Analytical techniques (Chapter 22)

- In paper chromatography, solvent is called the **mobile phase**, while the water trapped between the cellulose fibres of the paper is called **stationary phase**
- Different rates of movements are due to the mixtures' solubilities towards the solvent and the water – different partition coefficients
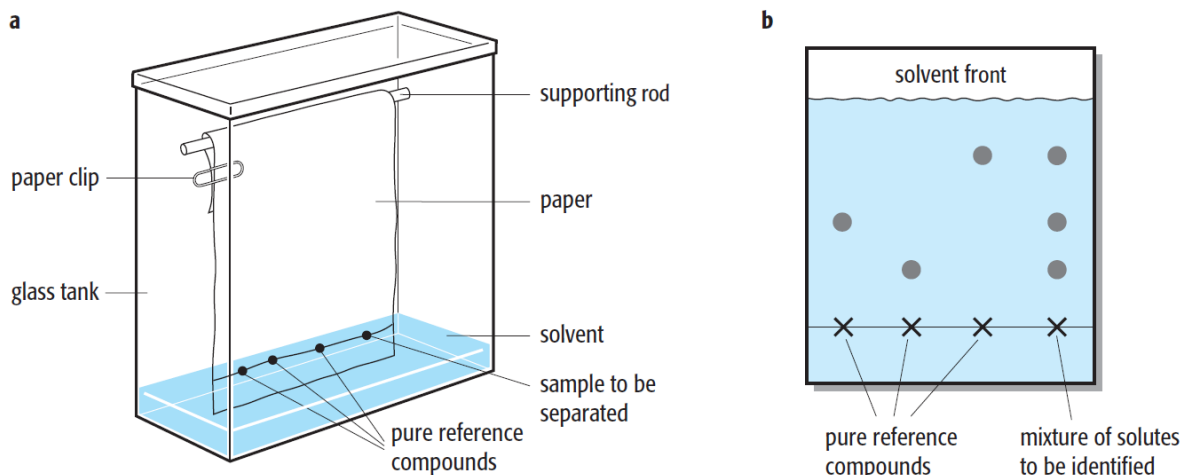
a

b

supporting rod

paper clip

paper

glass tank

solvent

sample to be separated

pure reference compounds

solvent front

pure reference compounds

mixture of solutes to be identified

Figure 29.2 a Paper chromatography. **b** The chromatogram produced. Components of the mixture can be identified by comparison with pure reference compounds or by calculating R_f values (see Figure 29.3) and comparing these values with those in tables of data.

- The R_f values (retardation factors) of substances, which is the ratio of the distance travelled by a particular component compared with the distance travelled by the solvent, are calculated in figure 29.3
- Sometimes two or more components in a mixture can have similar R_f values in a particular solvent, hence overlapping occurs; so **two-way chromatography** can be used (figure 29.4)
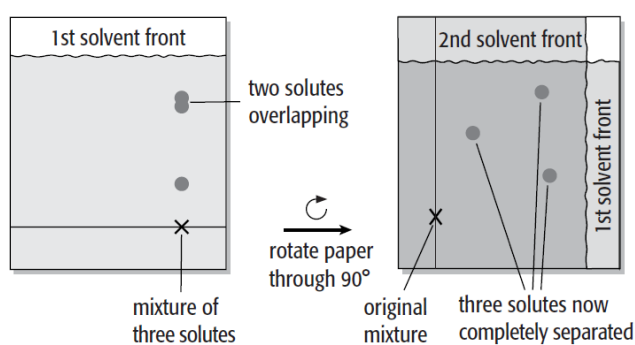
Making a two-way paper chromatogram

1st solvent front

two solutes overlapping

rotate paper through 90°

mixture of three solutes

original mixture

2nd solvent front

1st solvent front

three solutes now completely separated

Figure 29.4 Two-way paper chromatography to separate solutes with similar R_f values in a solvent. The technique can also be used with thin-layer chromatography (see page 436).
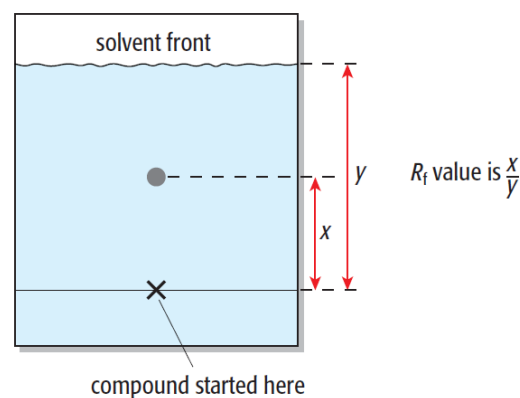

solvent front

y

x

R_f value is $\frac{x}{y}$

compound started here

Figure 29.3 How to calculate R_f values, which are then compared with reference values obtained under identical conditions.

