9 Mass spectrometry

KEYPOINTS

Principles

- Charged molecules or molecular fragments are generated in a high vacuum region or, immediately prior to a sample entering a high vacuum region, using a variety of methods for ion production. The ions are generated in the gas phase so that they can then be manipulated by the application of either electric or magnetic fields to enable the determination of their molecular weights.

Applications

- Mass spectrometry provides a highly specific method for determining or confirming the identity or structure of drugs and raw materials used in their manufacture.
- Mass spectrometry in conjunction with either gas chromatography (GC–MS) or liquid chromatography (LC–MS) provides a method for characterising impurities in drugs and formulation excipients.
- GC–MS and LC–MS provide highly sensitive and specific methods for determining drugs and their metabolites in biological fluids and tissues.

Strengths

- The best method for getting rapid identification of trace impurities, which should ideally be carried out using chromatographic separation in conjunction with high resolution mass spectrometry so that elemental compositions can be determined
- With the advent of electrospray mass spectrometry and the re-emergence of time of flight mass spectrometry, the technique will be of major use in the quality control of therapeutic antibodies and peptides.

Limitations

- Mass spectrometry is not currently used in routine quality control (QC) but is placed in a research and development (R & D) environment where it is used to solve specific problems arising from routine processes or in process development
- The instrumentation is expensive and requires support by highly trained personnel and regular maintenance. However, these limitations are gradually being removed.

Introduction

A mass spectrometer works by generating charged molecules or molecular fragments either in a high vacuum or immediately prior to the sample entering the high vacuum region. The ionised molecules have to be generated in the gas phase. In classical mass spectrometry there was only one method of producing the charged molecules but now there are quite a number of alternatives. Once the molecules are charged and in the gas phase they can be manipulated by the application of either electric or magnetic fields to enable the determination of their molecular weight and the molecular weight of any fragments which are produced by the molecule breaking up.

Instrumentation

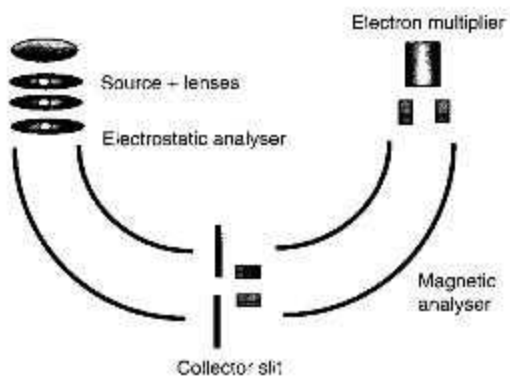


Fig. 9.1
Magnetic sector mass spectrometer.

A schematic view of a magnetic mass spectrometer is shown in Figure 9.1.

(i) The sample is introduced into the instrument source by heating it on the end of a probe until it evaporates, assisted by the high vacuum within the instrument.

(ii) Once in the vapour phase, the analyte is bombarded with the electrons produced by a rhenium or tungsten filament, which are accelerated towards a positive target with an energy of 70 eV. The analyte is introduced between the filament and the target, and the electrons cause ionisation as follows:

$$M + e \longrightarrow M^{+}$$

(iii) Since the electrons used are of much higher energy than the strength of the bonds within the analyte (4–7eV) extensive fragmentation of the analyte usually occurs.

(iv) Two types of system are commonly used to separate ions on the basis of their charge to mass ratio.

Magnetic sector instruments

In a magnetic sector instrument the ions generated are pushed out of the source by a repeller potential of same charge as the ion itself, and are then accelerated in an electric field of *ca* 3–8 kV and travel through an electrostatic field region so that they are forced to fall into a narrow range of kinetic energies prior to entering the field of a circular magnet. They then adopt a flight path through the magnetic field depending on their charge to mass (m/z) ratio; the large ions are deflected less by the magnetic field:

$$m/z = \frac{H^2 r^2}{2V}$$

where H is the magnetic field strength and r is the radius of the circular path in which the ion travels and V = the accelerating voltage.

At particular values for H and V, only ions of a particular mass adopt a flight path that enables them to pass through the collector slit and be detected. If the magnetic field strength is varied, ions across a wide mass range can be detected by the analyser, a typical sweep time for the magnetic field across a mass range of 1000 is 5–10 s but faster speeds are required if high resolution chromatography is being used in conjuction with mass spectrometry. The accelerating voltage can also be varied while the magnetic field is held constant in order to produce separation of ions on the basis of their kinetic energies.

Quadrupole instruments

A cheaper and more sensitive mass spectrometer than a magnetic sector instrument is based on the quadrupole analyser (Fig. 9.2), which uses two electric fields applied at right angles to each other, rather than a magnetic field, to separate ions according to their m/z ratios. One of the fields used is DC and the other oscillates at radiofrequency.

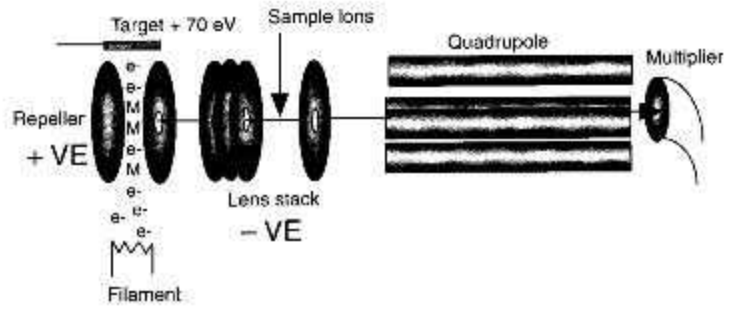


Fig. 9.2 Quadrupole mass spectrometer.

The effect of applying the two electrostatic fields at right angles to each other, one of which is oscillating, is to create a resonance frequency for each *m/z* value: ions which resonate at the frequency of the quadrupole are able to pass through it and be detected. Thus ions across the mass range of the mass spectrum are selected as the resonance frequency of the quadrupole is varied. A quadrupole instrument is more sensitive than a magnetic sector instrument since it is able to collect ions with a wider range of kinetic energies. The disadvantage of a simple quadrupole mass spectrometer is that it cannot resolve ions to an extent > 0.1 amu whereas a magnetic sector instrument can resolve ions to a level of 0.0001 amu or more. This enables the latter to be used to determine accurate masses for unknown compounds and thus assign their elemental compositions.

Mass spectra obtained under electron impact ionisation conditions

The original type of ionisation employed in mass spectrometry was electron impact (EI) ionisation as described earlier. This type of ionisation uses high energy electrons which produce extensive fragmentation of the bonds within the analyte. It is still very commonly used in standard chemical analyses but is not as readily applicable where molecules are very involatile or unstable. In these cases there is a range of other ionisation techniques which can be applied. These ionisation techniques, which are often used in conjunction with chromatography, are discussed in the sections on GC–MS and LC–MS. However, they may also be applied without prior chromatographic separation having been carried out.

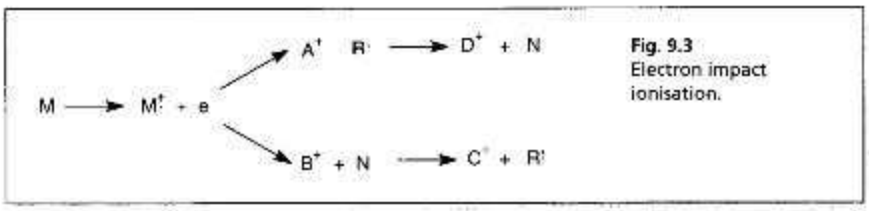


Fig. 9.3 Electron impact ionisation.

Figure 9.3 shows a generalised scheme for decomposition of a molecule under EI conditions. The principles of the scheme are as follows:

(i) $M^{+\cdot}$ represents the molecular ion which bears one positive charge since it has lost one electron and the unpaired electron which results from the loss of one electron is represented by a dot.

(ii) $M^{+\cdot}$ may lose a radical which, in a straightforward fragmentation not involving rearrangement, can be produced by the breaking of any single bond in the molecule. The radical removes the unpaired electron from the molecule leaving behind a cation A^+.

(iii) This cation can lose any number of neutral fragments (N) such as H_2O or CO_2 but *no further radicals*.

(iv) The same process can occur in a different order with a neutral fragment (H_2O, CO_2, etc.) being lost to produce $B^{+\cdot}$ and since this ion still has an unpaired electron it can lose a radical to produce C^+; this ion can thereafter only lose neutral fragments.

To summarise, the following rules apply to mass spectrometric fragmentation:

(i) The molecular ion can lose only *one* radical but any number of neutral fragments.

(ii) Once a radical has been lost only neutral fragments can be lost thereafter.

Molecular fragmentation patterns

Homolytic and heterolytic α-cleavage

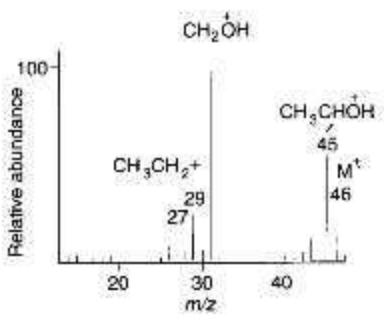


Fig. 9.4
EI mass spectrum of ethanol.

Under EI conditions the analyte develops a positive charge through the loss of one electron. If there is an electronegative atom in the structure of the molecule such as nitrogen or oxygen, this positive charge will be on the electronegative atom(s). If an electronegative atom is absent the charge is more difficult to locate with certainty. Figure 9.4 shows the EI spectrum of ethanol which provides an example of two types of fragmentation. The process is as follows:

(i) Homolytic α-cleavage (Fig. 9.5) is promoted by the presence of a hetero atom such as oxygen, nitrogen or sulphur and in molecules containing a hetero atom it often gives rise to the most abundant ion in the mass spectrum (the base peak).

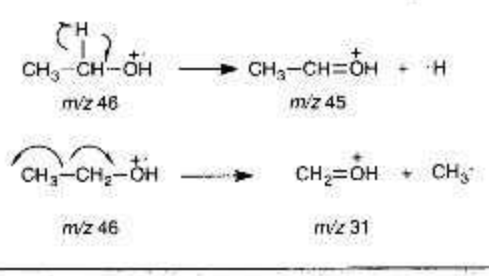


Fig. 9.5
Homolytic α-cleavage of ethanol.

(ii) One electron in the bond broken goes to the radical and the other combines with the unpaired electron on the hetero atom to produce a double bond; the hetero atom becomes positively charged.

(iii) Loss of the largest possible radical is most favoured. In the case of ethanol, loss of CH_3– gives rise to the base peak in the mass spectrum at *m/z* 31.

For many drug molecules this type of fragmentation dominates their mass spectra.

A minor ion in the spectrum of ethanol results from heterolytic α-cleavage (Fig. 9.6).

As is illustrated in Figure 9.7, homolytic α-cleavage is the major fragmentation mechanism for chains containing hetero atoms.

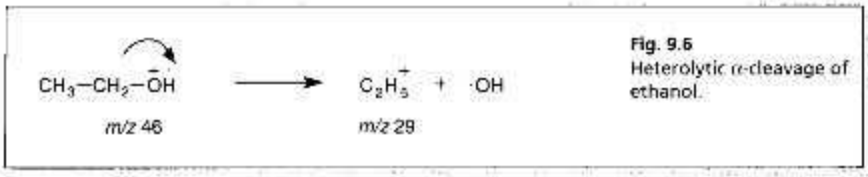


Fig. 9.6
Heterolytic α-cleavage of ethanol.

