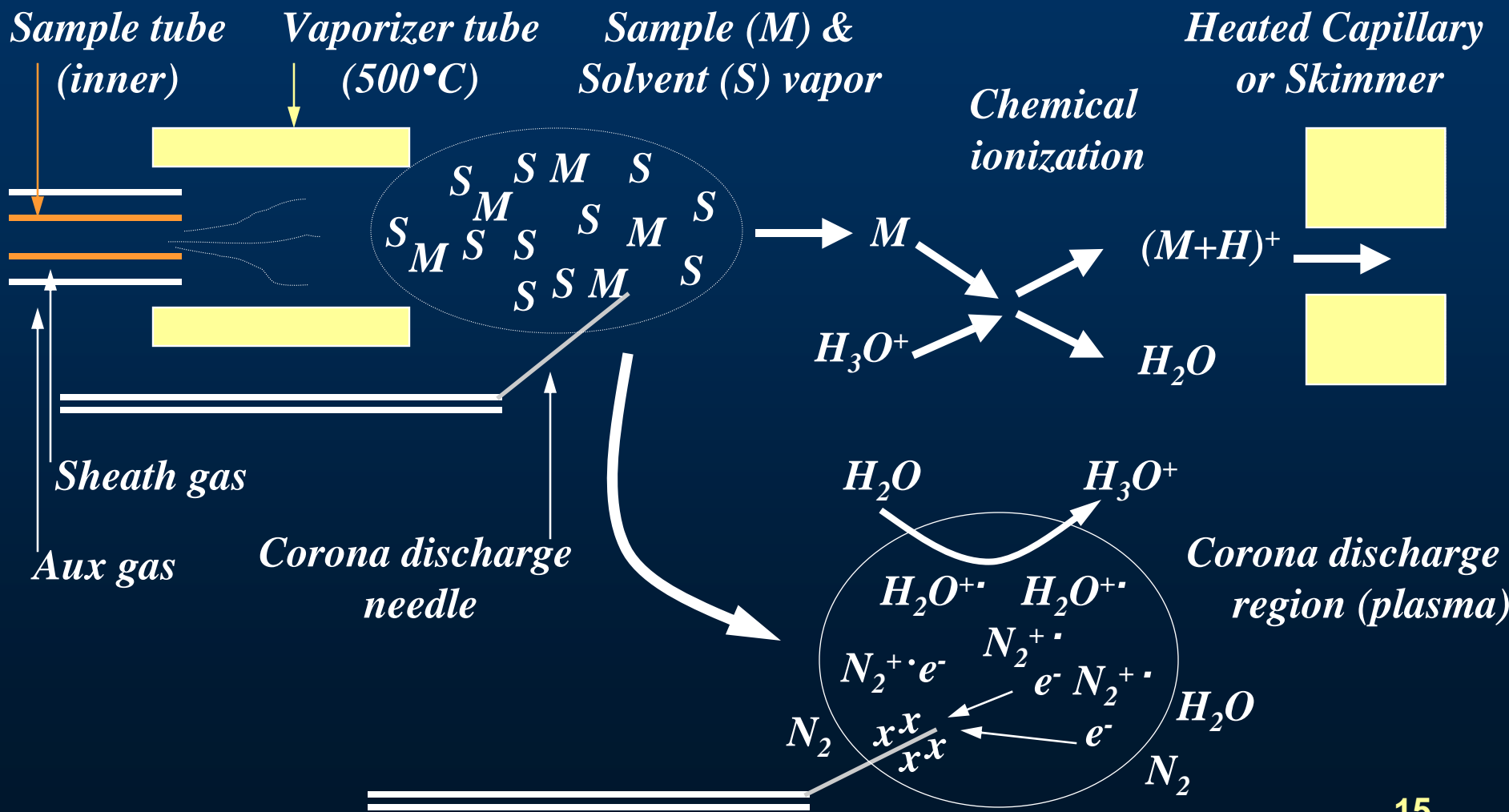


Analyte ion formation:



