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1. Mass spectrometry

Mass spectrometry in some form is familiar to many people whose chemistry education has gone beyond compulsory education.

The theory

Diagrams of simple mass spectrometers, such as *Fig. 1*, are common. The principles of such spectrometers are quite straightforward:

1   a sample is introduced into the spectrometer and vaporised;

2   ions of charge *z* (where *z* is a multiple of the charge on an electron) are produced by bombarding the sample with electrons in the ionisation chamber;

3   the ions are accelerated by an electric field so that they have similar kinetic energy;

4   the ions of mass *m* are deviated by a magnetic field such that ions of low or high m/z value strike the sides of the spectrometer, but ions of one particular m/z value continue along the spectrometer body;

5   by varying the magnetic field strength all ions are sequentially focused into the detector; and

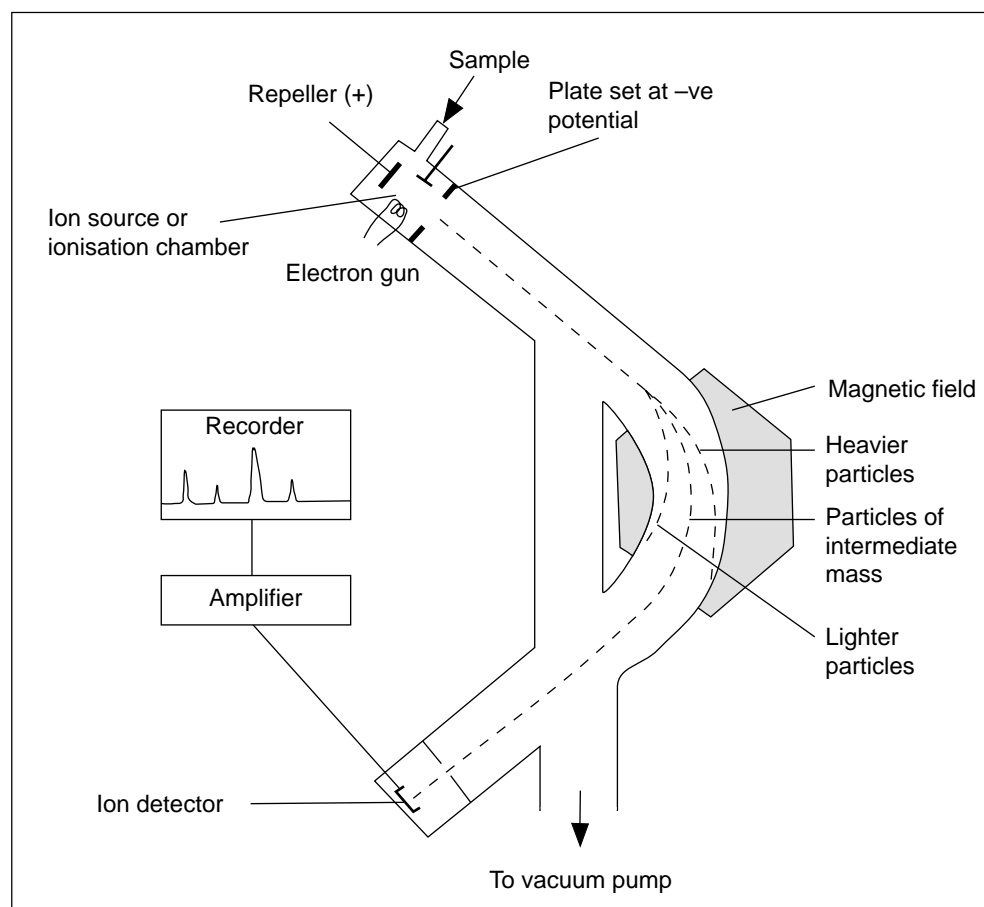**6**   the ions are detected and the mass spectrum plotted.

Figure 1   The mass spectrometer

Because the ions have to travel distances from 25 cm up to maybe 3 m inside the mass spectrometer it is important that they are not scattered or otherwise interfered with by other species – *ie* by reaction with neutral molecules. This is most likely to occur when air is present. This problem is overcome by subjecting the inside of the spectrometer to a high vacuum, typically 1.33×10^{-5} Nm^{-2} (10^{-7} mmHg) pressure. ($1\ Nm^{-2} \equiv 1\ Pa$; 1 torr $\equiv 1$ mmHg; 1 torr $\equiv 1.33 \times 10^{-2}$ Nm^{-2}). There are two ways of determining the *m/z* values of the ions produced: the accelerating electric field can be kept constant and the magnetic field scanned (in practice an electromagnet is used); or the magnetic field can be kept constant and the electric field scanned. It is usually the magnetic field that is scanned.

If masses are required to one atomic mass unit (one dalton) a single focusing instrument such as the one described will suffice. However, an accuracy of 1 in 10^6 – *ie* an accuracy that allows determination of precise atomic composition rather than nominal mass – can be achieved by using a double-focusing spectrometer. The principles for this type of spectrometer are identical to those for the single-focusing instrument, but include an electric sector before separation by a magnetic field *(Fig. 2)*. Ions with a similar kinetic energy (ke) enter the electrostatic analyser and are focused into a narrower energy range. The magnetic field can then separate the ions, which have a much smaller energy range, giving a much greater resolving power.
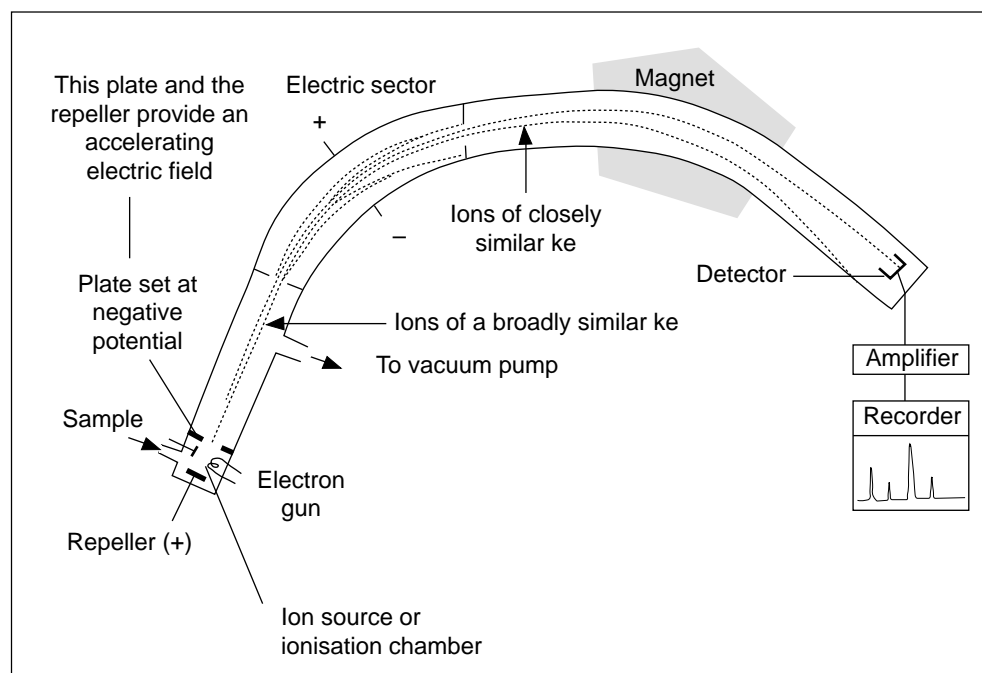

Figure 2   The double-focusing mass spectrometer

Practical considerations

The sample

Mass spectrometry is about 1000 times more sensitive than infrared (IR) or nuclear magnetic resonance (NMR) spectroscopy. Only a microgram or less is required to record a mass spectrum (many modern spectrometers require nanogram samples).

Different methods for introducing the sample in the vapour phase are used. Gaseous samples can be allowed to diffuse into the spectrometer – a 10^{-5} dm^3

(10 microlitre) sample would be ample for this. Volatile liquids can be injected in, but using rather smaller volumes, because the sample will vaporise under the low pressures present in the spectrometer.

Involatile liquids and solids are vaporised by placing the sample on a ceramic tip or in a glass capillary made of disposable soda-glass or reusable quartz (which has to be heated to very high temperatures to clean – *ie* to remove any residue) or in a metal crucible (typically 5 mm deep and 2 mm in diameter). These are inserted into the spectrometer (right up to, but not inside, the ion production area itself) and are subjected to temperatures of up to 300 °C (*Fig. 3*). The vapour is then allowed to diffuse into the ion production area. Solids dissolved in a solvent can be used by putting the solution into a crucible and allowing the solvent to evaporate.
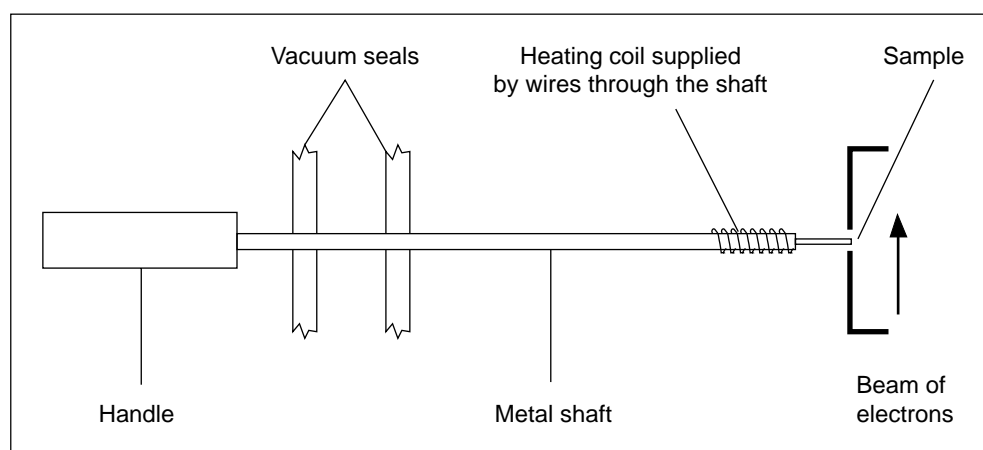

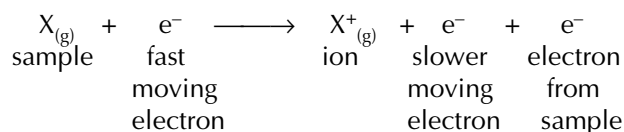**Figure 3**   Vaporisation of solid samples

The ionisation chamber of the mass spectrometer is heated to 150–250 °C to ensure that the vaporised sample remains in the gas phase. Thermally stable non-polar organic molecules – *eg* perfluorokerosene (fully fluorinated kerosene) – with masses up to 1000 daltons can generally be vaporised at temperatures below 300 °C. If polar groups are present (*eg* OH, COOH and NH_2 groups) the volatility decreases, and molecules with moderate polarities will only vaporise readily if their masses are below 500 daltons.

The rate of production of vapour is important – too slow and insufficient ions will be produced to obtain an appreciable signal; too rapid and the dominant ions will saturate the detector and information regarding relative ion intensities will be lost. If there are too many ions in the ionisation chamber of the spectrometer ion molecule interactions might occur, forming ions with a mass greater than the mass of the sample molecules.

Ion production

The method of ionisation most commonly discussed is electron impact (EI). Electrons are 'boiled off' from a heated filament, which is made the cathode with respect to an anode set typically at +10 to +70V (*Fig. 4*). As the electrons accelerate towards the anode they can collide with the vaporised sample. Their energies are therefore up to 70 eV (1 eV $\equiv$ 96.5 kJ mol^{-1}; 70 eV $\equiv$ 6750 kJ mol^{-1}).

Collision between the high energy electron and the sample 'knocks out' an electron from an electron orbital, generally from the highest energy level:

$$X_{(g)} \quad + \quad e^- \quad \longrightarrow \quad X^+_{(g)} \quad + \quad e^- \quad + \quad e^-$$

sample   fast   ion   slower   electron
           moving   moving   from
          electron   electron   sample

(Multiple charged ions can be formed. Doubly charged ions are detected at half their mass value on the final spectrum, which has mass/charge as its horizontal axis.)

Some negative ions are formed (less than 0.1 per cent of the positive ions formed), and those that are formed are attracted away from the electric sector and magnet. However, it is possible to set the spectrometer to monitor negative ions by reversing the polarity of the repeller.
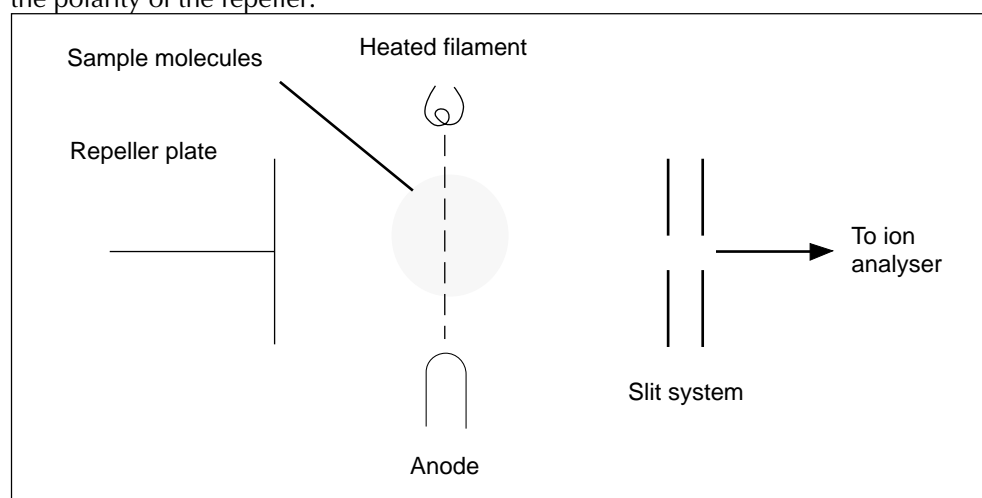

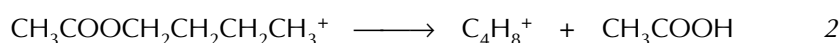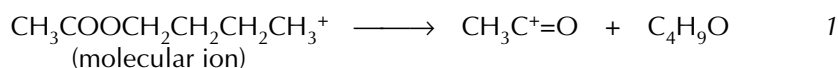**Figure 4**   Electron impact mechanism

Ionisation of an element requires from 381 kJ mol^{-1} (francium) to 2370 kJ mol^{-1} (helium) (*ie* 4–25 eV), and organic molecules need about 600–1000 kJ mol^{-1} (7–10 eV). With organic molecules the energy of the ionising electrons is so great compared with the bond energies in the molecules that fragmentation is possible – *nb* the strongest common single bond (bond energy 485 kJ mol^{-1}, 5 eV) is C–F. Some of the residual energy (possibly up to 600 kJ mol^{-1}) of the fast moving electron might also be transferred to the ion as internal energy.

