Ion fragmentation of small molecules in mass spectrometry

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Nomenclature: the main names and acronyms used in mass spectrometry

• **Molecular ion**: Ion formed by addition or the removal of one or several electrons to or from the sample molecules. **Electron Impact (EI-MS)**. \( M + e^- \rightarrow M^{+} + 2e^- \)

• **Adduct ion**: Ion formed through interaction of two species and containing all the atoms of one of them plus one or several atoms of them (e.g. alkali, ammonium).
**Contd..**

- **Pseudomolecular ion:** Ion originating from the analyte molecule by abstraction of a proton [M-H]- or addition of proton [M+H]+
- **Tandem mass spectrometry (Cooks, 1976): MS/MS (McLafferty, 1978), tandem in space or time**
- **Precursor ion/parent ion:** Ions undergoing fragmentation.
- **Product ion/daughter ion:** Ions resulting from parent/precursor ions.
- **Neutral loss:** Fragments lost as neutral molecules
- **In positive ionization mode,** a trace of formic acid is often added to aid protonation of the sample molecules; in **negative ionization mode** a trace of ammonia solution or a volatile amine is added to aid deprotonation of the sample molecules. Proteins and peptides are usually analysed under positive ionization conditions and polyphenols and acids under negative ionization conditions. In all cases, the \( m/z \) scale must be calibrated.

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**Terminology..**

- **Ionspray** denotes pneumatically assisted ESI operating at a flow rate of approximately 5 to 50 \( \mu \text{L}/\text{min} \).
- **Turboionspray** is ionspray with additional heated gas for flow rate of 0.1 to 2 ml/min.
- **Heated nebulizer** is the trade mark of AB Sciex for APCI.

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What is Collision Induced Dissociation (CID) or Collisionally Activated Dissociation (CAD)?

Precursor ion or parent ion

Activated ion

Collision gas

Fragmenting ion

Product ions

Neutral loss

Schematic of CID fragmentation

Other activation processes:
PSD (post source-decay)
ECD (electron capture dissociation)
SID (surface-induced dissociation)

Various types of MS/MS experiments

<table>
<thead>
<tr>
<th>Mode of operation</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1 Scan</td>
<td>Resolving (Scan)</td>
<td>RF-only</td>
<td>RF-only</td>
</tr>
<tr>
<td>Q3 Scan</td>
<td>RF-only</td>
<td>RF-only</td>
<td>Resolving (Scan)</td>
</tr>
<tr>
<td>Product Ion Scan (PIS)</td>
<td>Resolving (Fast)</td>
<td>Fragment</td>
<td>Resolving (Scan)</td>
</tr>
<tr>
<td>Precursor Ion Scan (PIS)</td>
<td>Resolving (Scan)</td>
<td>Fragment</td>
<td>Resolving (Fast)</td>
</tr>
<tr>
<td>Neutral Loss Scan (NLS)</td>
<td>Resolving (Scan)</td>
<td>Fragment</td>
<td>Resolving (Scan)</td>
</tr>
<tr>
<td>Selected Reaction Monitoring Mode (SRM)</td>
<td>Resolving (Fast)</td>
<td>Fragment</td>
<td>Resolving (Fast)</td>
</tr>
</tbody>
</table>

Table 1. Minimal 3Q QqQ Q q0 Q1 Q2 Q3 MS operation modes

N2 gas

Enhanced Q3 Single MS (EM-MS)
Enhanced Product Ion (EPI)
Enhanced Multistage (MSn)
Time delayed fragmentation (TDF)
Enhanced Resolution Q3 (ER-Q3)
Enhanced Multistage Charged (EM-C)

Figure 1. Schematic of QqLT (Q TRAP, AB/MS/MS, Sciex) and description of the various triple quadrupole and trap operation modes.

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Steps involved in fragmentation

Step # 1: Creation of ions

Step # 2: Add energy of activation

Step # 3: Charge directed fragmentation

Fragment ion + neutral molecule

Applications of MS/MS

• Identification and characterization drug metabolites

• Authentication and profiling of chemical components in a crude mixture

• Substructure analysis of unknown components

• Quantification of analytes in biological samples

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Ways to approach predicting MS/MS

- Likely sites of protonation or deprotonation.
- Likely leaving group.
- Mobility of protons
- Literature study

Where are the sites of Deprotonation/protonation? What is the most likely leaving Group in this molecule?

Fragmentation always follows the basic rules of chemistry

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Ion fragmentation for identification of phase II drug metabolites (glucuronide/sulfate conjugates)

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What fragment ions are characteristics for glucuronide conjugates?

