Advances in bioanalytical LC-MS using the Orbitrap mass analyzer

Dr. Michaela Scigelova
Bremen, Germany
Orbitrap by Application Areas

Application focus: Bioanalysis

- Key figures of merit
- Elemental composition determination
- Structural characterisation
- Quantitation

- Application areas
  - drug metabolism
  - doping control
  - food contaminants

BIOANALYSIS - Quantitative measurement of a drug, drug metabolite, or chemicals in biological fluids
Orbitrap Analyzer – Key Figures of Merit

- Mass accuracy
- Resolution
- Fidelity of isotope pattern abundancies
- Dynamic range
- Positive/negative switching
- Multiple levels of fragmentation
Accurate mass measurement is used to determine the elemental composition of an analyte:

- confirm the identification of target compounds
- eliminate false positive identification
- support the identification of unknowns
- separation of possible interferences

Example: mass 32
What can it be??

\[
\begin{align*}
S \\
O_2 \\
CH_3OH \\
N_2H_4
\end{align*}
\]
Accurate Mass Is a Powerful Filter

<table>
<thead>
<tr>
<th>Mass measured</th>
<th>Tolerance [Da]</th>
<th>Suggestions</th>
<th>Calc Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>32.0</td>
<td>+/- 0.2</td>
<td>O₂, CH₃OH, N₂H₄, S</td>
<td>31.9898, 32.0261, 32.0374, 31.9721</td>
</tr>
<tr>
<td>32.02</td>
<td>+/- 0.02</td>
<td>CH₃OH, N₂H₄</td>
<td>32.0261, 32.0374</td>
</tr>
<tr>
<td>32.0257</td>
<td>+/- 0.002</td>
<td>CH₃OH</td>
<td>32.0261</td>
</tr>
</tbody>
</table>
Accurate Mass

Makes Life Easier
RESOLUTION

- High resolution is necessary to separate peaks of one mass from those of another and ensure that ions of only one kind contribute to a particular measurement.

- Example of pirimicarb (m/z 239)

<table>
<thead>
<tr>
<th>Resolution</th>
<th>Mass tolerance (mmu)</th>
<th>Number of elemental composition suggestions*</th>
</tr>
</thead>
<tbody>
<tr>
<td>15,000</td>
<td>+/- 9</td>
<td>14</td>
</tr>
<tr>
<td>80,000</td>
<td>+/- 1.7</td>
<td>1</td>
</tr>
</tbody>
</table>

*Assuming CHNO elements
High resolution is necessary to separate peaks of one mass from those of another and ensure that ions of only one kind contribute to a particular measurement.

- Experiments involving complex mixtures
- Accurate mass determination
High resolution is necessary to separate peaks of one mass from those of another and ensure that ions of only one kind contribute to a particular measurement.

- Experiments involving complex mixtures
- Accurate mass determination
- Highly specific quantitation
Resolution

Enables Accurate Mass and Accurate Quantitation
Stable Mass Accuracy

- Stability of mass measurement
- Positive/negative acquisition modes
Dynamic Range of Mass Accuracy

- Azoxystrobin
- Resolution 15,000

<table>
<thead>
<tr>
<th>MH⁺</th>
<th>ΔM [ppm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>404.12390</td>
<td>-0.20</td>
</tr>
<tr>
<td>405.12723</td>
<td>5.27</td>
</tr>
<tr>
<td>406.12958</td>
<td>3.07</td>
</tr>
</tbody>
</table>

ThermoFisher Scientific
Dynamic Range of Mass Accuracy

- Azoxystrobin at resolution **80,000**
- The complete molecular ion cluster detected correctly.

