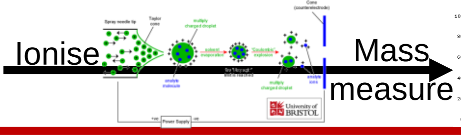
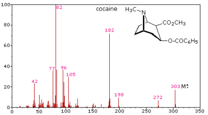
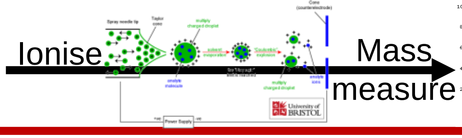
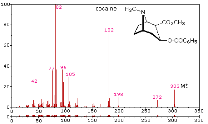




Mass Spectrometry

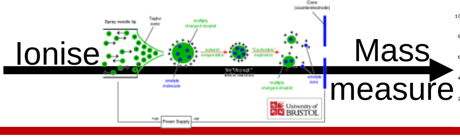
Flow	Introd^n	Ionise	Mass M'ment	Det / Record
Options	Options	Options	Options	Few Options
Gas	**Infusion**	EI	LRMS	Electron
Liquid	**GC-MS**	CI	HR(FT)MS	multiplier
Supercrit	**HPLC-MS**	Cold-EI	(AccMS)	Multichannel
fluid	SFE-MS etc.	FI	QMS	plate
	ICP-MS	FD	TOFMS	Faraday cup
	MALDI-MS	APCI	(LR/HR)	
	TGA-MS	APPI	Mag sector	
	Headspace	REMPI	IRMS	
	Ambient e.g.	…	Orbitrap	
	DESI-MS		Fast MS	
	DART-MS		Ion mobility	
	PTR-MS		MS/MS	
	SIFT-MS		…	
	…			

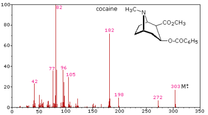




Background to Mass Spectrometry

- Briefly discuss the development of mass spectrometry… and some ionisation methods & mass analyser types
- Identify different uses and roles of mass spectrometry, focus on separation – MS; hyphenated instrumentation
- Interpret a typical analysis protocol for GC-MS ~ kerosene & an organic mix
- Identify several important MS techniques and interpretations – MS/MS; derivatisation; internal standards; library searching
- Discuss the role of MS in analytical chemistry ~ case studies
- Fast GC-MS





Orbitrap Exploris GC-MS

Mass Range m/z 30 to 3000
40 Hz scan @ 7,500 (m/z 200)
External calib < 3 ppm; Internal < 1 ppm
Resolution up to 30,000 at m/z 200

Development of MS – small footprint; Miniaturisation

R. Graham Cooks
Purdue Uni

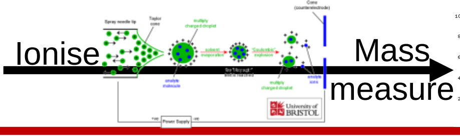
Torion ‘person portable GC-MS’. PerkinElmer. Milton Lee (BYU, Utah) (Here @ Botanic Garden Cranbourne)

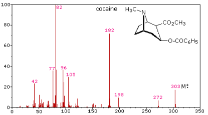
Nobel Prizes for (Analytical) Chemistry: 2002

Fenn

Tanaka

Wüthrich





2002 Nobel Prizes for MS

John Fenn

~ Research Professor of Analytical Chemistry

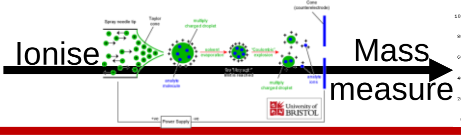
… for his work in MS, specifically electrospray ionization used to identify and analyze biological macromolecules.

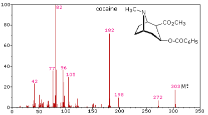
Koichi Tanaka

Shimadzu Corp

… soft desorption ionisation for MS analyses of biological macromolecules. Only person without post-bachelor's degree to have won a Nobel Prize in a scientific field.

@ Yale then Virginia Commonwealth University; (see Wikipedia update) Fenn's research into electrospray ionization found him at the center of a legal dispute with Yale University. He lost the law suit, - he misled the university about the potential usefulness of ESI. Yale awarded $500,000 in legal fees & $545,000 in damages. The decision .. provoked mixed responses from some at the institution; disappointed with the treatment of a Nobel Prize winner with such a long history at the school.

