Mass Spectrometry II

Alex J. Roche
Previously we talked about the ION SOURCE.

EI / CI

ESI / APCI
Ionization Techniques

Molecular Weight

Nonpolar

Very polar

API-Electrospray

APCI

Particle Beam

Thermospray

FAB

GC/MS

GC/MS = EI & CI
Mass Spectrometer

- Mass Spectrometer is an instrument designed to separate gas phase ions according to their m/z value.

- The mass analyzer is where the separation occurs.
Analyzers

• Moving charged particles (ionized species) can be deflected by electrical and/or magnetic fields.

• The motion and separation is dependant on the MASS to CHARGE ratio (Not just mass!)
• (Although in most cases, charge = 1)
• Exception is ESI and Peptides - deconvolution
Mass Analyzers

- Magnetic sectors
- Quadrupole (Q)
- Triple Quad (QQQ, QqQ or 3Q)
- Ion cyclotron with Fourier transformation (ICR-FT-MS)
- Ion trap (IT), linear trap (LT)
- Time-of-flight mass spectrometer (TOF)
- Reflection Time-of-flight mass spectrometer (ReTOF)
- Hybrids (TOF-TOF, QTOF, etc)
Important Analyzers

- **Quadrupole (LOWEST COST)**
  up to 4000 amu, accuracy 0.1 or 0.2 amu, scan speed up to 5000 amu per sec.
- **Ion Trap (LOW COST)**
  similar to quad, although can increase resolution if focus on a reduced mass range.
- **Triple Quad (MEDIUM COST)**
  same as quad, but much more sensitive (less noise) and approaching quantitative.
- **Time of Flight (HIGH COST)**
  upto to 500,000 amu, accuracy can be 5 parts per million, HRMS or accurate mass.
- **Fourier Transform – Ion Cyclotron Resonance MS (FTICR)**
  (MOST and VERY EXPENSIVE)
  Unsurpassed mass resolving power & accuracy (1 - 20 ppm)
# Accuracy and Resolution

<table>
<thead>
<tr>
<th></th>
<th>Mass range</th>
<th>Resolution</th>
<th>Accuracy (ppm)</th>
<th>cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quad ion traps</td>
<td>&lt;2000 or 4000</td>
<td>3000-5000</td>
<td>200-300</td>
<td>low</td>
</tr>
<tr>
<td>TOF</td>
<td>unlimited</td>
<td>10⁴-10⁵</td>
<td>20-100</td>
<td>medium</td>
</tr>
<tr>
<td>FT-ICR</td>
<td>&lt;2000 or 4000</td>
<td>10⁵-10⁷</td>
<td>1-50</td>
<td>high</td>
</tr>
</tbody>
</table>

**Full Width Half Maximum (FWHM)**  

\[ R = \frac{m}{\Delta m} \]

**Example Calculation**  

- True mass = 400.0000
- Measured mass = 400.0020
- Difference = 0.0020 or 2 mmu
- Error = \[ \frac{0.002}{400} \times 10^5 = 5 \text{ ppm} \]

Mass = 500  
Peak width (@ 50%) = 0.1  
Resolution (FWHM) = \[ \frac{500}{0.1} = 5000 \]

**LOW RES**  

**LOW RES and HRMS**  

**HRMS**
Agilent G6140A
Triple Quad

<table>
<thead>
<tr>
<th>Specification</th>
<th>G6140A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass Range</td>
<td>10-1350m/z</td>
</tr>
<tr>
<td>Scan Speed</td>
<td>10,000u/s, 2,500u/s</td>
</tr>
<tr>
<td>Mass Accuracy</td>
<td>+/-0.13u</td>
</tr>
<tr>
<td>Mass Axis Stab.</td>
<td>+/-0.13u 8hrs</td>
</tr>
</tbody>
</table>
Mass Spectrum

Ion signal vs. m/z

Ion signal is the charge or current generated for that m/z

Arbitrary units, normalized to the intensity of the dominant peak = 100%
Masses?

• When we say **atomic mass** and its sum to give **molecular weight**, it raises the questions of...

  ...decimal places?

  ...isotopes?

  what does the MS instrument actually detect?
Atomic Weight?

- How many decimals to use?
- $^1\text{H} = 1$ or $1.0079$ amu?
- Does it matter?

