Mass Spec

Sunday, 28 April 2019 3:03 pm

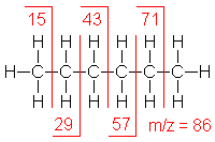
- Separates out the analytes by mass and the fragmentation patterns that occur
  - Can distinguish between individual isotopes
- Since fragmentation patterns can get quite complex, separation by HPLC can be necessary to get each analyte on its own

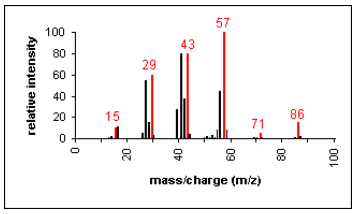
Ionisation

- MS relies on ionisation to create a charge that can propel the molecule
- The process typically occurs via bombardment with electrons and forms radicals in the process
- If the parent molecule isn't stable in a charge state, it can fragment as well and will not show up as a peak on the MS

Fragment pattern

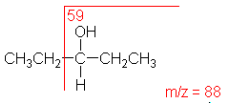
- Fragmentation is typically consistent for a particular molecule
- E.g. Hexane - MW=86

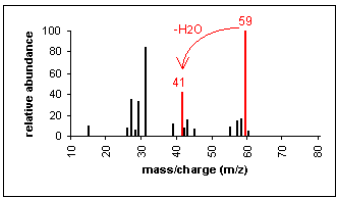




  - Fragments into 14 MW fragments (H-C-H)

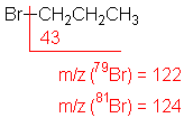
- E.g. 3-pentanol - MW=88

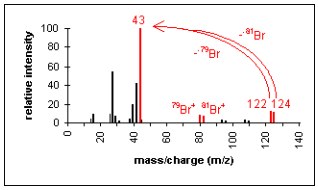




  - Upon ionisation the C-C bond next to the alcohol is cleaved
  - Sometimes a dehydration can also occur
  - The parent molecule is not stable in an ionised form and thus is not detected

- E.g. 1-bromopentane mw=123





  - Chlorine and bromine can often be detected by multiple isotopes being detected
    - ^{79}Br and ^{81}Br have a natural occurrence of 100:98

- Interpretation is based on identification of common molecular fragmentation patterns and their stable ions
  - Identifying what the resulting ion is, can tell you what the parent fragment may have looked like

Ionisation methods

- Different methods can have different results
- Different methods can be soft or hard
  - Soft methods result in less structural damage and thus less structural identification can be carried out
  - Hard methods are usually quite destructive and only useful for smaller mass molecules
- Since MS was originally invented for protein analysis, much larger potential methods have been created to ionise proteins.
  - Detection is still limited to a certain range however, so sometimes increasing the energy to decrease the m/z ratio is needed to obtain a satisfactory result.

Table 20.3
Comparison of Ionization Methods[a]

Ionization Method	Typical Analytes	Sample Introduction	Mass Range	Method Highlights
Electron impact (EI)	Relatively small volatile	GC or liquid–solid probe	To 1000 daltons	Hard method. Versatile, provides structure information
Chemical ionization (CI)	Relatively small, volatile	GC or liquid–solid probe	To 1000 daltons	Soft method. Molecular ion peak $[M + H]^+$
Electrospray (ESI)	Peptides, proteins, nonvolatile	Liquid chromatography or syringe	To 200,000 daltons	Soft method. Ions often multiply charged
Matrix-assisted laser desorption (MALDI)	Peptides, proteins, nucleotides	Sample mixed in solid matrix	To 500,000 daltons	Soft method, very high mass

[a]From Web page of Professor Vicki Wysocki, University of Arizona. Reproduced by permission.

Sample introduction and Ionisation

Electrospray Ionisation (ESI)

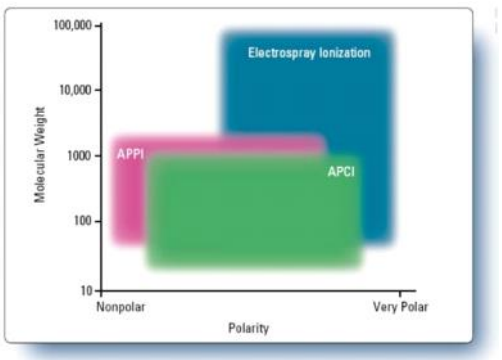
- An aerosol of the eluent is sprayed into the ioniser with a proton source such as acetic acid
- Thermal evaporation of the aerosol decreases the size of the droplets, but maintains the charged proton count
- As the droplets get smaller and smaller, the charges on the molecules and in the solvent increase in a "coulombic explosion" that breaks apart droplets further resulting in a gaseous spray of charged particles
- Useful for large molecules as they can become multiply charged

Atmospheric Pressure Chemical Ionisation (APCI)

- A plasma is formed with the eluent and a corona discharge (arcing electricity) is used to pull off electrons
- The corona discharge protonates the analytes by charging gasses to produce cations
- The charged gas charges the solvent molecules which in turn protonates the analytes in question
- Useful for smaller mass molecules, as there is less chance of multiple charges occurring

Atmospheric Pressure Photo Ionisation (APPI)

- High power UV is used to ionise the molecules in question
- When the UV wavelength is greater than the ionisation barrier of the molecule, the electron is liberated
- Useful where other methods of ionisation don't work, e.g. nonpolar analytes that won't hold a charge



Solute separation

Quadrupole

- Oscillates a magnetic filed between four electrodes down the path of the MS
- Only ions with a specific m/z will be resonant and allowed to pass down to the detector

Time of Flight (TOF)

- Filters ions based on the time it takes them to travel to the detector
- The ions are magnetically accelerated and will take a different amount of time based on the m/z

Solute detection

- MS detectors continuously scan the whole range of detectable m/z to generate a chromatogram
- Can be presented as:
  - Total Ion Count (TIC)
    - The m/z are accumulated into a single plot over all time
  - Show a single m/z over a period of time
- This is because the detector measures data in two dimensions and there are multiple ways to present the data in one dimension

Standardisation

- Isotopically enriched standards can be used to use MS quantitatively
  - The molecules will have the same physical properties and will be eluted at the same time, however will present with a different m/z
  - Comparison of quantity to the known spike can be made

Analysis

- Comparison/identification is made by both retention time and m/z
- Use of all standardisation methods (single point, calibration curve, standard addition) are possible