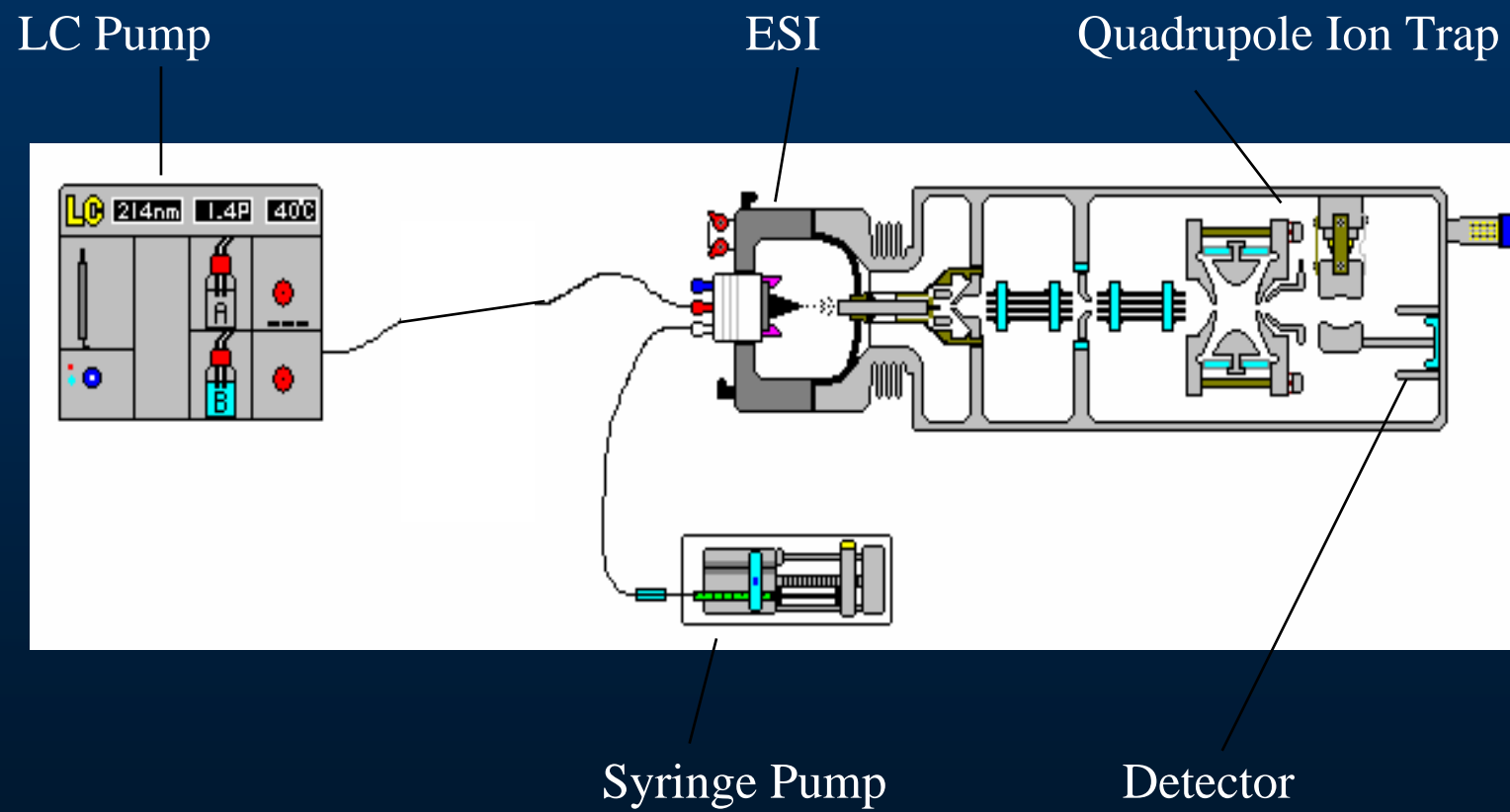


APCI - Basic Layout





Quadrupole Ion Trap



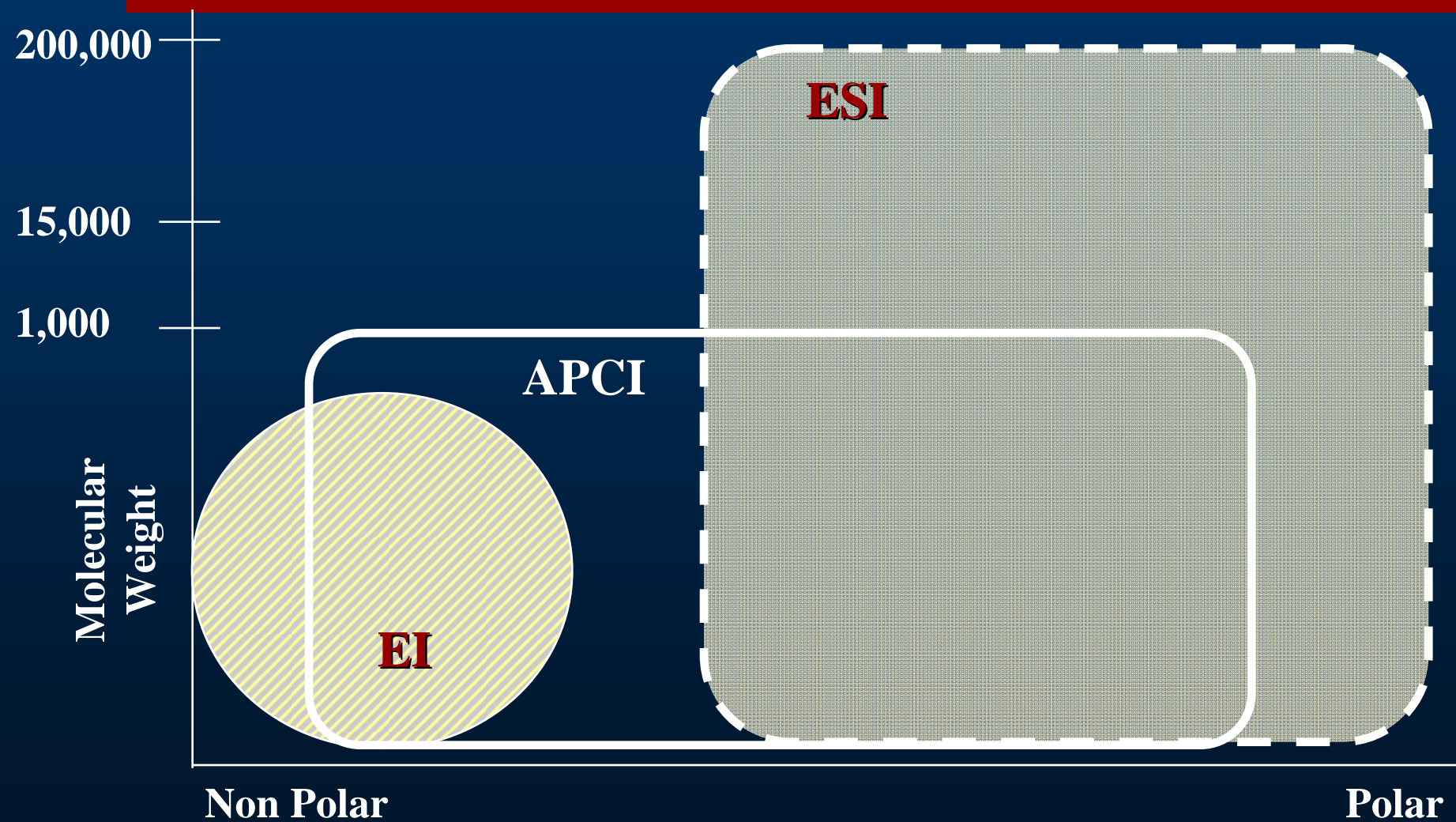


ESI or APCI ?

- Many compounds can be analyzed by both techniques with different sensitivities
- ESI is for highly polar compounds
- ESI is for molecular weights >1000 amu
- ESI is for thermally fragile compounds
- APCI generally gives more fragmentation



Analyte Compatibility





Qualitative Analysis



MW determination for Small Molecules



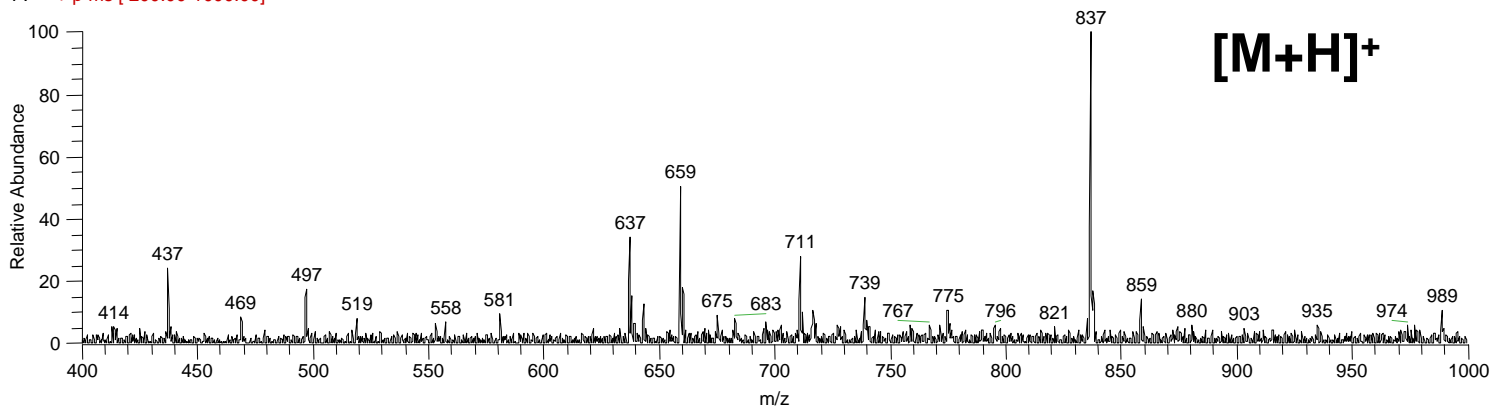
Commonly Observed Ions in ESI

D:\Xcalibur\data\Red_01

FD&C 3

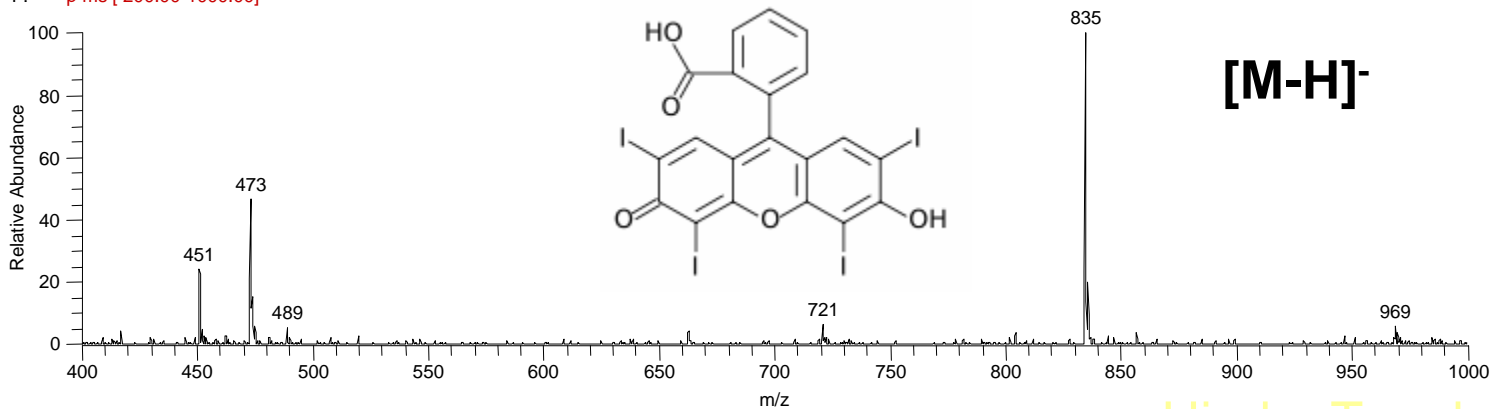
Red_01 # 306-324 RT: 4.86-5.09 AV: 19 NL: 4.14E5

F: + p ms [200.00-1000.00]



Red_01 # 1-17 RT: 0.01-0.23 AV: 17 NL: 1.86E5

F: - p ms [200.00-1000.00]



