INTERNATIONAL COLLEGE OF PHARMACEUTICAL INNOVATION

国际创新药学院

Fundamentals of Medicinal and Pharmaceutical Chemistry

FUNCHEM. 23 Analytics: Mass Spectrometry

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Learning outcomes

At the end of this lecture, the learner will be able to know

1. What is mass spectrometry
2. How mass spectrometry works
3. What information mass spectrometry gives
4. Fragmentation

Recommended reading

- Organic chemistry (John McMurry)
- Chapter 12 (See this chapter e-Book in the folder mass Spectrometry)

What is mass spectrometry

Mass spectrometry is a technique that measures the mass to charge ratio (m/z) of ions in the gas phase

1. In mass spectrometry, a substance is bombarded with an electron beam having sufficient energy to fragment the molecule.

$$M \xrightarrow{\text{electron beam}} M^{+}\bullet$$

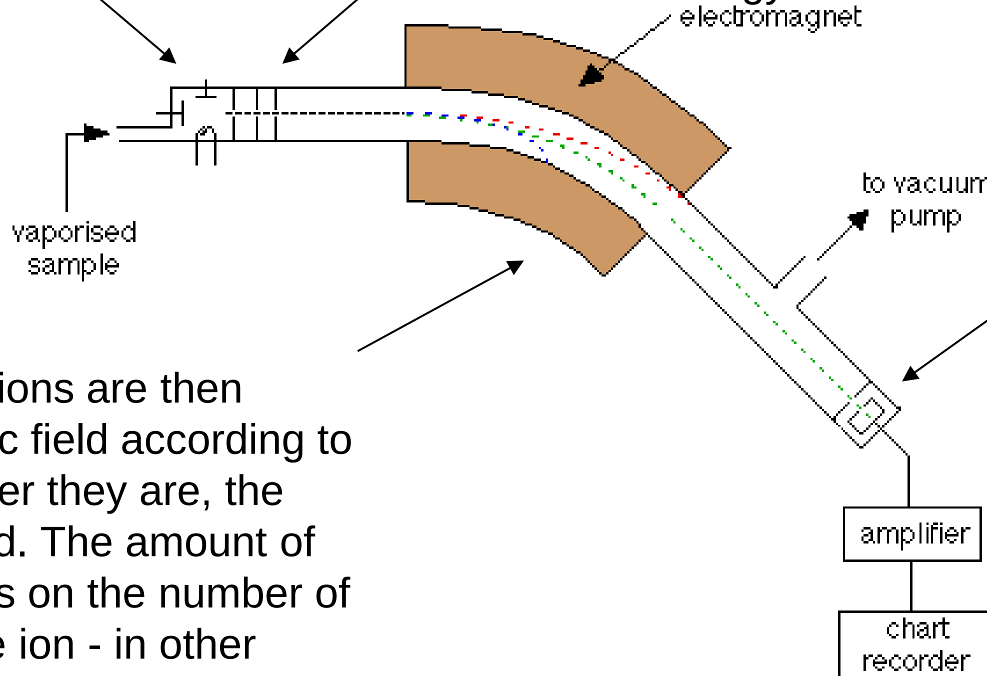
$$M \xrightarrow{\text{electron beam}} M^{+}$$

2. Since the bulk of the ions produced in the mass spectrometer carry the same charge (1+), the value m/e is equivalent to the molecular weight of the fragment.

How Mass spectrometry works

1. IONISATION: The atom is ionised by knocking one or more electrons off to give a positive ion. This is true even for things which you would normally expect to form negative ions (chlorine, for example) or never form ions at all (argon, for example). Mass spectrometers always work with positive ions

2. ACCELERATION: The ions are accelerated so that they all have the same kinetic energy



4. DETECTION: The beam of ions passing through the machine is detected electrically

3. DEFLECTION: The ions are then deflected by a magnetic field according to their masses. The lighter they are, the more they are deflected. The amount of deflection also depends on the number of positive charges on the ion - in other words, on how many electrons were knocked off in the first stage. The more the ion is charged, the more it gets deflected.

Mass spectrometry

3. The **positive** fragments which are produced (cations **M+** and radical cations **M•+**) are accelerated in a vacuum through a magnetic field and are sorted on the basis of mass-to-charge ratio.

