Chapter 2

Interpretation of Mass Spectra

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Additional information is available at the end of the chapter

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Abstract

The chapter includes an introduction to the main ionisation techniques in mass spectrometry and the way the resulting fragments can be analysed. First, the fundamental notions of mass spectrometry are explained, so that the reader can easily cover this chapter (graphs, main pick, molecular ion, illogical pick, nitrogen rule, etc.). Isotopic percentage and nominal mass calculation are also explained along with fragmentation mechanism. A paragraph emphasises the ionisation energy issues, the basics of ionisation voltage, the developing potential and the energy balance. A frame time of the main theoretical milestones in both theory and experimental mass spectrometry is highlighted here. In the second part of the chapter, the molecular fragmentation for alkanes, iso-alkanes, cycloalkanes, halogen, alcohols, phenols, ethers, carbonyl compounds, carboxylic acids and functional derivatives, nitrogen compounds (amines, nitro compounds), sulphur compounds, heterocycles and biomolecules (amino acids, steroids, triglycerides) is explained. Fragmentation schemes are followed by the simplified spectra, which help the understanding of such complex phenomena. At the end of the chapter, acquisition of mass spectrum is discussed. The chapter presented here is an introduction to mass spectrometry, which, we think, helps the understanding of the mechanism of fragmentation corroborating spectral data and molecular structures.

Keywords: mass spectra, ionisation techniques, detectors, fragmentation, organic molecules

1. Introduction

Mass spectrometry is a destructive method used to measure molecular weight and provide data on molecular structure; it differs from the other methods in that the sample is ionised and not subject to electromagnetic radiation. Ionised compounds are excited, which induces fragmentation. Analysis of such fragments provides information on the structure of molecules. Each fragment is characterised by the mass-to-charge ratio, m/z, and devices are able to separate and detect such ions. Mass spectrometers consist of three distinct parts (**Figure 1**):

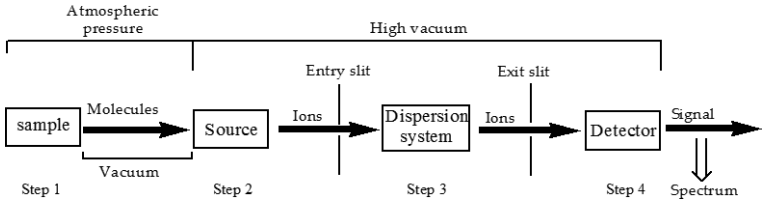


Figure 1. Schematics of a mass spectrometer [1].

- The source where ionisation of molecules and ion fragmentation occurs
- The dispersion system, the analyser, which ensures ion separation by mass/charge ratio
- The detector measuring the relative abundance of each ion

Traditionally limited to the study of classical small organic molecules ($M < 2000$), the scope of mass spectrometry has been recently also extended to the study of macromolecules ($M > 100{,}000$), polypeptides in particular.

The devices may be either used with a system for direct sample introduction (pure substances) or coupled with a chromatographic system. There are also devices (the MS-MS tandem) allowing for analysis of mixtures without prior chromatography. The first MS stage serves for selection of an ion, whereas the second analyses of ions result from fragmentation of the ion separated in the first stage [1, 2].

The first mass spectral measurements were undertaken by J.J. Thomson, in 1912, and, in 1918, F.L. Arnot and J.C. Milligan introduced the method of ionisation and pass through a magnetic sector. In 1946, W.E. Stephens assembled a time-of-flight (TOF) device, and, in 1953–1958, W. Paul used a quadrupole analyser. F.H. Field applied chemical ionisation in 1966, which was followed by the 'thermospray' method, introduced by the C.R. Blackley team in 1968. Coupling with gas chromatography was performed by C. Gohlke and F. McLafferty, the latter further introducing coupling with liquid chromatography in 1973. Since then, mass spectrometry techniques are continually improving (**Table 1**).

1.1. Ionisation methods

1.1.1. Electron impact (EI)

Electron impact ionisation occurs in a stainless steel chamber (**Figure 2**) at a pressure of less than 6×10^{-7} mmHg (i.e. vacuum conditions) achieved by means of a diffusion oil pump or a turbo-molecular pump. At 2000°C by thermoelectronic effect, electrons emitted by a rhenium filament are accelerated to the anode by a 5–100-V potential difference [1, 14].

To increase the electron-molecule impact probability, a magnetic field is applied, with the same direction as that of the electric field. The magnetic field induces a circular course to electrons,

The scientific term *spectrograph* starts being part of the scientific vocabulary (**1884**) [1, 3]

E. Goldstein studied 'canal rays' composed of positive ions. His work opened the gate to mass spectrometry (**1886**) [1, 3]

Wilhelm Wien showed that the mass-to-charge ratio of the positive ions (canal rays) has opposite polarity that of the electron (**1898**) [1, 3]

Francis William Aston and J. J. Thomson were the first to use mass spectrometry (**1897–1898**) [1, 3, 4]

F. W. Aston and A. J. Dempster introduced 'modern techniques' of mass spectrometry (**1918–1919**) [1, 3, 5, 6]

W. Kaufmann measured the relativistic mass increase of electrons using a mass spectrometer (**1901**) [1, 3]

J. J. Thomson separated the ^{20}Ne and the ^{22}Ne isotopes and assigned the m/z 11 signal to the doubly charged ^{22}Ne particle (**1913**) [1, 3, 4]

Construction of the first focusing mass spectrograph with a resolution of 130 (**1919**) [1, 3]

Nobel Prize in Chemistry (F. W. Aston)—mass spectrograph of isotopes for non-radioactive elements of the whole number rule (**1922**)

Double-focusing mass spectrograph developed by J. Mattauch and R. Herzog (**1934**)

A. J. Dempster—spark ionisation source (**1936**)

F. W. Aston—mass spectrograph with a resolution of 2000 (**1937**)

Westinghouse—new method for accurate gas analysis—a mass spectrometer (**1943**) [1]

W. Stephens introduces the time-of-flight (TOF) mass spectrometer (**1946**)

Nobel Prize in Physics for W. Paul—the quadrupole and quadrupole ion trap (**1953**)

The hydrogen transfer reaction or the 'McLafferty rearrangement'—A. J. C. Nicholson (**1954**)

Mass Spectrometric Analysis. Molecular Rearrangements by F. W. McLafferty (**1959**) [7]

Gas chromatograph coupled to a mass spectrometer the GS-MS technique introduced at Dow Chemical (**1959**)

World's first mass spectrometry society—British Mass Spectrometry Society (**1964**) [1, 3]

Chemical ionisation introduced by F. H. Field and M. S. B. Munson (**1966**) [8]

Electrospray ionisation (ESI)—M. Dole (**1968**)

Field desorption—H. D. Beckey (**1969**) [9]

Fourier transform ion cyclotron resonance mass spectrometry—M. B. Comisarow and A. Marshall (**1974**) [10]

Atmospheric pressure chemical ionisation (APCI) based on gas chromatography (GC)—Horning (**1974**) [11]

Plasma desorption mass spectrometry—R. MacFarlane (**1976**)

M. L. Vestal and C. R. Blakely's work with heating a liquid stream became known as thermospray. It became a harbinger of today's commercially applicable instruments (**1983**)

Electrospray ionisation, the technique for large molecules and liquid chromatography, coupled with mass spectrometry—J. B. Fenn Nobel Prize in Chemistry 2002 (**1984**) [12]

The term matrix-assisted laser desorption/ionisation (MALDI)—F. Hillenkamp and M. Karas (**1985**) [13]

The development of the ion trap technique—H. Dehmelt and W. Paul Nobel Prize in Physics (**1989**)

API III—the first commercial dedicated to LC-MS/MS for the pharmaceutical industry (**1989**)

The first low-cost high-performance MS/MS system—TurboIonSpray (**1997**)

Orbitrap mass spectrometer—A. Makarov (**1999**)

Development of electrospray ionisation (ESI) and soft laser desorption (SLD): mass spectrometric analyses of biological macromolecules (i.e. proteins)—J. B. Fenn and K. Tanaka Nobel Prize in Chemistry. Large-molecule laser desorption ionisation (**2002**)	
SCIEX Corporation—Single platform: quantitative and qualitative capabilities associated with triple quadrupoles and, respectively, with high-resolution accurate-mass system MS experiments for a broad spectrum of applications (**2010**)	
Waters Corporation—'quadrupole/time-of-flight' a hybrid mass spectrometer (**2016**)	

Table 1. A timeline in spectrometric measurement.

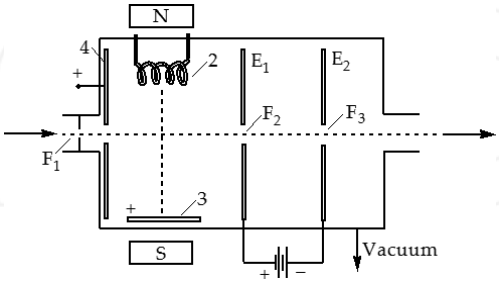


Figure 2. Schematics of an electron impact ionisation chamber (F1, entrance slit; F2, filament; F3, anode; F4,positive ion repeller; F2 and F3, slits; E1 and E2, electronic lenses) [1].

perpendicular to magnetic induction. The combination of the accelerated uniform rectilinear motion and the circular motion in the perpendicular plane lends electrons a helical movement of longer trajectory, thus increasing the likelihood of impact with the molecules. This results in ionisation with 0.01% yield, acceptable for an electron impact source [1, 15, 16].

The electron kinetic energy generates the pulling out of an electron, resulting in a positive molecular ion, also provided with a single electron:

$$M + e^- \rightarrow M^{+\bullet} + 2e^- \quad (1)$$

The molecular ion has the same empirical formula as the respective neutral molecule. The difference comes from one or several electrons. The molecular ion may be positive or negative.

The masses of these ions are equal with the sum of masses of abundant isotopes of the various atoms making up the molecule.

The symbol $M^{\bullet}$ (or $M^{+\bullet}$) does not refer to an added electron but to a post-ionisation unpaired electron. Addition of an electron (electron capture) to the neutral molecule yields a negative radical ion M^{-} [1, 17–19].

The ease of electron removal from the molecule depends on its nature, $n > \pi > \sigma$.

Molecule ionisation energy ranges from 8 to 12 eV; for electrons, the commonly used one is 70 eV, providing maximum ionisation efficiency.

In case of too much fragmentation, resulting in significant decrease of the M^+ molecular ion peak, electron energy may be reduced.

After crossing the source volume, electrons are trapped on a cathode (hatch), and impact-generated ions are expelled from the source by means of a plate (4) with a certain same-sign potential. Next, such electrons are accelerated at the source-analyser interface by a V_0 potential difference.

The positive plate (4) (**Figure 2**) is also meant to attract negative ions produced on sample projectile electron impact, such electrons being evacuated together with the other neutral particles after neutralisation of the negative electric charge [1, 20–22].

The excess energy is collected as internal energy by the molecular ion (from 12 to 70 eV). The molecular ion breaks into ion fragments, with sufficient internal energy to further break themselves, and the process continues.

A plasma ion is thereby obtained in the ionisation chamber, of which H^+ is the lightest and $M^{+\bullet}$ is the weightiest. All respective ions have a very short life span (milliseconds only), which requires their removal from the source as soon as possible, for analysis purposes.

Positive ions attracted to electrode E_1 enter through a slit in the area between E_1 and E_2, where an 8 kV accelerating magnetic field operates 1, 23].

• **Sample feed-in**

Solid, liquid or gaseous organic compounds can be analysed; however, inside the ionisation chamber, samples need to be in the gas phase.

The sample amount weighs microliters or micrograms and may be reduced to picograms when coupled with gas chromatography.

High-boiling solids and liquids are introduced into a quartz crucible (5 mm long/1 mm diameter) and transferred directly into the ionisation chamber, where they sublimate slowly depending on temperature.

Very volatile liquids are first vaporised and then introduced into the ionisation chamber. Gases are introduced with an accuracy valve [24–28].

1.1.2. Chemical ionisation (CI)

A reactant gas is introduced into the ionisation chamber, whose molecules become ionised on collision with a beam of electrons accelerated by a 400-V potential difference. Plasma formed is driven to the centre of the source by electrostatic lenses.

Positive chemical ionisation occurs when methane (or isobutane as a reactant gas) is used. For instance, ionised species may be generated where methane is used, such as

$$CH_4 + e^- \longrightarrow CH_4^+,\ CH_3^+,\ CH_2^{+\cdot},\ CH^+,\ C^{+\cdot},\ H^+ \qquad (2)$$

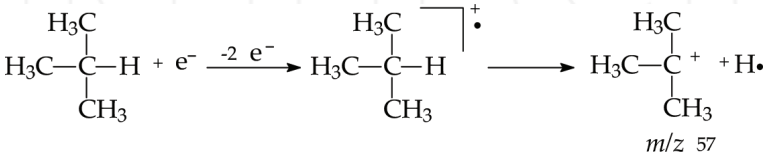
which may react with neutral methane molecules, e.g.

$$\begin{aligned} CH_3^+ + CH_4 &\longrightarrow C_2H_5^+ + H_2 \\ CH_4^{+\cdot} + CH_4 &\longrightarrow CH_5^+ + CH_3^{\cdot} \end{aligned} \tag{3}$$

The CH_5^+ ion, the plasma majority, is an acid (i.e. an electrophile) able to protonate most organic molecules by an exothermic reaction:

$$CH_5^+ + M \longrightarrow MH^+ + CH_4 \tag{4}$$

The MH^+ ion is a pseudo-molecular ion (peak at M+1), with weak internal energy and little potential for fragmentation. Therefore, molecular weight is easily determined, and its fragments provide information on the molecular structure. Isobutane is a 'milder' ionising agent than methane. The reactive entity is the emergent *tert*-butyl ion, $C_4H_9^+$:



(5)

In chemical ionisations with isobutene, a molecule M yields the pseudo-molecular ion MH^+ (peak at M+1) and isobutene:

$$C_4H_9^+ + M \longrightarrow MH^+ + C_4H_8 \tag{6}$$

However, the M+57 ion also frequently emerges:

$$C_4H_9^+ + M \longrightarrow M - C_4H_9^+ \tag{7}$$

The reaction of the $C_4H_9^+$-M molecular ion (peak at M+57) is less exothermic than for methane, resulting in almost no MH^+ ions.

Positive ionisation also occurs in the case of ammonia which at 20 Pa yields plasma mostly consisting of NH_4^+ ammonium ions of average acidity. Depending on the M basic (nucleophilic) nature, the result is either MH^+ ions (peak at M+1) or one MNH_4^+ ion (peak at M+18) [1, 29]:

$$\begin{aligned} NH_4^+ + M &\longrightarrow MH^+ + NH_3 \\ NH_4^+ + M &\longrightarrow M\text{-}NH_4^+ \end{aligned} \tag{8}$$

Less nucleophilic compounds such as hydrocarbons do not undergo ionisation.

Negative chemical ionisation occurs by bombarding the reaction gas, nitrogen, butane, or isobutene, with high-energy (240 eV) primary electrons, resulting in low-energy electrons, easy to capture by sample molecules. Capture may be non-dissociative or dissociative:

$$\begin{aligned} MX + e^- &\longrightarrow MX^- \\ MX + e^- &\longrightarrow M + X^- \end{aligned} \tag{9}$$

For compounds with an affinity for electrons, negative chemical ionisation is about three orders of magnitude more sensitive than positive chemical ionisation [30–32].

As highly dependent on experimental conditions (source temperature, pressure in the ionisation chamber, reactant gas purity), mass spectra resulting from chemical ionisation are less reproducible than those obtained by electron impact [1, 24].

1.1.3. 'Mild' electrospray ionisation (ESI)

The solution of macromolecular compounds such as polypeptides is introduced under pressure of a gas, N_2, into a capillary tube (50 μm, diameter), exiting in mist form (spray), further subjected to action of a powerful (4 kV) electric field (**Figure 3**).

Contrary to the other methods, ionisation occurs at atmospheric pressure and room temperature, the 'mildest' conditions possible. Methanol and water solutions (50%:50%) are used, as well as acetonitrile and water, in the same proportions [33–35].