Hiroko Tanaka

20

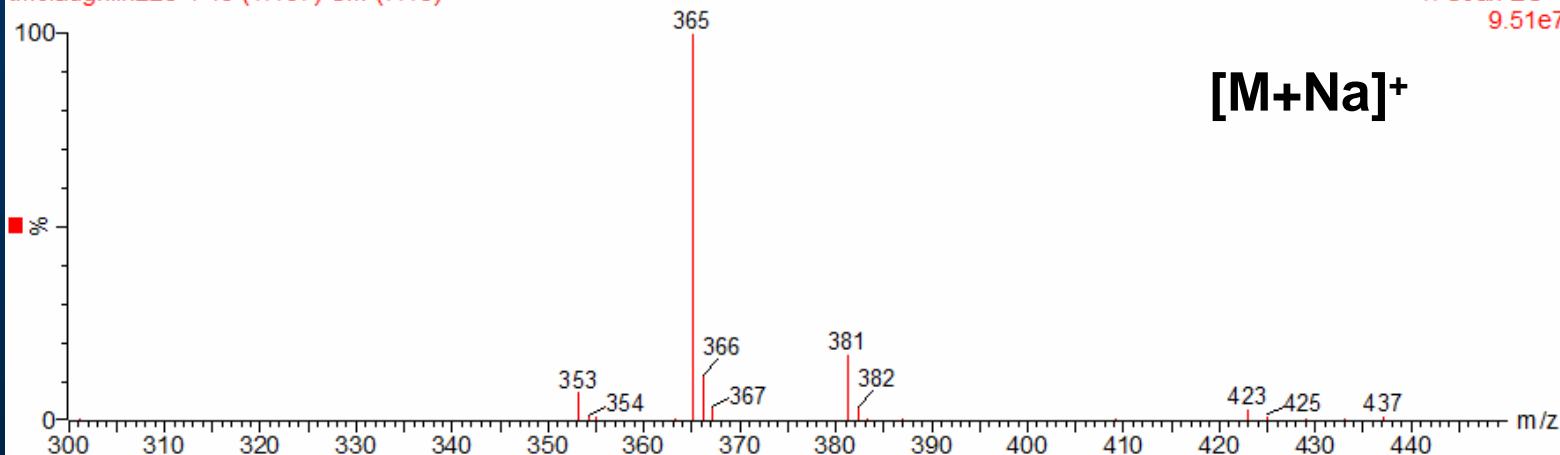


Commonly Observed Ions in ESI

Sucrose

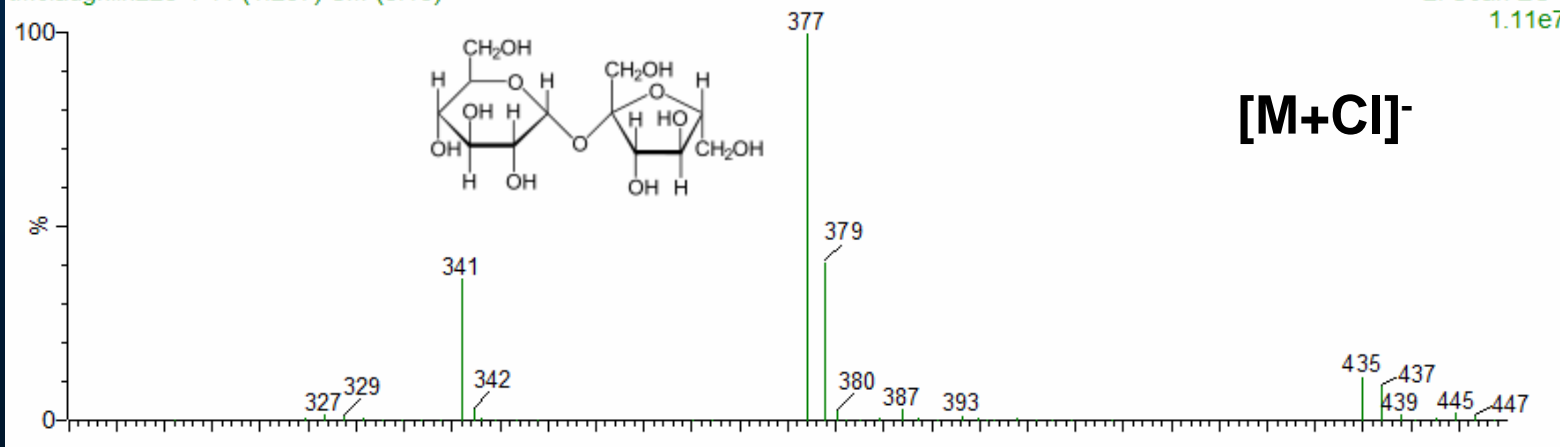
tmclaughlin226-1 13 (1.137) Cm (7:16)

1: Scan ES+
9.51e7



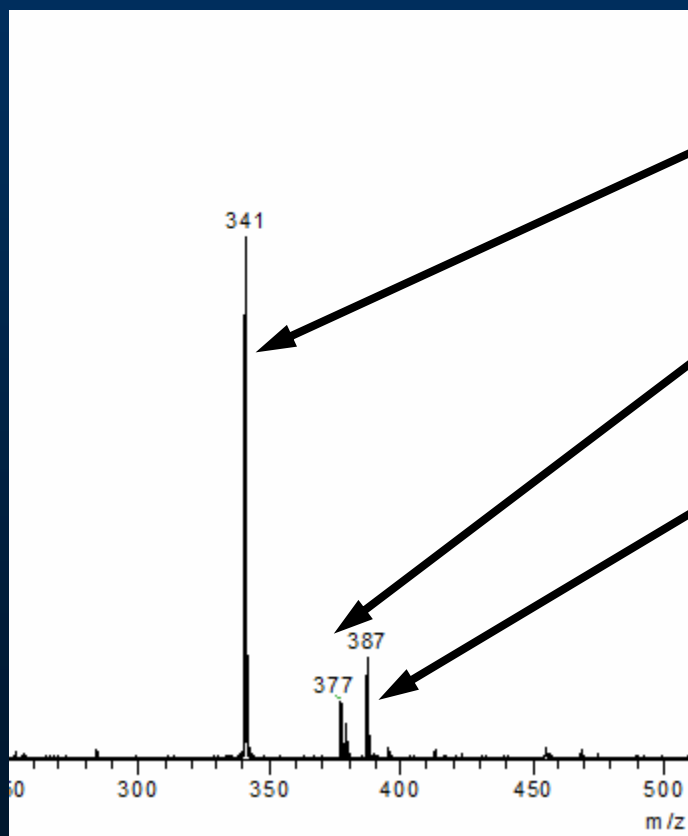
tmclaughlin226-1 14 (1.237) Cm (6:16)