When an organic molecule forms a positive ion, it becomes a radical-cation because one electron has been removed from a pair in a filled orbital.

$$M \quad \longrightarrow M^{\bullet+} + e^-$$
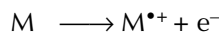
By convention, the radical electron is omitted from the representation of these ions in many texts.

If the radical-cation fragments, the molecular ion can lose either a radical or a neutral molecule *eg* the butyl ethanoate molecular ion (radical-cation) fragments as follows:

$$CH_3COOCH_2CH_2CH_2CH_3^+ \quad \longrightarrow \quad CH_3C^+\!\!=\!\!O \quad + \quad C_4H_9O \qquad \textit{1}$$
(molecular ion)

$$CH_3COOCH_2CH_2CH_2CH_3^+ \quad \longrightarrow \quad C_4H_8^+ \quad + \quad CH_3COOH \qquad \textit{2}$$

The remaining ions can fragment further to give the spectrum shown in *Fig. 5*. The molecular ion peak is not significant because the ion is unstable.

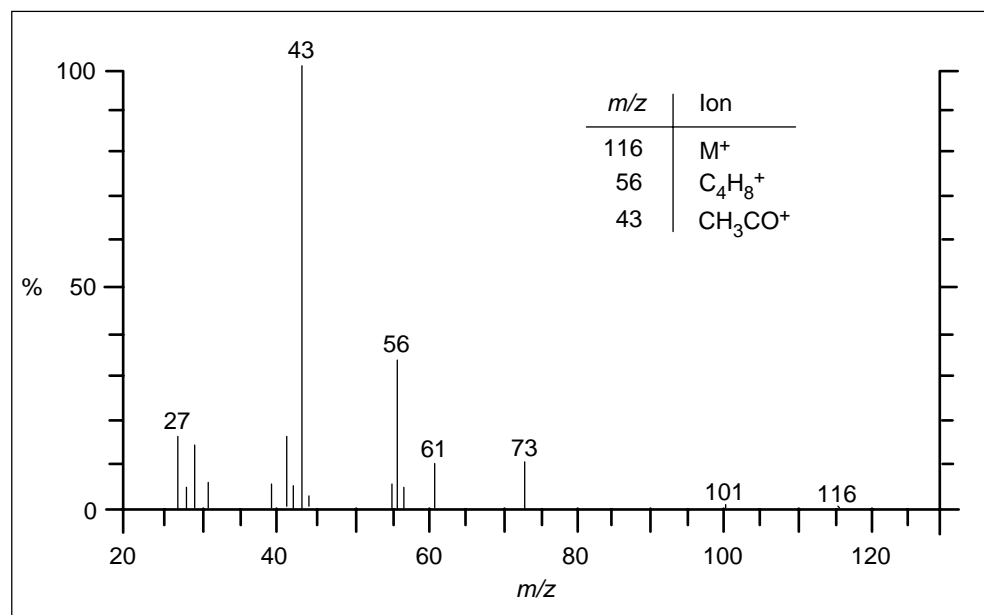| m/z	Ion
116	M^+
56	$C_4H_8^+$
43	CH_3CO^+

Figure 5   Mass spectrum of butyl ethanoate

The ionising electrons for this spectrum had energy 70 eV. These cause more fragmentation according to equation *1*. The peak at $m/z = 43$ is due to $CH_3C^+=O$, and the one at $m/z = 56$ due to $C_4H_8^+$. If ionising electrons of energy 15 eV had been used, the peak at $m/z = 56$ would have been the most abundant – *ie* fragmentation would predominantly have been according to equation *2*. However, in each case the molecular ion would not be significant.

So, although it is possible to obtain a unique spectrum which will act as a 'fingerprint' for an organic compound, all the operating conditions (including the energy of the ionising electrons) must be quoted. This is especially true if the spectrum obtained is to be compared with library spectra for identification.

Ionisation using lower potentials often gives more detailed information about the molecule because less energy is transferred to the sample and the molecular ion (M^+) can become more significant. A disadvantage of this method is that fewer ions are produced. One way of increasing the amount of molecular weight information obtained on a compound is to use a 'soft' ionisation technique such as chemical ionisation (CI).

The molecular ion and the arrangement and sizes of the peaks formed by the breaking apart (the fragmentation pattern) of an organic compound can often be used to identify it. However, there are cases when electron impact ionisation is inappropriate because it is impossible to ionise the molecule without it breaking up. The molecular ion can usually be observed by using other ionisation techniques, such as CI and FAB (see below). Other, more elaborate methods exist, such as laser desorption, secondary ion mass spectrometry (SIMS), electrospray and californium plasma desorption, which are compatible with large, polar molecules. (Descriptions of these techniques can be found in the bibliography.)

Deflection of the ions

The ions are accelerated through a series of plates set at increasingly negative potentials and emerge with a broadly similar energy, before passing into the electric

sector where they are deviated so that the emergent ions all have a more sharply defined range of energies. Deflection in the electric field is independent of mass, but for a given charge it is proportional to the energy of the ions.

In the radial electrostatic field within the analyser the ions follow a circular path of radius r, broadly given by the equation:

$$r = \frac{2V}{E}$$

where V = electrostatic potential
             E = energy of ion

The ions continue in a straight path outside the influence of the electric field, until they enter the magnetic field where they are deflected so that only the ions of a specific mass to charge ratio will continue on towards the detector, (Box).

Dependence of ion deflection on accelerating voltage and magnetic flux density

The kinetic energy of the ions after being accelerated is given by:

$$\tfrac{1}{2}\, mv^2 = zV \qquad\qquad\qquad 1$$

where m = mass of the ion
          z = charge on the ion
          v = velocity of the ion

The force on the ions in the magnetic field is described by:

$$\text{force} = Bzv \qquad\qquad\qquad 2$$

where B = magnetic flux density.

The force on a body as it accelerates towards its centre of curvature is:

$$\text{force} = \frac{mv^2}{r} \qquad\qquad\qquad 3$$

where r = radius of circular path.

Combining equations *2* and *3* we get

$$Bzv = \frac{mv^2}{r}, \text{ so } v = \frac{Bzr}{m} \qquad\qquad 4$$

Substituting equation *4* into equation *1*,

$$\tfrac{1}{2}\, m\, \frac{B^2z^2r^2}{m^2} = zV \quad \text{thus} \quad \frac{m}{z} = \frac{B^2r^2}{2V}$$

So, for a given *m/z* value, the radius of curvature of the deflected ion is dependent on the magnetic field strength and on the accelerating voltage.

The mass spectrum can be obtained by varying the accelerating voltage, *V*, or by varying the magnetic field (magnetic scanning). Voltage scanning can be done at high speed but this usually gives a distorted spectrum – the relative abundances of the fragments decreases as their mass increases. Magnetic scanning is the norm although it is a little slower, because it is restricted by the response time of the analyser magnet.

The double-focusing mass spectrometer is so precise that the relative mass of a compound can be determined to such an accuracy that its molecular formula can be assigned. The molecular ion peak of the unknown in *Fig. 8* is 122.036776, which can be assigned the molecular formula $C_7H_6O_2$ if it is assumed that only carbon, hydrogen, nitrogen and oxygen are present (Table 1). However without further information the precise isomer cannot be identified.

Table 1   Some formulae corresponding to nominal *m/z* = 122

Formulae	Actual mass
$C_4H_4N_5$	122.046668
$C_4H_{10}O_4$	122.057903
$C_6H_4NO_2$	122.024201
$C_6H_6N_2O$	122.048010
$C_6H_8N_3$	122.071819
$\underline{C_7H_6O_2}$	$\underline{122.036776}$
C_7H_8NO	122.060585
$C_7H_{10}N_2$	122.084394
$C_8H_{10}O$	122.073161
$C_8H_{12}N$	122.096970
C_9H_{14}	122.109545

These are based on the following relative atomic masses:

$$C = 12.0000000$$
$$H = 1.0078246$$
$$N = 14.0030738$$
$$O = 15.9949141$$

Ion detection

Many modern mass spectrometers use electron multipliers (*Fig. 6*). The ions strike a plate (the conversion dynode) made of a material (*eg* a copper/beryllium alloy) that emits electrons when struck by energetic particles . This is set at *ca* –1.4 kV. Secondary electrons are emitted from it, and they are accelerated and focused onto the second and subsequent dynodes, which are set at potentials progressively closer to earth. At each dynode there is an increase in the number of electrons emitted, such that at the end of the multiplier a gain of approximately 10^6 has been achieved. (A multiplier might contain 10 –14 dynodes.) Since ions are sharply focused onto one position the conversion dynode degrades after a period of time and must then be replaced.This occurs after perhaps two years on an instrument that is in daily use.
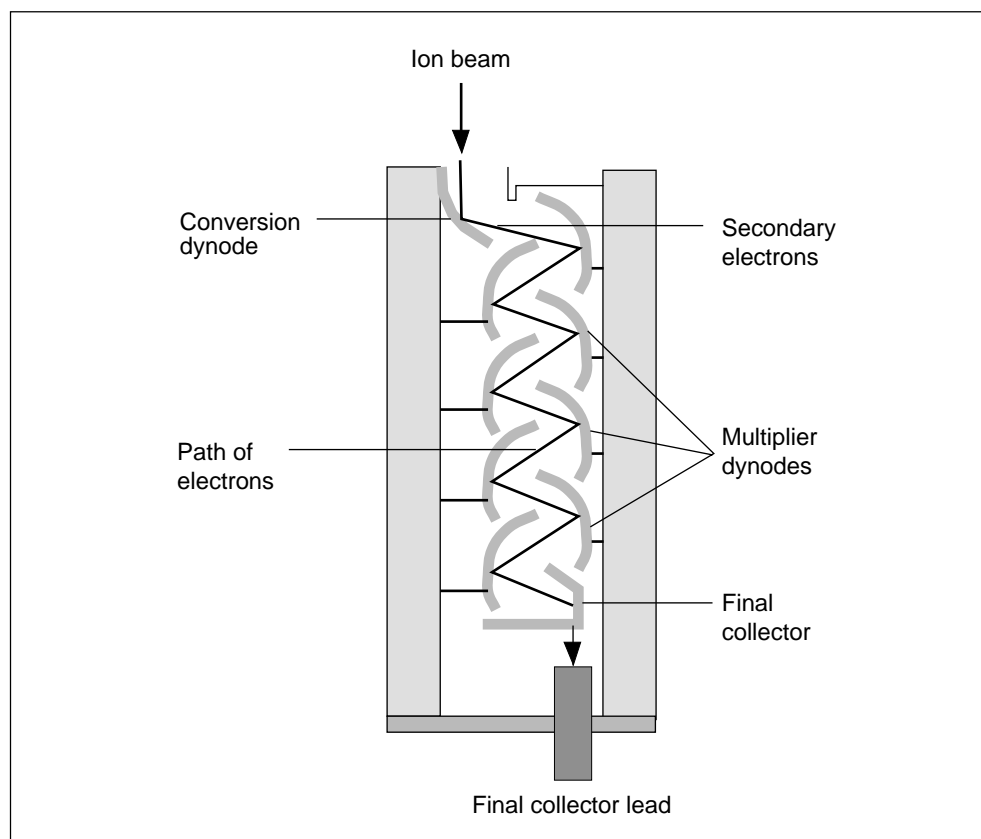
Figure 6   Schematic diagram of an electron multiplier

