Qualitative Analysis Workshop

Stanford MS users' meeting

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Thursday, August 21, 2008

Stanford University
MASS SPECTROMETRY

Qualitative Analysis Workshop

Brief introduction to ESI & APCI ionization

Qualitative Analysis

Stanford University
MASS SPECTROMETRY

SUMS

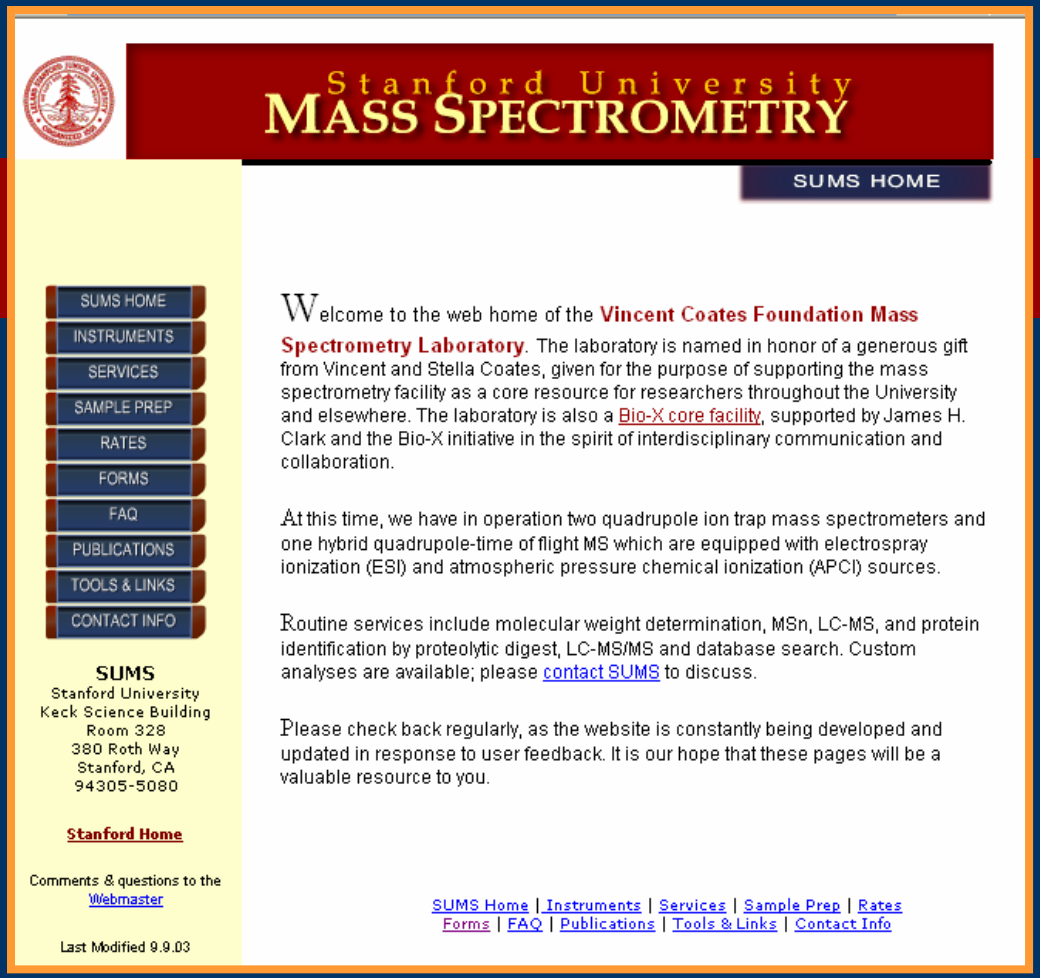
Stanford University Mass Spectrometry

Vincent Coates Foundation Mass Spectrometry Laboratory

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

Stanford University MASS SPECTROMETRY

http://mass-spec.stanford.edu



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

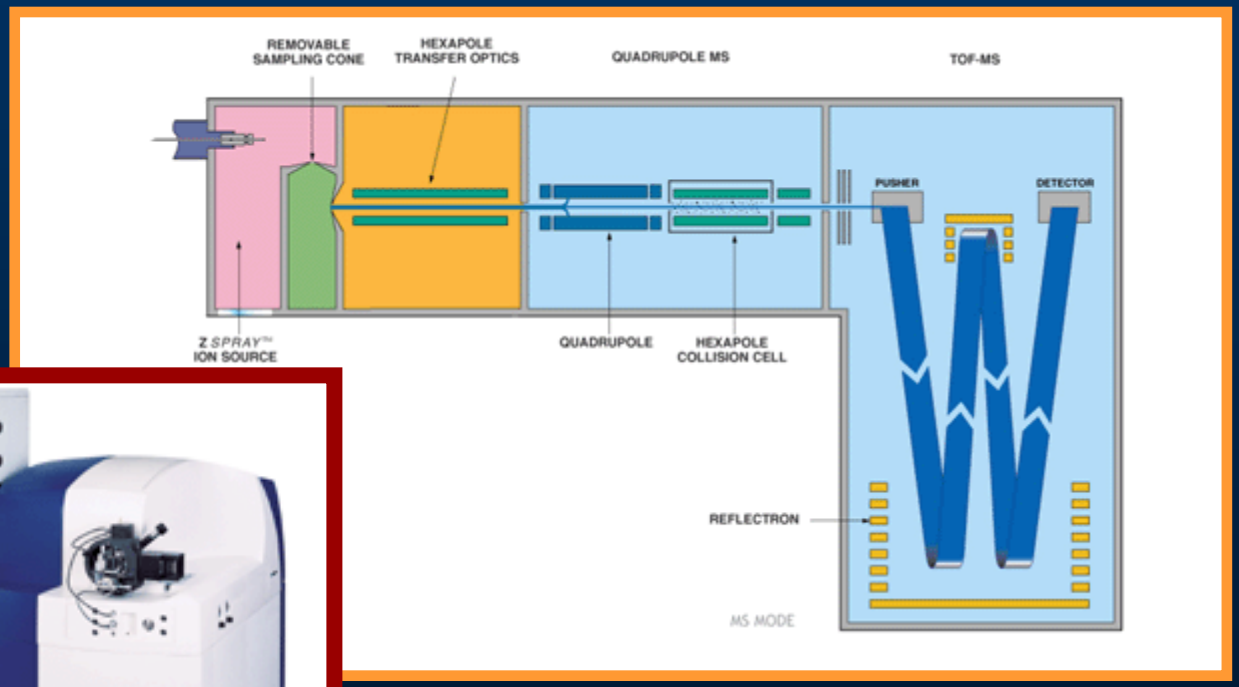
LCQ Classic MS

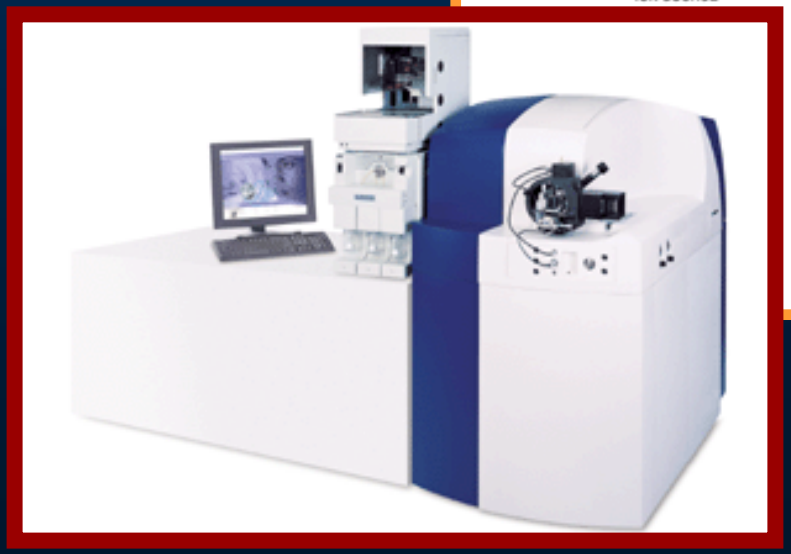
- Quadrupole Ion Trap LC-MS
- ThermoFinnigan Surveyor HPLC & LCQ “Classic” MS
  - MW determination
  - Analytical LC-MS
  - MS^n