The resulted mist is subjected to the electric field, and, after solvent removal in a nitrogen stream, ions with multiple charges remain, to be analysed next in a dispersive system of the spectrometer (**Figure 3**) [36–39].

1.1.4. Fast atom bombardment ionisation

Fast atom/ion bombardment (FAB) requires dissolution of the sample in a liquid, low-vapour-pressure matrix, able to yield protons, such as glycerol, thioglycerol and *m*-nitrobenzyl alcohol. Atoms accelerated to 10 keV (Xe, Ar, Kr) bombard the sample solution, and ionisation occurs at room temperature, producing an abundance of positive $(M+H)^+$ and negative $(M+H)^-$ pseudo-molecular ions [1].

1.1.5. Field desorption ionisation

The sample solution is deposited on a rhenium/tungsten filament covered with carbon needles, in a very high electric field of up to 10^8 volts/cm. The filament is heated to the sample melting point, and the ions migrate, accumulating at the end of the needles, finally desorbing and thus engaging sample molecules with molecular $M^{+\bullet}$ ions.

Field desorption ionisation is used for high molecular mass compounds and unstable or little volatile polar compounds such as carboxylic acids and sugars [1].

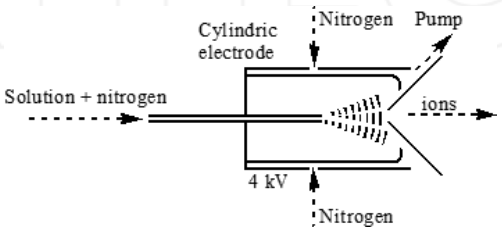


Figure 3. Schematics of an electrospray source where ions separate by the *m*/*z* ratio ($z \geq 1$). Ions detected are of the MH_n^{n+} type, where $n \geq 1$ [1].

1.1.6. MALDI 'mild' ionisation

MALDI is the abbreviation of matrix-assisted laser desorption/ionisation. The method uses a solid aromatic matrix, e.g. acids such as α-cyanocinnamic acid, sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid), picolinic acid, 3-aminopicolinic acid, etc., where the sample is dispersed in a definite proportion: one sample molecule per 10^4 matrix molecules, both in crystalline state and at room temperature and atmospheric pressure, in dry nitrogen atmosphere. By means of a microscope, a 3–10-ns-pulse UV laser irradiation is focused on a small matrix spot, 0.05–0.2 mm in diameter (**Figure 4**). An electronic and thermal excitation of molecules in the sample matrix occurs, able to yield protons causing ionisation. The matrix serves as an energy vector between the laser beam and the molecules of the analysed compound. Ionisation of organic molecules is 'mild', resulting in pseudo-molecular ions [40].

Ion and neutral molecule desorption occurs after laser irradiation, as a supersonic expansion jet. Desorbed ions are transferred to the analyser under vacuum by means of an interface. MALDI is the most used analytical method in modern biochemistry and polymer science [1, 41–43].

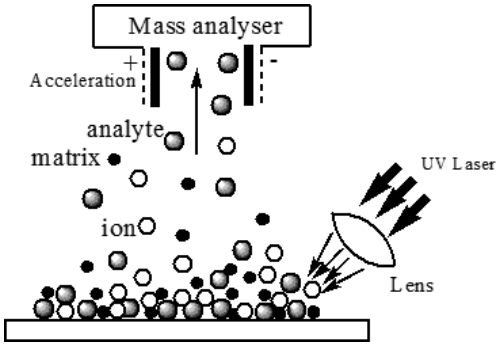


Figure 4. Schematics of the MALDI process [1].

2. Mass spectra

2.1. Mass spectral description: rules

Mass spectrum is the two-dimensional representation of signal intensity (peak) on the vertical axis versus the *m*/*z* ratio on the horizontal axis. Peak intensity directly reflects ionic species abundance with the respective *m*/*z* ratio [1].

The *m*/*z* ratio is dimension-free because it derives from the ion *m* mass number and the *z* number of elementary charges, which is equal to 1. Therefore, values on the horizontal axis are a direct reflection of *m* [1].

Data acquisition and processing are performed by a computer, in line with the diagram below:

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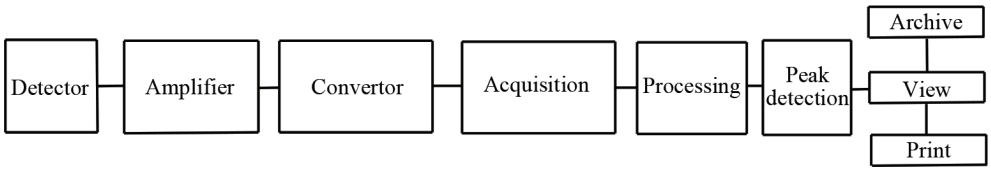

Figure 5 shows the spectrum of 2-methylimidazole. We may notice that the peak corresponds to the molecular ion (*m*/*z* 81.48), i.e. the *base peak* represents the greatest relative abundance (100%). Other peaks correspond to ion fragments.

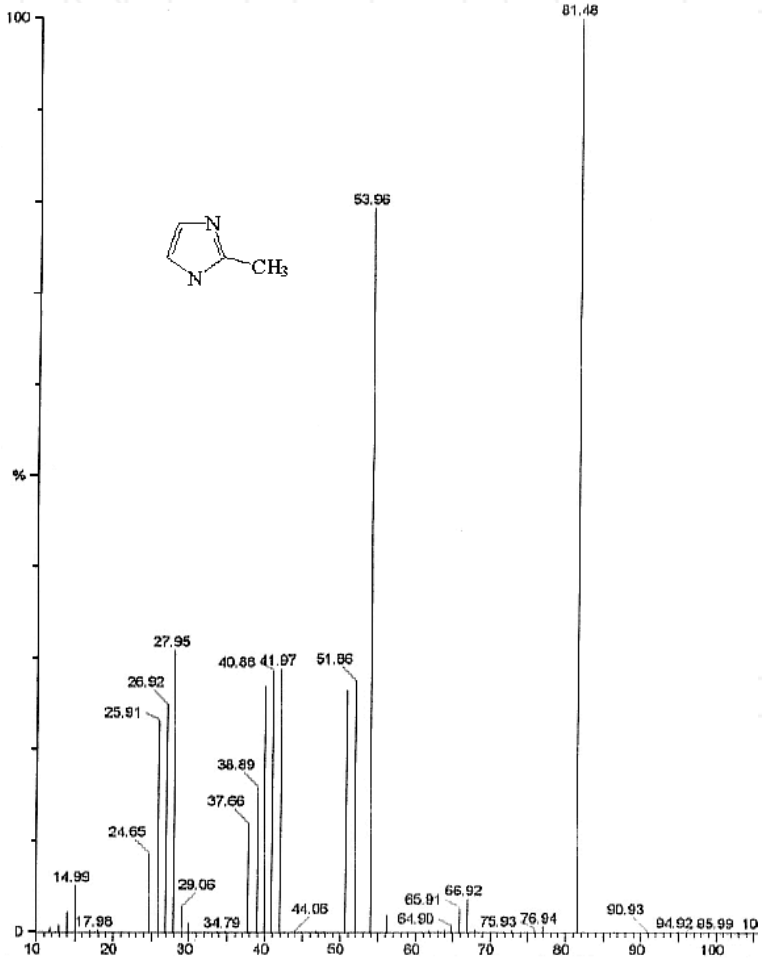

Figure 5. Mass spectrum for 2-methylimidazole [1].

The base peak results from spectrum *normalisation* consisting in selection of the most intense signal, which is assigned the 100% value. In relation to it, percent intensity is assigned to each signal, which represents the relative percent abundance of each ion fragment [1, 44, 45].

The molecular peak, the parent peak, corresponds to the $M^{+\bullet}$ molecular ion in pure compounds. This is the one that has the largest, easily identifiable *m*/*z* ratio. The existence of the molecular ion in the spectrum allows accurate determination of molecular mass. In certain compounds, the molecular ion is not present because it is very unstable and molecular mass cannot be determined [1, 44, 45].

Electron pair cations, resulting from fragmentation of the molecular ion, are usually more stable than the molecular ion and have hence greater abundance.

The nitrogen rule. *Molecular mass of organic compounds are even, except for those containing an odd number of nitrogen atoms.* When a compound is nitrogen-free, but an odd mass corresponds to the last peak, this is definitely not the molecular peak. The rule may be extended to fragmentation ions as well.

The nitrogen rule may be explained by the fact that elements contained in organic compounds have either even valence and atomic mass (O, C, S) or odd valence and atomic mass (H (halogen)) and by the fact that make the compounds containing only C, H, O, S and halogen show an even molecular mass only.

Illogical peaks. The difference between the predicted molecular mass and the immediately following fragment mass must correspond to the elimination of a hydrogen atom (mass 1) or one CH_3 group (mass 15). There are no fragments of masses between 3–14 and 21–25 mass units (*mu*). A smaller difference between those limits indicates that either the sample is impure or that greater mass peak is not the molecular peak [1, 44, 45].

2.1.1. Isotopes

An isotope is an element that has the same number of electrons in the electronic layer but a different number of neutrons in the nucleus. Therefore, isotopes have the same chemical properties and only differ in their mass. All elements have several natural-state isotopes [46–49].

Table 2 presents natural isotopes of the most common elements encountered in organic chemistry. One may note that the lightest isotope also has the greatest abundance [1, 50–52].

2.1.2. Molecular peaks of bromide compounds

When the molecule displays several isotopes such as those of bromine, of relatively close abundance, the dibromo-molecular ion, Br_2, has three peaks (**Figure 6**):

- One ^{79}Br–^{79}Br species of mass M = 158
- One ^{79}Br–^{81}Br species of mass M = 160
- One ^{81}Br–^{81}Br species of mass M = 162

Assuming for simplicity reasons that mass 79 and 81 isotopes have the same relative abundance, the likelihood of a mixed dibromo-^{79}Br–^{81}Br is two times higher than that of homogeneous

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Element	Isotopes/relative abundance					
Hydrogen	^{1}H	100	^{2}H	0.0151		
Carbon	^{12}C	100	^{13}C	1.112		
Nitrogen	^{14}N	100	^{15}N	0.37		
Fluorine	^{19}F	100				
Silica	^{28}Si	100	^{29}Si	5.10	^{30}Si	3.35
Phosphorus	^{31}P	100				
Chlorine	^{35}Cl	100	^{37}Cl	31.98		
Bromine	^{79}Br	100	^{81}Br	97.28		
Iodine	^{127}I	100				
Oxygen	^{16}O	100	^{17}O	0.04	^{18}O	0.20

Abundances are calculated by assigning the 100 values to the prominent isotope [1, 3].

Table 2. Natural isotope abundance of common elements.

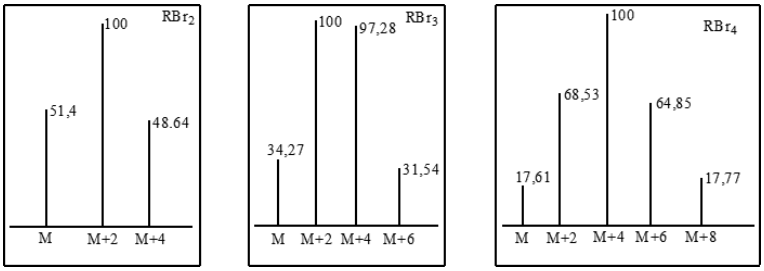


Figure 6. Molecular peaks of bromide compounds [1].

dibromo-^{79}Br–^{79}Br or ^{81}Br–^{81}Br. The molecular peak of the bromine molecule (Br_2) occurs in the form of a triplet of 1:2:1 intensities (**Figure 7**).

The abundance of these species corresponds to the binomial $(a+b)^n$ coefficient, where a is the relative abundance of the first isotope, b that of the second isotope and n the number of elements [3].

The exact calculation of peaks for brominated compounds is given in **Figure 6**. A similar calculation is possible for chlorinated compounds as well. Fluorine and iodine are isotopically pure. In halogenated compounds, carbon, hydrogen and oxygen isotopes are a minority. The ^{13}C isotopic contribution is 68 times higher than that of ^{2}H deuterium and 27 times higher than that of ^{17}O [1, 3].

Figure 8 represents the spectrum of bromo-chloromethane, with three prominent peaks for molecular ions. One may note that, the same as in all compounds with two bromine atoms, two chlorine atoms or one chlorine atom and one bromine atom in the molecule, the M+4 peak also appears in the spectrum.

High-resolution mass spectrometry is widely used to determine molecular formulas of certain unknown compounds. Spectrometers have a software that compares exact masses with those of various possible formulas [1, 3].

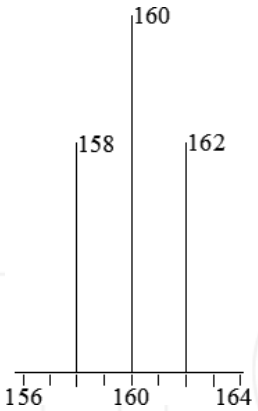


Figure 7. Simplified dibromo mass spectrum. [1].

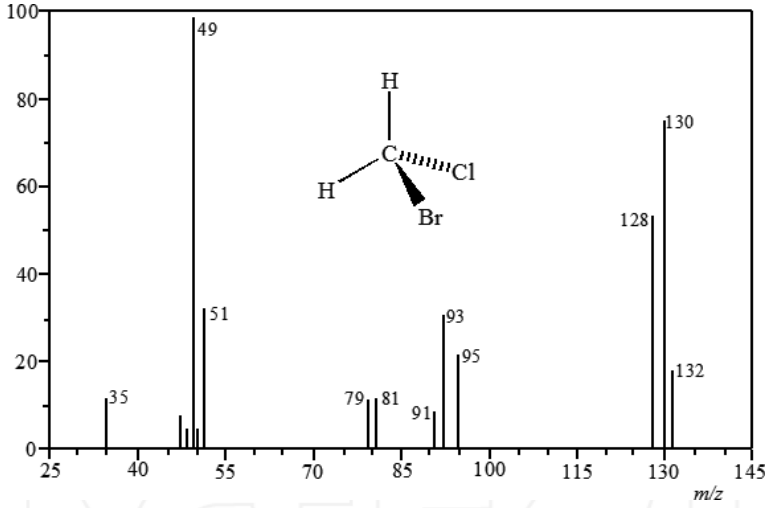


Figure 8. Simplified bromo-chloromethane mass spectrum [1].

2.1.3. Molecular formula

The molecular formula may often be obtained by high-resolution spectrometer measurements, because atomic weights are not integers. For example, a distinction among CO, N_2, CH_2N and C_2H_4 is possible for nominal weight[1] 28:

[1]*Nominal mass* corresponds to an integer which is the sum of the number of protons and neutrons contained in the atom. For example, ^{12}C contains six protons and six neutrons, and therefore its nominal weight is 12. The nominal molecular weight of ethene, CH_2=CH_2, is 28 Da (dalton). This mass is rendered by low-resolution spectrometers.

^{12}C 12.0000	$^{14}N_2$ 28.0062	^{12}C 12.0000	$^{12}C_2$ 24.0000
^{16}O 15.9949		$^{1}H_2$ 2.0116	$^{1}H_4$ 4.0312
27.9949		^{14}N 14.0031	28.0312
		28.0187	

Molecular mass observed for the CO molecular ion is the sum of exact masses of the most abundant carbon and oxygen isotope, which sum differs from the CO molecular mass based on atomic masses averaging the masses of all natural isotopes of an element (e.g. C = 12.01; O = 15.999).

Table 3 includes exact masses of isotopes of ordinary elements in organic compounds.

There are tables including formulas corresponding to molecules or fragments with their exact masses, obtained by addition of exact masses of the most abundant isotopes of each element.

