5. Mass spectrometry

Mass spectrometry

Introduction

- Mass spectrometry (MS) is an instrumental technique which generates a positively ions from a sample
- Uses high energy electrons to break a molecule into fragments
- This fragments the sample into ions of different masses and these fragments are then separated according to their mass-to-charge ratio (*m/z)*
- In MS, compounds are ionized, ionized molecule decomposes into smaller ions/radicals/radical ions/ neutrals.
- The positively charged fragments produced are separated, based on their mass/charge (m/z) ratio

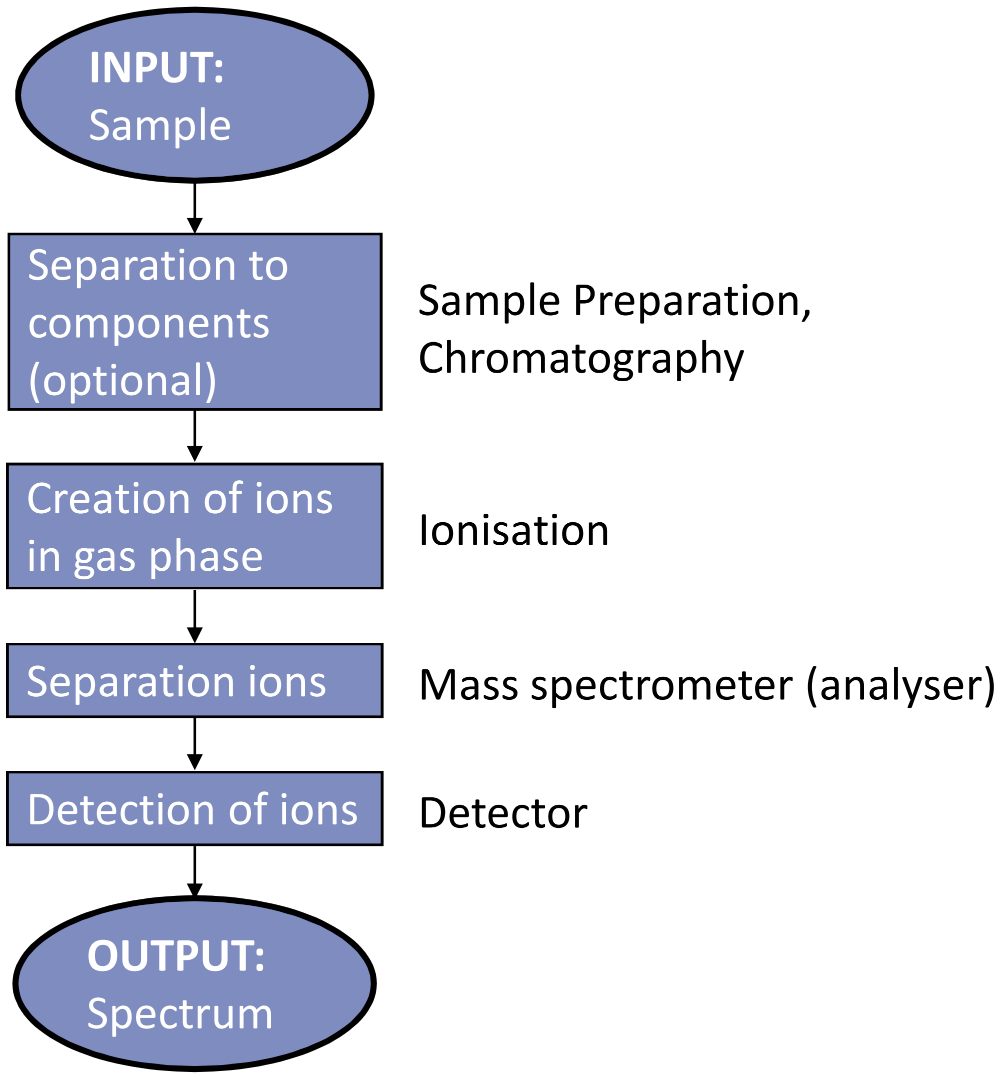
$$M \xrightarrow{\text{ionization}} M^{+\cdot} \xrightarrow{\text{fragmentation}} M^{+}_{1} + M^{+\cdot}_{2} + .. + N_1 + N_2\cdot + \ldots..$$

Parent ion — daughter ions, radicals, neutral, ...