<table>
<thead>
<tr>
<th>Element</th>
<th>Monoisotopic mass</th>
<th>Average mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1\text{H}$</td>
<td>1.007825</td>
<td>1.0079</td>
</tr>
<tr>
<td>$^{12}\text{C}$</td>
<td>12.000000</td>
<td>12.0110</td>
</tr>
<tr>
<td>$^{14}\text{N}$</td>
<td>14.003074</td>
<td>14.0067</td>
</tr>
<tr>
<td>$^{16}\text{O}$</td>
<td>15.994915</td>
<td>15.9994</td>
</tr>
<tr>
<td>$^{31}\text{P}$</td>
<td>30.973763</td>
<td>30.9738</td>
</tr>
<tr>
<td>$^{32}\text{S}$</td>
<td>31.972073</td>
<td>32.0660</td>
</tr>
<tr>
<td>$^{35}\text{Cl}$</td>
<td>34.968853</td>
<td>35.4527</td>
</tr>
</tbody>
</table>

most abundant natural isotope
MS or HRMS?

- Regular MS says MW = 98, which gives many possible formulae

- HRMS says MW = 98.0372 gives only one formula
- HRMS provides molecular formula
- (< 5ppm for publication!)
- HRMS machines are very expensive
Isotope Weights

- Which figures to use?

- Average Cl is 35.45, but, average Cl’s do not exist in RL!

Real Cl’s are either

\[ ^{35}\text{Cl} = 34.96885 \text{ (75.7%)} \]
\[ ^{37}\text{Cl} = 36.96590 \text{ (24.3%)} \]

- Mass spectrometer measures mass of real species (Not of averages)
Molecular Weight?

- MW of $\text{C}_{10}\text{H}_8\text{N}_3\text{OCl} = 221.6463$
  (e.g. if you wanted one mole of cpd)

- Mass spectrometer measures mass of real species

- HRMS of $\text{C}_{10}\text{H}_8\text{N}_3\text{OCl} = 221.0350$
  really the mass of:
  - $10 \times \text{^{12}C}$
  - $8 \times \text{^{1H}}$
  - $3 \times \text{^{14}N}$
  - $1 \times \text{^{16}O}$
  - $1 \times \text{^{35}Cl}$
Mass Spectrum Peaks

- Depending if we use positive or negative ionization, we may not see \( M^+ \), but \([M+H]^+\), or \([M-H]^-\)

\[ M^+ = C_{10}H_{8}N_{3}OCl = 221.03 \]

\[ [M+H]^+ = C_{10}H_{9}N_{3}OCl = 222.0 \]

(This shows typical quadrupole accuracy)

\[ [M-H]^- = C_{10}H_{7}N_{3}OCl = 220.0 \]
Isotope Peaks

Real Cl’s are either

\[ ^{35}\text{Cl} = 76\% \]
\[ ^{37}\text{Cl} = 24\% \]

\[ [\text{M+H}]^+ = \text{C}_{10}\text{H}_9\text{N}_3\text{O}^{^{35}\text{Cl}} = 222.0 \]
\[ \text{C}_{10}\text{H}_9\text{N}_3\text{O}^{^{37}\text{Cl}} = 224.0 \]

A 30% [X+2] is characteristic of a Chlorine being present
Mass Analyzers

Magnetic Sector
Quadrupole
Triple Quad
Magnetic Sector

• The ions are bent according to their m/z

• Varying V, different masses can be discerned by the focusing magnet
Quadrupole, Quad, Q

- Most widely used
- Two pairs of metallic rods (pen sized)
- Combination of DC and AC voltages applied
- For any given amplitude of DC/AC only ions with a specific \( m/z \) have a stable trajectory and make it to the detector

- Lifetime of an ion to detection is ~ 50 microseconds
• http://www.shsu.edu/~chm_tgc/sounds/flashfiles/GC-MS.swf
Quad Operation

• Either SCAN mode with ramping of DC/AC voltages, giving a range of mass detection.

  (generating a mass spectrum)

or

• SIM (Single Ion Monitoring) mode, using fixed DC/AC voltages, to only observe a specific mass (or masses).

  (This gives the most sensitivity. Sometimes used as Quantification Detector)
Triple Quad, QQQ, QqQ

Nowadays triple quad instruments are more common.

The 1st and 3rd quadrupoles are capable of mass selection.

The 2nd passes all masses – but has a gas inlet to cause Collision Induced Dissociation (CID) if desired. “Collision Cell”
QqQ

- Have two consecutive MS machines (mass analyzers / mass filters) connected by a place where you can induce some extra fragmentation if desired.

