Increasing metabolite coverage using +ve and –ve ion mode

Representative Q1 scans of a methanolic extract of human blood serum


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
Isotopic pattern and intensity of ions indicates the number of carbons and hetero atoms in the molecular ion

\[ ^1H = 99.9\%, \quad ^2H = 0.02\% \]
\[ ^{12}C = 98.9\%, \quad ^{13}C = 1.1\% \]
\[ ^{35}Cl = 68.1\%, \quad ^{37}Cl = 31.9\% \]

8-chlorodaidzein
m/z 287.011 (M-H)^{-}; 289.008 ([M-H+2]^+)

Ion fragmentation for identification of phase II drug metabolites (glucuronide/sulfate conjugates)
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 neutral loss of 176 Da is an indicative of glucuronide metabolites
MSMS of m/z 429 indicate that it may be daidzein glucuronide

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-.)

Among the annotated list of compounds by Metlin - phenylacetylglucose’s validation by MS/MS interpretation

Many metabolites, unidentified by the Metlin database
A medium chain dicarboxylic fatty acid with m/z 241.109 [M-H]⁻

C₁₂H₁₇O₅ [M-H]⁻
Calc. m/z 241.1081

Prasain et al., J Mass Spectrom. 2017
### 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>

NL = neutral loss.  
Kostiainen et al., 2003

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### Analysis of steroids by MS/MS

**Estradiol**  
m/z 273

![Estradiol diagram](image)

**Estrone**  
m/z 271

![Estrone diagram](image)
Derivatization of estradiol with dansyl chloride leads to the formation of E₂-dansyl (m/z 506)


Derivatization tremendously helps increase sensitivity of E2

MRM chromatogram (m/z 506/171) 50 picomole dansylated E2
MS based-Lipidomics

Profiling phospholipids and sphingosines in a complex mixture using MS/MS

PE
Neutral Loss scan 141

PC
Precursor ion scan 184

SM
m/z 184

PS
Neutral Loss scan 185
How to profile sphingolipids in a complex mixture using MS/MS?

$m/z$ 264 is a characteristic ion for all compounds containing a sphingosine backbone.

Phosphatidylcholine loses a methyl group to form a negatively charged, pseudomolecular ion.

Phospholipids may undergo demethylation and then the loss of the fatty acyl groups from glycerophosphocholine backbone.
Precursor ion scan m/z 264 in +ve ion mode is specific to identify ceramides in a sample

Precursor ion scan m/z 184.073 for PC/SM in a C. elegans lipid extract [A]; MS/MS of the precursor ion m/z 784 [B]
Several isobaric compounds - Identification by high resolution mass spectrometry

Product ion spectra of deprotonated arachidonic acid [AA] and its oxidation product 5-hydroxy-eicosatetraenoic acids [5-HETE]
Structure determination: Accurate mass of a precursor ion is not enough, but MS/MS is


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

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

Conclusions

• Identifying unknown metabolites is a significant analytical challenge in metabolomics and it requires integrated analytical and bio-informative approaches.

• The use of high-resolution MS and MS\textsuperscript{n} provides important information to propose structures of fragment and precursor ions.

• Only an integrated approach can describes multitude of metabolites present in a biological sample.