- In **thin-layer chromatography (TLC)**, the solid stationary phase adsorbs solute molecules onto its surface
  - ➢ Faster than paper chromatography and can be used on smaller samples

➤ Polar molecules have greater attraction towards the stationary phase (adsorbed more strongly onto its surface); therefore travels more slowly resulting to separations; identified by calculating the R_f values
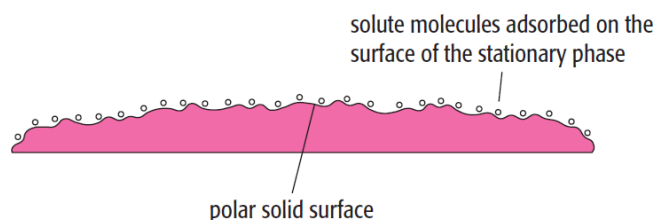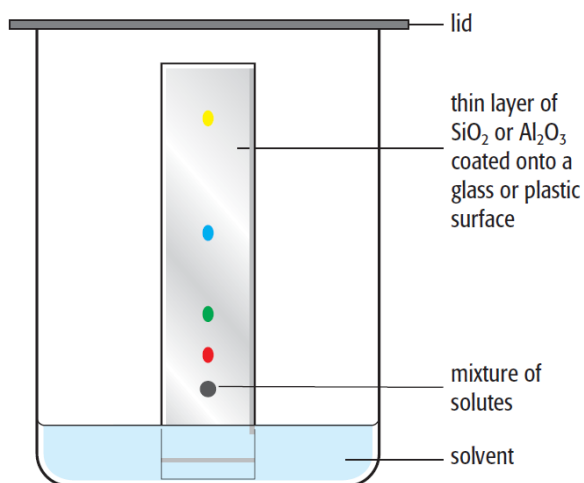

Figure 29.6 Adsorption chromatography. The mobile phase moves over the stationary solid phase.

- **Gas-liquid chromatography** (GLC) uses partitioning to separate and identify the components in a mixture; a gaseous sample enters the column, where the sample is moved through an inert gas carrier; method used for gases, liquids and volatile solids (vapour form)
  ➤ The detector detects the **retention time** (time taken for component to pass through the column); the area under each peak corresponds to the amount of solute emerging from the column
- The chromatogram obtained using the same carrier gas, flow rate, stationary phase and temperature
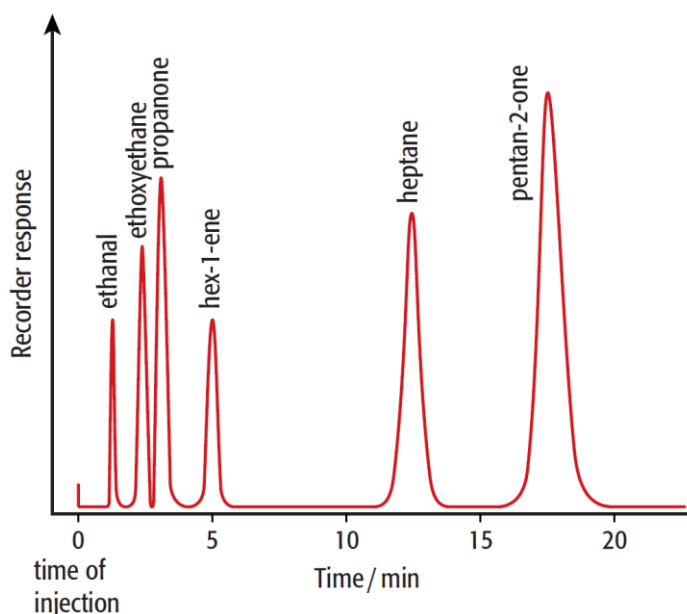- Figure 29.11 shows a chromatogram obtained using GLC:

Figure 29.11 A gas chromatogram from a mixture of volatile organic compounds.