The amount of information obtained from a mass spectrometer is so vast that it is only realistic to capture the data using a microprocessor. The signal from the electron multiplier is fed to an analogue to digital converter, then stored in a computer. The computer stores the mass value at the centre of each ion distribution, and a maximum value which corresponds to the number of ions of that mass (*Fig. 7*). The computer can then plot mass spectra. If plotted graphically the computer will set *m/z* values on the x-axis and relative abundance on the y-axis, setting the most abundant ion as 100 per cent abundance (*Fig. 8*). The intensities of other ions are then plotted relative to this peak.
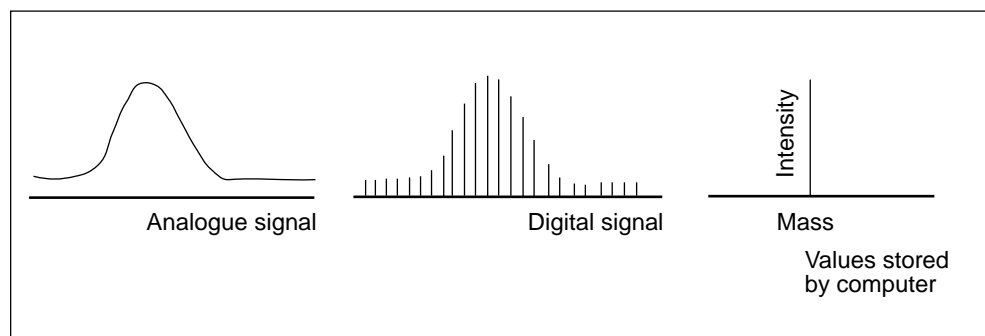
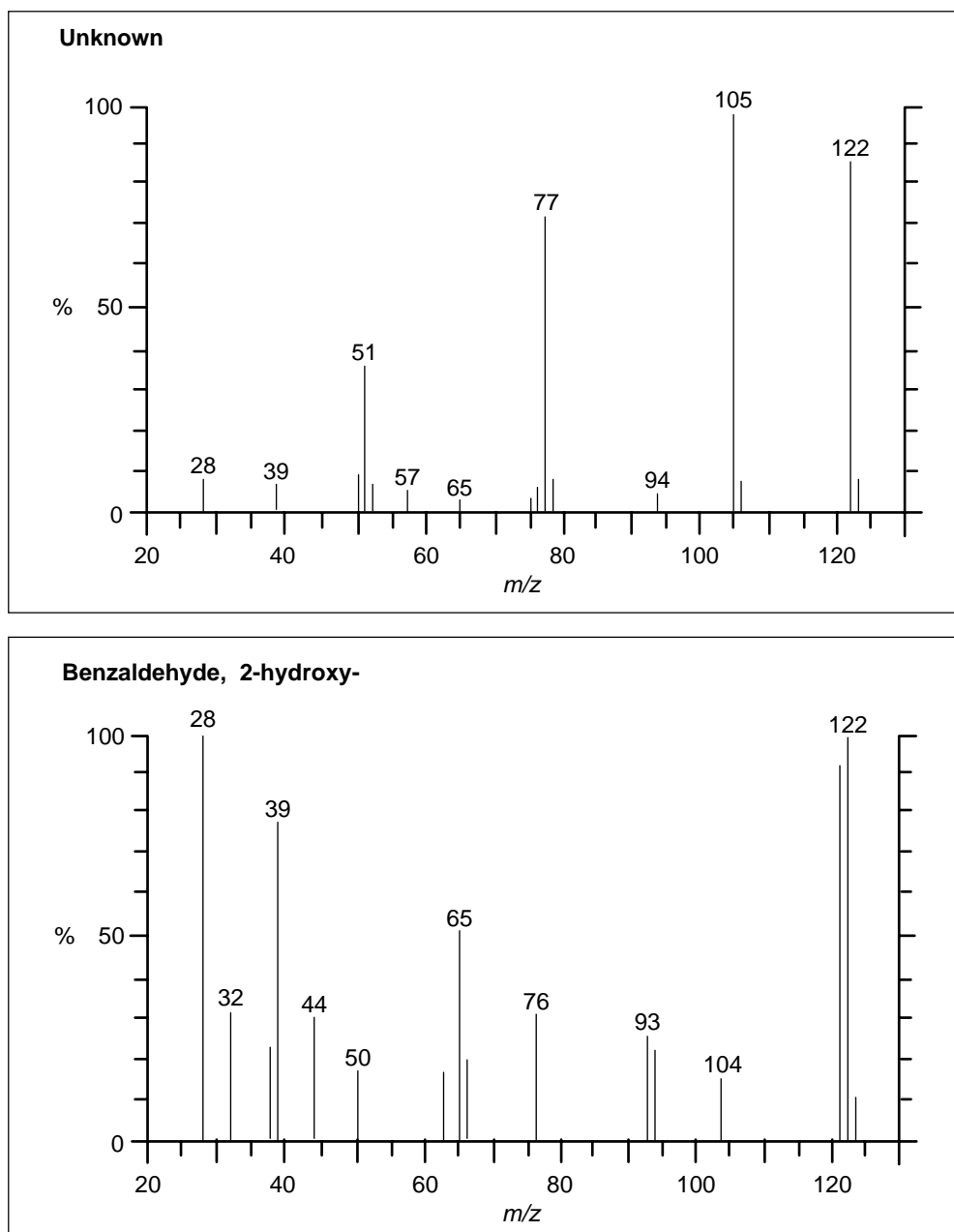**Figure 7**   Recording of data from the mass spectrometer

Figure 8   Mass spectra of an unknown and five isomers of the same molecular formula (continued overleaf)

The vacuum system

It is vital that the path of the ions in the spectrometer is not affected by residual gas molecules or by neutral species formed during the fragmentation or deflection of particles. The average distance travelled by ions between successive collisions is known as the mean free path, and is of the order of several hundred metres when the internal pressure of the spectrometer is 10^{-5} Nm^{-2}. In modern spectrometers two types of pumps are used to achieve this low pressure. A rotary, or backing, pump reduces the pressure to approximately 10^{-2} Nm^{-2}, in tandem with a diffusion pump (which traps gas molecules in the droplets as high speed jets of oil vapour condenses), or a turbomolecular pump (which has a powerful fan that turns very quickly).
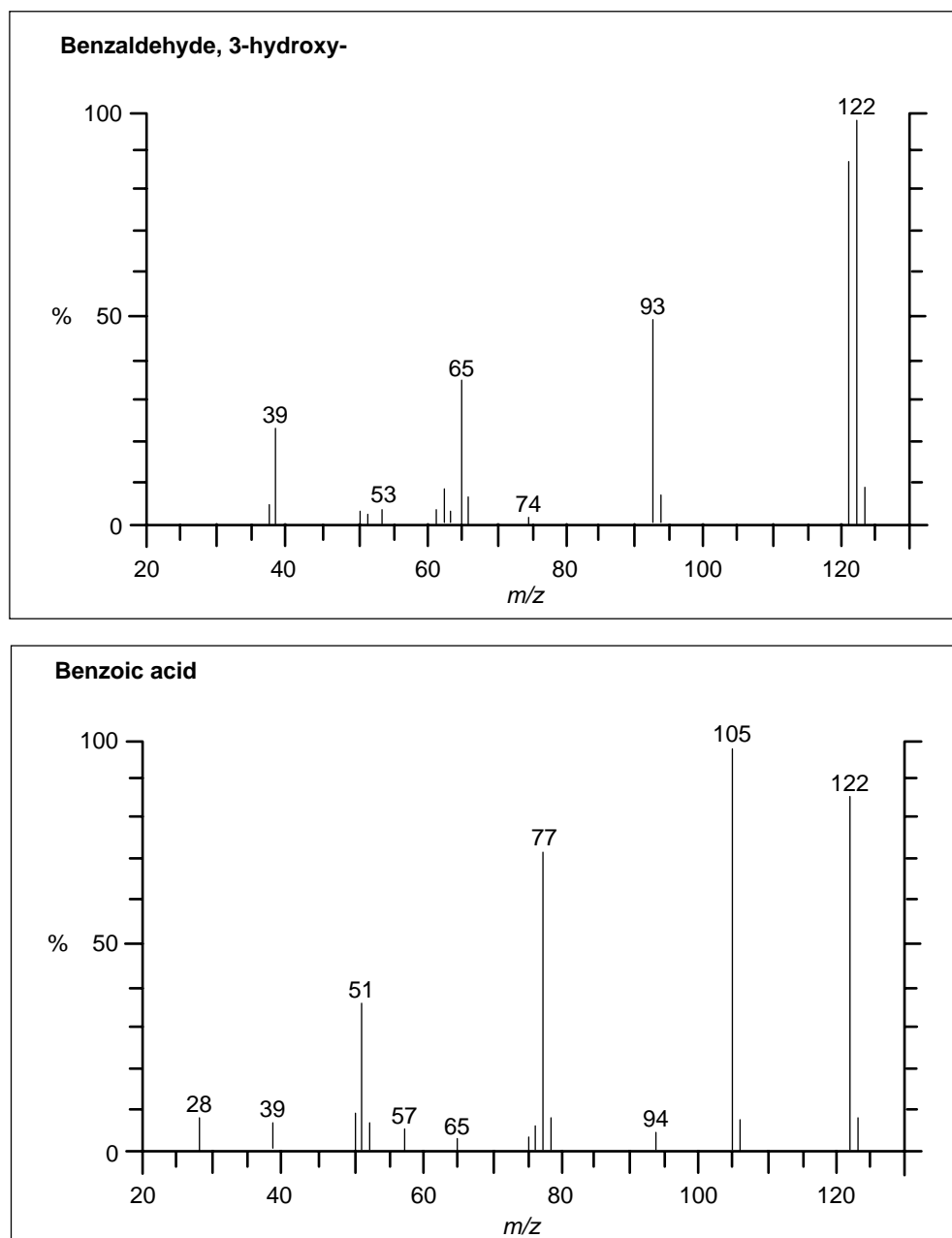
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Benzaldehyde, 3-hydroxy-

Benzoic acid

Figure 8   continued

Ions that do not reach the detector can pick up an electron from the walls of the spectrometer, and if of low relative mass they become gaseous and can be removed by the vacuum line, along with any particles that were not ionised in the first place. If the species are involatile, they condense on the inside walls of the spectrometer and are periodically removed by surrounding the instrument with electric band heaters and vaporising them off. Heavy or stubborn deposits have to be removed by mechanically stripping the instrument down and cleaning it. Fortunately, this does not happen very often.

The ionisation chamber of the spectrometer is particularly susceptible to contamination by condensed samples, therefore it is likely to need to be stripped down and mechanically cleaned every three months.
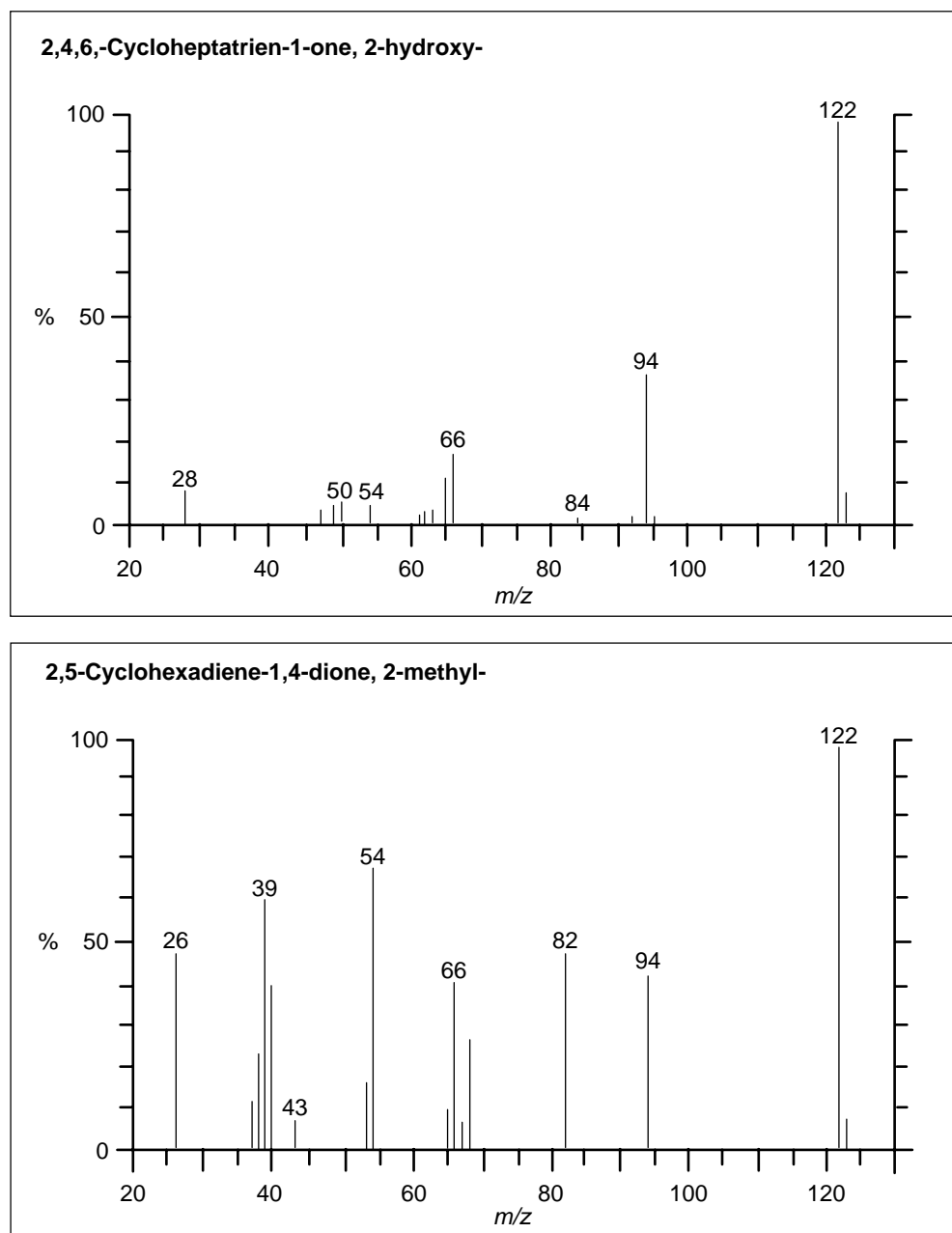
2,4,6,-Cycloheptatrien-1-one, 2-hydroxy-

2,5-Cyclohexadiene-1,4-dione, 2-methyl-

Figure 8   continued

Calibration

If low resolution data is sufficient, perfluorokerosene, for example, (containing fully fluorinated hydrocarbon molecules – *ie* no hydrogen atoms remain) can be used as a reference to calibrate the instrument. Many peaks are observed, separated by 12 or 50 mass units *(Fig. 9)*. Once calibrated, the spectrometer can function without recalibration for about one month.

