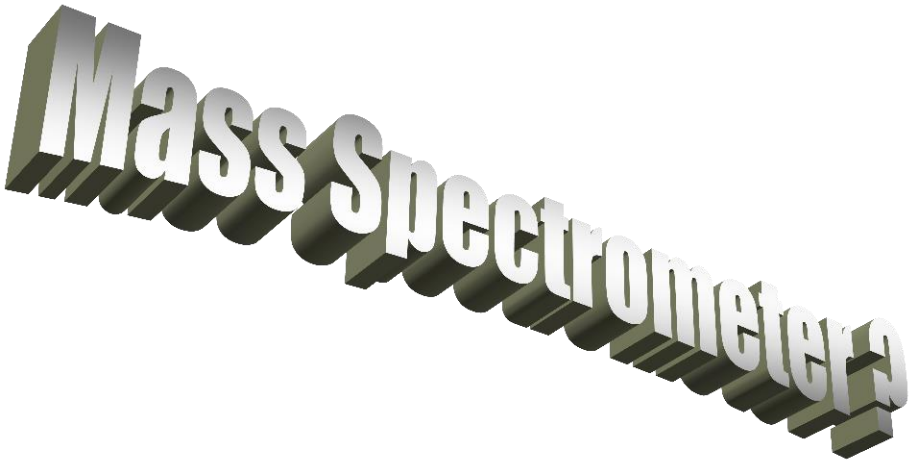
Umar Hafidz A.H.,M.Sc.

WHAT IS Mass Spectrometry ?



What For It is ?

How it is work ?

Mass spectrometry is an analytical technique used to measure the mass -to-charge ratio (m/z) of ions. It is most generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

A mass spectrometer is a device used for mass spectrometry, and produces a mass spectrum of a sample to find its composition.

This is normally achieved by ionizing the sample and separating ions of differing masses and recording their relative abundance by measuring intensities of ion flux.

E ⟶ Chemical compounds

In Mass Spectrometry

- It does not involve the absorption or emission of light.
- A beam of high-energy electrons breaks the molecule apart to fragments.
- The masses of the fragments and their relative abundance reveal information about the structure of the molecule.
- Molecules have distinctive fragmentation patterns that provide structural information to identify structural components.


GCD Plus


GCD
FLAMMABLE

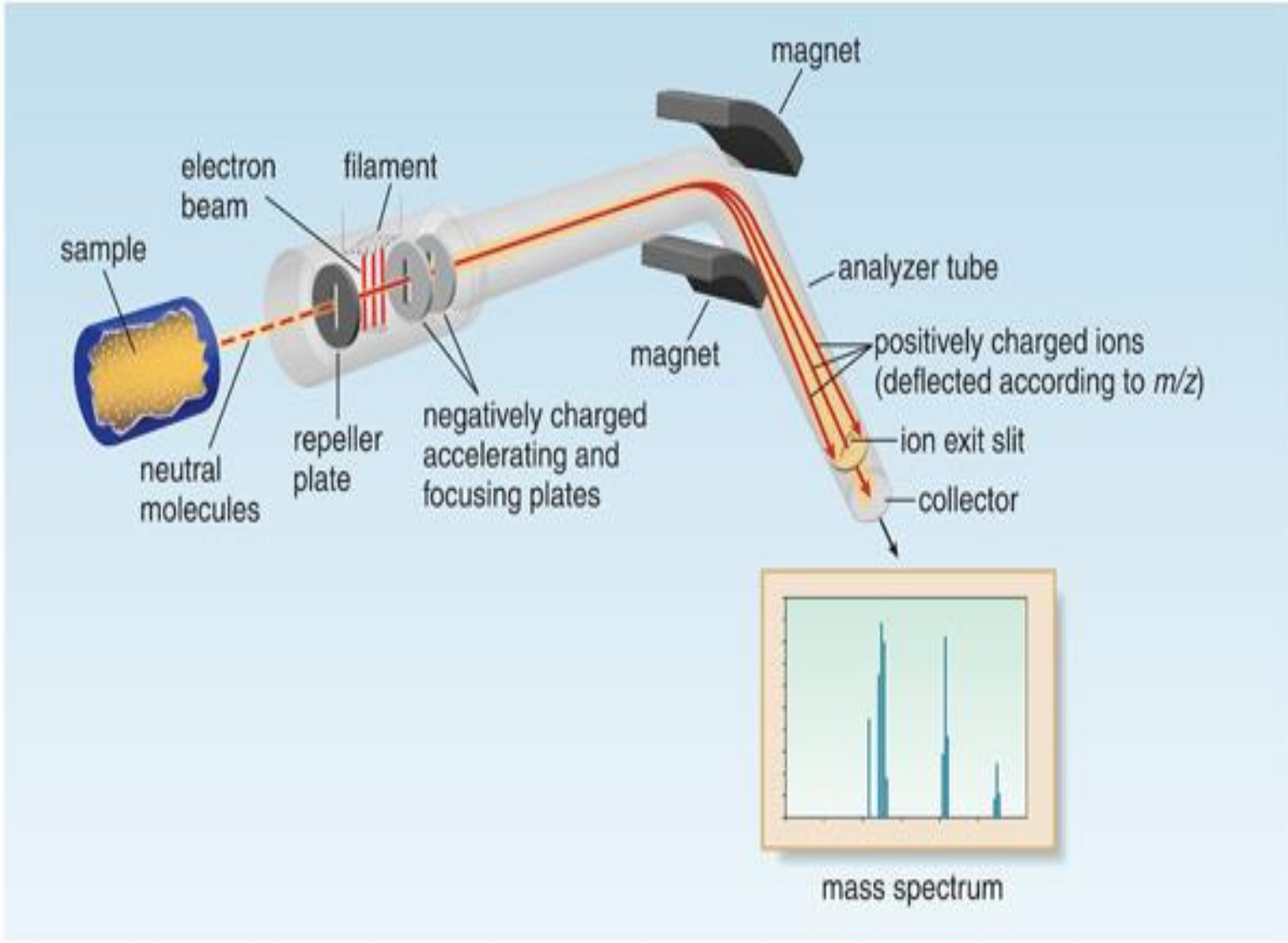
What for it is ?

The technique has several applications, including:

- identifying unknown compounds by the mass of the compound and/or fragments thereof.
- determining the isotopic composition of one or more elements in a compound.
- determining the structure of compounds by observing the fragmentation of the compound.
  studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in vacuum).
- determining other physical, chemical or even biological properties of compounds with a variety of other approaches.

- quantitating the amount of a compound in a sample using carefully designed methods (mass spectrometry is not inherently quantitative).

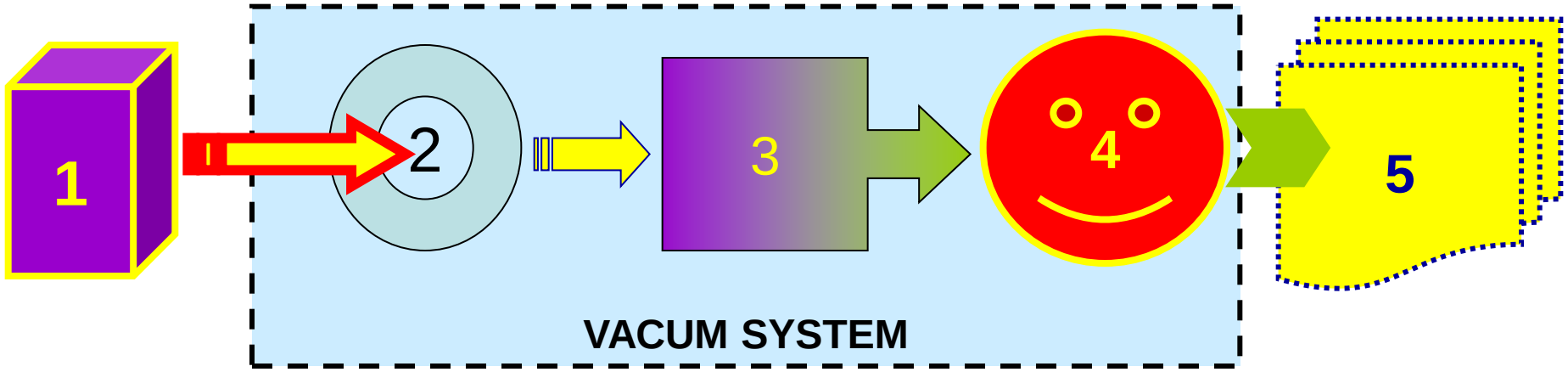
14.1 Schematic of a
ctrometer



In a mass spectrometer, a sample is vaporized and bombarded by a beam of electrons to form an unstable radical cation, which then decomposes to smaller fragments. The positively charged ions are accelerated toward a negatively charged plate, and then passed through a curved analyzer tube in a magnetic field, where they are deflected by different amounts depending on their ratio of mass to charge. A mass spectrum plots the intensity of each ion versus its *m/z* ratio.

Instrument Diagram

A typical mass spectrometer comprises three parts: sample inlet (1), ion source (2), a mass analyzer (3), detector (4) and reading system (5)



How about GC-MS ?

How it works ?

- **Different molecules have different masses, and this fact is used in a mass spectrometer to determine what molecules are present in a sample.**

- **Molecule sample is vaporized (turned into gas) and broken down (ionized) into electrically charged particles, called ions, in the first part of the mass spectrometer.**
- **These ions have specific molecular weights. They also have a charge, which means that they will be moved under the influence of an electric field.**

- **These ions are then sent into an ion acceleration chamber and passed through a slit in a metal sheet. A magnetic field is applied to the chamber, which pulls on each ion equally and deflects them (makes them curve instead of traveling straight) onto a detector.**
- **The lighter ions deflect farther than the heavy ions because the force on each ion is equal but their masses are not same.**
- **The detector measures exactly how far each ion has been deflected, and from this measurement, the ion's 'mass to charge ratio' can be worked out.**

- **From this information it is possible to determine with a high level of certainty what the chemical composition of the original sample was.**

SAMPLE INLET

1. Solid
2. Liquid
3. Gas
4. GC outlet
5. LC outlet

Pre-treatment ?

Pre-separation ?

Advantage ?

Disadvantage ?

ION SOURCE

The ion source is the part of the mass spectrometer that ionizes the material under analysis (the analyte). The ions are then transported by magnetic or electrical fields to the mass analyzer.

Techniques for ionization have been key to determining what types of samples can be analyzed by mass spectrometry.

- **Electron ionization and chemical ionization are used for gases and vapors. In chemical ionization sources, the analyte is ionized by chemical ion-molecule reactions during collisions in the source.**
- **Electrospray ionization and matrix-assisted laser desorption/ionization Two techniques often used with liquid and solid biological samples**
- **Inductively coupled plasma sources are used primarily for metal analysis on a wide array of samples types.**
- **OTHERS: Fast atom bombardment (FAB), thermospray, atmospheric pressure chemical ionization (APCI), secondary ion mass spectrometry (SIMS) and thermal ionisation**

IONIZATION METHODS COMPARISON

IONIZATION METHODS	TYPICAL ANALYTES	SAMPLE INTRODUCTION	MASS RANGE	METHODS HIGHLIGHTS
Electron Impact (EI)	Relatively small volatile	GC or liquid/solid probe	to 1,000 Daltons	Hard method versatile provides structure info
Chemical Ionization (CI)	Relatively small volatile	GC or liquid/solid probe	to 1,000 Daltons	Soft method molecular ion peak [M+H]+
Electrospray (ESI)	Peptides Proteins Non volatile	Liquid Chromato graphy or syringe	to 200,000 Daltons	Soft method ions often multi ply charged
Fast Atom Bombardment (FAB)	Carbohydrates, Organometallics, Peptides non volatile	Sample mixed in viscous matrix	to 6,000 Daltons	Soft method but harder than ESI or MALDI
Matrix As-sisted Laser Desorption (MALDI)	Peptides Proteins Nucleotides	Sample mixed in solid matrix	to 500,000 Daltons	Soft method very high mass

Mass Analyzer

Analyzer	System Highlights
Quadrupole	Unit mass resolution, fast scan, low cost
Sector (Magnetic and/or Electrostatic)	High resolution, exact mass
Time-of-Flight (TOF)	Theoretically, no limitation for m/z maximum, high throughput
Ion Cyclotron Resonance (ICR)	Very high resolution, exact mass, perform ion chemistry

Detector

The final element of the mass spectrometer is the detector.

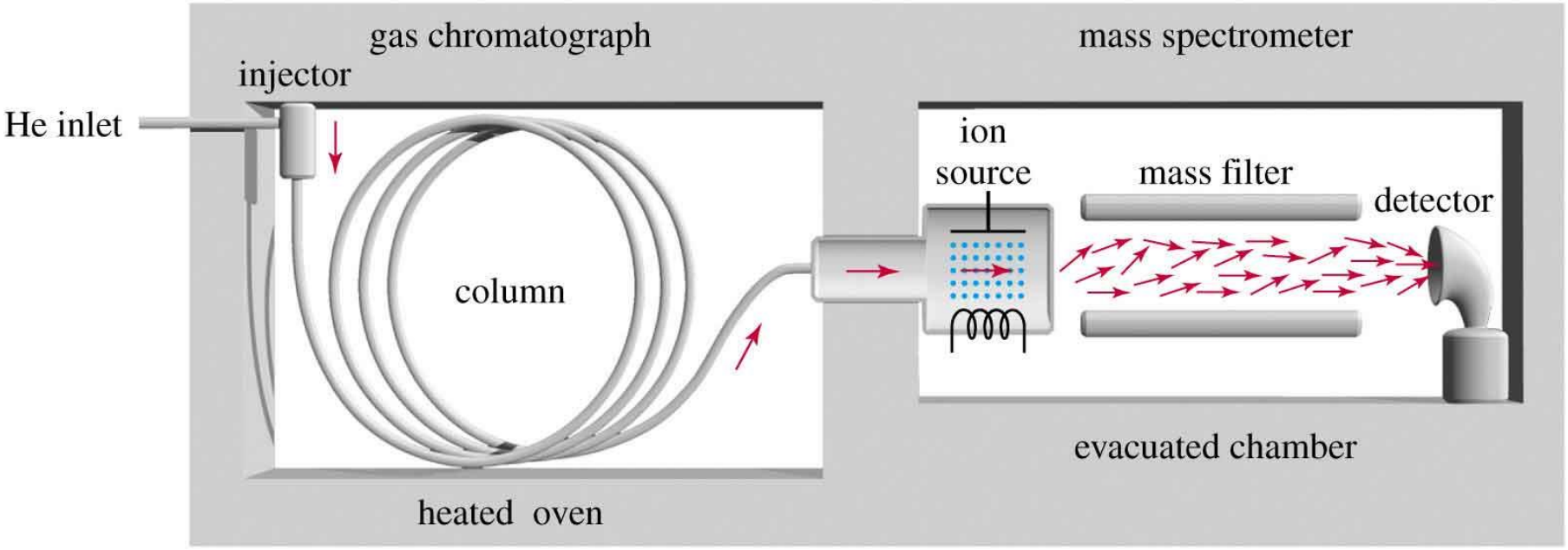
The detector records the charge induced or current produced when an ion passes by or hits a surface.

In a scanning instrument the signal produced in the detector during the course of the scan versus where the instrument is in the scan (at what m/z) will produce a mass spectrum, a record of how many ions of each *m/z* are present.

- Electron multiplier
- Microchannel Plate Detectors
- FTMS

The GC-MS

A mixture of compounds is separated by gas chromatography, then identified by mass spectrometry.



What is GC-MS ?