- Determination of percentage composition of a mixture by GLC:
  ➤ The composition peaks are identified
  ➤ The areas under each peak recorded is proportional to the amount of solute emerging from the column
  ➤ The area of each is measured by (as they are roughly triangular):

$\frac{1}{2} \times \text{base} \times \text{height}$ (i.e. the area of a triangle)

➤ The percentage composition given by:

(approx.) % of ester A

$$= \frac{\text{peak area (or height) of A}}{\text{sum of the areas (or heights) of A, B and C}} \times 100$$

- **Low-resolution proton NMR spectrum**:
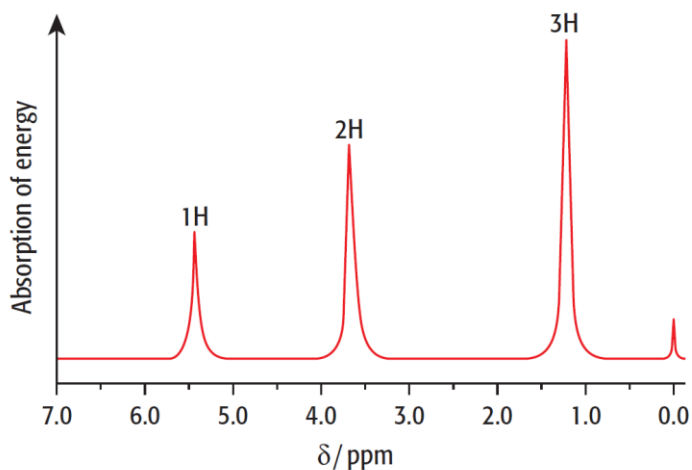 ➤ Shows a single peak for each non-equivalent hydrogen atom

Figure 29.17 The low-resolution NMR spectrum of ethanol, CH_3CH_2OH.

  ➤ The area under each peak tells us the relative number of equivalent ^1H atoms responsible for the particular chemical shift
  ➤ Largest peak: $-CH_3$ hydrogen atoms; middle peak: $-CH_2$ hydrogen atoms; smallest peak: $-OH$ hydrogen atoms
  ➤ The type of H atom present can be checked against tables of data (ppm value)
- **High-resolution proton NMR spectrum**:
  ➤ The splitting pattern of a peak depends on the number of hydrogen atoms on the adjacent carbon atom(s); using the $n + 1$ rule, where n is the number of ^1H atoms on the adjacent carbon atom
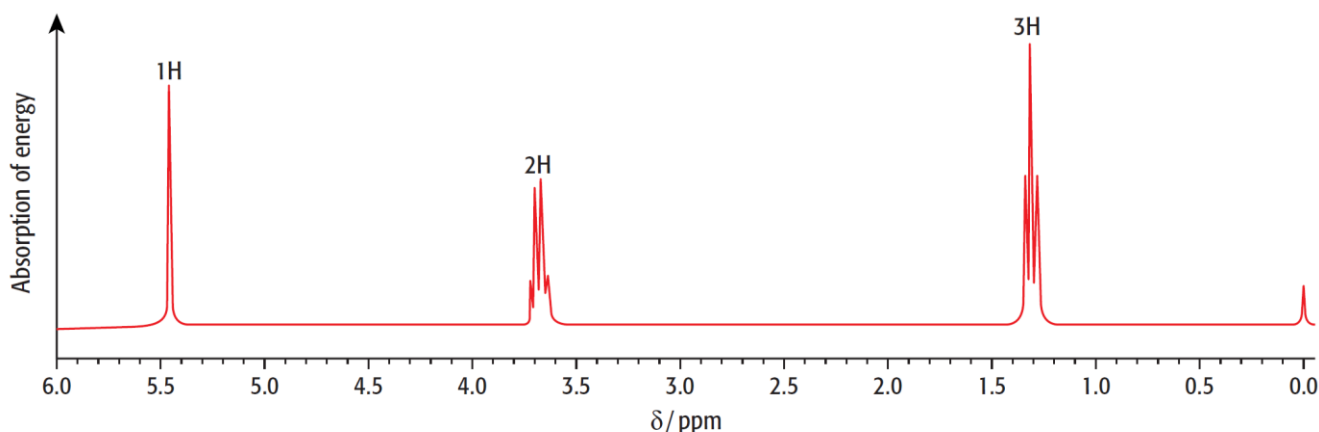 ➤ E.g. ethanol (CH_3CH_2OH)

Figure 29.18 The high-resolution NMR spectrum of ethanol, showing the splitting pattern in two of the peaks. The area under each series of peaks still represents the number of equivalent ^1H atoms in the molecule, as in low-resolution NMR.