More detailed information (higher resolution data) can be obtained by putting a reference material in with the sample so that the instrument can be calibrated at the same time as analytical data are obtained. The reference will depend on the mass of the compound, but is likely to be perfluorokerosene with a mass range appropriate to the approximate mass of the unknown. (Mass ranges of perfluorokerosene are

available in the same way as boiling point ranges of petroleum ether.) The peaks due to the reference material are used for calibration, and are then subtracted from the final spectrum by a computer (the data system). It is unlikely that the reference peaks will coincide with the sample peaks because fluoro compounds tend to have masses lower than the nominal mass, whereas most other compounds have higher masses.
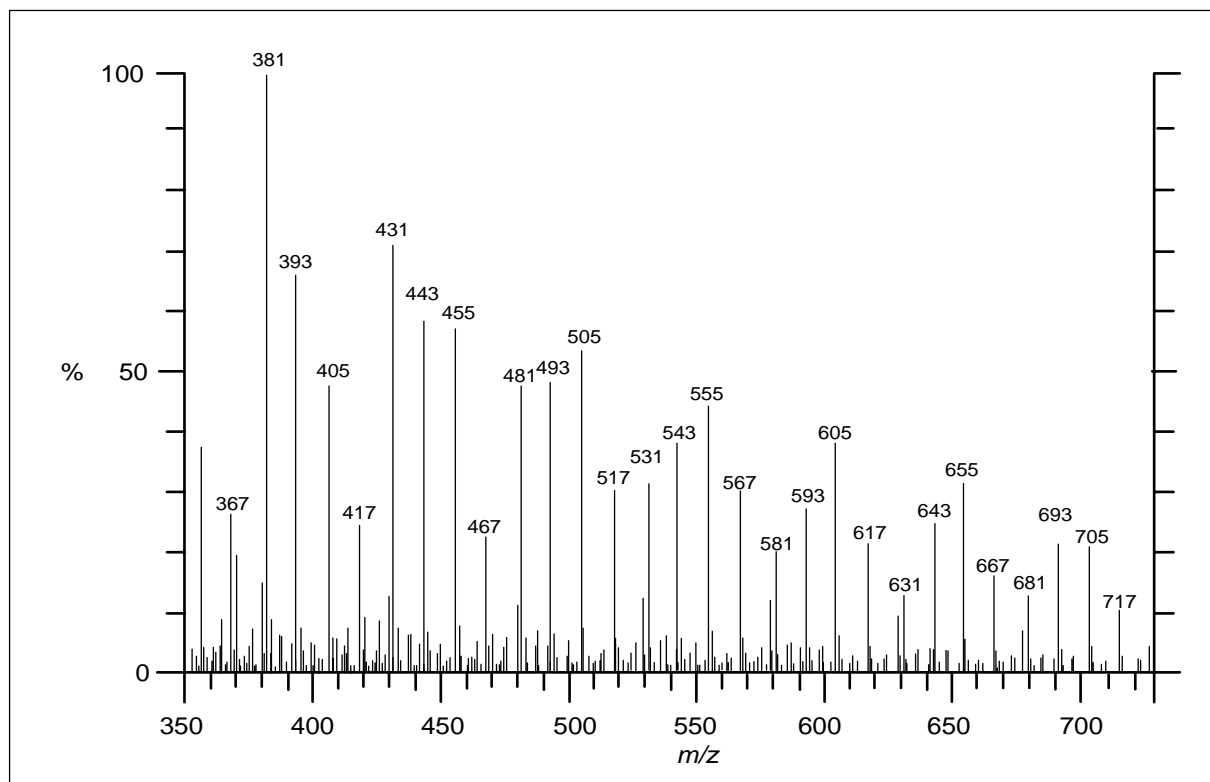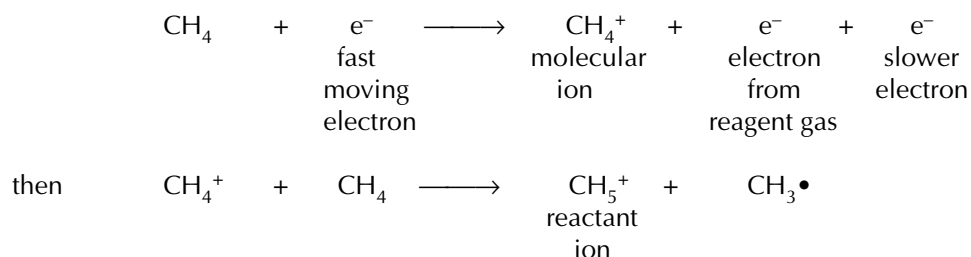

Figure 9   Mass spectrum of a perfluorokerosene, used for calibrating a mass spectrometer

Other ionisation techniques

Electron impact frequently fails to yield the information required from substances. A variety of alternative methods for ionising substances exist.

Chemical ionisation (CI)

In this technique a reagent gas such as methane, methylpropane or ammonia is ionised by electron bombardment and is then allowed to react with a neutral molecule to produce a molecular ion, *eg*

$$CH_4 \quad + \quad e^- \quad \longrightarrow \quad CH_4^+ \quad + \quad e^- \quad + \quad e^-$$

		fast		molecular		electron		slower
		moving		ion		from		electron
		electron				reagent gas		

then $CH_4^+ \quad + \quad CH_4 \quad \longrightarrow \quad CH_5^+ \quad + \quad CH_3\bullet$

reactant
ion

The reactant ion, CH_5^+ in this case, then reacts with the sample gas and protonates it:

$$M \;+\; CH_5^+ \longrightarrow MH^+ \;+\; CH_4$$
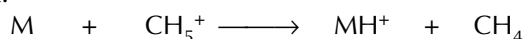
In this way positive ions are produced, but these are one mass unit higher than the parent molecule. The ions produced almost always have less internal energy than ions formed by electron impact, and thus fragment less as a consequence.

In practice the reagent gas is allowed into the ion chamber at a partial pressure of 10^2 Nm^{-2} (Pa). The amount of sample in the ion source can be very small – on the nanogram scale. The bombarding electrons come from a hot filament, and their energy is in the range 9650–48250 kJ mol^{-1} (100–500 eV) – higher than for electron impact. Statistically the reagent gas is more likely to be ionised than the sample.

The choice of reagent gas depends on the ease of fragmenting the sample. The internal energy of the species MH^+ decreases in the order $CH_5^+ > C_4H_9^+ > NH_4^+$. This results from the relative strength of the bonding between the reagent gas and the proton it transfers to the sample: ammonia bonds most strongly and methane least. Consequently, if the ammonium ion is the protonating agent it transfers very little energy to the sample. Conversely, the CH_5^+ ion readily protonates samples and the energy liberated is transferred as the internal energy of the sample – it is then likely to fragment more extensively. However, one disadvantage of using methane or methylpropane as the reagent gas is that the spectra obtained are complicated above the $(M+H)^+$ molecular ion because adducts such as $(M+CH_3)^+$ and $(M+C_2H_5)^+$ are also formed.

The difference between the spectra obtained by electron impact and chemical ionisation is immediately apparent in *Figs. 11 a* and *b*, where the spectra of Tenormin (*Fig. 10*) a drug produced by ICI, used for treating heart conditions are shown.
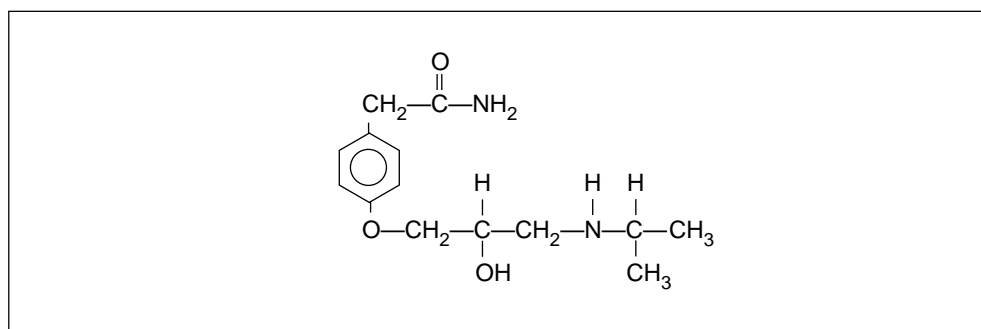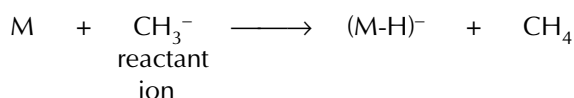

Figure 10   Structure of Tenormin

Under CI conditions equal abundances of positive and negative ions are usually produced. The processes involved in negative ion formation are complicated, but one way that they can be formed is:

$$M \;+\; \underset{\substack{\text{reactant}\\ \text{ion}}}{CH_3^-} \longrightarrow (M\text{-}H)^- \;+\; CH_4$$

The molecular ion peaks obtained by CI are predominantly $(M+H)^+$ and $(M–H)^-$. One major advantage of negative ion chemical ionisation is that some compounds can be detected at pico- (10^{-12}) or femto- (10^{-15}) gram levels.

Clearly the potentials inside the spectrometer have to be altered to enable negative ion spectra to be measured, but good, reproducible data can be recorded.
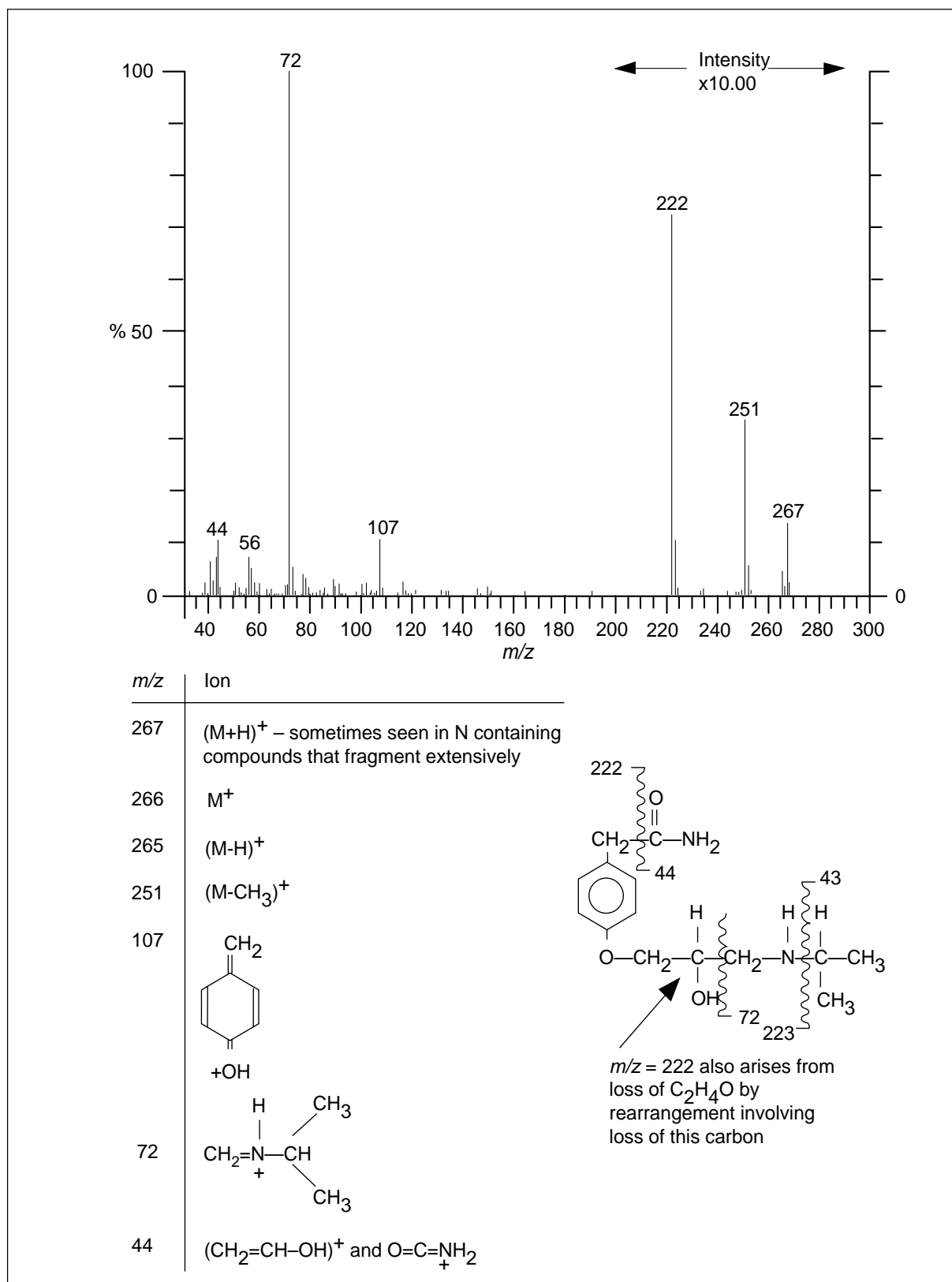
m/z	Ion
267	$(M+H)^+$ – sometimes seen in N containing compounds that fragment extensively
266	M^+
265	$(M-H)^+$
251	$(M-CH_3)^+$
107	
72	
44	$(CH_2=CH-OH)^+$ and $O=\underset{+}{C}=NH_2$

m/z = 222 also arises from loss of C_2H_4O by rearrangement involving loss of this carbon

Figure 11a   Mass spectrum of Tenormin using electron impact.

