Liquid Chromatography Coupled to Tandem Mass Spectrometry

Processes Needed to Generate a Mass Spectrum

1) Generate desolvated ions (Sources)
2) Transfer ions into mass spectrometer vacuum (Interface Region)
3) Mass analyze ions (Quadrupoles)
4) Detect ions (Detector)
5) Process the data (Software)
The Source
The Source
Ionization Sources

- **Turbo IonSpray** - IonSpray (pneumatically assisted Electrospray) option which utilizes a heated auxiliary gas flow.
- **Heated Nebulizer** - Atmospheric Pressure Chemical Ionization (APCI) source which ionizes compounds in the gas phase.
Ion Evaporation and Formation
IonSpray (ESI)

IonSpray:
- Electrospray ionization (ESI)
- Gentlest ionization technique
- Applicable to polar and ionic substances
Multiply Charged Ions

Molecules which can be multiply protonated or deprotonated can exist in more than one charge state.

Peaks at $[M \pm nH]^{n \pm}$ ($n = \text{number of charges}$)
Nebulizer

Cool Zone

Primary Flash Zone

Secondary Flash Zone
Heated Nebulizer (APCI)

Heated Nebulizer: - Atmospheric Pressure Chemical Ionization (APCI)
- corona discharge
- polar to non-polar thermally stable compounds
Atmospheric Pressure Chemical Ionization (APCI)

Corona Discharge Chemistry – Positive Ions

1) EI on atmosphere causes e⁻ removal from
   N₂ & O₂ forming N₂⁺⁺ & O₂⁺⁺ (primary ions)

2) In a complex series of reactions N₂⁺⁺ & O₂⁺⁺ react with H₂O & CH₃OH forming
   H₃O⁺ & CH₃OH₂⁺ as reagent ions for Cl.

3) H₃O⁺ & CH₃OH₂⁺ donate protons to analyte forming [M+H]⁺
GC-MS vs. LC-MS

- IonSpray
- APCI
- GC-MS

Analyte Polarity

- Ionic
- Non-Ionic

Molecular Weight

- 10^1
- 10^2
- 10^3
- 10^4
- 10^5
Ion Production

API Chemistry and Example Spectra
**GAS PHASE Acid-Base Scale**

for **Positive Ions & Neutral Molecules**

<table>
<thead>
<tr>
<th>Neutral Molecules</th>
<th>Reagent Ions</th>
<th>Increasing Acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong acid</td>
<td>Weak Base</td>
<td></td>
</tr>
<tr>
<td>formic acid</td>
<td>HCOOH$_2^+$</td>
<td></td>
</tr>
<tr>
<td>acetic acid</td>
<td>CH$_3$COOH$_2^+$</td>
<td></td>
</tr>
<tr>
<td>water</td>
<td>H$_3$O$^+$</td>
<td></td>
</tr>
<tr>
<td>methanol</td>
<td>CH$_3$OH$_2^+$</td>
<td></td>
</tr>
<tr>
<td>phenol</td>
<td>C$_6$H$_6$OH$_2^+$</td>
<td></td>
</tr>
<tr>
<td>formaldehyde</td>
<td>HCHOH$^+$</td>
<td></td>
</tr>
<tr>
<td>N$_2$O</td>
<td>N$_2$OH$^+$</td>
<td></td>
</tr>
<tr>
<td>methane</td>
<td>CH$_5^+$</td>
<td></td>
</tr>
<tr>
<td>ethane</td>
<td>C$_2$H$_5^+$</td>
<td></td>
</tr>
<tr>
<td>benzene</td>
<td>C$_6$H$_6$H$^+$</td>
<td></td>
</tr>
<tr>
<td>acetonitrile</td>
<td>CH$_3$COC$_2$H$_6^+$</td>
<td></td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>NH$_4^+$</td>
<td></td>
</tr>
<tr>
<td>ammonia</td>
<td>C$<em>6$H$</em>{15}$NH$^+$</td>
<td></td>
</tr>
<tr>
<td>triethylamine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Addition of acetic acid or formic acid will increase intensity in positive ion mode by higher concentration of H$^+$.
- Trifluoroacetic acid (TFA) works well in positive ion mode at a concentration up to 0.05%, but lingers for a long time in negative ion mode. (TFA ions are at m/z 113 and m/z 227—dimer)
- Changing from acetonitrile to methanol may increase signal intensity.
GAS PHASE Acid-Base Scale for Negative Ions & Neutral Molecules