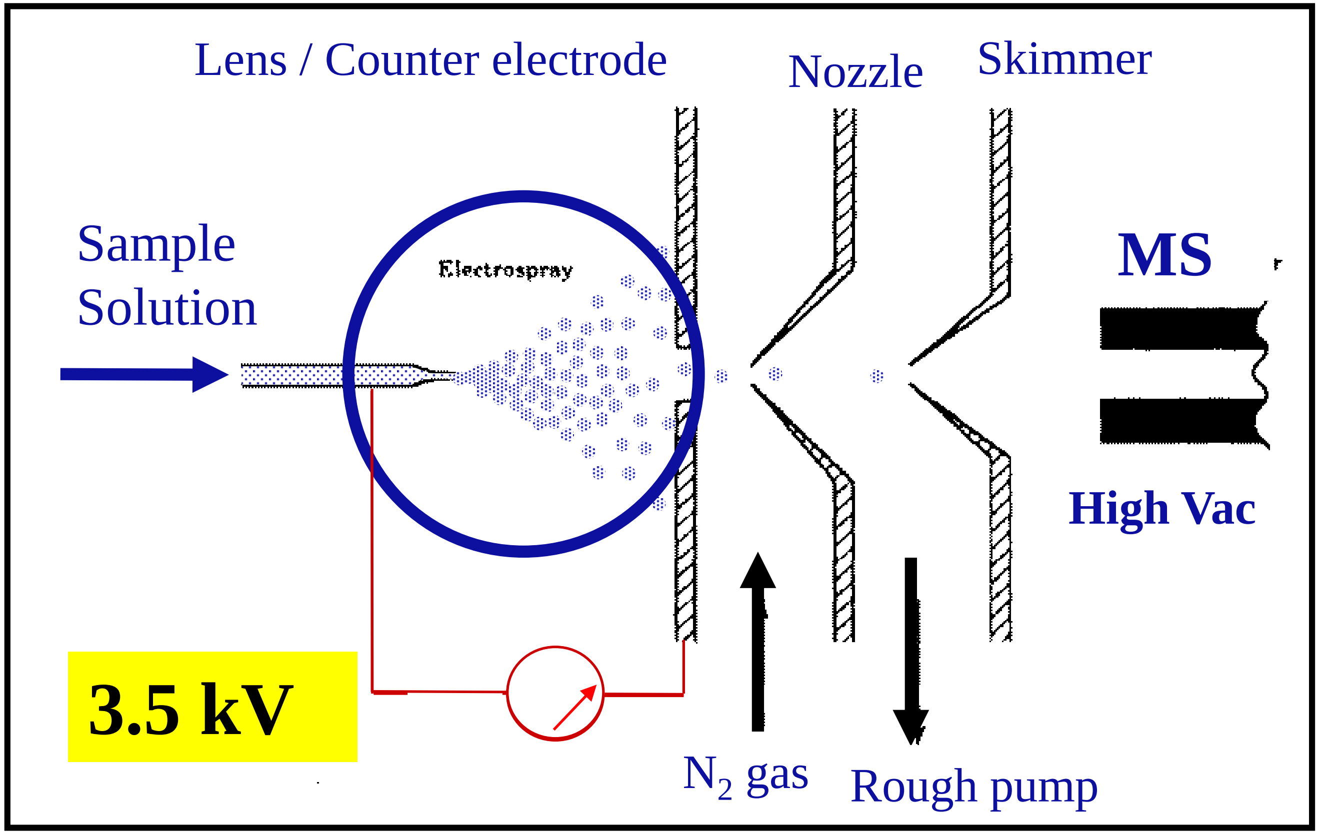


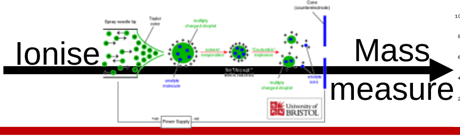


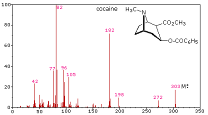
Electrospray Ion (ESI) Formation

for liquid phase introduction – e.g. HPLC to access MS info

Form charged droplets - Apply large potential gradient to create spray of fine charged droplets ‘single ion in droplet’ from the tip of the needle (electrohydrodynamic flow)

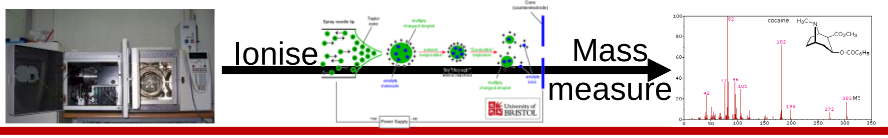




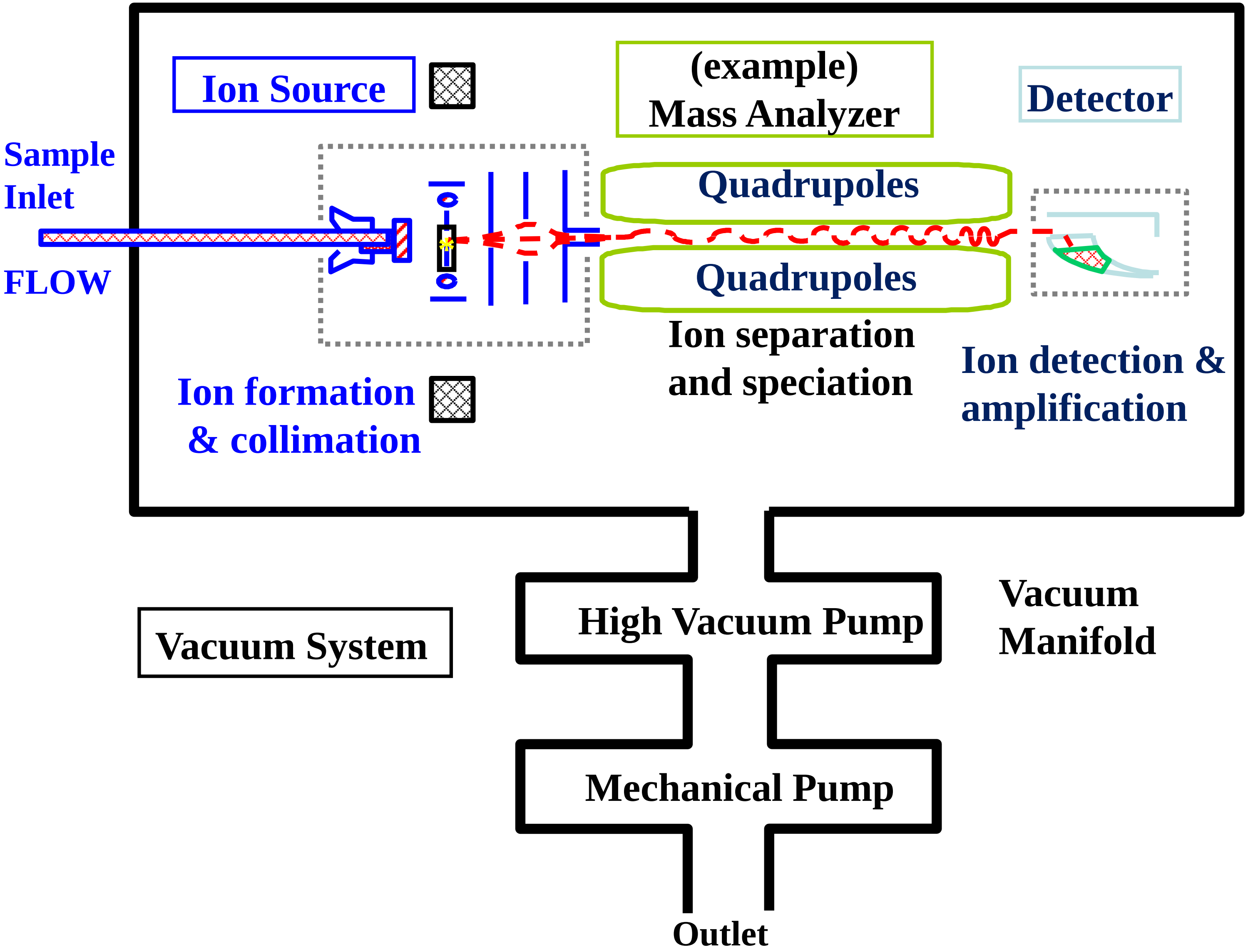


Characteristics of ESI

- Ionisation in a solution phase – charge exchange process – e.g. H^+ addition or loss, H^- addition
- Little, or ***<u>no</u>*** fragmentation
- Often multiple transfer of charge (large molecules)
- Cationisation, capture of metal ions and other cations (e.g. NH_4^+) is common– e.g. Na^+ is ubiquitous
- Formation of ionic clusters is frequently observed
- Solvent – moderately polar; electric field forms charged drops.
- Solvent must be sufficiently volatile; add formate and trifluoroacetate / acids if needed; not phosphates
- Samples should not be too strongly solvated.
- Avoid systems with strong clustering or where aggregation is promoted.