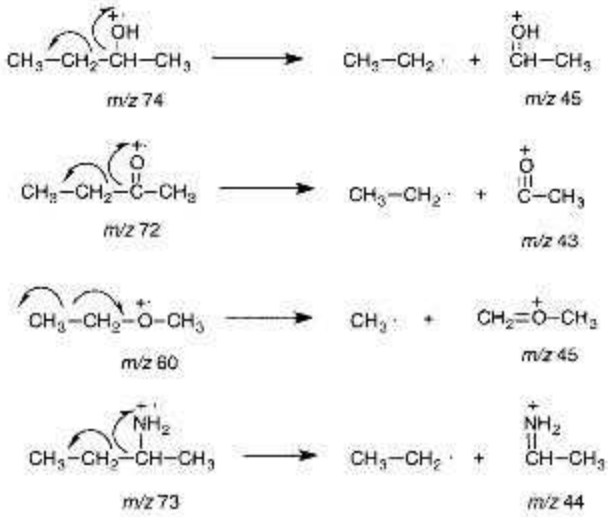
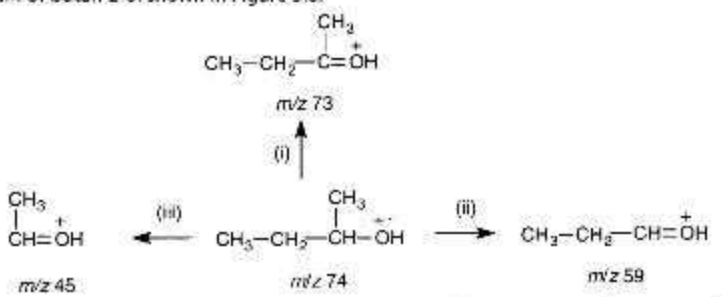


Fig. 9.7
Some further examples of homolytic α-cleavage.

Self-test 9.1

Indicate the type(s) of cleavage and the fragments lost which gives rise to the major ions in the spectrum of butan-2-ol shown in Figure 9.8.



Answers: (i) Homolytic α-cleavage with loss of H·; (ii) Homolytic α-cleavage with loss of CH_3·; (iii) Homolytic α-cleavage with loss of C_2H_4.

Figure 9.9 shows the spectrum of n-butanol. In this case homolytic α-cleavage, which gives rise to the ion at *m/z* 31, does not completely dominate the spectrum and the spectrum produced is more complex as a result. Loss of the neutral fragment H_2O occurs via a 1,4 elimination (Fig. 9.10); this produces an ion at *m/z* 56 which, since it is still a radical cation, gives rise to the fragment at *m/z* 41 via loss of CH_3· and followed by loss of H_2 as a neutral fragment to give *m/z* 39.

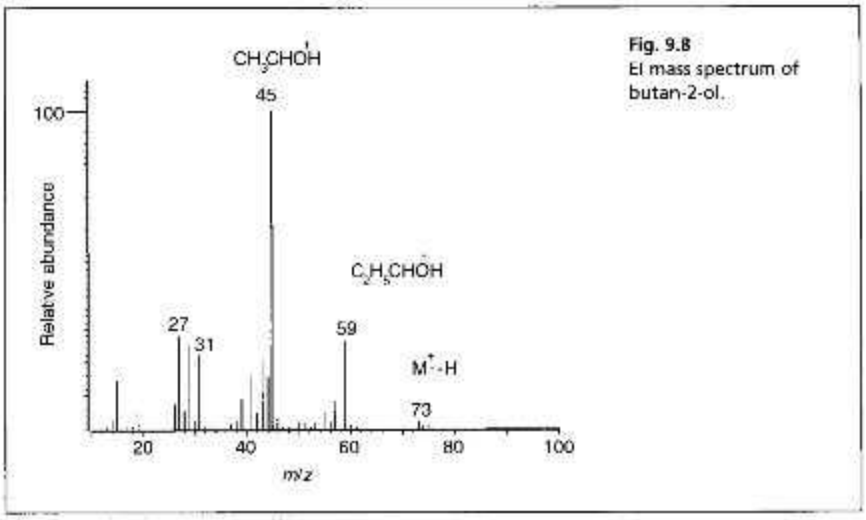


Fig. 9.8
EI mass spectrum of butan-2-ol.

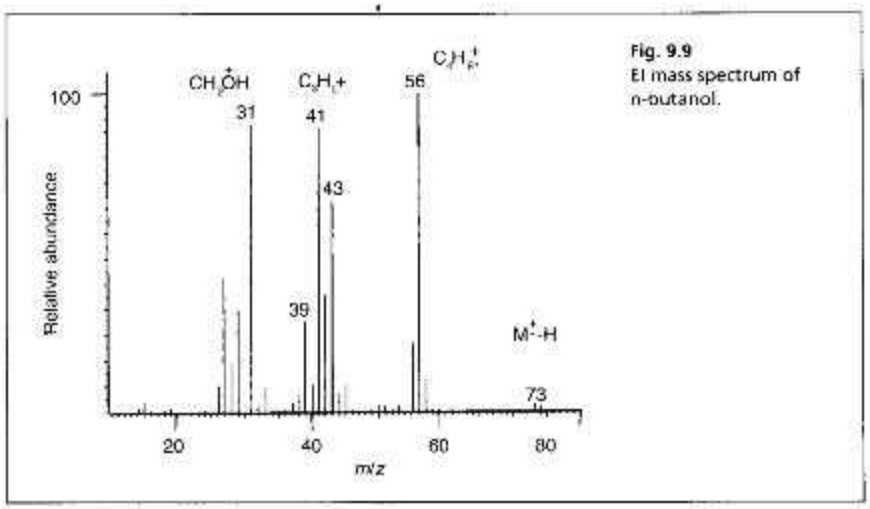


Fig. 9.9
EI mass spectrum of n-butanol.

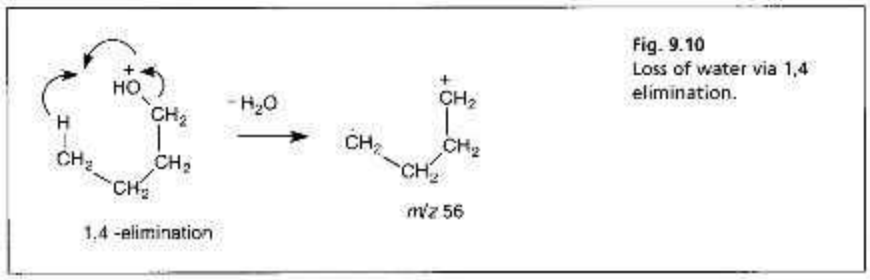


Fig. 9.10
Loss of water via 1,4 elimination.

Cleavage with proton transfer

Cleavage with proton transfer is also common in the mass spectra of drug molecules. In the first two examples the initial step is homolytic α-cleavage as shown in Figure 9.11; this is followed by loss of a neutral hydrocarbon fragment.

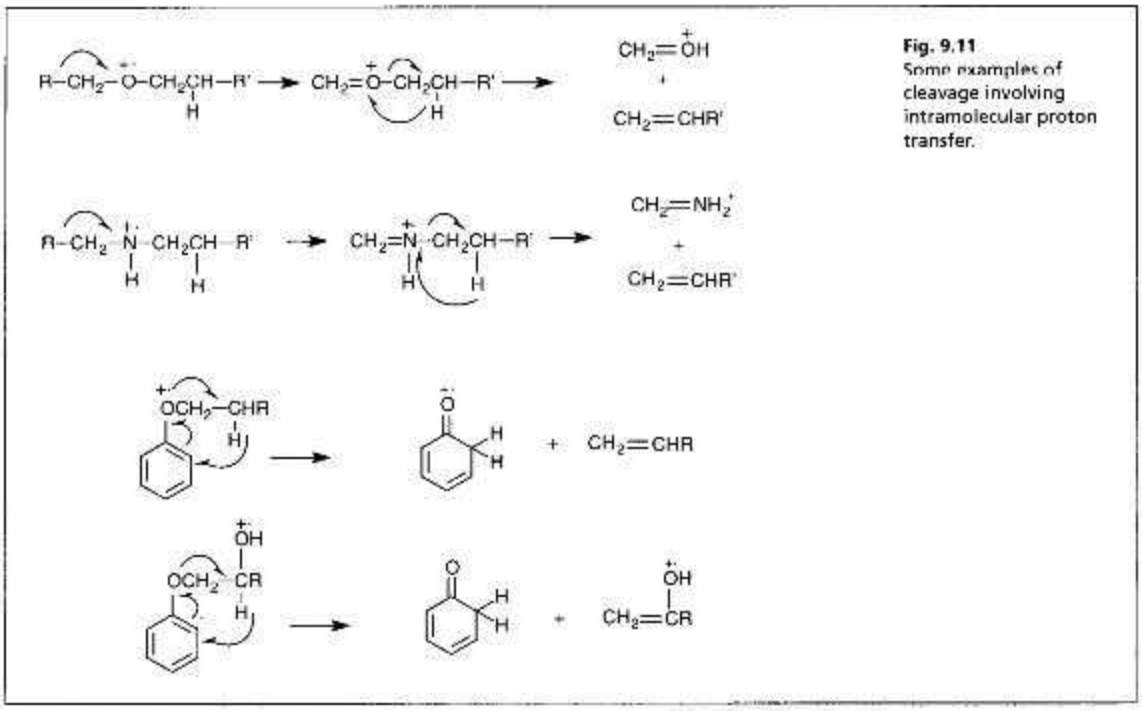


Fig. 9.11 Some examples of cleavage involving intramolecular proton transfer.

Table 9.1 shows some typical small fragments which are lost from the molecular ions of molecules.

Table 9.1 Common losses from a molecular ion

Loss amu	Radicals/neutral fragments lost	Interpretation
1	H·	Often a major ion in amines, alcohols and aldehydes
2	H_2	
15	CH_3·	Most readily lost from a quaternary carbon
17	OH· or NH_3	
18	H_2O	Readily lost from secondary or tertiary alcohols
19/20	F·/HF	Fluorides
28	CO	Ketone or acid
29	C_2H_5·	
30	CH_2O	Aromatic methyl ether
31	CH_3O·	Methyl ester/methoxime
31	CH_3NH_2	Secondary amine
32	CH_3OH	Methyl ester
33	$H_2O + CH_3$·	
35/36	Cl·/HCl	Chloride
42	$CH_2=C=O$	Acetate
43	C_3H_7·	Readily lost if isopropyl group present
43	CH_3CO·	Methyl ketone
43	$CO + CH_3$·	
44	CO_2	Ester
45	CO_2H·	Carboxylic acid
46	C_2H_5OH	Ethyl ester
46	$CO + H_2O$	
57	C_4H_9·	
59	CH_3CONH_2	Acetamide
60	CH_3COOH	Acetate
73	$(CH_3)_3Si$·	Trimethylsilyl ether
90	$(CH_3)_3SiOH$	Trimethylsilyl ether

Fragmentation of ring structures

More complex types of fragmentation involve rearrangement of the structure of a molecule prior to fragmentation and this is more likely to occur if the molecule has a ring in its structure. Figure 9.12 shows the EI spectrum of cyclohexanol.