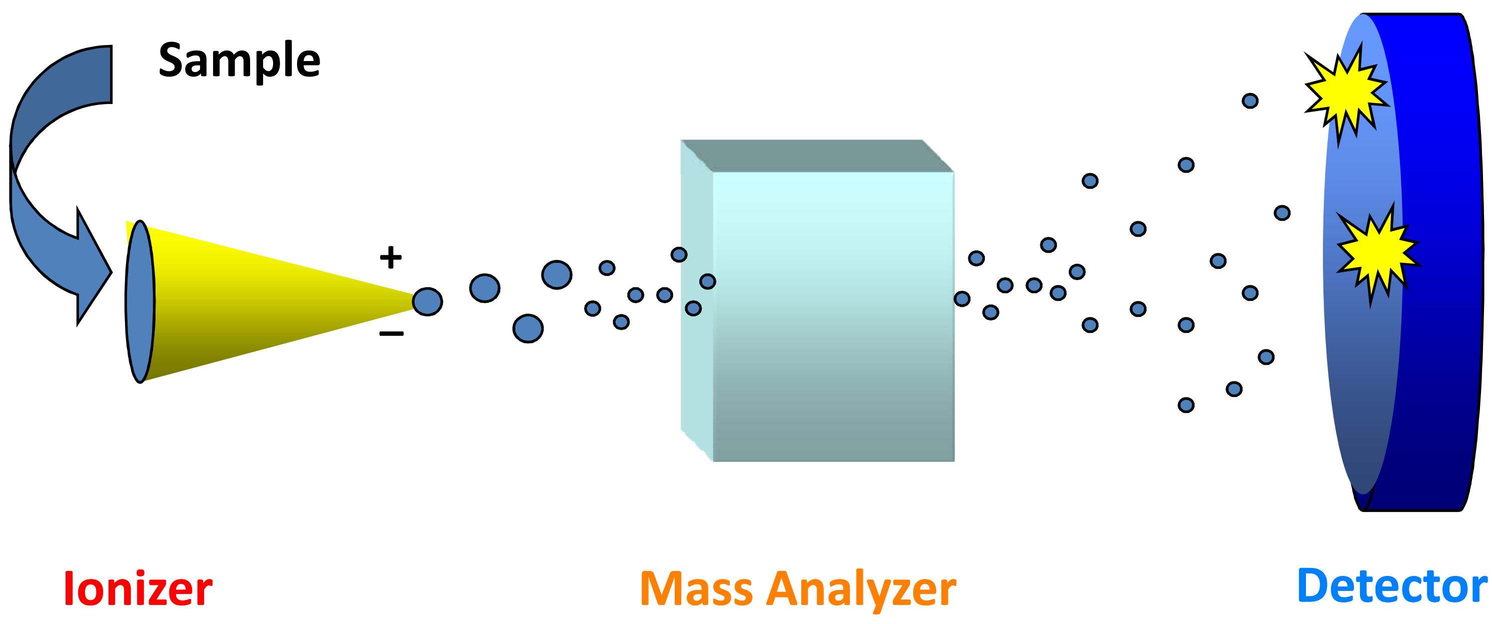
Principle of Mass Spectrometry

- Molecules are ionised
  - Ionisation produces several ions and neutral fragments
- Ions are separated and measured according to their mass-to-charge ratios (m/z)
- This is often written as the m/z ratio. Since the charge, z = 1 for most ions, the value of m/z is equal to the mass of the ion
- Ionisation of a molecule produces typical pattern of ions which can be used to identify the molecule
- Ions are easier to detect than neutral molecules

Mass spectrometry as process



Mass Spec Principles



Instrumentation

- Mass spectrometers consist of three basic parts:
  - an ion source
  - a mass analyzer
  - and a detector system
- The stages within the mass spectrometer are:
  - Production of ions from the sample (ionization)
  - Separation of ions with different masses (deflection)
  - Detection of the number of ions of each mass produced
  - Collection of data to generate the mass spectrum

1. Ion source

- The part of the mass spectrometer that ionizes the analyte, usually to cations by loss of an electron, because ions are easier to manipulate than neutral molecules
- The ions are then transported by magnetic or electric fields to the mass analyzer

Methods of sample ionization (Ionization techniques):

- Electron Ionization
- Chemical Ionization
- Matrix-Assisted Laser Desorption Ionisation (MALDI)
- Fast Atom Bombardment (FAB)

Ionization methods

Methods of sample ionization (Ionization techniques):

Hard *ionization technique:*

- a lot of energy involved
- extensive fragmentation of analyte molecules
- Electron Impact (EI) most common, uses an electron beam (70 eV electrons) to ionize samples