Mass Spectrometer Hardware



Ion Source

**(example)
Mass Analyzer**

Detector

**Sample
Inlet**

FLOW

Quadrupoles

Quadrupoles

**Ion formation
& collimation**

**Ion separation
and speciation**

**Ion detection &
amplification**

High Vacuum Pump

**Vacuum
Manifold**

Vacuum System

Mechanical Pump

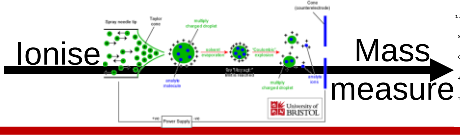
Outlet

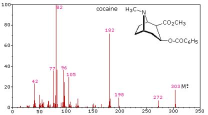
Ion Source
Mechanisms of Sample Ionisation

- Protonation: $M + H^+ \rightarrow MH^+$
- Cationisation: $M + Cat^+ \rightarrow MCat^+$
- Deprotonation: $MH \rightarrow M^- + H^+$
- Electron Ejection: $M \rightarrow M^{+\cdot} + e-$
- Electron Capture: $M + e- \rightarrow M^{-\cdot}$

e.g. $[M+Na]^+$ - sodiation

Typical ionisation mechanisms leading to ion formation. Others can occur e.g. photoionization (REMPI) ; normally one mechanism arises depending on the ion source process



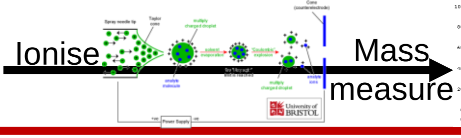


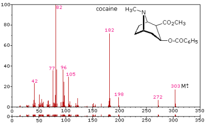
Named Methods of Sample Ionisation

• Electron (**Not** <u>Electron Impact</u>!) Ionisation (EI)	**H**
• Chemical Ionisation (CI)	**S**
• Atmospheric Pressure Chemical Ionisation (APCI)	**S**
• Electrospray Ionisation (ESI)	**S**
• Fast Atom Bombardment (FAB)	**H**
• Field Desorption / Field Ionisation (FD/FI)	**S**
• Matrix Assisted Laser Desorption Ionisation (MALDI)	**S**
• Thermospray Ionisation (TSP)	**S**

S: SOFT; H: HARD

Depends on the energy imparted to molecule during ionisation, and tendency towards fragmentation

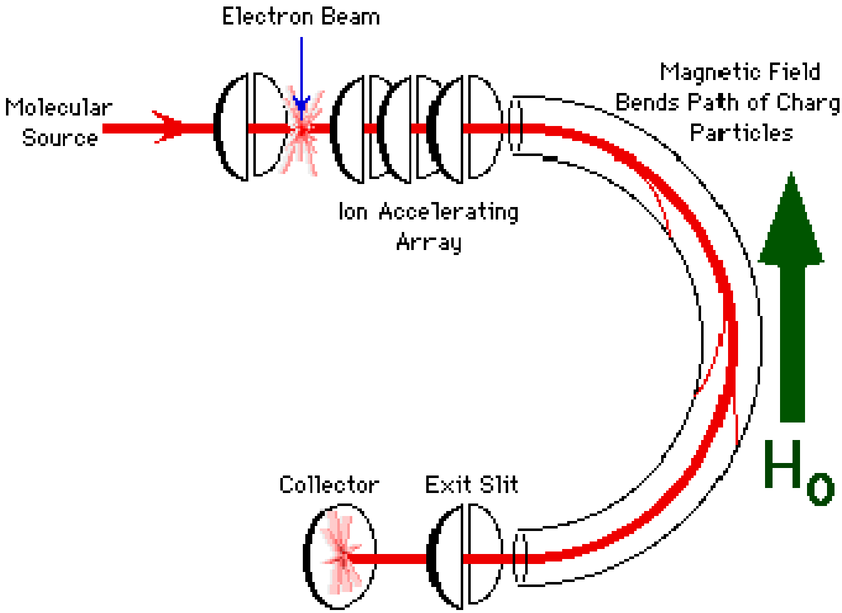




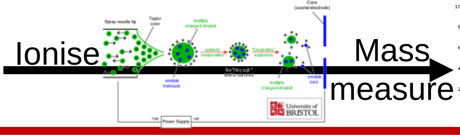
Analysers

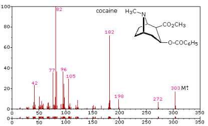
Electric Fields vs Magnetic Fields

- Magnetic field deflection
- Time-of-flight (TOFMS)
- RF fields (i.e. quadrupoles); Ion Trap



Magnetic field deflection



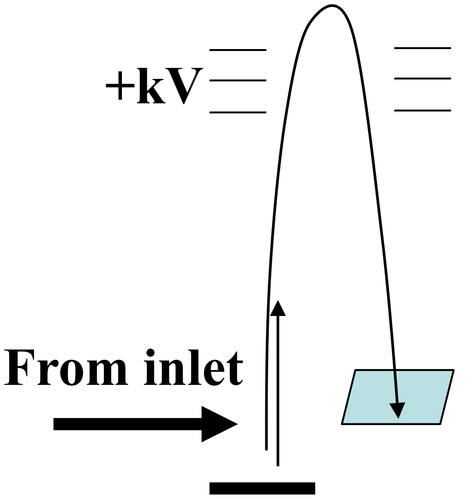


Analysers

Electric Fields vs Magnetic Fields

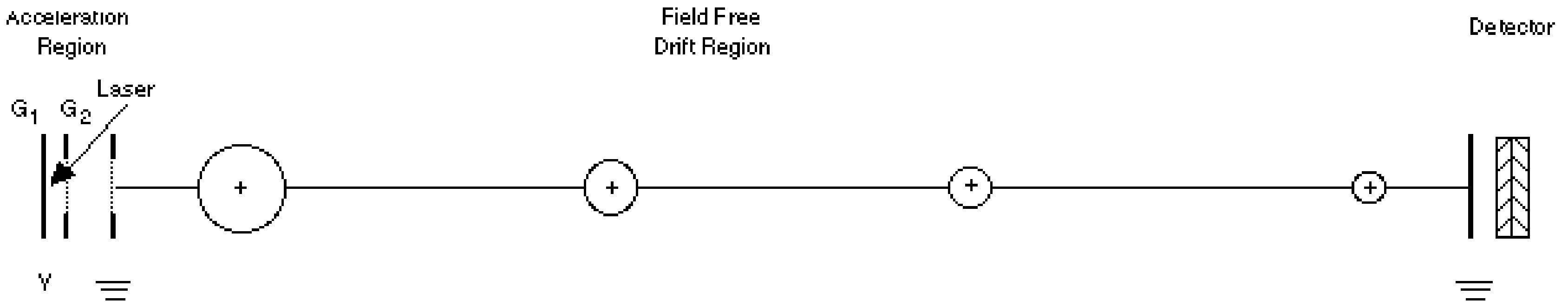
- Magnetic deflection
- Time-of-flight (TOFMS)
- RF fields (i.e. quadrupoles); Ion Trap

