Extension 3: More About Mass Spectrometry and the Identification of Organic Molecules

1. Combining a mass spectrometer with a gas chromatograph

In Unit 3, we introduced the mass spectrometer. Putting aside the technology, the output of a MS is very simple: a plot of ion intensity against the mass/charge value (m/z or m/e, where z or e is the charge on the ion). However, if the sample mixture that is being injected or sprayed into the mass spectrometer contains many different substances, mass spectrometry may not be able to identify all or even most of the components present as the mass spectrum of the mixture will contain hundreds or thousands of peaks, some overlapping. One way of tackling this is to first use another analytical instrument – such as a gas chromatograph (GC) – to separate the mixture *before* it enters the mass spectrometer so that a fraction of the original mixture (containing substances with the same retention time) enter the mass spectrometer. The mass spectrum of each fraction emerging from the GC contains fewer compounds and so the mass spectrum is easier to interpret. The combined instrument is then called a Gas Chromatograph Mass Spectrometer, GC-MS (see Fig. 3.1(a)).
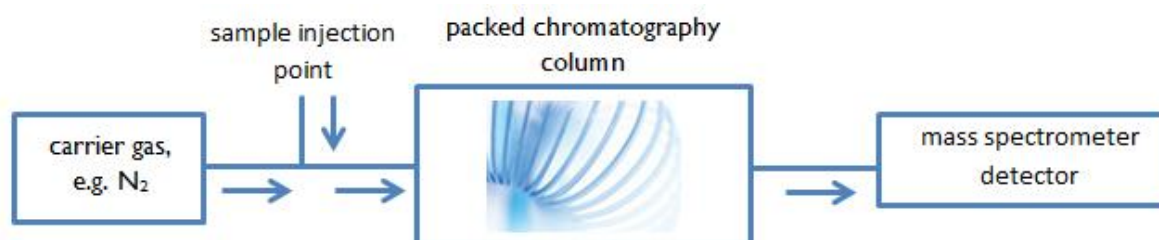

Fig. 3.1(a) Essential parts of a Gas Chromatograph – Mass Spectrometer (GC-MS)
The gas (He, N_2 etc) carries the injected sample into the column of the GC and the separated fractions are then fed into the mass spectrometer.

The GC works by passing the sample, contained in a carrier gas such as hydrogen, through a column of specially selected material. Different molecules stick to the material to different extents, so slowing them down to different degrees and producing different column 'retention times'. Under known conditions (e.g. temperature, column type), retention times are characteristic of particular molecules or types of molecules in the sample and in GC-MS these molecules are fed sequentially into the mass spectrometer for further analysis. For example, if three compounds with a retention time of 3.2 minutes in the GC flow into the MS, the mass spectrum is a superimposition of the mass spectra of the three molecules and not (say) of the one thousand molecules in the original sample. This greatly simplifies the appearance and interpretation of the m/z spectrum. It also allows conclusive identifications to be made – something which is very important in forensic investigations where evidence is presented in court.

Fig 3.1(b) A modern GC-MS

The RHS part of the instrument is the oven, containing the narrow column through which the sample passes to be separated. The buttons allow the operator to control the temperature of the oven. The LHS is the mass spectrometer, in this case one using a quadrupole to analyse ions. The top of the instrument is an automated sampler, which can sequentially inject different samples for GC-MS analysis in the absence of the operator.

Photograph courtesy of Agilent Technologies.

Suppose that in an experiment with a particular GC column at 100°C, ethanol (C_2H_5OH) was found to have a retention time of 90 seconds. An unknown sample was then injected into the same GC and column at 100°C. The GC separated the mixture into fractions which emerge from the column at different retention times. One such fraction had a retention time of 90 seconds (Fig. 3.2(a)). When this fraction was injected into the MS, the resulting mass spectrum contained the features of the reference mass spectrum of ethanol (Fig. 3.2(b)), with a strong *m/e* peak at 46. The fact that the fraction with the retention time of ethanol also yielded the mass spectrum of ethanol shows that the original mixture contained ethanol.
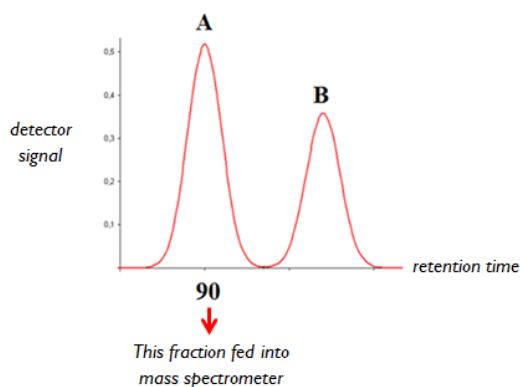