Gas chromatography-mass spectroscopy (GC-MS) is one of the so-called hyphenated analytical techniques. As the name implies, it is actually two techniques that are combined to form a single method of analyzing mixtures of chemicals.

Gas chromatography separates the components of a mixture and mass spectroscopy characterizes each of the components individually.

By combining the two techniques, an analytical chemist can both qualitatively and quantitatively evaluate a solution containing a number of chemicals.

The mass spectrometer includes:

- A vacuum system
- Tools to introduce the sample (Direct, LC, GC …)
- Tools to produce the gas phase ions from the sample molecules
- Tools to fragment the ions, in order to obtain structural information, or to get more selective detection
- A detection system
- Software and computing

The sequence is :

Stage 1: Ionisation

The atom is ionised by knocking one or more electrons off to give a positive ion. This is true even for things which you would normally expect to form negative ions (chlorine, for example) or never form ions at all (argon, for example). Mass spectrometers always work with positive ions.

Stage 2: Acceleration

The ions are accelerated so that they all have the same kinetic energy.

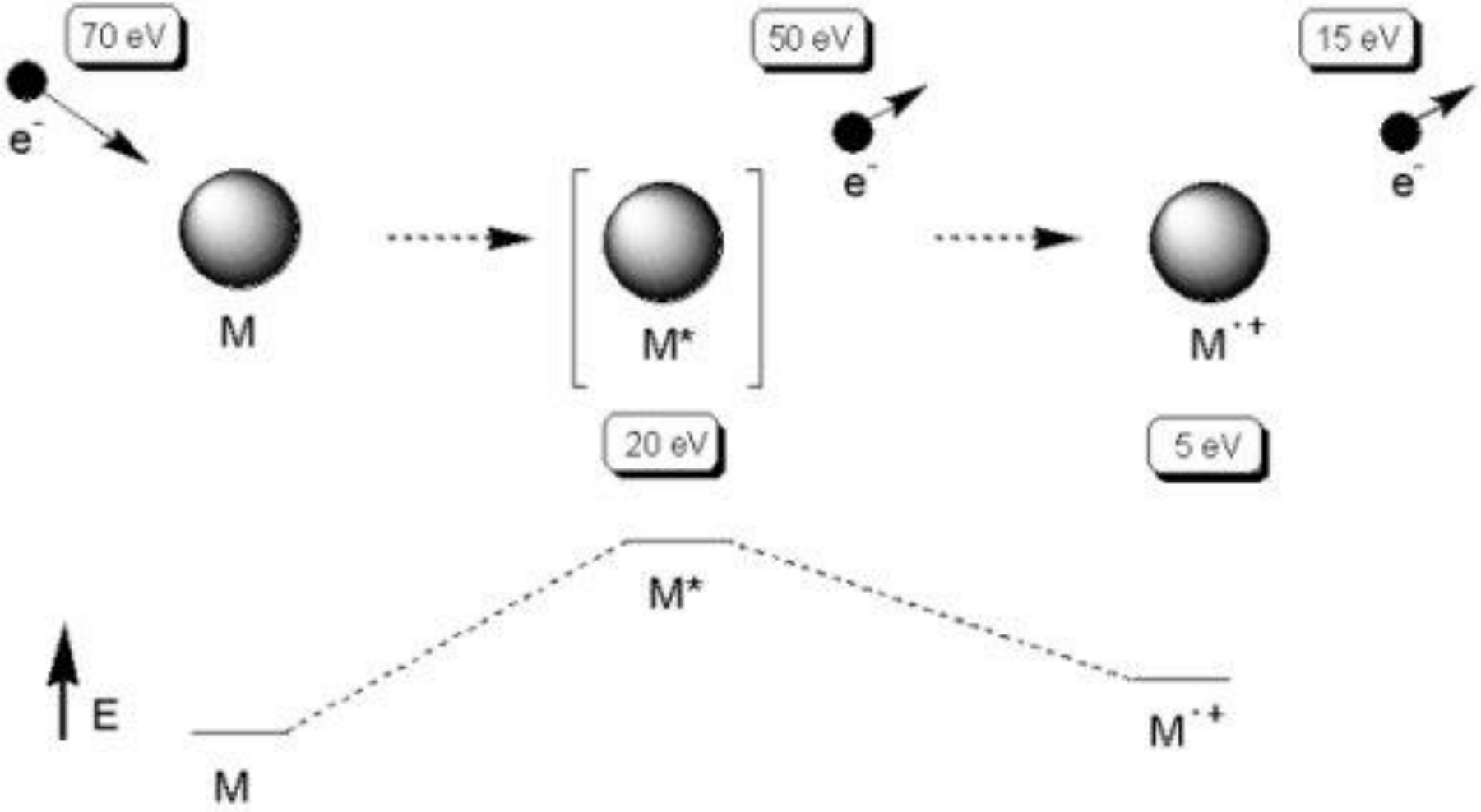
Stage 3: Deflection

The ions are then deflected by a magnetic field according to their masses. The lighter they are, the more they are deflected.

The amount of deflection also depends on the number of positive charges on the ion - in other words, on how many electrons were knocked off in the first stage. The more the ion is charged, the more it gets deflected.

Stage 4: Detection

The beam of ions passing through the machine is detected electrically.



Overall reaction

$$M + e^- \longrightarrow M^{\cdot+} + 2\,e^-$$

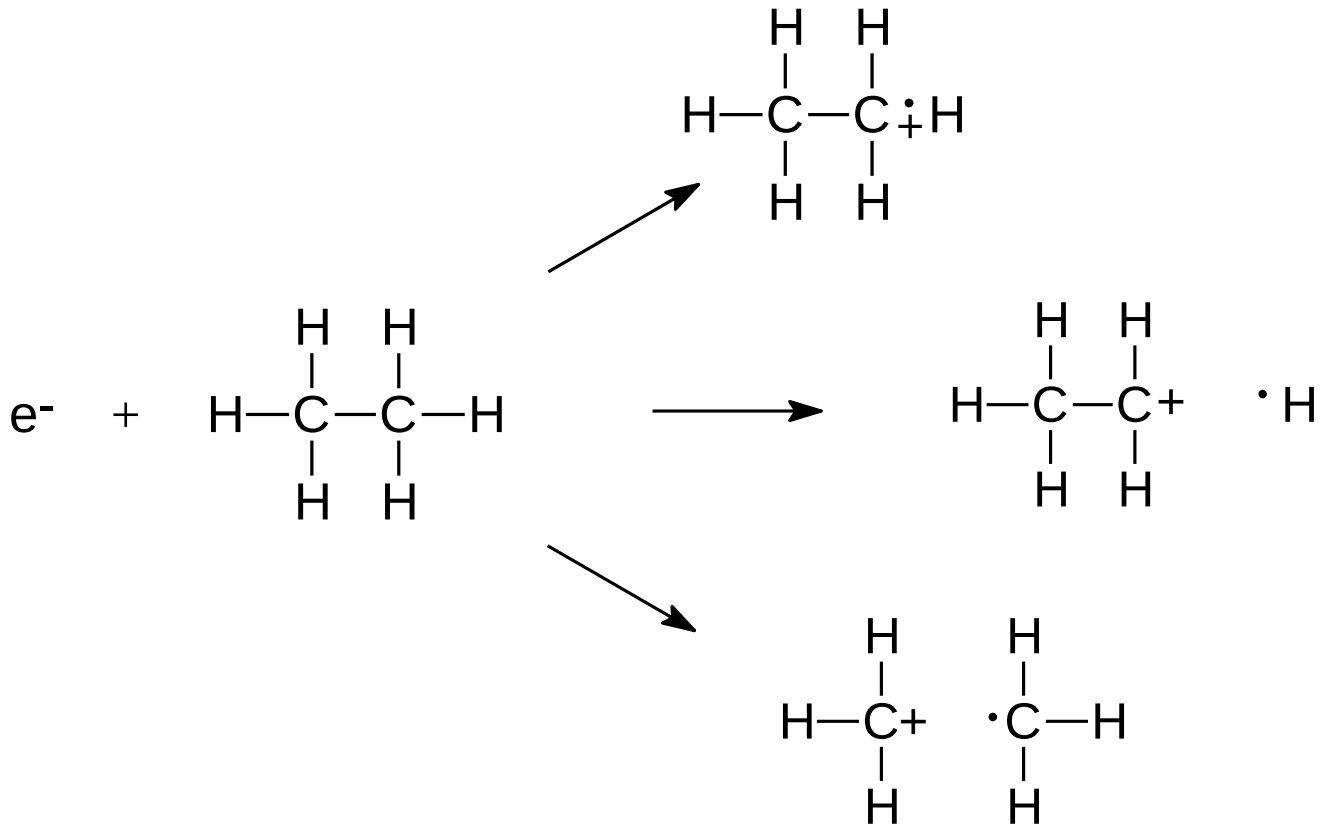
The gas molecules are bombarded by a high-energy electron beam (70eV). An electron which strikes a molecule may impart enough energy to remove another electron from that molecule.

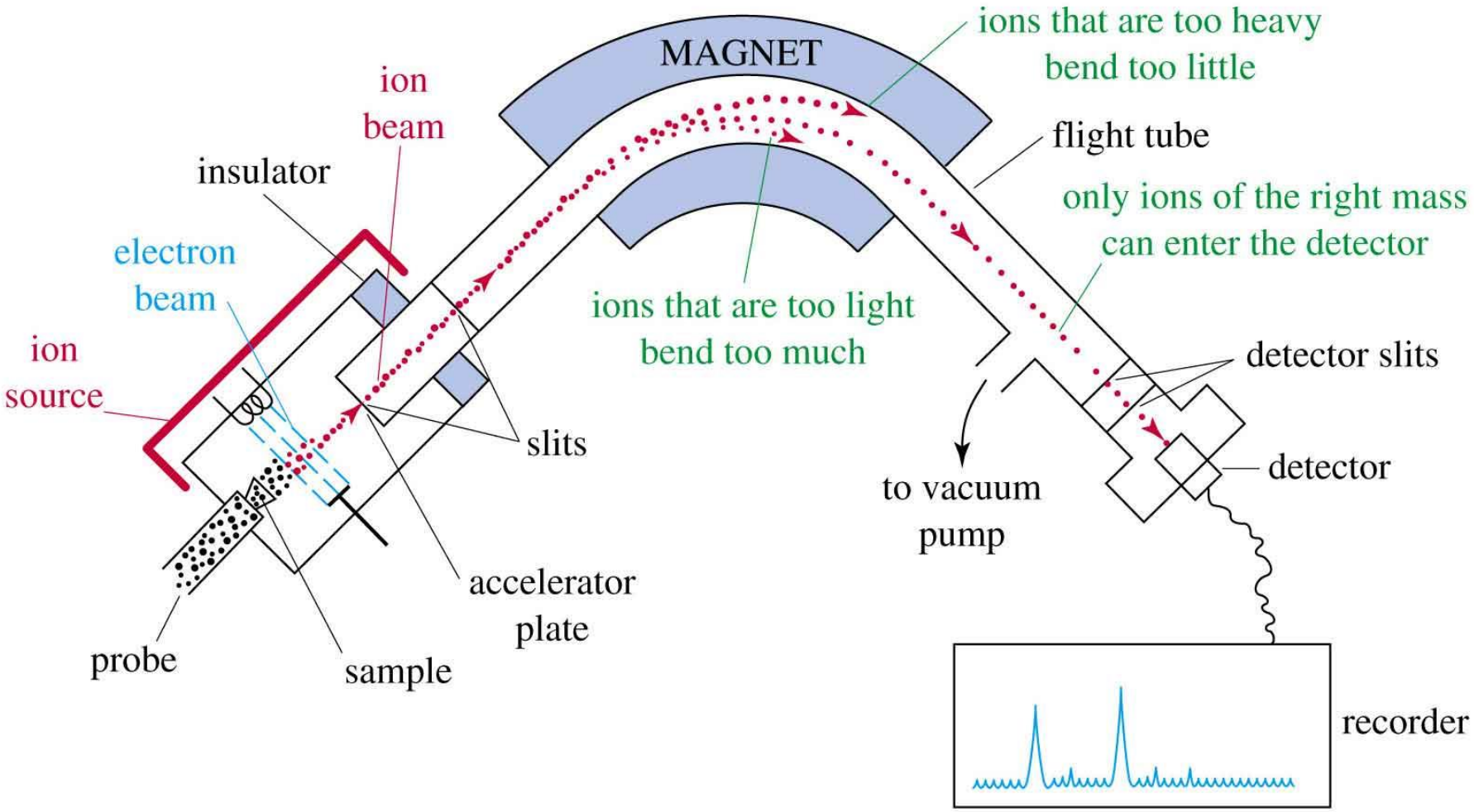
Methanol, for example, would undergo the following reaction in the ionizing region:

$$CH_3OH + 1\ \text{electron} \longrightarrow CH_3OH^{+\bullet} + 2\ \text{electrons}$$

(note: the symbols $^{+\bullet}$ indicate that a radical cation was formed)

A high-energy electron can dislodge an electron from a bond, creating a radical cation (a positive ion with an unpaired e^-).

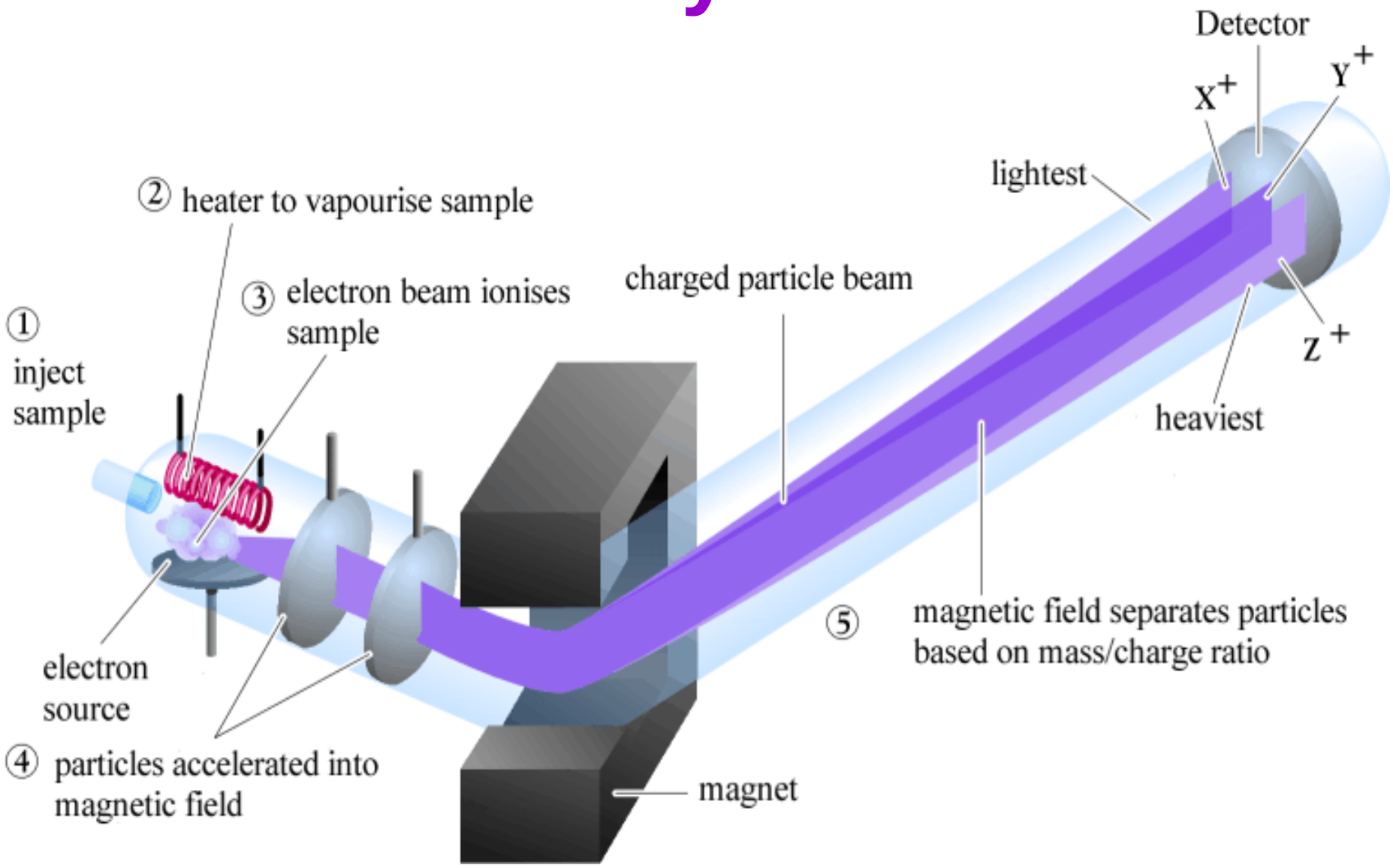



MAGNET
ions that are too heavy
bend too little
ion
beam
flight tube
insulator
only ions of the right mass
can enter the detector
electron
beam
ions that are too light
bend too much
ion
source
detector slits
slits
to vacuum
pump
detector
accelerator
plate
probe
sample
recorder