= MS.MS
= MS\(^2\)
= tandem MS
= tandem in space MS
Things you can do with QQQ

More than just ‘better’ MS

1 normal use and
3 main common tandem experiments

(there are a few others, e.g. MRM, but these are the most simple and commonly run)

Multi-dimensional MS

Get more information!
(A) Full Scan

- Use it as a normal MS machine
- functions just like a “longer” quadrupole instrument (so more accurate)
- Usually your first run
- Provides a ‘normal’ mass spectrum
(B) Product Ion Scan

- Or “Daughter Ion” spectrum

- A single m/z ion is selected using Q1
- In Q2 it interacts with the (inert) collision gas causing fragmentation
- The fragments are analyzed by scanning in Q3
- “Fragmentation of a fragment” (or Desired Molecular ion)
- Truly MS²
Product Ion Scan (contd.)

This is the most common use of a tandem instrument. It generates very clean mass spectra, since (almost) all of the chemical noise is removed in Q1.

→ Structural Elucidation
“Classic” MS$^2$ Protocol

• Run full MS

• Identify the desired ion (usually Molecular ion)

• Run MS$^2$ selecting molecular ion in Q1 and watching it fragment in Q3

• → Beautifully clean / informative mass spectra

• (Reduction of noise = improvement in S/N ratio, thus can use this for Quantitative analysis).
(C) Precursor Ion Scan

• Or the “Parent Ion” spectrum

- Q3 is held to measure the occurrence of a particular ion, and Q1 is scanned
- This generates a spectrum of precursor ions that result in a specific product ion
- “Identifies the ions that give a particular fragment ion”
Precursor Ion Scan

- E.g. which ions contain a trifluoromethyl group? $[\text{CF}_3]^+ = 69$

- Set Q3 to detect 69, it will tell us which ions from Q1 generate a 69 fragment ion
- Which implies that they contained a trifluoromethyl group

- Separates out your peaks / ions of interest – additional purification
(D) Neutral Loss Scan

- Q1 is scanned and CID occurs in Q2

- Q3 is scanned to produce a spectrum of ions that undergo a designated neutral mass loss.

- E.g. if we set the mass loss to 119 will reveal which ions lose a $\cdot$CF$_2$CF$_3$ (pentafluoroethyl radical – not MS active)

- → Tell us which ions contained a pentafluoroethyl group
MS$^2$ Method Recap

- Run simple MS

- Use that data to devise more elaborate MS$^2$ expt (decide what your ‘handle’ or extra dimension is)

- → Cleaner /more informative MS info

- This extra separation means you can get clean MS without perfect LC separation
Quantification

- Quantification means a response from only your compound.
- The industry / legal standard is (MRM) Multiple Reaction Monitoring.
- Court of Law / Pharmaceutical lab – drugs of abuse / steroid athlete testing.

- MRM is a double mass filtering, which only gives a signal from a specified ion that produces a specified fragment.
Quantification

• You need to already know your desired ion, and how it fragments.

• Q1 only passes desired ion.
• There is fragmentation (in the CC), and Q3 only passes desired fragment ion.
• If you chose correctly, only your desired cpd will make it through = quantitative process, on pM \((10^{-12}\text{M})\) scale!
Explained:

Ion Sources
Mass Analyzers
(Types of MS² runs)

$227,952.53 = LC.QQQ.MM.ESI.APCI$
## Order Confirmation

### Customer Information

Alex Roche  
Rutgers University  
1 University Dr  
Camden NJ 08102

### Order Details

<table>
<thead>
<tr>
<th>Product/Description</th>
<th>Qty/Unit</th>
<th>Unit List Price</th>
<th>Discount Amount</th>
<th>Extended Net Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1621A</td>
<td>1.000 EA</td>
<td>0.00 USD</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>PSO Organization Package Service -002 Travel for 2 Days of consulting.</td>
<td>1 EA</td>
<td>1,200.00 USD</td>
<td></td>
<td>1,200.00</td>
</tr>
</tbody>
</table>

**Item Total**: 1,200.00

**Gross Amount**: $297,713.31  
**Total Discount**: $69,760.75  
**Total**: $227,952.53

- **Estimated delivery**: 01/25/2007
Thanks to the National Science Foundation

• Award Number: 0541663
• Award Title: Acquisition of a High Performance Liquid Chromatography - Mass Spectrometry (HPLC - MS) System
• PI/Co-PI Name: Alex Roche
• $228,703