<table>
<thead>
<tr>
<th>Neutral molecules</th>
<th>Reagent Ions</th>
<th>Increasing Basicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak Acid</td>
<td>Strong Base</td>
<td></td>
</tr>
<tr>
<td>NH₃</td>
<td>NH₂⁻</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>OH⁻</td>
<td></td>
</tr>
<tr>
<td>Toluene</td>
<td>C₆H₅CH₂⁻</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>CH₃O⁻</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>C₂H₅O⁻</td>
<td></td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>CH₂CN⁻</td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>CH₃COCH₂⁻</td>
<td></td>
</tr>
<tr>
<td>CH₃SH</td>
<td>CH₃S⁻</td>
<td></td>
</tr>
<tr>
<td>CH₃NO₂</td>
<td>CH₂NO₂⁻</td>
<td></td>
</tr>
<tr>
<td>HCN</td>
<td>CN⁻</td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td>C₆H₅O⁻</td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>CH₃COO⁻</td>
<td></td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>C₆H₅COO⁻</td>
<td></td>
</tr>
<tr>
<td>HCl</td>
<td>Cl⁻</td>
<td></td>
</tr>
</tbody>
</table>

- For R-COOH compounds use NH₄ac buffer (pH = 6.4).
- Try addition of NH₃ in case of neutral analytes in negative ion mode.
- Stay away from triethylamine, it collects in the source!
Ionization Suppression

- **Compounds That Cause Sensitivity Suppression**
  - Salts can interfere with ionization and can cluster to complicate spectrum (but also aid in identification)
  - Strong bases or quaternary amines can interfere with positive mode analytes, e.g. Triethylamine (TEA)
  - Acids - Sulfuric/Sulfonic acids and TFA interfere in negative mode experiments
  - Phosphate buffer and non-volatile ion pairing agents (e.g. SDS) can cause severe suppression and complex spectra

- **Dimerization** ([2M+H]^+) can occur at high concentration, leading to non-linearity during quantitation
  - Dimer Signal at m/z = (MW*2)^+1
  - Can cause non-linearity at high concentrations
Progesterone in Positive Ion ESI-MS

TIS POSITIVE MODE−Progesterone
ACN/H2O=50:50
0.0 mM NH4Ac−high orifice

[\text{[M+ Na]}^+]$

+Q1 MCA (10 scans): from Q1−PROSTRN−0 mM

Intensity, cps

Intensity, cps

Intensity, cps

Intensity, cps

+Q1 MCA (10 scans): from Q1−PROSTRN−2.0 mM

+Q1 MCA (10 scans): from Q1−PROSTRN−20.0 mM

+Q1 MCA (10 scans): from Q1−PROSTRN−100.0 mM

5.92e5 cps

1.34e7 cps

8.06e6 cps

5.80e6 cps
Effect of Phosphate Buffer

CCK dissolved in Phosphate Buffer

Phosphate Buffer removed from CCK sample
Adducts & Clusters formed in LC/MS

<table>
<thead>
<tr>
<th>Adduct Ion Formed</th>
<th>Cause</th>
<th>Adduct*/ Ionic</th>
<th>APCI</th>
<th>ESI</th>
<th>+/-</th>
<th>m/z of Ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium [M+Li]⁺</td>
<td>Lithium Salts</td>
<td>Ionic</td>
<td>✓</td>
<td>✓</td>
<td>+</td>
<td>[M + H + 6]⁺</td>
</tr>
<tr>
<td>Ammonia [M + NH₄]⁺</td>
<td>Ammonia/ NH₄OH</td>
<td>Adduct</td>
<td>✓</td>
<td>✓</td>
<td>+</td>
<td>[M + H + 17]⁺</td>
</tr>
<tr>
<td>Water [M + H₂O]⁺</td>
<td>Water/ Acids</td>
<td>Adduct</td>
<td>✓</td>
<td>✓</td>
<td>+</td>
<td>[M + H + 18]⁺</td>
</tr>
<tr>
<td>Sodium [M + Na]⁺</td>
<td>Sodium salts</td>
<td>Ionic</td>
<td>✓</td>
<td>✓</td>
<td>+</td>
<td>[M + H + 22]⁺</td>
</tr>
<tr>
<td>Chloride [M + Cl]⁻</td>
<td>Chlorinated solvent</td>
<td>Ionic</td>
<td>✓</td>
<td>✗</td>
<td>-</td>
<td>[M + 35 (&amp; 37)]⁻</td>
</tr>
<tr>
<td>Potassium [M + K]⁺</td>
<td>Potassium salts</td>
<td>Ionic</td>
<td>✓</td>
<td>✓</td>
<td>+</td>
<td>[M + H + 38]⁺</td>
</tr>
<tr>
<td>Acetonitrile [M + H + CH₃CN]⁺</td>
<td>Acetonitrile in solvent</td>
<td>Adduct</td>
<td>✓</td>
<td>✓</td>
<td>+/-</td>
<td>[M + H + 41]⁺ [M - H + 41]⁻</td>
</tr>
<tr>
<td>Formic Acid [M - H + HCO₂H]⁻</td>
<td>Formic Acid</td>
<td>Adduct</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>[M - H + 46]⁻</td>
</tr>
<tr>
<td>Acetic Acid [M - H + CH₃CO₂H]⁻</td>
<td>Acetic Acid</td>
<td>Adduct</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>[M - H + 60]⁻</td>
</tr>
<tr>
<td>TFA [M - H + CF₃CO₂H]⁻</td>
<td>TFA</td>
<td>Adduct</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>[M- H + 114]⁻</td>
</tr>
</tbody>
</table>