2. Reflectron



Pulsed ‘Push’ plate

1. Linear TOF MS

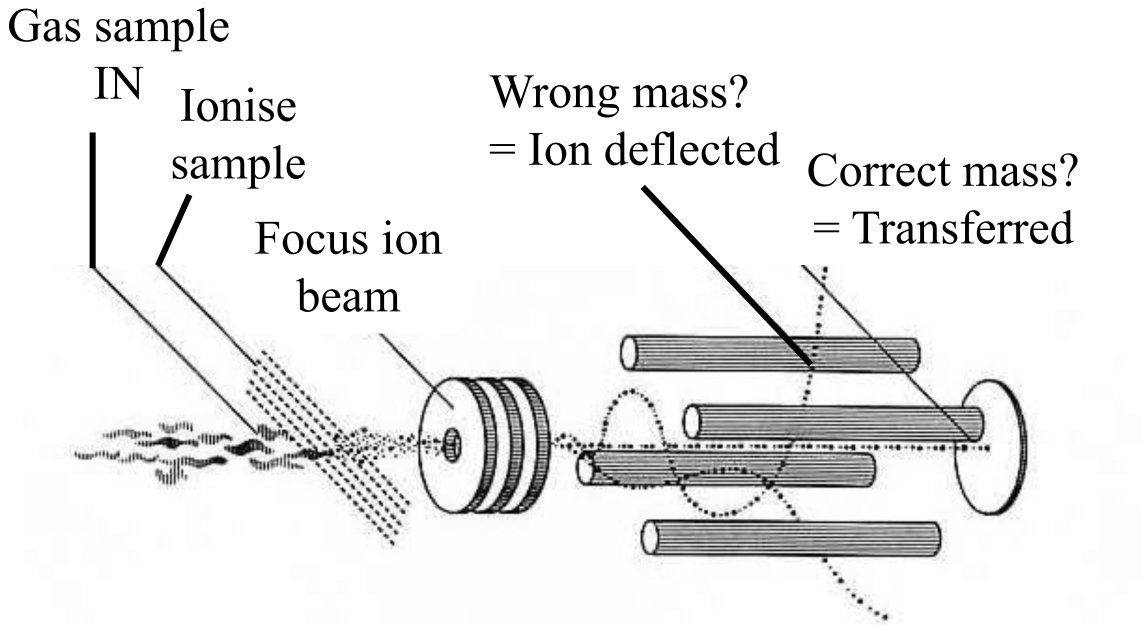


Time-of flight (TOFMS)

Analysers

Electric Fields vs Magnetic Fields

- **Magnetic deflection**
- **Time-of-flight (TOFMS)**
- **RF fields (i.e. quadrupoles); Ion Trap**



Gas sample IN

Ionise sample

Focus ion beam

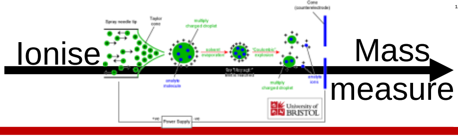
Wrong mass? = Ion deflected

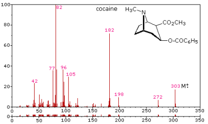
Correct mass? = Transferred

Quadrupole

Mass Spectrometer qMS (single quad; triple quad)

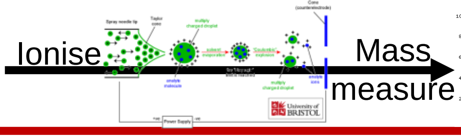
Quadrupole mass filter

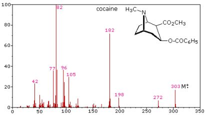




Chromatography-MS ("Hyphenated") Techniques

- Chromatographic methods coupled directly to MS instruments
- Ideal for
  - impure mixtures
  - multicomponent samples
- The current popularity of MS is largely due to GC and LC methods – “MS for the Masses”

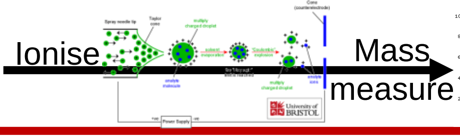


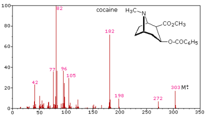


Electron Ionisation (EI); Supersonic Beam EI (Cold EI); Chemical Ionisation (CI) &

Learning Objectives;

- Understand the mechanism of EI, CI, ESI
- Discuss some factors involved in identifying a molecular ion
- Understand the mechanisms of chemical ionisation.