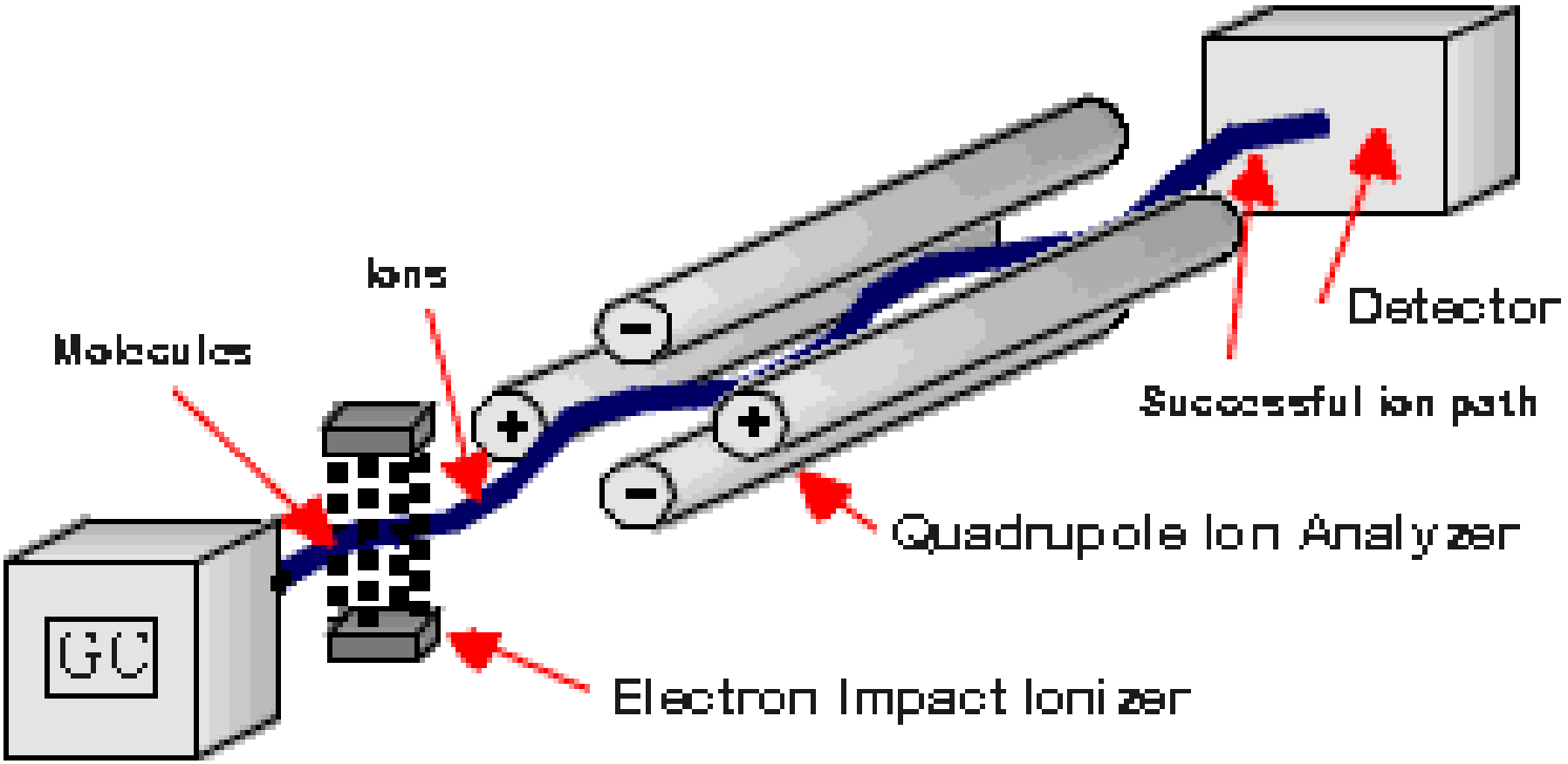
Separation of Ions

- Only the cations are deflected by the magnetic field.
- Amount of deflection depends on ***m/z***.
- The detector signal is proportional to the number of ions hitting it.
- By varying the magnetic field, ions of all masses are collected and counted. =>

Mass Analyzer



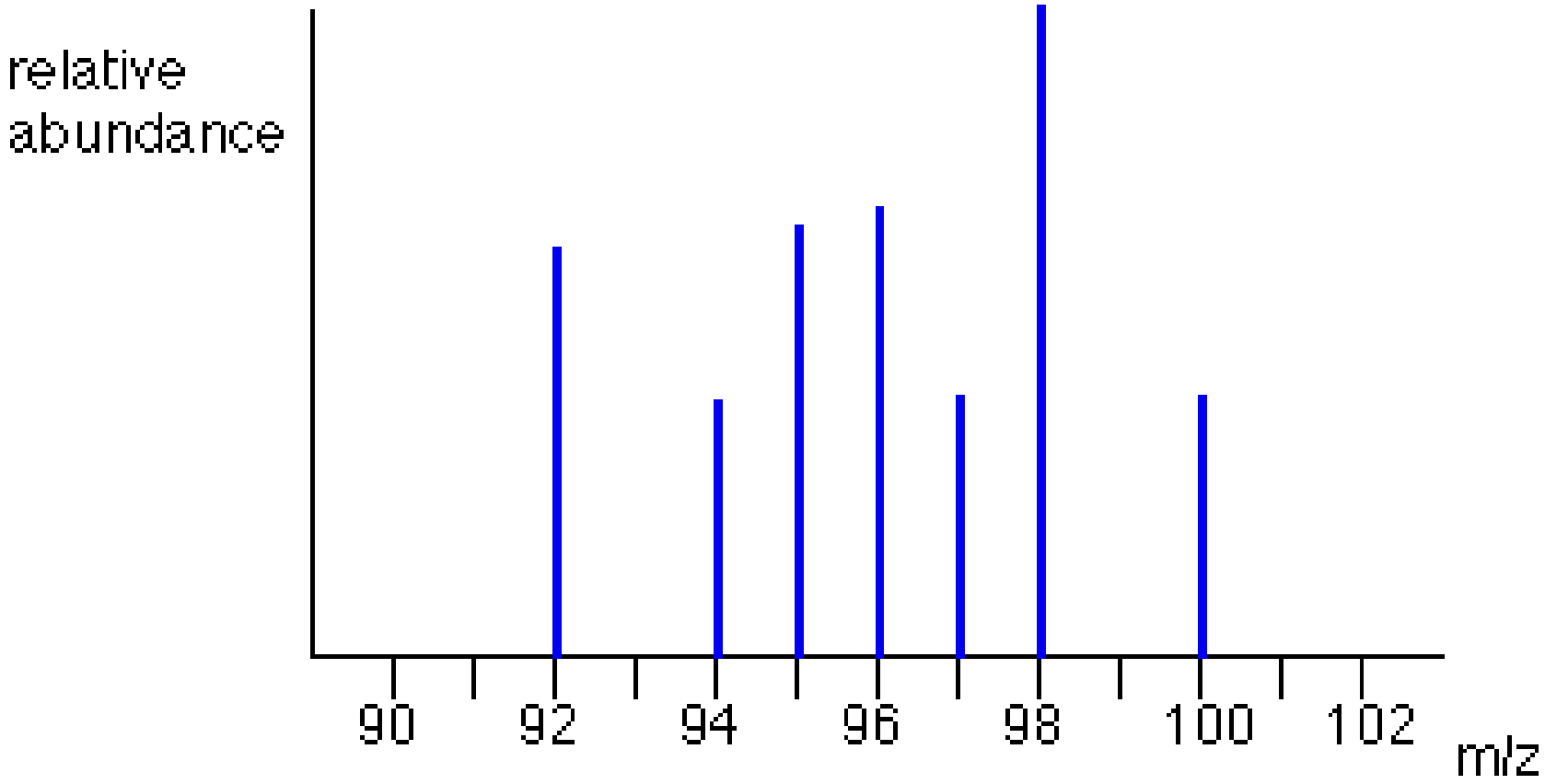
Mass Analyzer

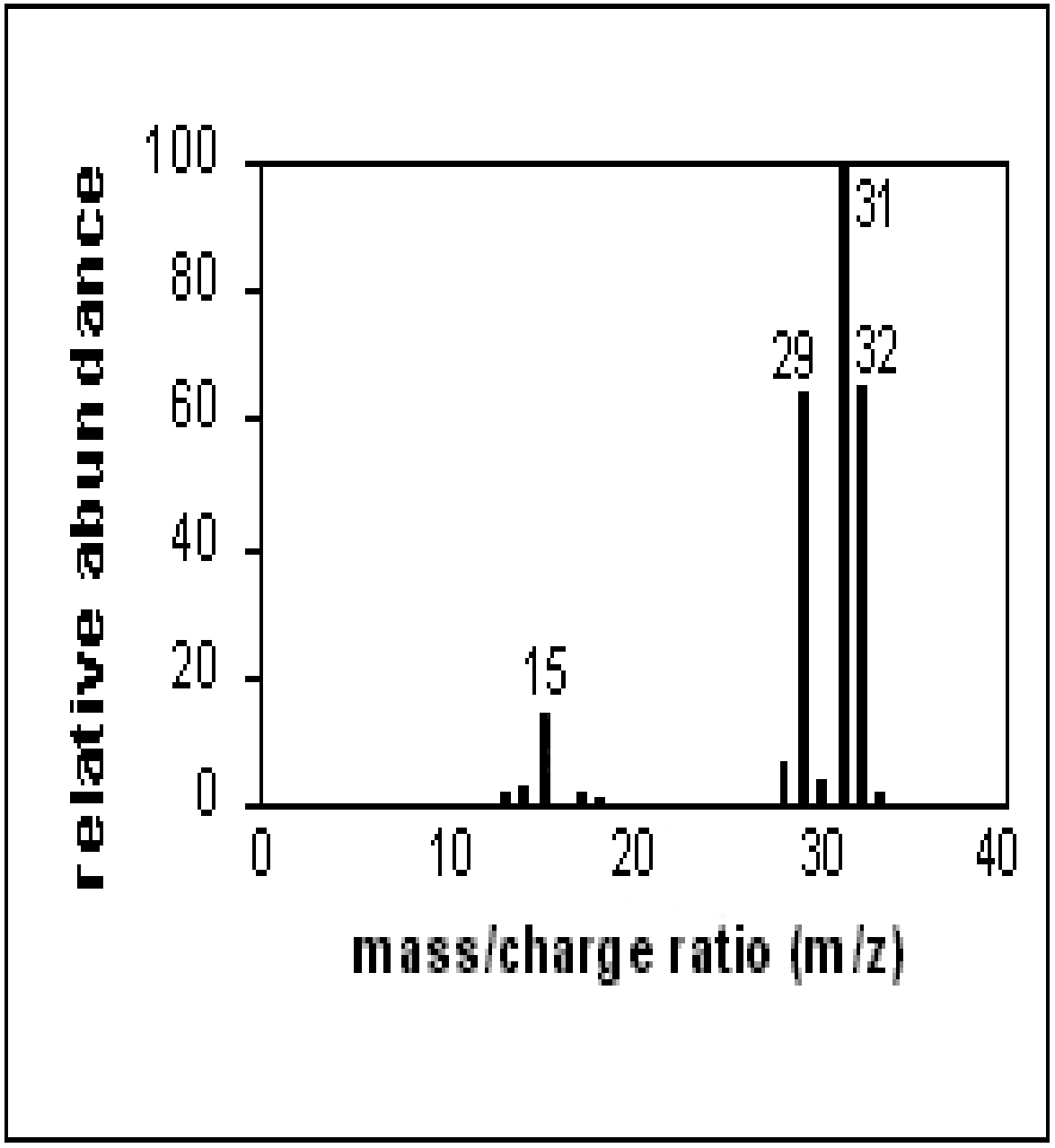


What the mass spectrometer output looks like

The output from the chart recorder is usually simplified into a "stick diagram". This shows the relative current produced by ions of varying mass/charge ratio.