* Adducts can often be broken up by using a higher DP voltage.
Ion Transfer
Lens (IQn) and rod (ROn) voltages are linked

Keep ions moving at correct speed through system For best sensitivity, proper mass filtering
MS & MS/MS (tandem)
Quadrupole Theory

* Quadrupole acts as a **mass filter**
  - Separates ions based on $m/z$ ratio
* Quad. made of 4 rods
  - “A” pole - vertical rods; “B” pole - horizontal rods (by convention)
* DC & RF voltages are imposed
  - $U = (DC)_A - (DC)_B$ (FDC)
  - $V = \text{RF voltage (RF}_{p-p})$
  - $V = \text{Const.} \cdot M \cdot r_0^2 \cdot \nu^2$
    
    **Note:** $M \propto V$

- $\nu$ = RF frequency
- $r_0 = \frac{1}{2}$ distance between rods
Stability Diagram
DC/RF

Operating Line
(through the apices)

V (RF)

U (DC)
MS Operation

Scan Modes
Q1 MS Scans

Start/Stop Mode
Q3 Single-Stage MS Scans

Start/Stop Mode

CAD GAS (Setting = 1)

CAD GAS (Setting = 1)
Available Scan Modes
- Product Ion
  - Provides structural information
- Precursor Ion
  - Provides structural information complementary to Product Ion
- Neutral Loss
  - Provides compound class specificity
- MRM
  - Used for Quantitation

In all MS/MS modes, Q3 is a mass filter
- Q3 may scan or be fixed at a given m/z
- Q2 is “source” of product ions entering Q3
MS/MS – Product Ion Scan

$m_1^+$ fixed

$m_3^+$ scanned
MS/MS – Precursor Ion Scan

$m_1^+$ scanned

$m_3^+$ fixed
MS/MS – Neutral Loss Scan

\( m_1^+ \text{ scanned} \)

\( \delta m \)

\( m_3^+ \text{ scanned} \)
MS/MS – MRM
(Multiple Reaction Monitoring)

Precursor ion fixed
Fragmentation (CAD)
Product ion fixed
Fragmentation

- When an ion fragments the following formulas apply:
  - Positive mode: \([\text{Precursor}]^+ \rightarrow \text{Product}^+ + \text{Neutral}\)
  - Negative mode: \([\text{Precursor}]^- \rightarrow \text{Product}^- + \text{Neutral}\)

- Two modes of fragmentation on Sciex Instruments:
  - CAD = Collisionally Activated Dissociation
    * Energy controlled by CE; occurs in Q2 (collision cell)
    * Ions collide with \(N_2\) molecules (CAD gas), collision energy is converted into vibrational energy, and bonds break
  - CID = Collision-Induced Dissociation
    * Energy controlled by DP; occurs between orifice and skimmer
    * Ions collide with \(N_2\) (from curtain gas) and solvent molecules, collision energy is converted...
I on Detection
Detector & Signal Handling

Ion Path  →  Deflector

→

CEM

→

CEM Horn Voltage (-)

CEM Bias Voltage (+)

1 MΩ

Signal Output

1000 pF
10 kV

→

Signal Handling Board

System Controller (in System Electronics Box)