Soft *ionization techniques:*

- Less energy is used to ionize the analyte molecule
- Less fragmentation occurs

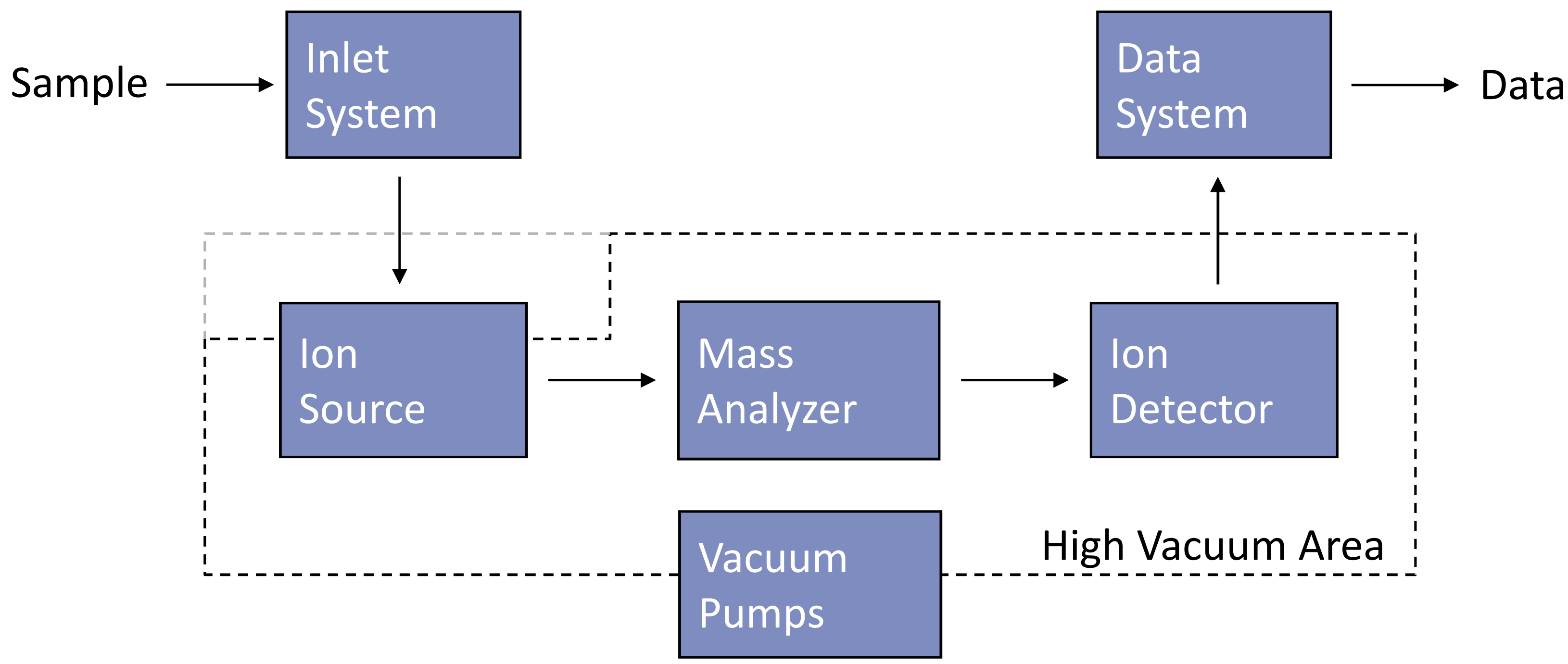
Ionization methods

- Soft ionization methods include the following:
  - Chemical Ionisation (CI) most common
  - Fast Atom Bombardment (FAB)
  - Matrix Assisted Laser Desorption Ionisation (MALDI)
  - Advantage: they are less likely to break the sample into small fragments and are more likely to produce a molecular ion

MASS ANALYZERS:

- After ions are formed in the source region, they are accelerated into the mass analyzer by an electric field
- The mass analyzer separates the ions according to their *m/z value*
- The heart of the mass spectrometer is the mass analyzer , which is the device that separates the ions created in the ion source according to their m/z ratios

Diagram of mass spectrometer instrument



VACUUM SYSTEM

- All mass spectrometers operate at very low pressure (high vacuum)
- This reduces the chance of ions colliding with other molecules in the mass analyzer. Any collision can cause the ions to react, neutralize, scatter, or
    - All these processes will interfere with the mass spectrum
- To minimize collisions, experiments are conducted under high vacuum conditions, typically 10^{-2} to 10^{-5} Pa