The stick diagram for molybdenum looks lilke this:





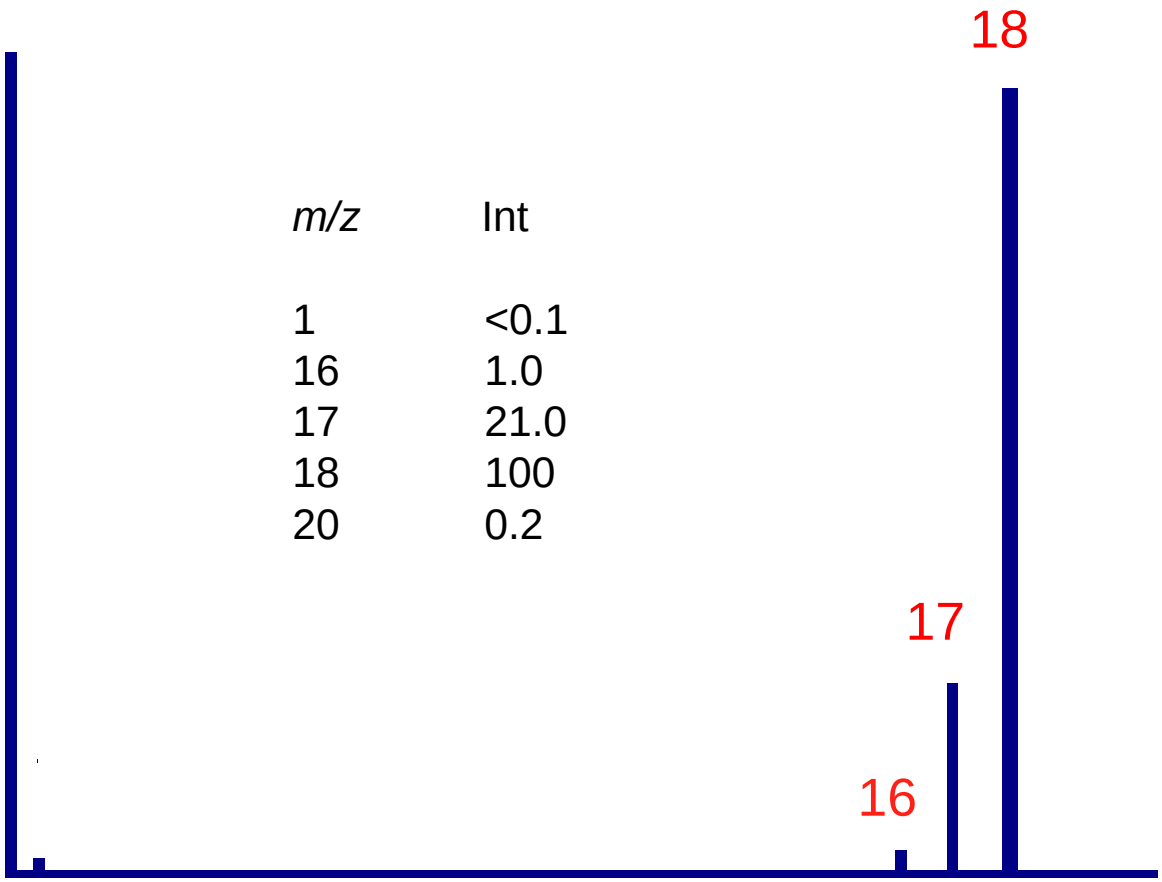
ions	m/z
$CH_3OH^{+\bullet}$	32
$H_2C{=}OH^{+}$	31
$HC{\equiv}O^{+}$	29
H_3C^{+}	15

Mass Spectrum of Water

Unknown 1

m/z	Int.
1	<0.1
16	1.0
17	21.
18	100.
20	0.2

relative abundance

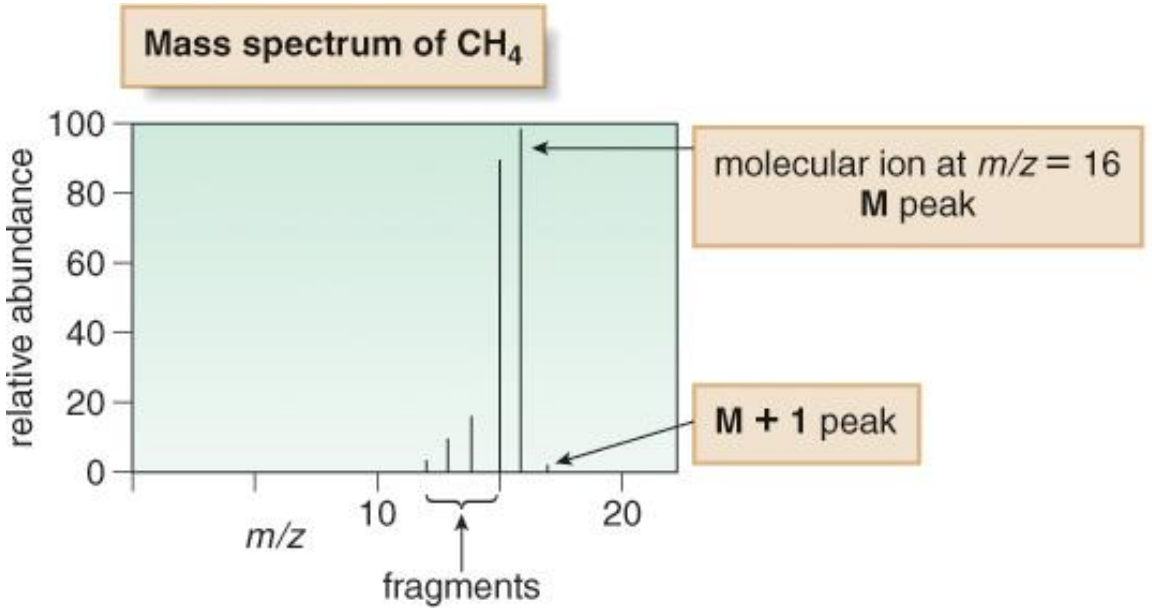


m/z	Int
1	<0.1
16	1.0
17	21.0
18	100
20	0.2

Mass
(mass-to-charge ratio)

Mass Spectrometry

Consider the mass spectrum of CH_4 below:



m/z	relative abundance	
17	1.2	
16	100.0	← base peak
15	85.9	
14	16.1	
13	8.1	
12	2.8	

- The tallest peak in the mass spectrum is called the base peak.
- The base peak is also the M peak, although this may not always be the case.
- Though most C atoms have an atomic mass of 12, 1.1% have a mass of 13. Thus, $^{13}CH_4$ is responsible for the peak at *m/z* = 17. This is called the M + 1 peak.

CH_3OH

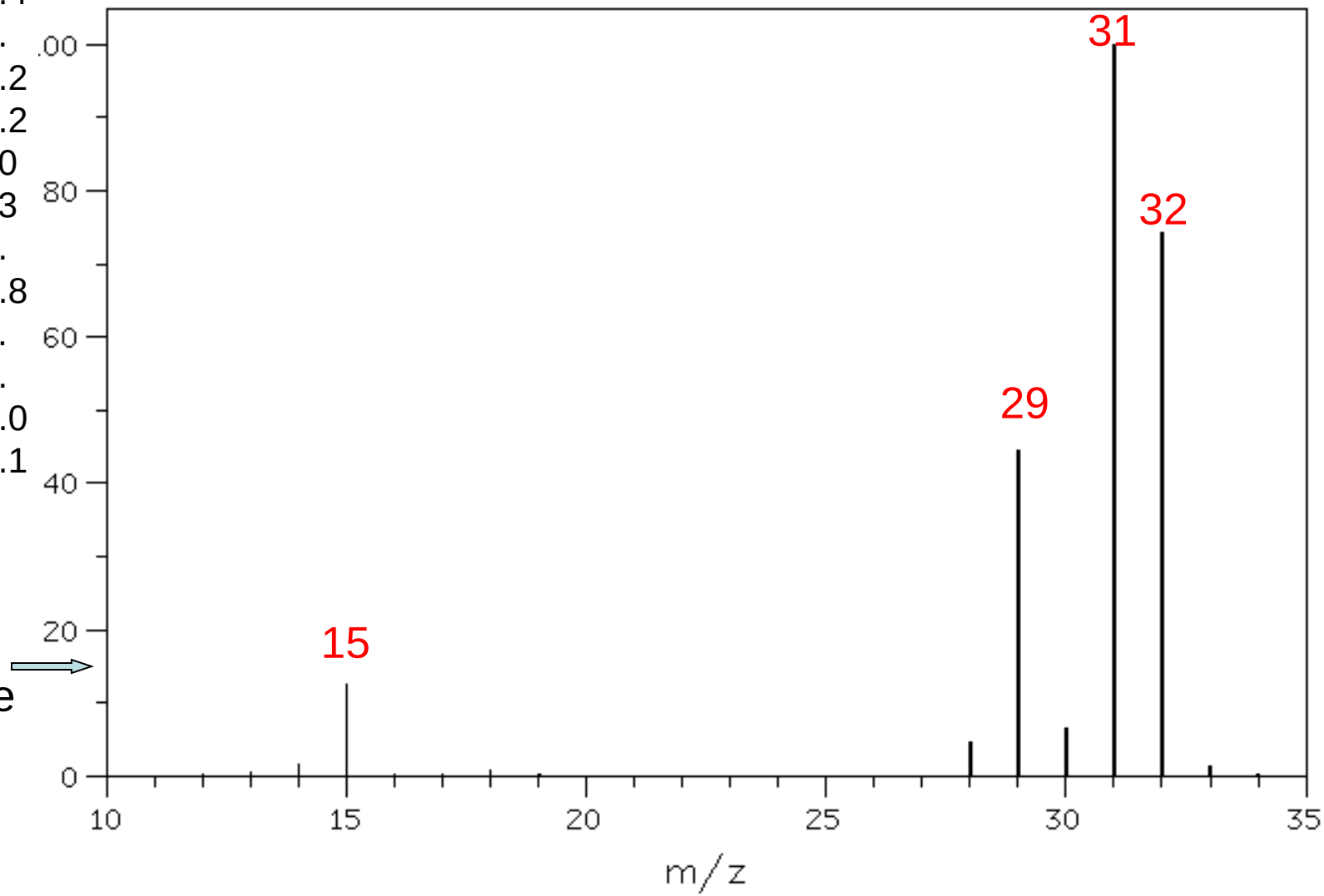
m/z	Int
12	0.3
13	1.7
14	2.4
15	13.
15.5	0.2
16	0.2
17	1.0
28	6.3
29	64.
30	3.8
31	100.
32	66.
33	1.0
34	0.1

$CH_3OH + e^- \rightarrow CH_3OH^{+\bullet}$ (*m/z* 32) + $2e^-$

$CH_3OH^{+\bullet} \rightarrow CH_2OH^+$ (*m/z* 31) + H•

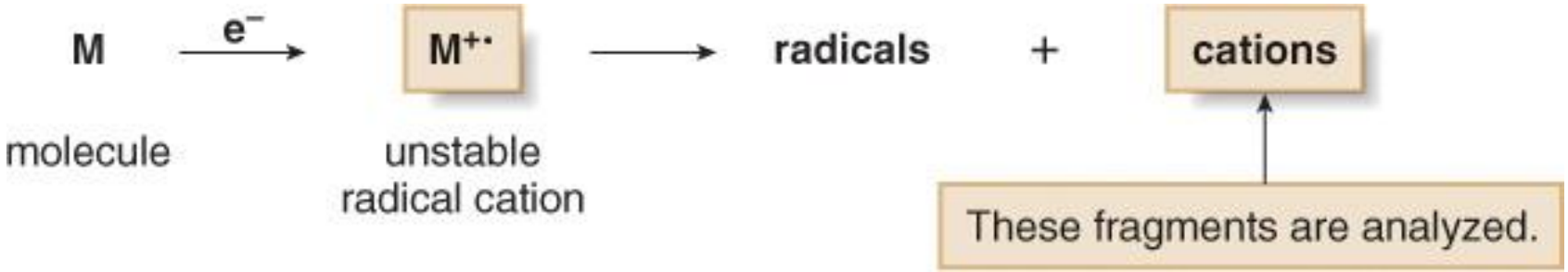
$\rightarrow CH_3^+$ (*m/z* 15) + HO•

$CH_2OH^+ \rightarrow CHO^+$ (*m/z* 29) + H_2



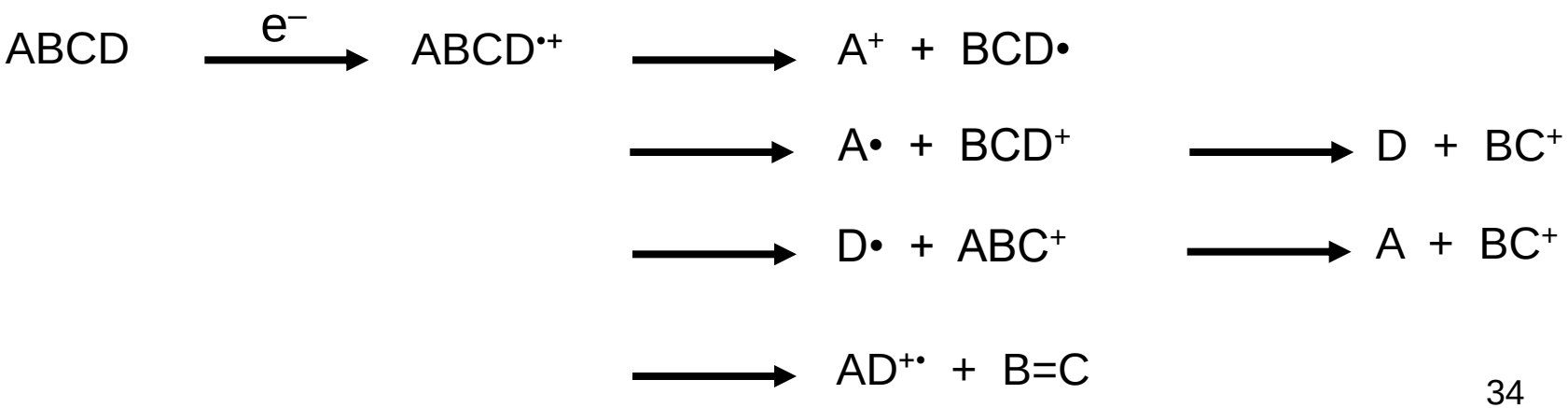
Mass Spectrometry

- 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 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.
- A mass spectrum is a plot of the amount of each cation (its relative abundance) versus its mass to charge ratio (*m/z*, where m is mass, and z is charge).
- Since z is almost always +1, m/z actually measures the mass (m) of the individual ions.



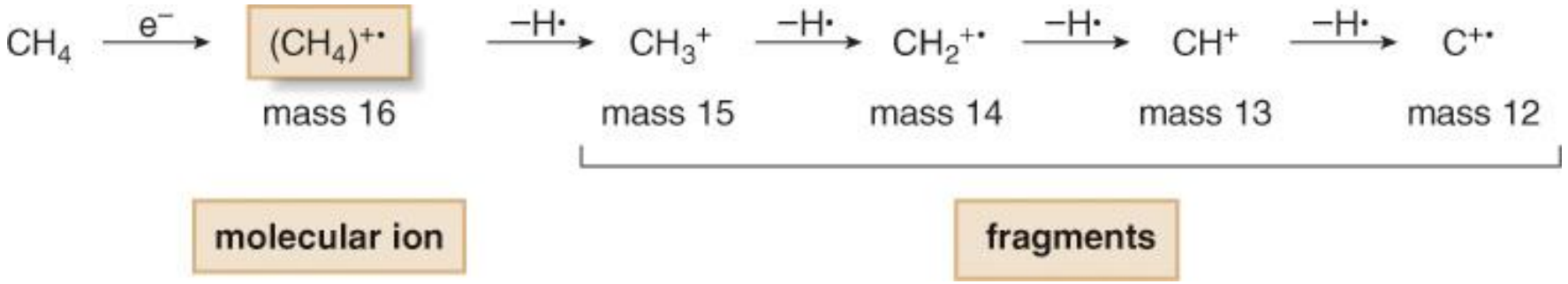
Basic Mechanisms of Fragmentation

- Mass spectral reactions are unimolecular; the sample pressure in the EI source is kept sufficiently low so that bimolecular (ion-molecule) or other collisions are usually negligible. If sufficiently excited, the $M^{+\bullet}$ ions can decompose by a variety of energy dependent mechanisms each of which results in the formation of an ion and a neutral species (radical). This primary product may have sufficient energy to decompose further.
- In the MS of ABCD, the abundance of BCD^+ will depend on the average rates of its formation and decomposition, whereas $[BC^+]$ will depend upon the relative rates of several competitive reactions. There are several types of unimolecular reactions that can take place:

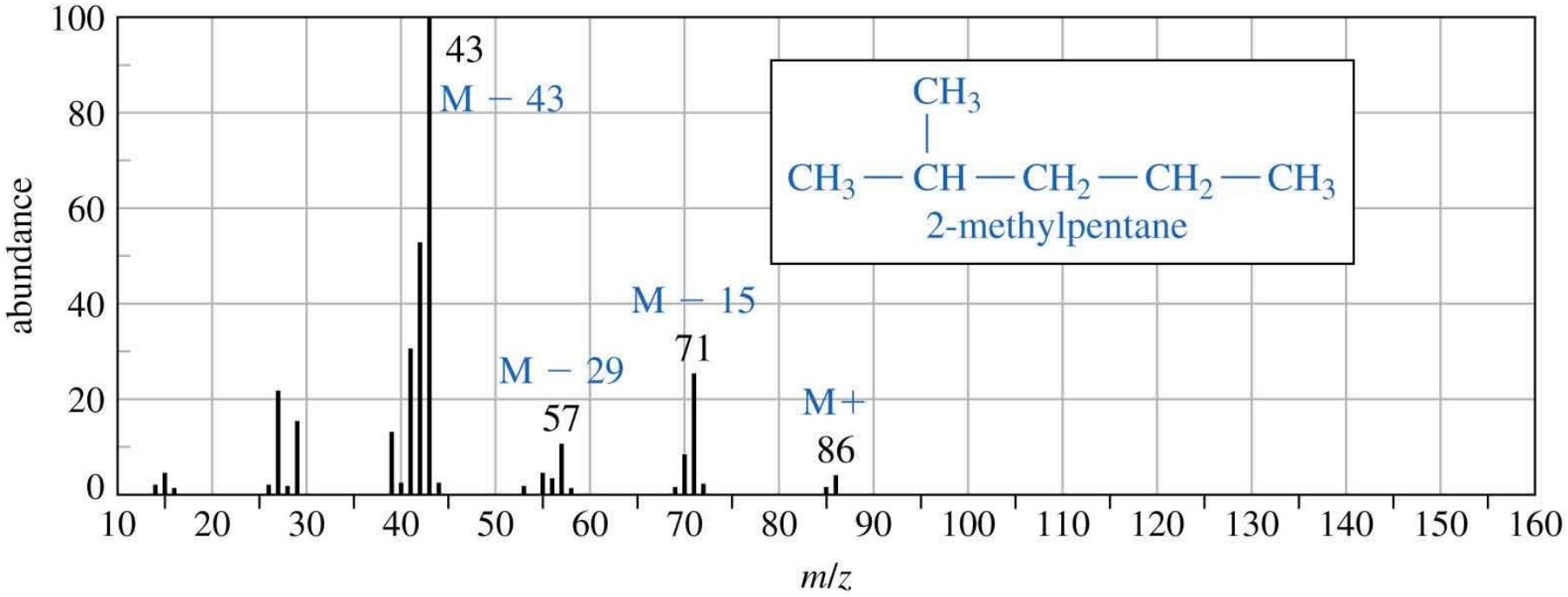


Mass Spectrometry

- The mass spectrum of CH_4 consists of more peaks than just the M peak.
- Since the molecular ion is unstable, it fragments into other cations and radical cations containing one, two, three, or four fewer hydrogen atoms than methane itself.
- Thus, the peaks at m/z 15, 14, 13 and 12 are due to these lower molecular weight fragments.

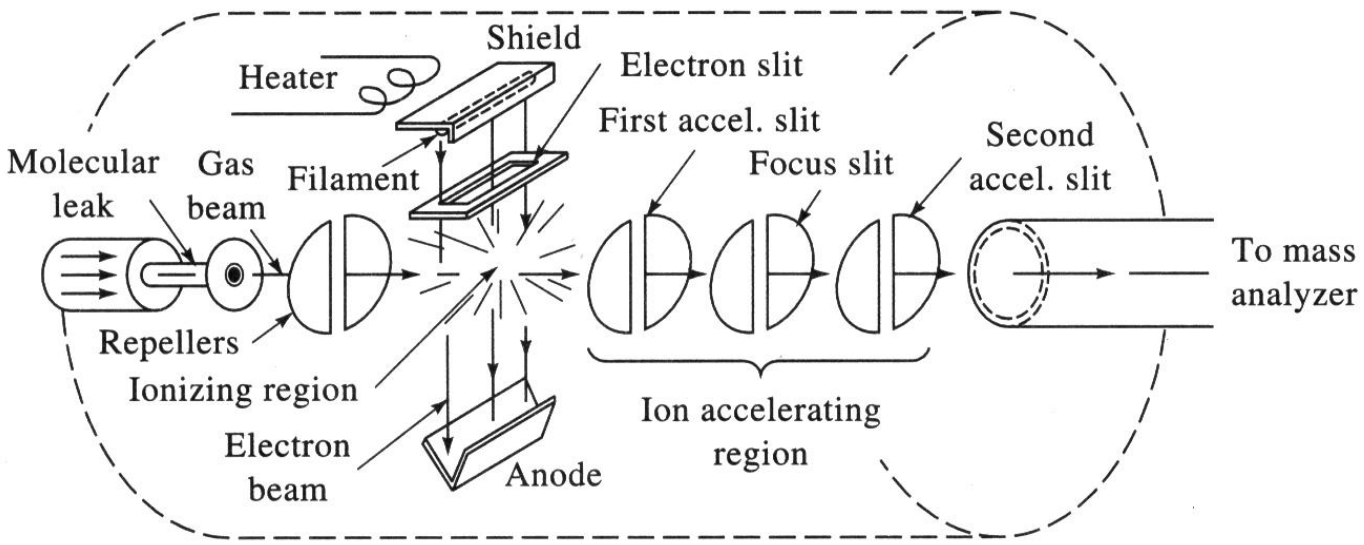


Mass Spectra of Alkanes



A. Electron Impact (EI) Ionization

- A hard, gas phase ion source.



- The analyte molecules pass through a stream of electrons which bombard the molecule and dislodge an electron.

$$M + e^- \rightarrow M^{\bullet +} + 2e^-$$

- The positive ions are attracted to the accelerator plate by a potential (≈ 70 V) applied between the accelerator plate and the repeller.

- Results in a highly excited $M^{\bullet +}$, which undergoes fragmentation and rearrangement.

B. Chemical Ionization (CI)

- Same setup as EI except the ionization chamber is pressurized with a reagent gas.
- The reagent gas is present in a 10^3 to 10^4 excess over the analyte.
- The reagent gas, usually methane, is preferentially ionized.

$$CH_4 + e^- \rightarrow CH_4^{\bullet+} + 2e^-$$

- The primary ions $CH_4^{\bullet+}$ and CH_3^+ form, which go on to give secondary ions.

$$CH_4^{\bullet+} + CH_4 \rightarrow CH_5^+ + CH_3^{\bullet}$$
$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$$
$$CH_4 + C_2H_5^+ \rightarrow C_3H_5^+ + 2H_2$$

- Collisions between the reagent ions and the analyte cause ***proton transfer*** and ***hydride transfer*** to occur.

$CH_5^+ + MH \rightarrow MH_2^+ + CH_4$
$C_2H_5^+ + MH \rightarrow MH_2^+ + C_2H_4$
} **Proton Transfer**

$C_2H_5^+ + MH \rightarrow M^+ + C_2H_6$ **Hydride Transfer**

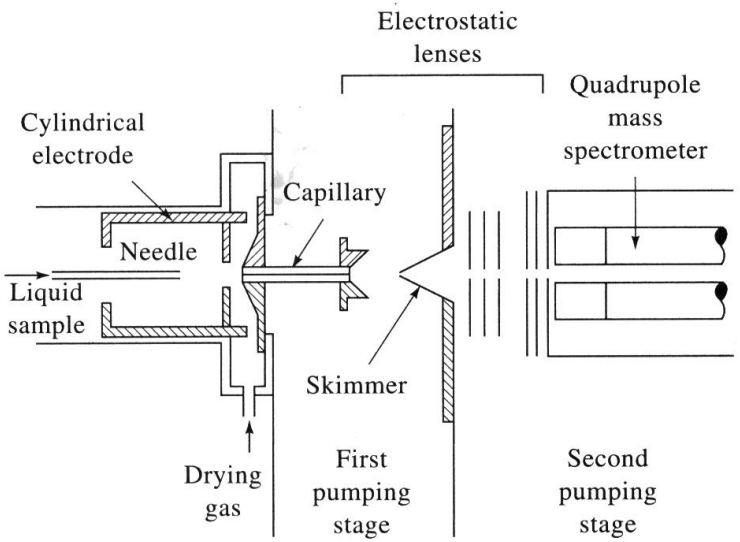
- Spectra contain M+1 & M-1 molecular ion peaks.

C. Desorption Techniques

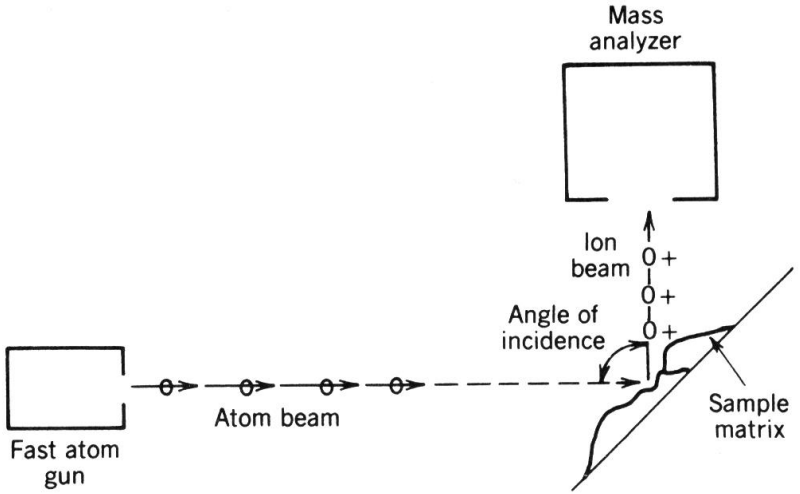
- Soft ionization techniques that usually result in spectra that consist of only M or M+1 peaks.
- Commonly used for biological samples (i.e. proteins & DNA) or thermally unstable molecules and can measure molecular weights that exceed 10,000 amu.

1. Electrospray Ionization (ESI)

- The most common ionization technique used to analyze biomolecules.
- Can be used to analyze biological macromolecules > 100,000 amu.
- The sample is pumped through a needle surrounded by several kV of potential.
- The charged spray of ultra-fine droplets of sample then passes into a capillary.
- While in the capillary the solvent evaporates and the charge is attached to the analyte.



2. Fast Atom Bombardment (FAB)



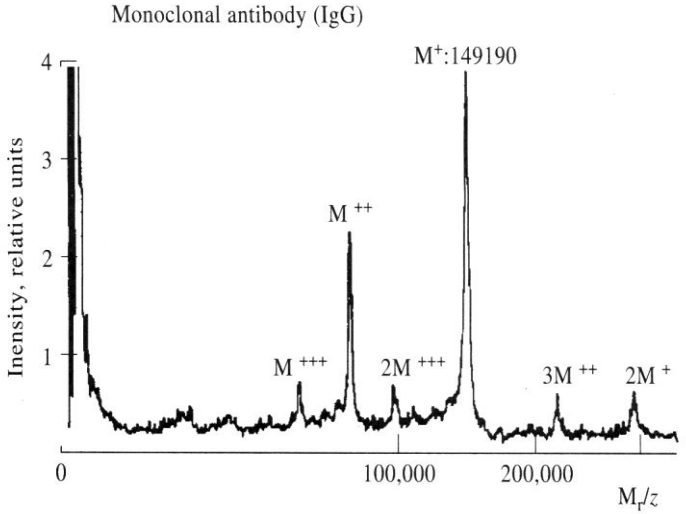
- The sample is prepared in a glycerol matrix and bombarded with high velocity argon or xenon atoms.
- Analyte anions and cations sputter off the sample, but only cations enter the mass analyzer due to a negatively charged accelerator/repeller plate at the analyzer inlet.
- The matrix reduces fragmentation of the analyte by absorbing most of the vibrational energy imparted by the fast atom stream.
- Used primarily for high molecular weight polar compounds.

3. Matrix Assisted Laser Desorption/Ionization (MALDI)

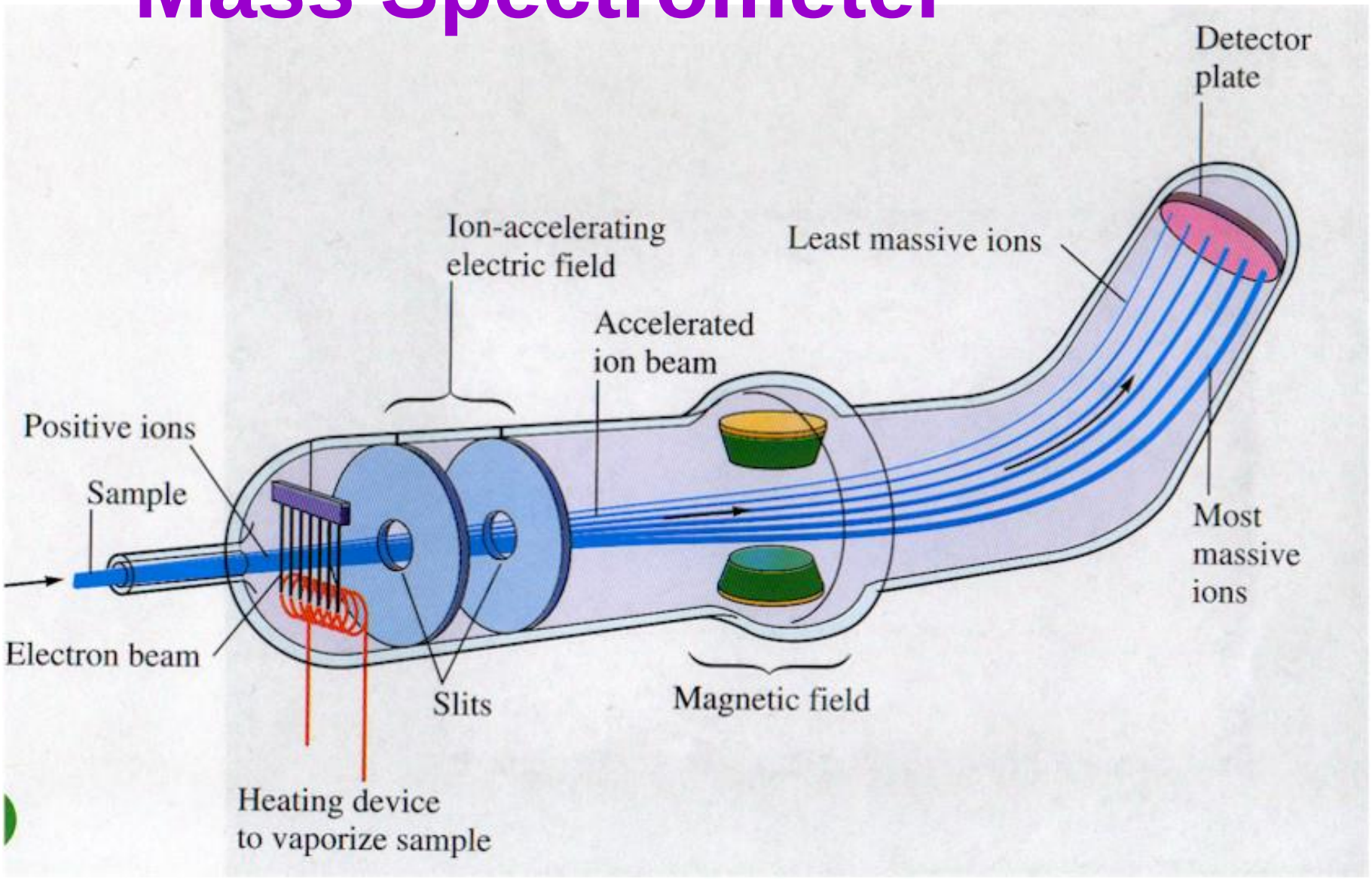
- The sample is prepared in an aqueous/alcohol solution and mixed with a large excess of a radiation-absorbing matrix material.
- The sample matrix is then dried (evaporated) on the surface of a metallic probe.
- The mixture is then irradiated with a pulsed laser beam of the same wavelength that the radiation-absorbing matrix absorbs. Analyte cations are released from the mixture and enter a time-of-flight mass analyzer.
- The entire mass spectrum is obtained between laser pulses.
- MALDI has found widespread application for large (mw > 100,000) biological macromolecules since its inception in 1988.