2: Scan ES-
1.11e7





Commonly Observed Ions in ESI



$[M-H]^-$

$[M+Cl]^-$

$[M-H+HCOOH]^-$



Commonly Observed Ions in ESI

ESI+ adducts

- $M+H^+$
- $M+Na^+$
- $M+NH_4^+$
- $2M+H^{+n}$
- $M+nH^{n+}$

ESI- adducts

- $M-H^-$
- $M+Cl^-$
- $M-H+acid^-$
- $2M-H^-$
- $M-nH^{n-}$



Qualitative Analysis



MW determination for
Biomolecules



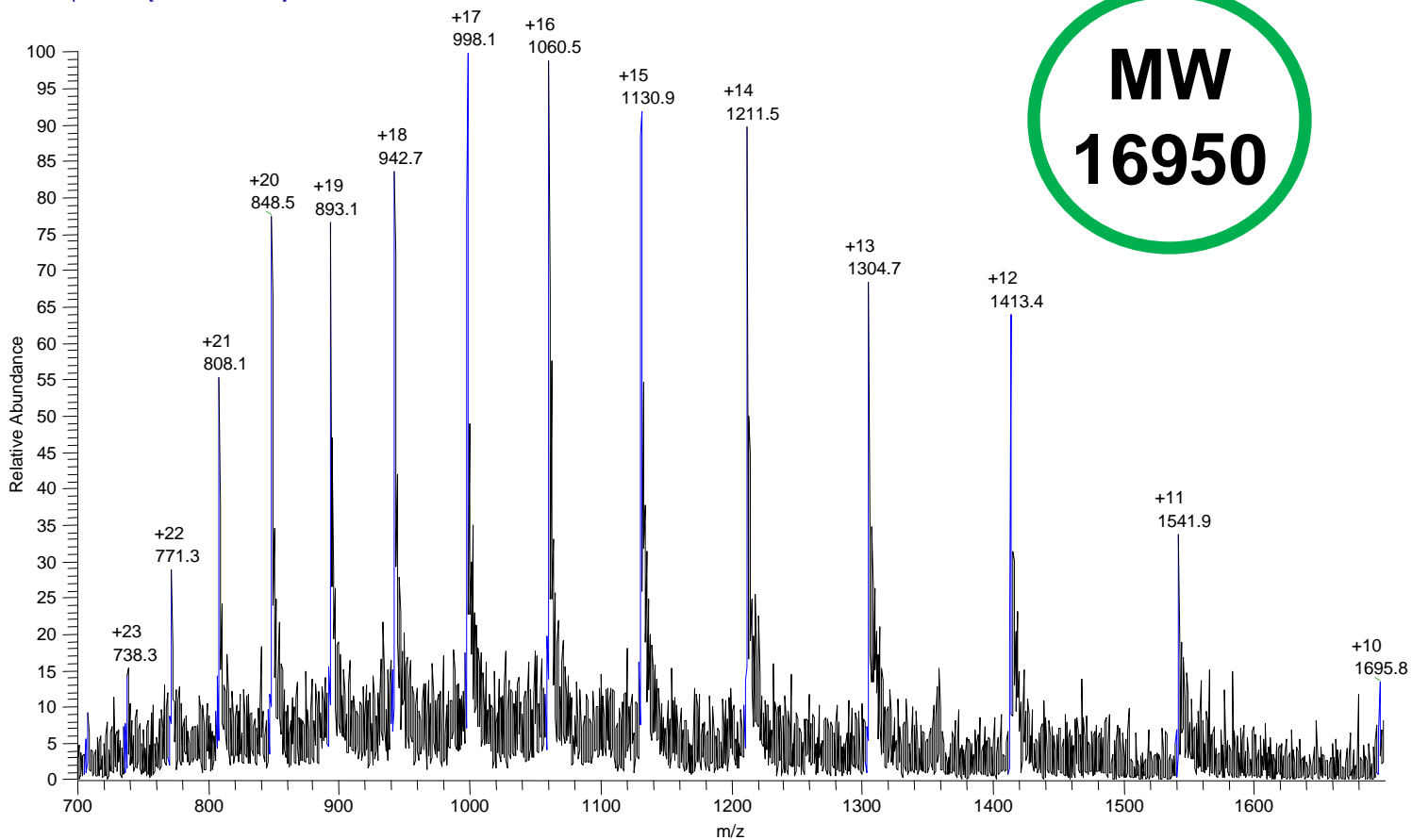
Commonly Observed Ions in ESI

D:\Xcalibur\data\Mb_01

horse heart myoglobin

Mb_01 # 747-800 RT: 14.28-15.98 AV: 54 NL: 8.25E5

T: + p Full ms [600.00-1800.00]





Commonly Observed Ions in ESI

Formula to
calculate charge of
an ion in the
distribution

$$\frac{M_n}{(M_n - M_{n+1})} = n+1$$

Example calculation

$$\frac{1541.9}{(1541.9 - 1413.4)} = 12$$

$$12 \times 1413.4 = 16961 - 12 = 16949$$



Auto Deconvolution Step 2

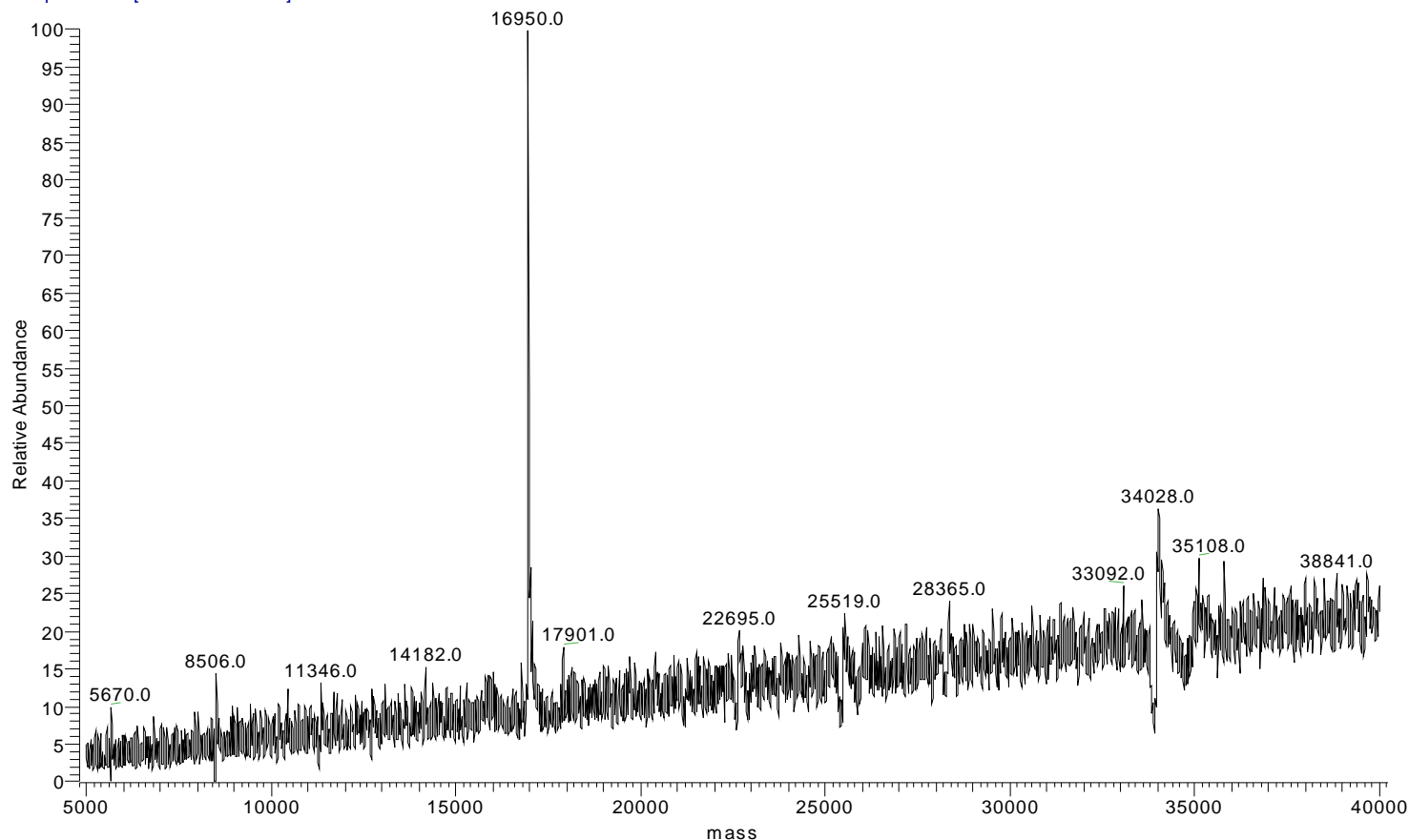
D:\Xcalibur\data\Mb_01

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horse heart myoglobin

1 RT: 0.00 P: + NL: 7.00E6

T: + p Full ms [600.00-1800.00]