  ➤ The CH_3 peak is split into three because there are two ^1H atoms on the adjacent CH_2 group; $n + 1 = 3$ (triplet)

> ➤ The CH_2 peak is split into four as there are three ^1H atoms on the adjacent CH_3 group; $n + 1 = 4$ (quartet)
> ➤ The –OH signal apeears as a single peak, as the –OH proton excahngws rapidly with protons in water present:

$$CH_3CH_2OH + H_2O \rightleftharpoons CH_3CH_2OH + HOH$$
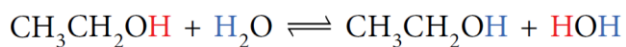
- ▪ Exchanges also occur with amines and amides which contain –NH– group
- ▪ These peaks will disappear from the spectra by the addition of deuterium oxide, D_2O, to the sample
  - • Exchanges reversibly with the protons in the –OH or –NH– groups
  - • D_2O atoms do not absorb in the same region of the electromagnetic spectrum as protons, hence the disappearance
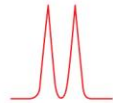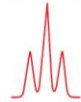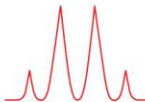
$$-OH + D_2O \rightleftharpoons -OD + HOD$$
$$-NH-CO- + D_2O \rightleftharpoons -ND-CO- + HOD$$
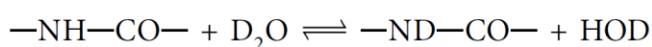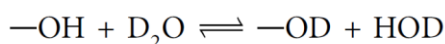
Number of adjacent ^1H atoms	Using the $n + 1$ rule, the peak will be split into ...	Relative intensities in the splitting pattern	Observed on the NMR spectrum as ...
0	1 peak, called a singlet	1	
1	2 peaks, called a doublet	1:1	
2	3 peaks, called a triplet	1:2:1	
3	4 peaks, called a quartet	1:3:3:1	

Table 29.2 Splitting patterns in high-resolution NMR spectra.

- • **Carbon-13 NMR spectroscopy**:
  - ➤ Analysis of carbon-13 NMR spectra is similar to that of proton NMR, looking to match different chemical shifts to characteristic molecular environments
  - ➤ Signals produced are discrete vertical lines on the spectra (no splitting patterns)
  - ➤ Heights of the lines are not proportional to the number of equivalent ^{13}C atoms present
  - ➤ The solvent used to prepare the samples for ^{13}C NMR analysis is $CDCl_3$
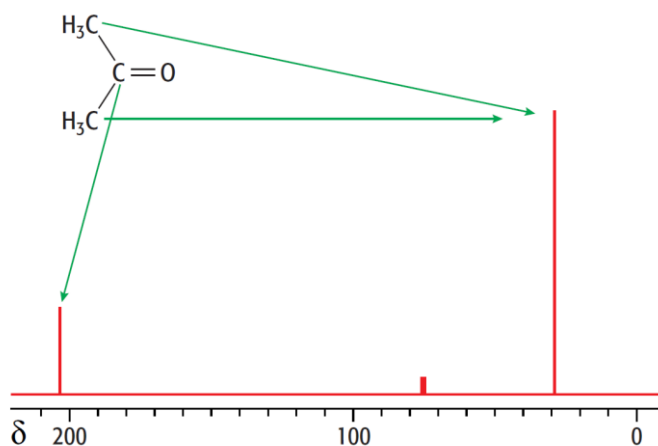 - ➤ E.g. propanone $(CH_3)_2CO$

Figure 29.21 The carbon-13 NMR spectrum of propanone.