<table>
<thead>
<tr>
<th>MH⁺</th>
<th>ΔM [ppm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>404.12390</td>
<td>-0.39</td>
</tr>
<tr>
<td>405.12723</td>
<td>-0.45</td>
</tr>
<tr>
<td>406.12958</td>
<td>-0.98</td>
</tr>
</tbody>
</table>
On Isotopes, Their Abundancies, and How Can That Be Useful
Fidelity of Isotope Pattern Abundancies

- Identification of an ‘unknown’
- Accurately determine the mass of compound X and determine sum formulae proposals
- *Exclude* false positive hits by comparing the proposed sum formulae to the theoretical isotope patterns

For details see Thermo Application note 30130
GOAL: Unique Elemental Composition

- Assuming better than 1 ppm mass deviation, generally, a unique elemental composition can be obtained for compounds < 300 Da*

- But with an additional information from Isotopic Abundance Ratios, unique elemental composition can be obtained for compounds up to 2200 Da

Kind T, Fiehn O: Metabolomic database annotations via query of elemental compositions: Mass accuracy is insufficient even at less than 1 ppm. *Bioinformatics* 7, 234-244 (2006)*

Elemental Composition

Structural Elucidation
LTQ Orbitrap Velos Technology

- Parent isolation
- Fragmentation
- Fast ion trap detection
- Fragmentation of ions
- Higher energy

- High accuracy/resolution detection
- ETD module
- Peptides with PTM
Fragmentation ‘Menu’

CID
- In an ion trap
- ‘Resonance’ fragmentation
- Detection in ion trap (fast)
- Detection in Orbitrap (high resolution/accurate mass)

HCD
- In multipole collision cell
- Multiple collisions possible
- Detection in Orbitrap (high resolution/accurate mass)
- No low mass cut-off

Each fragmentation technique has its pros and cons
Select what best suits your application
You have a choice 😊
Roxithromycin $\text{C}_{41}\text{H}_{76}\text{N}_2\text{O}_{15}$ $m/z$ 837.53185
Quercetin – Higher energy fragmentation HCD
Multiple Levels of Fragmentation - MS<sup>n</sup>

- Traditional library search only useful for compounds represented in the library
- Unknown compounds not in the library can not be identified
- Use MS<sup>n</sup> to obtain structural arrangements of unknowns

Level = MS stage
Node = product ion spectrum
Branch = connects precursor and its product ion spectrum

Group of compounds with structural similarity

- Different structures
- Different masses
- Different MS/MS spectra

BUT

- Share some structural features
- Consistent CID behaviour
- Important structural information

Identical $\text{MS}^n$ spectra of analogues

Getting to Know the ‘Unknown’

- **Apomorphine** not in the database
- **MS^n n = 1 - 4**
- Data indicated two precursor ions m/z 239 and 193 common to heroin, codeine and morphine

Substructures identified by comparison to existing ‘spectra trees’ in the library

Concept of ‘Spectral Trees’

- Identification of ion structures and sub-structures
- Subsequent reconstruction of the molecular structure of small organic compounds
- Characterisation of structurally similar compounds
- Identification of designer drugs
- Novel chemical analogues

Mass Frontier sw for creating spectral tree libraries
SUMMARY

- Accurate mass is useful
- For precursor and fragment ions
- **Useful only if reliable**
  - Calibration stability (days)
  - Dynamic range (> 3 orders of magnitude)
  - Stable in alternating positive/negative mode

- **Reliable only if measured at adequate resolution**
Orbitrap in Bioanalysis

- Published research articles (2006-2008)

- Drug metabolism, 17
- Doping control, 16
- Food contaminants/residue analysis, 5
- Drug metabolism, 17
Doping control

Doping control

Drug metabolism


Drug metabolism


Drug metabolism


(direct comparison of quantitative performance between API 4000 triple quadrupole and the LTQ Orbitrap)


Food contaminants/residue analysis


- Entry of the Exactive instrument in late 2008
Executive Summary

- Mass spectrometric detection will be employed in most bioanalytical assays.

- The Orbitrap mass spectrometer has been used for a wide range of applications: in the discovery phase and during the preclinical and clinical stages of drug development, for detection of food contaminants, and in doping control.

- The desirable attributes of the Orbitrap-based analyzers most quoted in published literature are:
  - reliable high mass accuracy and its dynamic range
  - very high resolving power
  - MS/MS or MS\(^n\) fragmentation capabilities with accurate mass

- Development continues in the areas of Orbitrap design, mass analyzer hybridization, coupling to novel ionization techniques, and advancing data processing tools