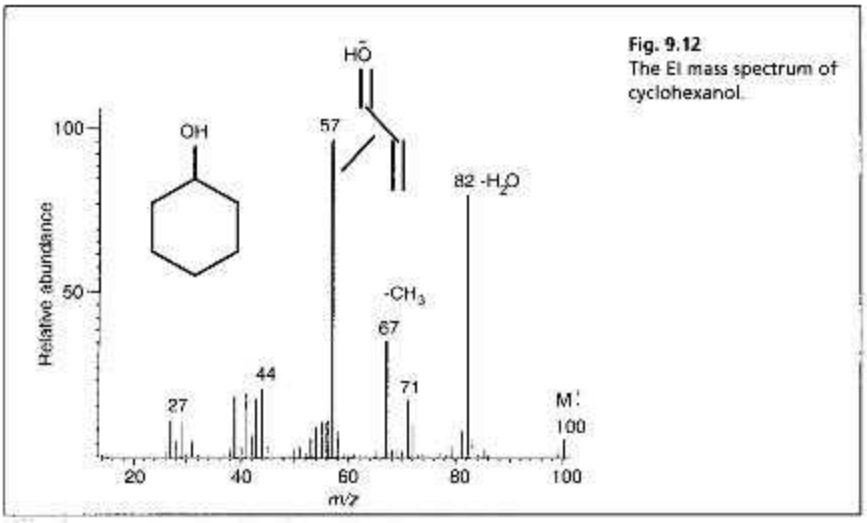


Fig. 9.12
The EI mass spectrum of cyclohexanol.

The major ions in the spectrum are due to the loss of the neutral fragment water, as in the case of n-butanol 1,4 elimination is probably involved.The base peak is formed via homolytic cleavage next to the OH group followed by proton transfer (Fig. 9.13). The base peak of the mass spectrum is formed as shown in Figure 9.13.

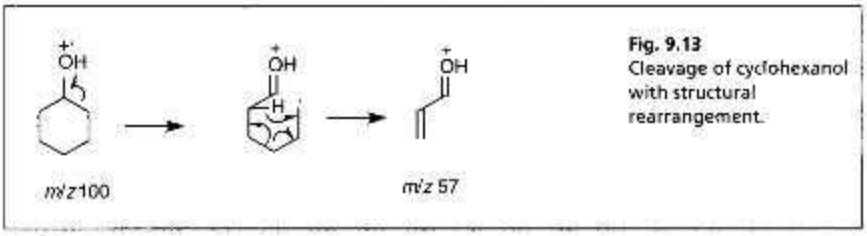


Fig. 9.13
Cleavage of cyclohexanol with structural rearrangement.

Retro Diels–Alder fragmentation is another type of fragmentation which occurs in compounds with ring systems.

The peak at *m/z* 68 in the mass spectrum of limonene results from the molecule breaking in half as shown in Figure 9.14.

EI mass spectra of some drug molecules

Examples where the molecular ion is abundant

In the case of some drugs the molecular ion may be abundant in the mass spectrum. Figure 9.15 shows the mass spectrum of codeine, where the molecular ion at *m/z* 299 is the base peak. The extended ring structure of the molecule means that apart from the abundant molecular ion, the fragmentation of codeine is not easy to interpret because of the structural rearrangements which occur. The only other ion in the mass spectrum of codeine closely related to the molecular ion is at *m/z* 229 and formation of this ion involves some rearrangement of the ring structure.

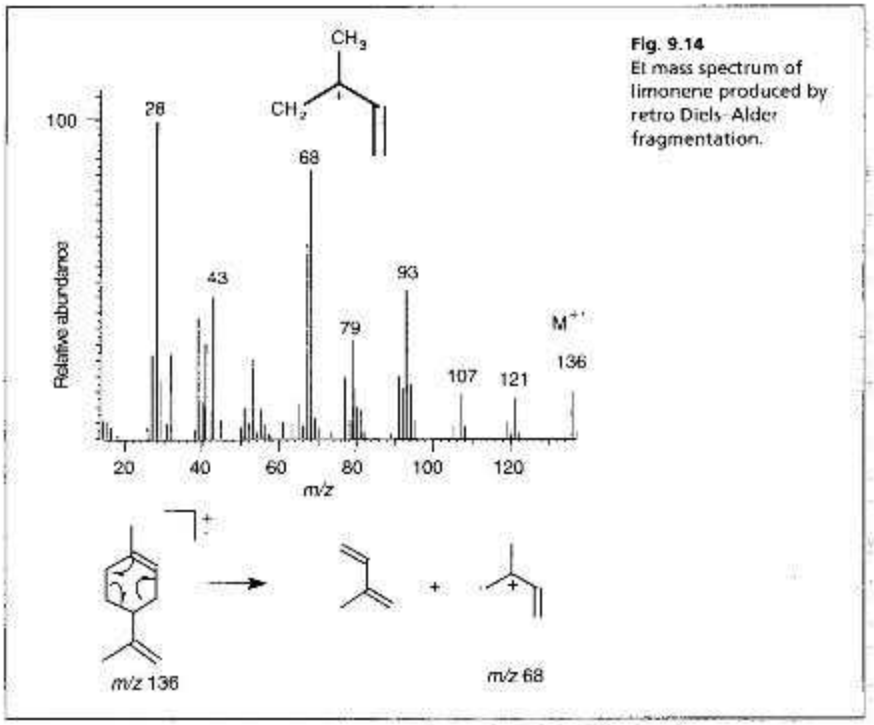


Fig. 9.14
EI mass spectrum of limonene produced by retro Diels–Alder fragmentation.

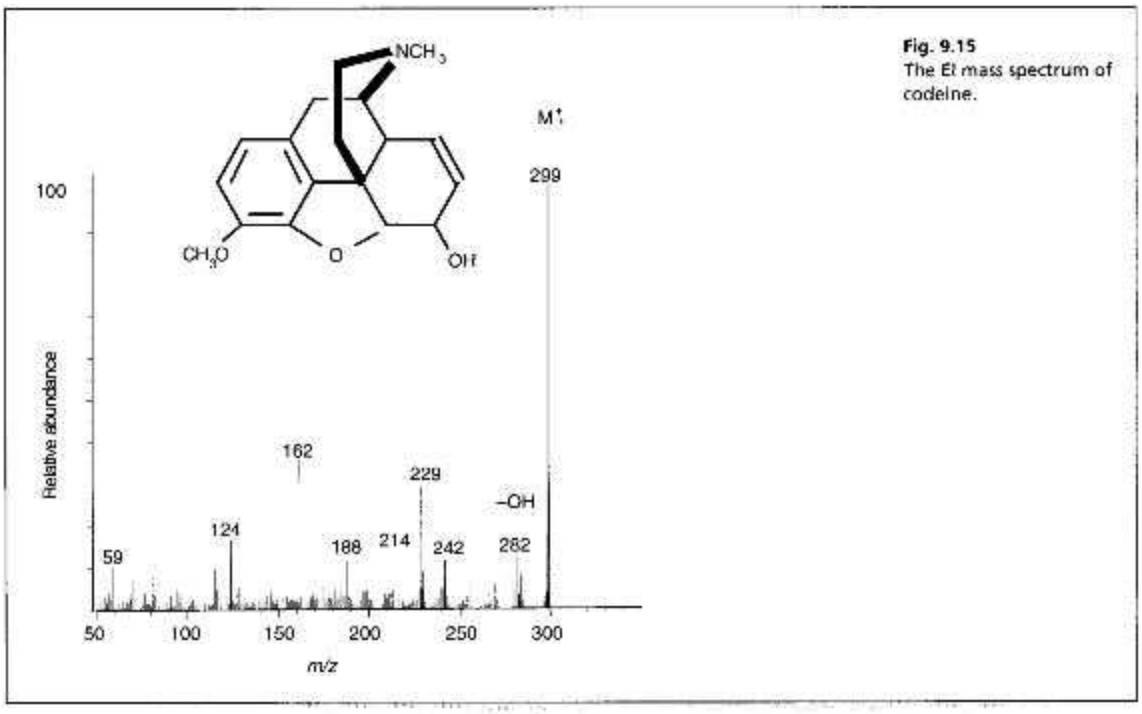


Fig. 9.15
The EI mass spectrum of codeine.

Drugs which yield abundant molecular ions under EI conditions include: caffeine, coumatetralyl, cyclazocine, dextromethorphan, dichlorphenamide, diflunasil, dimoxyline, fenclofenac, flurbiprofen, griseofulvin, harmine, hydralazine, hydroflumethiazide, ibogaine, ketotifen, levallorphan, methaqualone, nalorphine. These drugs are characterised by having ring structures without extensive side chains, or if side chains are present, they do not contain hetero atoms which would direct cleavage to that part of the molecule.

Drug molecules in which homolytic α-cleavage dominates the spectrum

Since many drugs contain hetero atoms the fragmentation of drug molecules is often directed by α-homolytic cleavage adjacent to these atoms. Figure 9.16 shows the mass spectrum of bupivacaine, where homolytic α-cleavage is directed by the nitrogen atom in the heterocyclic ring resulting in an ion at *m/z* 140, which dominates the spectrum.

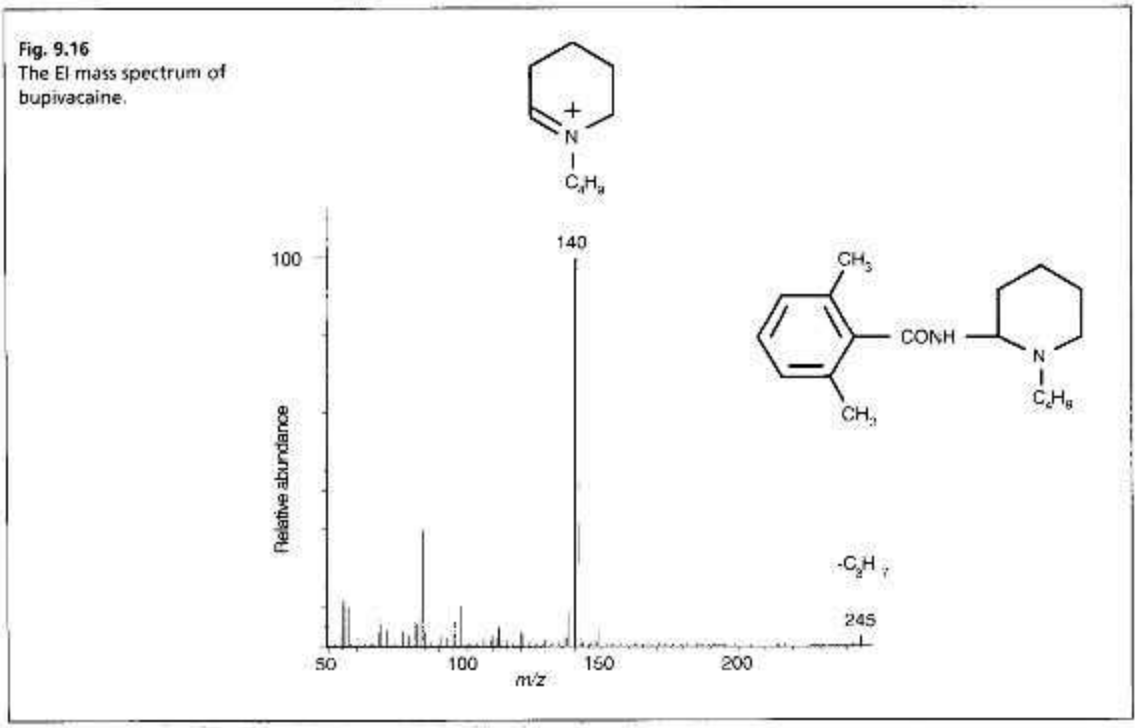


Fig. 9.16
The EI mass spectrum of bupivacaine.