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m/z	Ion
323	$(M+C_4H_9)^+$
309	$(M+C_3H_7)^+$
267	$(M+H)^+$
72	

323 and 309 — Adducts formed between Tenormin and the reagent gas, methylpropane (isobutane)

For m/z 72:

$$CH_2=\overset{H}{\underset{+}{N}}-\underset{\diagdown}{\overset{\diagup}{CH}}\begin{matrix}CH_3\\CH_3\end{matrix}$$
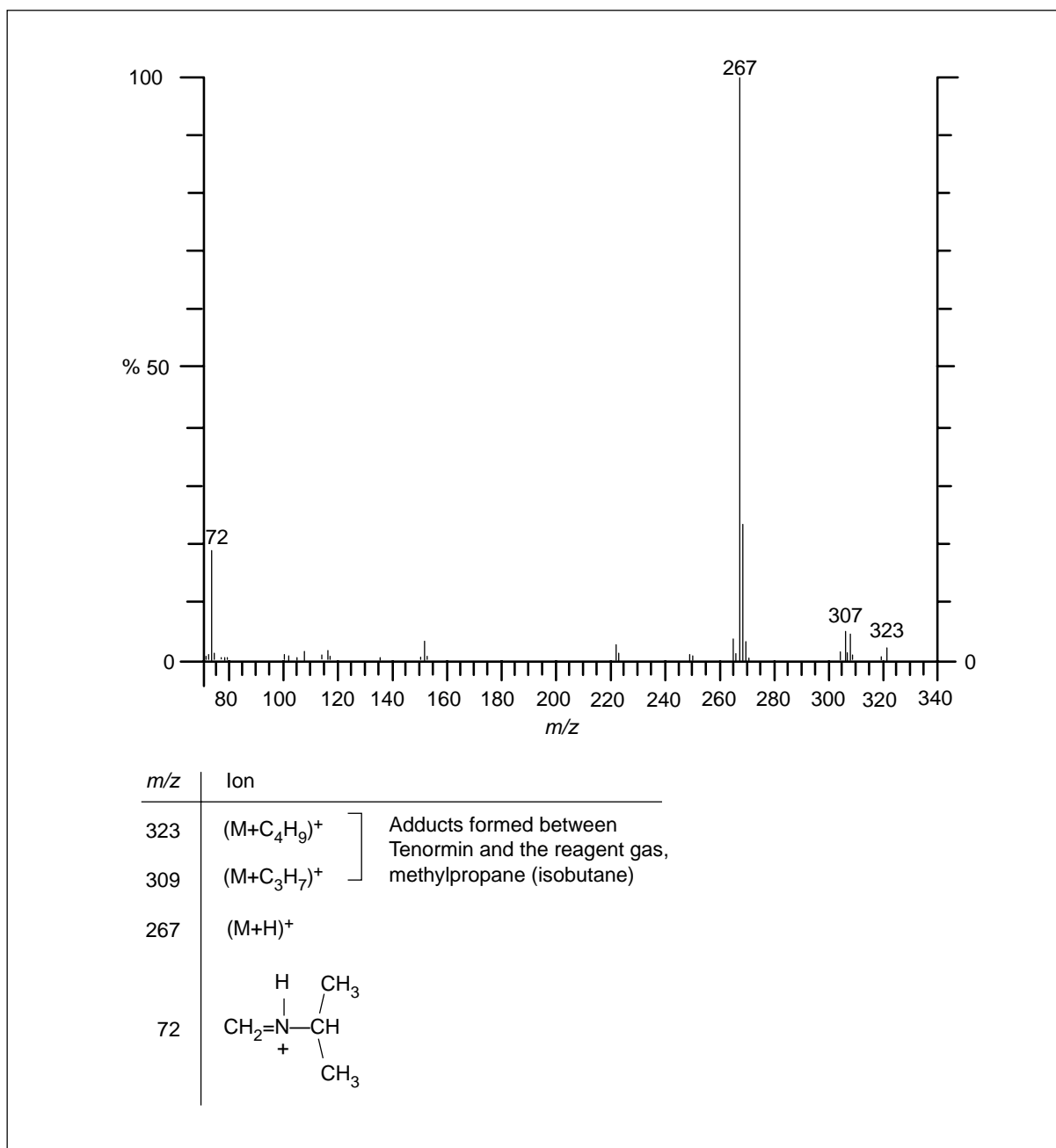
Figure 11b Mass spectrum of Tenormin using chemical ionisation
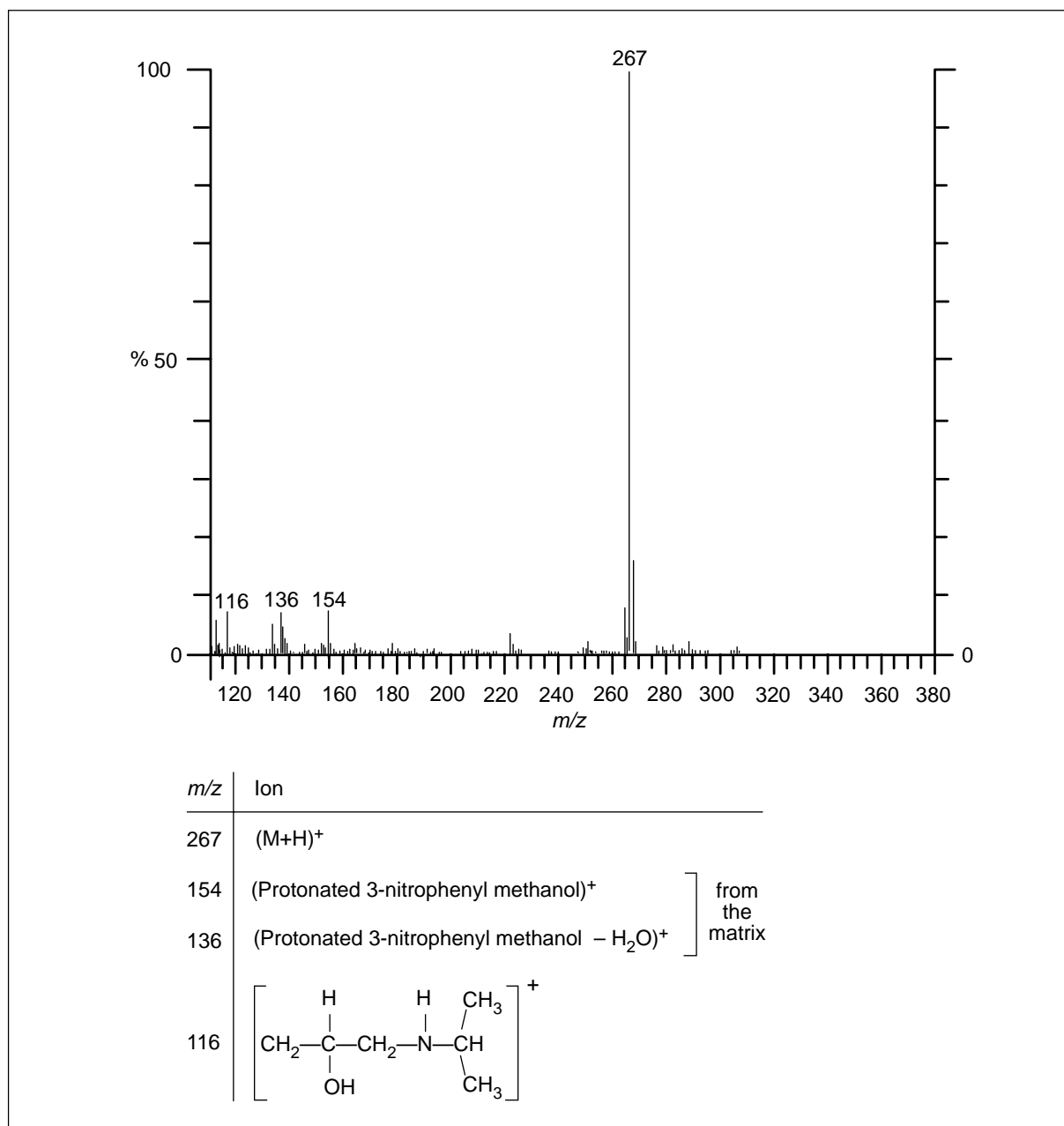
Figure 11c   Mass spectrum of Tenormin using fast atom bombardment

Fast atom bombardment (FAB)

If the sample is susceptible to decomposition when heated, FAB is a useful technique because it involves no heating at all. A beam of atoms with high kinetic energy, usually xenon atoms with several keV energy, is used to strike a solution of the sample in a 'matrix' compound such as glycerol (propan-1,2,3-triol) or 3-nitrophenyl methanol (*m*-nitrobenzyl alcohol), on the end of a probe (*Fig. 12*).
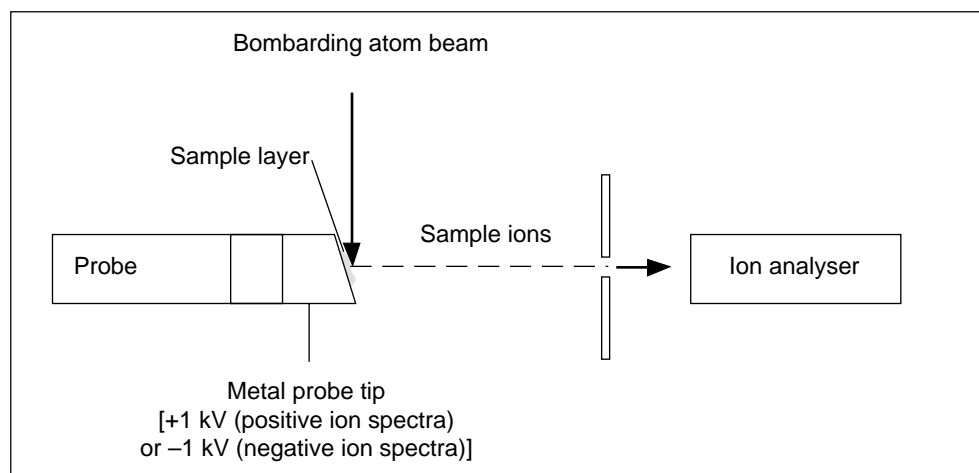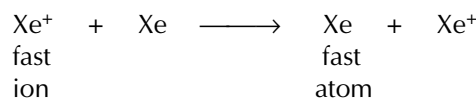
Bombarding atom beam

Sample layer

Sample ions

Probe

Ion analyser

Metal probe tip
[+1 kV (positive ion spectra)
or −1 kV (negative ion spectra)]

Figure 12   Fast atom bombardment – the ion source

The fast atoms are generated by accelerating xenon ions to 6–9 keV, then neutralising them as they pass xenon atoms at low pressure. Neutralisation takes place by electron transfer:

$$Xe^+ \quad + \quad Xe \quad \longrightarrow \quad Xe \quad + \quad Xe^+$$
$$\text{fast} \qquad\qquad\qquad\qquad\qquad \text{fast}$$
$$\text{ion} \qquad\qquad\qquad\qquad\qquad\; \text{atom}$$

When the sample is struck by the fast atoms, it is desorbed from the surface of the probe by the transfer of momentum, usually as an ion. In common with chemical ionisation the sample molecule in FAB is usually detected as $(M+H)^+$ or as $(M–H)^-$. The ions produced are analysed in the mass spectrometer in the same way as ions produced by other methods. The FAB mass spectrum of Tenormin is shown in *Fig. 11c*.

Salts of the type AX can be examined by FAB, and the A^+ ions are detected (usually as AXA^+) in the positive ion mode, and the X^- ions in the negative ion mode.

Interpretation of the mass spectrum of a compound

With the aid of a computer it is possible to obtain information from the spectrum quickly – *eg* the mass of the molecular ion peak can be measured with sufficient precision to be able to assign the molecular formula directly. Libraries of mass spectra are commercially available on computer software, and the spectra of all the library compounds with the same molecular formula can be called up for comparison. The first spectrum in *Fig. 8* is of an unknown compound with a molecular ion peak corresponding to the formula $C_7H_6O_2$, and with it are the library spectra of five isomers of this formula. Although the computer can be used to compare one spectrum with another it is not necessary in this case. The peak at *m/z* = 105 (corresponding to the $C_6H_5CO^+$ ion) is sufficient to be able to eliminate all the isomers except benzoic acid. However, the spectrum of the unknown does not always correspond exactly to the library spectrum. This is entirely normal because the operating conditions under which the sample and the library compound were recorded might not have been exactly the same. Make, and to a greater extent, type of instrument, can influence the final spectrum. Unless there is a significant difference in the two spectra the major peaks should be sufficient to identify the compound.

If the spectrum is of a new or commercially sensitive compound then it will not

appear in a commercial library, although the elemental composition can be determined. In these cases it is usual to confirm a suspected structure rather than try to determine the structure from first principles.

In confirming a suspected structure, or trying to elucidate a completely unknown structure, a rather different approach is used. The ratio of the two isotopes carbon-12 to carbon-13 ($^{12}C{:}^{13}C$) is 100:1.1, which is significantly greater than the isotopic ratios for hydrogen, nitrogen and oxygen. Therefore the isotopic peak at $(M+1)^+$ will be 1.1 per cent of the height of the molecular ion peak for every carbon atom present in the molecule. In the spectrum of benzoic acid *(Fig. 14)* the $(M+1)^+$ peak at $m/z = 123$ is approximately 8 per cent (7.7 per cent) of the height of the molecular ion peak. This is consistent with there being seven carbon atoms in the sample.