Product ion spectrum of genistein glucuronide in ESI-MS/MS

Glucosides/glucuronides conjugates are easily cleaved off by higher potential at orifice

The loss of 80 Da from the parent ion and the presence of m/z 80 in the product ion spectra are the indicative of sulfate conjugates of phenolic compounds like daidzein [A] and equol [B]
What happens with aliphatic sulfates in MS/MS?

Aliphatic and aromatic sulfate conjugates behave differently in MS/MS aliphatic typically show $m/z$ 97 (HSO$_4^-$) and $m/z$ 80 (SO$_3^-$).

The absence of the $m/z$ 97 fragment with the base peak $m/z$ 80 makes the distinction between aromatic and aliphatic sulfates.

**Change in mass is associated with possible metabolic reaction**

<table>
<thead>
<tr>
<th>Metabolic rxn</th>
<th>Change in mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylation</td>
<td>+14</td>
</tr>
<tr>
<td>Demethylation</td>
<td>-14</td>
</tr>
<tr>
<td>Hydroxylation</td>
<td>+16</td>
</tr>
<tr>
<td>Acetylation</td>
<td>+42</td>
</tr>
<tr>
<td>Epoxidation</td>
<td>+16</td>
</tr>
<tr>
<td>Desulfuration</td>
<td>-32</td>
</tr>
<tr>
<td>Decarboxylation</td>
<td>-44</td>
</tr>
<tr>
<td>Hydration</td>
<td>+18</td>
</tr>
<tr>
<td>Dehydration</td>
<td>-18</td>
</tr>
</tbody>
</table>

**Characteristic fragmentation of drug conjugates by MS/MS**

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>Ionization mode</th>
<th>Scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucuronides</td>
<td>pos/neg</td>
<td>NL 176 amu</td>
</tr>
<tr>
<td>Hexose sugar</td>
<td>pos/neg</td>
<td>NL 162 amu</td>
</tr>
<tr>
<td>Pentose sugar</td>
<td>pos/neg</td>
<td>NL 132 amu</td>
</tr>
<tr>
<td>Phenolic sulphate</td>
<td>pos</td>
<td>NL 80 amu</td>
</tr>
<tr>
<td>Phosphatate</td>
<td>neg</td>
<td>Precursor of m/z 79</td>
</tr>
<tr>
<td>Aryl-GSH</td>
<td>pos</td>
<td>NL 275 amu</td>
</tr>
<tr>
<td>Aliphatic-GSH</td>
<td>pos</td>
<td>NL 129</td>
</tr>
<tr>
<td>taurines</td>
<td>neg</td>
<td>Precursor of m/z 124</td>
</tr>
<tr>
<td>N-acetylcysteins</td>
<td>neg</td>
<td>NL 129 amu</td>
</tr>
</tbody>
</table>

NL = neutral loss.

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How steroids get fragmented in MS/MS?

Estradiol m/z 273

Estrone m/z 271

Estradiol Standard Curve 0.05 – 25 µM

\[ r = 0.9959 \]

Sensitivity is an issue in quantification of steroids
Derivatization of estradiol with dansyl chloride leads to the formation of \( \text{E}_2\text{-dansyl} \) (\( m/z \) 506)


Does derivatization help increase sensitivity?

Representative MRM chromatogram (mass transition 506/171) obtained from 50 picomole concentration of dansylated E2
Calibration curve for dansylated E2 showing linearity from 50 picomole to 100 nanomole concentration range ($r = 0.999$)

Can MS/MS analysis help distinguish isoflavone glucosides?

The Kudzu as a source of isoflavones
O- and C-glucosides fragment differently in ESI-MS/MS

Possible product ions of puerarin in ESI-MS/MS in negative ion mode

Neutral losses (162 and 180) is useful in deciding whether sugar is attached to an aromatic ring or not.

Possible structure:

Hexose attached To the ring B

162 loss is an indicative of aromatic attachment

Isomers like genistein and apigenin are readily separated by tandem mass spectrometry.

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Neutral loss of HCl (36 Da) is diagnostic for 3’-chloro derivative of genistein and daidzein in ESI-MS/MS but not in 8-chloro derivatives.

Comparison of product ions help elucidate the unknown structures.
Fragmentation of taxoids in ESI-MS/MS

Fragmentation of basic taxoids from *T. Wallichiana* extract

*Prasain et al. Anal Chem, 2001*
ESI-MS/MS spectra of taxoids (1-3). Peaks m/z 194 and 210 represent the intact alkaloid side chain.

Loss of 60 or 42

Diterpenoid Scaffold

Alkaloid Side chain m/z 210

References