Element	Atomic mass	Nucleus	Exact mass
Hydrogen	1.00794	^{1}H	1.00783
		^{2}H	2.01410
Carbon	12.01115	^{12}C	12.00000
		^{13}C	13.00336
Nitrogen	14.0067	^{14}N	14.0031
		^{15}N	15.0001
Oxygen	15.9994	^{16}O	15.9949
		^{17}O	16.9991
		^{18}O	17.9992
Fluorine	18.9984	^{19}F	18.9984
Silica	28.0855	^{28}Si	27.9769
		^{29}Si	28.9765
		^{30}Si	29.9738
Phosphorus	30.9738	^{31}P	30.9738
Sulphur	32.066	^{32}S	31.9721
		^{33}S	32.9715
		^{34}S	33.9679
Chlorine	35.4527	^{35}Cl	34.9689
		^{37}Cl	36.9659
Bromine	79.9094	^{79}Br	78.9183
		^{81}Br	80.9163
Iodine	126.9045	^{127}I	126.9045

Table 3. Exact masses of certain isotopes [1, 3].

The mass of the molecular ion is the sum of the most abundant isotope (^{12}C, ^{1}H, ^{16}O, etc.) in the molecule [1, 3].

In the case of methane, the molecular ion occurs by *m*/*z* 16 corresponding to the formula $^{12}C^{1}H_4$. However, there are also molecular species containing less abundant isotopes: $^{13}C^{1}H_4$ (*m*/*z* 17, peak M+1), $^{12}C^{2}H^{1}H_3$ (*m*/*z* 17, peak M+1), $^{13}C^{2}H^{1}H_3$ (*m*/*z* 18, peak M+2) and so on [1, 3].

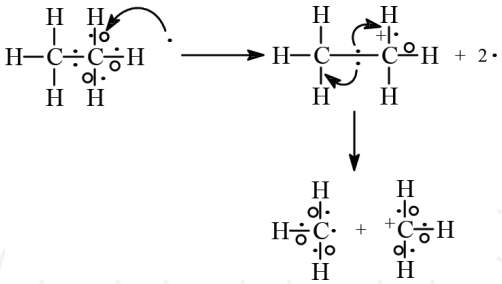
In the spectrum of methane presented below in tabular form, the M+1 peak represents 1.14% of the M (basic) peak, and the M+2 peak is negligible:

m/*z*	1	2	12	13	14	15	16	17
Relative abundance	3.4	0.2	2.8	8.0	16.0	86.0	100	1.14

Intensity of isotope peaks is lower than the molecular M peak, except for cases when chlorine or bromine is present (**Figure 8**).

2.2. Fragmentation: mechanism

The impact of a very energetic electron with a molecule turns the latter into a cation radical, with loss of an electron. A range of rearrangements or fragmentations follow, which depend on the molecule nature and structure [1, 3]:



(10)

Straight-chain or branched hydrocarbon fragmentation occurs, resulting in formation of more stable carbocations; their stability increases in the order:

$$^{+}CH_3 < RCH_2^{+} < R_2CH^{+} < R_3C^{+} < CH_2{=}CH{-}CH_2^{+} < C_6H_5{-}CH_2^{+}$$

2.3. Energy aspects

In ionisation chambers, about one molecule in 10,000 is ionised. This requires 8–12 eV, i.e. 800–1200 KJ mol^{-1}. This energy is known as *ionisation potential (IP) (M).*

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Depending on electron impact conditions, molecular ions have internal energy, E_{int}, ranging from 0 to 10 eV. In the case of too weak internal energy, the $M^{+\bullet}$ molecular ion does not undergo breakdown, generating the molecular peak; for energies over 1 eV, the $M^{+\bullet}$ ion undergoes breakdown, resulting in formation of primary ion fragments [53]:

$$M^{+\bullet} \rightarrow A^{+} + B^{\bullet} \tag{11}$$

The difference in enthalpy required to produce the A^+ ion is known as the A^+ *potential occurrence*, i.e. *PO* (A^+).

In cases of high internal energy, $M^{+\bullet}$ decomposes, in formation of both primary and secondary fragments;

$$M^{+\bullet} \longrightarrow A^{+} + B^{\bullet} \qquad A^{+} + B^{\bullet} \longrightarrow C^{+} + D^{\bullet} \ldots\ldots \text{etc.} \tag{12}$$

At one point, *the balance of all fragments from ions of different internal energies is the mass spectrum* achieved by electron impact at 70 eV.

In line with the diagram in **Figure 9**,

$$IP(M) = \Delta fH^{0}(M^{+}) - \Delta fH^{0}(M) \tag{13}$$

Considering that the energy of the reverse reaction is close to 0, the (E_a) activation energy is

$$E_a = \Delta fH^{0}(A^{+}) + \Delta fH^{0}(B\cdot) - \Delta fH^{0}(M^{+}) \tag{14}$$

Therefore, the potential of A^+ is equal to

$$PO(A^{+}) = IP(M) + E_a \tag{15}$$

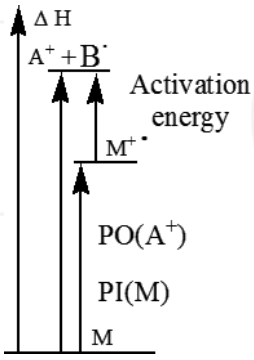


Figure 9. Energy diagram of a fragmentation [1].

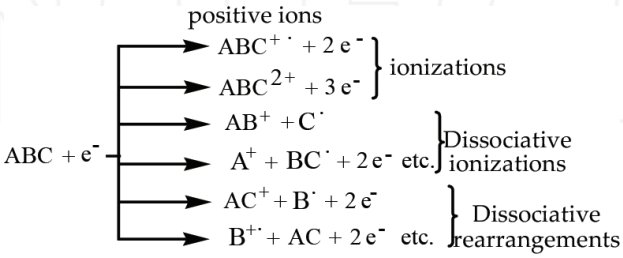
$$PO(A^+) = \Delta fH^0(A^+) + \Delta fH^0(B\cdot) - \Delta fH^0(M) \tag{16}$$

ΔfH^0 is the standard enthalpy for formation in the gas phase.

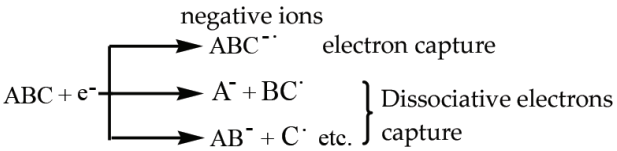
Mass spectrum is the balance of a series of competing and consecutive reactions [54–56].

2.4. Processes under electronic ionisation conditions

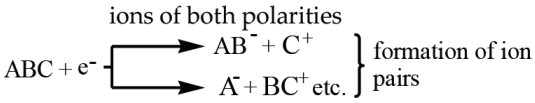
The following types of ions are produced during electronic ionisation: molecular ions, fragmentation ions, multiple charge ions, metastable ions, rearrangement ions and pair ions [1, 3, 57, 58]:



(17)

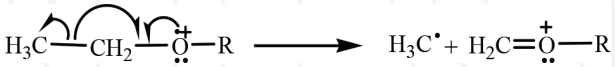


(18)



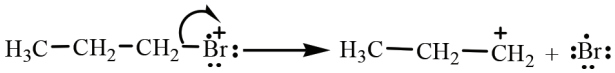
(19)

Most processes are very rapid, occurring within a few nano- or microseconds. Fragmentation of a molecular ion $M^{+\bullet}$ may be performed by homo- or heterolytic cleavage of a single bond. In homolytic cleavage, each electron moves independently. One fragment is an even-electron cation and another free radical with an unpaired electron [1, 3, 54]:



(20)

In heterolytic cleavage, an electron pair moves together to the charged atom. Once again, fragments are an even-electron cation and a radical. The charge is placed on the alkyl group:



(21)

Further fragmentation of such a cation generally results in another even-electron cation and a fragment or even-electron molecule:

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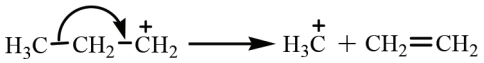

(22)

Multiple bond consecutive or simultaneous fragmentations may occur when an energy advantage exists, deriving from formation of a very stable cation and/or a stable radical or a neutral molecule.

Fragmentation of a certain bond is related to bond strength, to the possibility of low-energy transition and to the stability of arising fragments.

Given the greatly reduced pressure of a spectrometer, the likelihood of collisions is low, and therefore unimolecular breakdowns occur [57–59].

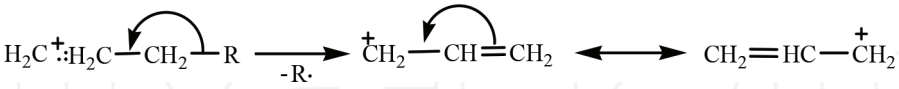
The following general rules have been established to predict prominent peaks in electronic impact mass spectra:

- The relative height of molecular ion peaks is greater for straight-chain ions and smaller than that of branched-chain ones.
- Generally, the relative height of the molecular ion peak decreases with increase of the molecular mass in a homologous series. Fatty ethers may be an exception.
- Due to an increased stability of tertiary carbocations as compared to secondary and primary ones, likelihood of cleavage increases as the carbon atom is more substituted.

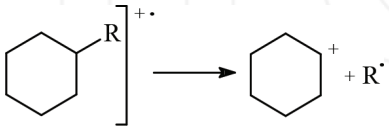
$$^{+}CH_3 < RCH_2^{+} < R_2CH^{+} < R_3C^{+}$$

The longest chain may be eliminated as a radical, because such a radical may be stabilised by stabilisation of the lone pair ion:

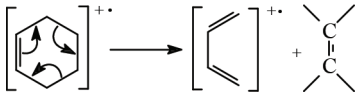
- Double bonds and cyclic structures, particularly aromatic ones, stabilise the molecular ion and increase its likelihood.
- Double bonds favour allylic cleavage, resulting in formation of resonance-stabilised allylic cations:


(23)

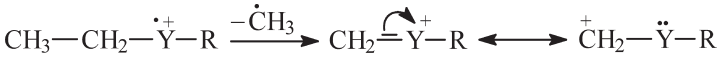
- Cyclic alkanes with side chains cleave at α, and the positive charge tends to remain on the cyclic fragment:


(24)

Unsaturated rings may undergo a retro-Diels-Alder reaction:


(25)

- Alkyl arenes cleave at the β to the aromatic ring and give rise to a resonance-stabilised benzyl ion or a tropylium ion.
- Heteroatom-containing compounds cleave at the C–C bond next to the heteroatom, passing the charge over to the heteroatom-containing fragment:



(Y = O, NH, S) (26)

- Cleavage often associates with the elimination of small, neutral, stable molecules such as CO, H_2O, NH_3, H_2S, HCN, olefines, mercaptans, ketone or alcohols [60–62].

2.5. Derivatisation

In the case of difficult-to-volatilise compounds or compounds whose molecular peak cannot be determined, a derivative which can be prepared that is more volatile, has a predictable cleavage pattern, a simplified fragmentation pattern and a better stability of the molecular ion. A low-volatility polar group of compounds such as carbohydrates, dicarboxylic acids and peptides become volatile and able to render characteristic peaks by acylation of the –OH or $–NH_2$ groups or methylation of the –COOH groups.

Trimethylsilylating of the same groups allows passage of corresponding compounds through the chromatographic column (GC).

Reducing ketones to hydrocarbons allows elucidation of their carbonate skeletons. Reducing polypeptides to more volatile poly-amino alcohols also allows prediction of the fragmentation pattern [1, 28].

2.6. Qualitative and quantitative analysis

Mass spectrometry is particularly important to organic chemistry because it allows acquisition of information about the composition and particularly about the structure of molecular compounds. Mass spectra provide data for structural assessments, fragmentation being performed by semi-empirical rules serving to the study of unknown compounds.

The identified molecular ion must correspond to spectrum ions produced by loss of fragments.

High intensity of the molecular ion indicates stable molecular structure.

A multiplet in the molecular ion area indicates the presence of a specific isotopic structure heteroatom, such as silica, sulphur, chlorine and bromine.

Proportionality of the intensity of the signal with the analyte amount allows the use of mass spectrometry in quantitative assays. For this purpose internal standard methods are used. As standard a compound similar to the analyte is employed provided that the ionisation produces easily to monitor ions different from those of the analyte. As analyte similar chemical compound,

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such as a deuterated isotope or analogue whose ionisation produces easily to monitor ions, different from those of the analyte can be employed [1, 3, 63].

2.7. Mass spectra of the main classes of organic compounds

2.7.1. Alkanes

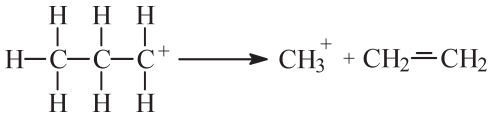
Hydrocarbon mass spectra are easy to interpret because hydrocarbons have C–C and H–H bonds only. Taking into account molecule dissociation enthalpies, one finds that C–C bonds are the easiest to break:

Bond	C–C	C–H	C=C
Δ in kJ	340	420	660

In straight-chain alkanes, fragmentation occurs through loss of a methyl, leading to fragments of *m*/*z* = molecular mass 15. For instance,

$$\underset{m/z\ 58}{\left[CH_3\text{-}CH_2\text{-}CH_2\text{-}CH_3\right]^{+\cdot}} \longrightarrow \underset{m/z\ 43}{CH_3\text{-}CH_2\text{-}CH_3^{+}} + \underset{m\ 15}{CH_3^{\cdot}} \quad (27)$$

In general, fragments correspond to *m*/*z* 29, 43, 57, 71, 85, 99, etc., i.e. to molecular mass −15 and −14 × n or C_nH_{2n+1} ions separated by 14 mass units. Ethene neutral molecules may also form:



$$\longrightarrow CH_3^{+} + CH_2{=}CH_2 \quad (28)$$

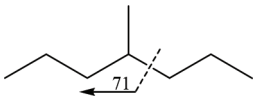
Ions 43 and 57 are among the most stable of the spectrum (with the highest peaks), consistent with their standard formation enthalpy. Unlike higher mass ions, they do not undergo secondary fragmentation [3]:

R^+ ion	CH_3^+	CH_3–CH_2^+	CH_3–CH_2–CH_2^+	C_3H_7–CH_2^+
m/*z*	15	29	43	57
ΔfH^0 in kJ/mol	1086	915	873	911

Compounds with more than eight carbon atoms show similar spectra (**Figure 10**). Their identification depends on the molecular ion peak.

Branched alkane spectra are largely similar to those of straight-chain alkanes, but fragment abundance does not decrease evenly. Fragmentation preferentially occurs at branching points [64].

For example, in the case of 4-methylheptane, the same fragment with *m*/*z* 71 as in the case of *n*-octane is produced, but the relative abundance of that resulting from the fragmentation of 4-methylheptane is higher. So you can distinguish the *n*-alkanes from branched alkanes [1, 3].



Cyclohexane undergoes complex fragmentation requiring much energy on cycle break. The mass spectrum (**Figure 11**) shows a more intense molecular ion than those of acyclic compounds,

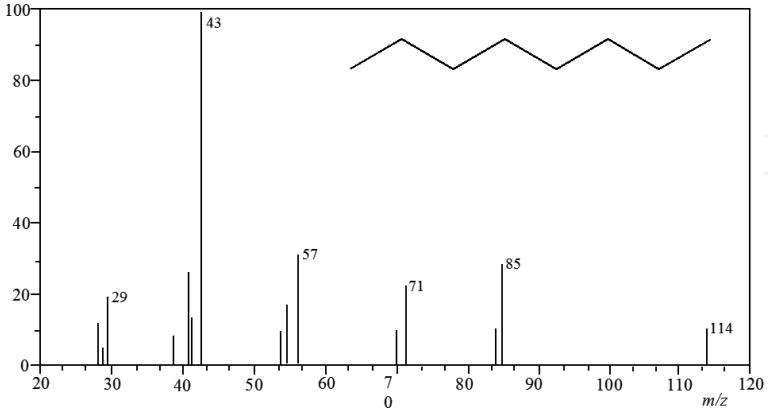


Figure 10. *n*-Octane-simplified mass spectrum [1].