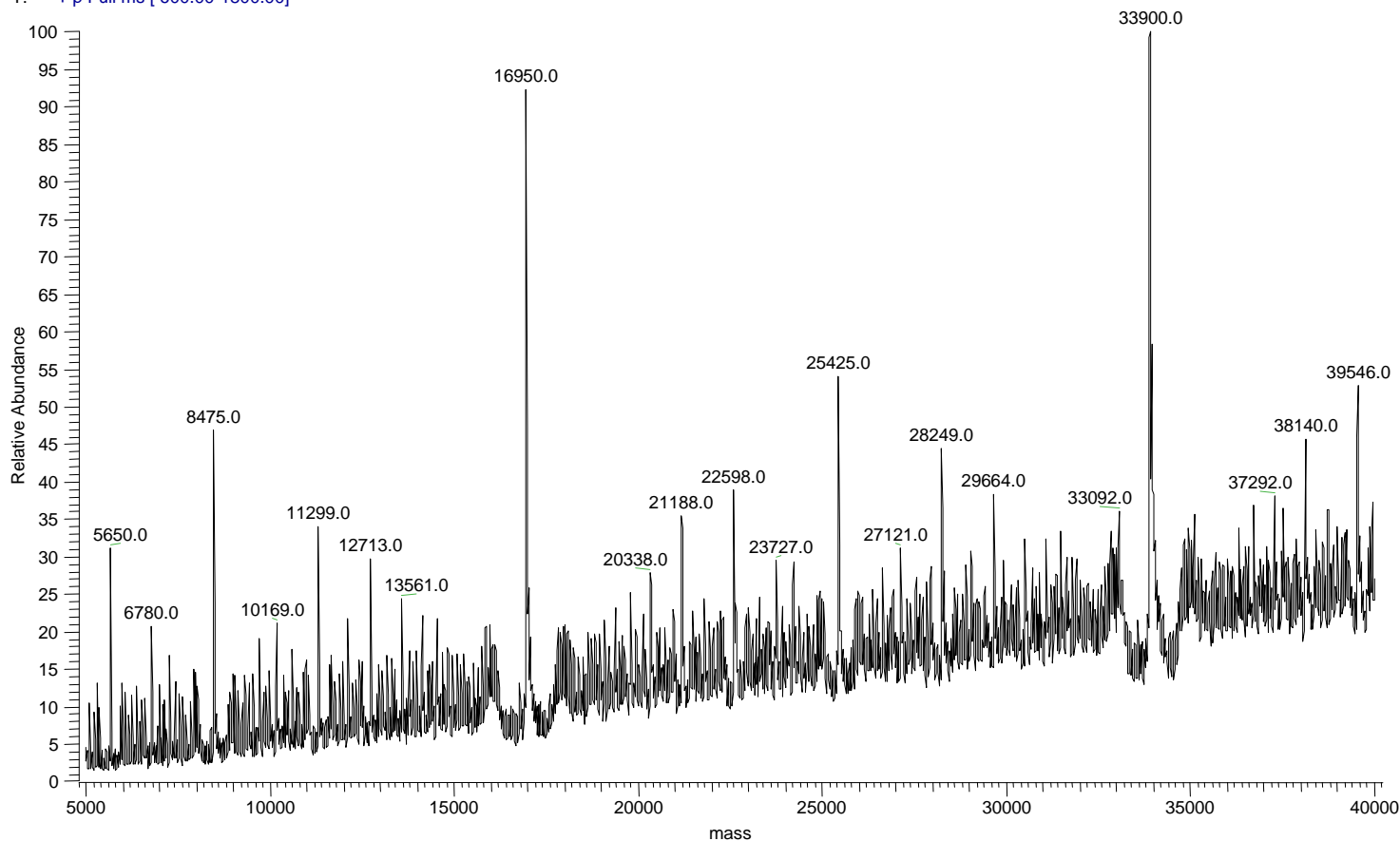
Auto Deconvolution Step 1

D:\Xcalibur\data\Mb_01

horse heart myoglobin

1 RT: 0.00 P: + NL: 7.51E6

T: + p Full ms [600.00-1800.00]



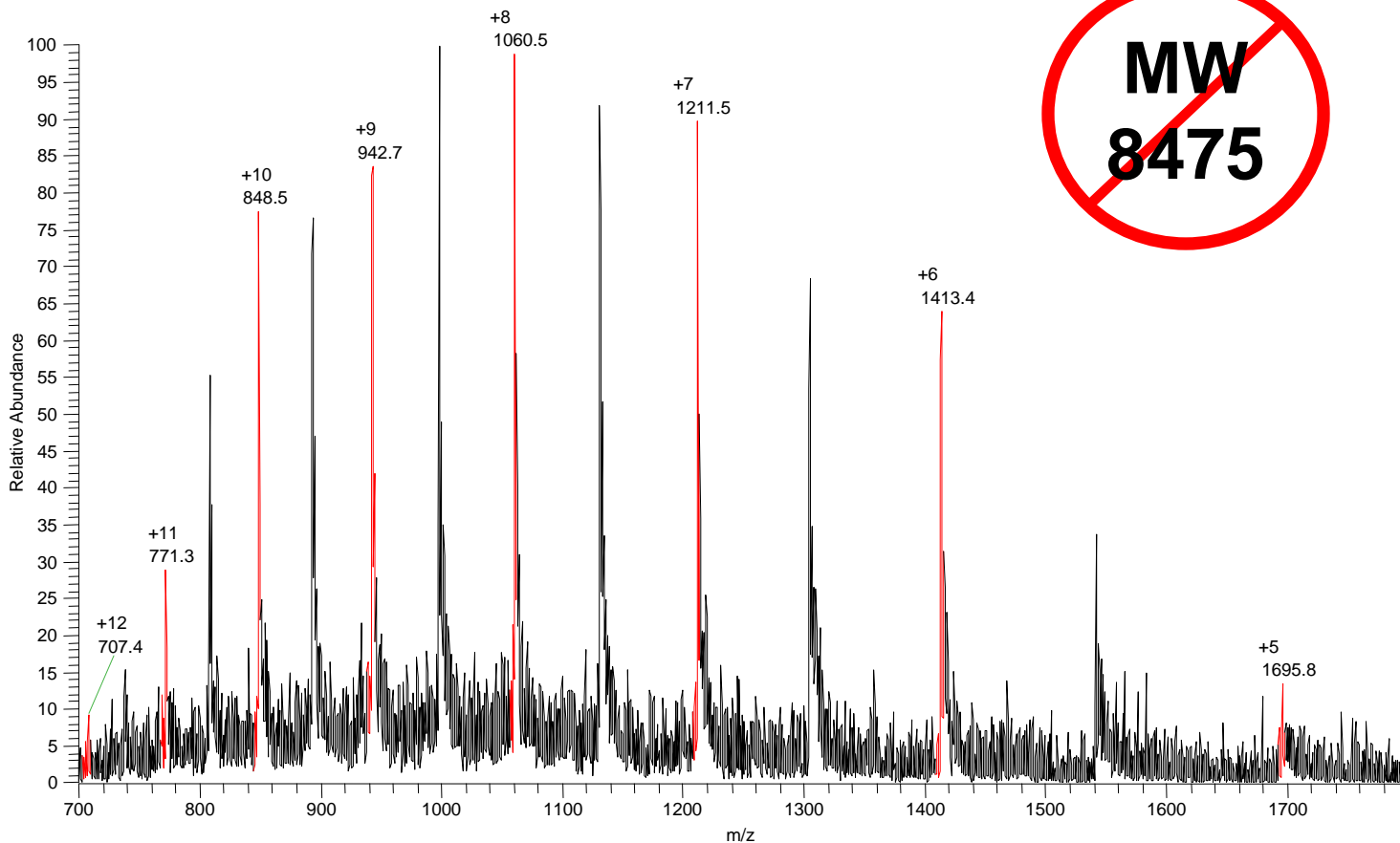


Commonly Observed Ions in ESI

D:\Xcalibur\data\Mb_01

horse heart myoglobin

Mb_01 # 747-800 RT: 14.28-15.98 AV: 54 NL: 8.25E5
T: + p Full ms [600.00-1800.00]