4. Only the cations are deflected by the magnetic field.

5. Amount of deflection depends on ***m/z***.

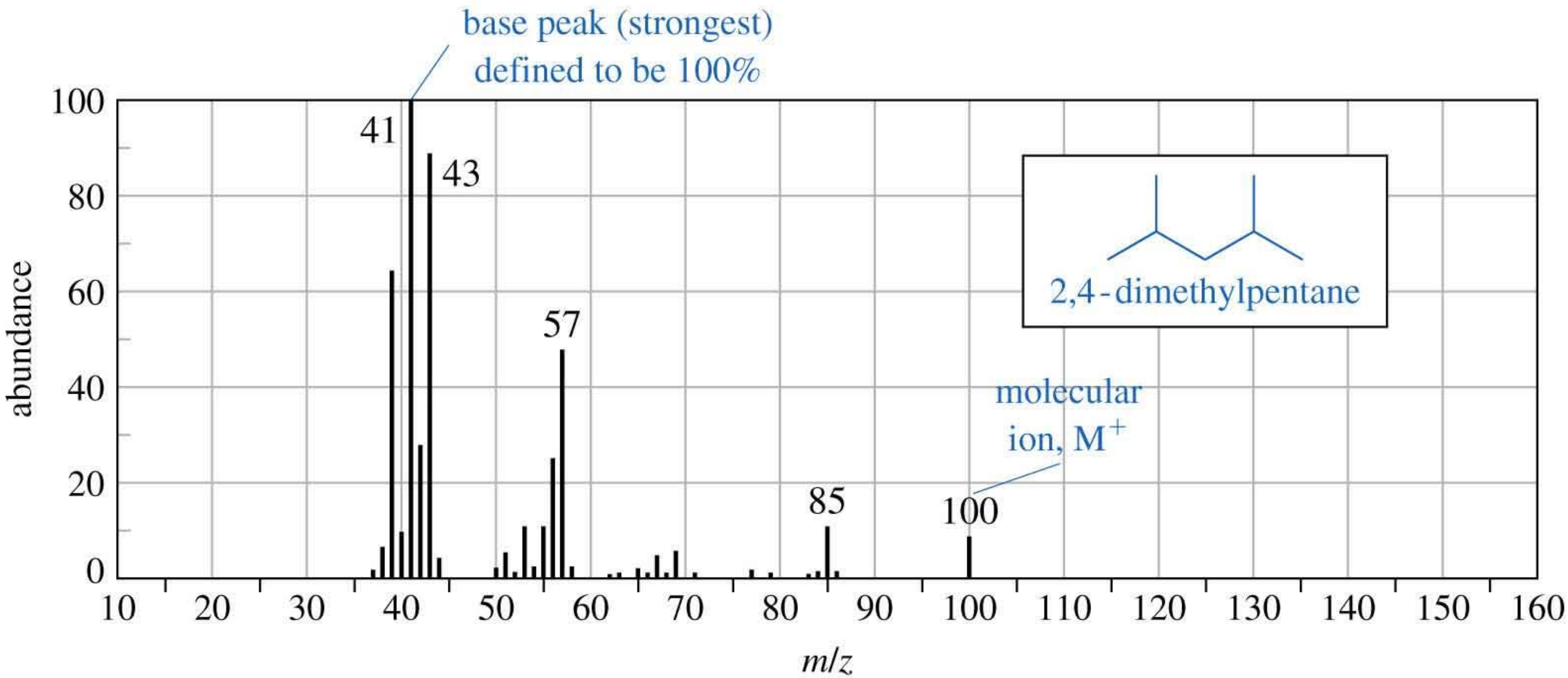
6. By varying the magnetic field, ions of all masses are collected and counted.

7. The detector signal is proportional to the number of ions hitting it.

$$M \xrightarrow{\text{electron beam}} M^{\overset{+}{\bullet}} \longrightarrow X^{+} + Y^{\bullet}$$

The Mass Spectrum

Mass spectrum is a plot of Masses are graphed according to their relative abundance.



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NOTE: The mass spectrum is populated by several peaks = fragmentation

Fragmentation

When the vaporised organic sample passes into the ionisation chamber of a mass spectrometer, it is bombarded by a stream of electrons. These electrons have a high enough energy to knock an electron off an organic molecule to form a positive ion. This ion is called the ***molecular ion*** (M+ or M•+) or sometimes the ***parent ion***

The molecular ions (M+ or M•+) are energetically unstable, and some of them will break up into smaller pieces.

$$M \xrightarrow{\text{electron beam}} M^{\overset{+}{\bullet}} \longrightarrow X^{+} + Y^{\bullet}$$

The uncharged free radical won't produce a line on the mass spectrum. Only charged particles will be accelerated, deflected and detected by the mass spectrometer. These uncharged particles will simply get lost in the machine - eventually, they get removed by the vacuum pump.

The ion, X+, will travel through the mass spectrometer just like any other positive ion - and will produce a line on the stick diagram.