The presence of two other elements, chlorine and bromine, is usually easy to determine. The two isotopes of chlorine have mass numbers 35 and 37, and occur naturally in the ratio 76:24. Thus the appearance of two molecular ion peaks differing by 2 mass units, and in the approximate ratio 3:1 suggests the presence of a chlorine atom in the compound. Bromine has isotopes of relative masses 79 and 81 in approximately 1:1 ratio (50.5:49.5), so two molecular ion peaks of similar height differing by 2 mass units indicates the presence of a bromine atom. Compounds with more than one chlorine and/or bromine atom give the molecular ion and ion fragment peaks with the patterns shown in *Fig. 13*. All the peaks differ by 2 mass units.
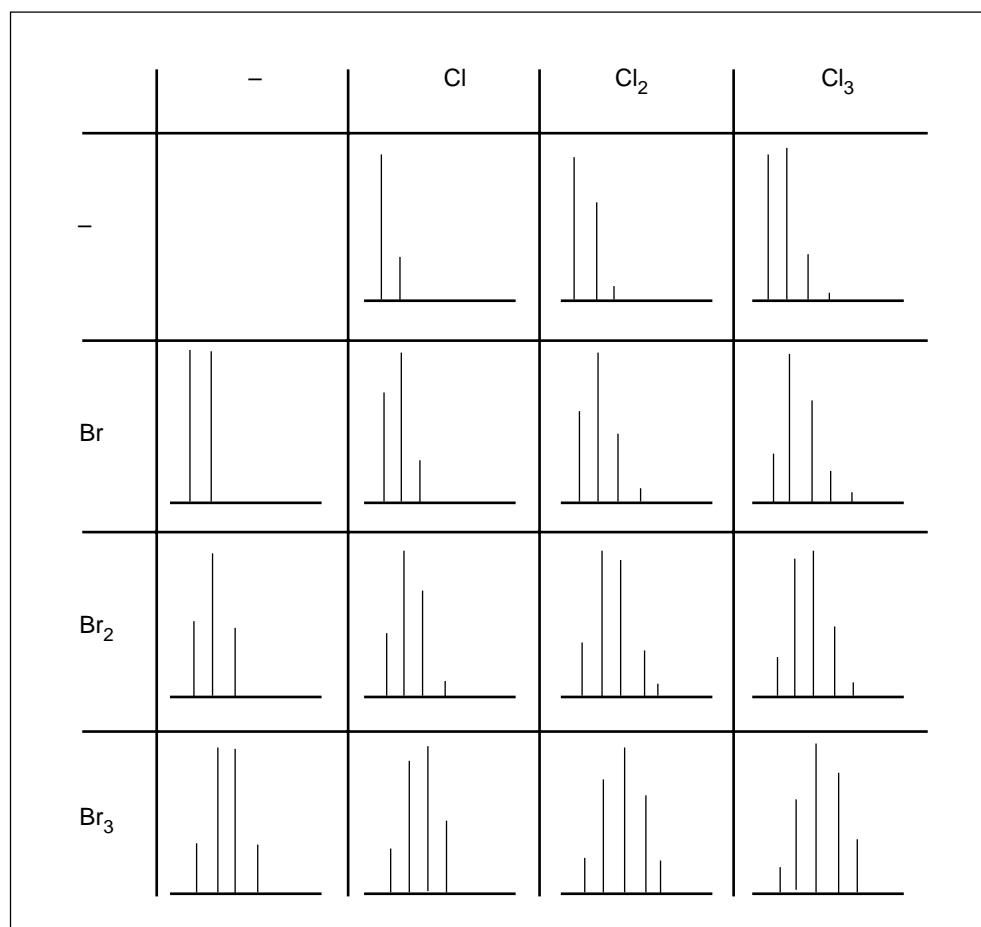

Figure 13   Peak patterns due to the presence of chlorine and bromine atoms in molecular ions and ion fragments

In the absence of chlorine and bromine atoms, if the relative mass of the molecular ion is odd, then probably an odd number of nitrogen atoms is present; and if the relative mass is even then either nitrogen is not present, or there is an even number of nitrogen atoms.

Once some idea of the molecular composition has been calculated an attempt can be made to determine the structure of the compound. This is not always easy because without some prior knowledge of the structure it is difficult to work out exactly what has been lost from the molecular ion. However, there are some groups that are commonly lost from the molecular ion and some of these are shown in Table 2.

For compounds containing a benzene ring, monosubstituted compounds might be expected to give a peak at $m/z = 77$, corresponding to $C_6H_5^+$. While such a peak is often observed, bond fission occurs more frequently one bond away from the benzene ring.

Figures 14 and *15* show the mass spectra of benzoic acid and methyl benzoate with the assignment of the major peaks.
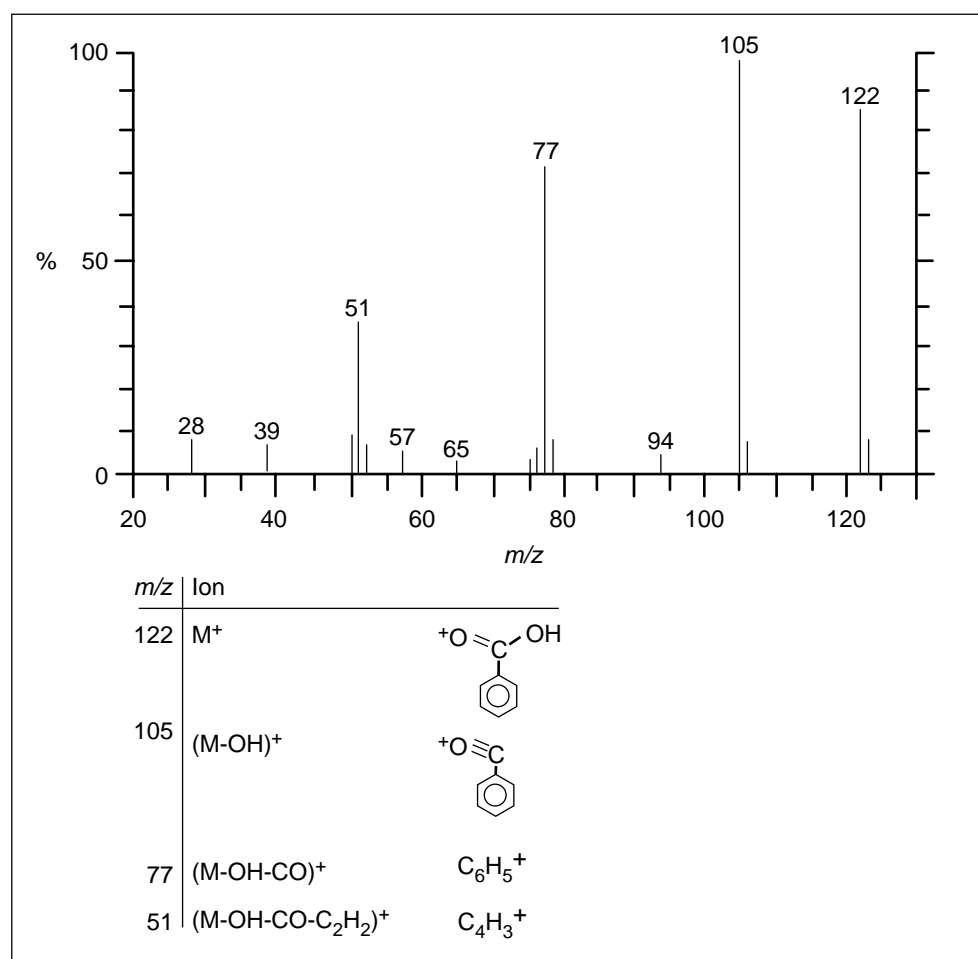

m/z	Ion	
122	M^+	$^+O \equiv C \diagup OH$ (benzene ring)
105	$(M-OH)^+$	$^+O \equiv C$ (benzene ring)
77	$(M-OH-CO)^+$	$C_6H_5^+$
51	$(M-OH-CO-C_2H_2)^+$	$C_4H_3^+$

Figure 14   Mass spectrum of benzoic acid
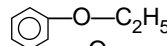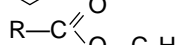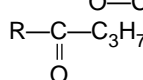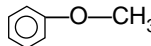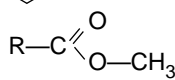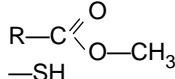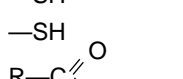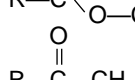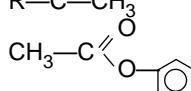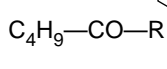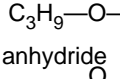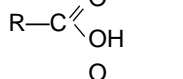
Table 2   Common losses from molecular ions

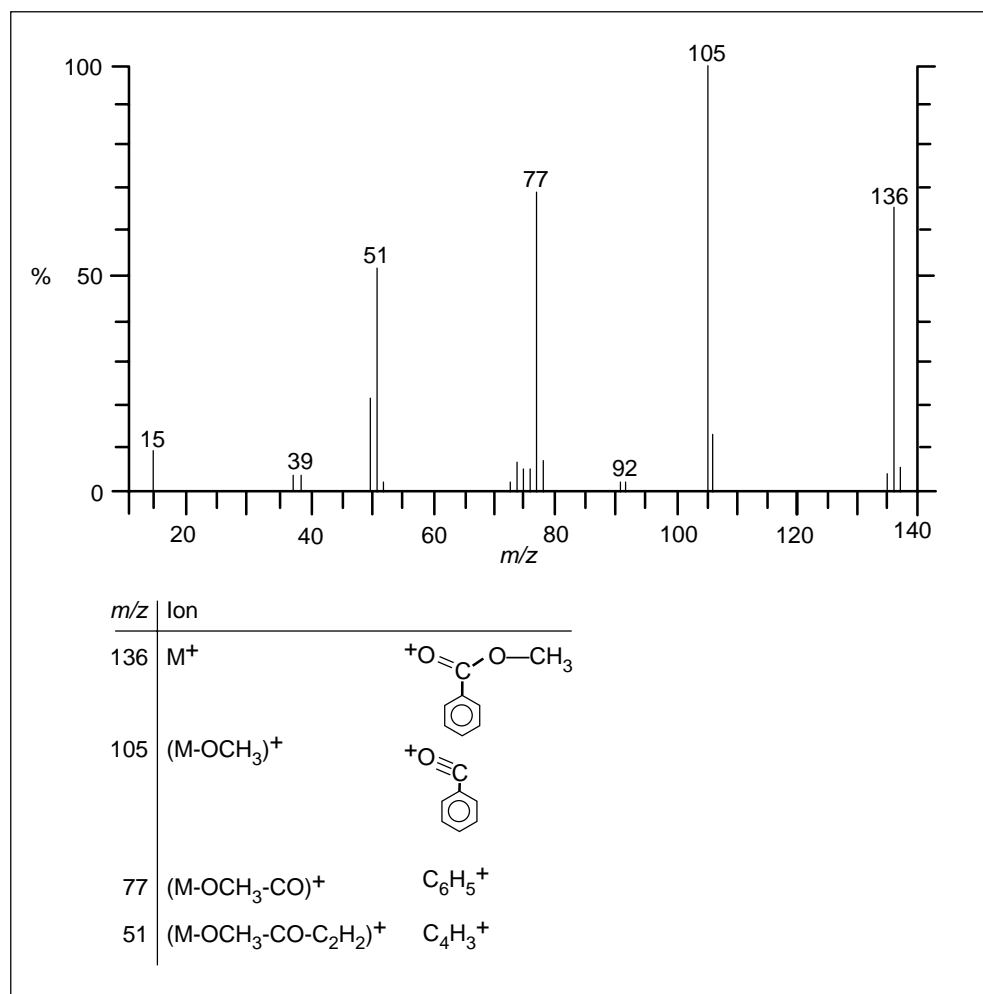| Ion	Groups commonly associated with the mass lost	Possible inference
M − 16	O	$\overset{+}{-}NO_2$, $=\overset{+}{N}-\overset{-}{O}$, $>S=O$
M − 16	NH_2	$ArSO_2NH_2$, $-CONH_2$
M − 17	NH_3	
M − 17	OH	Alcohol
M − 18	H_2O	Ketone, aldehyde, alcohol
M − 28	CO	Quinone
M − 28	C_2H_4	Aromatic ethyl ether $\bigcirc\!\!\!-O-C_2H_5$
		Ethyl ester $R-C\!\!\underset{O-C_2H_5}{\overset{O}{\diagup}}$
		Propyl ketone $R-\underset{O}{\overset{\|}{C}}-C_3H_7$
M − 29	CHO	Aldehyde $R-CHO$
M − 29	C_2H_5	Ethyl ketone C_2H_5-CO-R
M − 30	C_2H_6	
M − 30	OCH_2	Aromatic methyl ether $\bigcirc\!\!\!-O-CH_3$
M − 30	NO	$Ar-NO_2$
M − 31	OCH_3	Methyl ester $R-C\!\!\underset{O-CH_3}{\overset{O}{\diagup}}$
M − 32	CH_3OH	Methyl ester $R-C\!\!\underset{O-CH_3}{\overset{O}{\diagup}}$
M − 33	HS	Thiol $-SH$
M − 34	H_2S	Thiol $-SH$
M − 41	C_3H_5	Propyl ester $R-C\!\!\underset{O-C_3H_7}{\overset{O}{\diagup}}$
M − 42	CH_2CO	Methyl ketone $R-\underset{O}{\overset{\|}{C}}-CH_3$
		Aromatic ethanoate $CH_3-C\!\!\underset{O-\bigcirc}{\overset{O}{\diagup}}$
M − 42	C_3H_6	Butyl ketone C_4H_9-CO-R
		Aromatic propyl ether $C_3H_9-O-\bigcirc$
M − 44	CO_2	Carboxylic acid, ester, anhydride
M − 45	CO_2H	Carboxylic acid $R-C\!\!\underset{OH}{\overset{O}{\diagup}}$
M − 46	C_2H_5OH	Ethyl ester $R-C\!\!\underset{O-C_2H_5}{\overset{O}{\diagup}}$
M − 46	NO_2	$Ar-NO_2$
M − 48	SO	Aromatic sulphoxide

Figure 15   Mass spectrum of methyl benzoate

Applications of mass spectrometry

So far discussion of the application of mass spectrometry has been restricted to determining relative atomic/isotopic mass and identification of organic compounds. However, there are other applications. The mass spectrometer is sufficiently sensitive to measure the isotopic composition of substances accurately. Although the isotopic composition of elements is usually regarded as constant, there are some variations. Atmospheric oxygen, for instance, is richer in ^{18}O than fresh surface water. Hydrogen, carbon, oxygen and sulphur are just four elements whose isotopic compositions in different locations have been found to vary, and these variations have been studied using the mass spectrometer.

