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Qualitative Analysis Workshop

Brief introduction to ESI & APCI ionization

Qualitative Analysis
Stanford University Mass Spectrometry

Vincent Coates Foundation Mass Spectrometry Laboratory

Core resource for Stanford community. Also serve external academic institutions and industry researchers.
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, MSe, 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.
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
  - $\text{MS}^n$
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

ESI Needle
+/- 5 kV

Heated Capillary or Skimmer

Solvent evaporation and ion release

Taylor Cone
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

![Diagram showing the process of ion ejection from highly charged droplets](image-url)
APCI: Atmospheric Pressure Chemical Ionization

Mechanism for positive ion formation

Primary ion formation:

\[ \text{N}_2 + e^- \rightarrow \text{N}_2^+\cdot + 2e^- \]
\[ \text{H}_2\text{O} + e^- \rightarrow \text{H}_2\text{O}^{+\cdot} + 2e^- \]

Secondary ion formation:

\[ \text{H}_2\text{O}^{+\cdot} + \text{H}_2\text{O} \rightarrow \text{H}_3\text{O}^+ + \cdot\text{OH} \]

Analyte ion formation:

\[ \text{H}_3\text{O}^+ + \text{Analyte} \rightarrow [\text{Analyte} + \text{H}]^+ + \text{H}_2\text{O} \]
APCI - Basic Layout

Sample tube (inner) → Vaporizer tube (500°C) → Sample (M) & Solvent (S) vapor → Heated Capillary or Skimmer

Chemical ionization:

- \[ \text{Sample (M) \& Solvent (S) vapor} \]
- \[ \text{Corona discharge region (plasma)} \]
- \[ \text{Sheath gas} \]
- \[ \text{Aux gas} \]

Corona discharge needle:

- \[ H_3O^+ \]
- \[ H_2O \]
- \[ (M+H)^+ \]

H2O

N2

H3O+

H2O

M

N2

Corona discharge region (plasma): \[ N_2^+ \cdot e^- \]

H3O+

H2O

N2

H2O

Chemical ionization:

- \[ H_3O^+ \]
- \[ H_2O \]
- \[ (M+H)^+ \]

Corona discharge region (plasma): \[ N_2^+ \cdot e^- \]

H3O+

H2O

N2

Corona discharge region (plasma): \[ N_2^+ \cdot e^- \]

H3O+

H2O

N2

Corona discharge region (plasma): \[ N_2^+ \cdot e^- \]
Quadrupole Ion Trap

LC Pump

ESI

Quadrupole Ion Trap

Syringe Pump

Detector
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

Molecular Weight

Non Polar  

Polar

ESI

APCI

EI

Stanford University

Mass Spectrometry
Qualitative Analysis

MW determination for Small Molecules
Commonly Observed Ions in ESI

- \([\text{M+H}]^+\)
- \([\text{M-H}]^-\)

Hiroko Tanaka
Commonly Observed Ions in ESI

Sucrose

1: Scan ES+ 9.51e7
2: Scan ES- 1.11e7

[M+Na]^+ 

[M+Cl]^-
Commonly Observed Ions in ESI

- \([\text{M-H}]^-\)
- \([\text{M+Cl}]^-\)
- \([\text{M-H+HCOOH}]^-\)
Commonly Observed Ions in ESI

ESI+ adducts
- M+H⁺
- M+Na⁺
- M+NH₄⁺
- 2M+H⁺ⁿ⁺
- M+nHⁿ⁺

ESI- adducts
- M-H⁻
- M+Cl⁻
- M-H+acid⁻
- 2M-H⁻
- M-nHⁿ⁻
Qualitative Analysis

MW determination for Biomolecules
Commonly Observed Ions in ESI

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

MW 16950
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 08/19/08 05:47:09 PM 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]

Relative Abundance

5000 10000 15000 20000 25000 30000 35000 40000

mass

5000 10000 15000 20000 25000 30000 35000 40000

mass

5650.0 6780.0 10169.0 13561.0 20338.0 21188.0 22598.0 23727.0 25425.0 27121.0 28249.0 29664.0 33092.0 33900.0 37292.0 38140.0 39546.0
Commonly Observed Ions in ESI

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T: + p Full ms [600.00-1800.00]

MW 8475
Commonly Observed Ions in ESI
Qualitative Analysis

High Resolution MW determination or accurate mass determination
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)
C₁₉H₂₉NO₈
MW 399.1893

[M+Na]⁺
C₁₉H₂₉NO₈Na
MW 422.1791

Andrew Hinman
Centroided Spectrum

M (neutral)
C_{19}H_{29}NO_{8}
MW 399.1893

[M+Na]^+
C_{19}H_{29}NO_{8}Na
MW 422.1791

Andrew Hinman

34
### 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)

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<thead>
<tr>
<th>Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>PPM</th>
<th>DBE</th>
<th>Formula</th>
<th>Score</th>
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<th>H</th>
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**[M+Na]^+**

C_{19}H_{29}NO_{8}Na

MW 422.1791

Andrew Hinman
Elemental Composition Report

422.1791 amu, 1.7 ppm, C₁⁹H₂⁹NÖ₈Na

Single Mass Analysis
Tolerance = 5.0 PPM  /  DBE: min = -1.5, max = 50.0
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Monoisotopic Mass, Odd and Even Electron Ions
810 formula[e] evaluated with 8 results within limits (up to 50 closest results for each)

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AHXII-20
031103_12403_AH132 (2.257) AM (Cen,4, 80.00, Ar,5000.0,0.00,1.00); Sm (SG, 2x3.00); Sb (5,40.000
2.26e4

[M+Na]⁺
C₁₉H₂₉NÖ₈Na
MW 422.1791

Andrew Hinman
Qualitative Analysis

LC-MS and LC-MSn
LC-MS of cough syrup

D:\Xcalibur\data\Rt_07

08/20/08 03:47:54 PM

t0

NL: 8.83E7  
Base Peak F: +
c ESI Full ms [100.00-800.00]
MS Rt_07

NL: 3.98E7  
Base Peak F: +
c d Full ms2  
MS Rt_07

NL: 6.25E5  
Channel A UV  
Rt_07

Time (min)

0 1 2 3 4 5 6 7 8 9 10 11

Relative Absorbance

0 20 40 60 80 100

Relative Abundance

0 20 40 60 80 100

Relative Abundance
Data dependent MS²

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
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• Rohan Thakur

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