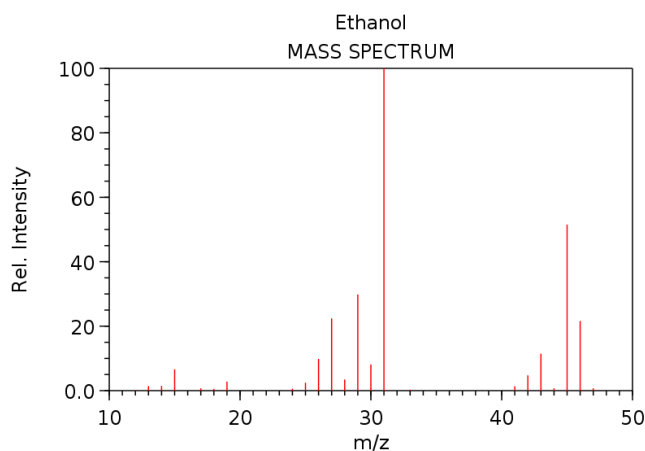**Fig. 3.2 (a)** Two fractions separated by a GC column

Fig. 3.2 (b) Reference Mass Spectrum of ethanol, C_2H_5OH. Source: NIST Chemistry WebBook, NIST Standard Reference Database Number 69, Eds. P.J. Linstrom and W.G. Mallard.

2. The use of mass spectrometry in the identification of organic molecules

In Unit 3 of the book we explained how ions are formed in the chamber of the mass spectrometry. Let's start by explaining the mass spectrum of ethanol, C_2H_5OH (i.e. CH_3CH_2OH), obtained by electron ionization (EI) and shown in Fig 3.2(b). The main peaks are at m/z 14, 15, 17, 29, 31, 45 and 46. We will assume that all the ions have $z = +1$ i.e. that they are singly positively charged ions: unless you are told otherwise, you may assume this in interpreting mass spectra. We will also use approximate (whole number) atomic masses in our calculations, although with larger molecules errors can occur if accurate atomic masses are not used.

Consider the peak at $m/z = 46$. The molecular mass of ethanol is $(2 \times 12) + (6 \times 1) + 16 = 46$ u. This makes it likely that the peak is caused by the parent molecular ion $C_2H_5OH^+$. The process occurring within the MS chamber to produce this ion is:

$$C_2H_5OH + e^- \rightarrow C_2H_5OH^+ + 2e^- \qquad \dots (1)$$
$$m/z = 46$$
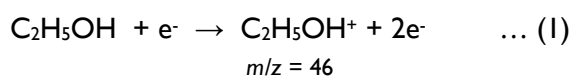
A useful tip in interpreting mass spectra is to start with the line with the highest m/z. Assuming that $z = +1$, the mass of the ion producing that line is m. In the absence of more information we generally work on the basis that this line is produced by an unfragmented 'parent molecule', here C_2H_5OH.

We now go through the other peaks suggesting likely ways in which the ions would be formed. In order to produce masses below that of the parent ion (here, below $m/z = 46$) fragmentation of the ions must have occurred: the image to keep in mind is that of highly energetic ions falling apart to produce smaller ions or molecules.