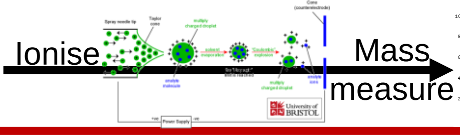


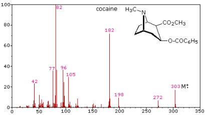


Mass Spectrometry

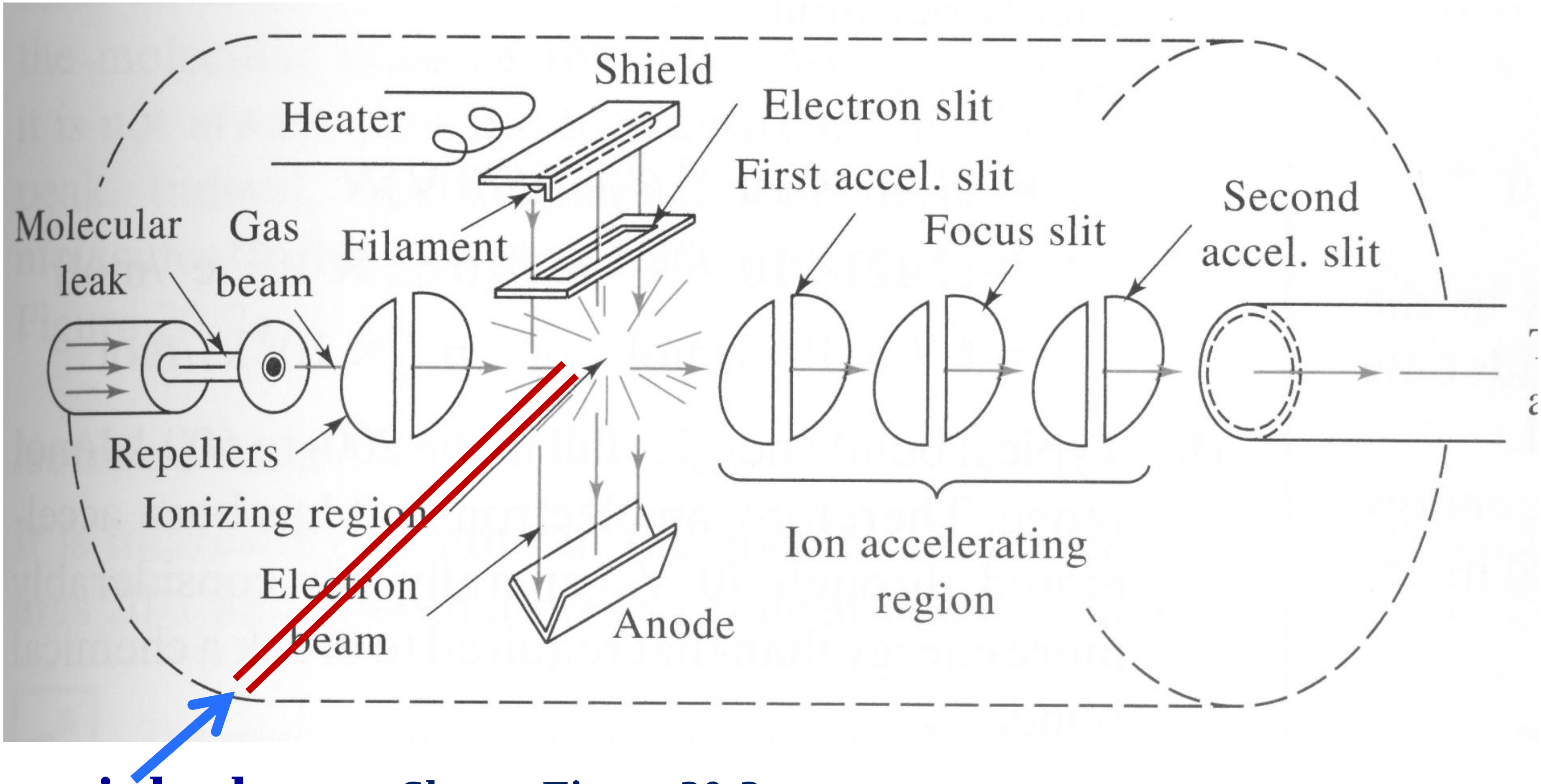
What information do we get from a mass spectrum?

1. **The Molar Mass** The single most important piece of information; allows us to narrow the list of possible compounds down considerably.
2. **The Functional Groups** What groups of atoms are present in our compound? What is attached to what? ***Fragment ions*** and ***neutral losses*** inferred from the mass spectrum tell us what our molecule consists of.
3. **The Chemical Nature of the Molecule** Each peak in the mass spectrum has a characteristic intensity. This gives an indication of how easy it is to form a particular fragment, or how stable the fragment ion is.



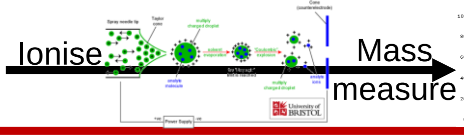


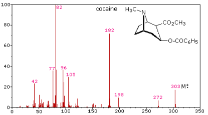
Electron Ionisation EI-MS



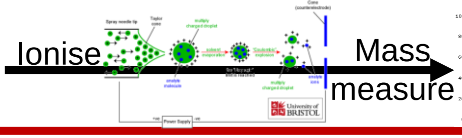
or inlet here

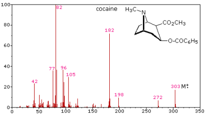
Skoog; Figure 20-3



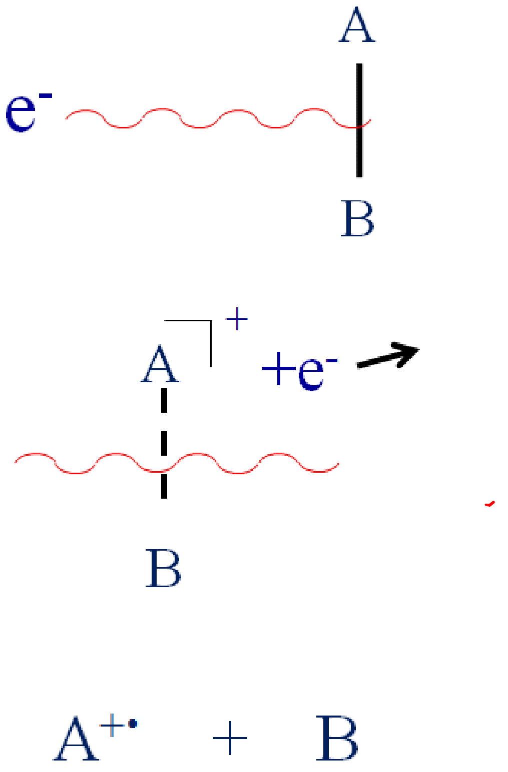


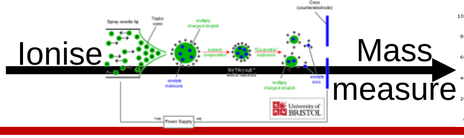
1. Electrons are emitted (thermionic emission) from a heated filament (wire; usually tungsten/rhenium); accelerated by applying ~70 eV between the filament and the ‘trap electrode’. **(WHY 70 eV?? If < 70 eV?)**
2. Can use lower eV – gives less fragment^{n}; ‘Select eV’ @ ~ 14 eV is used by the company “Markes” with GC.
3. The path of the molecules (left-to-right above) is at right angles to the electron beam. Ionisation occurs here; some collisions can also arise.
4. “ion-optics” focus the ion beam; an acceleration potential accelerates the ions into the mass analyser

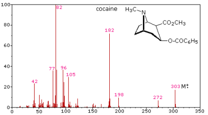




1. Molecule vaporises
2. Energetic electrons: energy transferred to M (e.g. to A-B bond)
   $M + e^- \rightarrow M^+ + e^- + e^-$
3. Molecular ion undergoes fragmentation > secondary ions
   $M^+ \rightarrow S^+ + N$ (secondary ion + neutral)
4. Charged ions separated; based on *m/z*:
   e.g. m/z=18 could be $[H_2O]^+$
   m/z=46 $[CH_3CH_2OH]^+$ *or* $[NO_2]^+$ etc.