Mass analyzers

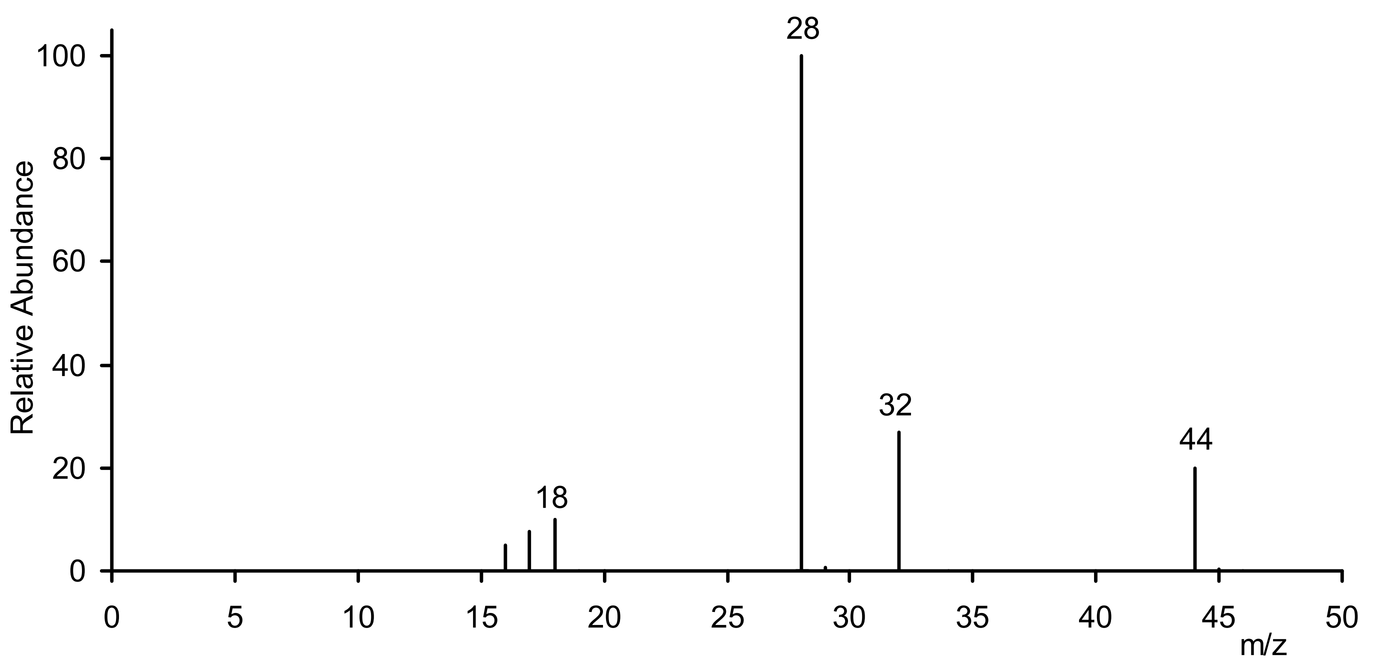
- The main types of analyzers used in the mass spectroscopy:
  - Quadrapole mass analyzer
  - Time of fight mass analyzer
  - magnetic sector analyzer
  - Ion trap mass analyzer

THE DETECTOR

- The detector records either the charge induced or the current produced when an ion passes by or hits a surface
- The choice of ion beam detection depends on a number of factors, but the most important is the ion beam intensity
- The common detectors used in mass spectrometers are
  - electron multiplier (EM)
  - Faraday's cup, and
  - Photomultiplier detectors

Basic Mass Spectrum

- The mass spectrum is presented in terms of ion abundance vs. m/e ratio (mass)
- Y-axis:Relative abundance: highest ion is set to 100%
- X-axis:mass/charge (m/z);