A similar type of fragmentation dominates the spectrum of other local anaesthetics such as prilocaine and procaine; and sympathomimetics such as ephedrine, salbutamol and terbutaline and β-adrenergic blockers such as propranolol and oxyprenolol.

Isotope peaks

Figure 9.17A shows the mass spectrum of chloroquine under EI conditions. This molecule gives a spectrum typical of a basic compound with a side chain. In this case homolytic α-cleavage takes place adjacent to the nitrogen in the side chain with

the charge being retained on the smaller portion of the molecule resulting in an ion at *m/z* 86. Another feature of the spectrum which can be noted is the double molecular ion which occurs at *m/z* 319 and *m/z* 321, and which arises from the presence of a chlorine atom in the molecule. Chlorine has two common isotopes with atomic masses 35 and 37 in the ratio 3:1, and the small molecular ion for chloroquine at *m/z* 319 has a peak associated with it in a 3:1 ratio at *m/z* 321. This is seen more clearly in the negative ion chemical ionisation (NICI) spectrum of chloroquine (Fig. 9.17B). NICI is a soft ionisation technique which produces little, if any, fragmentation in a molecule and results in an abundant molecular ion. In this case, the chlorine isotope peak for chloroquine at *m/z* 321 can be seen clearly. Bromine in the structure of a molecule will also produce an isotope pattern since it has isotopes with atomic weights of 79 and 81 which occur in more or less a 1:1 ratio. These typical patterns can be useful in characterisation of impurities in synthetic drugs because reactive intermediates which are used in drug synthesis may contain bromine or chlorine. If more than one chlorine or bromine atom is present, then the isotope pattern is more complex.

The isotopes of all the other elements commonly found in drug molecules are much less abundant, although because of the number of carbon atoms in large

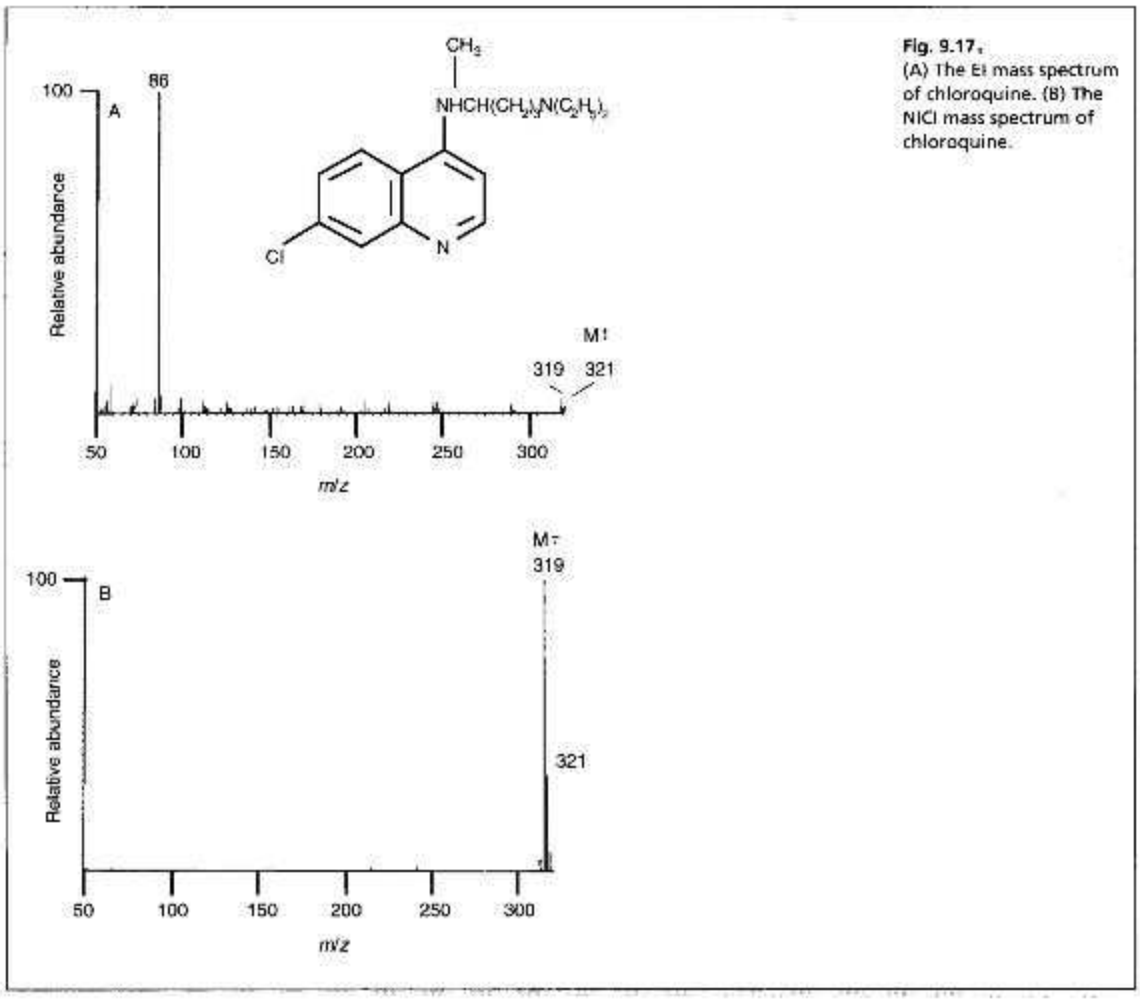
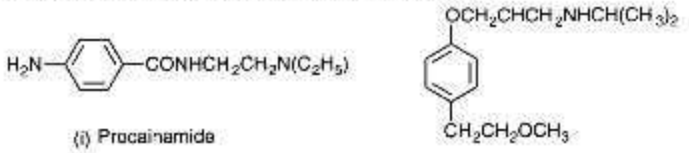


Fig. 9.17. (A) The EI mass spectrum of chloroquine. (B) The NICI mass spectrum of chloroquine.

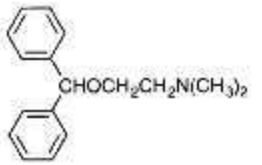
Self-test 9.2

Predict the base peak in mass spectra of the following molecules:

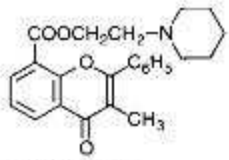


(i) Procainamide

(ii) Metoprolol



(iii) Diphenhydramine



(iv) Flavoxate

Answers: The mass spectra of all these molecules are dominated by homolytic α cleavage next to the nitrogen atom in their side chains. (i) *m/z* 86; (ii) *m/z* 72; (iii) *m/z* 58; (iv) *m/z* 98.

molecules, the M+1 ion for carbon is usually substantial. Although the abundance of carbon-13 is only 1.1% compared with carbon-12, the presence of 40 carbon atoms in a molecule would give rise to an M+1 ion with a 44% abundance since there is a 44% probability that the molecule will contain a carbon-13 atom.

Tropylium ion

Another type of directed fragmentation occurs in molecules which have a benzyl group. For instance, the spectrum of levodopa (L-dopa) is dominated by an ion at *m/z* 123 which arises from the formation of a tropylium ion as shown in Figure 9.18. This ion is readily formed in any compound with a benzyl group. The benzyl CH_2 becomes incorporated into a seven-membered ring structure in which the positive charge on the ion is delocalised around the ring structure, giving a very stable cation. Of course other types of fragmentation can compete; in the case of L-dopa, homolytic cleavage next to the nitrogen atom is possible which gives rise to a fragment at *m/z* 74, but this fragment is of lower abundance than the fragment at *m/z* 123.

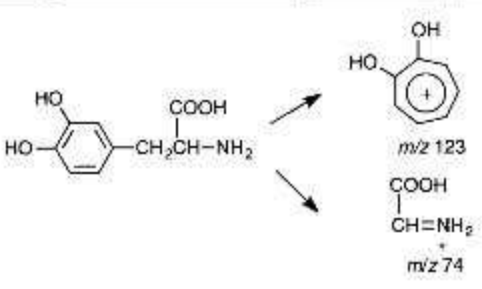
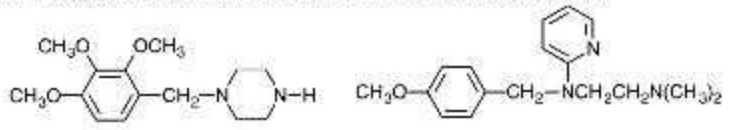


Fig. 9.18
Two routes of fragmentation in the mass spectrum of L-dopa.

Self-test 9.3

Predict the likely base peak in the mass spectra of the following molecules:



(i) Trimetazidine (ii) Mepyramine

Answers: Both molecules yield tropylium ions as the base peak in their mass spectra: (i) $C_9H_{13}O_3^+$, m/z 181; (ii) $C_8H_9O^+$ m/z 121

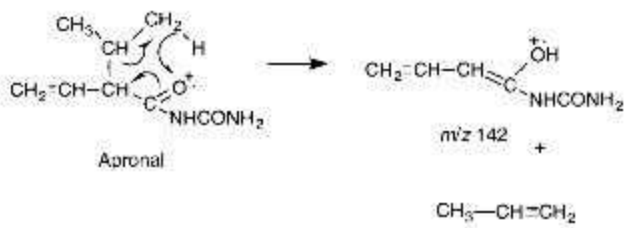
McLafferty rearrangement

McLafferty rearrangement can occur in carboxylic acids, esters, ketones and amides which have a side chain containing at least three carbon atoms. The generalised fragmentation is shown in Figure 9.19. For example, the sedative drug apronal has a McLafferty fragment as a major ion in its mass spectrum arising as shown in Figure 9.20.

R_1 = CH_2R, OH, OR, NHR.

R_2 = H or alky

Fig. 9.19 The McLafferty rearrangement.



Apronal

Fig. 9.20 McLafferty fragmentation of apronal.

This type of fragmentation is not particularly common in drug molecules, often because where it is possible homolytic α-cleavage dominates the spectrum, but it is a feature of long chain lipid molecules such as fatty acid esters.

Gas chromatography–mass spectrometry (GC–MS)

For the chromatographic aspects of GC–MS, refer to Chapter 11. Gas chromatography (G–C) was the earliest chromatographic technique to be interfaced to a mass spectrometer. The original type of gas chromatograph had a packed GC column with a gas flow rate passing through it at *ca* 20 ml/min and the major problem was how to interface the GC without losing the mass spectrometer vacuum.

This was solved by use of a jet separator where the column effluent was passed across a very narrow gap between two jets and the highly diffusable carrier gas was largely removed, whereas the heavier analyte molecules crossed the gap without being vented. The problem of removing the carrier gas no longer exists since GC capillary columns provide a flow rate of 0.5–2 ml/min, which can be directly introduced into the mass spectrometer without it losing vacuum.

Ionisation techniques used in GC–MS

There are three main types of ionisation used with GC–MS, which will be discussed in turn.