TABLE 20-4 Matrices Most Frequently Used for MALDI Together with the Usable Wavelengths*

Matrix	Wavelength (nm)
Nicotinic acid	266, 220–290
Benzoic acid derivatives:	
2,5-Dihydroxybenzoic acid	266, 337, 355
Vanillic acid	266
2-Amino-benzoic acid	266, 337, 355
Pyrazine-carboxylic acid	266
3-Aminopyrazine-2-carboxylic acid	337
Cinnamic acid derivatives:	
Ferulic acid	266, 377, 355
Sinapinic acid	266, 337, 355
Caffeic acid	266, 337, 355
3-Nitrobenzylalcohol	266

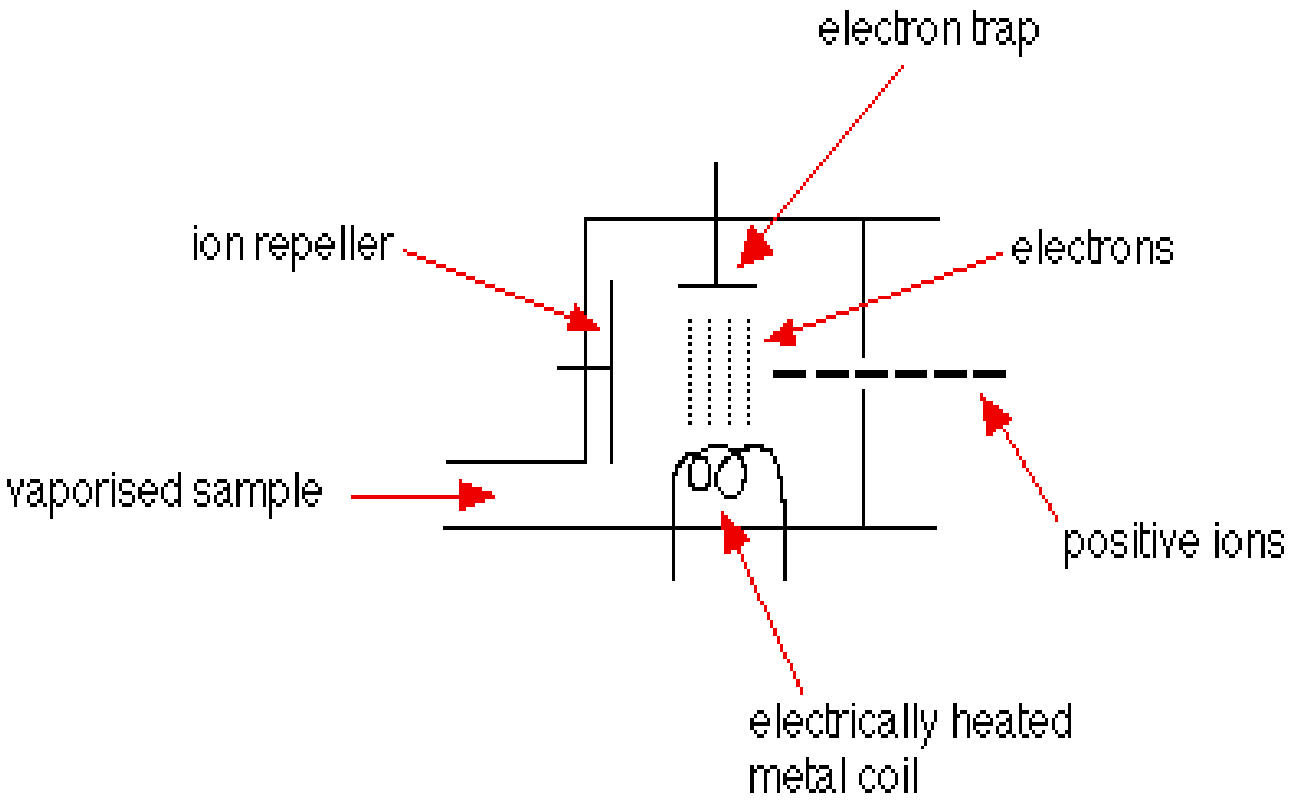


Mass Spectrometer



Stage 1: Ionisation

The atom is ionised by knocking one or more electrons off to give a positive ion. This is true even for things which you would normally expect to form negative ions (chlorine, for example) or never form ions at all (argon, for example). Mass spectrometers always work with positive ions.



IONIZATION

The vaporised sample passes into the ionisation chamber. The electrically heated metal coil gives off electrons which are attracted to the electron trap which is a positively charged plate.

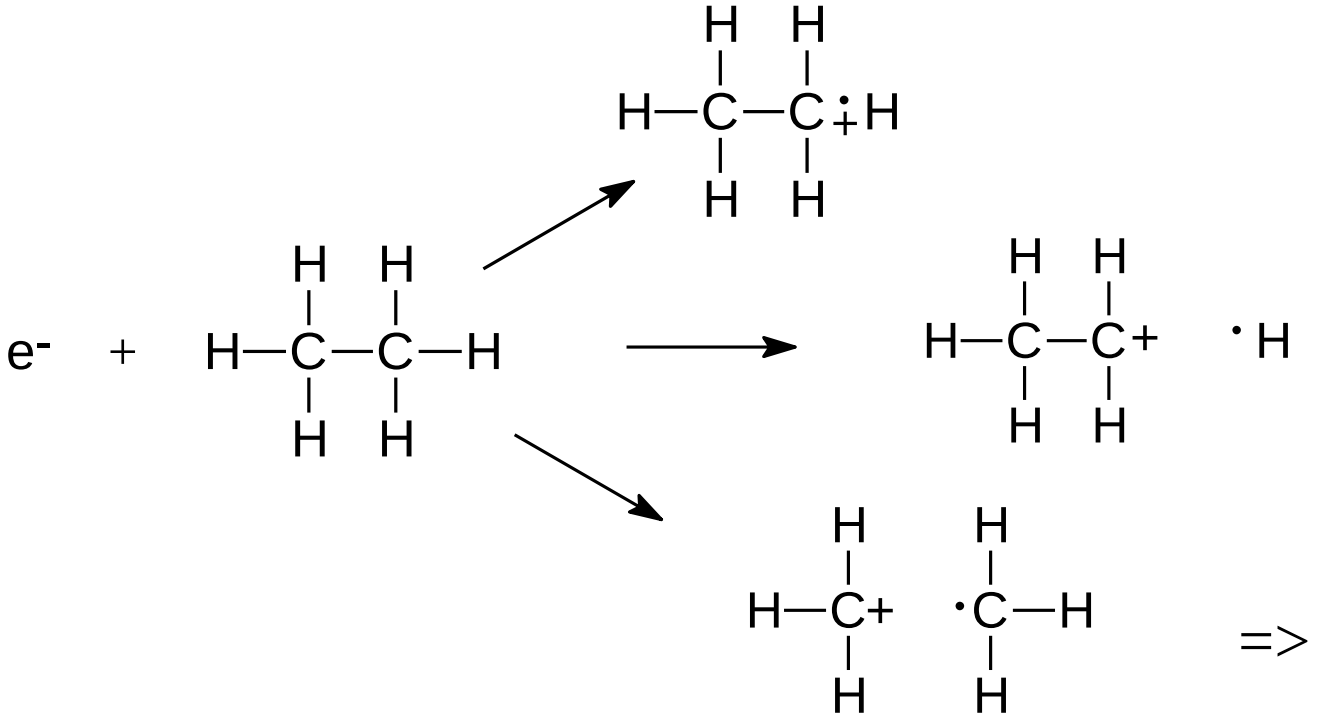
The particles in the sample (atoms or molecules) are therefore bombarded with a stream of electrons, and some of the collisions are energetic enough to knock one or more electrons out of the sample particles to make positive ions.

Most of the positive ions formed will carry a charge of +1 because it is much more difficult to remove further electrons from an already positive ion.

These positive ions are persuaded out into the rest of the machine by the ion repeller which is another metal plate carrying a slight positive charge.

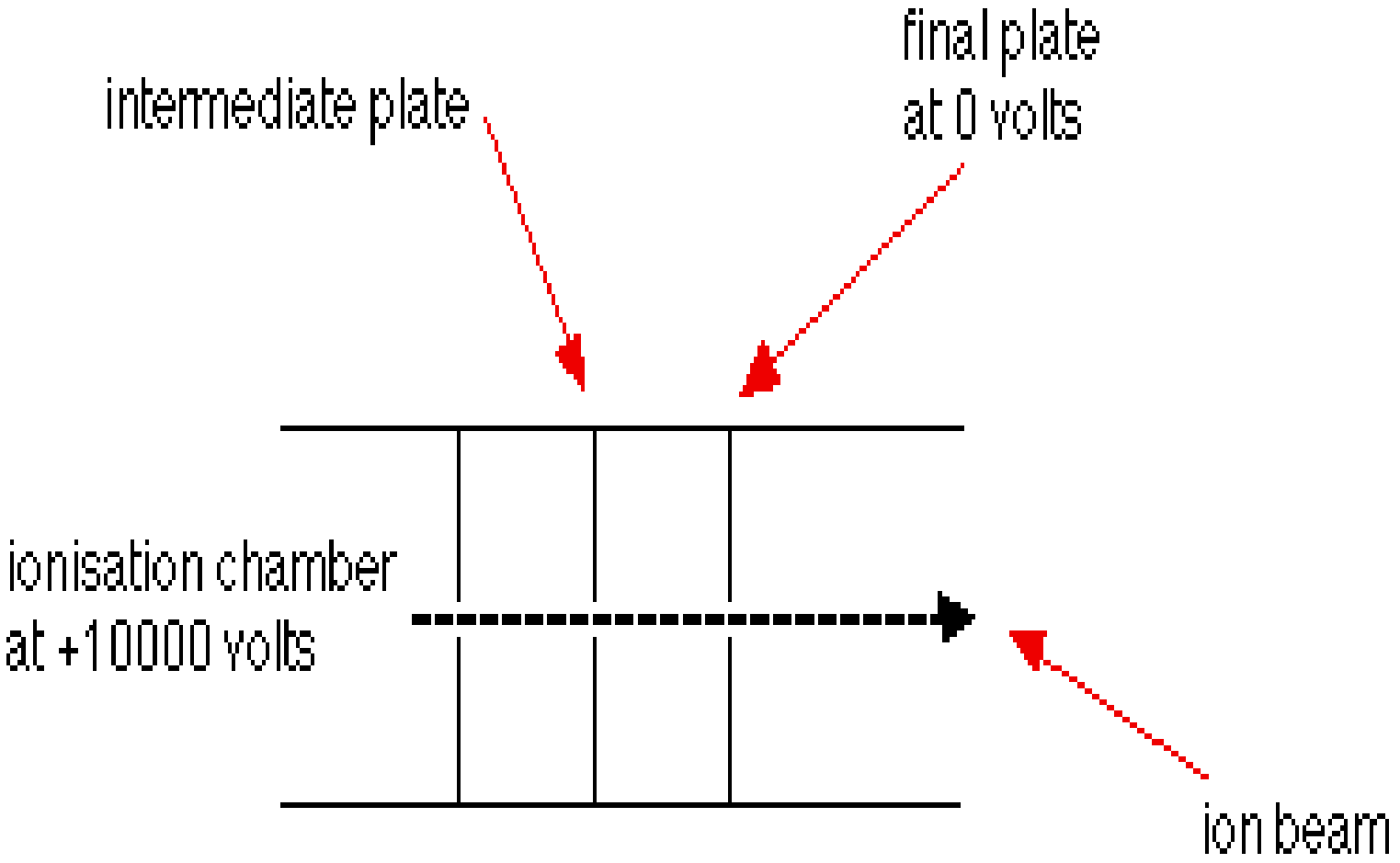
Electron Impact Ionization

A high-energy electron can dislodge an electron from a bond, creating a radical cation (a positive ion with an unpaired e^-).

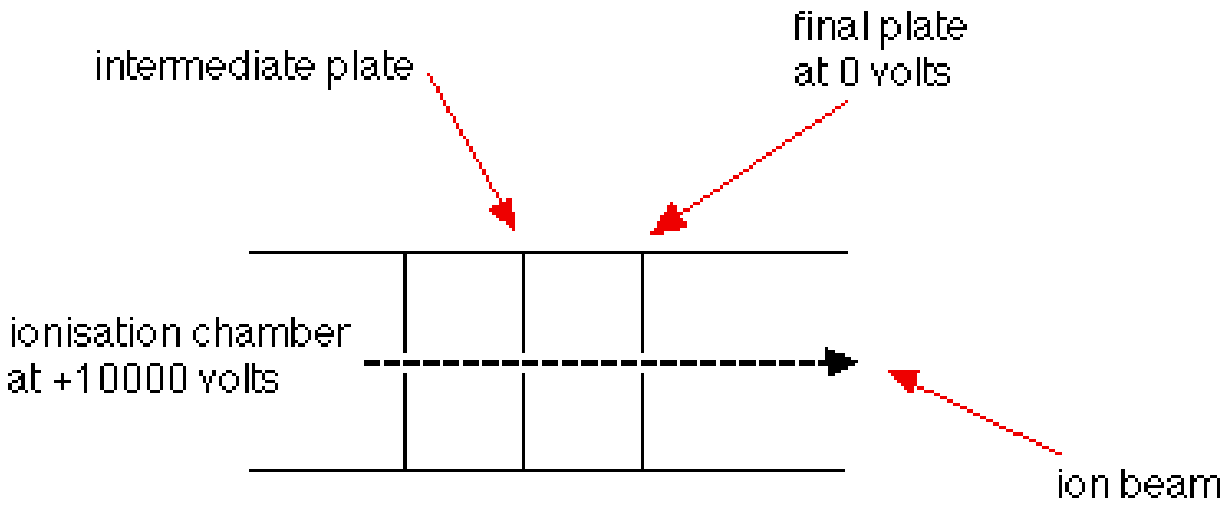


Stage 2: Acceleration

The ions are accelerated so that they all have the same kinetic energy.