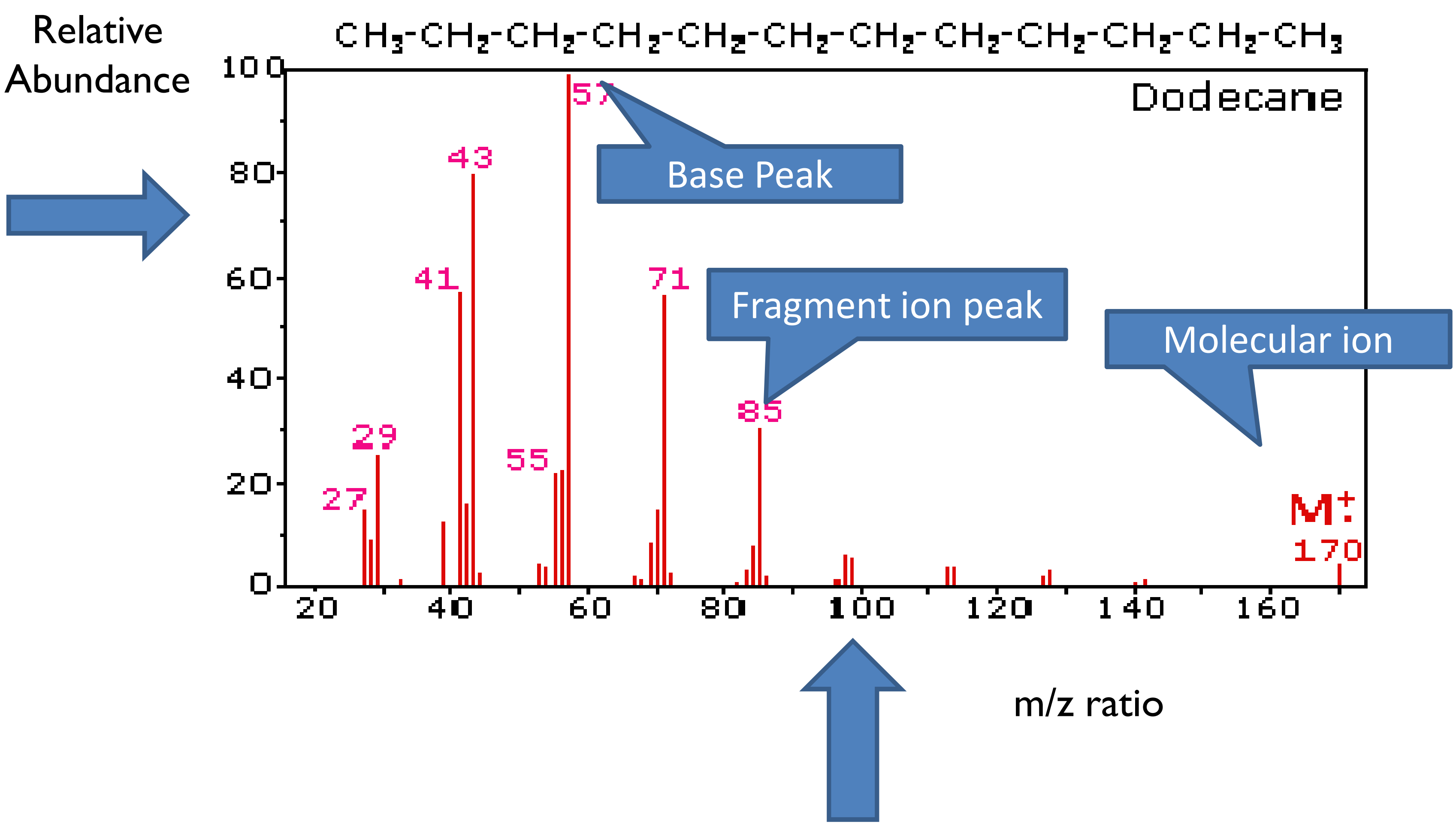


Interpreting mass spectrum

Terminology:

- **Molecular ion (M+):** The ion obtained by the loss of an electron from the molecule and represent the molecular weight of the compound.
- **Base peak:** The most intense peak in the MS,
  - it is assigned a value of 100% intensity and the intensities of other peaks are reported as percentages of the base peak
- **Fragment ion peaks–** peaks with smaller m/z values which represent the positively charged fragments of the molecule
- Spectrum shows fragmentation patterns
- The base peak is not necessarily the same as the parent ion peak.
- **The m/z values and the fragmentation pattern** are used to determine the molecular weight and structure of organic compounds