Electron impact

This type of ionisation has been discussed earlier in this chapter. The mass spectrum of psoralen under EI conditions is shown in Figure 9.21 along with the corresponding GC trace, which is produced by the total ion current (TIC) across the scan range of the mass spectrum. The molecular ion $M^{+\cdot}$ is in this case in good abundance at *m/z* 186 and other ions arise as follows: *m/z* 158 ($M^{+\cdot}$–CO), *m/z* 130 ($M^{+\cdot}$–2CO), *m/z* 102 ($M^{+\cdot}$–3CO).

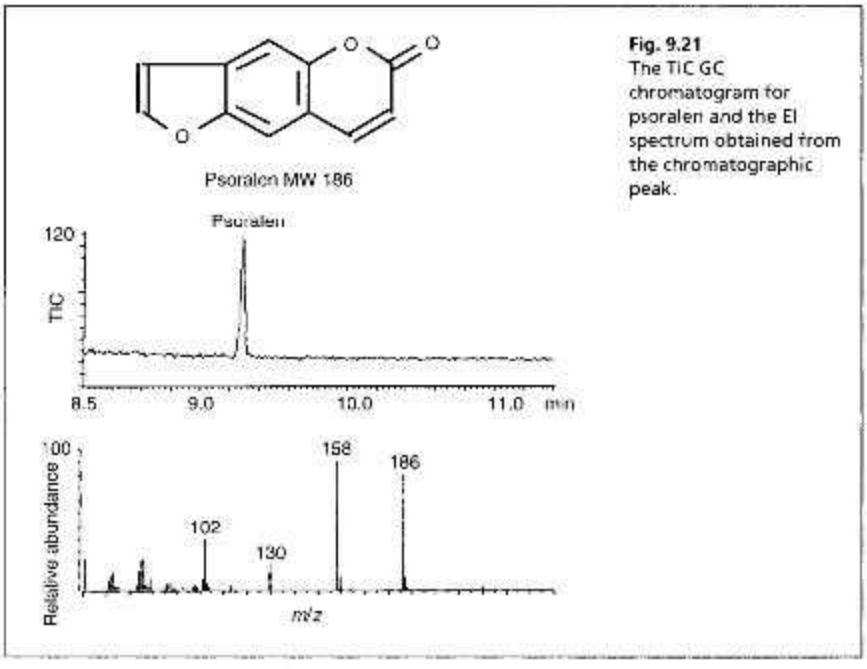


Fig. 9.21 The TIC GC chromatogram for psoralen and the EI spectrum obtained from the chromatographic peak.

Positive ion chemical ionisation (PICI)

In the PICI mode a reagent gas is continuously introduced into the ion source, e.g. methane (isobutane and ammonia are also used). The gas interacts with electrons produced by the filament to produce a series of ions shown in Figure 9.22.

The positively charged ions can either associate with the analyte or can transfer a proton to the analyte. The most commonly observed adduct ions are illustrated by the PICI spectrum of psoralen (Fig. 9.23). In this case, the ions arise via addition

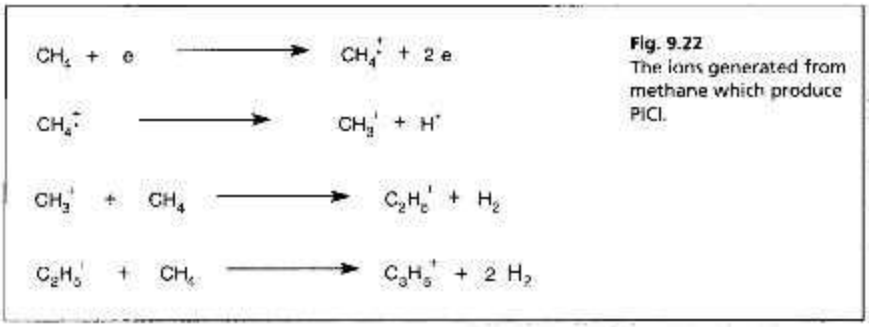


Fig. 9.22
The ions generated from methane which produce PICI.

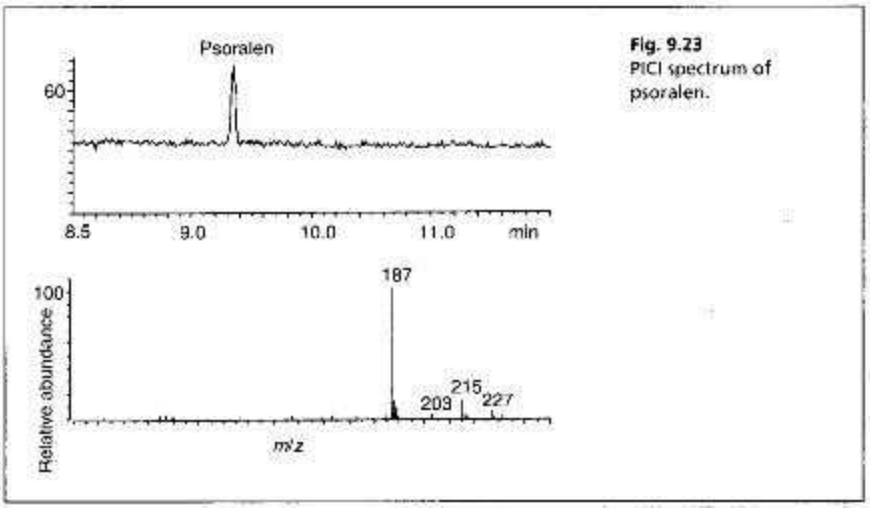


Fig. 9.23
PICI spectrum of psoralen.

of: H^- (*m/z* 187), $C_2H_5^+$ (*m/z* 215) and $C_3H_5^+$ (*m/z* 227) to the molecular ion. The signal:noise ratio indicated by the baseline of the corresponding GC trace shows that, while the fragmentation of the molecule is reduced, ionisation efficiency is also reduced in comparison with EI.

Negative ion chemical ionisation (NICI)

The most common form of ionisation occurring in the case of negative ion spectra is electron capture ionisation. Again a reagent gas is used and the electrons collide with it so that their energies are reduced to < 10eV. Molecules with a high affinity for electrons are able to capture these low energy thermal electrons. This is often loosely called NICI but since it does not involve the formation of a chemical adduct it is not strictly chemical ionisation. The two commonly observed types of electron capture are shown in Figure 9.24.

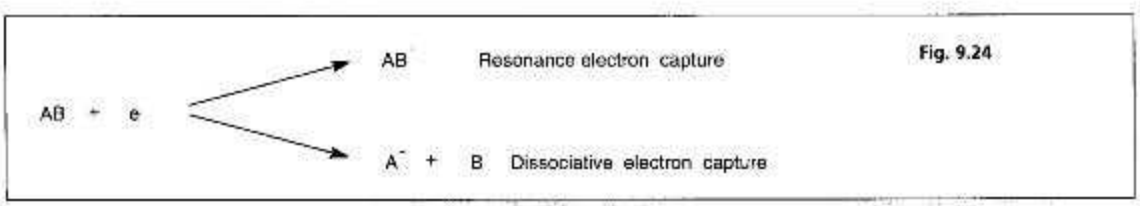


Fig. 9.24

The NICI spectrum of psoralen (Fig. 9.25) indicates that resonance capture is occurring so that most of the ion current is carried by the molecular ion at *m/z* 186. The associated GC trace indicates that ionisation is *ca* 10 times more efficient for the same amount of psoralen in comparison with EI. In addition, since most of the ion current is channelled into the molecular ion, an analytical method which selectively monitored the molecular ion of psoralen would be *ca* 40 times more sensitive if NICI conditions were used rather than EI. The technique only works for compounds which are electron capturing. The small ion at *m/z* 218 indicates that the psoralen has also formed an adduct with traces of oxygen present in the instrument, i.e. true chemical ionisation.

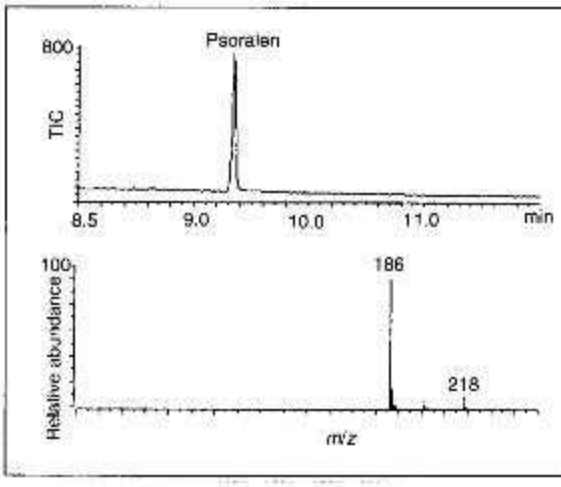


Fig. 9.25
NICI spectrum of psoralen.

Applications GC–MS to impurity profiling

With the advent of requirements by the American Food and Drug Administration (FDA) for identification of any impurity at a level of > 0.1% in pharmaceuticals, mass spectrometry with chromatography has found a role in impurity identification. Such impurities can arise either from the manufacturing process or from degradation of the drug. Figure 9.26 shows a GC–MS trace for a commercial sample of the β-blocker propranolol. The synthetic route leading to propranolol is shown in Figure 9.27.

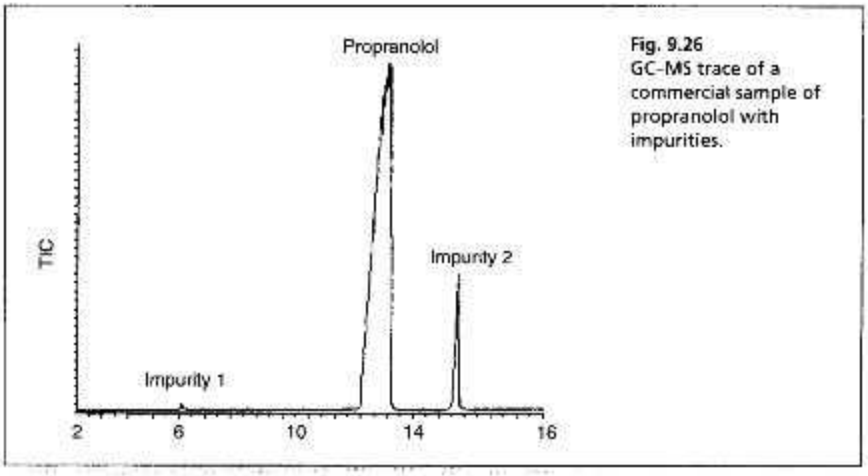


Fig. 9.26
GC–MS trace of a commercial sample of propranolol with impurities.