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

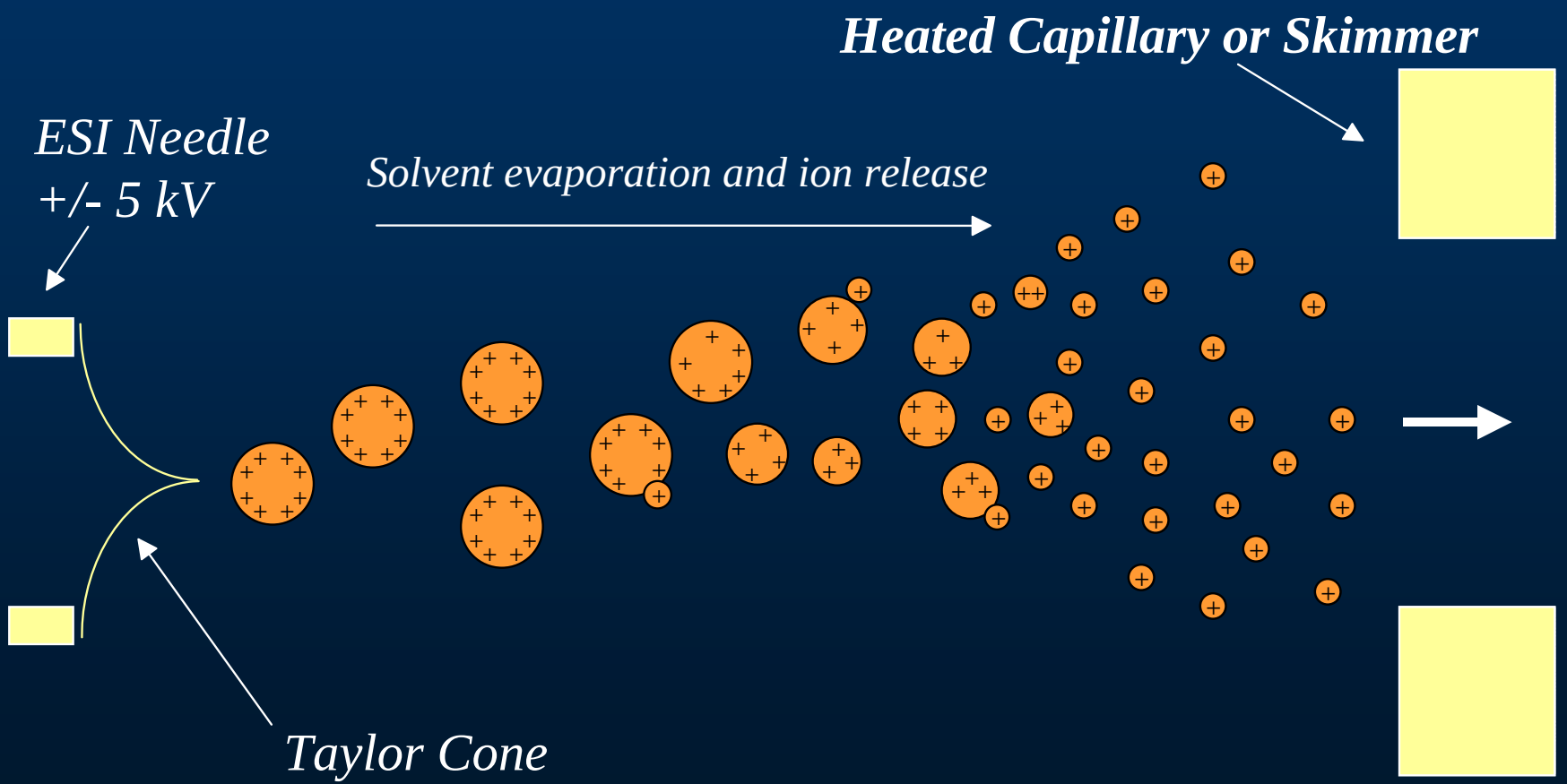
The Mass Spectrometer: **Components**

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

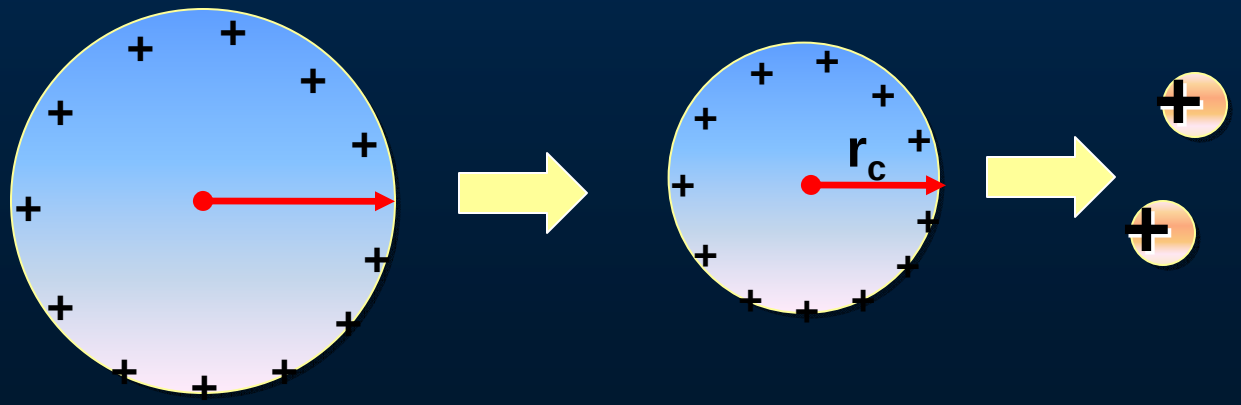
Ion evaporation **- Dole Model (1968)**

- Studied/Supported by Röllgen et al. 1989
- Requires formation of extremely small droplets (r~1nm) 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=8~10nm & n~70+ charges)
- Does not require formation of very small droplets (r~1nm) that contain only one charge



APCI: Atmospheric Pressure Chemical Ionization

Mechanism for positive ion formation

Primary ion formation:

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

$$H_2O + e^- \rightarrow H_2O^{+\bullet} + 2e^-$$

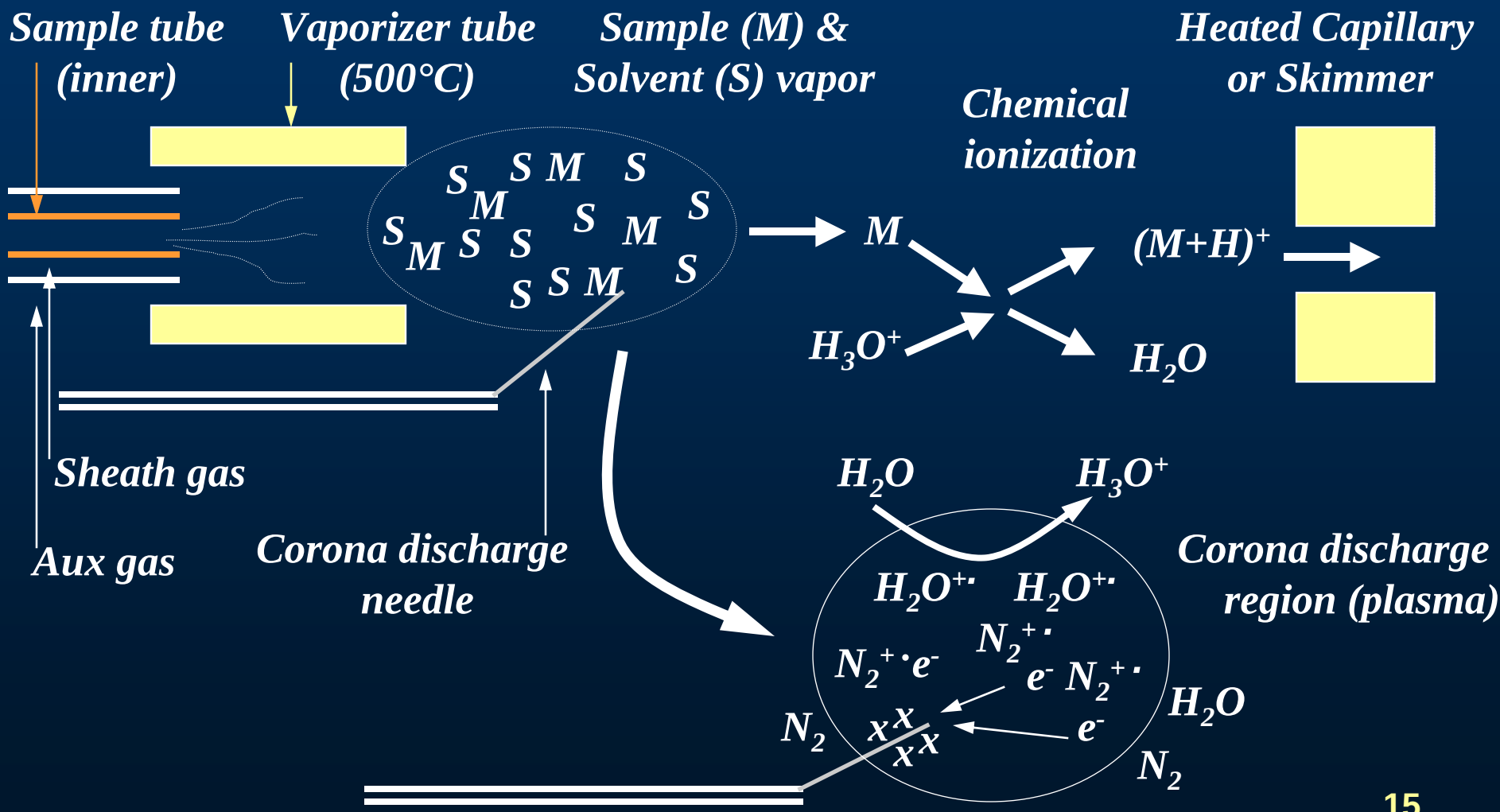
Secondary ion formation:

$$H_2O^{+\bullet} + H_2O \rightarrow H_3O^+ + {}^{\bullet}OH$$

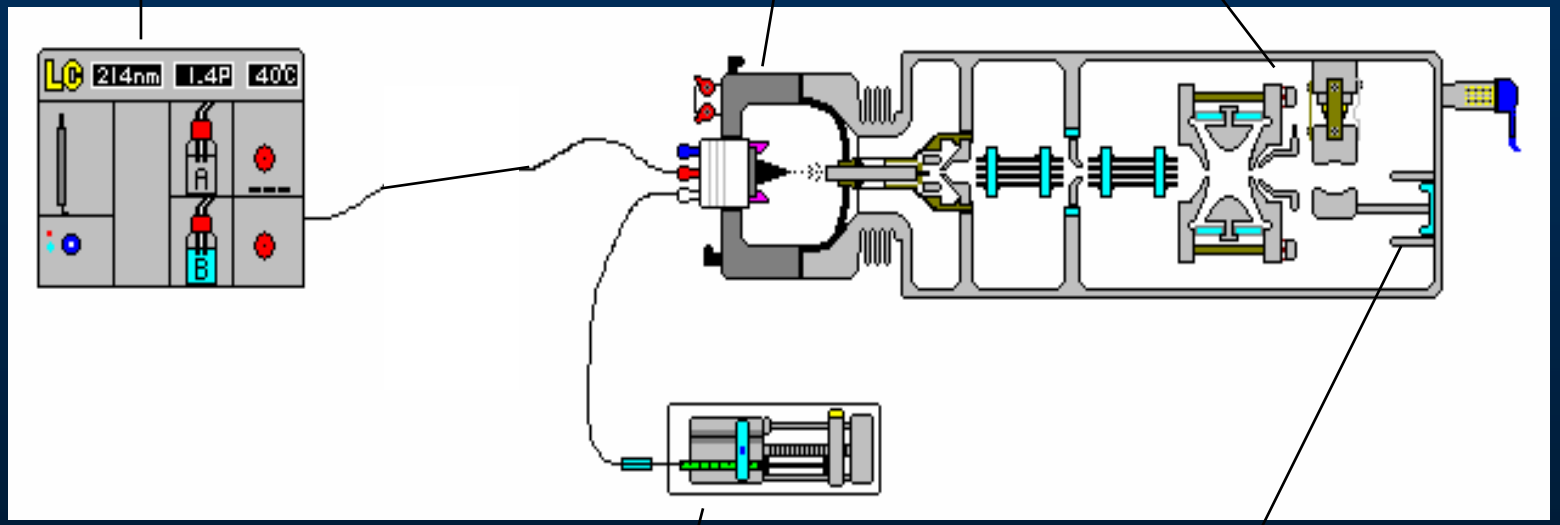
Analyte ion formation:

$$H_3O^+ + \textit{Analyte} \rightarrow [\textit{Analyte} + H]^+ + H_2O$$

APCI - Basic Layout



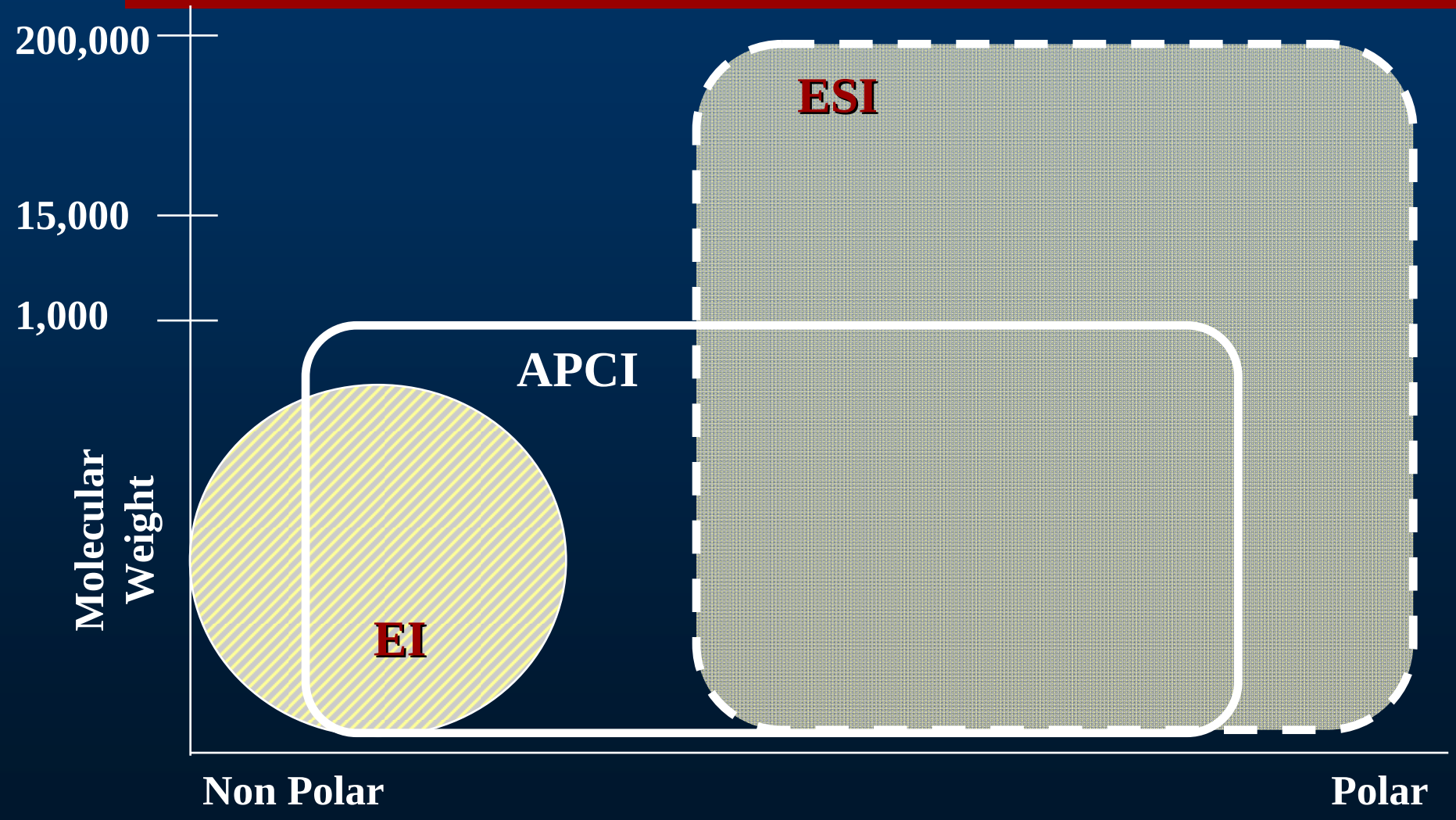
Quadrupole Ion Trap



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

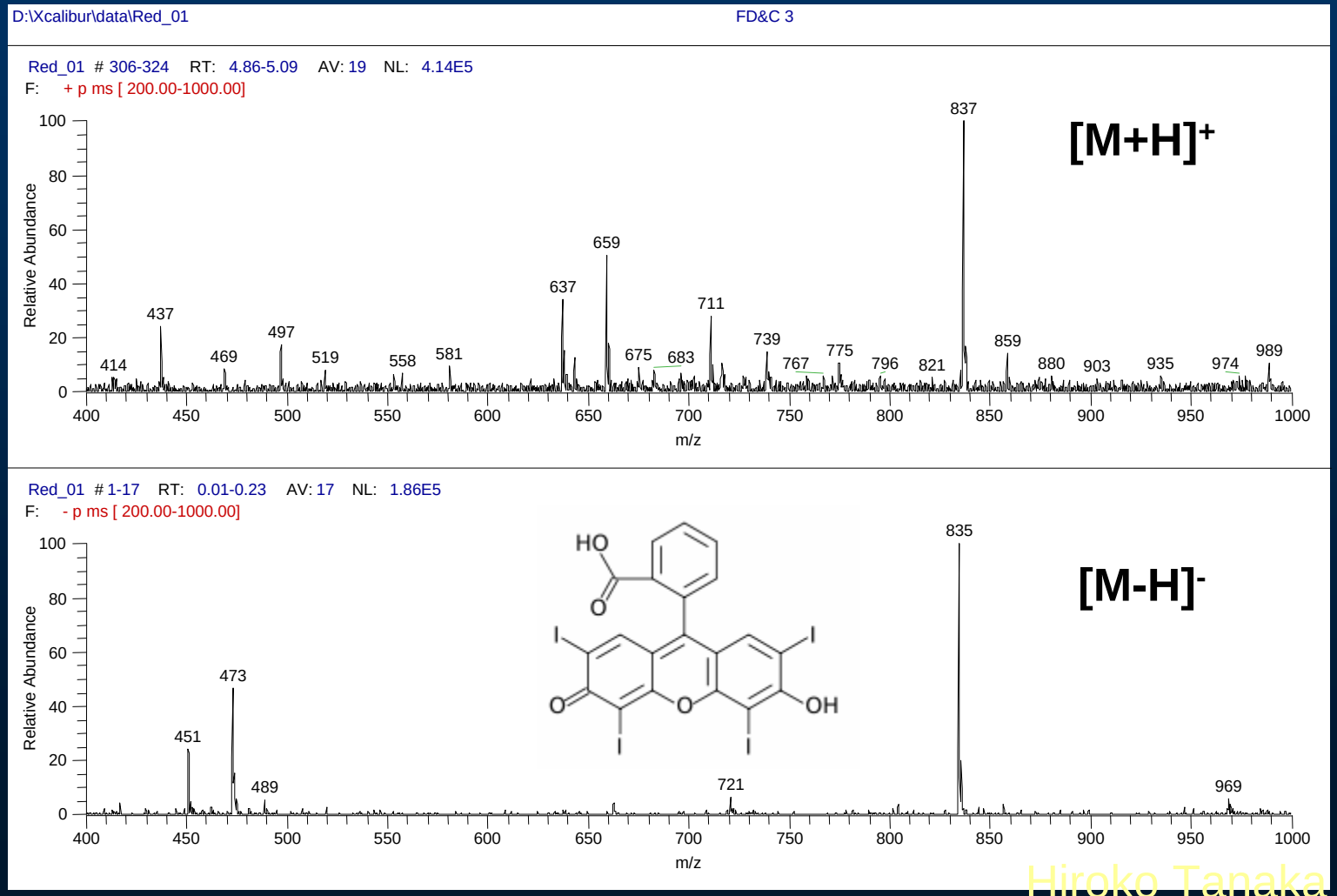
Analyte Compatibility



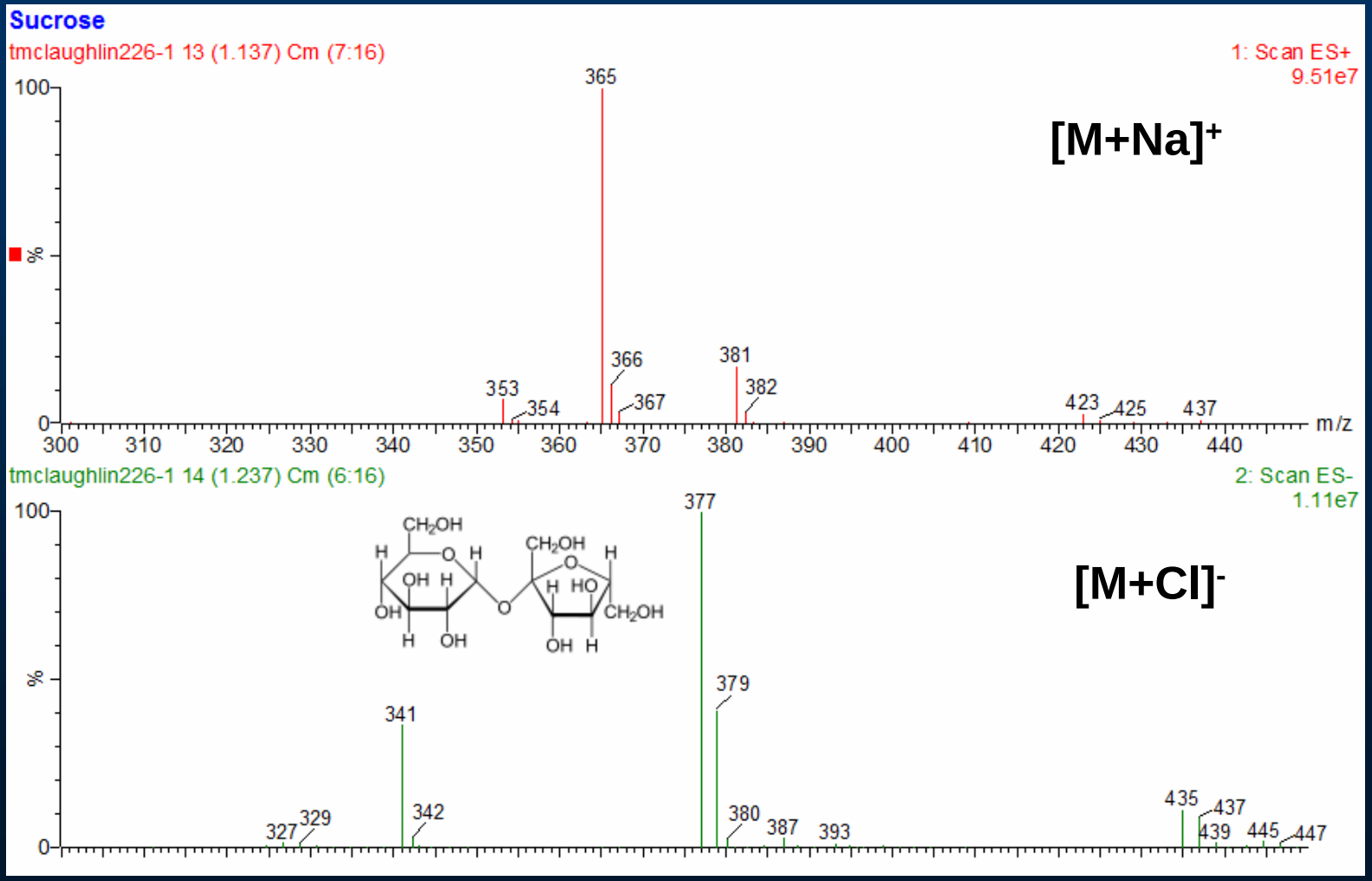
Qualitative Analysis

MW determination for Small Molecules

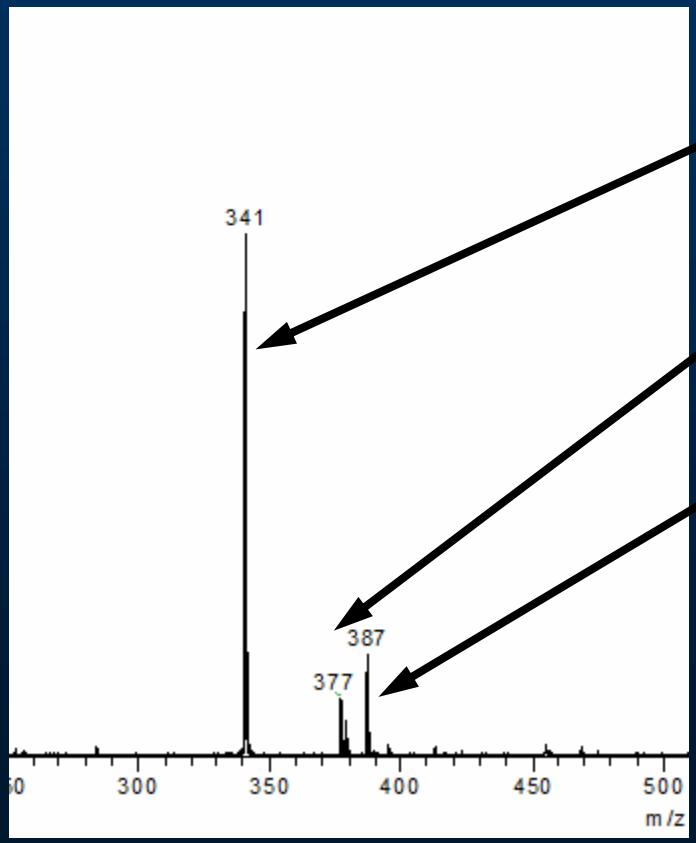
Commonly Observed Ions in ESI



Commonly Observed Ions in ESI



Commonly Observed Ions in ESI



[M-H]$^-$

[M+Cl]$^-$

[M-H+HCOOH]$^-$