Mass Spectra



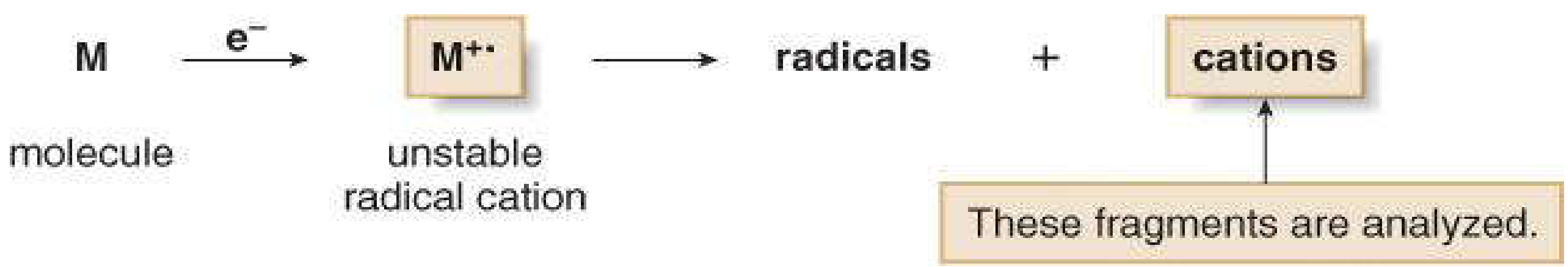
Mass Spectrometry

The Mass Spectrum

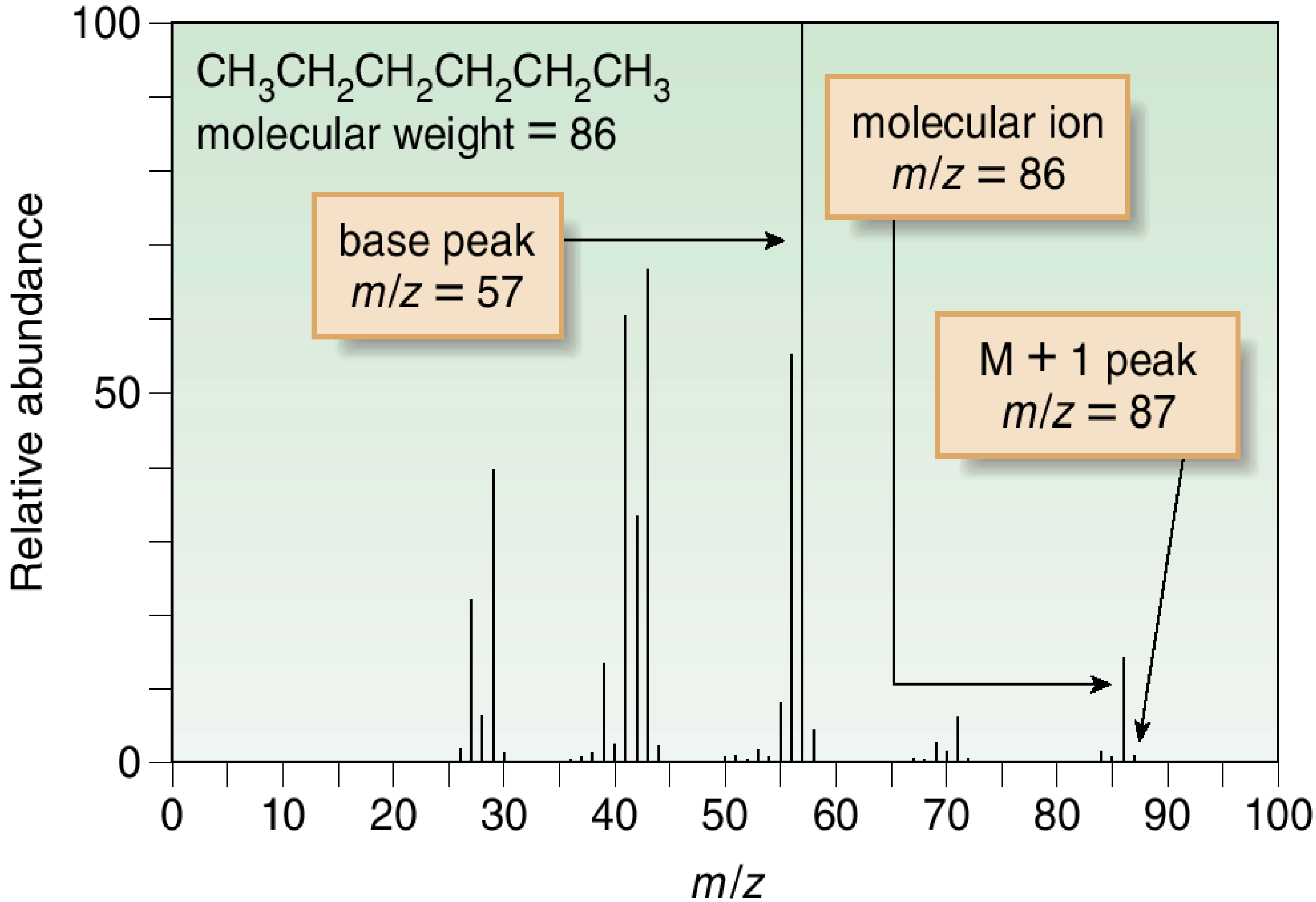
- The mass-to-charge ratio of this ion is important as it usually provides the relative molecular mass of the sample molecule.
- The most abundant ion formed in ionization gives rise to the tallest peak on the mass spectrum – this is the *base peak*
- The molecular ion peak corresponds to the loss of one electron from the intact molecule *and is usually the fragment of highest mass in the* spectrum
- If a molecule loses only one electron in the ionization process, a ***molecular ion*** is observed that gives its molecular weight – this is designated as **M^+** on the spectrum

The mass spectrum………

- The resulting mass spectrum is a graph of the mass (m/z) of each cation vs. its relative abundance
  - The peaks are assigned abundance as a percentage of the base peak (the most intense peak in the spectrum). The base peak is scaled to 100
- When the electron beam ionizes the molecule, the species that is formed is called a radical cation, and symbolized as $M^{+\bullet}$
  - The radical cation $M^{+\bullet}$ is called the molecular ion or parent ion
  - The mass of $M^{+\bullet}$ represents the molecular weight of M.
  - Because $M^{+\bullet}$ is unstable, it decomposes to form fragments of radicals and cations that have a lower molecular weight than $M^{+\bullet}$
  - The mass spectrometer analyzes the masses of cations



Example 2: consider mass spectrum of hexane ($CH_3CH_2CH_2CH_2CH_2CH_3$)