Consider the peak at $m/z = 45$. This is consistent with the fragmentation:

$$C_2H_5OH^+ \rightarrow C_2H_5O^+ + H \qquad \dots (2)$$
$$m/z = 45$$
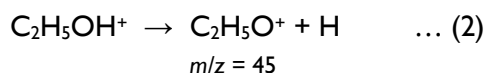
Similarly, the ion responsible for the peak at $m/e = 31$ is likely to be CH_2OH^+, produced by the breakage of the $CH_3\text{-}CH_2$ bond:

$$C_2H_5OH^+ \rightarrow CH_3 + CH_2OH^+ \qquad \dots (3)$$
$$m/z = 31$$
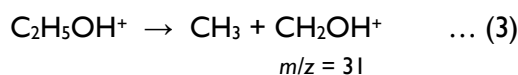
The molecular fragment CH_3 (better represented as a radical, $CH_3\cdot$) is not detected because it is uncharged but if it subsequently becomes ionized in the mass spectrometer chamber, the CH_3^+ ion will produce a peak at $m/z = 15$ – as seen in Fig 3.2(b).

In summary, we can assign the ion that is likely to be responsible for a peak by working out its mass. Sometimes, there might be several fragmentation routes by which the ion in question is created, but our interest is in working out the identity of the ions rather than how they are formed. Using this procedure, we complete the ion identification as in Table 1.

Table 1 Identification of ions responsible for peaks in MS spectrum of ethanol

m/z	ion responsible for peak
14	CH_2^+
15	CH_3^+
17	OH^+
29	$CH_3CH_2^+$
31	CH_2OH^+
45	$C_2H_5O^+$
46	$C_2H_5OH^+$

In practice, we use the reference MS spectrum to identify an 'unknown'. If a compound is injected into a mass spectrometer and produces the peaks listed in Table 1, it is likely to be ethanol. If the unknown has a retention time in a GC column equal to that of an ethanol reference as well as displaying the ions in Table 1, the identification of the molecule is confirmed.

Revision Questions

1. The mass spectrum of propanone (acetone), CH_3COCH_3, contains strong peaks at $m/z =$ 58, 43 and 15. What ions are likely to be responsible for these peaks?

2. The spectrum of benzene sulphonic acid, $C_6H_5SO_3H$:
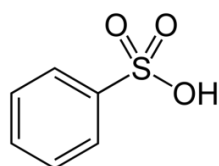
   displays peaks at $m/z = 158$ and 77. What ions are responsible for these peaks?

3. (a) A sample of compound 'B', molecular mass 78.5 u, displays a prominent C=O absorption in its infrared spectrum. Its chemical reactions suggest the presence of the –CO-Cl group. The EI mass spectrum of 'B' shows strong peaks at $m/z = 15$ and 43 and two further peaks at $m/z = 63$ and 65 (with the ratio of the intensities of the 63 to 65 peaks being 3:1). What ions are responsible for the four peaks in the mass spectrum? Suggest the likely molecular structure of 'B'.

   (b) The mass spectrum of 'B' contains virtually no peak of the parent ion. Why is this and how can the molecular mass of the parent ion be confirmed?

Answers

1. $CH_3COCH_3^+$ (58, the parent molecular ion), CH_3OO^+ (43) and CH_3^+ (15).

2. Molecular mass of benzene sulfonic acid = 158 so parent ion at $m/z = 158$. Ion at $m/z = 77$, probably due to the benzene ring $C_6H_5^+$ ($m = 77$ u).

3. (a) The peaks at m/z 15 and 43 are likely to be due to CH_3^+ and CH_3CO^+ respectively, making it very likely that 'B' contains the component CH_3CO. The ratio of the two peaks at 63 and 65 is 3:1, suggesting chlorine is present in its two natural isotopic forms Cl-35 and Cl-37 and the corresponding ions producing these peaks are due to the species –$COCl^+$

with the Cl in this unit being present as the Cl^{35} and Cl^{38} isotope respectively. Since we are provided with the molecular mass of 'B' we may now confirm its molecular formula as CH_3COCl. [Note that the given molecular mass of CH_3COCl contains the average mass of chlorine ($m = 35.5$ u) whereas specific peaks for chlorine-containing ions in the MS spectrum are caused by species either containing the isotope Cl-35 or Cl-38].

(b) The parent ion CH_3COCl^+ (in its two isotopic forms with masses 78 and 81 u respectively) fragments so easily that it is almost undetectable in an electron ionization mass spectrum. The NIST MS spectrum confirms this (see https://webbook.nist.gov/chemistry/form-ser/, search for acetyl chloride and select mass spectrum). It would be useful to use a softer ionization technique such as ESI, which would contain more intense parent ion peaks.