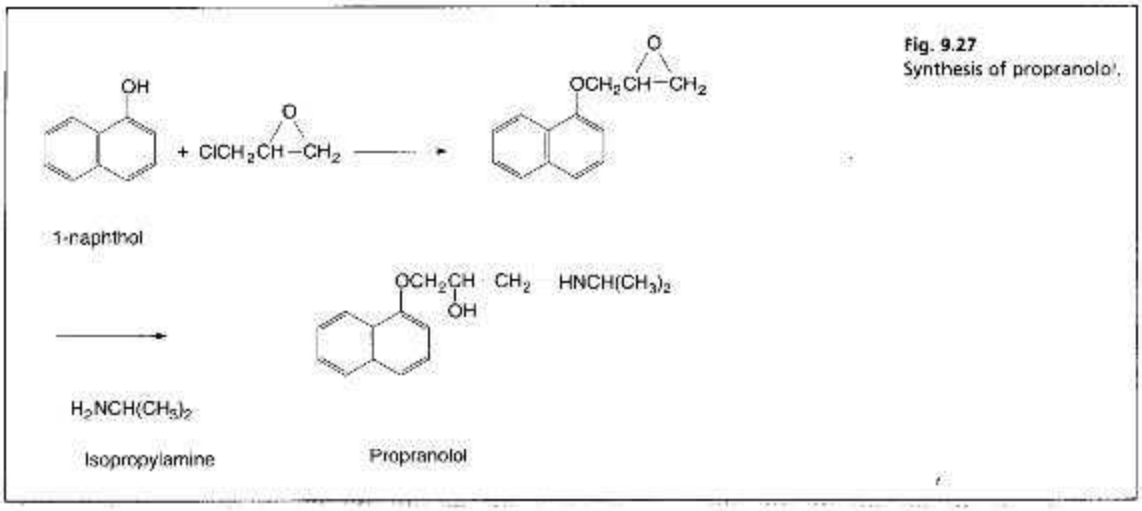


Fig. 9.27
Synthesis of propranolol.

There is one major additional peak in the sample which runs after propranolol (impurity 2). The mass spectrum for propranolol is shown in Figure 9.28.

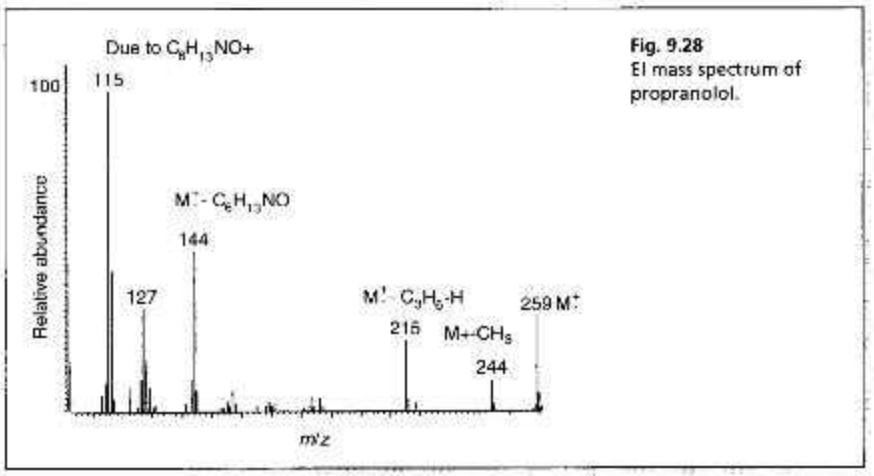


Fig. 9.28
EI mass spectrum of propranolol.

The molecular ion can be seen at *m/z* 259 and the two major fragment ions at *m/z* 144 and 115 arise as shown in Figure 9.29.

The impurity peak shows a major fragment at *m/z* 158 (Figure 9.30). This compound must have a molecular weight higher than that of propranolol since its GC retention time is longer. However, there is no substantial ion of molecular weight higher than that of propranolol in its mass spectrum. The mass spectrum of the impurity under PCI conditions is shown in Figure 9.31.

Impurity 2 gives an apparent molecular ion at *m/z* 302 and also shows a major fragment at *m/z* 158. This information is consistent with an additional isopropyl being attached to the nitrogen in propranolol as shown in Figure 9.32. The fragment at *m/z* 158 observed both under EI and PCI conditions arises as shown in Figure 9.32. Thus, this extra peak is manufacturing impurity, probably arising from a small amount of disopropylamine being present in isopropylamine used as a reagent in one

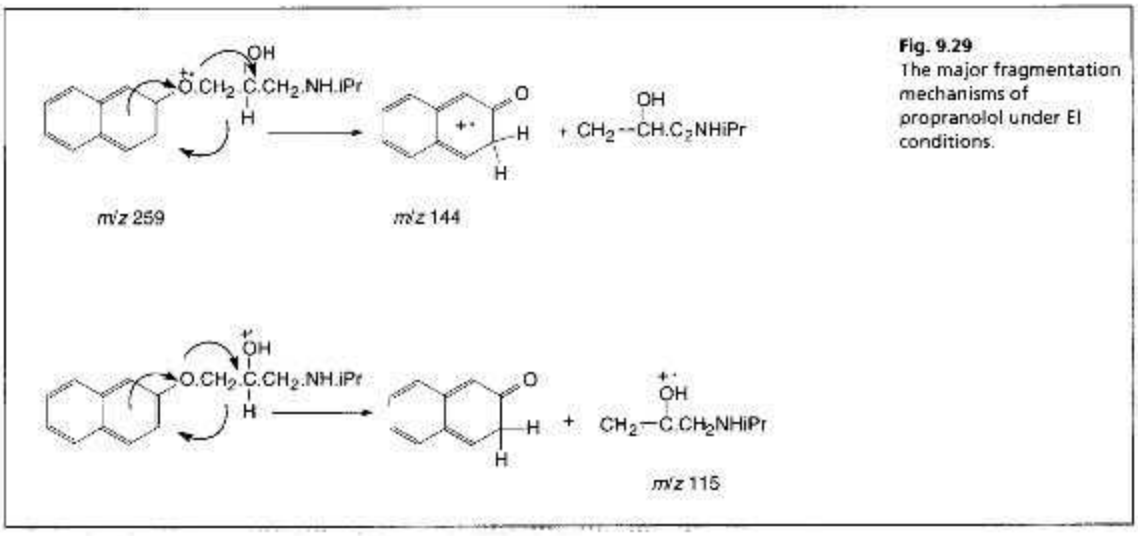


Fig. 9.29
The major fragmentation mechanisms of propranolol under EI conditions.

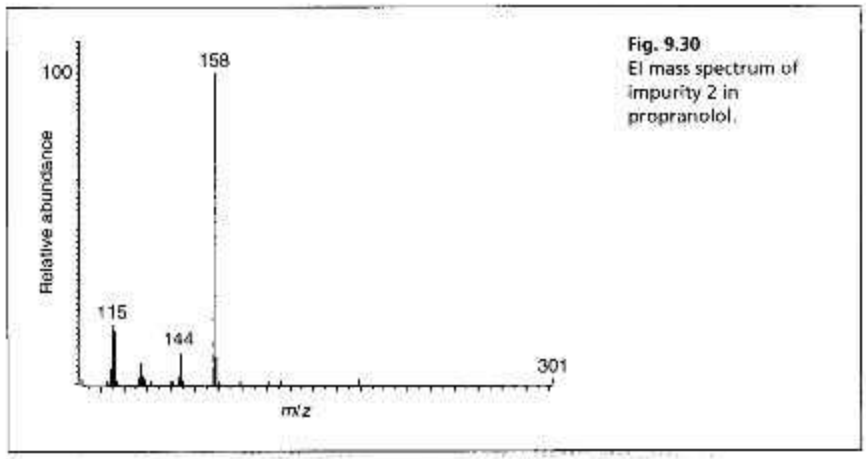


Fig. 9.30
EI mass spectrum of impurity 2 in propranolol.

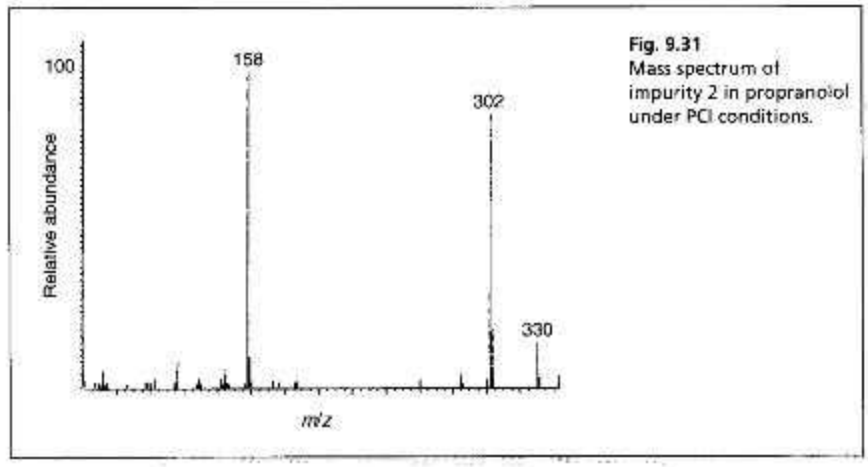


Fig. 9.31
Mass spectrum of impurity 2 in propranolol under PCI conditions.

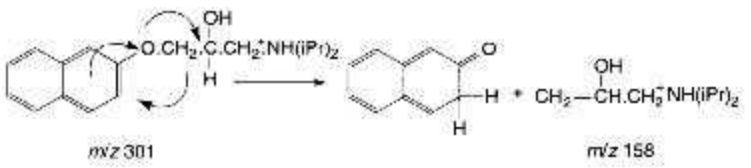


Fig. 9.32
MS fragmentation of the impurity peak in propranolol.

of the later stages of the synthesis. It is also possible to detect in the propranolol sample a very minor amount of 1-naphthol, impurity I (Fig. 9.26), which is used in an earlier stage of its synthesis.

Liquid chromatography–mass spectrometry (LC–MS)

The interfacing of a liquid chromatograph to a mass spectrometer proved much more difficult than interfacing a gas chromatograph since each mole of solvent introduced into the instrument produces 22.4 l of solvent vapour, even at atmospheric pressure. The technique has made huge advances in the last 10 years and there are many types of interface available, the most successful of which are the electrospray and atmospheric pressure ionisation sources. Table 9.2 summarises the major types of LC–MS interface which are available. In many cases, LC flow rates have to be in the range 10–100 μl per min so that either splitting of the eluent from a normal column is required or microbore chromatography is used. Table 9.3 summarises some additional ion separation methods which are used in conjunction with liquid chromatography.

Table 9.2 LC–MS interfaces

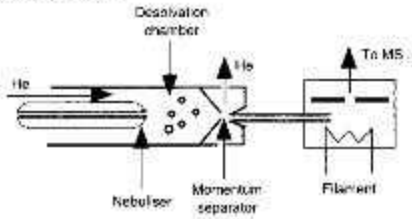
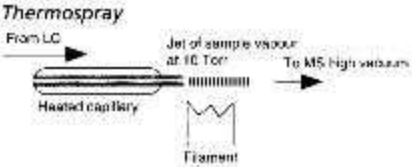
Interface	Comments
Particle beam  	Useful interface which is applicable to a wide range of molecules. The volatile solvent molecules are stripped from the sample and lost in a process similar to that used in the early jet separators used in GC–MS. The heavier sample molecules enter the MS and can be ionised by the standard methods of EI, PICI or NICI. Gives spectra with EI fragmentation which can be referred for identification to EI spectral libraries built up over many years. No solvent background thus sensitive to the 10^{-12} g level. Solvent flow rate up to 1 ml/min, mass range up to 1000 amu
Thermospray  	The eluent from the column is vapourised and a portion of the vapour (ca 1%) is transferred to the mass spectrometer and the rest of the vapour is pumped to waste. The spectra produced are like CI spectra since the presence of solvent vapour with the sample reduces the energy of the ionisation process and adducts can be formed with the solvent. Sensitive to the 10^{-9} g level; mass range up to 2000 amu

Table 9.2 LC–MS interfaces (Cont.)