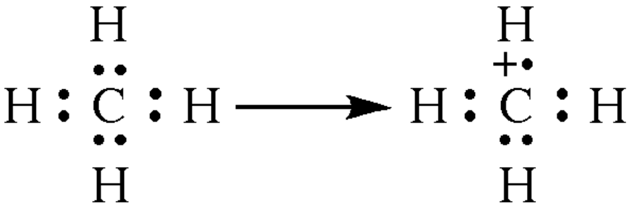




Identifying the parent ion; M^+

1. It must be the ion of highest mass in the spectrum. (*But remember isotopes and the possibility of a contaminant being present.)*
2. The parent ion must be an *odd electron* ion:

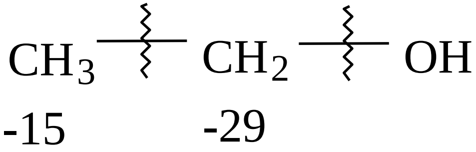
eg.



$$H:\overset{H}{\underset{H}{\ddot{\underset{\cdot\cdot}{C}}}}:H \longrightarrow H:\overset{H}{\underset{H}{\overset{+\cdot}{\underset{\cdot\cdot}{C}}}}:H$$

ie. $CH_4^{+\bullet}$

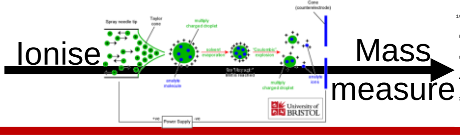
3. The parent ion must be capable of yielding the important ions in the high mass region of the spectrum by loss of logical neutral species.

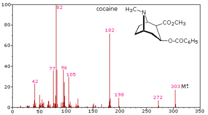


CH_3 – CH_2 – OH

-15 -29

M.M. = 46





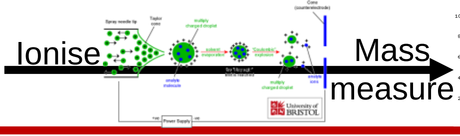
Which peak is the molecular ion, M^+ ??

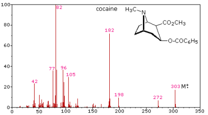
1. It is the peak with the highest mass *(BUT)*

2. It is the peak from the most abundant isotopes of the combining atoms *(BUT)*

3. All the fragment ions should arise from the molecular ion

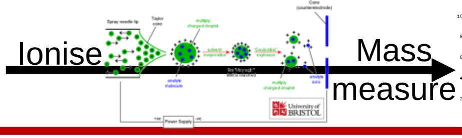
4. It is not always present …

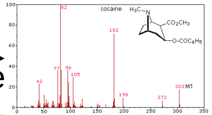




Isotope Patterns in MS

- Most commonly (but not exclusively), mass spectrometry is used to study organic compounds consisting of combinations of
  C, H, O, N, S, P, F, Cl, Br, I and Si
- From this list, all except P, F and I possess stable isotopes in adequate abundance to appear in mass spectra.
- The presence of isotopes makes it possible to calculate elemental compositions for many peaks in a mass spectrum.
- Especially useful in ACCURATE MASS MS analysis

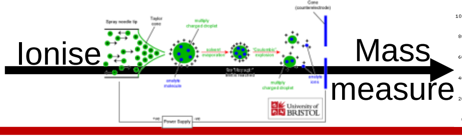


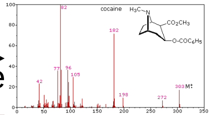


Cold EI

- Developed by Aviv Amirav, Tel Aviv University
- Commercialised by PerkinElmer – the GC-iQT system in PE Lab, Lvl 2. It still uses EI, BUT …
- … supersonic molecular beam **cools the analyte** so with EI and an elevated gas flow, the vibrationally cold molecular ion increases in intensity. Especially for saturated compounds – GOOD! Since these have poor EI $M^{+\cdot}$.
- Useful for GC since saturates are usually analysed by GC
- BUT – spectra are different from usual EI spectra – so can affect library match & searching…

http://www.avivanalytical.com/Supersonic-GC-MS.aspx





Chemical Ionisation

- CI ion source very similar to EI. But it may allow higher pressure of reagent gas…
- Principal difference Use of reagent gas (typically ammonia, methane or isobutane).
- Electrons (filament) ionise the CI gas, which then ionises the sample.
- Sample is introduced from a reservoir or heated probe, or as the eluent from a gas chromatograph (GC).

Chemical Ionisation (CI)

- Positive Ion Mode (PCI)

  $GH^+ + M \rightarrow G + MH^+$

  (GH^+ = protonated reagent gas)

  $G\text{-}H^+ + M \rightarrow G + M\text{-}H^+$

  ($G\text{-}H^+$ = reagent gas with loss of H^-)

Transfer of a 'group' to M

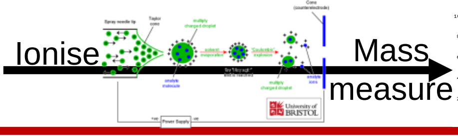
- Negative Ion Mode (NCI)

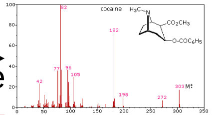
  $G\text{-}H^- + M \rightarrow G + M\text{-}H^-$

  ($G\text{-}H^-$ = deprotonated reagent gas)

Transfer of a 'group' from M

- MH^+ and $M\text{-}H^-$ are *"pseudo-molecular ions"*
- So the mechanism of ion formation is very different to EI!





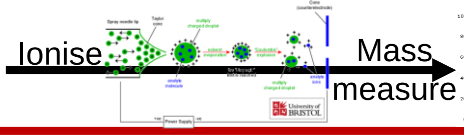
Chemical Ionisation (CI)

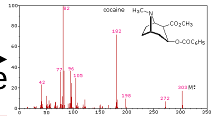
- Reduced Fragmentation
- Sometimes EI yields no molecular ion at all, EVEN with reduced e- energy, i.e. < 70 eV
- CI can be mild enough to give very little fragmentation – JUST pseudo Molec ion.
- Molecular Mass information
- Increased Sensitivity
- Increased Selectivity