➢ E.g. propanone $(CH_3)_2CO$
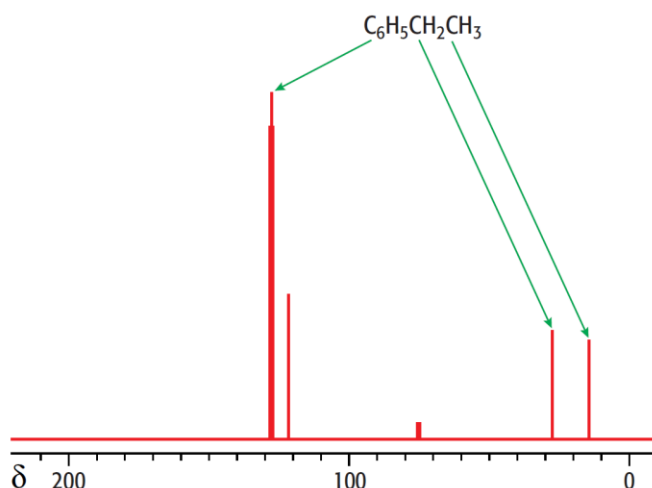
$C_6H_5CH_2CH_3$

Figure 29.22 The carbon-13 NMR spectrum of ethylbenzene.

- Tetramethylsilane, TMS, as the standard for chemical shift measurements at 0 ppm in both proton NMR and ^{13}C NMR
- Deuterated solvents, e.g. CDCl3, when obtaining an NMR spectrum as it will not interfere with the spectrum, no peaks produced from the solvent
- **Mass spectroscopy**:
  ➢ The peak at the highest mass-to-charge ratio is caused by the **molecular ion (M^+)**, giving the relative molecular mass of the sample
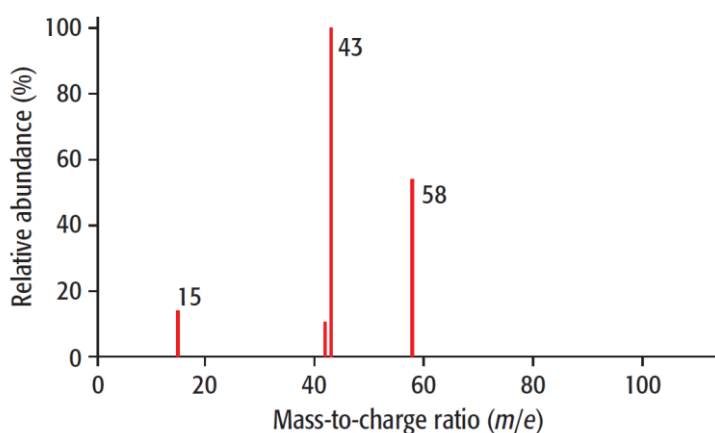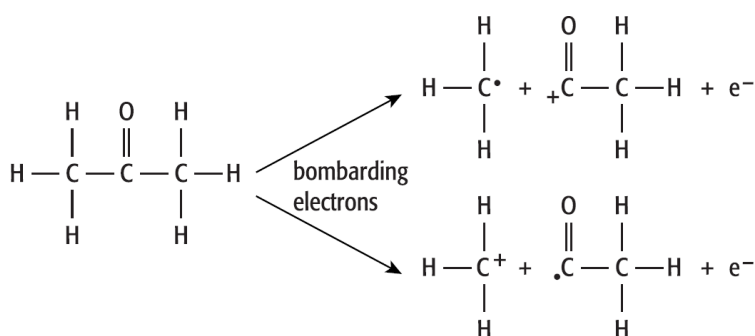

Figure 29.23 The mass spectrum of propanone.

Figure 29.24 The fragmentation of propanone: $^+CH_3$ causes the peak at 15 and CH_3C^+O causes the peak at 43.

➢ Very small peak beyond the molecular ion peak at a mass of [$M + 1$] caused by the 1.10% carbon-13 present in any organic compound, hence number of carbon atoms (n) in a molecule is given by:

$$n = \frac{100}{1.1} \times \frac{\text{abundance of } [M+1]^+ \text{ ion}}{\text{abundance of } M^+ \text{ ion}}$$

An unknown compound has a molecular ion peak, M^+, with a relative abundance of 54.5% and has an $[M+1]^+$ peak with a relative abundance of 3.6%. How many carbon atoms does the unknown compound contain?