Mass spectrometer applications are also found in medicine and biochemistry. Many of the substances found in living systems are complex mixtures and are sensitive to fragmentation by using EI. Separation of individual compounds is done by using gas chromatography (GC) or high performance liquid chromatography (HPLC) and the mixture from the column (the eluate) is fed into a mass spectrometer (see chapter 5). Once inside the spectrometer soft ionisation methods are frequently used to ionise the sample. For example, FAB is often used to determine the residue sequence of peptides. Similarly, radioactively labelled compounds and compounds labelled with stable isotopes such as ^{13}C are used to determine the fragmentation

mechanisms of organic molecules and extended studies have led to the identification of metabolic pathways. Labelled drugs allow pathways of drug metabolism to be studied on a picomole scale.

Examples of GC-MS studies (gas chromatography interfaced to a mass spectrometer) include:

1   drug abuse by athletes; oestrogens in the urine of pregnant females;

2   determination of prostaglandins in human semen;

3   the presence of ergotamine (an alkaloid) and other hallucinogens in blood;

4   the separation and identification of the compounds responsible for giving foodstuffs their aromas and tastes;

5   proteins labelled with ^{15}N-glycine;

6   the identification of bile acids in the gall bladder of an Egyptian mummy;

7   characterising tar from the Mary Rose;

8   the separation and detection of urinary acids thought to be connected with sudden infant death syndrome (SIDS) or cot death; and

9   the highly specific and sensitive quantitative procedure for the determination of residues or contaminants in foodstuffs thereby facilitiating increased safety.

Mass spectrometry is a powerful technique but it does have some disadvantages; for example: optical isomers cannot be distinguished from their spectra (although *cis/trans* isomers sometimes can); and although only small samples are required for analysis the technique is destructive, and the sample cannot be recovered.

Applications also exist in environmental analysis. Although concentration methods are sometimes required to supply the spectrometer with sufficient sample to analyse, GC-MS is sensitive enough to detect pollutants such as polychlorinated biphenyls (PCBs), which are found in surface water in concentrations on the nanogram per dm^3 (ng l^{-1} or 10^{-9} g l^{-1}) scale. It is important that they are detected because they do not degrade in nature and build up in the food chain. Some seals, for example, have been found to have several per cent PCBs in their total fat content.

Monitoring for dioxin can also be done by using mass spectrometry. Dioxin can be found in the effluent stacks of incinerators, and is a most undesirable substance in the environment because of its toxicity and stability.

Mass spectrometers have been adapted for use on board spaceships, where less attention has to be paid to the inclusion of vacuum pumps because the atmosphere is already at low pressure. Indeed, in some cases the atmosphere has been sampled using an open source. The exhaust gases of the space rocket must be avoided, of course, as must the emissions from manned modules. The Viking space craft that landed on Mars was equipped with GC-MS, and both the Martian atmosphere and soil were analysed (it was found that the partial pressures of water, nitrogen and carbon dioxide are very low, and that the soil contained a lot of chlorine and sulphur).

Biomarkers in the petrochemical industry

Biomarkers are organic compounds whose carbon skeletons provide an unambiguous link with a known natural product, and these are particularly useful in the petrochemical industry.

When the marine organisms that formed crude oil died, their bodies were subjected to extreme heat and pressure, resulting in the formation of the oil. In the chemically reducing environment present many of the natural products – *eg* steroids

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and terpenoids – were saturated. These saturated products are now present in crude oil, and serve as biomarkers. It is thus possible to identify the organisms from which the oil was formed, enabling the terrain at the time of formation to be deduced. Once the biomarkers from a particular oilfield have been identified it is also possible to determine where an oil sample originated. Thus, if an oil spillage occurs at sea and nobody admits responsibility, the biomarkers could be used to identify its source. It may then be a simple matter to find out which tankers were carrying oil from that field at the time of the incident, and so identify the culprit.

Identifying the biomarkers is done by separating the components of the crude oil using gas chromatography (see page 123) and feeding them directly into the chamber of a mass spectrometer. Once the components have been identified, by their retention times on the chromatography column, and their fragmentation patterns, the biomarkers can be detected on subsequent occasions by selective ion monitoring, in which only the characteristic peaks are looked for.

Geological dating

Naturally occurring potassium contains 0.01167 per cent of the radioactive isotope ^{40}K. One of its daughter products is ^{40}Ar. If a rock sample contains potassium, and the argon formed by the decay of ^{40}K is unable to diffuse out of the crystal, it is possible to determine the age of the rock sample.

The argon content of a sample is determined by using a mass spectrometer to analyse the gas released when the sample is heated to melting in a vacuum. It is assumed that none of the argon produced *in situ* diffuses out of the crystal lattice of the rock, and that there is no loss or gain of potassium – *ie* it is assumed that the sample has remained a closed system since its formation.

A tracer of ^{38}Ar is used to determine the ^{40}Ar in a method known as isotope dilution, in which the unknown has a reference sample added against which it can be measured. The total remaining potassium can be measured by using several methods, including flame photometry, atomic absorption spectrometry and isotope dilution.

It is possible that some atmospheric argon is trapped in geological specimens, and this will register on the mass spectrum once it has been released from the crystal lattice. Atmospheric argon consists predominantly of three isotopes, ^{36}Ar, ^{38}Ar and ^{40}Ar. The latter isotope will clearly interfere with the results expected from the rock sample, but this can be compensated for from a knowledge of the natural abundances of the isotopes – the ratio of ^{40}Ar:^{36}Ar is 295.5:1. Thus the peak at m/z = 40, due to the argon in the rock, can be calculated.

By using the half life of ^{40}K and the radiogenic ^{40}Ar value, the amount of potassium that has decayed can be determined. From a knowledge of the ratio of naturally occurring isotopes it is then possible to determine how much ^{40}K was originally present. From this the proportion that has decayed can be calculated, and hence the age of the sample can be found. This is known as potassium argon dating (*Fig. 16*).

Recent trends

Improved spectrometer technology over the past decade has made detection of smaller and smaller concentrations of materials possible. Electron multipliers in the detectors of spectrometers can now achieve a gain of 10^7 electrons per ion, and instruments are available that can routinely measure elemental compositions down to $10^{-12}g\ g^{-1}$ or $10^{-12}g\ cm^{-3}$ (pg g^{-1} or pg cm^{-3}) of sample. By interfacing other techniques with mass spectrometry it is possible to analyse solid, liquid and gaseous samples – often automatically.

Mass spectrometers employing a magnetic field are now being complemented, and in some cases superseded, by quadrupole spectrometers. The ions produced
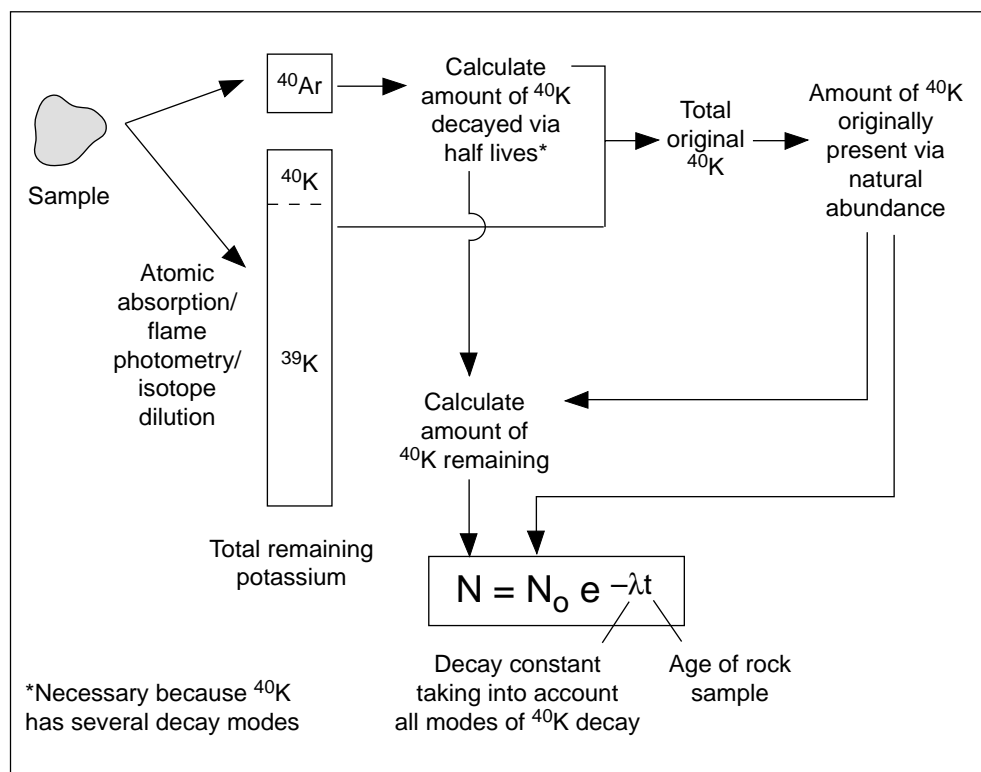
Figure 16   Schematic diagram of the potassium-argon dating method

travel down the gap between four rods through which a radiofrequency and a dc current are passed. The ion path in such spectrometers is helical. These have the advantage of being cheaper than magnetic sector instruments, but are not capable of such a high resolution. In theory, magnetic sector instruments will differentiate between ions of m/z values of 60 000 and 60 001, although in practice are likely to resolve 600.00 from 600.01 or 60.000 from 60.001. However, quadrupole spectrometers can only produce unit resolution (*ie* 60 and 61).

The size and cost of quadrupole instruments has decreased significantly in recent years, and benchtop models are now available. The lower potentials necessary in these instruments mean that they are much less prone to arcing inside the spectrometer. They are frequently interfaced to other techniques, such as gas chromatographs, where the presence of ions at particular nominal mass values is sought.

In the future this technique will probably be more accessible to non-specialists who need specific data quickly, perhaps without the detailed interpretation a skilled spectrometrist could give.

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Exercises

Below are the mass spectra of some organic compounds. Try to determine their structures. Interpretations are given.
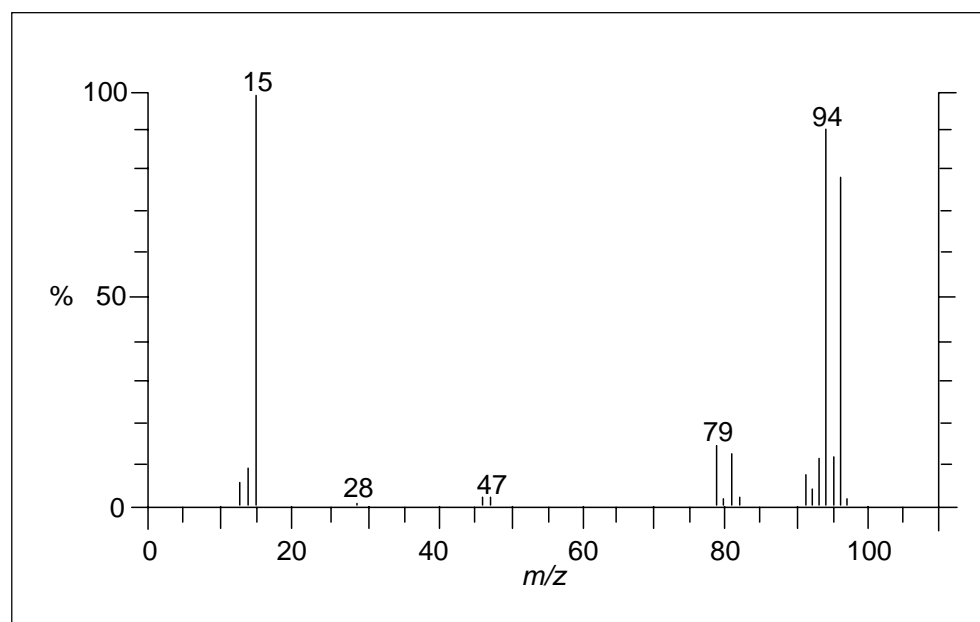
Exercise 1

Figure 17

The peaks of similar abundance, at m/z = 94 and 96, are likely to be isotope peaks. Their relative proportions and their mass difference of two would suggest Br. This is supported by the peaks at m/z = 79 and 81. Subtracting 79 from 94 or 81 from 96 leaves 15, which corresponds to CH_3. The peaks at m/z = 12-14 correspond to the loss of successive protons from this group, and the peaks at m/z = 91, 93 and 95 correspond to the loss of protons from the molecular ion. The peak at m/z = 94 is larger than the peak at 96 because it comprises $CH^{81}Br^+$ and $CH_3^{79}Br^+$, as well as the ^{79}Br isotope being very slightly more abundant. Therefore, we can conclude that the compound is bromomethane, CH_3Br.