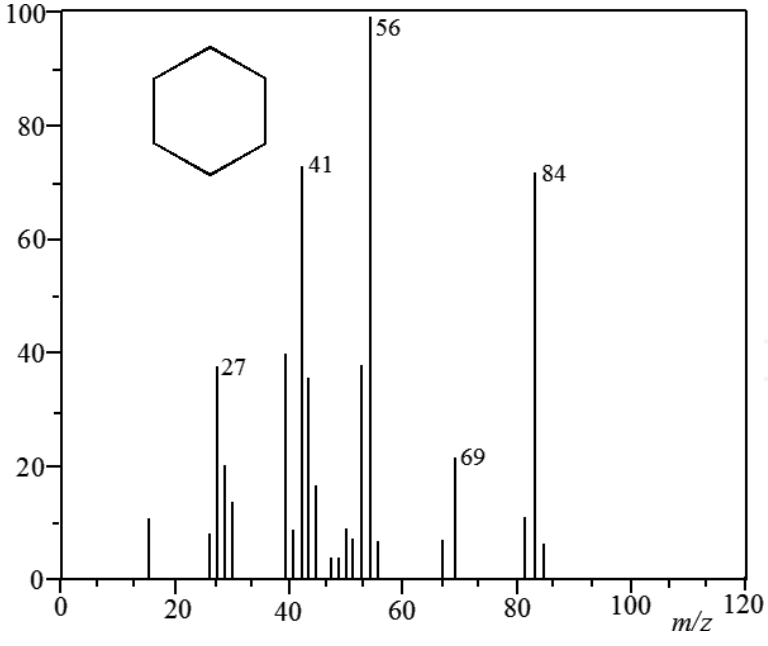


Figure 11. Cyclohexane mass spectrum [1].

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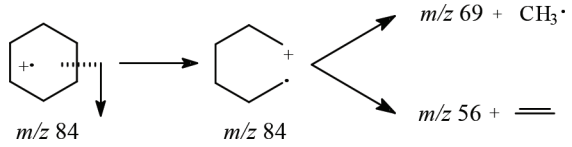
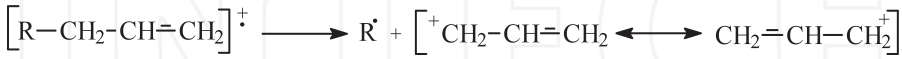
because fragmentation involves a break of two C–C bonds. The base peak is at *m/z* 56, following ethene elimination [54]:



(29)

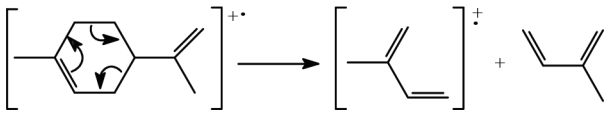
2.7.2. Alkenes

By ionisation and fragmentation, alkenes produce a fragment *m/z* 41, corresponding to the allyl carbocation:



(30)

The molecular ion peak is visible in alkenes. It is difficult to locate the double bond in acyclic alkenes since it easily migrates from one fragment to the other. Location of the double bond in cyclic alkenes results from the tendency to allylic cleavage without double-bond migration. Limonene shows a unique, retro-Diels-Alder cleavage pattern:



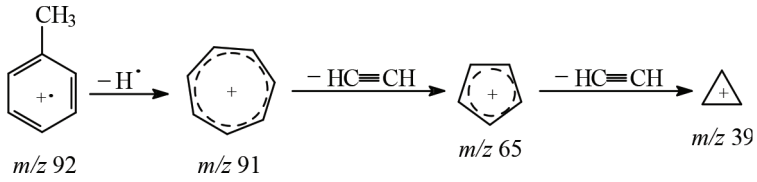
(31)

Similar to saturated hydrocarbons, acyclic alkenes are characterised by a number of peaks separated by 14 unit intervals. Among them, C_nH_{2n-1} and C_nH_{2n} peaks are more intense than C_nH_{2n+1} peaks [1, 3, 54].

2.7.3. Arenes

These compounds render easy-to-interpret spectra. The molecular peak is intense because the aromatic ring is very stable (**Figure 12**). Accurate measures can be performed for peaks M+1 and M+2.

Although reduced, molecular ion fragmentation can produce characteristic ions: *m/z* 77 $(M-H)^+$, *m/z* 51 ($C_4H_3^+$), *m/z* 91–26 (HC≡CH) and *m/z* 39 ($C_3H_3^+$) aromatic ions. The alkyl radical substituted benzene undergoes cleavage at β to the aromatic ring, the so-called benzyl fragmentation, leading to formation of peak *m/z* 91, C_6H_5–CH_2^+, often the base peak. Toluene also converts to the *m/z* 91 ion, known as the *tropylium ion*, isolated at low temperatures where its NMR spectrum was recorded [1, 3]:



(32)

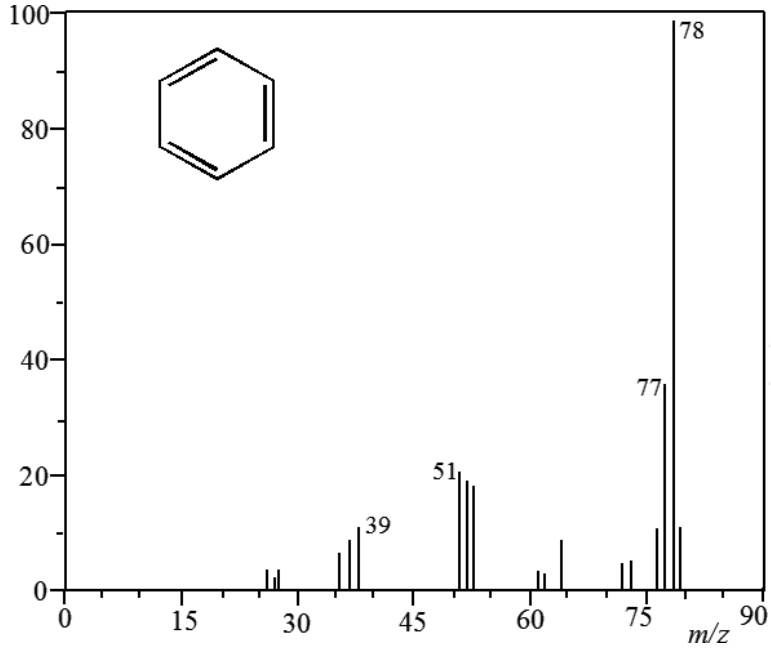
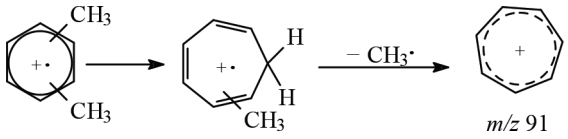


Figure 12. Benzene-simplified mass spectrum [1].

In xylenes, a methyl group is lost to reach the very stable tropylium ion:



(33)

By elimination of an acetylene molecule, the tropylium ion converts to *m*/*z* 65, which in turn loses an acetylene molecule, converting to the 'aromatic ion' *m*/*z* 39 [54].

2.7.4. Halogenated compounds

Bromine and chlorine compounds are mainly distinguishable by appearance of molecular peak of their natural isotopes. Based on dissociation energies of their molecule bonds, fragmentation patterns of molecular ions can be predicted:

Bond	C–C	C–H	C–F	C–Cl	C–Br	C–I
ΔH_0 in kJ/mol	340	420	456	334	268	230

Characteristic fragmentation of brominated and iodised compounds consists of cleavage of the C–Br and C–I bonds at low dissociation energy. Two patterns of fragmentation are then possible:

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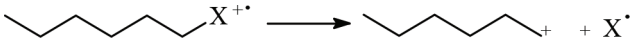
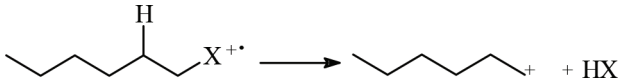


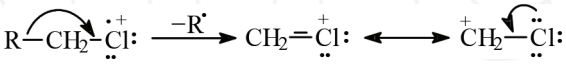
(34)

accompanied by the rearrangement of hydrogen at β, observed particularly in chlorinated compounds:



(35)

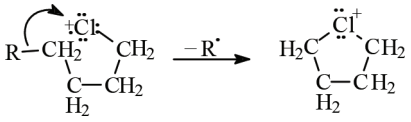
Low-molecular-mass monochlorinated alkanes show a detectable molecular peak. Although the chlorine atom does occur in fragmentation of the molecular ion, its intervention is much smaller than in oxygen-, nitrogen- or sulphur-containing compounds. Cleavage at adjacent C–C in a monochlorinated chain results in a small peak *m*/*z* 49 as well as in the isotope peak *m*/*z* 51 [1, 3, 54, 65]:



(36)

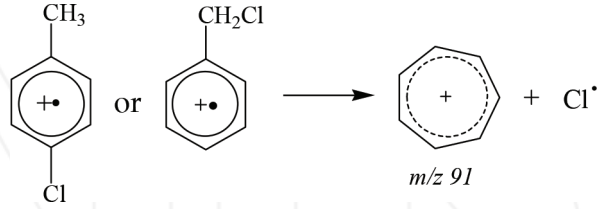
Cleavage of the C–Cl bond results in a small Cl^+ peak and an R^+ peak, dominant in low-molecular-mass halogens but very weak when the chain is greater than C_5.

Here, the chain > C_5-chlorinated compounds render $C_3H_6Cl^+$, $C_4H_8Cl^+$ and C_5HCl^+ ions, of which $C_4H_8Cl^+$ shows the most intense peak (the base peak sometimes, because of its cyclic structure):



(37)

HCl elimination occurs probably at 1,3 position, resulting in formation of a weak peak (on average) M–36. Conduct of brominated compounds is similar to that of chlorinated ones [65]:



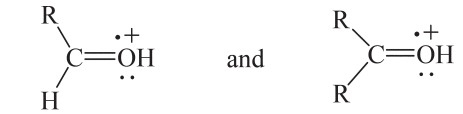
(38)

Halogenated aromatic compounds have a prominent peak M–X when X is directly related to the cycle. When possible, the tropylium ion is easily formed [1, 3, 60].

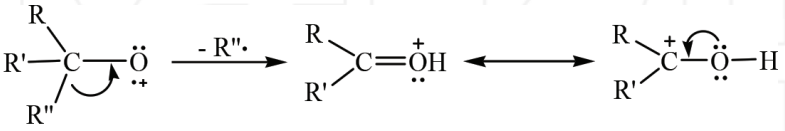
2.7.5. Hydroxy compounds

Alcohols easily losing one water molecule, their molecular ion, are almost non-existent. Water loss may occur under the influence of heat even before fragmentation. Therefore, in this case, the spectrum resembles that of an alkene.

Generally, the break occurs in the bond next to the oxygen atom. Primary alcohols mainly show a predominant peak due to the CH_2=OH+ (*m*/*z* 31). Secondary and tertiary alcohols cleave with formation of ions:

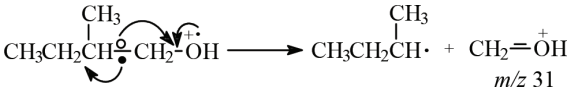


(*m*/*z* 45, 59. 73 ...) (*m*/*z* 59, 73, 87 ...) , respectively (39)



(R''>R') (40)

When R and/or R′=H, a peak may show at M−1. Analysis of branched alcohols is more difficult [3]:



(41)

The mass spectrum of 2-methyl-1-butanol also renders the peak at *m*/*z* 57 (**Figure 13**) corresponding to the $CH_3CH_2^+CHCH_3$ fragment, whose rise is difficult to explain [66].

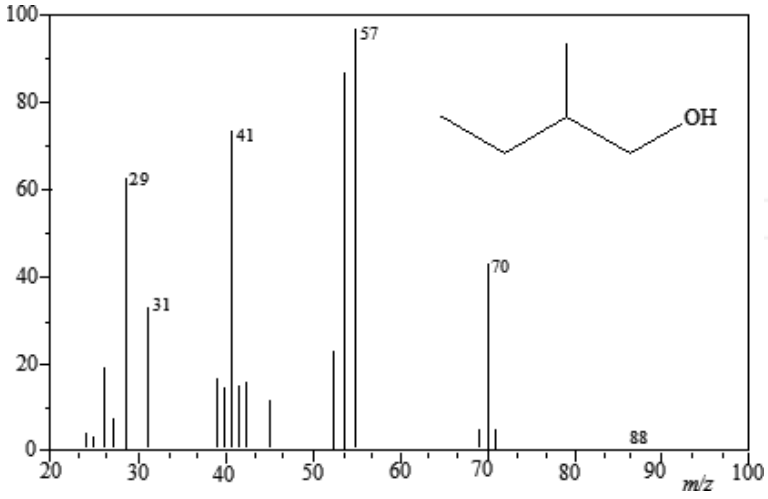
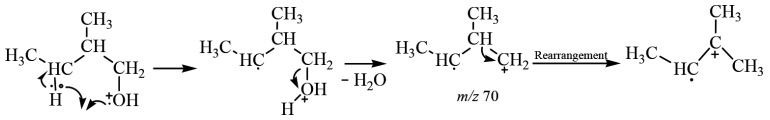


Figure 13. 2-Methyl-1-butanol-simplified mass spectrum. The *m*/*z* 88 molecular peak cannot be observed.

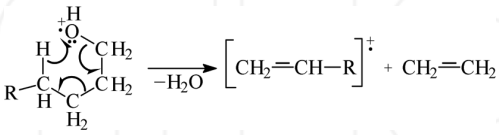
http://dx.doi.org/10.5772/intechopen.68595

The peak at m/z 70 corresponds to a dehydration (M−18), which is supposed to occur according to the following mechanism:



(42)

In over C_6-chain primary alcohols, the break of C–C bonds results in spectra similar to those of alkenes. Concomitant elimination of water and an alkene gives rise to a peak M–(alkene + water) [3]:

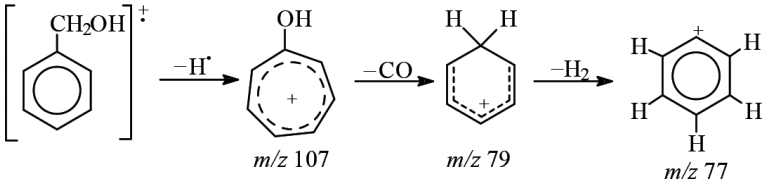


(43)

The alkene ion undergoes breakdown by successive elimination of ethylene.

Cycloalkanes undergo complex fragmentation. Cyclohexanol (M^+ = *m*/*z* 100), by elimination of one hydrogen at α, forms the $C_6H_{11}O^+$ ion, by elimination of water forms the $C_6H_{10}^+$ ion and by complex cycle cleavage results in the $C_3H_5O^+$ ion.

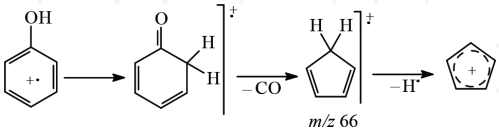
Benzyl alcohol and substitution counterparts form a prominent parent peak. Following a cycle cleavage at β, an average abundance peak (M–OH) is also present [60]:



(44)

The sequence of M−1, M−2 and M−3 peaks should be noted. Also, the $C_6H_7^+$ ion is formed by the elimination of CO, as well as the $C_6H_5^+$ ion, by elimination of hydrogen.

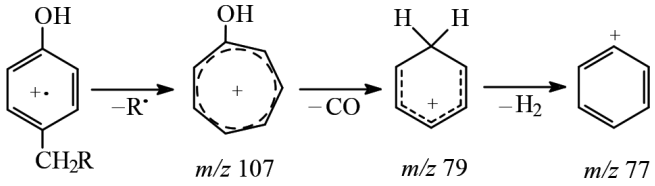
Phenols are characterised by abundant molecular peak as well as by the M–CO (M−28) fragment arising from formation of a cyclohexadienyl intermediate:



(45)

The molecular ion peak in ordinary phenol is the base peak, and the M−1 peak is weak. In cresols, as a result of a slight benzylic C–H cleavage, the M−1 peak is more prominent than the molecular ion peak.

Fragmentation of alkylphenols is similar to that of alkylbenzenes, which further cleave in the same way as un-alkylated phenols [61, 62]:



(46)

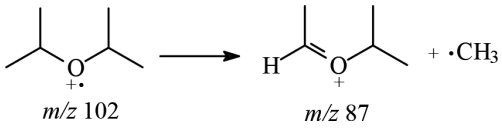
2.7.5.1. Ethers

The molecular ion peak is weak in *aliphatic ethers* (**Figure 14**) and intense in aromatic ones. The M+1 peak (resulted on H· collision with the molecular ion) is more visible.