Acceleration



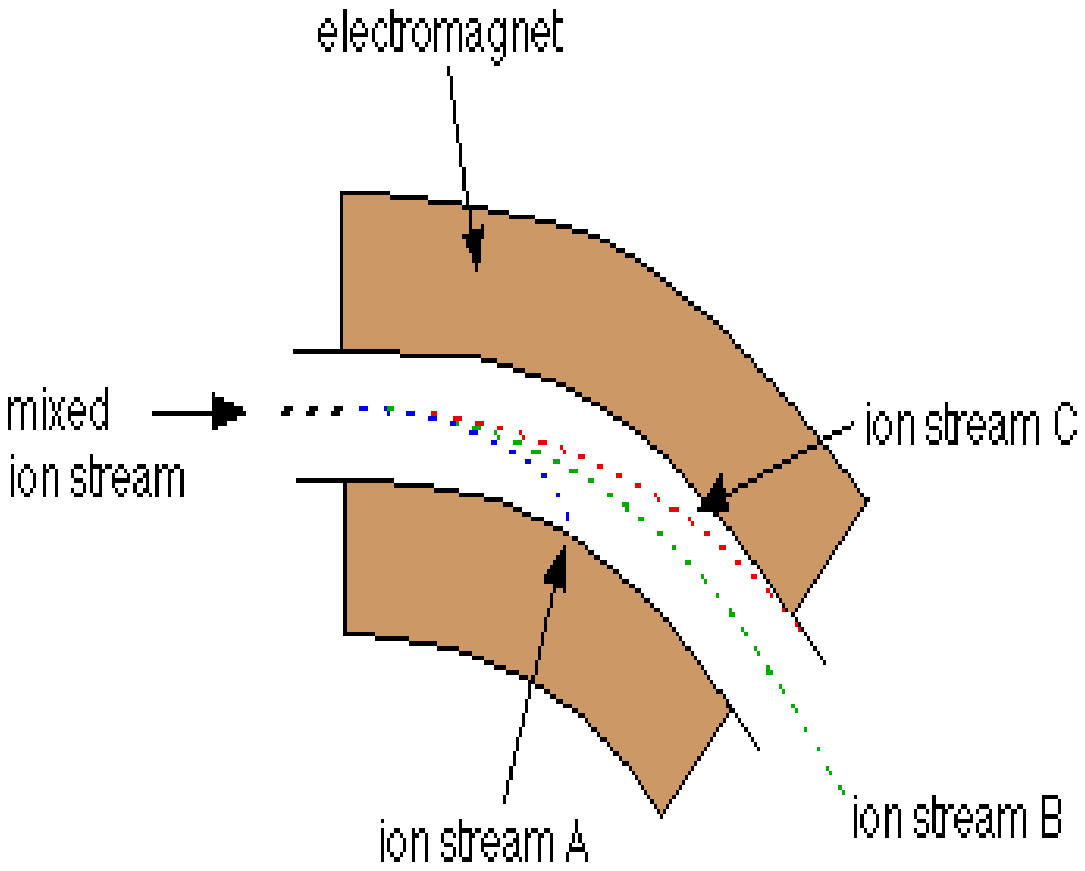
The positive ions are repelled away from the very positive ionisation chamber and pass through three slits, the final one of which is at 0 volts. The middle slit carries some intermediate voltage. All the ions are accelerated into a finely focused beam.

Stage 3: Deflection

The ions are then deflected by a magnetic field according to their masses.

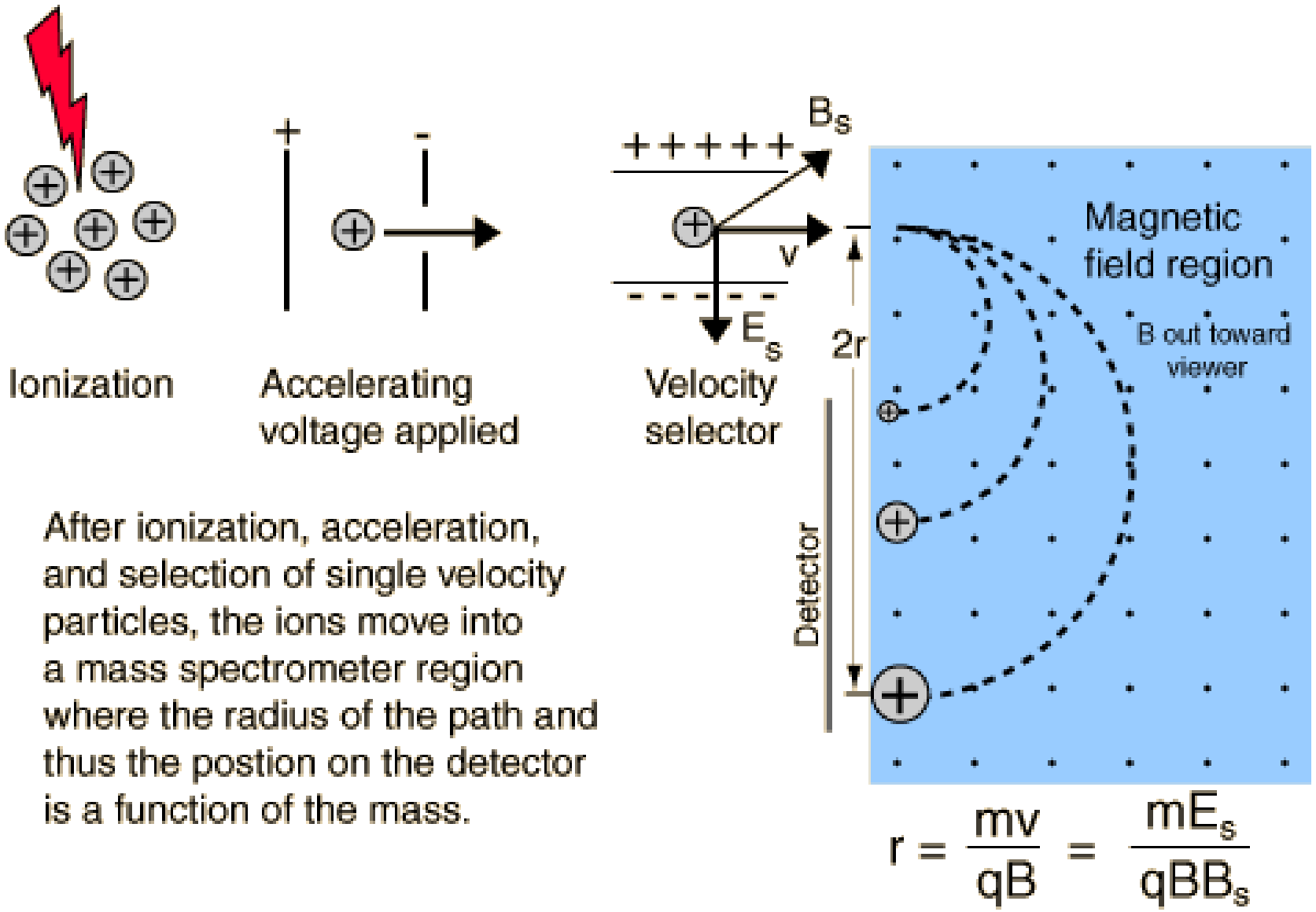
The lighter they are, the more they are deflected.

The amount of deflection also depends on the number of positive charges on the ion – in other words, on how many electrons were knocked off in the first stage. The more the ion is charged, the more it gets deflected.



Separation of Ions

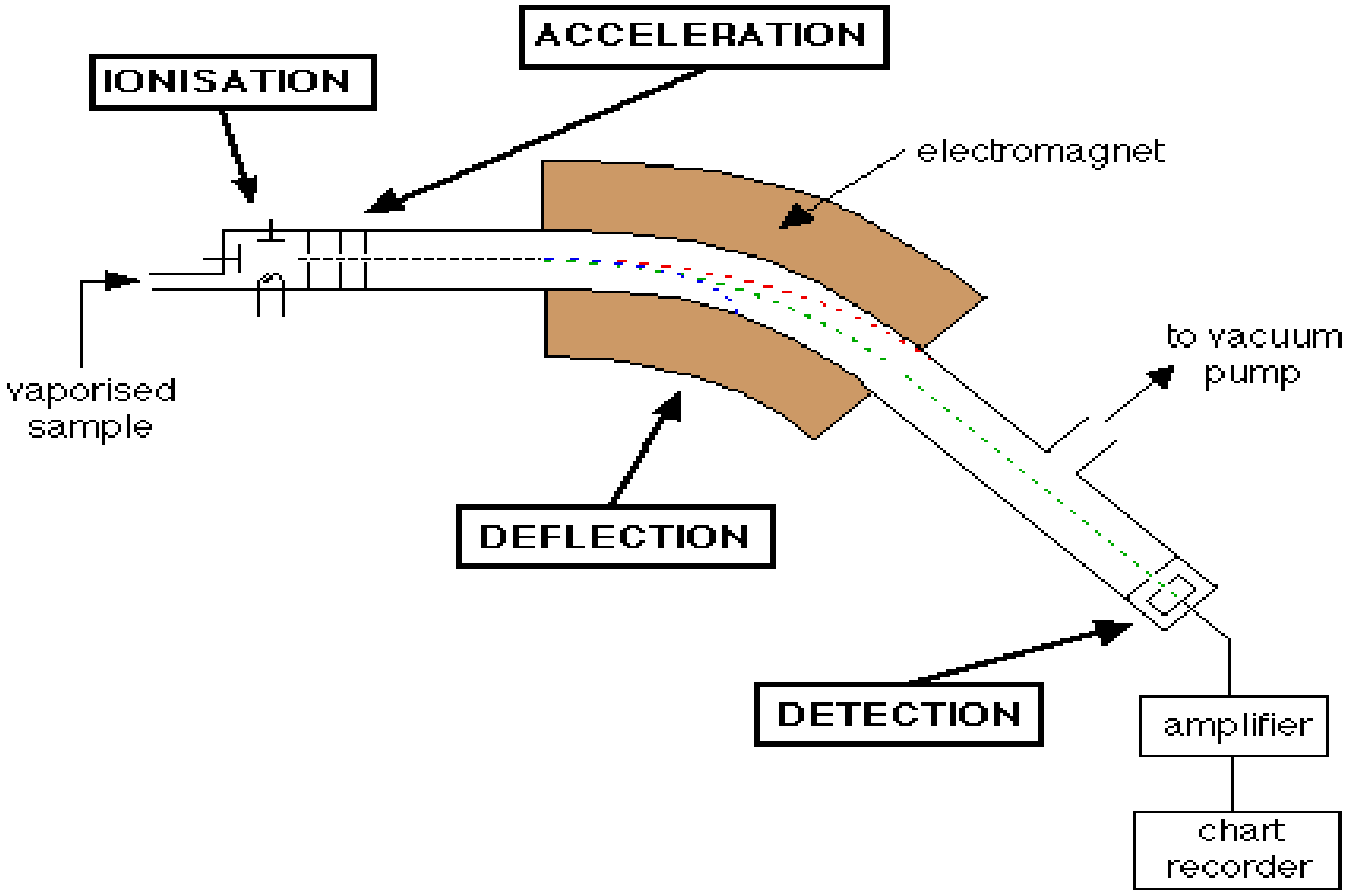
- Only the cations are deflected by the magnetic field.
- Amount of deflection depends on ***m/z***.
- The detector signal is proportional to the number of ions hitting it.
- By varying the magnetic field, ions of all masses are collected and counted. =>



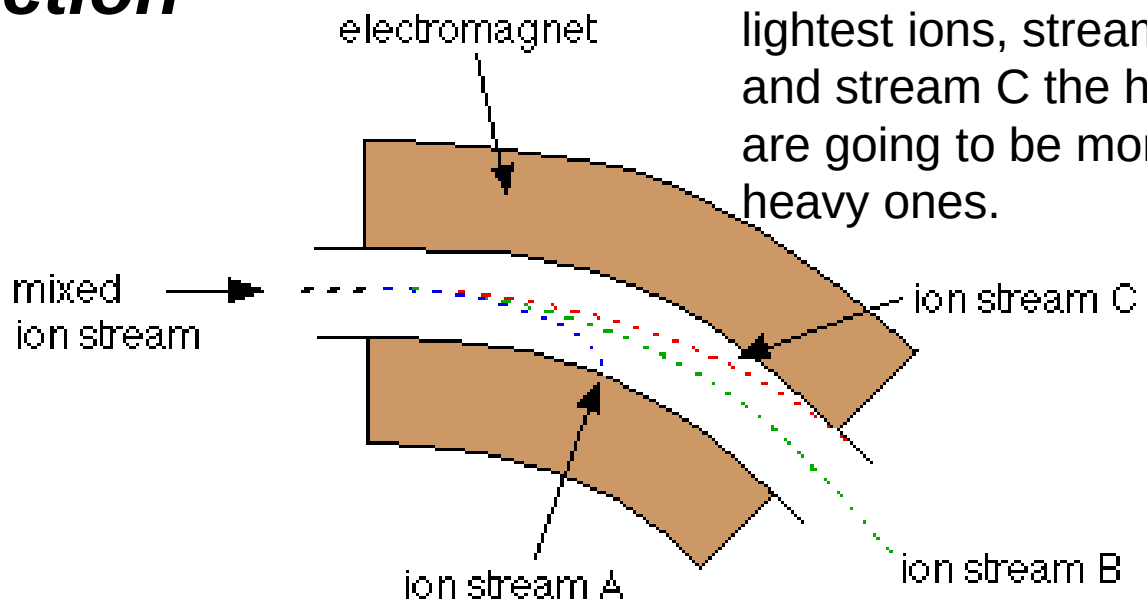
After ionization, acceleration, and selection of single velocity particles, the ions move into a mass spectrometer region where the radius of the path and thus the postion on the detector is a function of the mass.