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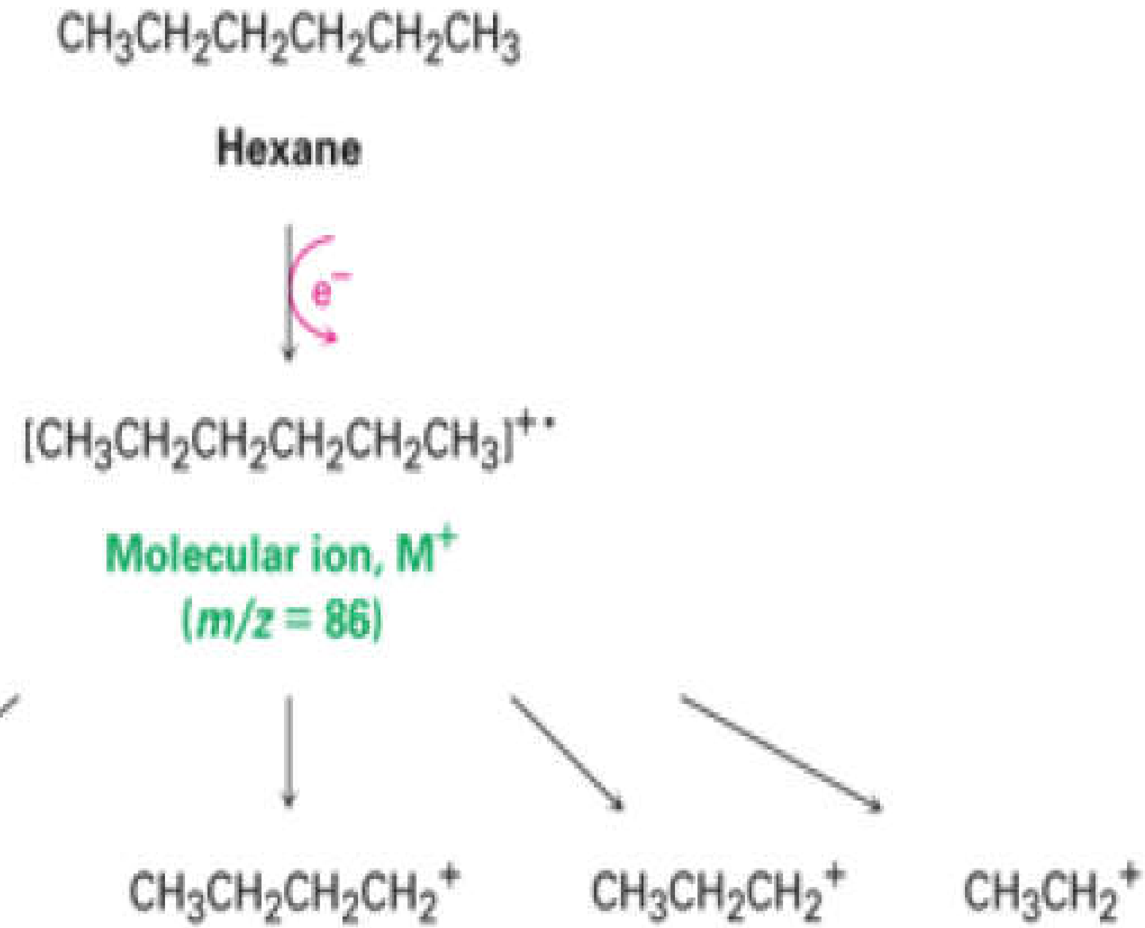


m/z:	71	57	43	29
Relative abundance (%):	10	100 (base peak)	75	40

Cont...

- The molecular ion $M^{+\cdot}$ is at m/z = 86 for hexane and the base peak whose relative abundance =100 occurs at m/z 57 and arises from loss of ethyl radical from hexane cation radical
- The peaks at m/z 71, 43, and 29 are due to loss of methyl radical from hexane cation radical, loss of propyl radical from hexane cation radical, and loss of butyl radical from hexane cation radical, respectively.

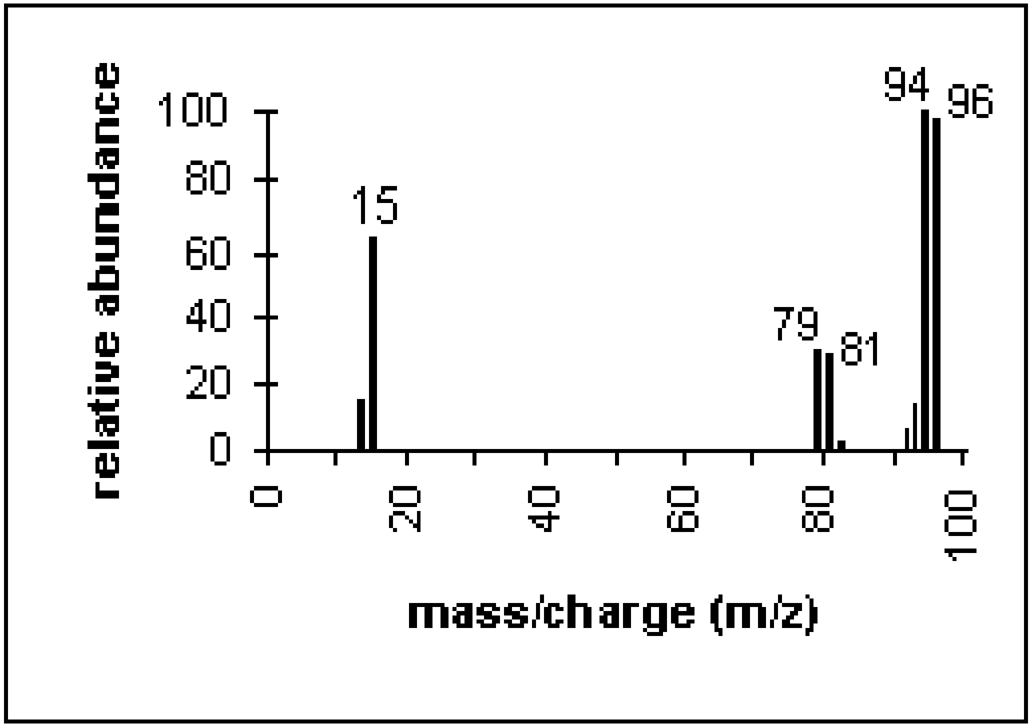
Mass Spectrometry...........

