Chapter

1

Mass Spectrometry: Introduction and its Application

Mass spectrometry is a sophisticated analytical technique used to:

- quantify known materials,
- identify unknown compounds within a sample, and
- elucidate the structure and chemical properties of different molecules.

Mass spectrometer works by converting the molecule into gaseous ions, with or without fragmentation, which are separated and then characterized by their mass to charge ratios (*m*/*e*) and relative abundances. With the help of masses of fragment, the mass/original structure of molecule is predicted. Two key components in this process are the ion source, which generates the ions, and the mass analyzer, which sorts the ions. Each ion then goes to the detector and recorded its presence in the form streak line depending on its relative abundance.

To illustrate the function of mass spectrometry (Fig 1.1), imagine a stone being projected from a catapult towards a delicate vase (I). On impact, the vase is shattered (II). If the pieces are carefully collected (III), the vase can be reconstructed from the fragments (IV). In this example, the vase represents the molecule, the catapult and stone represent the device for making the fragments.

Mass spectrometry is unlike most other forms of spectroscopy or spectrometry that are concerned with non-destructive interactions between molecules and electromagnetic radiation,

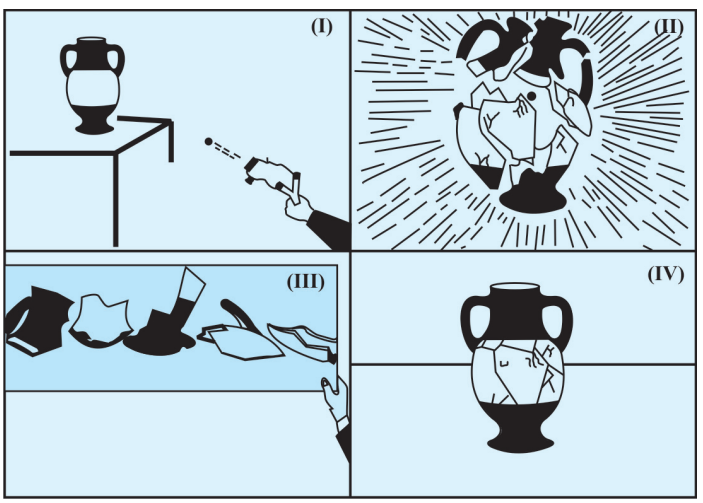

Fig. 1.1: Illustration of the principle of mass spectrometry by electron bombardment

is a destructive technique. This is because mass spectrometry is the study of the effect of ionizing energy on molecules. It depends upon chemical reactions in the gas phase in which sample molecules are consumed during the formation of ionic and neutral species. Although sample is consumed destructively by the mass spectrometer, the technique is very sensitive and only trace amounts of material are used in the analysis. A mass spectrometer converts sample molecules into ions in the gas phase, separates them according to their mass to charge ratio (*m*/*e*) and sequentially records the individual ion fragment in the form of the mass spectrum. The mass spectrum (Fig. 1.2) usually presented as a vertical bar graph in which bar represent an ion (fragment ion) having specific mass to charge ratio and length of bar indicates the relative abundance or relative intensity. The highest peak intensity is counted 100%, and rest of peaks is measured with respect to 100% intensity peak (base peak). For example, in mass spectrum of methyl-t-butyl ketone, the highest peak (base peak) at *m*/*e* 57 is taken as 100% intensity peak. The rest of fragment ions peaks, e.g. *m*/*e* 100, 85, 43, 41, 29 are measured with respect to the highest peak, *m*/*e* 57.