Commonly Observed Ions in ESI

ESI+ adducts

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

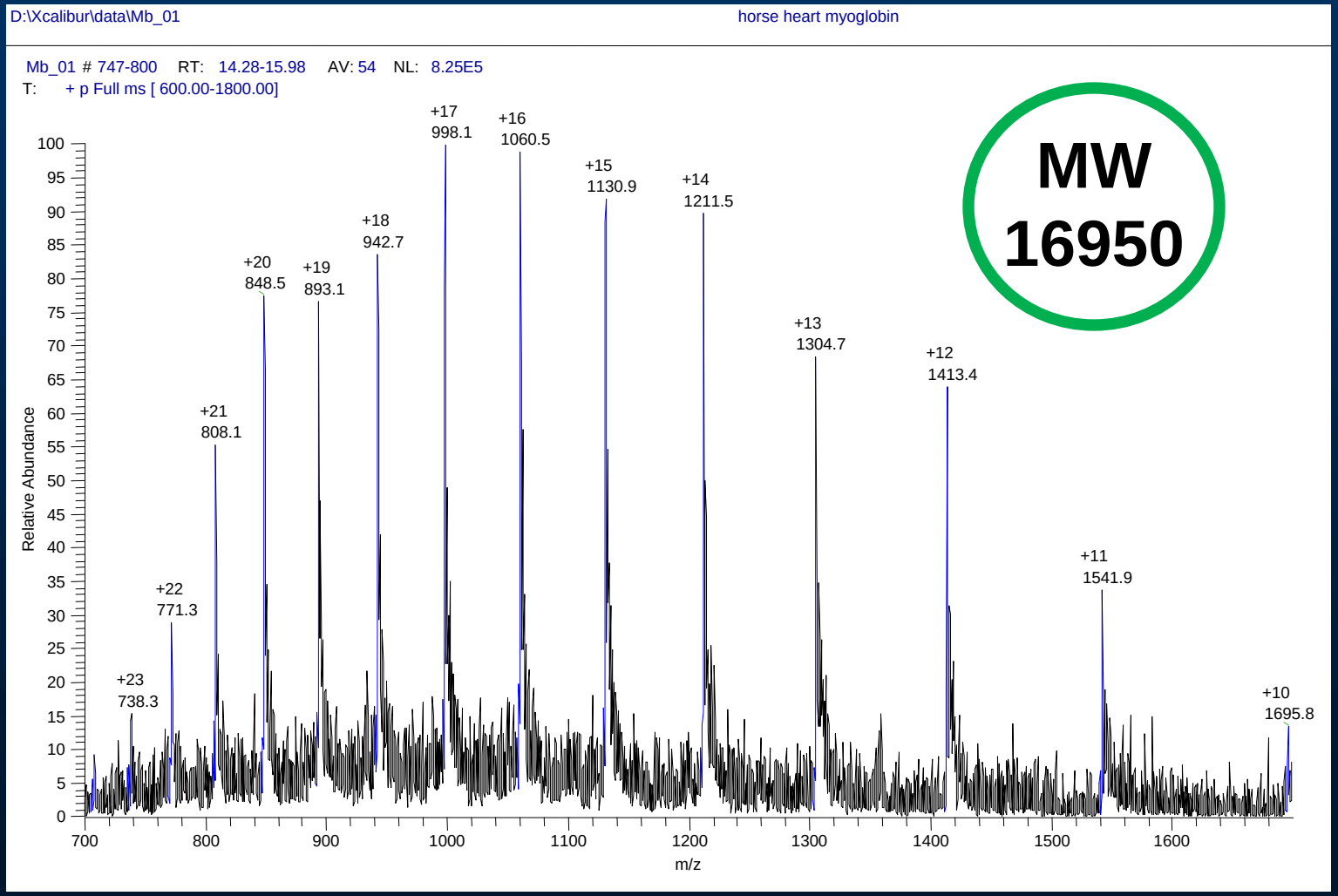
ESI- adducts

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

Qualitative Analysis

MW determination for Biomolecules

Commonly Observed Ions in ESI



Commonly Observed Ions in ESI

Formula to calculate charge of an ion in the distribution

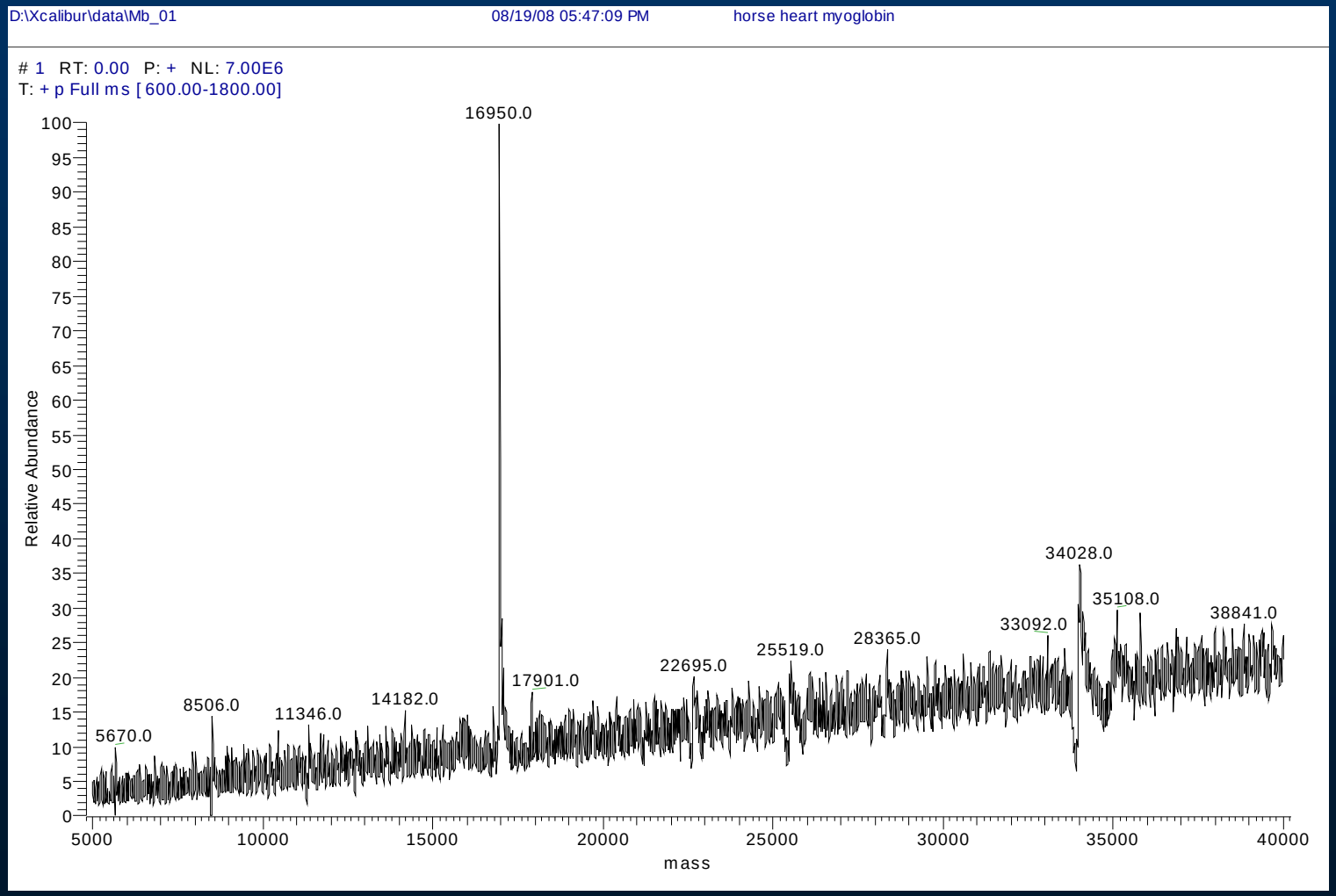
$$\frac{M_n}{(M_n - M_{n+1})} = n+1$$

Example calculation

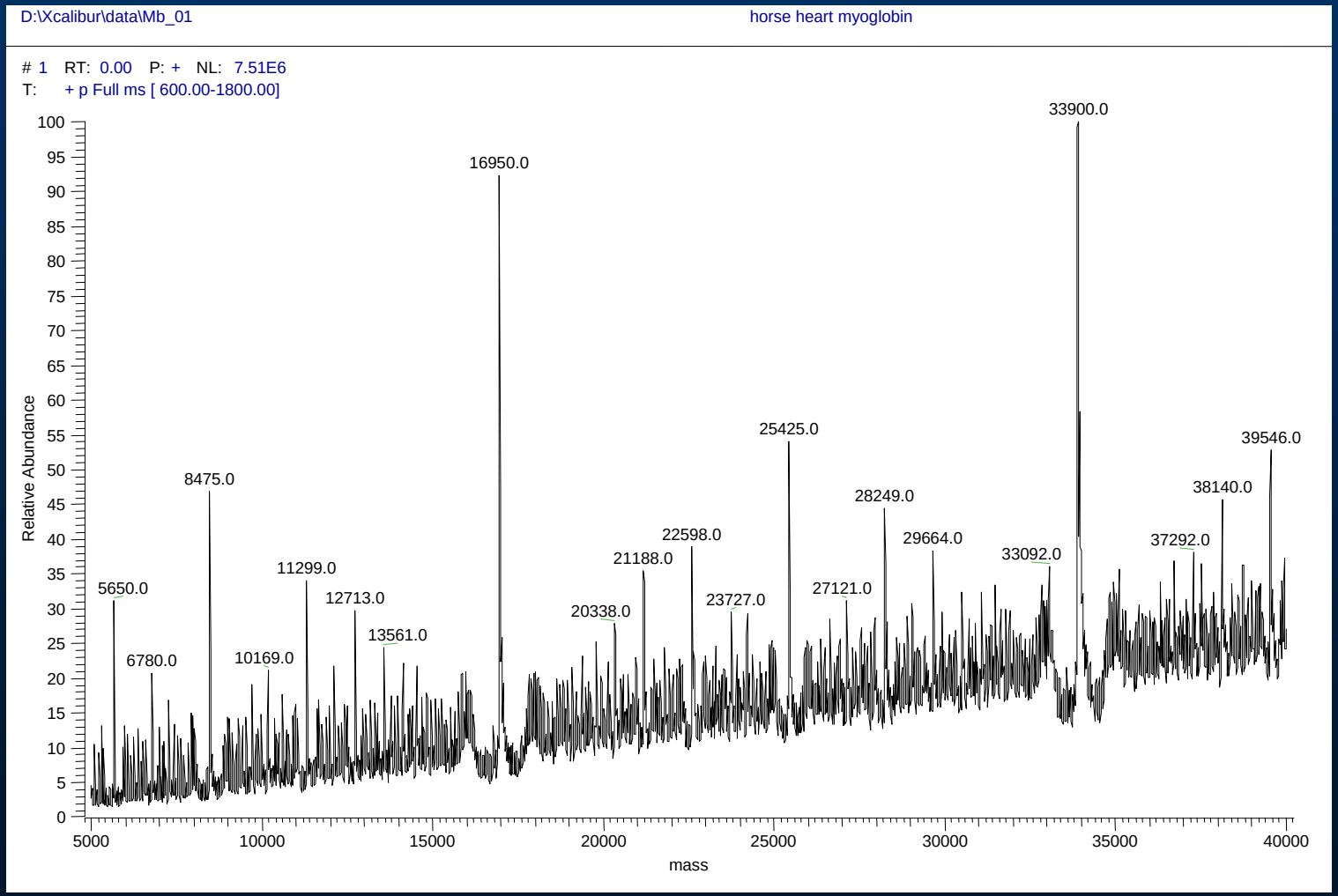
$$\frac{1541.9}{(1541.9 - 1413.4)} = 12$$