CI - Reagent Gases

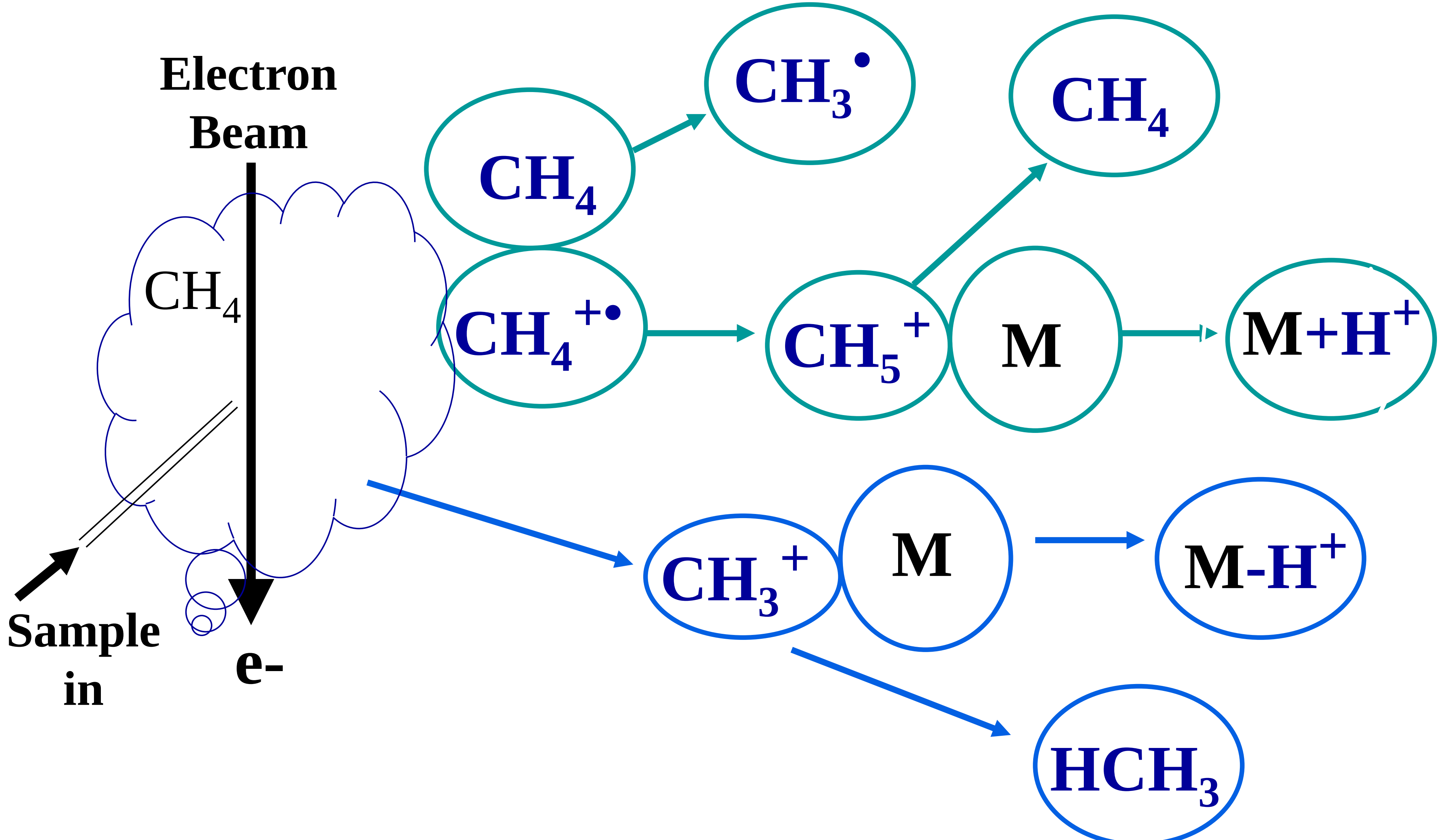
- Methane:
- $CH_4 + e^- \rightarrow CH_4^{+\cdot} + 2e^- \rightarrow CH_3^+ + H\cdot$
- $CH_4^{+\cdot} + CH_4 \rightarrow CH_5^+ + CH_3^\cdot$
- $CH_4^{+\cdot} + CH_4 \rightarrow C_2H_5^+ + H_2 + H\cdot$
- Isobutane:
- $i\text{-}C_4H_{10} + e^- \rightarrow i\text{-}C_4H_{10}^{+\cdot} + 2e^-$
- $i\text{-}C_4H_{10}^{+\cdot} + i\text{-}C_4H_{10} \rightarrow i\text{-}C_4H_9^+ + C_4H_9 + H_2$
- Ammonia:
- $NH_3 + e^- \rightarrow NH_3^{+\cdot} + 2e^-$
- $NH_3^{+\cdot} + NH_3 \rightarrow NH_4^+ + NH_2\cdot$
- $NH_4^+ + NH_3 \rightarrow N_2H_7^+$

These charged species transfer proton H^+ or other groups (e.g. NH_4^+) to molecule ..