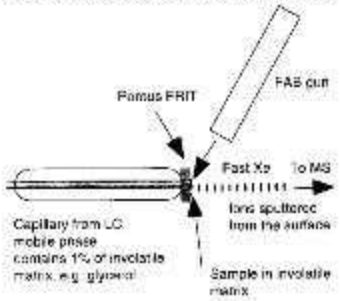
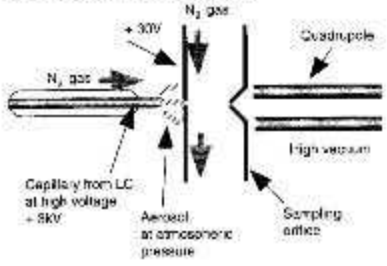
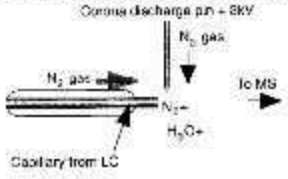
Interface	Comments
FRIT–FAB (fast atom bombardment) 	The mobile phase enters the instrument directly so that the flow rates can only be ca 10 μl/min. The mobile contains 1% of an involatile matrix, e.g. glycerol. The sample flows out onto the centre of a porous disc and the solvent, apart from the involatile matrix, evaporates. The sample in the matrix is struck by fast atoms (Xe or Cs) from a FAB gun and the high energy of the atoms generates ions from the sample. Soft ionisation technique produces limited fragmentation. Sensitive to 10^{-9} g level for lipophilic compounds, mass range up to 2000 amu or more
Electrospray (ES) ionisation 	The most common LC–MS interface. Flow rates up to 1 ml/min but best at 200 μl/min or below. A charged aerosol is generated at atmospheric pressure and the solvent is largely stripped away with a flow of N_2 gas. The charged molecules are drawn into the MS by electrostatically charged plates. Can determine both small molecules and molecules up to 200 000 amu. Spectra can be simple, containing molecular ion only, or fragmentation can be induced by varying the cone voltage. More suitable for polar molecules
Atmospheric pressure ionisation (API) 	Very similar to ES but can operate at normal LC flow rates 0.2–2 ml/min. ES instruments can be simply converted to run this technique. Ionisation is more analogous to CI with the corona discharge producing ions such as H_3O^+ and N_2^+, which promote the ionisation of the sample. Complementary to ES since this interface will ionise less polar molecules

Table 9.3 Additional ion separation methods

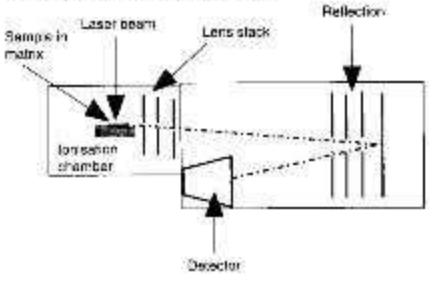
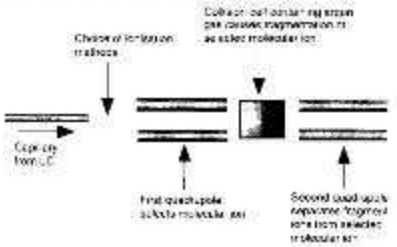
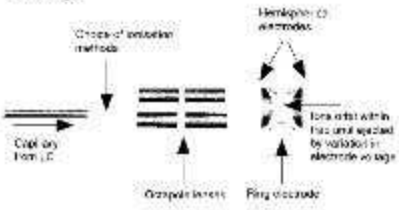
Matrix-assisted laser desorption with time of flight (MALDI–TOF) 	Can be used for very large proteins > 200 000 amu. The sample is dissolved in a light-absorbing matrix, soft ionisation is promoted by a pulsed laser and ions are ejected from the matrix and accelerated using an electrostatic field into a field-free region. The lighter ions travel fastest. In order to improve resolution a device called a 'reflectron' is used to focus the kinetic energies of a population of a particular ion prior to its entering a field-free region. The length of time taken for ions to reach the detector gives their molecular weight (MW). The pulsed nature of the ionisation ensures there is no overlap between spectra. Ideal technique for characterisation of the MW of large proteins

Table 9.3 Additional ion separation methods (*Cont.*)

Tandem mass spectrometry 	Commonly known as MS–MS. One quadrupole is used to filter out the molecular ion of the compound of interest which is introduced into a collision cell where argon gas is used to induce futher diagnositic fragmentation of the molecular ion. The secondary fragments can then be separated with a second quadrupole. Three or four quadrupoles can be used to produce even more filtering. The technique can remove the need for a chromatographic step replacing it with high-speed ion filtering
Ion trap 	The ion trap separates ions by capturing them within a circular electrode where they orbit until they are ejected by a variation in voltage. The technology is developing rapidly and has advantages over a quadrupole in that ions can be trapped while tandem MS-type fragmentation is produced. Can filter out background while the ion of interest is retained in the trap before being further fragmented and ejected

Applications of LC–MS in pharmaceutical analysis

Determination of impurities in insulin-like growth factor with ES–MS

ES–MS provides an excellent means for quality control of recombinant proteins, some of which are now used as drugs, e.g. human insulin, interferons, erythropoietin and tissue plasminogen activating factor.[1] The advantage of ES–MS in the determination of proteins is that multiple charges can be formed on a protein to bring it within range of standard mass spectrometers which have a mass range of 1000–2000 amu. A protein with a charge of 10+ and a MW of 10 000 would show up at 1000 amu. It would be further characterised by having ions in a series bearing different charges, e.g. 909 (11+), 1000 (10+), 1111 (9+), 1250 (8+), etc. The simplicity of the single ion spectra for each charge number means that small amounts of related proteins that may contaminate the main protein show up quite clearly. Thus, variations in protein structure such as degree of glycosylation or in the terminal amino acids of the protein can be seen quite clearly.

An example of how ES–MS can be used to determine minor impurities in a recombinant protein is shown in Figure 9.33, where some small additional ions in the mass spectrum of recombinant insulin-like growth factor (IGF) can be seen. The major ions in the spectrum are due to IGF itself bearing varying charge but the minor impurities also give rise to peaks and these can be interpreted as shown in Table 9.4.

Before the advent of this technique the determination of protein molecular weight was a laborious process and control and identification of minor impurities more or less impossible.

Characterisation of a degradant of famotidine

Tablets of famotidine, an anti-ulcer compound, were subjected to stress conditions in pack.[2] Figure 9.34 indicates the profile obtained from analysis of an extract from the stressed tablets by LC-atmospheric pressure chemical ionisation mass spectrometry (APCIMS). The structure of famotidine is shown in Figure 9.35.

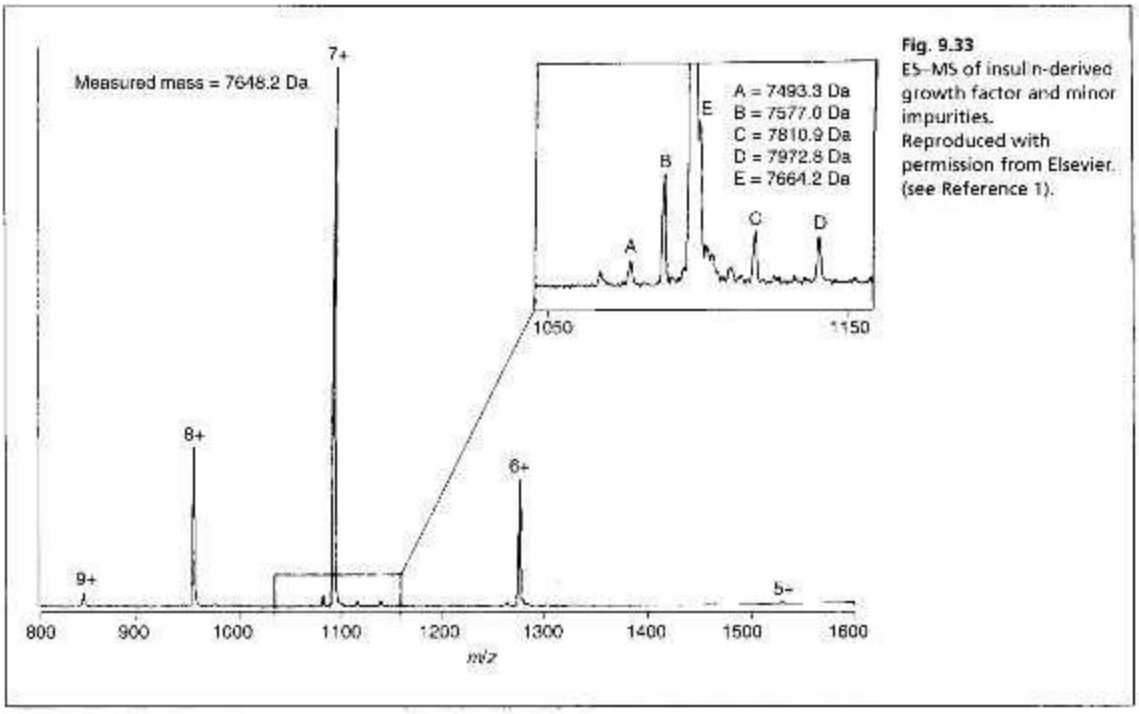


Fig. 9.33
ES–MS of insulin-derived growth factor and minor impurities. Reproduced with permission from Elsevier. (see Reference 1).

Table 9.4 Minor impurities in IGF

Assignment	MW Da
IGF	7648
IGF-N terminal glycine-proline	7494
IGF-C terminal alanine	7577
IGF oxidised methionine	7664
IGF + hexose	7810
IGF + 2 × hexose	7972

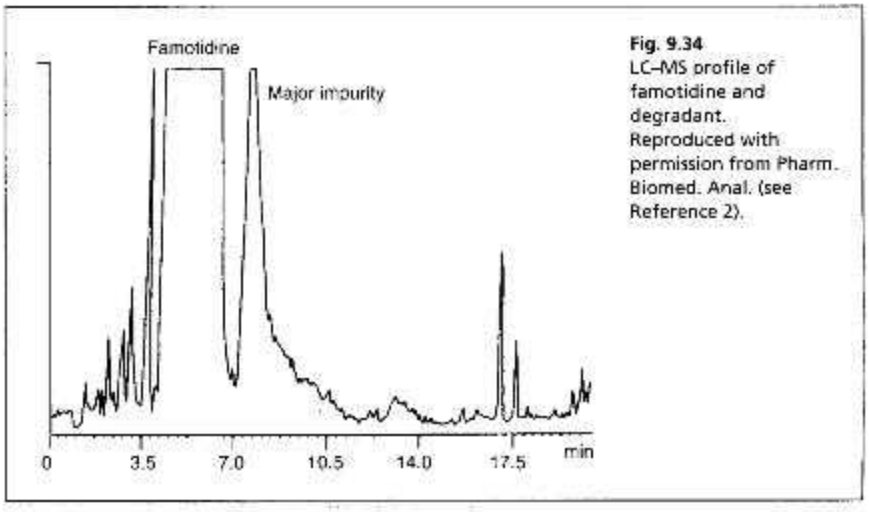


Fig. 9.34
LC–MS profile of famotidine and degradant. Reproduced with permission from Pharm. Biomed. Anal. (see Reference 2).