12 x 1413.4 = 16961-12 = 16949

Auto Deconvolution Step 2



Auto Deconvolution Step 1



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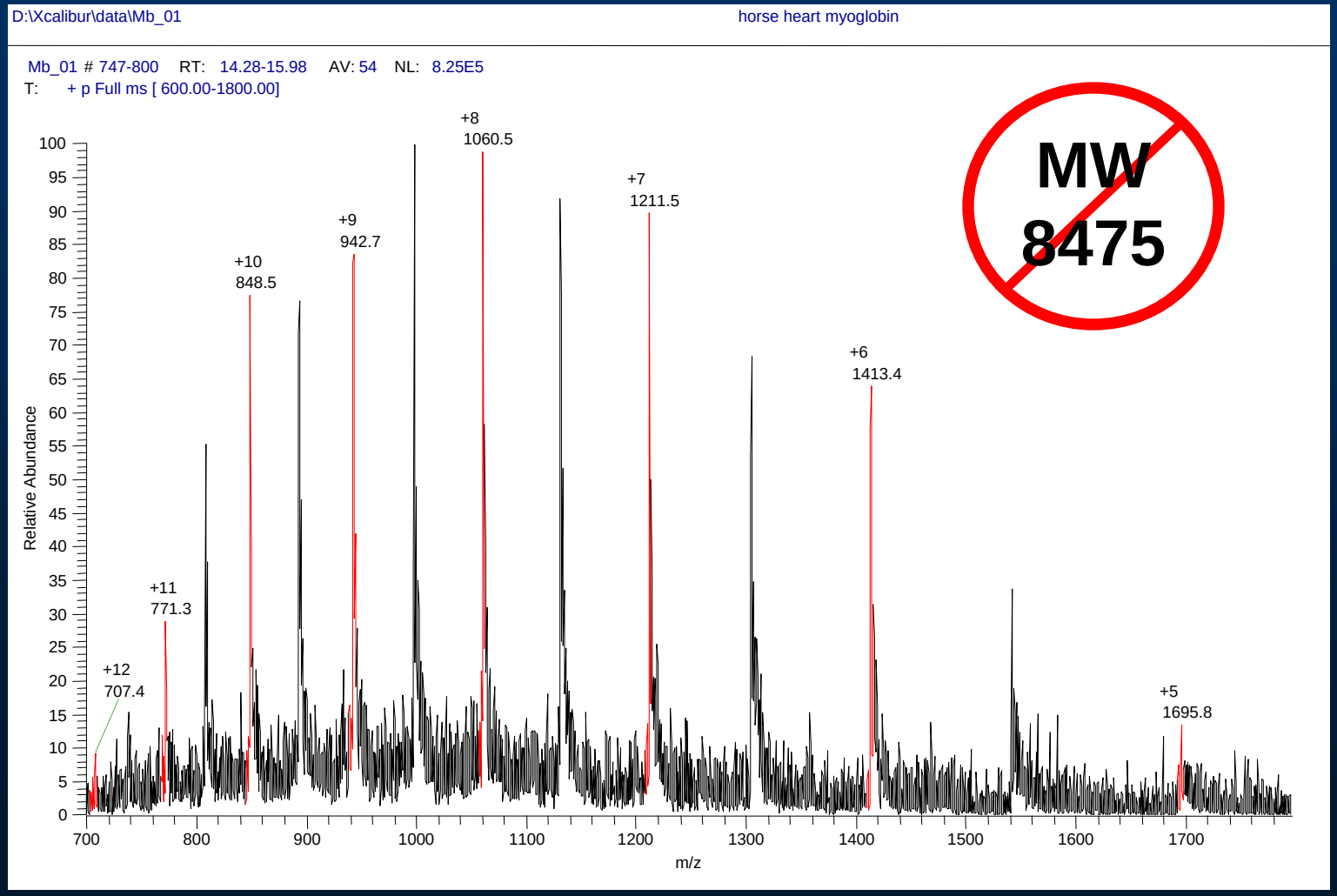

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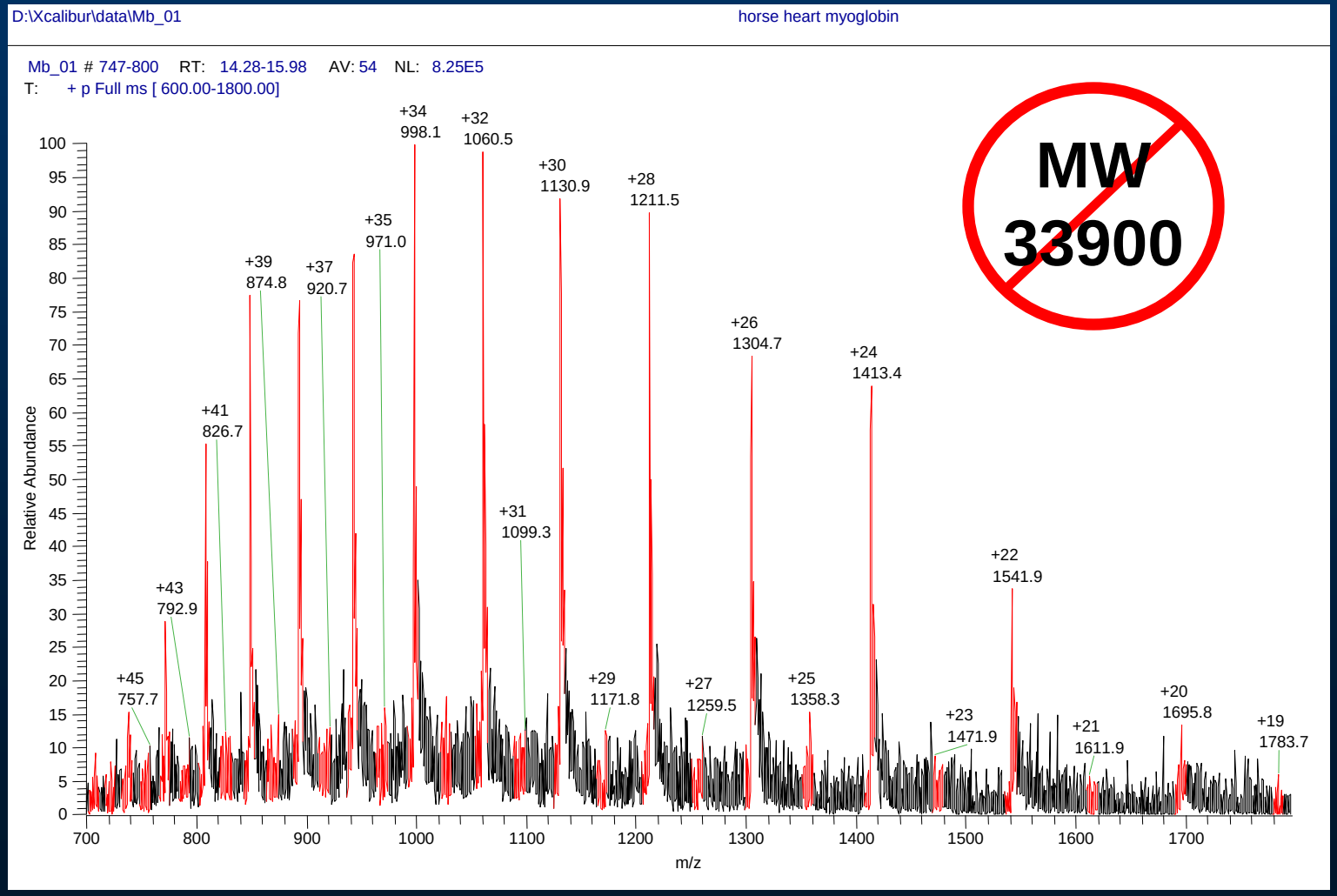


Qualitative Analysis

High Resolution MW determination or accurate mass determination

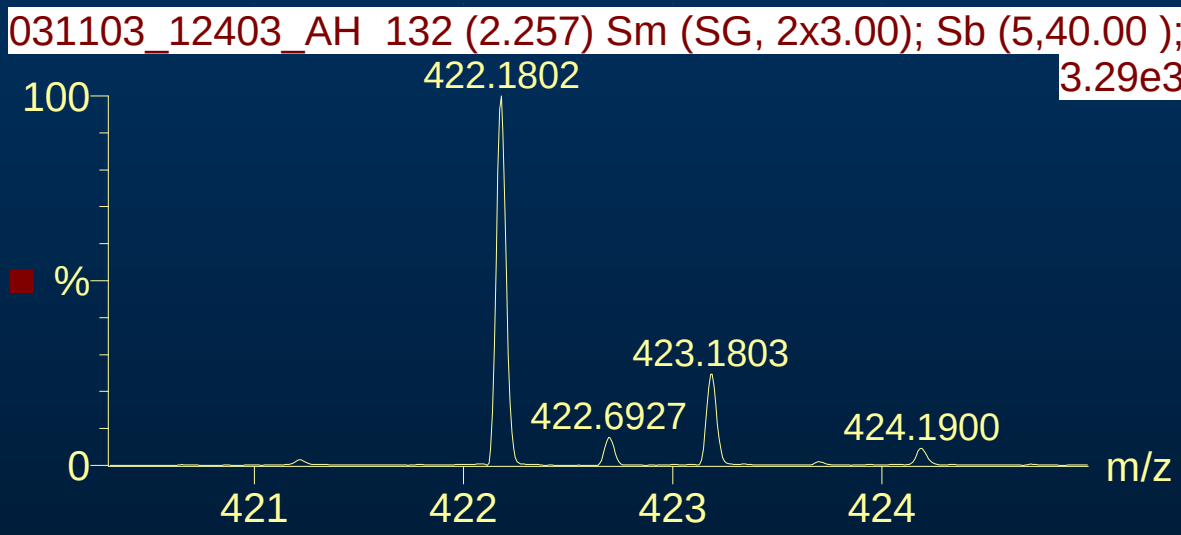
High Resolution MS

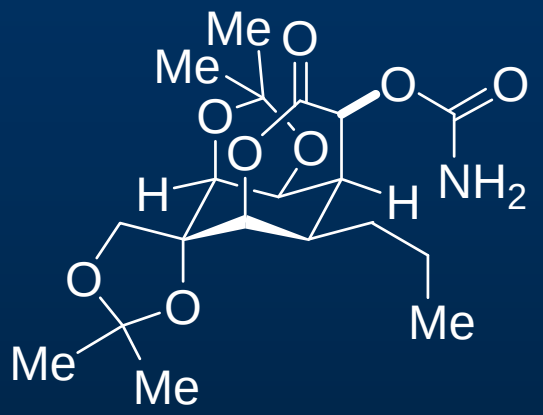
The Journal of Organic Chemistry compound characterization checklist:

"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





M (neutral)

$C_{19}H_{29}NO_8$

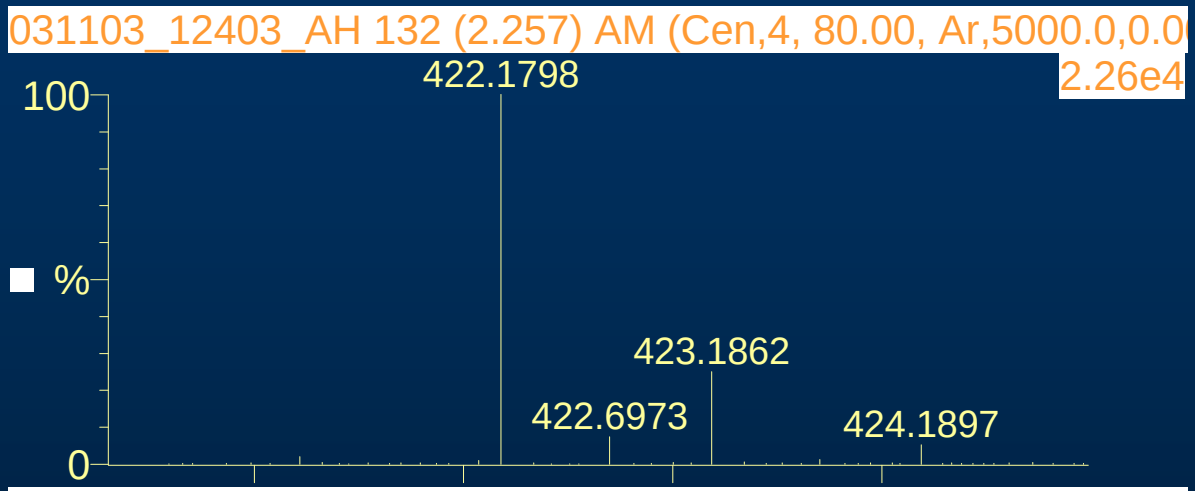
MW 399.1893

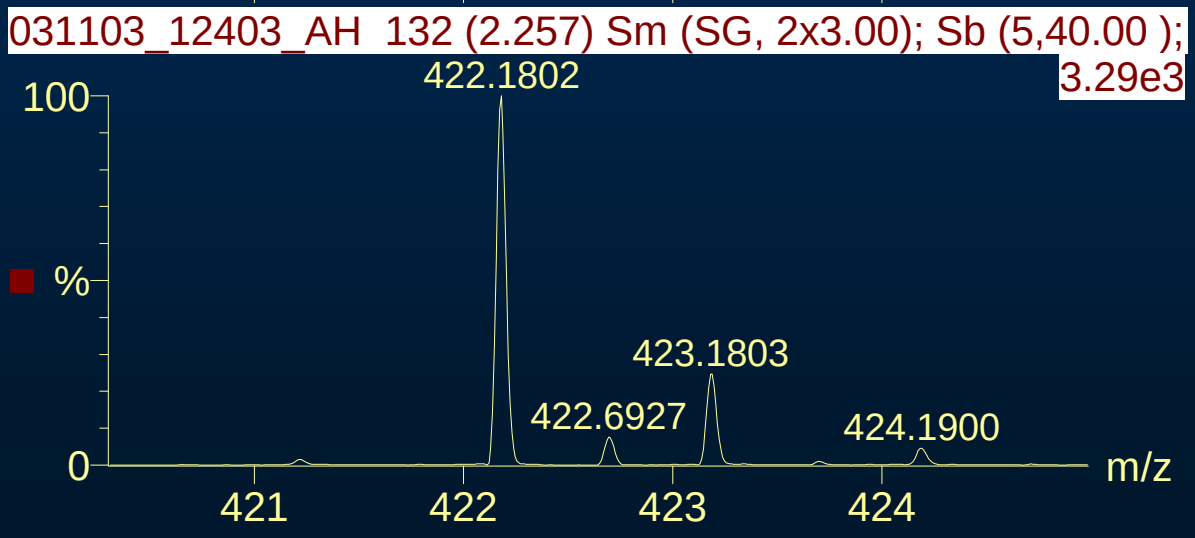
$[M+Na]^+$

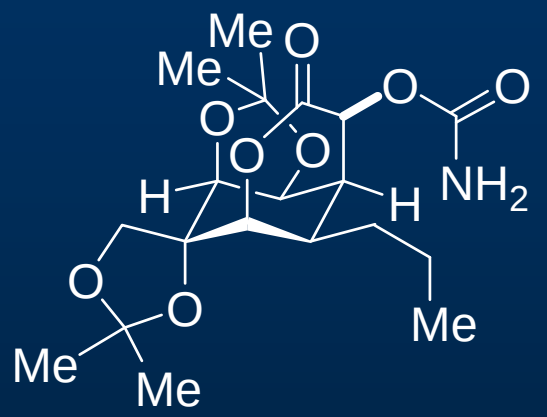
$C_{19}H_{29}NO_8Na$

MW 422.1791

Centroided Spectrum







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Elemental Composition Report

Single Mass Analysis

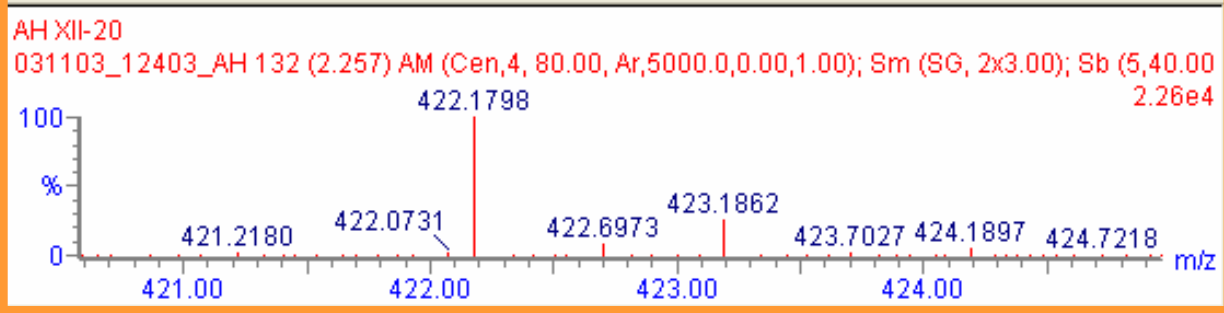
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

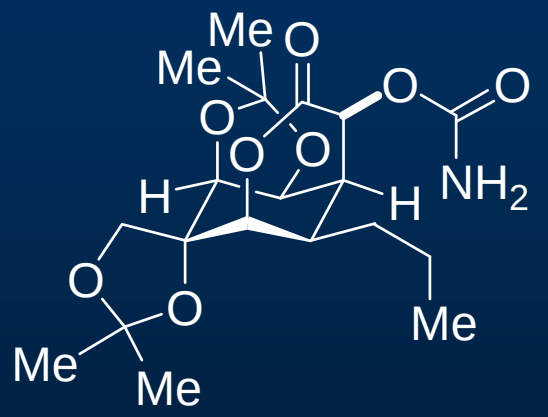
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

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	-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