CI - Methane Reagent Gas



Electron Beam

CH_4

Sample in

e-

CH_4

$CH_3^{\bullet}$

$CH_4^{+\bullet}$

CH_5^{+} M

CH_4

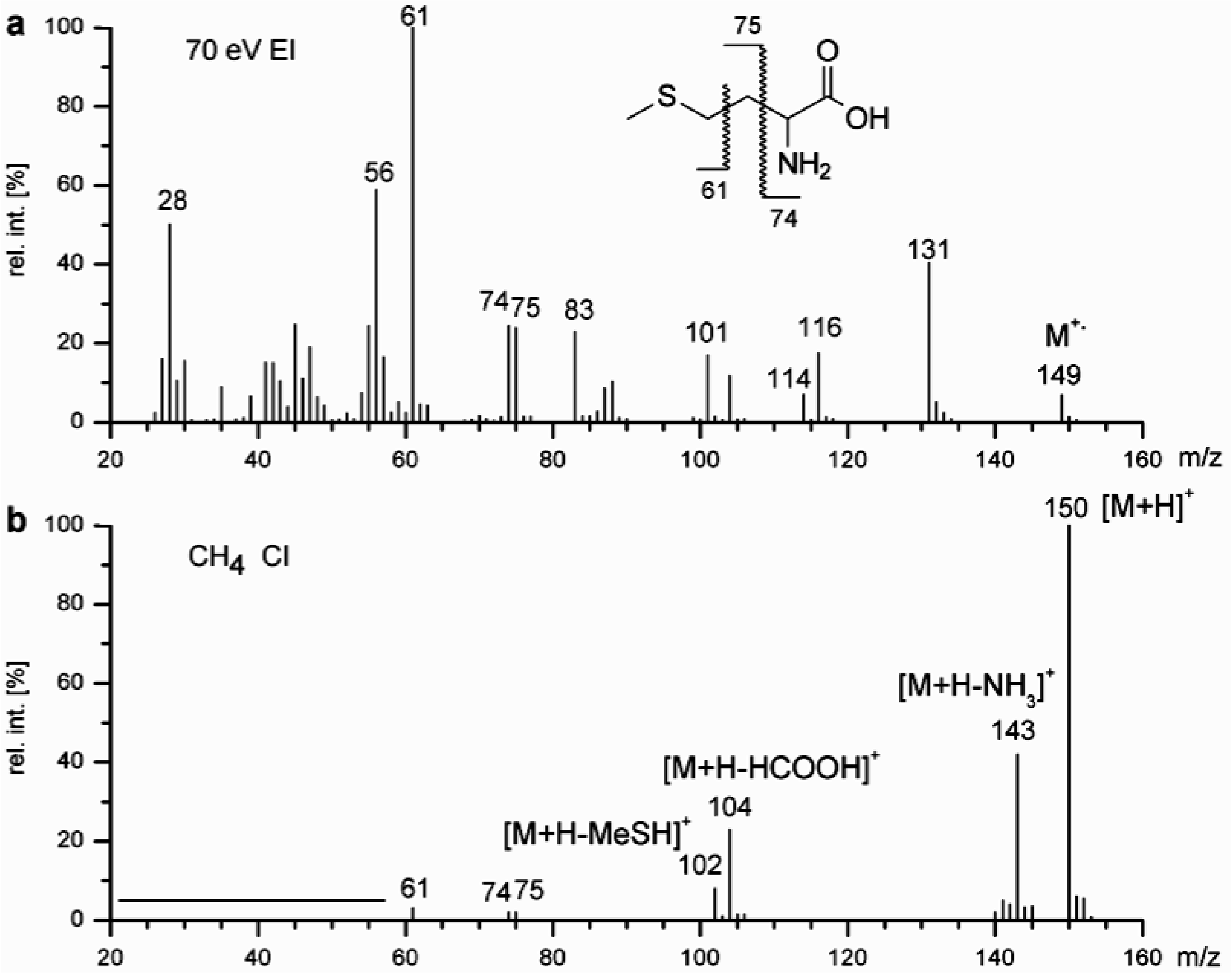
$M{+}H^{+}$

CH_3^{+} M

$M{-}H^{+}$

HCH_3

EI vs CI – Methionine; MM = 149

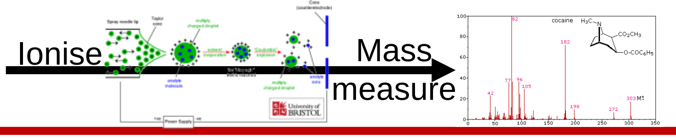


EI

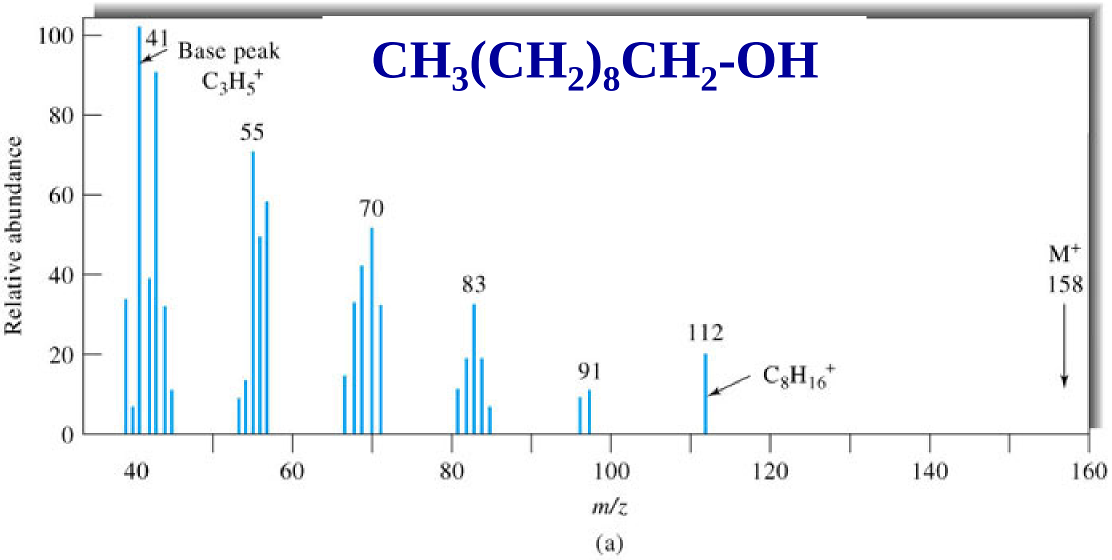
Almost complete fragmentation of $M^{+\cdot}$

CI (Methane)

Strong $[M+H]^+$ ion, limited fragmentation

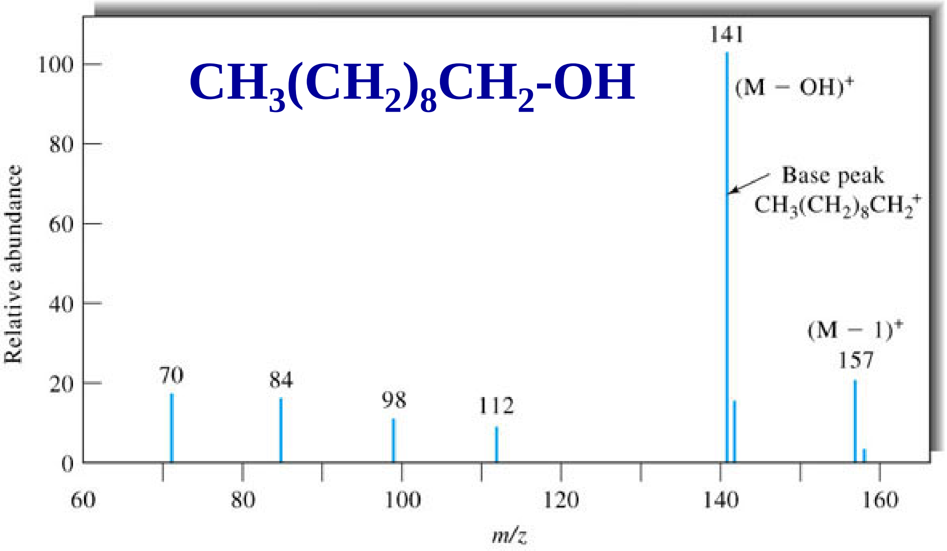


EI vs CI – decan-1-ol



$CH_3(CH_2)_8CH_2$-OH

EI;
Total fragmentation of $M^{+\cdot}$



$CH_3(CH_2)_8CH_2$-OH

CI;
Strong high mass ion $[M-OH]^+$; much less fragmentation than EI

From Skoog; Figure 20-2