The presence of a hydrogen atom can be inferred from the presence of prominent peaks by m/z 31, 45, 59, 73, etc., representing RO^+ and $ROCH_2^+$ fragments [3].

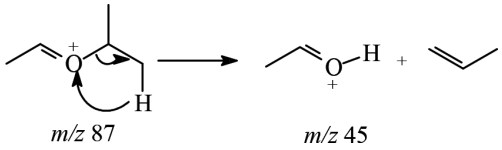
Compared to alcohols, ethers do not support fragmentation with water elimination.

Ethers are characterised by fragmentation of the C–C bond at β to oxygen [67–71]:



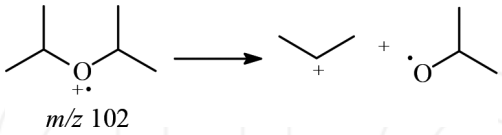
(47)

For a possible H at β to O^+, secondary fragmentation then follows:



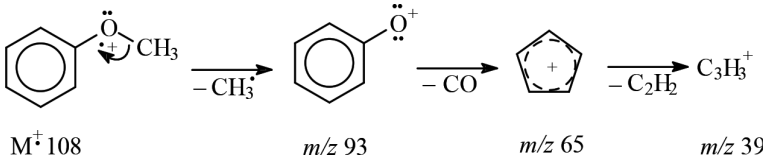
(48)

Cleavage of the simple C–O bond, sometimes observed in simple ethers, gives rise to branched ions:



(49)

The molecular peak is predominant in *alkyl aryl ethers*. The bond at β to the cycle is the first to break, followed by further breakdown of the resulting fragment. Anisole with M^+ by *m/z* 108 converts to *m/z* 93, *m/z* 65 and *m/z* 39 ions [1, 3, 67–71]:



(50)

http://dx.doi.org/10.5772/intechopen.68595

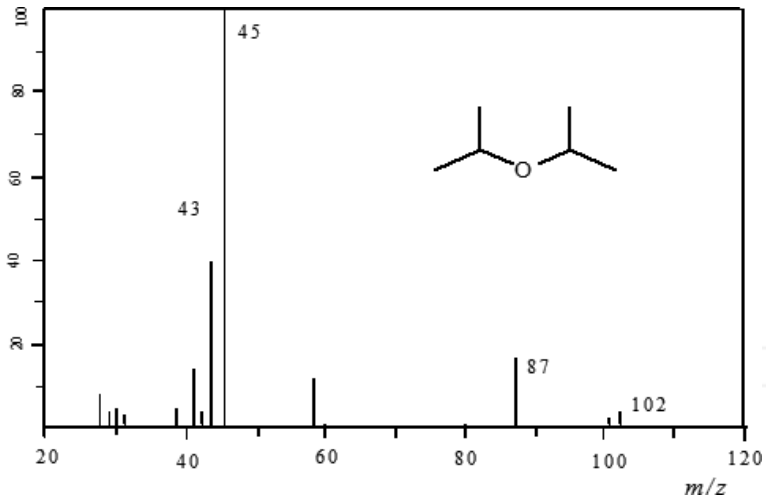
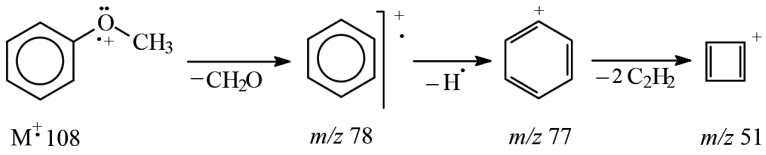


Figure 14. Di-isopropyl ether-simplified mass spectrum [1].

Concomitant loss of formaldehyde results in formation of *m*/*z* 78, m/z 77 and *m*/*z* 51 ions (**Figure 15**):



(51)

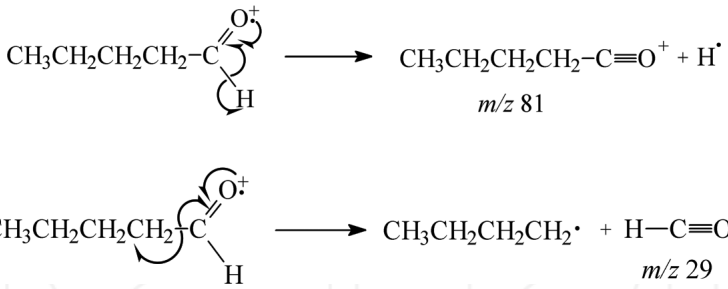
Due to complex rearrangements, *diphenyl ethers* display peaks at M–H, M–CO and M–CHO.

2.7.6. Carbonyl compounds

2.7.6.1. Aldehydes

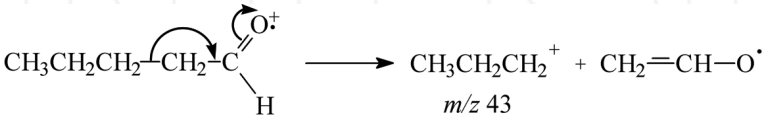
Generally, the aldehyde molecular peak may be identified. Break of the C–H and C–C bonds next to the oxygen atom renders an M−1 peak and an M–R peak (*m*/*z* 29, CHO^+). The M−1 peak is characteristic even for long-chain aldehydes, but the *m*/*z* 29 peak in > C_4 aldehydes may also result from the $C_2H_5^+$ hydrocarbon ion [1, 3, 72].

McLafferty fragmentation of the C–C α,β bond occurs in these aldehydes, resulting in formation of a prominent peak by *m*/*z* 44, 58 or 72, etc., depending on substituents in α position (**Figure 16**). This resonance-stabilised ion arises in cyclic transition state. Based on pentanal as reference, there are four fragmentation patterns:



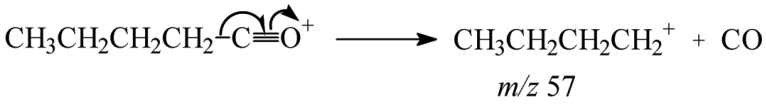
(52)

Cleavage of the bond in β position:



(53)

Cleavage of the bond in α position:



(54)

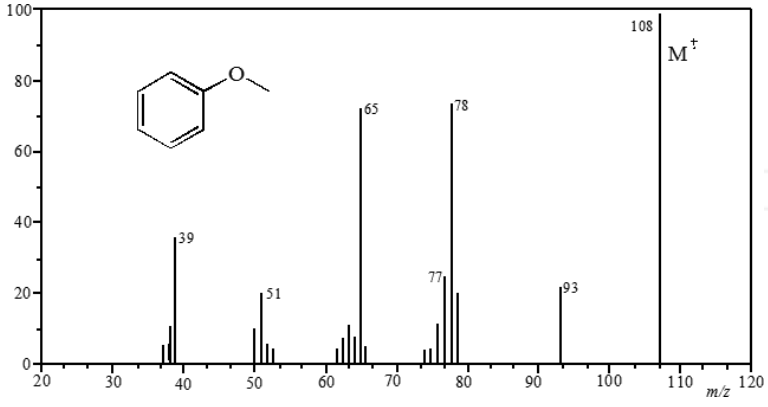


Figure 15. Anisole-simplified mass spectrum with intense peak [M–CH_2O] by *m/z* 78 [1].

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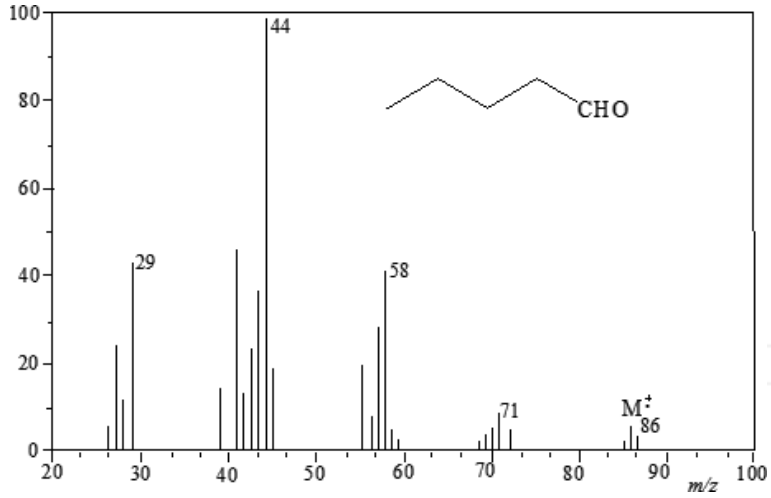
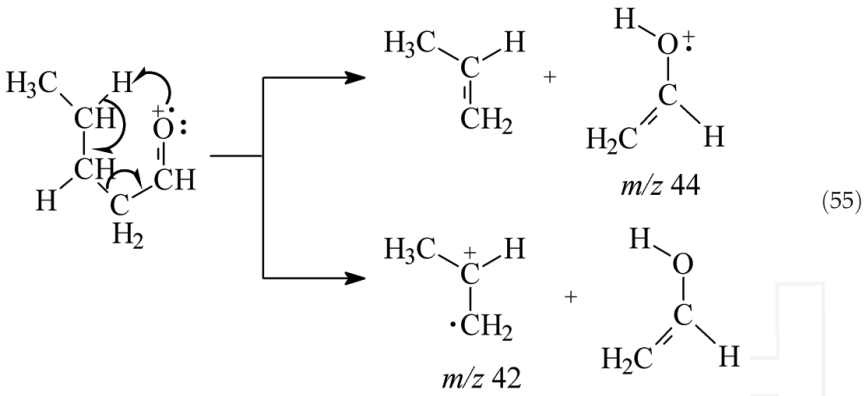


Figure 16. Simplified mass spectrum of pentanal [1].

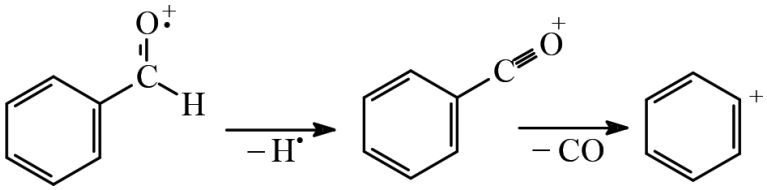
McLafferty rearrangement:



(55)

Spectra of straight-chain aldehydes show other characteristic peaks as well, e.g. M−18 (water loss), M−28 (ethene loss) and M−43 (loss of CH_2=CH–OH). The hydrocarbon-fragmentation-like pattern becomes more prominent with an increase in the chain [3].

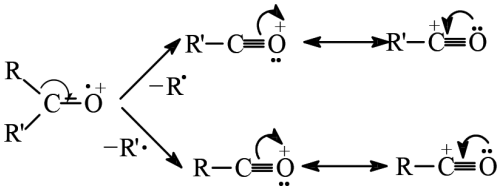
Aromatic aldehydes lose one hydrogen and convert to the benzoyl ion, peak by M−1, further converting to the phenyl cation by CO elimination:



(56)

2.7.6.2. Ketones

Ketone molecular ion peak is generally sufficiently prominent. One exception is that of the 3-methyl-2-pentanone, whose spectrum does not display the molecular ion peak by *m*/*z* 100 (**Figure 17**). The most frequent R′–CO–R ketone fragmentation pattern results in formation of resonance-stabilised $R'–CO^+$ or $R–CO^+$ acylium ions [1, 3, 73]:



(57)

Fragmentation generates the peaks by *m*/*z* 43, 57 or 71. The base peak commonly results by loss of the most important alkyl group.

For an alkyl chain bound to the CO group with three or more carbon atoms, cleavage of the C–C bond at α–β occurs, accompanied by hydrogen migration and formation of a prominent peak [60–62].

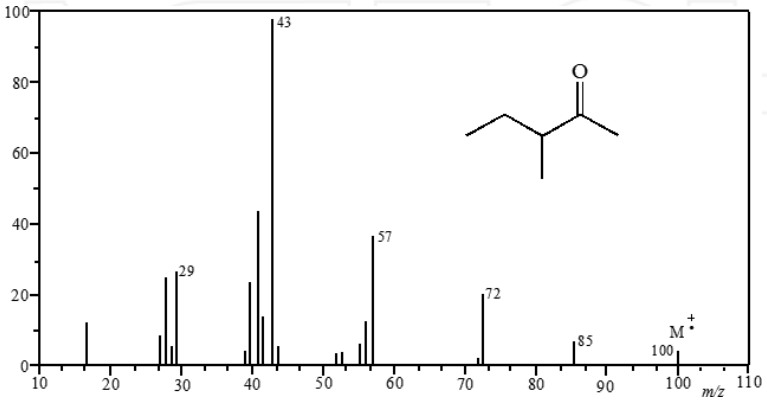
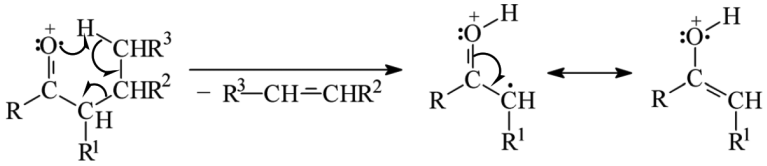


Figure 17. 3-Methyl-2-pentanone-simplified mass spectrum [1].

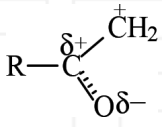
http://dx.doi.org/10.5772/intechopen.68595

McLafferty rearrangement occurs:



(58)

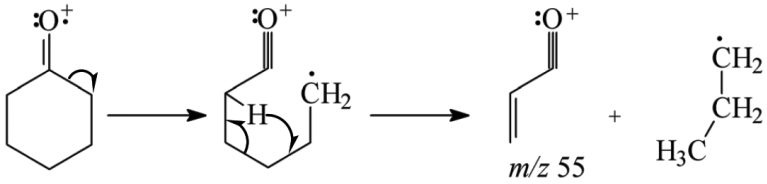
Cleavage of the α–β bond does not occur because an unstable ion would otherwise result, with two adjacent positive cores:



(59)

Unless high-resolution techniques are used, in long-chain ketones, hydrocarbonated peaks may not be distinguished from acylated ones because the mass of one C=O unit (28) equals that of two CH_2 units.

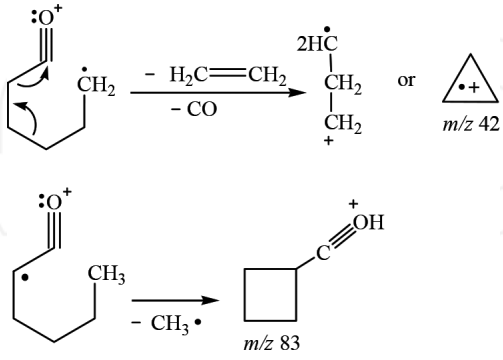
The molecular ion of *cyclic ketones* is predominant. Similar to aliphatic ketones, the first fragmentation occurs at the bond adjacent to the carbonyl group [53]:



(60)

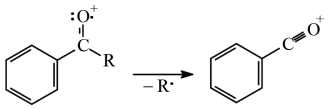
The resulting ion by *m*/*z* 55 is the base peak. The same ion results in cyclopentanone as well, on elimination of an ethyl radical (instead of a propyl radical, the same as in cyclohexanone) [53].

Distinctive peaks by *m*/*z* 42 and 43 in the cyclohexanone spectrum arise from the following fragmentations:



(61)

The molecular ion peak is predominant in *aromatic ketones*. Fragmentation of alkyl aryl ketones occurs at the bond at β to the cycle, resulting in formation of the benzoyl Ar–C≡O^+ cation, usually the base peak. This may lose CO, resulting in formation of one aryl ion (*m*/*z* 77 for acetophenone) [1, 53, 73]:

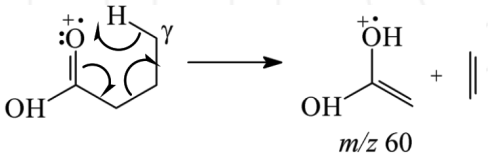


(62)

In the case of R with one hydrogen at γ, a McLafferty rearrangement occurs, similar to aliphatic ketones, resulting in formation of one ion by *m/z* 120 [3, 53, 73].