$[M+Na]^+$

$C_{19}H_{29}NO_8Na$

MW 422.1791

Elemental Composition Report

422.1791 amu, 1.7 ppm, $C_{19}H_{29}NO_8Na$

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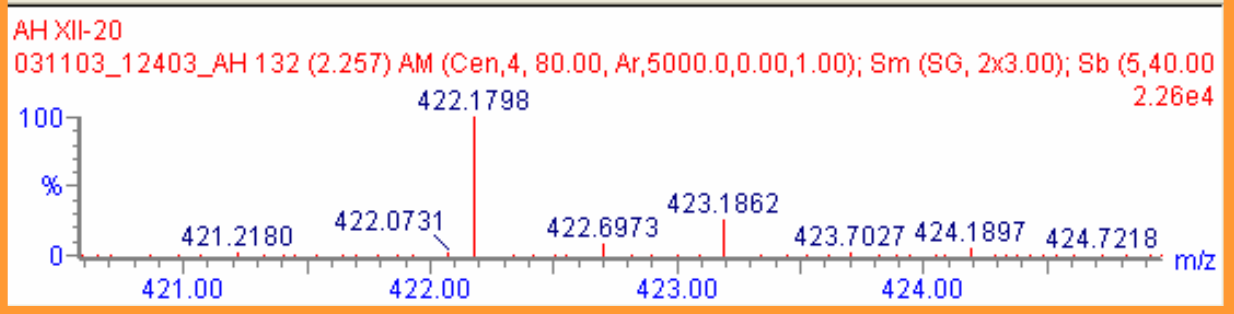
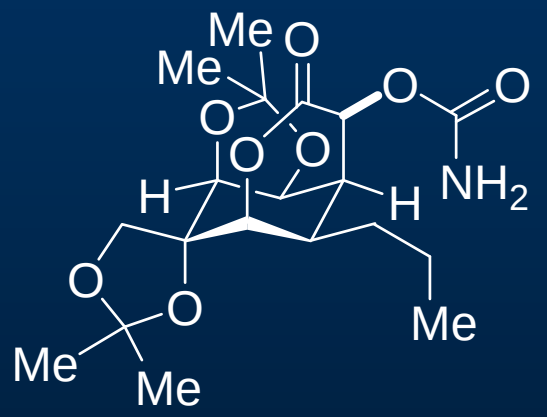
	422.1791	0.7	1.7	5.5	C19 H29 N O8 Na	5	19	29	1	8	1
	422.1788	1.0	2.3	4.0	C18 H30 O11	6	18	30		11	
	422.1783	1.5	3.6	22.0	C31 H22 N2	8	31	22	2		
	422.1815	-1.7	-4.0	8.5	C21 H28 N O8	1	21	28	1	8	
	422.1818	-2.0	-4.7	10.0	C22 H27 N2 O5 Na	2	22	27	2	5	1
	422.1777	2.1	4.9	6.0	C17 H27 N4 O7 Na	7	17	27	4	7	1





$[M+Na]^+$

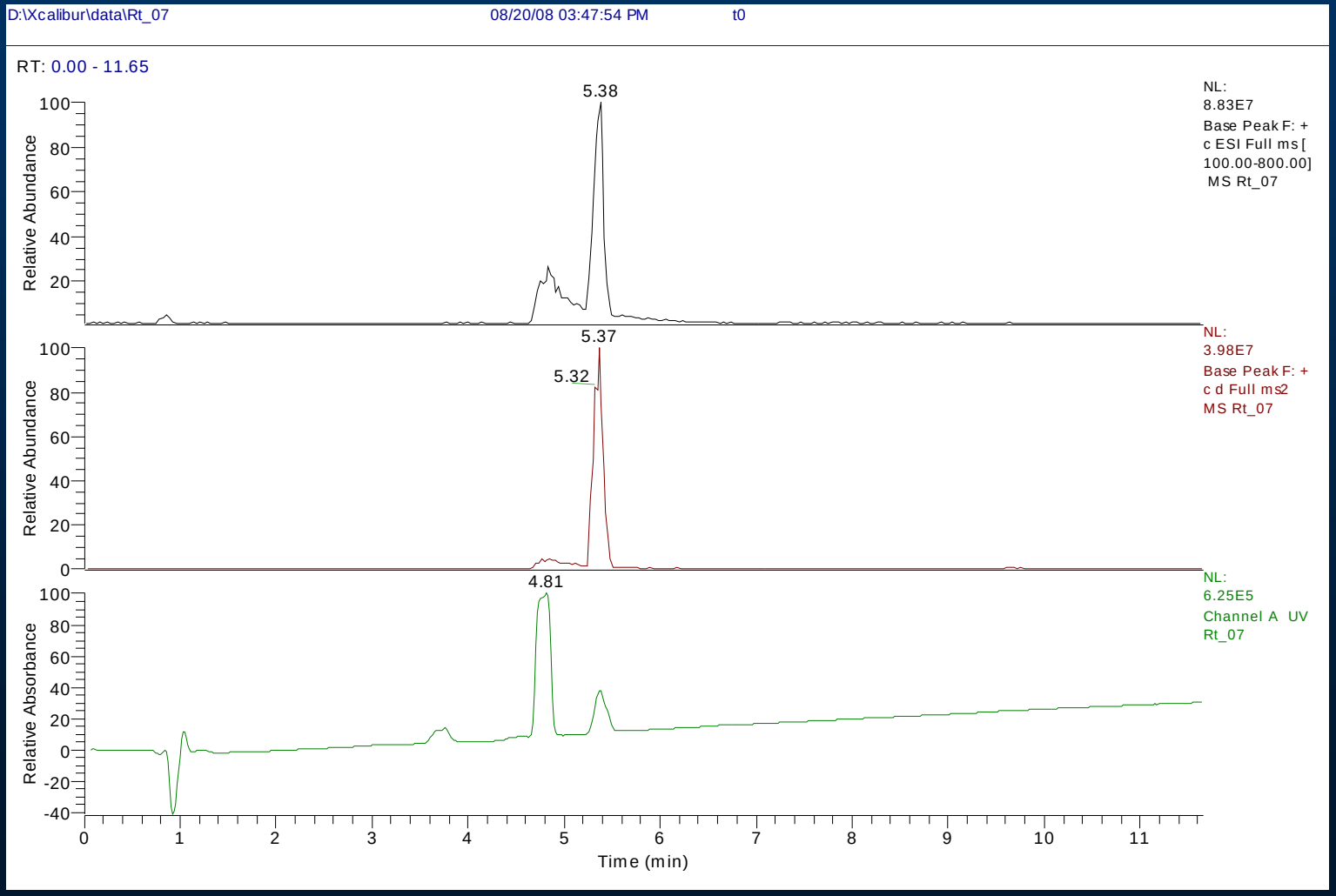
$C_{19}H_{29}NO_8Na$

MW 422.1791

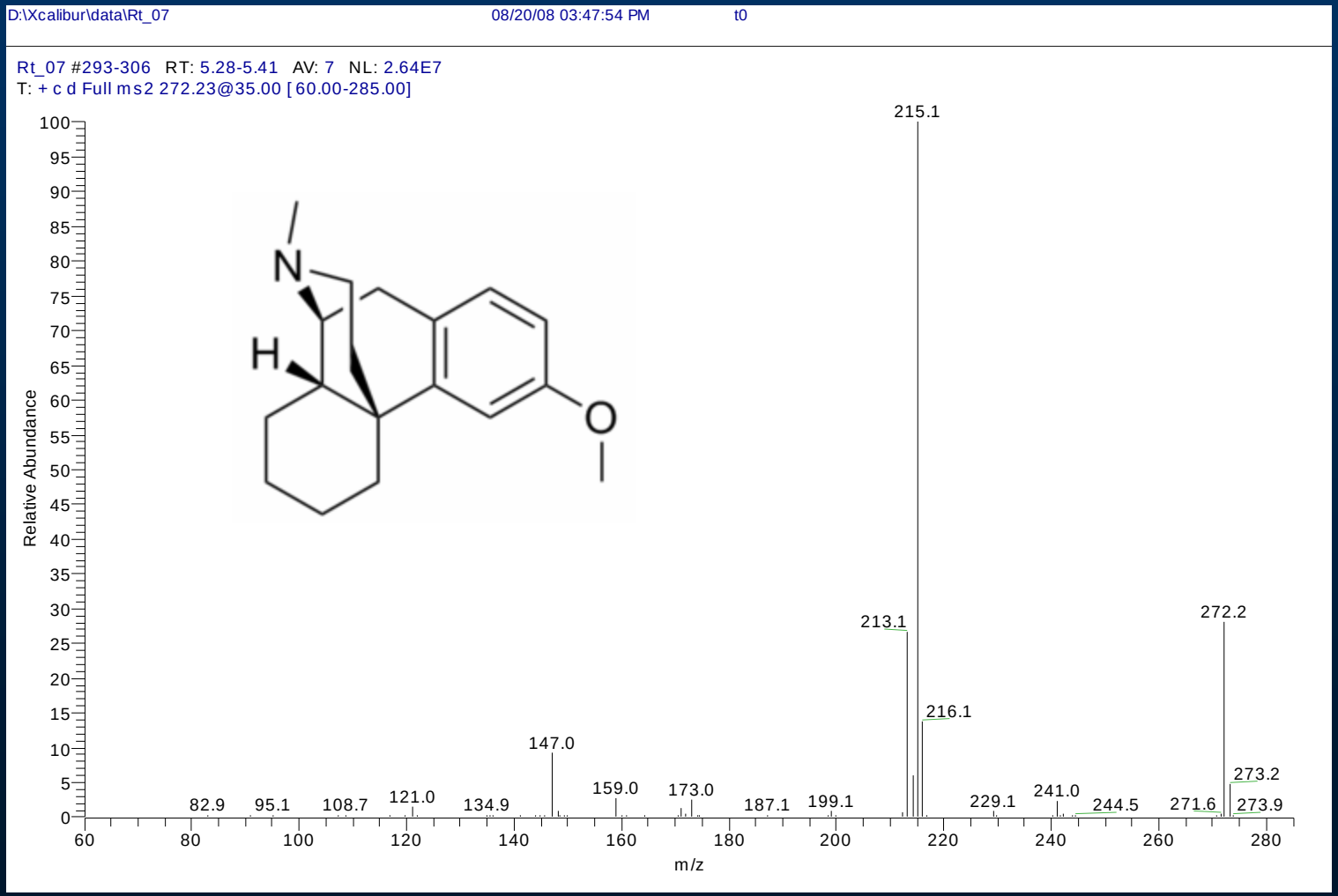
Qualitative Analysis

LC-MS and LC-MSn

LC-MS of cough syrup



Data dependent MS^2



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

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- Chris Adams
- Karolina Krasinska
- Pavel Aronov

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- Rohan Thakur

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