Introduction to Tandem Mass Spectrometry

Dr Santiago Vazquez
Drug Toxicology Unit
Overview

• Fundamentals
• Terminology
• Mass Analysers
• Tandem Mass Spectrometry
Rocker Box- Gold Cradle
What Does a Mass Spectrometer Do?

• Measures mass at the molecular level

• Analytical balances
  0.001g to 1g ± 0.0001g

• Mass spectrometers
  1x $10^{-24}$g to 1x $10^{-19}$g ± 1x $10^{-25}$g
  Or
  1 Da to 100 000 Da ± 0.1 Da
What Are Mass Spectrometers Good For?

• Structural information

• Elemental composition

• Identification

• Quantitation

• Scaring the pants off your management accountant
Application of Mass Spectrometry

- **Biotechnology**: the analysis of proteins, peptides, oligonucleotides
- **Pharmaceutical**: drug discovery, combinatorial chemistry, pharmacokinetics, drug metabolism, drug degradation product analysis
- **Clinical**: TDM, neonatal screening, haemoglobin analysis, drug testing
- **Environmental**: Pesticides on foods, PAHs, PCBs, water quality, food contamination
- **Forensic**: Toxicology, identification of drugs
Moore’s Law for MS- Increase in Sensitivity
The First Mass Spectrometer

- 1907, J.J. Thomson (Cavendish Laboratory) built a parabola spectrograph a predecessor to a mass spectrometer

- 1913, refined this to produce the first mass spectrum that demonstrated different isotopes of neon
Components of a Mass Spectrometer

- **Sample inlet**, through which sample molecules are introduced into the mass spectrometer
- **Source**, where the molecules are converted to ions
- **Mass analyser**, where ions are separated according to their $m/z$
- **Detector**, which converts the ion energy into electrical signals for transmission to a computer
- **Key specifications**: resolution, mass measurement accuracy, and sensitivity
Components of a Mass Spectrometer

- **Inlet**
  - LC
  - GC
  - Plate

- **Ion Source**
  - ESI
  - APCI
  - MALDI
  - EI
  - CI

- **Mass Analyser**
  - quadrupole
  - ToF
  - Magnetic Sector
  - Ion Trap
  - FTMS
  - Orbitrap

- **Detector**
  - EM
  - MCP
  - Faraday Cup

- Mass Spectrum
Which Mass?

• **Average Mass**
  
The sum of the atomic weights of all the atoms in a molecule, e.g. C = 12.0111, H = 1.0079, O = 15.9994.

• **Monoisotopic Mass**
  
The sum of the exact masses of the most abundant stable isotope of the atoms that make up a molecule, e.g. C = 12.0000, H = 1.00783, O = 15.9949

• **Nominal Mass**
  
The sum of the integer mass of the most abundant naturally occurring stable isotopes of the atoms in a molecule, e.g. C = 12, H = 1, O = 16
The Mass of Angiotensin I

Nominal mass = 1296

Average mass = 1297.4799

Monoisotopic mass = 1296.6852

$^{12}$C$_{62}$H$_{90}$N$_{17}$O$_{14}$

Resolution = 1000

$^{12}$C$_{61}^{13}$C$_1$H$_{90}$N$_{17}$O$_{14}$

Resolution = 5000

$^{12}$C$_{60}^{13}$C$_2$H$_{90}$N$_{17}$O$_{14}$
Accurate Mass Measurements

• Used to determine/confirm the elemental composition of unknown

• The better the precision/accuracy of measurement the less the ambiguity

• Factors affecting accurate mass measurements
  – Temperature (particularly with ToF)
  – Humidity
  – Vibrations
  – Can all affect instrumental stability and reproducibility of results
Accurate Mass & Exact Mass

Measured accurate mass
• An experimentally determined mass that allows the elemental composition to be determined by comparison with the calculated exact mass