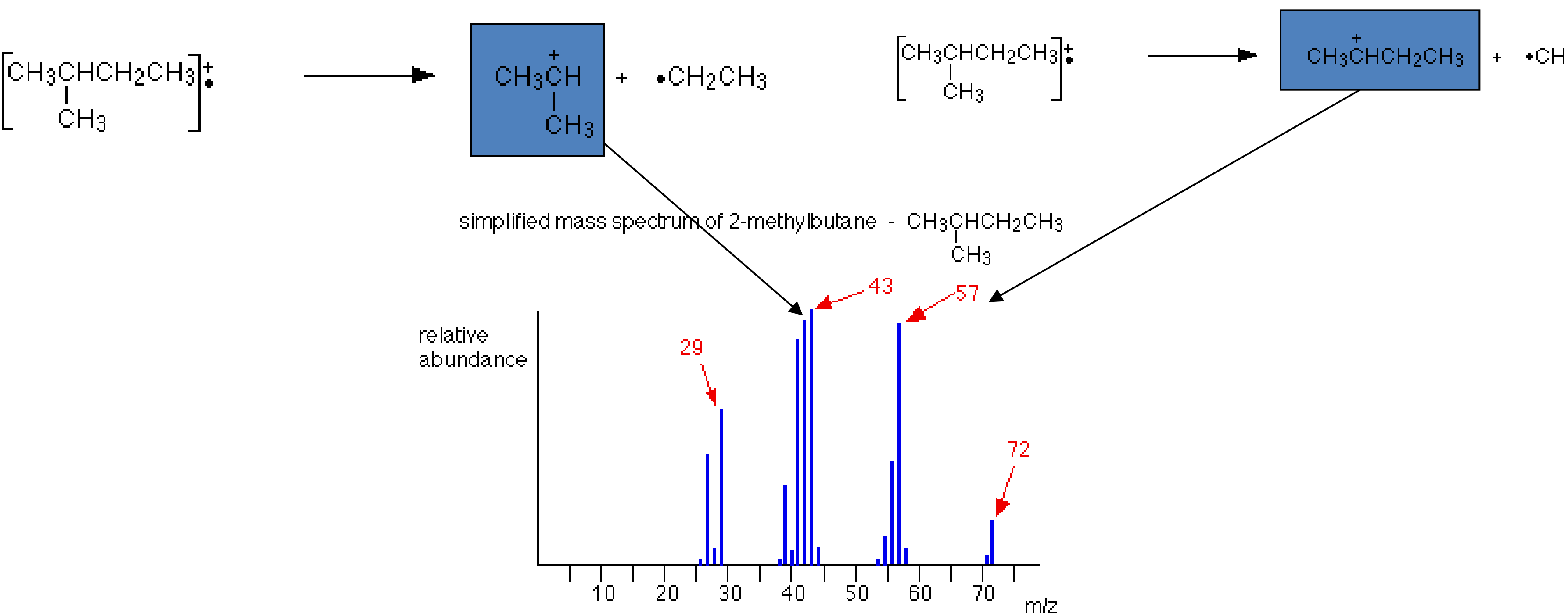
All sorts of fragmentations of the original molecular ion are possible - and that means that you will get a whole host of lines in the mass spectrum.

Rules for predicting fragmentation

(Limited and very general!!!)

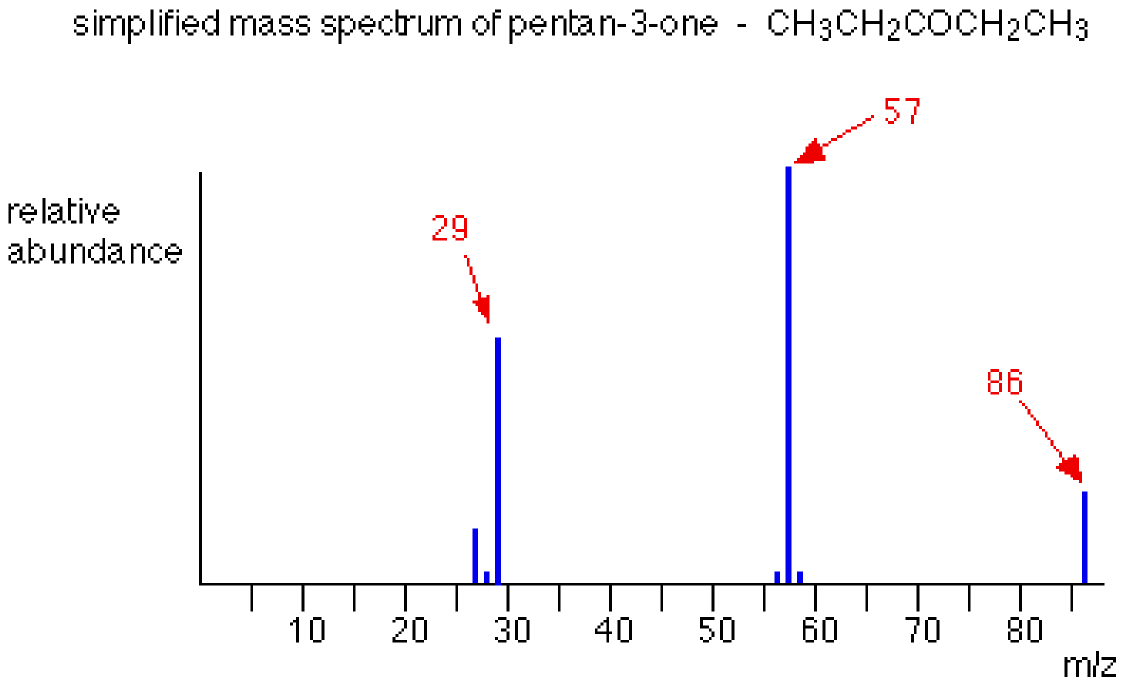
The fragmentation deals with formation of carbocations and radicals, therefore in general terms the most abundant fragment is the most stable carbocation or radical. Therefore any consideration valid to discuss the stability of carbocation could be used to predict fragmentation:

For example: 2-methylbutane: The most abundant ions formed (57 and 43) are secondary carbocations – they have two alkyl groups attached to the carbon with the positive charge. As such, they are relatively stable compared to primary ones

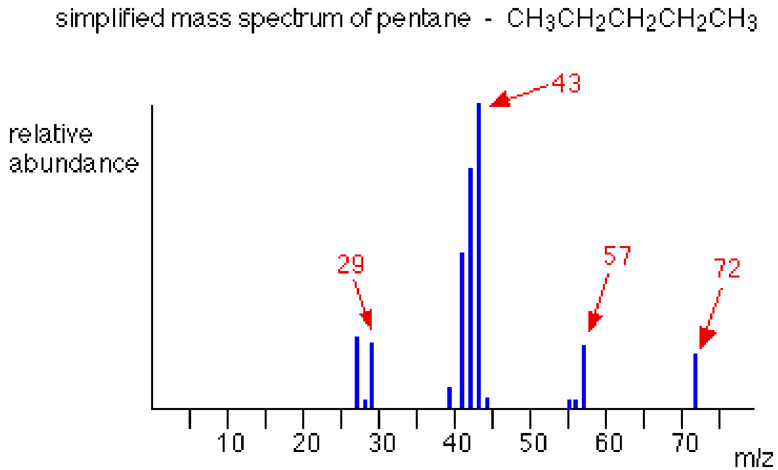


Rules for predicting fragmentation!