$$r = \frac{mv}{qB} = \frac{mE_s}{qBB_s}$$

Mass Analyzer



Deflection

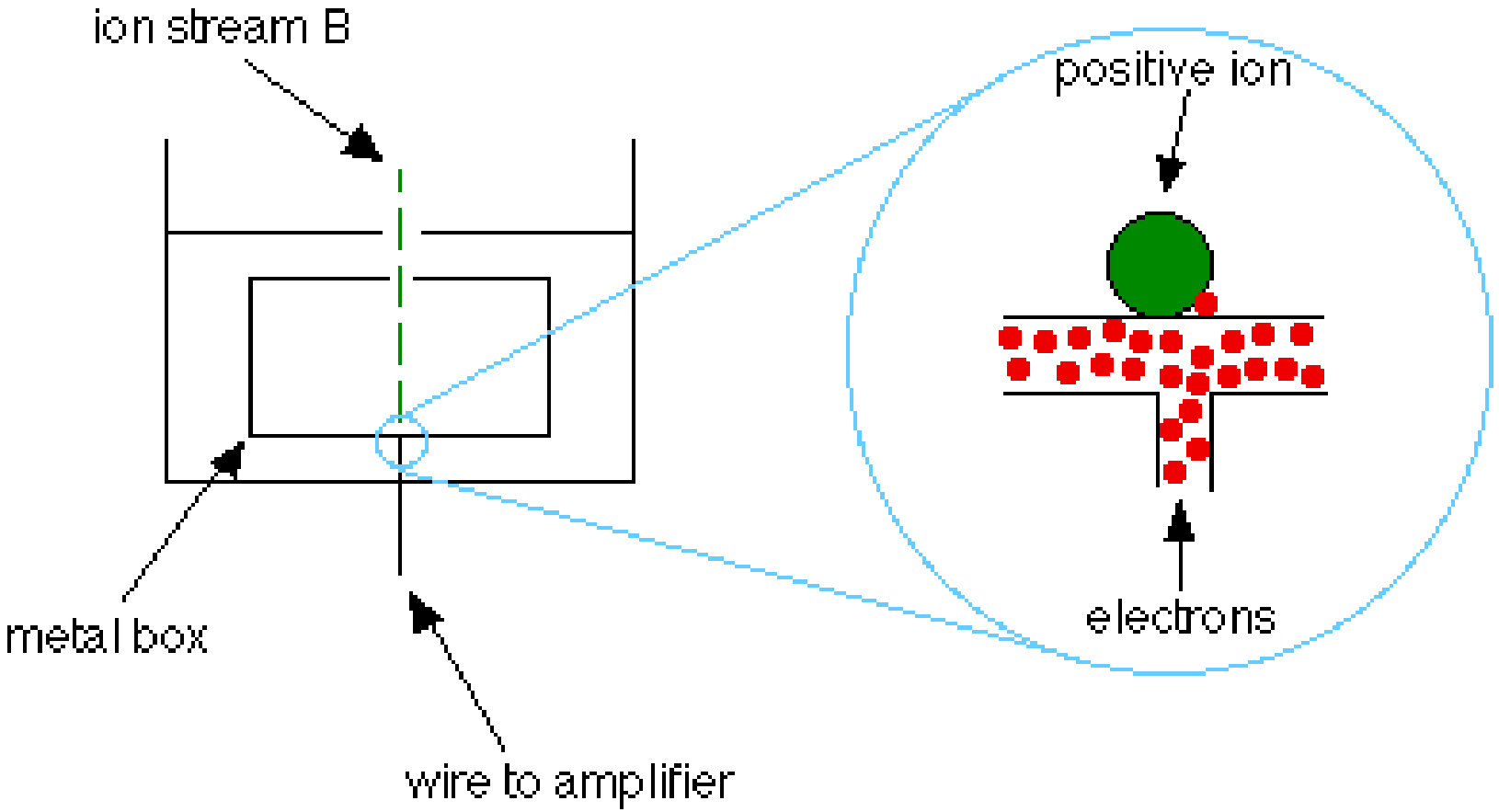


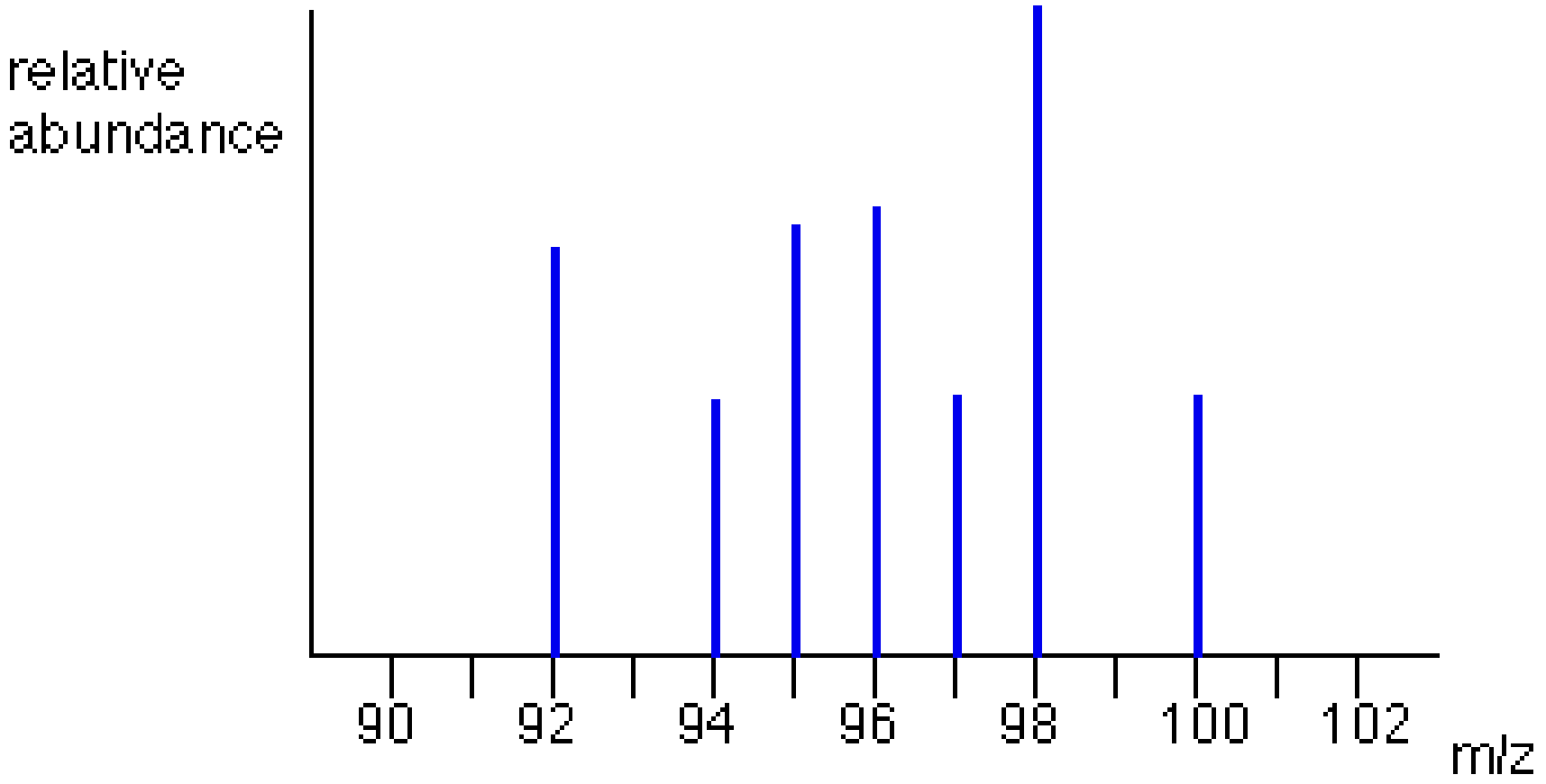
Assuming 1+ ions, stream A has the lightest ions, stream B the next lightest and stream C the heaviest. Lighter ions are going to be more deflected than heavy ones.

Different ions are deflected by the magnetic field by different amounts. The amount of deflection depends on:
the mass of the ion. Lighter ions are deflected more than heavier ones.
the charge on the ion. Ions with 2 (or more) positive charges are deflected more than ones with only 1 positive charge

Stage 4: Detection

The beam of ions passing through the machine is detected electrically.



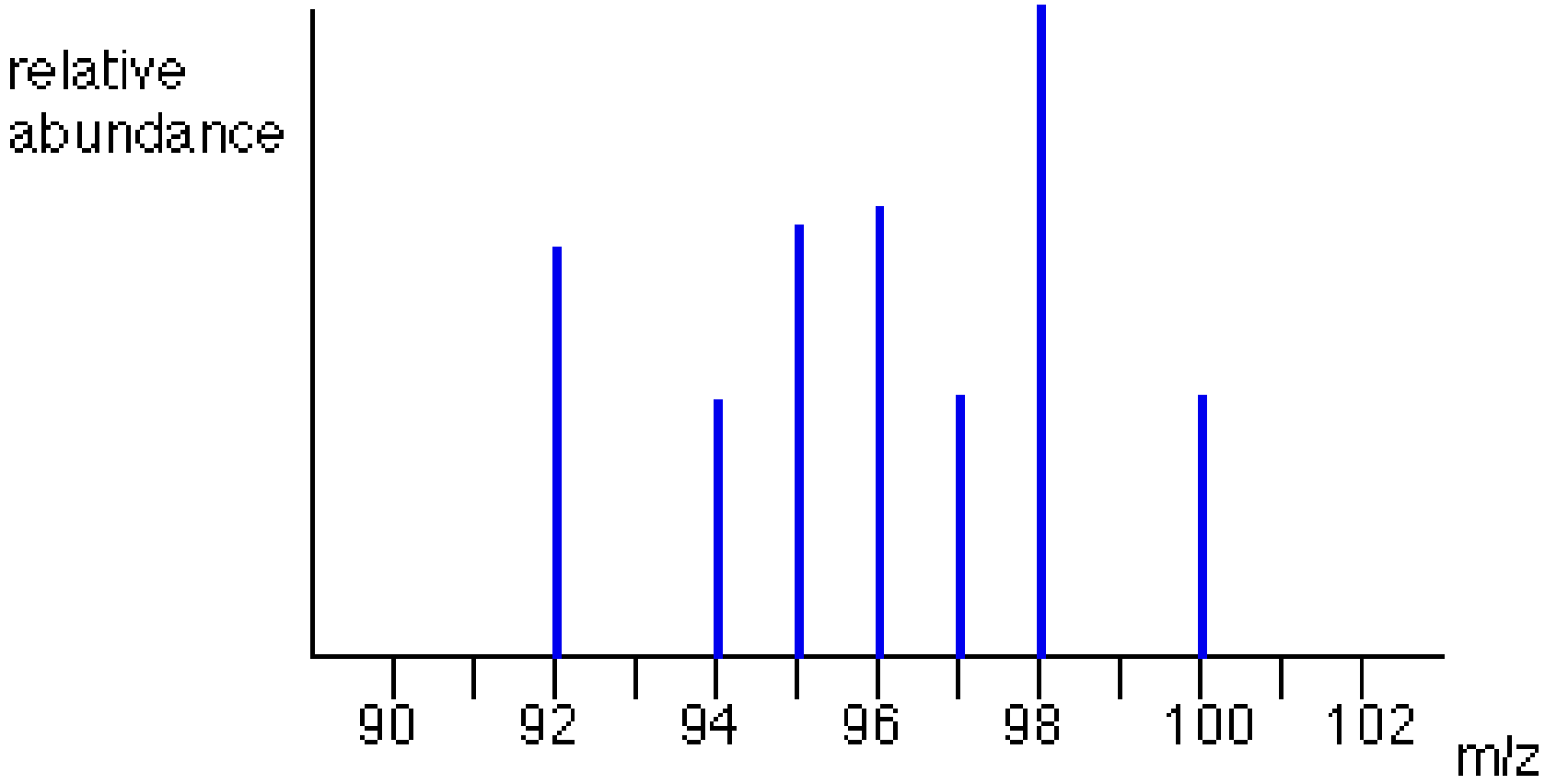


The plot of the mass-to-charge ratio (m/z) of these ions, as a function of abundance, is a mass spectrum.

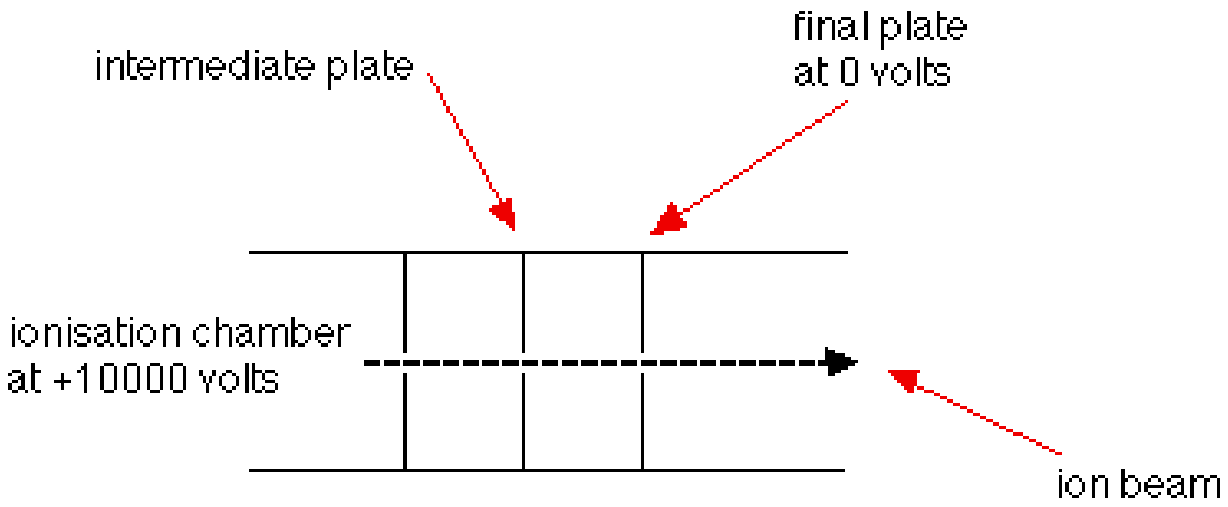
What the mass spectrometer output looks like

The output from the chart recorder is usually simplified into a "stick diagram". This shows the relative current produced by ions of varying mass/charge ratio.

The stick diagram for molybdenum looks lilke this:



Acceleration



The positive ions are repelled away from the very positive ionisation chamber and pass through three slits, the final one of which is at 0 volts. The middle slit carries some intermediate voltage. All the ions are accelerated into a finely focused beam.

Mass analyzer

Mass analyzers separate the ions according to their mass per charge (*m/z*). There are many types of mass analyzers. Usually they are categorized based on the principles of operation.

Sector MS: It uses an electric and/or magnetic field to affect the path and/or velocity of the charged particles in some way. The force exerted by electric and magnetic fields are defined by the Lorentz force law:

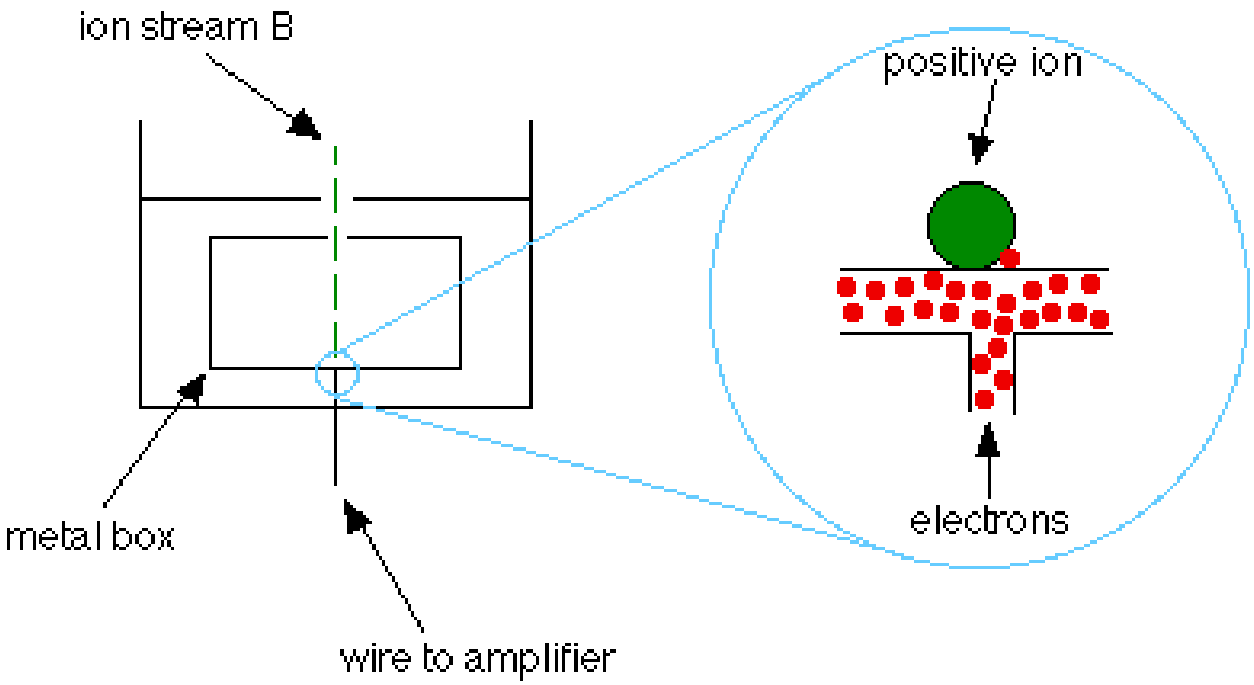
$$\mathbf{F} = q(\mathbf{E} + \mathbf{v} \times \mathbf{B})$$

where **E** is the electric field strength, **B** is the magnetic field induction, *q* is the charge of the particle, **v** is its current velocity (expressed as a vector), and × is the cross product. All mass analyzers use the Lorentz forces in some way either statically or dynamically in mass-to-charge determination.

As shown above, sector instruments change the direction of ions that are flying through the mass analyzer. The ions enter a magnetic or electric field which bends the ion paths depending on their mass-to-charge ratios (*m/z*), deflecting the more charged and faster-moving, lighter ions more. The ions eventually reach the detector and their relative abundances are measured. The analyzer can be used to select a narrow range of m/z's or to scan through a range of m/z's to catalog the ions present.

Besides the original magnetic-sector analyzers, several other types of analyzer are now more common, including time-of-flight, quadrupole ion trap, quadrupole and Fourier transform ion cyclotron resonance mass analyzers.

Detection



Only ion stream B makes it right through the machine to the ion detector. The other ions collide with the walls where they will pick up electrons and be neutralised. Eventually, they get removed from the mass spectrometer by the vacuum pump.