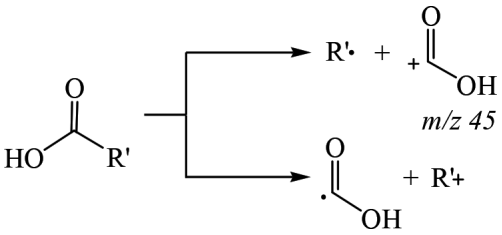
2.7.7. Carboxylic acids

In *straight-chain monocarboxylic acids*, although weak, the molecular peak can generally be observed. The *m/z* 60 peak is characteristic and sometimes the base peak, due to the McLafferty rearrangement [53, 73]:



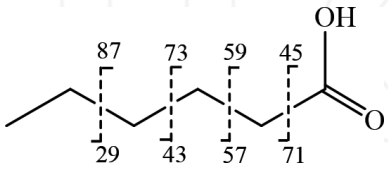
(63)

Short-chain acids display predominant peaks by M–OH and M–COOH. The $COOH^+$ ion by *m/z* 45 is low in intensity, and M–OH^+, by *m/z* M–17, may only rarely be observed:



(64)

Long-chain acids display spectra (**Figure 18**) with two peak series resulting from cleavage of each C–C bond whose charge is either on the oxygen fragment (*m/z* 45, 59, 73, 87, etc.) or the alkyl fragment (by *m/z* 27, 28, 41, 42, 55, 56, 69, 70, etc.) [3, 53, 73]:



(65)

Dicarboxylic acids are termed into esters to increase their volatility. Trimethylsilyl esters[2] are very suitable.

[2]Similar to trimethylsilyl ethers, trimethylsilyl esters are used for volatilisation of carboxylic acids or alcohols in electronic impact MS (EI-MS) or GC-MS. In EI-MS, a clear peak, $[M–CH_3]^+$and often $[Si(CH_3)_3]^+$ results by *m/z* 73, as base peak.

http://dx.doi.org/10.5772/intechopen.68595

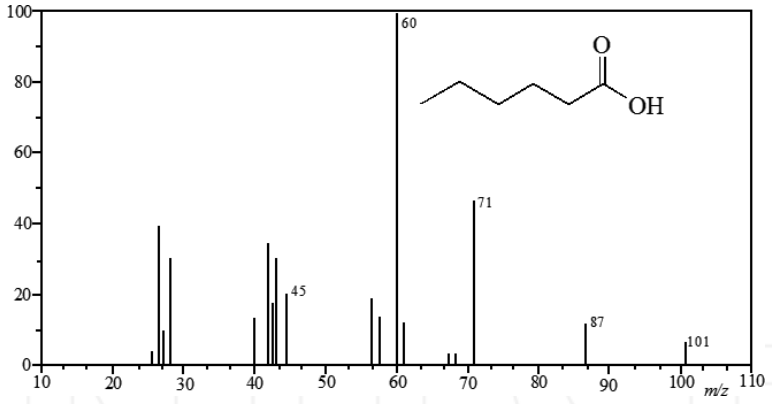
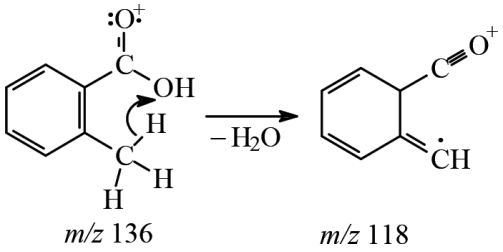


Figure 18. Hexanoic acid-simplified mass spectrum [1].

Aromatic acids display easily noticeable molecular peaks. Other peaks result by OH loss (M−17) and COOH loss (M−45). Water loss (M−18) occurs if there exists one in orthohydrogen or a hydrogen group.

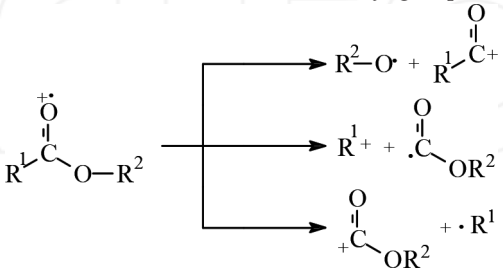
One instance of *ortho-effect* is that of *o*-methylbenzoic (*o*-toluic) acid [1, 3, 53]:



(66)

2.7.8. Carboxylic esters

Esters of aliphatic acids, even soaps, usually display one noticeable molecular peak. The following ions may result by break of the bonds at α to the carbonyl group:

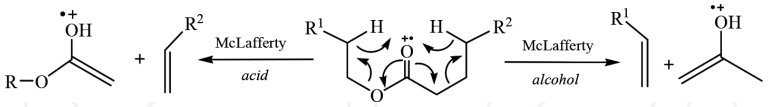


(67)

In a $R^1CO–OR^2$ ester, simple fragmentations in the α position of the carbonyl group give rise to several ions: R^{1+}, R^1CO^+ and $COOR^{2+}$.

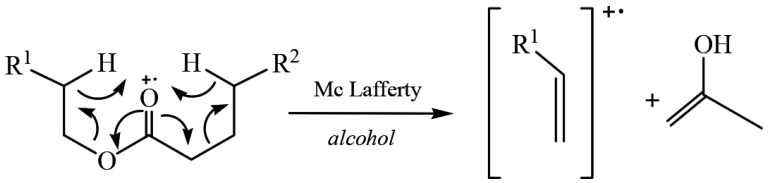
The characteristic peak occurs due to common McLafferty rearrangement, with cleavage of a bond not directly adjacent to the carbonyl group.

In case of multiple rearrangement possibilities, there is a tendency to favour that of the 'acid' part as compared to the 'alcohol' part [1, 3]:



(68)

The McLafferty rearrangement of the *alcohol* part does not occur unless there is a competition with rearrangement of the *acid* part or the arising ion is stabilised, e.g. by an aromatic substituent. Instead, rearrangement of the *alcohol* part to acetals often occurs, where the $[M-60]^+$ ion is important, whereas the ion by *m/z* 60 $[C_2H_4O_2]^+$ is virtually non-existent [3]:



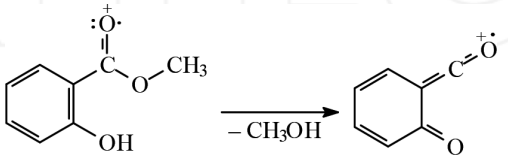
(69)

$R^1CO–OR^2$ esters, where R^2 has more than two carbon atoms, also produce an $R^1C(OH)_2^+$ ion originating from a double rearrangement of the alcohol part.

In $R^1CO–OR^2$ esters, there are characteristic ionic series, allowing for unambiguous determination of the 'acid' part (**Table 4**) and the 'alcohol' part.

For example, the ethyl propanoate spectrum (**Figure 19**) includes R^{1+} ions (where R^1 is ethyl) by *m/z* 29, R^1CO^+ by *m/z* 57 and $R^1C(OH)_2^+$ by *m/z* 75 [3, 53].

Esters of aromatic acids display a predominant molecular peak. Alkyl benzoates eliminate alcohol by *ortho-effect*, similarly to aromatic acids. For example, in the spectrum of methyl salicylate, the base peak ion is that of the ion by *m/z* 120. The ion *m/z* 92 results by CO elimination:

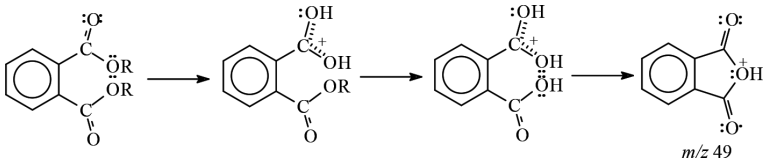


m/z 152 *m/z* 120

(70)

In phthalic esters, widely used as plasticisers, there is a prominent peak by *m/z* 149, likely resulted from fragmentation of two ester groups and finally of a water molecule [74]:

http://dx.doi.org/10.5772/intechopen.68595



(71)

R^1 'acid' part	CH_3	C_2H_5	C_3H_7	C_4H_9	C_5H_{11}
m/z R^{1+}	15	29	43	57	71
m/z R^1CO^+	43	57	71	85	99
$R^1C(OH)_2^+$	61	75	89	103	117
R^2 'alcohol' part	CH_3	C_2H_5	C_3H_7	C_4H_9	
m/z R^2OCO^+	59	73	87	101	
m/z McLafferty	74	88	102	116	

Table 4. Ion characteristic to the ester 'acid' and 'alcohol' parts, respectively [1, 3].

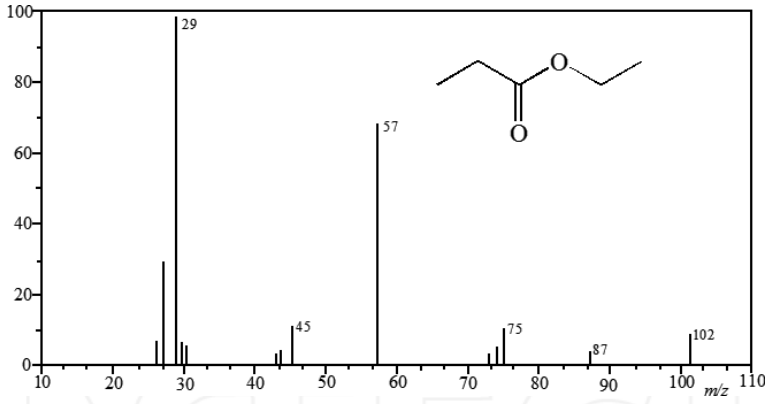


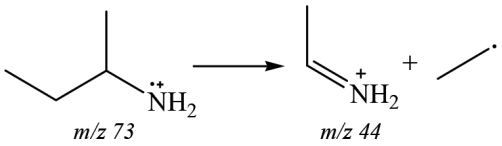
Figure 19. Ethyl propanoate-simplified mass spectrum [1].

2.7.9. Amines

The molecular ion peak of *aliphatic amines* is odd, generally weak, unnoticeable even in long chain or strongly branched amines. The base peak results from a C–C (α,β) fragmentation next to the nitrogen atom.

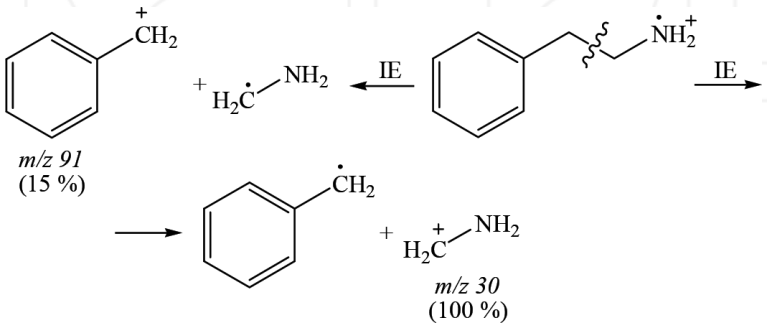
Mass spectra reveal the presence of *iminium* ions due to nitrogen, which is a very good stabiliser of adjacent ions.

Primary amines undergo break of the β bond to the NH_2 group [1, 3]:



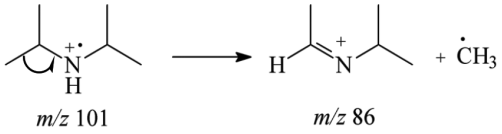
(72)

For instance, 2-phenylethylamine, found in chocolate, red wine, cheese and also involved in migraine, provides a molecular ion of relatively low abundance on electron impact, which undergoes fragmentation in the β position resulting in formation of two carbocations:



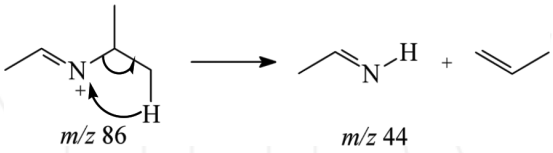
(73)

Secondary amines have the same degradation pathway as esters (**Figure 20**). Fragmentation of the C–C bond at β to nitrogen occurs [1, 3, 75]:



(74)

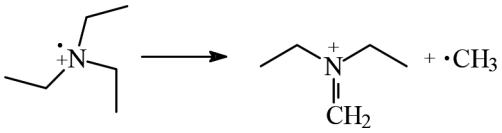
In case of one H^+ at C at β, ion rearrangement occurs:



(75)

The *m*/*z* 44 ion is the base peak in the *di*-isopropylamine spectrum.

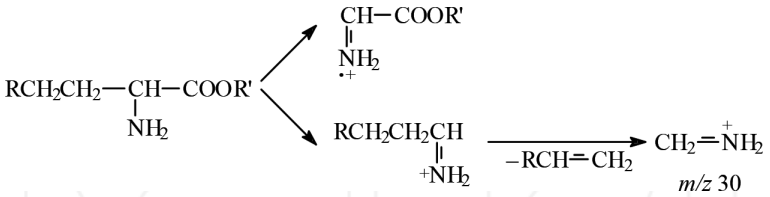
Tertiary amines lose an alkyl radical, resulting in formation of a resonance-stabilised iminium radical. For instance, the base peak of *m*/*z* 101 molecular peak triethylamine is by *m*/*z* 86 [1, 3]:



(76)

http://dx.doi.org/10.5772/intechopen.68595

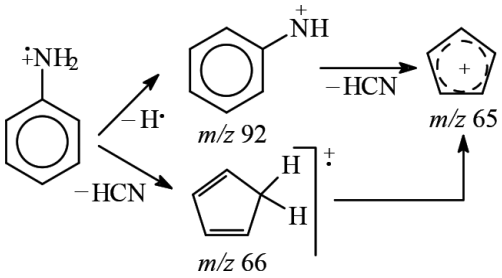
Amino acids may undergo fragmentation in two C–C bonds next to nitrogen, favouring the one arising from loss of the carboxyl group. The aliphatic amine-containing fragment undergoes breakdown, resulting in a peak by *m/z* 30 [76]:



(77)

Unlike acyclic amines, *cyclic amines* have intense molecular peaks. The first cleavage occurs at either the carbon at α with loss of a hydrogen atom, resulting in formation of a prominent peak M−1, or by cycle opening, followed by ethene elimination (for pyrrolidine) to form $CH_2N^+H{=}CH_2$ (*m/z* 43, the base peak). Further, $CH_2{=}N^+{=}CH_2$ (*m/z* 42) results by loss of a hydrogen atom.

Monomolecular *aromatic amines* (with odd number of nitrogen atoms) display an intense molecular ion [76]:



(78)

By loss of a proton of the amino group, aniline converts into an M−1 peak ion, which, by subsequent loss of one HCN molecule, renders the predominant peak by *m/z* 65 (the *cyclopentadienyl ion*).

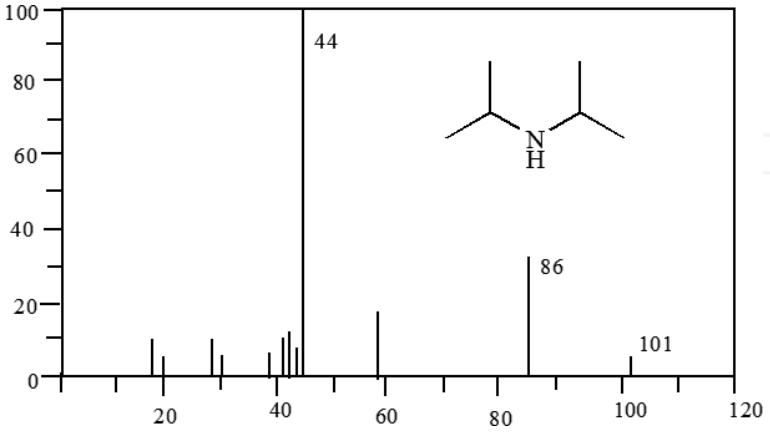
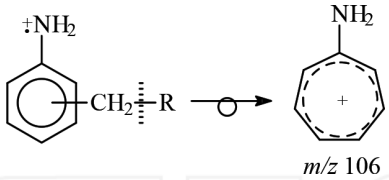


Figure 20. Di-isopropylamine-simplified mass spectrum [1].