The most abundant peak in the fragmentation of pentan-3 one (base peak) is at *m/z* =57, and is due to the **Acylium ion** $[CH_3CH_2CO]^+$. Acylium species are carbonyls containing a positive charge. These species are relatively stable and therefore could be formed easily under the experimental conditions present inside the mass spectrometer.

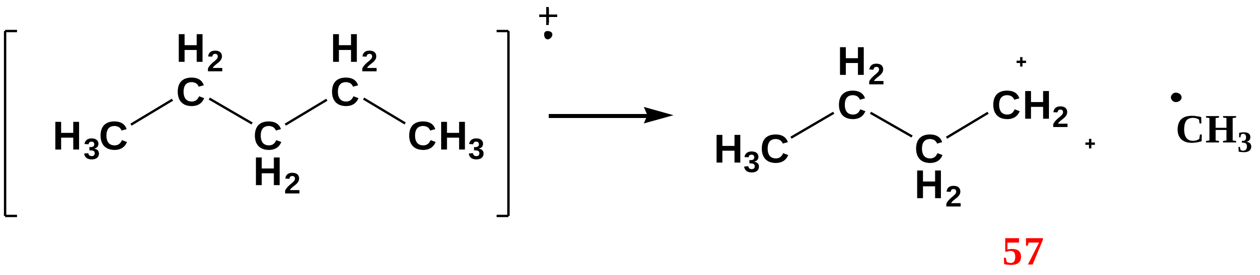


Mass spectrum of pentane 1/2

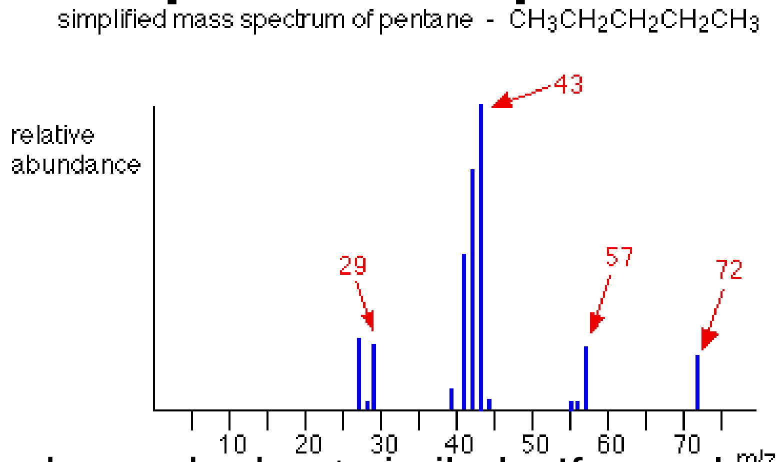


1. In the stick diagram showing the mass spectrum of pentane, the line produced by the heaviest ion passing through the machine (at ***m/z* = 72**) is due to the molecular ion.

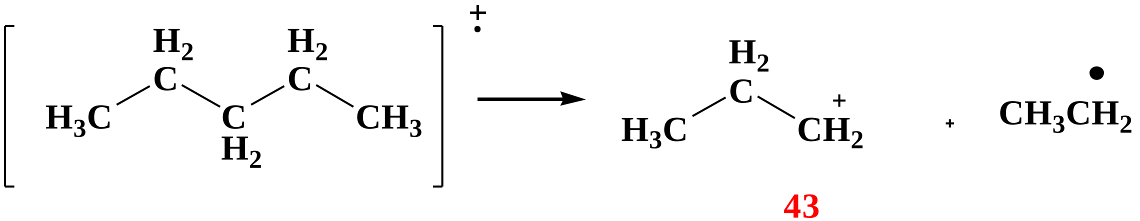
2. *What causes the line at m/z = 57?*
How many carbon atoms are in this ion? There can't be 5 because 5 x 12 = 60. What about 4? 4 x 12 = 48. That leaves 9 to make up a total of 57. How about $C_4H_9^+$ then? $C_4H_9^+$ would be $[CH_3CH_2CH_2CH_2]^+$, and this would be produced by the following fragmentation:



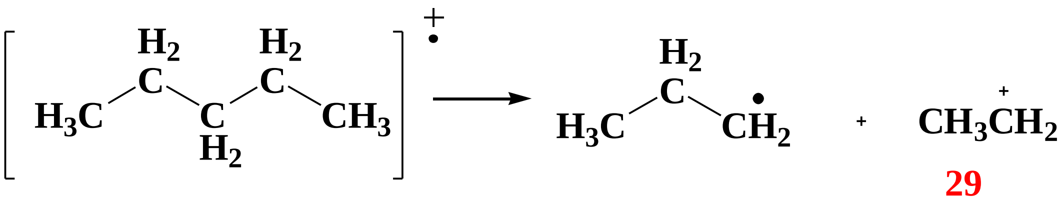
Mass spectrum of pentane 2/2



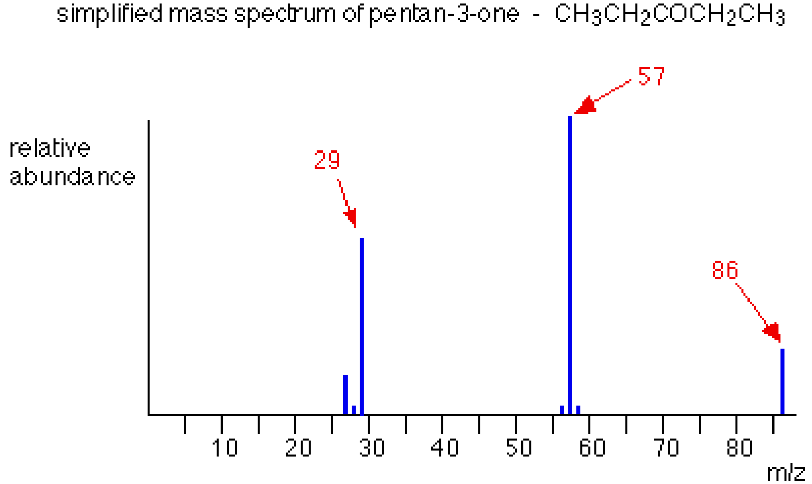
3. The line at *m/z* = **43** can be worked out similarly. If you play around with the numbers, you will find that this corresponds to a break producing a 3-carbon ion



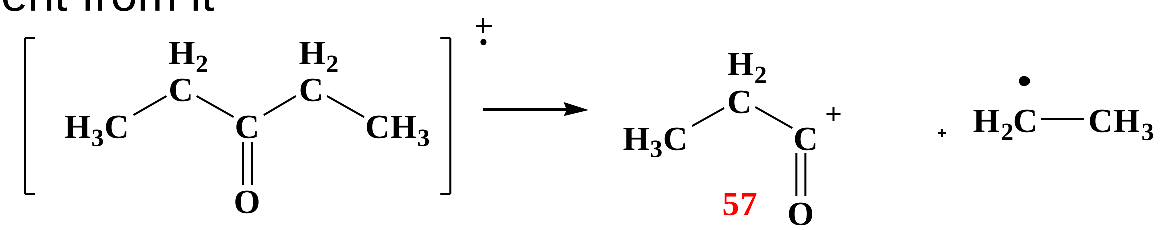
4. The line at *m/z* = **29** is typical of an ethyl ion, $[CH_3CH_2]^+$



Mass spectrum of pentanone 1/2

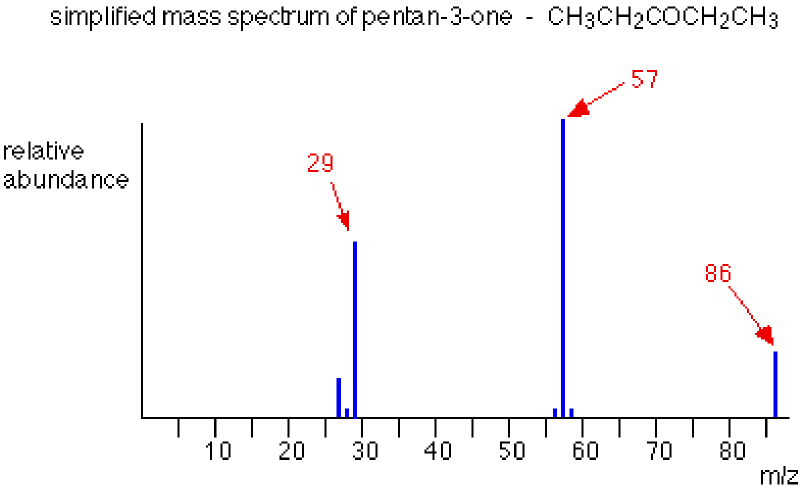


1. This time the base peak (the tallest peak - and so the most common fragment ion) is at *m/z* = **57**. But this isn't produced by the same ion as the same *m/z* value peak in pentane. If you remember, the *m/z* = 57 peak in pentane was produced by $[CH_3CH_2CH_2CH_2]^+$. If you look at the structure of pentan-3-one, it's impossible to get that particular fragment from it

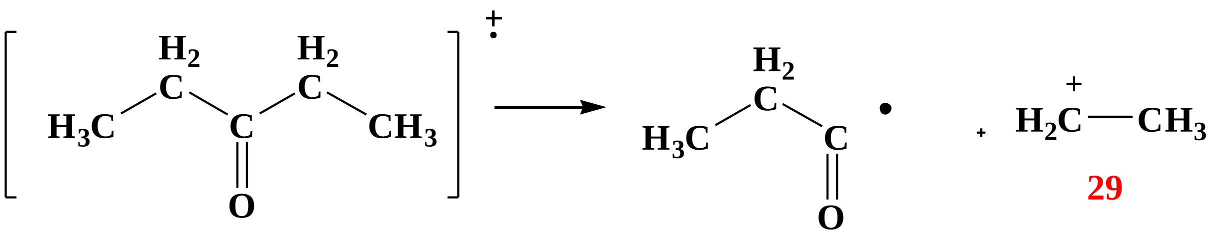


2. Work along the molecule mentally chopping bits off until you come up with something that adds up to **57**. With a small amount of patience, you'll eventually find $[CH_3CH_2CO]^+$ which is produced by this fragmentation

Mass spectrum of pentanone 2/2



You would get exactly the same products whichever side of the CO group you split the molecular ion. The *m/z* = **29** peak is produced by the ethyl ion which once again could be formed by splitting the molecular ion either side of the CO group.