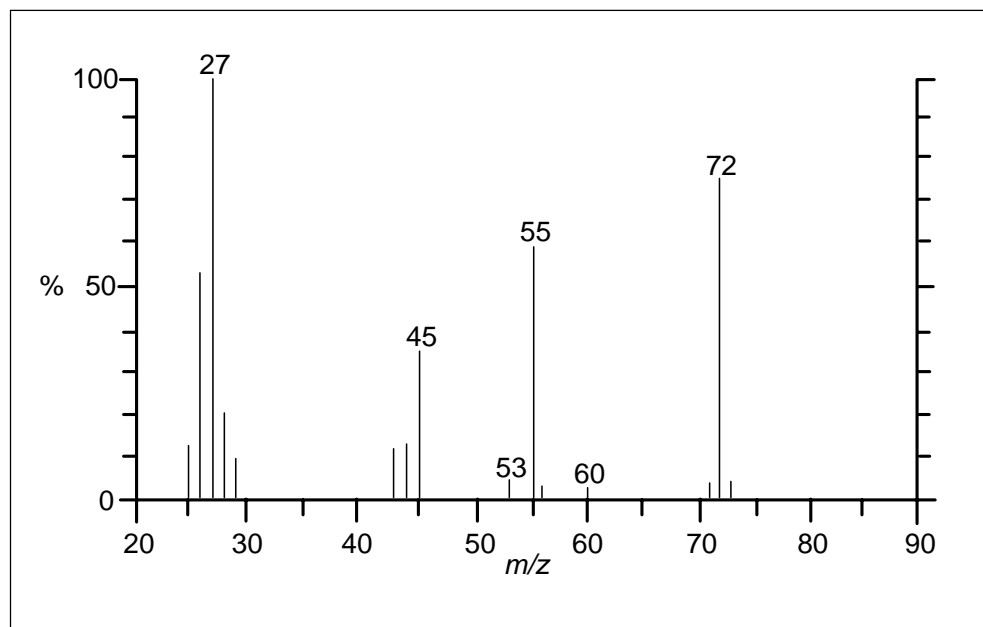The peak at m/z = 97 is due to the $^{13}CH_3\ ^{81}Br^+$ ion.

Exercise 2

Figure 18

No isotopic cluster due to a halogen is present, and the peak at $m/z = 73$, *ie* $(M+1)^+$, suggests that 2.7 per cent ÷ 1.1 per cent = 2.5 carbon atoms are present. If three carbon atoms are present, 36 of the 72 mass units remain and either two oxygens, two nitrogens or one of each is likely to be present. One nitrogen is unlikely because the relative formula mass is even. Formulae which fit are $C_3H_8N_2$ and $C_3H_4O_2$. The absence of a peak at M-16 eliminates a primary amine (NH_2 group). Cyclic and secondary amine structures are also unlikely because the peaks in the spectrum are not abundant enough to suggest either CH_3–NH masses or losses. This leaves us with two oxygen atoms to try to account for. The peak at $m/z = 55$ is M-17, which would correspond to the loss of an OH group. The peak at $m/z = 27$ corresponds to C_2H_3, CH_2=CH, and its abundance suggests its relative stability. This leaves us with C_2H_3, C, OH, and O to piece together. The possibility of a carboxylic acid group is confirmed by the peak at $m/z = 45$ ($COOH^+$). This suggests that the compound is propenoic acid (acrylic acid), CH_2=CHCOOH.
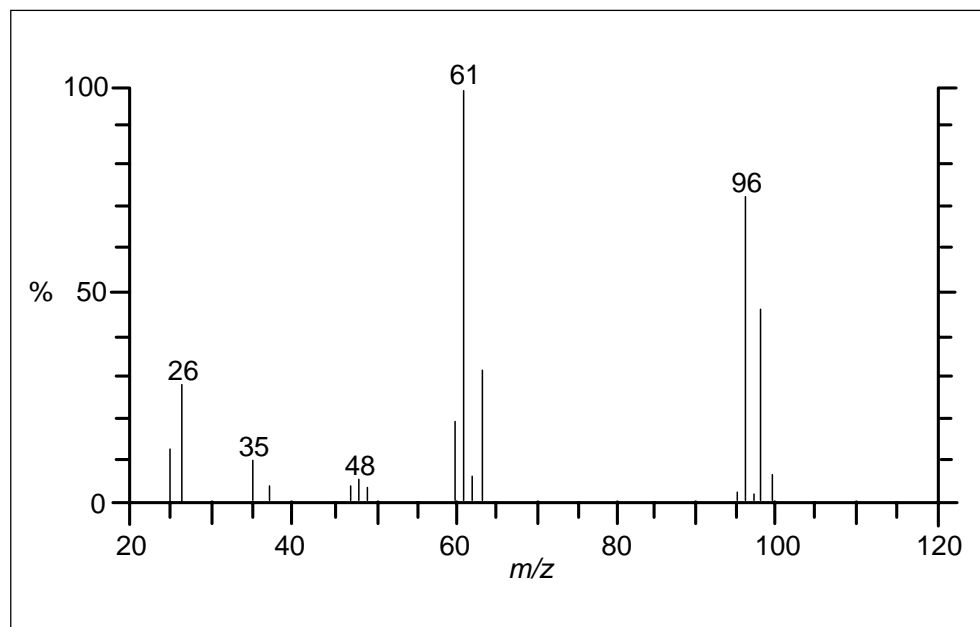
Exercise 3

Figure 19

The abundance of the peaks at m/z = 96 and 98 lead us to consider whether the peak at 100 is truly the molecular ion peak, rather than an isotopic peak. A glance at m/z = 35 and m/z = 37 shows the presence of peaks in the ratio 3:1, strongly suggesting chlorine. The abundance of the peaks at m/z = 96, 98 and 100 in the approximate ratio 9:6:1 suggests that two chlorine atoms are present. The peaks at m/z =61 and m/z = 63 (formed by the loss of one chlorine atom) have heights in the ratio of 3:1 – typical of the pattern expected for a single chlorine atom remaining in the molecule. This accounts for all but 26 mass units. The group C_2H_2 fits this, so the compound must be dichloroethene, $C_2H_2Cl_2$, but without further information it is impossible to decide which isomer it is (actually it is *trans*-1,2-dichloroethene).

Exercise 4

The two compounds are known to be 1-aminobutane, $CH_3CH_2CH_2CH_2NH_2$, (*A*) and
N,*N*-dimethyl aminoethane, $CH_3CH_2N(CH_3)_2$,(*B*). Try to decide which spectrum
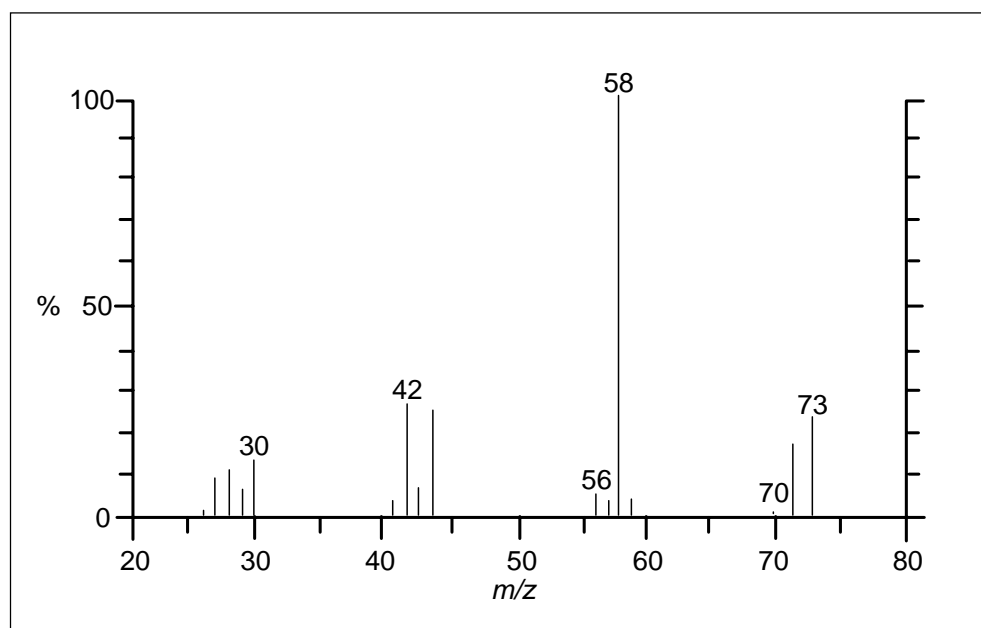corresponds to which isomer, and explain why the spectra appear as they are.
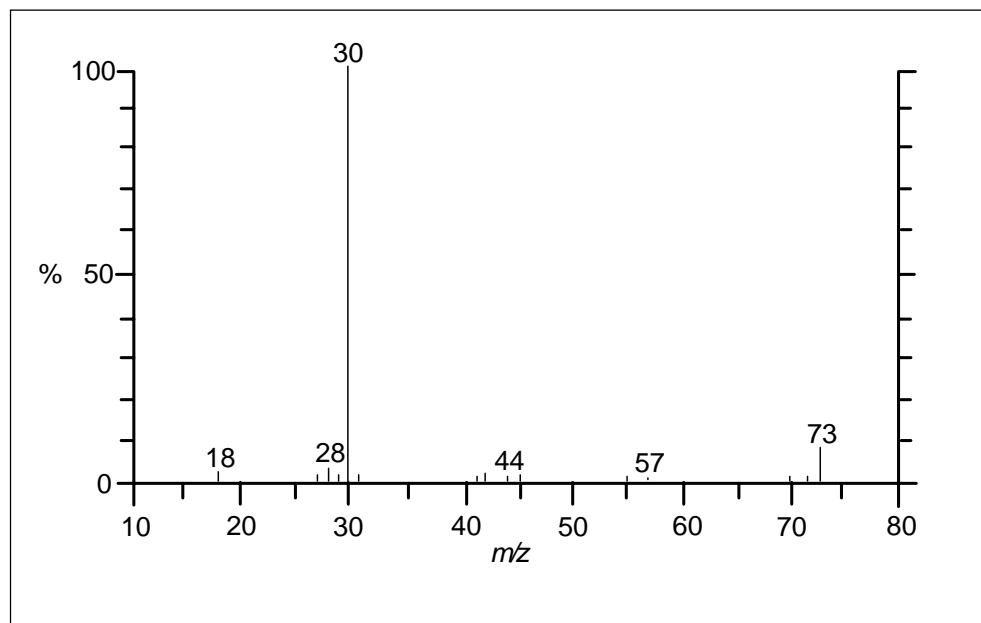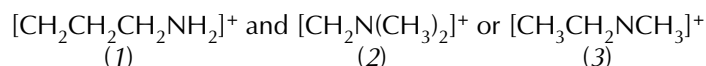

Figure 20

Figure 21

Although it might appear difficult to assign the spectra, it is not. What must be
considered is the relative stabilities of the ions that might be formed. Basically, a
positive charge on a highly substituted ion will be more stable than an ion with a

straight chain because the effect of the positive charge can be reduced by the inductive effect of the side chain groups. *Figure 20* shows a major peak at (M-15) – *ie* at $m/z = 58$. This corresponds to loss of a CH_3 group because loss of an NH group is highly unlikely in the case of (A) and impossible in the case of (B). The remaining ions would be:
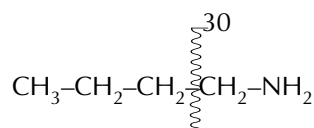
$$[CH_2CH_2CH_2NH_2]^+ \text{ and } [CH_2N(CH_3)_2]^+ \text{ or } [CH_3CH_2NCH_3]^+$$
$$(1) \qquad\qquad\qquad (2) \qquad\qquad\qquad (3)$$

Ion (*1*) would be less stable than ions (*2*) and (*3*), although it is not possible to say which of these two ions gives the peak on this information alone. A tentative guess at this stage would be that *Fig. 20* is the mass spectrum of the tertiary amine.

Figure 21 shows only one significant peak, at $m/z = 30$. The only sensible ions that can give this peak are $[CH_2NH_2]^+$, and $[CH_3NH]^+$. In neither case can (*B*) give these ions because it has no hydrogen atoms bonded to the nitrogen atom. Thus, the peak at $m/z = 30$ must be due to the $[CH_2NH_2]^+$ ion because there is no methyl group bonded to the nitrogen in (*A*) so *Fig. 21* is the mass spectrum of (*A*).

An experienced mass spectrometrist would be able to interpret the spectra directly from the knowledge that amines are susceptible to fragmentation one bond away from the C–N bond (the bond to the nitrogen) – *ie* (*B*) would fragment as follows:

$$
\begin{array}{c}
58 \qquad CH_3 \\
\quad / \\
CH_3{-}CH_2{-}N \\
\quad \backslash \\
CH_3
\end{array}
$$
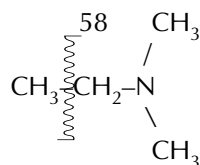
The peak at $m/z = 58$ in *Fig. 20* would then correspond to ion (*2*) above. Similarly (*A*) would fragment here:

$$CH_3{-}CH_2{-}CH_2{-}CH_2{-}NH_2 \qquad (30)$$

giving the peak at $m/z = 30$.