Alkyl anilines undergo tropylic cleavage, resulting in formation of amino-tropylium ion, peaking by *m/z* 106 [76]:



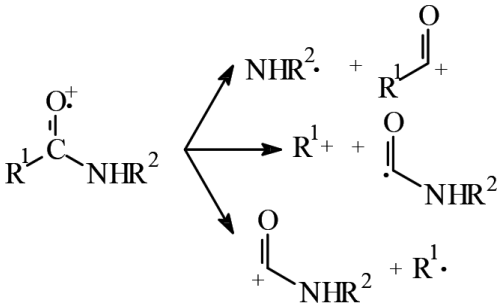
m/z 106

(79)

2.7.10. Amides

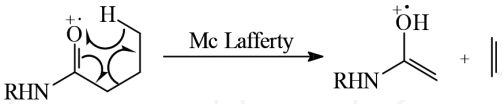
In *aliphatic amides*, the molecular ion of a monoamide is generally identifiable. Dominant fragmentation patterns depend on chain length as well as on the number and length of nitrogen-bound alkyl groups [76].

Simple fragmentations at α to the carbonyl result in the following ions:



(80)

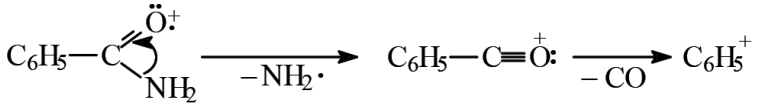
The base peak of primary amides with straight chain longer than that of propionamide results from McLafferty rearrangement [1, 3, 54, 77]:



(81)

Secondary and tertiary amides with one hydrogen at γ of the acyl part and methyl groups at the nitrogen atom undergo McLafferty rearrangement, producing a dominant peak.

Aromatic amides are typically represented by benzamide (**Figure 21**). The molecular ion loses NH_2, resulting in a resonance-stabilised benzoyl cation which then undergoes fragmentation, leading to the phenyl cation [1, 3, 77]:



m/z 121 *m/z* 105 *m/z* 77

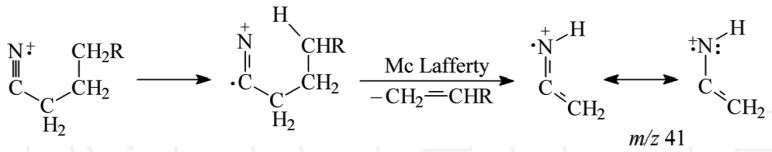
(82)

http://dx.doi.org/10.5772/intechopen.68595

2.7.11. Nitriles

Except for acetonitrile and propionitrile, molecular peaks of aliphatic nitriles are weak. They are often accompanied by M+1 or M−1 peaks.

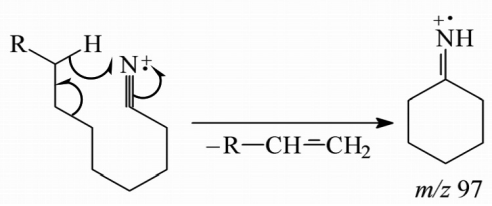
In C_4–C_9 straight-chain nitriles, rearrangement to transition status with a six-atom cycle results in ion *m/z* 41, the base peak [1, 3]:



(83)

This peak is no certain indication because of the presence of another peak $C_3H_5^+$ (*m/z* 41), in all aliphatic chain hydrocarbons.

Following McLafferty rearrangement, straight-chain nitriles of more than seven carbon atoms render a characteristic peak by *m/z* 97:



(84)

Simple fragmentation of the C–C bond, aside from the one next to nitrogen, renders a series of homologous peaks of even mass along the hydrocarbon chain by *m/z* 40, 54, 68 and 82, due to the $(CH_2)_nC{\equiv}N^+$ ions, similar to hydrocarbons [1, 3].

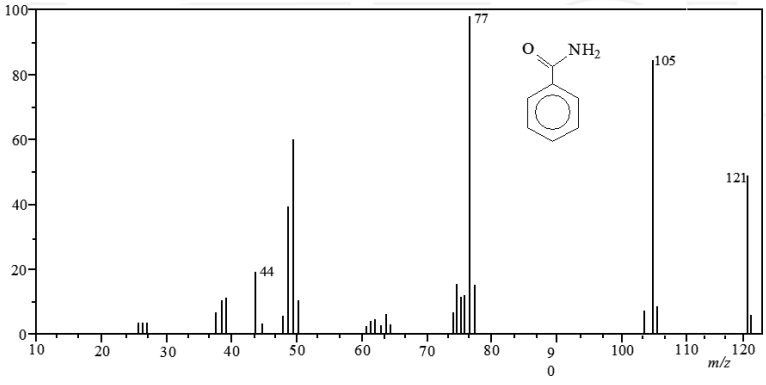
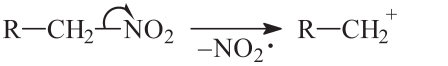


Figure 21. Benzamide-simplified mass spectrum [1].

2.7.12. Nitro compounds

Aliphatic nitro-derivatives have (odd) weak or absent molecular peaks. As the nitro group produces sharp polarisation of the C–N bond, the latter is broken, giving rise to hydrocarbon characteristic fragments:



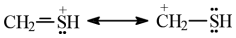
(85)

The presence of the NO_2 group is shown by a weak peak by *m/z* 46 (NO_2^+) and a sizeable one by *m/z* 30 (NO^+).

Aromatic nitro-derivatives have a prominent molecular peak. Predominant peaks result from elimination of an NO_2 radical (M−46) and a neutral NO molecule with rearrangement for formation of the phenoxy cation [1, 3].

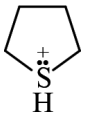
2.7.13. Sulphur-containing compounds

Mercaptan and thio-ether compounds render more intense molecular ion peaks than corresponding oxygen compounds. *Mercaptan* fragmentation is similar to that of alcohols. Cleavage of the C–C bond (αβ) results in a characteristic ion:



(86)

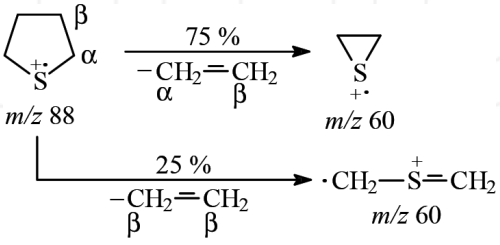
by *m/z* 47. Fragmentation of the β–γ bond gives rise to an average intensity peak by *m/z* 61 and γ–δ fragmentation results in a peak by *m/z* 75. Fragmentation at the δ–ε bond renders a more intense, cyclisation-stabilised peak by *m/z* 89 [1, 3, 54]:



(87)

Similar to alcohols, primary mercaptans lose H_2S, resulting in a prominent peak by *m/z* 34.

Aliphatic *sulphides* render an intense peak by M+2. Fragmentations occur similarly to ethers. Cyclic sulphides fragment differently from cyclic ethers. For instance, similarly to tetrahydrofuran, in addition to hydrogen fragmentation at α and β, tetrahydrothiophene undergoes ethene fragmentation in different positions, resulting in formation of the *m/z* 60 ion, the base ion [75]:

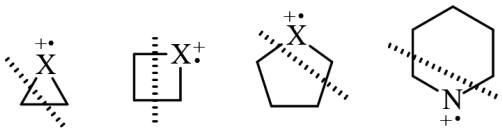


(88)

2.7.14. Heterocyclic compounds

Molecular ions of saturated heterocyclic compounds show a strong tendency to transannular rearrangement, often rendering the base peak.

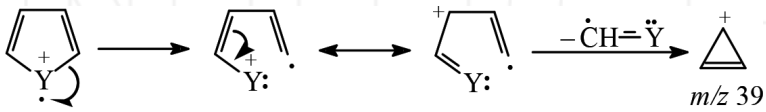
http://dx.doi.org/10.5772/intechopen.68595



(89)

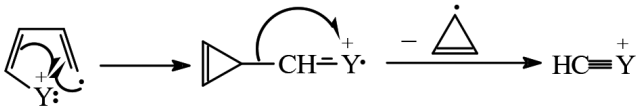
X=O, S, NH

Whether alkylated or not, *heteroaromatic compounds* render an intense molecular peak. Cleavage of the bond at β occurs as for alkylbenzenes. The charge of the molecular ion is mainly localised on the heteroatom and not on the aromatic ring. Five-atom aromatic heterocycles display similar fragmentation patterns. The first step consists in the cleavage of the carbon–heteroatom bond:



m/z 39

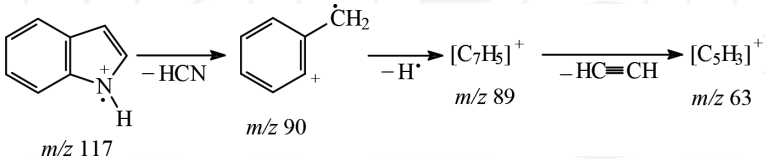
(90)



Y=O, S, NH

Furan displays two main peaks $C_3H_3^+$ (*m/z* 39) and $HC{\equiv}O^+$ (*m/z* 29). *Thiophene* shows three peaks: $C_3H_3^+$ (*m/z* 39), $HC{\equiv}S^+$ (*m/z* 45) and $C_2H_2S^{+\bullet}$ (*m/z* 58) [1, 3, 54, 78, 79].

Pyrrole displays three peaks as well: $C_3H_3^+$ (*m/z* 39), $HC{\equiv}NH^{+\bullet}$ (*m/z* 28) and $C_2H_2N^{+\bullet}H$ (*m/z* 41). It also eliminates one neutral HCN molecule, producing an intense peak by *m/z* 40. *Indole* as well eliminates hydrocyanic acid, and the ion fragment *m/z* 90 is stabilised by hydrogen elimination, resulting in formation of a dehydrotropylium ion:

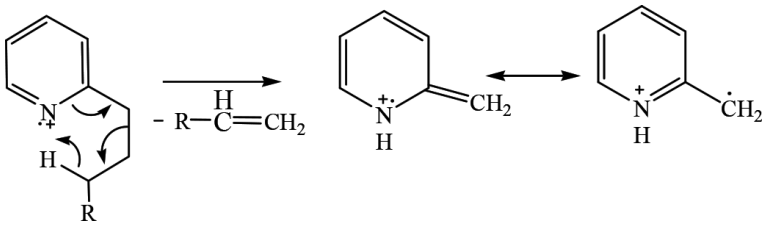


m/z 117 *m/z* 90 *m/z* 89 *m/z* 63

(91)

The pentatomic poly-heterocycles such as oxazoles, imidazoles, pyrazoles, etc. fragment more easily. In the case of an N heteroatom, elimination of HCN is preferred. For three or more directly bonded carbon atoms, the characteristic peak arises—$C_3H_3^+$ (*m/z* 39). Unsubstituted pyridines eliminate HCN, resulting in a base peak by *m/z* 52. Similarly to toluene, β- and γ-picolines also render intense peaks by M–HCN, *m/z* 66, and l M−1, *m/z* 78.

Substituted pyridines with large alkyl groups undergo fragmentation at β, γ, δ as well as McLafferty rearrangements, resulting in a peak by *m/z* 93 [1, 3]:



(92)

Ease of rearrangement depends on substituent position, decreasing in the order 2 > 4 > 3. Pyrazines undergo similar fragmentations because all substituents are in ortho to nitrogen atoms [1, 3].

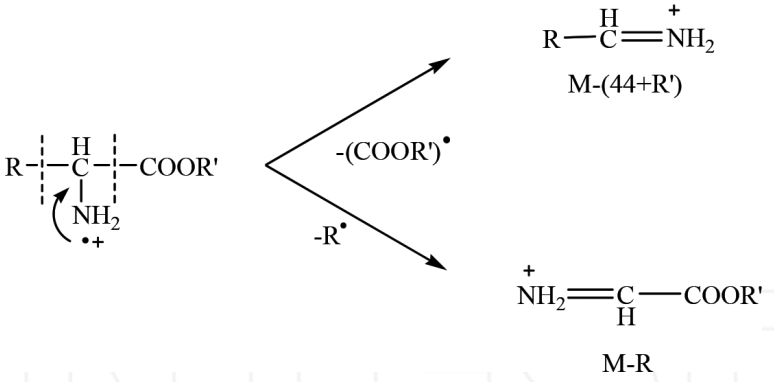
2.7.15. Natural compounds

2.7.15.1. Amino acids

As amino acids are zwitterionic compounds, often non-volatile, their methyl esters are studied instead. The spectra produced by electron impact ionisation display weak or non-existent molecular peaks, because of amino acid capacity to easily lose their carboxyl group and of their esters to easily lose their carboalkoxyl group on electronic impact [76, 77]:

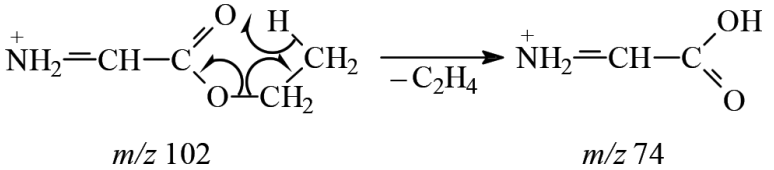
(93)

Esters of amino acids basically have two fragmenting patterns:



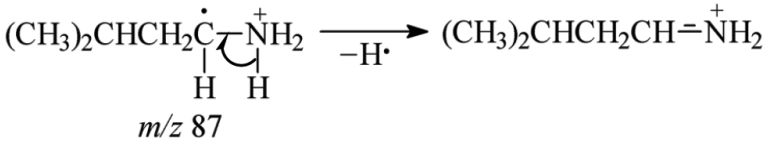
(94)

There is an average or high peak intensity of iminium ions, $RCH{=}N^{+}H_2$, as well as of the $N^{+}H_2{=}CHCO_2R'$ ion. The [M–R] ester group ion may undergo McLafferty rearrangement:



(95)

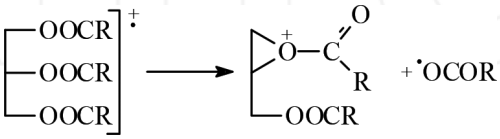
If field desorption (FD) ionisation is used, as for leucine, for instance, spectra show the MH^+ *m/z* 132 ion, which eliminates a carboxyl group to convert to the *m/z* 87 ion, which in turn eliminates a hydrogen atom, resulting in formation of the *m/z* 86 ion [76, 77]:



(96)

2.7.15.2. Triacylglycerols

Triglyceride molecular ions convert to characteristic ions [$M–O_2CR$] formed by stabilising the positive charge of the neighbouring oxygen:

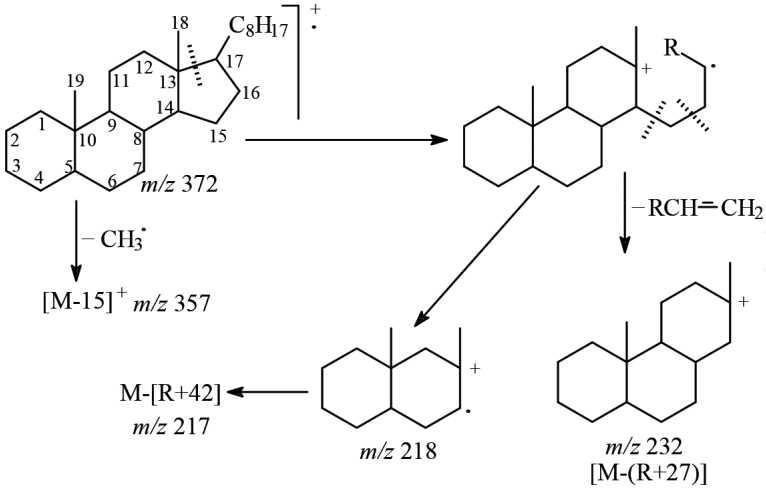


(97)

The OCOR fragment converts to the $[RCO_2H + H]^+$ fragment, allowing for identification of fatty acids. By the electron impact ionisation technique and by chemical ionisation, high-molecular-mass and low-volatility glycerides render weak or non-existent molecular peaks (MH^+) [1, 3, 77].