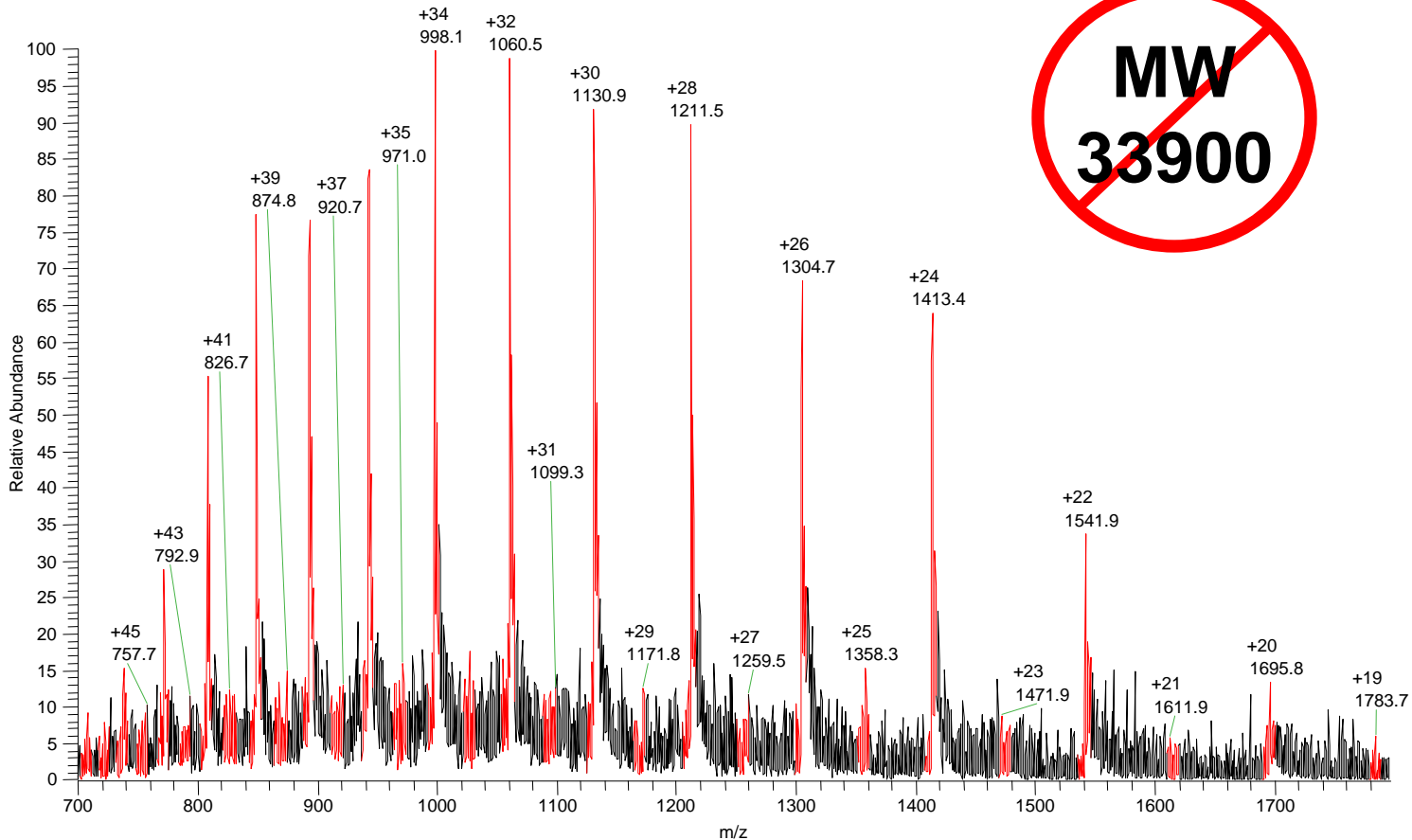
Commonly Observed Ions in ESI

D:\Xcalibur\data\Mb_01

horse heart myoglobin

Mb_01 # 747-800 RT: 14.28-15.98 AV: 54 NL: 8.25E5

T: + p Full ms [600.00-1800.00]





Qualitative Analysis



High Resolution MW determination or
accurate mass determination



High Resolution MS

The Journal of Organic Chemistry compound characterization checklist:

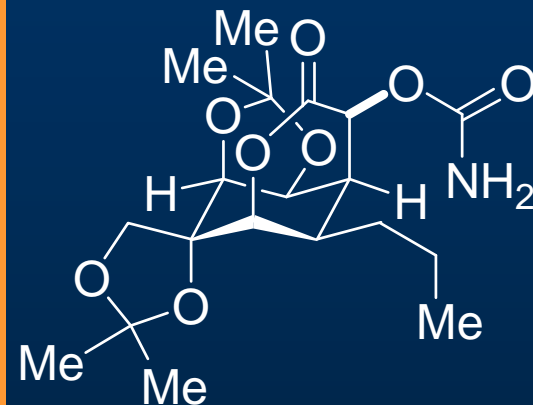
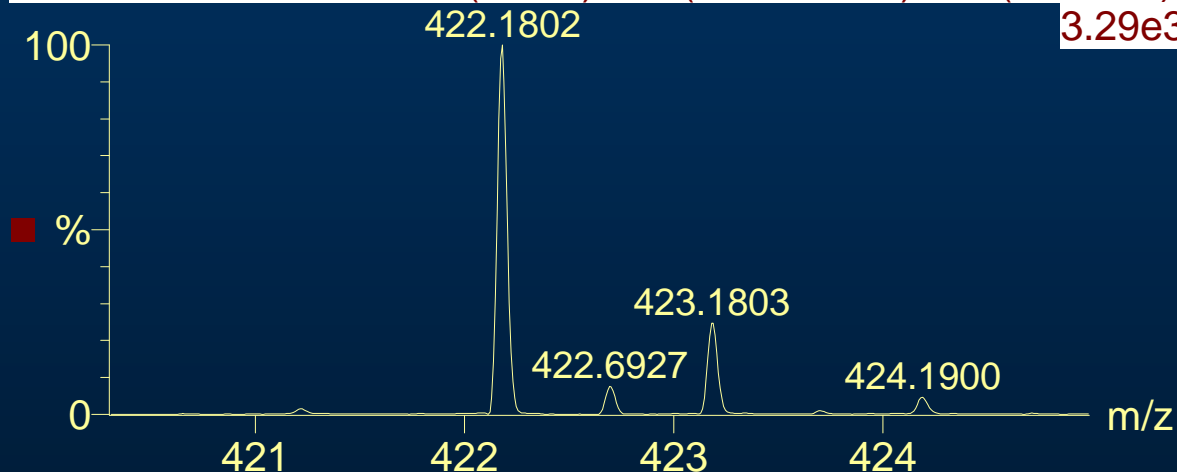
“For most new compounds, the data should include...”

- *HRMS or elemental analysis data, and*
- *a copy of a proton NMR spectrum in the supporting information.”*
- Synthetic pathway is documented
- Purified material is used for analysis



High-Resolution MS - Q-ToF

031103_12403_AH 132 (2.257) Sm (SG, 2x3.00); Sb (5,40.00); 3.29e3



M (neutral)
 $C_{19}H_{29}NO_8$
MW 399.1893

$[M+Na]^+$
 $C_{19}H_{29}NO_8Na$
MW 422.1791

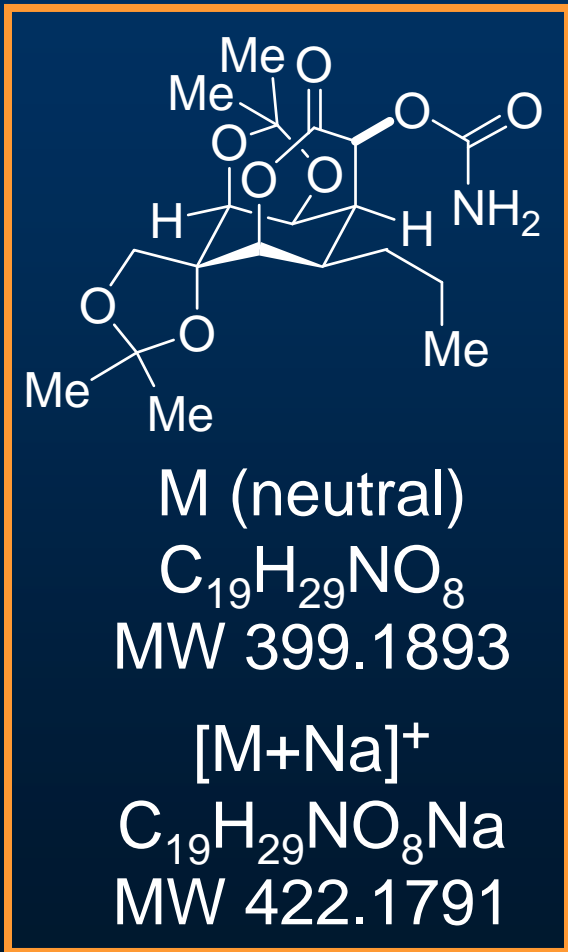
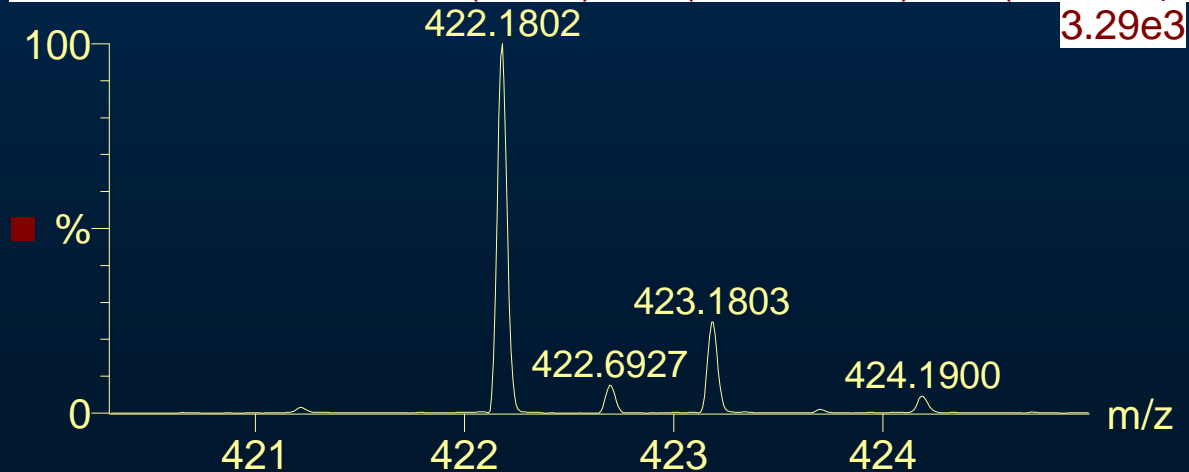