Substituting the values of relative abundance into the equation:

$$n = \frac{100}{1.1} \times \frac{\text{abundance of } [M+1]^+ \text{ ion}}{\text{abundance of } M^+ \text{ ion}}$$

we get:

$$n = \frac{100}{1.1} \times \frac{3.6}{54.5} = 6.0$$
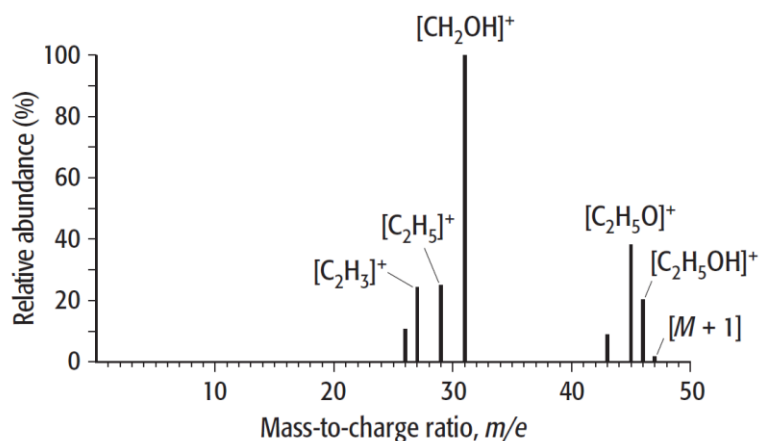
There are **6 carbon atoms** in each molecule.

Figure 29.25 The mass spectrum of ethanol, showing the $[M+1]$ peak.

➤ A sample containing chlorine or bromine atoms will form $[M+2]$ peak which is beyond the molecular ion peak, due to their isotopes

Isotopes	Approximate %
^{35}Cl	75
^{37}Cl	25
^{79}Br	50
^{81}Br	50

Table 29.6 Naturally occurring isotopes of chlorine and bromine.

❖ Imagine a sample of chloromethane, CH_3Cl; molecules of $CH_3{}^{35}Cl$ (75%) and molecules of $CH_3{}^{37}Cl$ (25%). The molecular ion will be $CH_3{}^{35}Cl^+$, and two units beyond that on the mass spectrum will be the peak for $CH_3{}^{37}Cl^+$
❖ The peak for $CH_3{}^{37}Cl^+$ will be one-third the height of the molecular ion – the $[M+2]$ peak
❖ In the mass spectrum of bromomethane, CH_3Br, we will have two molecular ion peaks of approximately the same height – one for $CH_3{}^{79}Br^+$ and the other for $CH_3{}^{81}Br^+$ – the $[M+2]$ peak
  ➤ If the $[M+2]$ peak is one-third the height of the M peak, this suggests the presence of one chlorine atom per molecule
  ➤ If the $[M+2]$ peak is the same as the height of the M peak, this suggests the presence of one bromine atom per molecule
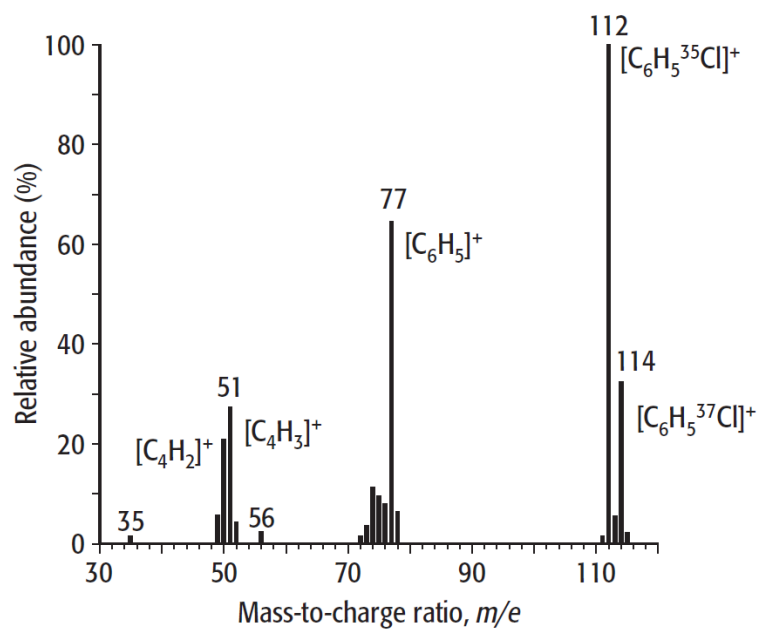
Figure 29.26 The mass spectrum of chlorobenzene, showing the [M + 2] peak. (Note that there are also tiny [M + 1] and [M + 3] peaks corresponding to ^{13}C in the molecule.)