2.7.15.3. Steroids

Cholestane is a typical representative of steroids [80]. The intense peak molecular ion undergoes four fragmentation patterns:



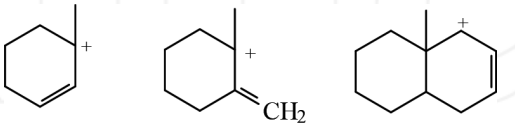
(98)

a. Fragmentation of an angular methyl resulting in formation of the *m/z* 357 peak fragment [31, 32].

b. Fragmentation of the C_{13}–C_{18} bond, favoured by C_{13} being tertiary, resulting in formation of the intermediary ion which is able to fragment in various ways. Fragmentation of the C_{15}–C_{16} bond, with elimination of an olefine and formation of the *m*/*z* 232 ion of mass M–(C_8H_{17}+27).

Cleavage of the C_{14}–C_{15} bond with formation of the resonance-stabilised *m*/*z* 217 ion followed by elimination of a hydrogen atom gives rise to the m/z 217 peak ion the most intense of the spectrum.

c. Fragmentation of the C_9–C_{10} bond, resulting in formation of a cation radical undergoing fragmentation of the C_5–C_6 or C_6–C_7 bonds and transfer of one hydrogen atom with generation of stable ions by *m*/*z* 95 and *m*/*z* 109 [80]:



m/*z* 95 *m*/*z* 109 *m*/*z* 149 (99)

d. Fragmentation of C_8–C_{14} and C_9–C_{11} bonds, followed by transfer of a hydrogen atom to form the ion peak at *m*/*z* 149.

Polyhydroxylated steroids such as cholesterol have spectra with weak or non-existent molecular peaks. Dehydrations occur on heating. Chemical ionisation cannot be used, and the protonated molecular ion dehydrates quickly. Spectra obtained by field desorption (FD) mass spectrometry does not show dehydrations, and the molecular peak is present [1, 3, 81].

2.8. Recommendations for a mass spectrum analysis

Identification of the molecular ion is the first stage for mass spectrum interpretation because the molecular ion is the source of information on molecular composition.

If the electron impact ionisation-rendered spectrum does not allow identification of the molecular ion, other ionisation methods might be used.

Prominent peaks in a mass spectrum are generally those resulting from **primary fragmentations**. Secondary fragmentations may be used as aids for spectrum analysis. Fragment ions of higher mass close to that of the molecular ion are easy to identify because they correspond to formation of a small neutral entity such as CH_3, CH_2=CH_2, etc. Even if at low intensity, they have a key contribution to establishing the structure.

Mass differences between the molecular ion and the fragments must correspond to an actual chemical composition.

The molecular formula inferred must comply with the nitrogen rule.

Contrary to interpretation of NMR spectra, interpretation of all peaks in a mass spectrum is less interesting; only important characteristic peaks are considered. Small mass and low intensity ions are generally not significant.

Systematic spectrum interpretation requires compliance with the following *recommendations*:

- Gathering of basic information such as sample origin, alleged class of compounds, solubility, thermal stability and other spectrometric data.
- Writing down of *m*/*z* values for all relevant peaks and calculation of mass differences between prominent peaks.
- Consideration of the ionisation method used and examination of the general spectrum appearance. Other aspects to consider are whether there is an intense molecular ion (as in aromatic, heterocyclic, polycyclic compounds) or a weak molecular ion (as in aliphatic and multifunctional compounds) or the presence of impurities (solvents, lubricants, plasticisers) or occurrence of basic signals from residual air or wash fractions from chromatographic column.
- Investigation of the presence or absence of functional groups.
- Consultation of tables showing possible structures of different ions and potential structures of neutral fragments (**Tables 5** and **6**).
- Assignment of the unknown compound structure using known structures. Analyte structures can sometimes only arise partly from the known structure, or isomers cannot be determined.
- The proposed molecular structure is correlated with mass spectrum data [82–87].

2.9. Anomalies

Spectra may occur in mass spectrometry that are difficult to define, giving rise to confusion, which can only be avoided by appropriate preparation of samples or change of working conditions [1, 3].

2.9.1. The presence of impurities

Small amounts of impurities may produce peaks in the regions in which the MS spectrum should be white. Such peaks make it difficult to determine the *m*/*z* of the molecular ion. GC-MS impurities may result from residues of previous samples or degradation of the chromatography column. Small peaks may occur at values higher than *m*/*z* of the molecular mass. Sufficient time is necessary between injections into the chromatograph to evacuate previous samples. A background scan can be used to identify peaks due to residual material in the mass spectrometer [1, 3].

2.9.2. Metastable ions

Under normal conditions, the ion arising in the source is sufficiently stable to reach the detector and determine occurrence of a peak. If its life is less than a few μs, the ion is *metastable* and undergoes partial breakdown in its path, in line with a first-order kinetic [81, 87]:

Fragment (*m*/*z*)	Potential structure
15	CH_3^+
17	OH^+
18	H_2O^+; NH_4^+
19	F^+
26	CN^+
27	$C_2H_3^+$
28	$C_2H_4^{+\bullet}$; $CO^{+\bullet}$
29	$C_2H_5^+$; CHO^+
30	CH_4N^+ (amine)
31	CH_2OH^+ (alcohol, ether)
33	CH_2F^+
35	$^{35}Cl^+$ (together with $^{37}Cl^+$ by *m*/*z* 37)
39	$C_3H_3^+$ (aromatic)
41	$C_3H_5^+$; $C_2H_3N^{+\bullet}$ (nitrile)
42	$C_3H_6^{+\bullet}$
43	$C_3H_7^+$; CH_3CO^+ (carbonyl)
44	$C_2H_6N^+$ (amine); $C_2H_4O^{+\bullet}$ (McLafferty: aldehyde)
45	CH_3–CH–OH^+ (alcohol); CH_3–O–CH_2^+ (ether); $COOH^+$ (acid)
49	$CH_2{}^{35}Cl^+$
51	$CH_2F_2^+$; $C_4H_3^+$ (aromatic)
53	$C_4H_5^+$
54	NC–CH_2–CH_2^+ (nitrile); $C_4H_6^{+\bullet}$
55	$C_4H_7^+$; CH_2=CH–CO^+ (unsaturated ester, cyclic ketone)
56	$C_4H_8^{+\bullet}$ (cycle)
57	$C_4H_9^+$; C_2H_5–CO^+
58	$C_3H_6O^{+\bullet}$; (McLafferty); $C_3H_8N^+$ (amine)
59	$C_3H_7O^+$ (alcohol, ether); CH_3–OCO^+ (ester); $C_2H_5NO^{+\bullet}$ (amide)
60	$CH_3COOH^{+\bullet}$ (McLafferty: acetate)
61	$C_2H_5O_2^+$ (double rearrangement: acetate)
65	$C_5H_5^+$ (aromatic)
68	$C_5H_8^{+\bullet}$; $C_4H_6N^+$ (nitrile)
69	$C_5H_9^+$; CF_3^+; $C_4H_5O^+$
71	$C_5H_{11}^+$; C_3H_7–CO^+
72	$C_4H_8O^{+\bullet}$ (McLafferty); $C_4H_{10}N^+$ (amine), $C_3H_6NO^+$
73	$C_4H_9O^+$ (alcohol, ether); C_2H_5–OCO^+ (ester); $C_3H_7NO^{+\bullet}$
74	$C_3H_6O_2^{+\bullet}$ (McLafferty: ester, acid)

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Fragment (*m*/*z*)	Potential structure
75	$C_3H_7O_2^+$ (double rearrangement: propionate)
77	$C_6H_5^+$ (aromatic)
79	$C_6H_7^+$ (aromatic); $^{79}Br^+$ (together with $^{81}Br^+$ by *m*/*z* 81)
80	$C_4H_3NHCH_2^+$ (pyrrole)
81	$C_4H_3O–CH_2^+$ (furan)
82	$C_6H_{11}^{+\bullet}$ (alkene, cyclane); $CH_2{}^{35}Cl_2^{+\bullet}$
85	$C_6H_{13}^+$; $C_4H_9–CO^+$
86	$C_5H_{10}O^{+\bullet}$; $C_5H_{12}N^+$
87	$C_5H_{11}O^+$ (alcohol, ether); $C_3H_7–OCO^+$ (esters); $C_4H_9NO^{+\bullet}$
88	$C_4H_8O_2$ (McLafferty: ester, acid)
89	$C_4H_9O_2^+$ (double rearrangement: butanoate)
91	$C_7H_7^+$ (aromatic)
92	$C_7H_8^{+\bullet}$ (McLafferty: aromatic)
93	$CH_2{}^{79}Br^+$; $C_6H_5O^+$ (phenol); $C_7H_9^+$ (terpene)
94	$C_6H_6O^+$ (Mc Lafferty: phenyl ether)
95	$C_4H_3O–CO^+$ (furan)
97	$C_7H_{13}^+$
98	$C_6H_{10}O^{+\bullet}$
99	$C_7H_{15}^+$; $C_6H_{11}O^+$
100	$C_6H_{14}N^+$
101	$C_4H_9–OCO^+$
103	$C_6H_5–CH=CH^+$; $C_5H_{10}O_2^+$
104	$C_6H_5–CH=CH_2^{+\bullet}$ (Mc Lafferty: ester and aromatic ketone)
105	$C_6H_5–C_2H_4^+$; $C_6H_5–CO^+$
107	$C_6H_5–OCH_2^+$; $C_6H_5–CH_2–O^+$
108	$C_6H_5–OCH_3^{+\bullet}$; $C_6H_5–CH_2–OH^{+\bullet}$ (benzyl ester)
117	$C_6H_5–C_3H_4^+$
119	$C_6H_5–C_3H_6^+$; $C_6H_5–C_2H_2O^+$
120	$C_7H_4O_2^{+\bullet}$
121	$C_7H_5O_2^{+\bullet}$; $C_8H_9O^+$; $C_9H_{13}^+$ (terpene)
127	I^+
131	$C_3F_5^+$; $C_6H_5–CH=CH–CO^+$
149	$C_8H_5O_3^+$ (phthalate)
152	$C_6H_4= C_6H_4^{+\bullet}$
154	$C_6H_5=C_6H_5^{+\bullet}$

Table 5. Ion fragments and potential structures [1, 3, 78, 79].

Fragment (*m*/*z*)	Potential structure
1	H•
15	CH_3•
17	•OH (small weight acid)
18	H_2O (alcohol, aldehyde, ketone)
19	F•
20	HF
26	HC≡CH; •CN
27	HCN
28	CH_2=CH_2; CO (aldehyde)
29	C_2H_5•; HCO•
30	NH_2CH_2•; CH_2=O; NO•
31	CH_3O•; •CH_2OH; NH_2CH_3
32	CH_3OH; S
33	CH_3• and H_2O (alcohol); SH•
34	H_2S
35	^{35}Cl• together with ^{37}Cl•
36	H^{35}Cl together with H^{37}Cl
40	CH_3C≡C–H
41	CH_2=CH–CH_2•
42	CH_2=C=O; CH_2=CH–CH_3
43	CH_3–CO•; C_3H_7•
44	CO_2; CH_2=CH–OH; N_2O; NH_2–CO•
45	•COOH; C_2H_5O•; $C_2H_5NH_2$
46	C_2H_5OH; •NO_2
49	•$CH_2{}^{35}$Cl
51	•CH_2F_2
54	CH_2=CH–CH=CH_2
55	CH_2=CH–CH_2–CH_2•
56	C_4H_8
57	C_4H_9•; C_2H_5–CO•
58	C_3H_6O
59	CH_3OCO•; CH_3COO•; CH_3CONH_2
60	CH_3COOH (acetate); C_3H_7OH
63	^{35}Cl–CH_2CH_2•
64	SO_2
68	C_5H_8

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Fragment (*m*/*z*)	Potential structure
69	CF_3•
70	C_5H_{10}
71	C_5H_{11}•; C_3H_7–CO•
73	CH_3CH_2–O–CO•
74	C_4H_9OH; CH_3CH_2COOH
77	C_6H_5•
78	C_6H_6
79	^{79}Br• together with ^{81}Br•
80	H^{79}Br
100	CF_2=CF_2
119	CF_3CF_2•
122	C_6H_5COOH
127	I•
128	HI

Table 6. Neutral fragments and potential structures [1, 3, 78, 79].

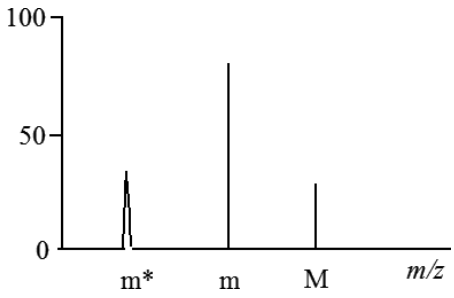

Figure 22. Three peaks of a metastable transition [1].

$$M^{\bullet +} \rightarrow m^{+} + m'^{\bullet} \quad (100)$$

In case the fin m^+ ion occurs in a dual-focus device before exiting the electrostatic sector, because of its insufficient kinetic energy, it is wasted on the walls of the device. Instead, if the breakdown occurs between the outlet of the electrostatic reactor and the entrance to the magnetic reactor, the trace of the m^+ ion can be observed at a pseudo-mass m^* not corresponding to an actual mass.

A metastable transition leads to occurrence of three peaks in the mass spectrum (**Figure 22**). The metastable peak, also known as the *diffuse peak*, is less definitely shaped, and its position

does not necessarily correspond to a mass close to an integer. Metastable ion peaks appear flattened, of low intensity and sometimes concealed by normal peaks. Metastable peak detection can be achieved inter alia by reducing the acceleration potential of ions for their longer maintenance in the ionisation chamber, with reduction of those fragmenting in flight [1, 3].

2.9.3. The absence of the molecular ion

Many compounds such as tertiary alcohols and alkyl halides fragment so easily that the molecular ion cannot be identified in the mass spectrum.

Tertiary alcohols dehydrate easily, and bromides and chlorides can easily lose halogens by fragmentation. Even without the molecular ion peak, the use of the spectra library may predict molecular structure [1, 75, 78].

2.9.4. Complex fragmentations

Mass spectra of pure compounds show difficult-to-interpret peaks. Corresponding fragments thereof may result from multiple-stage fragmentations or certain complex rearrangements (**Tables 5** and **6**). Such peaks should not be insisted upon [1, 3, 78, 79, 88].

3. Conclusion

Mass spectrometry is currently used both in research and development of new molecular structures in industry and other related fields. This method along with NMR, IR, XRD and UV-Vis has become indispensable to any research laboratory in the field of organic chemistry. It has many uses in pharmaceutical (drug design, combinatorial chemistry, pharmacokinetics, drug metabolism, etc.) in the clinical field (neonatal screening, haemoglobin analysis, drug abuse, doping), environmental protection (water quality, food contamination) in geology (oil composition) and of course in biotechnology (analysis of proteins, polypeptides, hormones, etc.).

Pharmacokinetics needs the use of mass spectrometry because of the complex nature of the matrix (often blood or urine). Pharmacokinetics deserves high sensitivity to observe low dose and long-time data point. Commonly used for this application is liquid chromatography-mass spectrometry (LC-MS) with a triple-quadrupole mass spectrometer. A specificity of the experiment is added by tandem mass spectrometry.

Mass spectrometry has utility both in quantitative and qualitative analysis. The method is used to identify the isotopic species and calculation of nominal weight in organic compounds. The development of detectors and ionisation techniques along with its coupling with chromatography greatly widened the scope of combinations including ionic compounds or macromolecules.

Mass spectrometry is also used in protein characterisation and sequencing. Methods for protein ionisation are electrospray ionisation (ESI) and matrix-assisted laser desorption/ionisation (MALDI). Here, two approaches are used for protein characterisation. First, intact proteins are ionised via two techniques, ESI and MALDI, followed by passing them to a mass spectrum analyser. This approach in protein analysis is commonly referred to as the 'top-down' strategy.

Mass spectrometry is also a complementary method to HPLC in glycan analysis. Intact glycan molecules is to be detected directly as single charged ions via MALDI or, after permethylation or peracetylation, via fast atom bombardment (FAB) mass spectrometry. Smaller glycans give good signals in electrospray ionisation mass spectrometry (ESI-MS).

The Universe has also been hit by mass spectrometry via the Viking programme as a standard method for analysis. A specialised GC-MS instrument aboard the Huygens probe entered the atmosphere of Titan in early 2005.

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