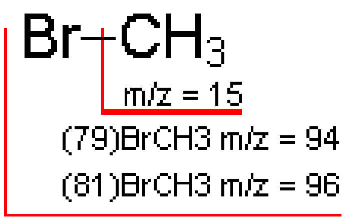
For molecules that contain Cl or Br, the isotopic peaks *are* diagnostic

- The presence of a large M+2 peak is evidence of a compound containing either chlorine or bromine, because each of these elements has a high percentage of a naturally occurring isotope two unit heavier than the most abundant isotope
- If the M+2 peak is one-third the height of the molecular ion peak, the compound contains a chlorine atom
  - because the natural abundance of ^{37}Cl is one-third that of the ^{35}Cl
- If the M and M+2 peaks are of about the same height, the compound contains **a bromine atom**
  - because the natural abundances of ^{79}Br and ^{81}Br are of about the same

Easily Recognized Elements in MS

- Bromine:
  - $M^+ \sim M+2$ (50.5% ^{79}Br/49.5% ^{81}Br)

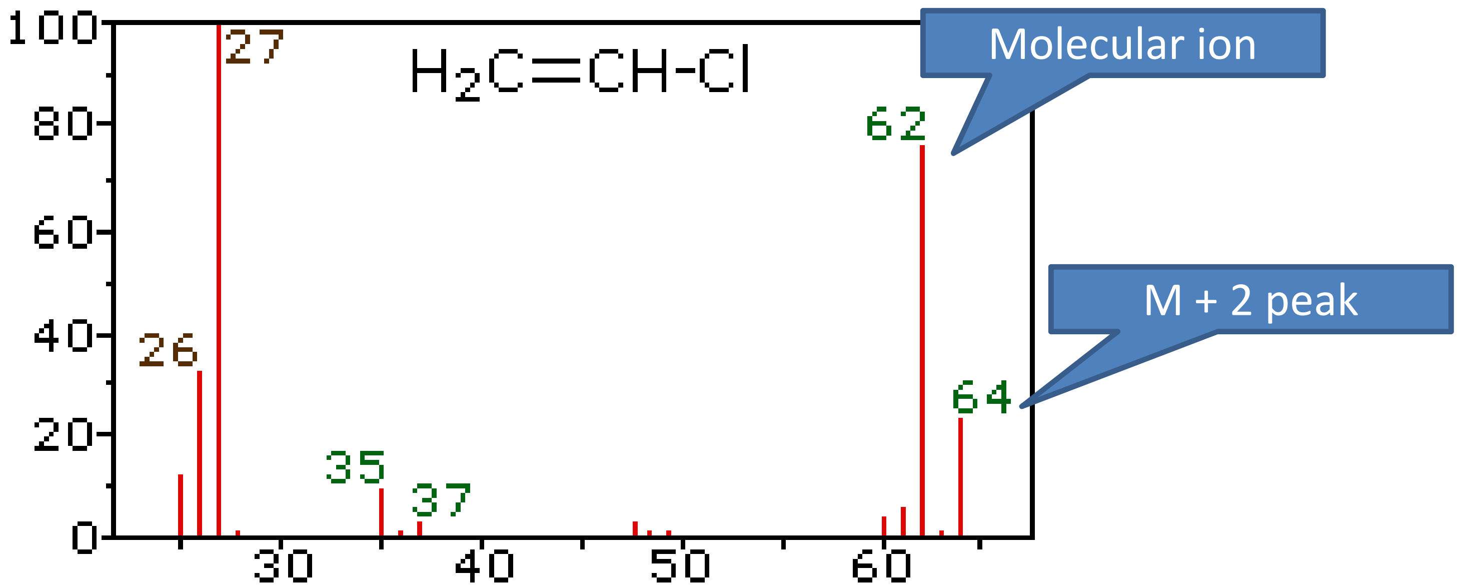




Mass Spectrum of Bromo methane

Easily Recognized Elements in MS

- Chlorine:
  - M+2 is ~ 1/3 as large as M^+



Mass spectrometry……cont'd

- Another recent development in mass spectrometry is the so-called 'hyphenated' techniques
- a mass spectrometer is coupled to another analytical technique such as gas or liquid chromatography
- In gas chromatography–mass spectrometry (GC-MS) a gas chromatograph is used to separate a mixture into individual components which are then injected directly into the mass spectrometer for detection and analysis
- Liquid chromatography–mass spectrometry (LC-MS) is a similar technique in which liquid chromatography is used to separate the components of a mixture prior to introduction into the mass spectrometer

Mass Spectroscopy

What information can be determined

- Molecular weight
- Structure (from fragmentation fingerprint)
- Isotopic incorporation / distribution

Applications

- Mass spectrometry provides a highly specific method for determining or confirming the identity or structure of drugs and raw materials used in their manufacture
- Mass spectrometry in conjunction with either gas chromatography (GC–MS) or liquid chromatography (LC–MS) provides a method for characterizing impurities in drugs and formulation excipients