Calculated exact mass
• Is a theoretical value calculated by summing the masses of the individual isotopes of the molecule

\[ ppm = \frac{|m_{\text{exact}} - m_{\text{accurate}}| \times 10^6}{m_{\text{exact}}} \]
Effect of ppm & Mol. Wt. on Potential Formulae

![Graph showing the effect of ppm and molecular weight on the number of potential formulae for compounds of different mass accuracies. The graph includes lines for 300 Da, 600 Da, and 1000 Da, illustrating an increase in the number of formulae with increasing ppm accuracy.](image-url)
Resolution

- Ability of a mass spectrometer to distinguish between ions of different $m/z$ ratios
- This is measured by:
  - The 10% valley definition
    - relies on two adjacent peaks being just resolved
  - The full width at half maximum (FWHM) definition
    - is most commonly used today, determined by the peak width at 50% of height
Calculating Resolution

10% Valley Definition

Resolution = \frac{m_1}{m_2 - m_1}

FWHM Definition

\Delta m/z at 50% Intensity

Resolution = \frac{m_1}{\Delta m/z_{(FWHM)}}
Glucagon at Different Resolutions

Resolution (FWHM)

- 1000
- 3000
- 10000
MASS ANALYSERS
Quadrupole Mass Analyser

- Most common analyzer- rugged, inexpensive, and compact
- Has four parallel metal rods with RF and DC voltages applied to them
- For a given ratio of DC to RF at a fixed frequency only ions of a given $m/z$ value will pass through the quadrupole- mass filter
- Various ion transition modes- scan through all masses or sit at one fixed mass
- $m/z$ range is only up to ca. 1000 Da
Quadrupole Mass Analyser
Quadrupole Mass Spectrometer (MS)

$Q_1$

Single mass transition mode
Time-of-Flight Mass Analyser

• The least complex mass analyser
• Ions are given a defined kinetic energy and allowed to drift through a field-free region (0.5 to several meters)
• Time taken for ions to arrive at the detector is related to the $m/z$
• Wide range of $m/z$ can be measured with good sensitivity
• Moderate to high resolving powers (5,000-20,000 FWHM)
• Relatively high duty cycle
ToF Mass Spectrometer (MS)

Detector

ToF
TANDEM MASS SPECTROMETRY
What is a Tandem MS

- Two mass analyzers combined in one instrument linked with a collision cell
- Different analyzers can be combined to create “hybrid” instruments e.g. QToF
- Depending on the mass analyser different scanning modes and MS experiments can be performed
## QQQ MS/MS Scanning Modes

<table>
<thead>
<tr>
<th>Mode</th>
<th>MS 1</th>
<th>Collision Cell</th>
<th>MS 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product Ion Scan</strong></td>
<td>Selected m/z</td>
<td>CID</td>
<td>Scanned</td>
</tr>
<tr>
<td><strong>Precursor Ion Scan</strong></td>
<td>Scanned</td>
<td>CID</td>
<td>Selected m/z</td>
</tr>
<tr>
<td><strong>Neutral Loss Scan</strong></td>
<td>Scanned</td>
<td>CID</td>
<td>Stepped Scan</td>
</tr>
<tr>
<td><strong>SRM or MRM</strong></td>
<td>Selected m/z</td>
<td>CID</td>
<td>Selected m/z</td>
</tr>
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</table>
Tandem mass spectrometry (MS/MS) - Advantage of MS/MS and Beyond

- MS Signal
- S/N Ratio
- Chemical Noise

Number of MS Stages
QQQ Tandem Mass Spectrometer (MS/MS)
QToF Tandem Mass Spectrometer (MS/MS)
Product Ion Spectrum: Progesterone

Mass Spectrum from MS1

Precursor ion

Product ion spectrum from MS2

Product ions
Conclusion

• Mass spectrometry is a versatile technique that has found wide application in a wide range of fields

• Modern tandem MS systems are robust and sensitive

• Get one if you can!!!!!