Centroided Spectrum

031103_12403_AH 132 (2.257) AM (Cen,4, 80.00, Ar,5000.0,0.0)



031103_12403_AH 132 (2.257) Sm (SG, 2x3.00); Sb (5,40.00);





Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions

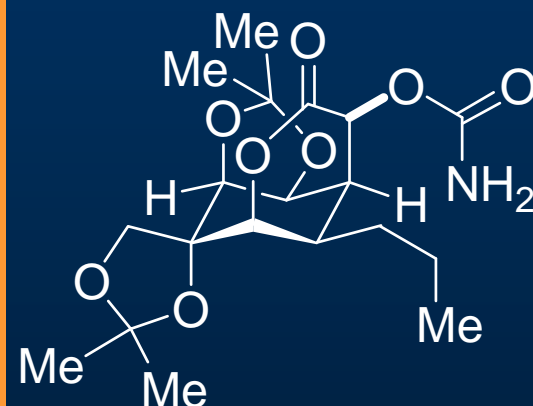
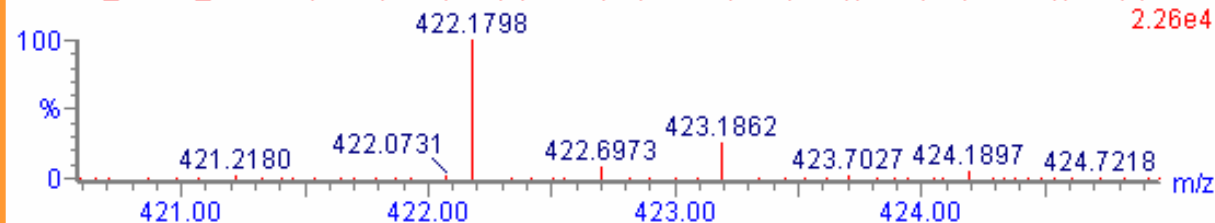
810 formula(e) evaluated with 8 results within limits (up to 50 closest results for each)

Mass	Calc. Mass	mDa	PPM	DBE	Formula	Score	C	H	N	O	Na
422.1798	422.1801	-0.3	-0.8	9.0	C19 H26 N4 O7	4	19	26	4	7	
422.1804	422.1804	-0.6	-1.5	10.5	C20 H25 N5 O4 Na	3	20	25	5	4	1
422.1791	422.1791	0.7	1.7	5.5	C19 H29 N O8 Na	5	19	29	1	8	1
422.1788	422.1788	1.0	2.3	4.0	C18 H30 O11	6	18	30		11	
422.1783	422.1783	1.5	3.6	22.0	C31 H22 N2	8	31	22	2		
422.1815	422.1815	-1.7	-4.0	8.5	C21 H28 N O8	1	21	28	1	8	
422.1818	422.1818	-2.0	-4.7	10.0	C22 H27 N2 O5 Na	2	22	27	2	5	1
422.1777	422.1777	2.1	4.9	6.0	C17 H27 N4 O7 Na	7	17	27	4	7	1



AH XII-20

031103_12403_AH 132 (2.257) AM (Cen,4, 80.00, Ar,5000.0,0.00,1.00); Sm (SG, 2x3.00); Sb (5,40.00 2.26e4



$[M+Na]^+$
 $C_{19}H_{29}NO_8Na$
MW 422.1791



Elemental Composition Report

422.1791 amu, 1.7 ppm, C₁₉H₂₉NO₈Na

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

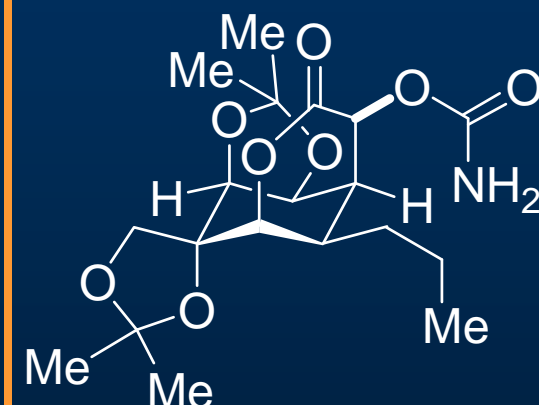
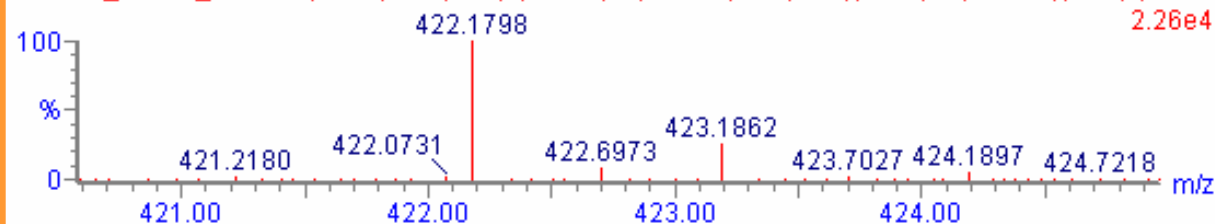
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions

810 formula(e) evaluated with 8 results within limits (up to 50 closest results for each)

Mass	Calc. Mass	mDa	PPM	DBE	Formula	Score	C	H	N	O	Na
422.1798	422.1801	-0.3	-0.8	9.0	C19 H26 N4 O7	4	19	26	4	7	
	422.1804	-0.6	-1.5	10.5	C20 H25 N5 O4 Na	3	20	25	5	4	1
	422.1791	0.7	1.7	5.5	C19 H29 N O8 Na	5	19	29	1	8	1
	422.1788	1.0	2.3	4.0	C18 H30 O11	6	18	30		11	
	422.1783	1.5	3.6	22.0	C31 H22 N2	8	31	22	2		
	422.1815	-1.7	-4.0	8.5	C21 H28 N O8	1	21	28	1	8	
	422.1818	-2.0	-4.7	10.0	C22 H27 N2 O5 Na	2	22	27	2	5	1
	422.1777	2.1	4.9	6.0	C17 H27 N4 O7 Na	7	17	27	4	7	1

AH XII-20
031103_12403_AH 132 (2.257) AM (Cen,4, 80.00, Ar,5000.0,0.00,1.00); Sm (SG, 2x3.00); Sb (5,40.00