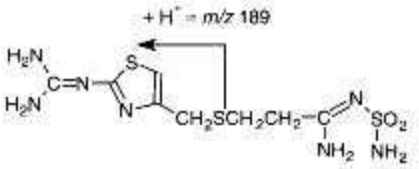


Fig. 9.35 Fragmentation of famotidine.

The mass spectra obtained for famotidine and its degradant by APCIMS and APCIMS–MS are shown in Figures 9.36 and 9.37.

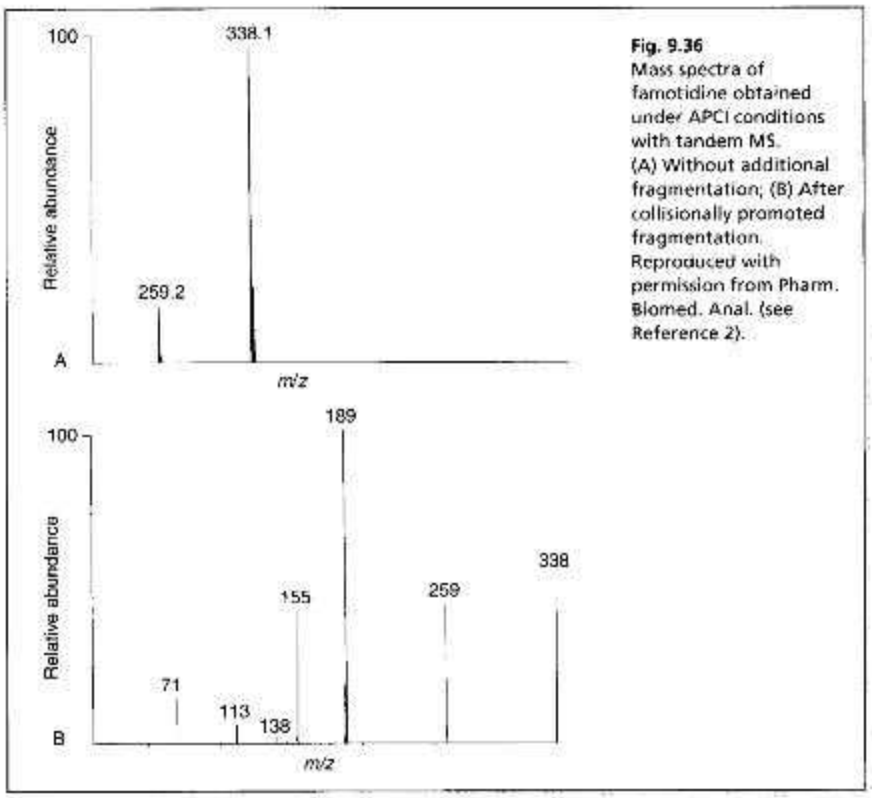


Fig. 9.36 Mass spectra of famotidine obtained under APCI conditions with tandem MS. (A) Without additional fragmentation; (B) After collisionally promoted fragmentation. Reproduced with permission from Pharm. Biomed. Anal. (see Reference 2).

The degradant had a MW 12 amu higher than that of famotidine. The fragment at *m/z* 189 was common to both spectra, indicating that the two molecules were similar in structure. The degradant was found to have the structure shown in Figure 9.38 and was proposed to result from reaction of famotidine with formaldehyde residues present in the packaging. The structure of the degradant was confirmed by synthesis of a standard for the degradant by reaction of famotidine with formaldehyde.

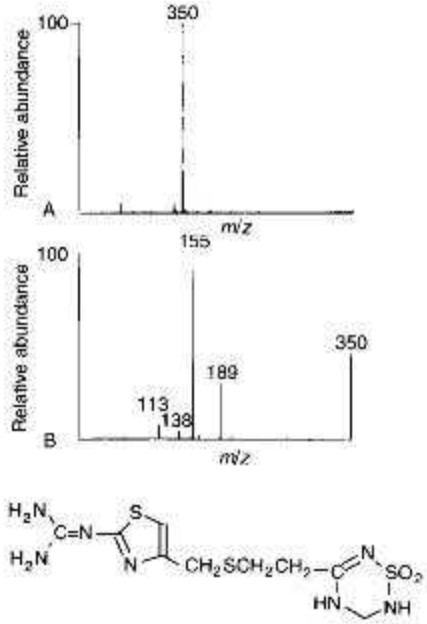


Fig. 9.37
Mass spectra of a famotidine degradant obtained under APCI conditions with tandem MS. (A) Without additional fragmentation; (B) After collisionally promoted fragmentation. Reproduced with permission from Pharm. Biomed. Anal. (see Reference 2).

Profiling impurities and degradants in butorphanol tartrate

HPLC coupled to an ES–MS was used to elucidate the structure of a number of degradants in butorphanol[3] following its storage in aqueous solution. Figure 9.38 shows the LC–MS profile of the degradants which were detected in butorphanol.

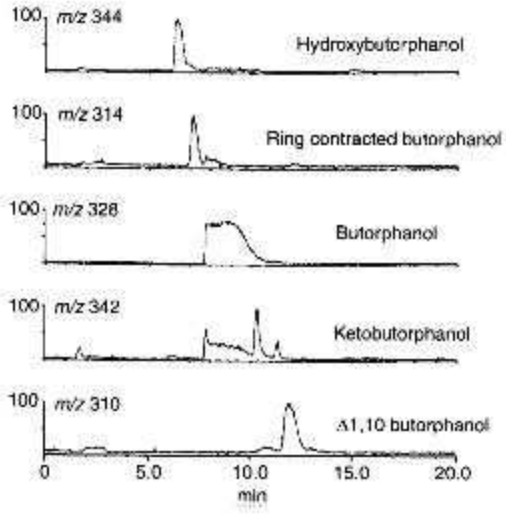


Fig. 9.38
LC–MS selected ion profile of the degradants formed from butorphanol. Reproduced with permission from Pharm. Biomed. Anal. (see Reference 3).

Figure 9.39 shows the APCI MS–MS spectrum of butorphanol, where additional fragmentation of the molecule was produced in a collision cell as an aid to identification.

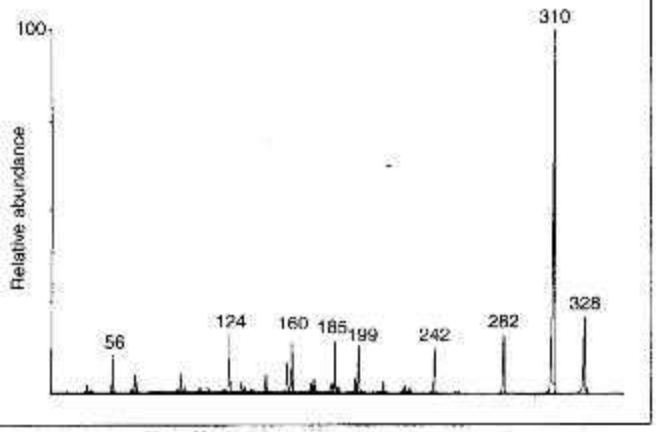


Fig. 9.39
MS–MS spectrum of butorphanol ionised using APCI. Reproduced with permission from Pharm. Biomed. Anal. (see Reference 3).

Some of the ions present in butorphanol shown in Figure 9.39 can be explained by the following losses: 310 ($MH-H_2O$), 282 ($MH-H_2O-C_2H_4$) and 242 ($MH-H_2O-C_5H_8$). An ion at *m/z* 199 formed by rearrangement of the structure prior to fragmentation with loss of one of the rings was assigned the following structure (Fig. 9.40):

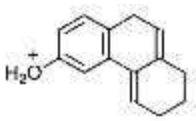


Fig. 9.40
Characteristic fragment ion generated from butorphanol and related degradants.

The fragment in Figure 9.40 was characteristic of the nucleus of the molecule and provided an important pointer in the elucidation of structure of the other degradants. By comparison with mass spectrum obtained for butorphanol, the degradants shown in Figure 9.41 were found to be present.

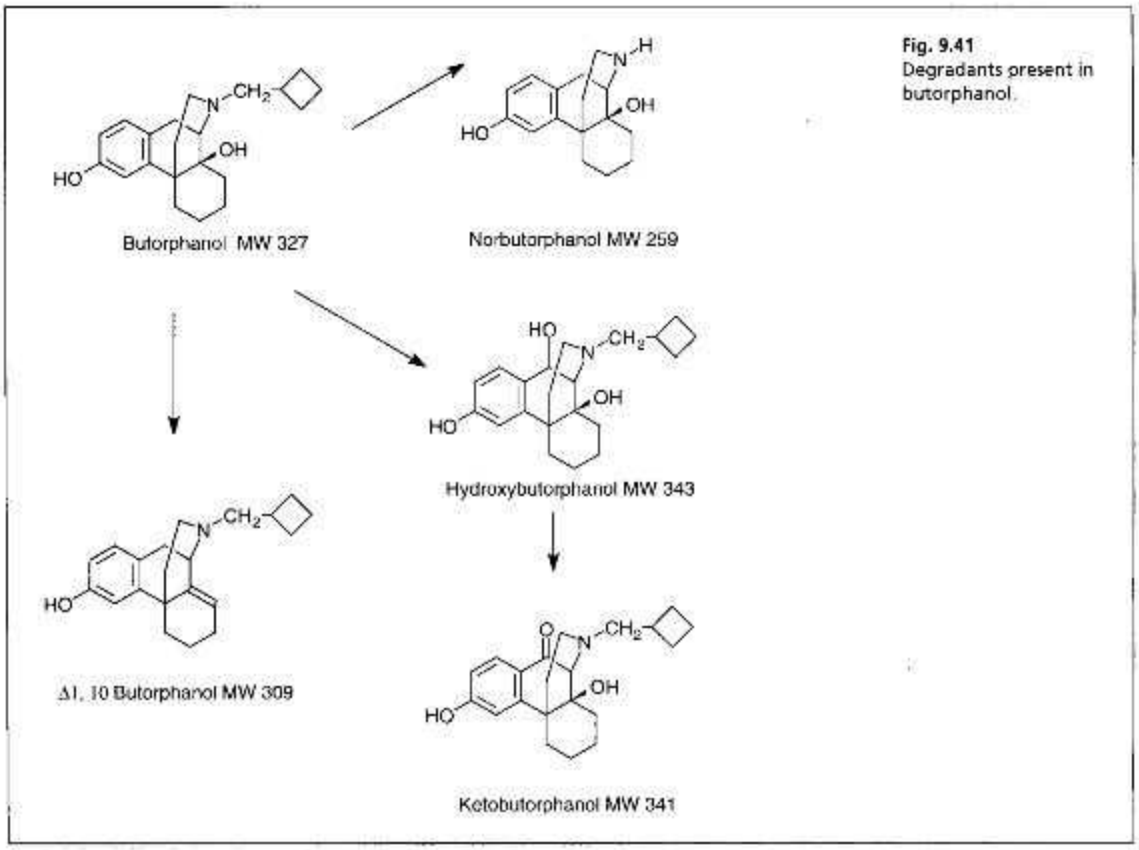


Fig. 9.41
Degradants present in butorphanol.

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3. K.J. Volk, S.E. Klohr, R.A. Rourick, E.H. Kerns and M.S. Lee. J. Pharm. Biomed. Anal. 14, 1663–1674 (1996).

Further reading

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