Using mass spectra to distinguish between compounds

Suppose you had to suggest a way of distinguishing between pentan-2-one and pentan-3-one using their mass spectra.

pentan-2-one	$CH_3COCH_2CH_2CH_3$
pentan-3-one	$CH_3CH_2COCH_2CH_3$

Each of these is likely to split to produce ions with a positive charge on the CO group. In the pentan-2-one case, there are two different ions like this (remember the stability of acylium ions)

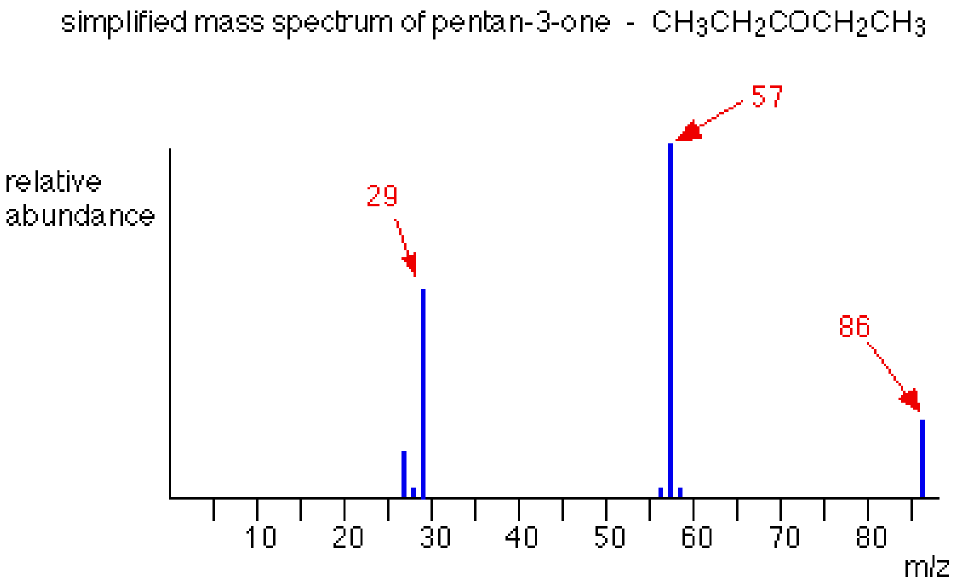
In the pentan-2-one case, there are two different ions like this:

$[CH_3CO]^+$ giving a strong line at m/z = **43**

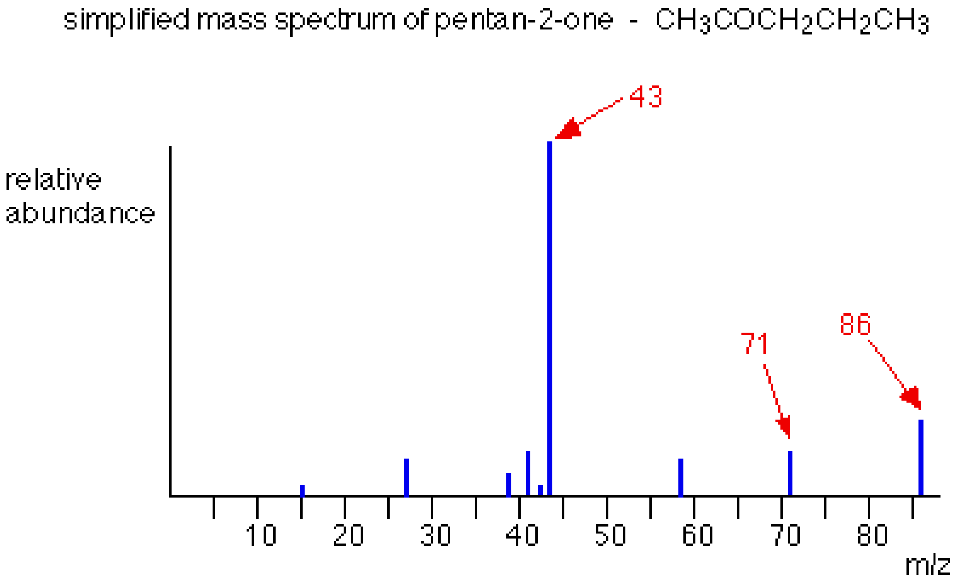
$[COCH_2CH_2CH_3]$+ giving a strong line at m/z = **71**

In the pentan-3-one case, you would only get one ion of this kind:

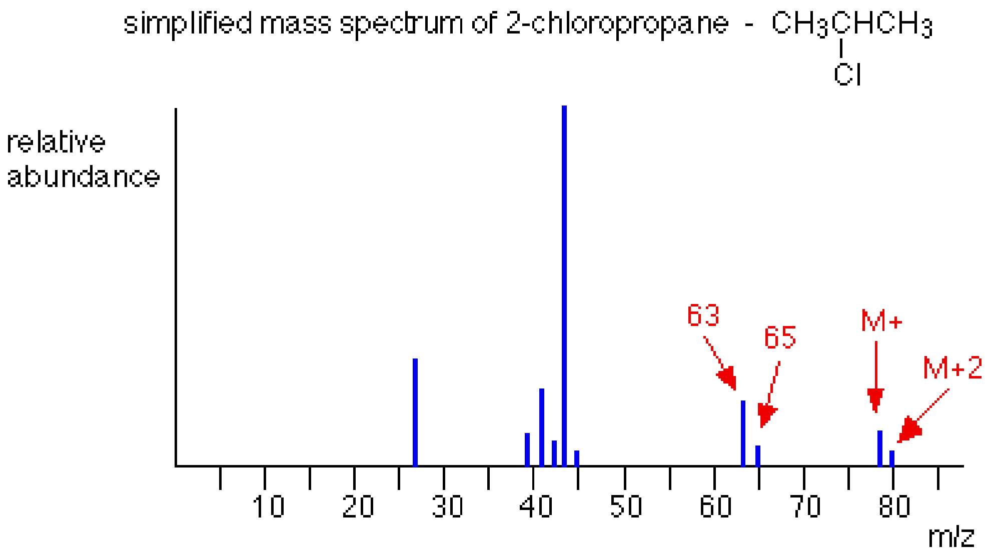
$[CH_3CH_2CO]^+$ giving a strong line at m/z = **57**



You don't need to worry about the other lines in the spectra the 43, 57 and 71 lines give you plenty of difference between the two. The 43 and 71 lines are missing from the pentan-3-one spectrum, and the 57 line is missing from the pentan-2-one.

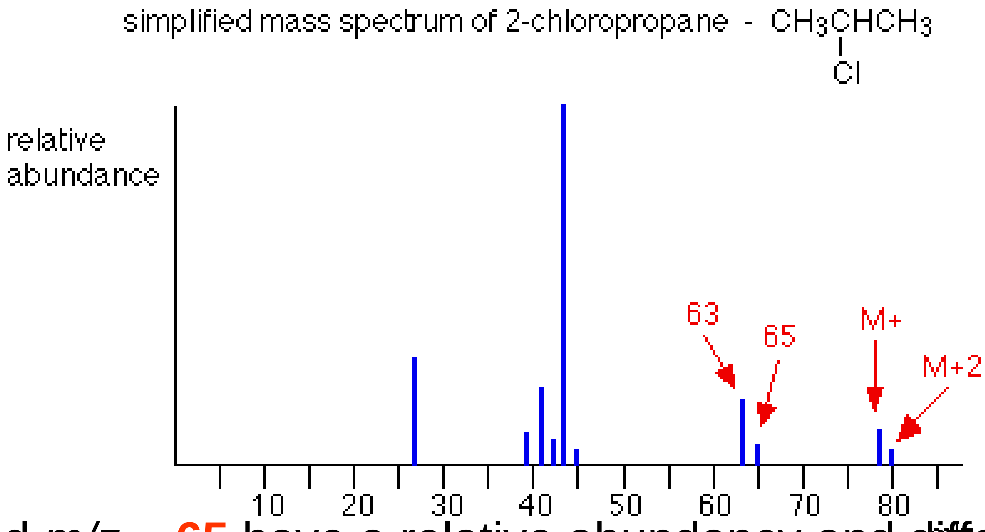


Compounds containing chlorine atoms 1/2

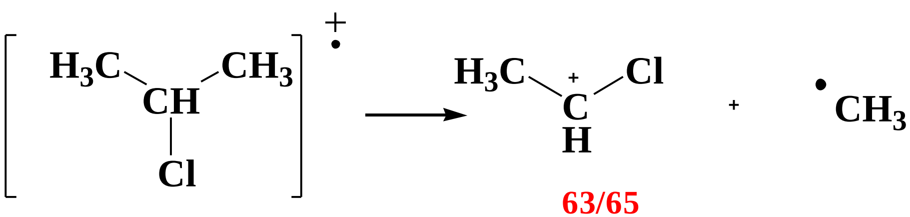


The molecular ion peaks (M^+ and M^++2) each contain one chlorine atom but the chlorine can be either of the two chlorine isotopes, ^{35}Cl and ^{37}Cl.
The molecular ion containing the ^{35}Cl isotope has a relative formula mass of 78. The one containing ^{37}Cl has a relative formula mass of 80 hence the two lines at *m/z* = 78 and *m/z* = 80. *Notice that the peak heights are in the ratio of 3 : 1*. This reflects the fact that chlorine contains 3 times as much of the ^{35}Cl isotope as the ^{37}Cl one. That means that there will be 3 times more molecules containing the lighter isotope than the heavier one. So . . . if you look at the molecular ion region, and find two peaks separated by 2 *m/z* units and with a ratio of 3 : 1 in the peak heights, you are looking at a molecule containing 1 chlorine atom.