[M+Na]⁺
C₁₉H₂₉NO₈Na
MW 422.1791



Qualitative Analysis



LC-MS and LC-MSn



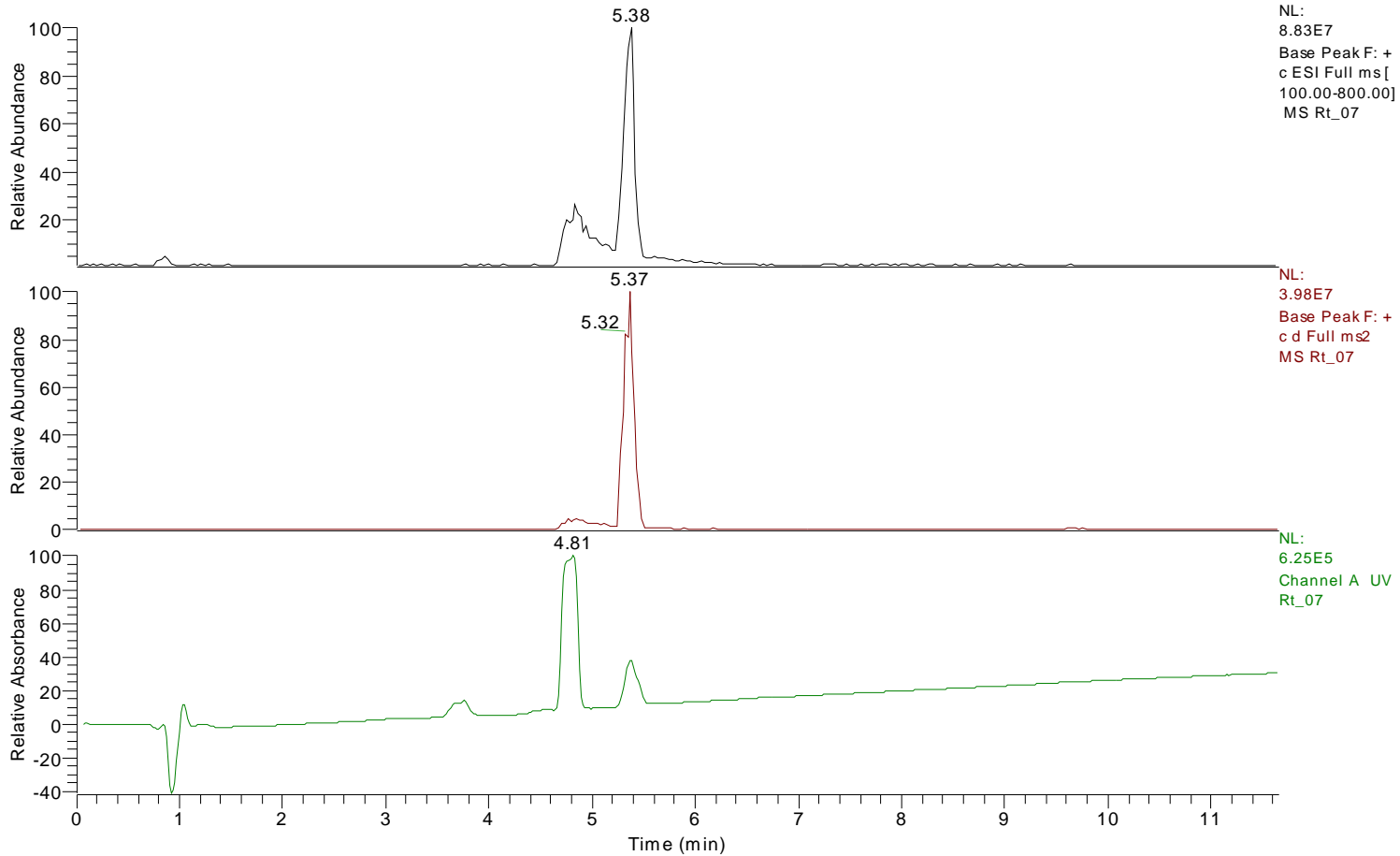
LC-MS of cough syrup

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t0

RT: 0.00 - 11.65





Data dependent MS²

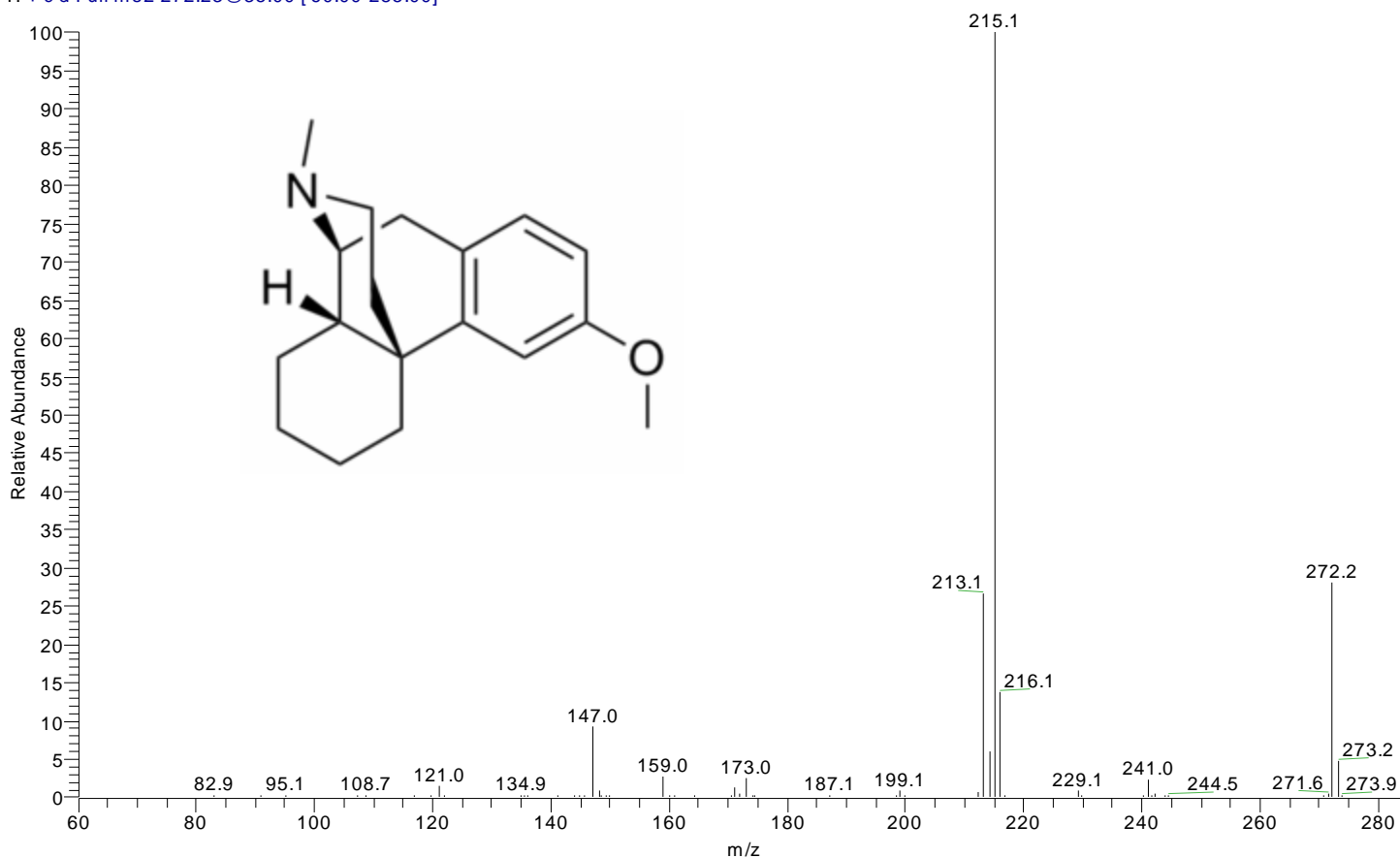
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08/20/08 03:47:54 PM

t0

Rt_07 #293-306 RT: 5.28-5.41 AV: 7 NL: 2.64E7

T: + c d Full ms2 272.23@35.00 [60.00-285.00]





Tips for Analysis of Unknowns

If there is a proposed structure, provide a standard along with the unknown sample whenever possible.

If no standard is available, isolation of the peak of interest to obtain H-NMR or synthesis of the proposed structure may still be necessary for definitive identification.



Conclusion



Acknowledgements



Acknowledgements

SUMS:

- Allis Chien
- Chris Adams
- Karolina Krasinska
- Pavel Aronov

Thermo Fisher Scientific

- Rohan Thakur

Funding:

- Stanford Bio-X Initiative
- Vincent Coates Foundation