Ion fragmentation of small molecules in mass spectrometry

Jeevan Prasain

jprasain@uab.edu

6-2612

Feb 3, 2012

Small molecules are important!!

- 89% of all known drugs and 50% of all drugs are derived from pre-existing metabolites.
- Small molecules are cofactors and signalling molecules to 1000's of proteins.
- 100,000 (lipidome)

Feb 3, 2012
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. 
  
  \[ M + e^- \rightarrow M^{\text{+}} + 2e^- \]

- **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).

Adduct formation in +/-ve ion modes

Nielsen et al., J Nat Prod. 2011
Molecules with inherent positive charge: molecular weight and m/z are same

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.
Isotopic distribution and MS

- $^{1}\text{H} = 99.9\%$, $^{2}\text{H} = 0.02\%$
- $^{12}\text{C} = 98.9\%$, $^{13}\text{C} = 1.1\%$
- $^{35}\text{Cl} = 68.1\%$, $^{37}\text{Cl} = 31.9\%$
- Monoisotopic mass - the mass of the most abundant isotope
- Average mass - the abundance weighted mass of all isotopic components.

Feb 3, 2012

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

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

Feb 3, 2012
Various types of MS/MS experiments

<table>
<thead>
<tr>
<th>Mode of operation</th>
<th>Q1</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>
<td>RF-only</td>
</tr>
<tr>
<td>Q3 Scan</td>
<td>RF-only</td>
<td>RF-only</td>
<td>Resolving (Scan)</td>
<td></td>
</tr>
<tr>
<td>Product ion scan (PI)</td>
<td>Resolving (Ised)</td>
<td>Fragment</td>
<td>Resolving (Scan)</td>
<td></td>
</tr>
<tr>
<td>Precursor ion scan (PC)</td>
<td>Resolving (Scan)</td>
<td>Fragment</td>
<td>Resolving (Ised)</td>
<td></td>
</tr>
<tr>
<td>Neutral Loss Scan (NL)</td>
<td>Resolving (Ised)</td>
<td>Fragment</td>
<td>Resolving (Scan Offset)</td>
<td></td>
</tr>
<tr>
<td>Selected Reaction Monitoring mode (SRM)</td>
<td>Resolving (Ised)</td>
<td>Fragment</td>
<td>Resolving (Ised)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Schematic of QqQ (Q-TRAP, AB/MS, Scien) and description of the various triple quadrupole and trap operation modes.

Hopfgartner et al. J. Mass Spectrom, 2004

Applications of MS/MS

- **Pharmaceuticals** - Identification and quantification of drug metabolites, PK/PD
- **Academic/biotechnology** - analysis of protein/peptides, authentification and profiling of chemical components in a crude mixture, substructure analysis of unknown components
- **Clinical** - eg. neonatal screening, steroids in athletes etc.
- **Environment** - eg. dioxins in fish...
- **Geological** - eg. oil compositions...

Feb 3, 2012
Interpreting MS/MS spectra

- Likely sites of protonation or deprotonation.
- Likely leaving group.
- 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

Feb 3, 2012

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

O- and C-glucosides fragment differently in ESI-MS/MS

Feb 3, 2012

Possible product ions of puerarin m/z 415 in MS/MS

Feb 3, 2012

Intensity of product ions indicates their stability - ions bearing aromatic ring are more intense
Ion fragmentation for identification of phase II drug metabolites (glucuronide/sulfate conjugates)

Feb 3, 2012

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

Feb 3, 2012
MSMS of m/z 429 indicate that it may be daidzein glucuronide

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 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 (HSO4-) and m/z 80 (SO3-). The absence of the m/z 97 fragment with the base peak m/z 80 makes the distinction between aromatic and aliphatic sulfates.


Feb 3, 2012
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>

Feb 3, 2012

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>Phosphate</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>Pos</td>
<td>Precursor of m/z 126</td>
</tr>
<tr>
<td>N-acetylcysteins</td>
<td>neg</td>
<td>NL 129 amu</td>
</tr>
</tbody>
</table>

Feb 3, 2012

NL = neutral loss. Kostiainen et al., 2003
Analysis of steroids by 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

Feb 3, 2012
Derivatization of estradiol with dansyl chloride leads to the formation of \text{E}_2\text{-dansyl} (m/z 506)


Feb 3, 2012

Derivatization tremendously helps increase sensitivity of E2

MRM chromatogram (m/z 506/171) 50 picomole dansylated E2

Feb 3, 2012
Calibration curve for dansylated E2 showing linearity from 0.005-100 nM concentration range ($r = 0.999$)

Substructure analysis in ESI-MS/MS (dereplication and partial identification of natural products)
Fragmentation of basic taxoids from *T. Wallichiana* extract


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

Feb 3, 2012
MS/MS precursor-scan spectra of typical alkaloid side chains to identify the basic taxoids compounds in an ethyl acetate extract of *T. wallichiana*.

**Comparison of precursor scan spectra obtained from the scaffold m/z 309 and side chain m/z 194, 210 and 252**

**Taxoids with scaffold m/z 309 and alkaloid side chains are shown by dashed lines**