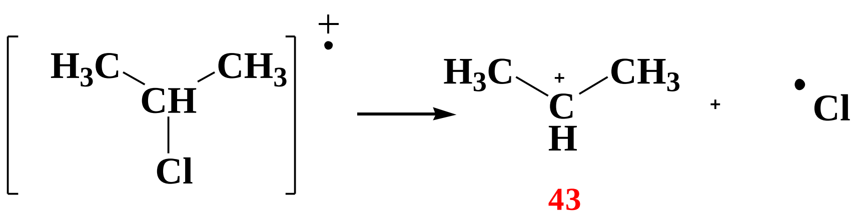
Compounds containing chlorine atoms 2/2



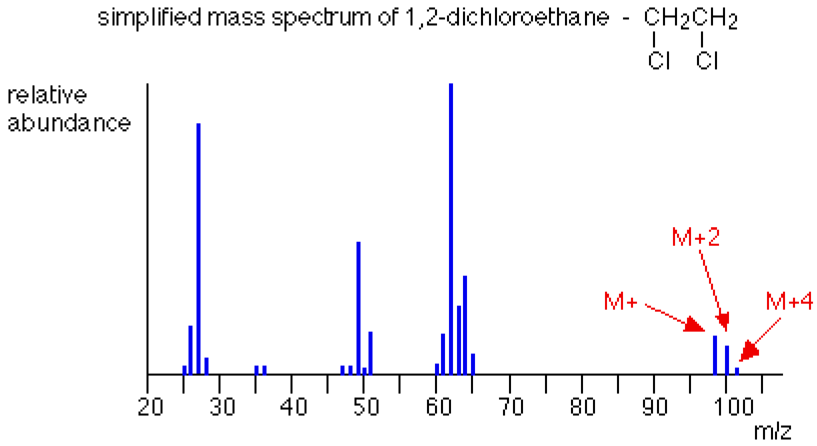
Ions at *m/z* = **63** and *m/z* = **65** have a relative abundancy and difference of 2 *m/z* units. This pattern is due to the presence of either ^{35}Cl or ^{37}Cl in this fragment. The fragmentation that produced those ions was:



…and that the most abundant fragment is at **43** *m/z* and that does not contain the halogen



Two chlorine atoms in a compound



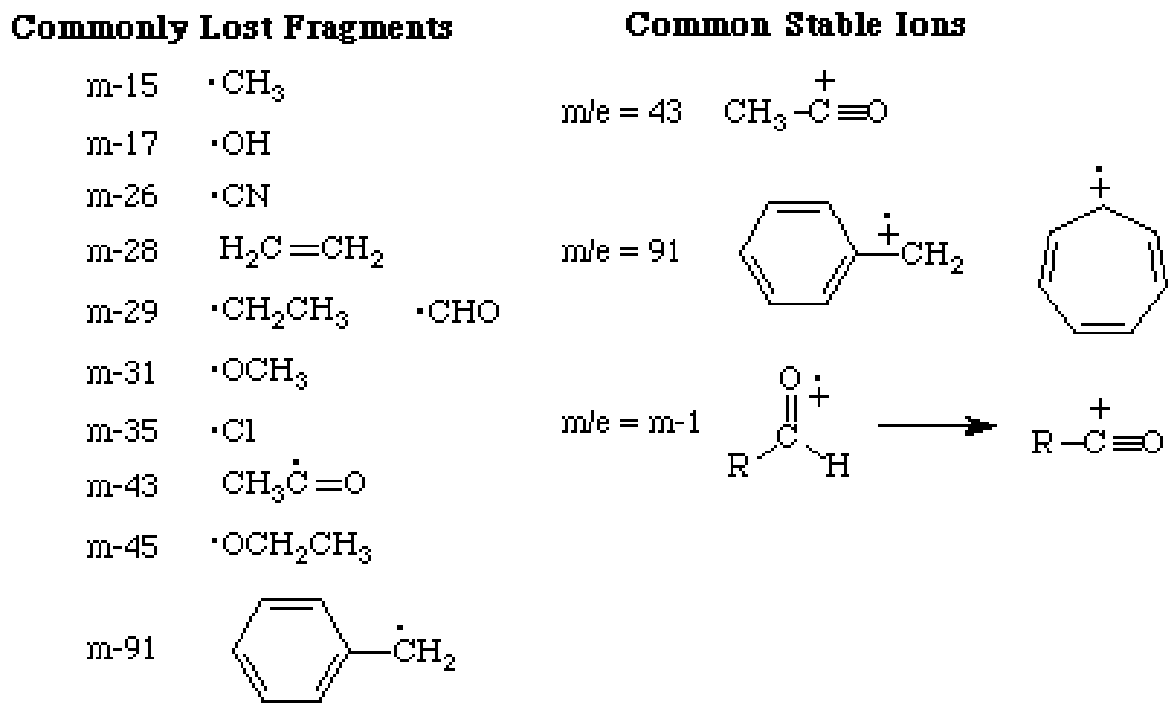
The lines in the molecular ion region (at *m/z* values of **98**, **100** ands **102**) arise because of the various combinations of chlorine isotopes that are possible. The carbons and hydrogens add up to 28 (CH_2CH_2 fragment) so the various possible molecular ions could be:

$$28 + 35 + 35 = 98$$
$$28 + 35 + 37 = 100$$
$$28 + 37 + 37 = 102$$

If you have the necessary maths, you could show that the chances of these arrangements occurring are in the ratio of 9 :6 : 1 and this is the ratio of the peak heights.

So if you have 3 lines in the molecular ion region (M+, M+2 and M+4) with gaps of 2 m/z units between them, and with peak heights in the ratio of 9:6:1, the compound contains 2 chlorine atoms.

Commonly lost fragments/ stable ions



The lists given above are by no means exhaustive and represents only the simplest and most common fragments seen in the mass spectrum;

HINT: just spend a little time with calculator and periodic table and have fun!!

Learning outcomes

At the end of this lecture, the learner will be able to know

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