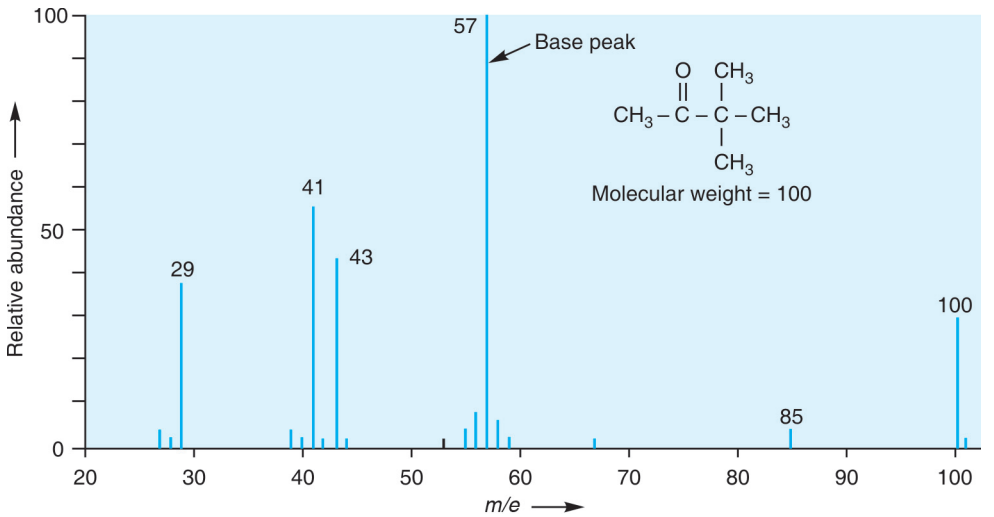

Fig. 1.2: Mass spectrum of methyl-*t*-butyl ketone

Special advantages of mass spectrometry

- High sensitivity
- High accuracy
- Coupling of chromatographic techniques such as GC, HPLC, etc. It means analysis can be done in a mixture or impure sample for particular component.

Disadvantages of mass spectrometry

- Destructive technique
- Samples cannot be collected for further study

History of mass spectrometry

Goldstein discovered the positively charged 'rays' in 1886. Later on, Wilhelm Wien found that strong electric or magnetic fields deflected the canal rays and, in 1899, constructed a device with perpendicular electric and magnetic fields that separated the positive rays according to their charge-to-mass ratio (*Q*/*m*). Wien found that the charge-to-mass ratio depends on the nature of the gas in the discharge tube. He deflected a beam of positive ions in electric and magnetic fields. In 1912, **J.J. Thomson** (Father of Mass Spectrometry) built his 'parabola

mass spectrograph' to measure the charge-to-mass ratio (z/m) for several ionic species. He was able to demonstrate the existence of two isotopes of neon, masses 20 and 22, using a magnetic deflection instrument. In the expression z/m, z is the charge number, i.e. the total charge on an ion divided by the elementary charge (e), and m is the nucleon number, i.e. the sum of the total number of protons and neutrons in an atom, molecule or ion. In modern mass spectrometry, the parameter measured is m/z, rather than z/m.

Thomson's student, **Aston** continued the work at Cambridge and built instruments that helped him to establish the presence of isotopes. He was subsequently able to measure the atomic mass of most elements with sufficient accuracy to be able to calculate the 'packing fraction' of their atomic nuclei. The packing fraction is the difference between the accurate atomic mass of the isotope and the nearest whole number divided by the mass number, also known as the mass defect. His work on isotopes also led to his formulation of the **Whole Number Rule** which states that "the mass of the oxygen isotope being defined [as 16], all the other isotopes have masses that are very nearly whole numbers," a rule that was used extensively in the development of nuclear energy.

Aston also obtained accurate measurements of the ratios of the stable isotopes of many of the known elements. At the end of this exciting period of development, Aston was convinced that much of the potential of mass spectrometry had been exploited. It was not until the 1940s that the technique was put to work in elucidating organic structures in the petroleum industry. Ionization was effected by electron 'impact' [now called electron ionization (EI)] for those molecules that could withstand vaporization into the heated and evacuated ion source without decomposition. This limited the practical mass range to less than 1000 Daltons (Da)* but yielded useful fragmentations for structure elucidation (*see* Chapter 2).

By choosing to work with 70 electron volt (eve), many ions were formed with internal energies far in excess of the ionization energy (IE). These ions decompose rapidly to produce lower mass (fragment) ions and neutral radicals or molecules. During the 1950s, commercial instruments were being built and new applications discovered. One of the earliest of these was the identification of low molecular weight volatile food flavor compounds. Ten years later, the powerful combination of electron ionization mass spectrometry (EIMS) with gas chromatography (GC/MS) led to an explosion of applications where mass spectrometry was used in qualitative and quantitative, chemical and biochemical studies. GC/MS instruments produced enormous amounts of data, which were best handled by computers data acquisition methods. In 1966, **Munson and Field** described chemical ionization (CI) technique. This technique increased the yield of ions representative of the molecular weight of volatile molecules through interactions with reagent gas ions (e.g. CH_5^+ ions from methane) with little excess energy. Other 'soft' ionization techniques such as field desorption (FD) and particle desorption methods based upon ion generation by Cf-252 fast fission products [plasma desorption, (PDMS)] were introduced during the 1970s for nonvolatile compounds. At the same time (and in response to these developments) the instrumental mass range was increased to cope with the larger sample molecule ions now entering the gas phase. This process accelerated in the 1980s with the introduction of Fast Atom Bombardment (FAB) ionization. FAB was the first ionization technique to enable biologists and biochemists routinely to obtain molecular weight information on complex, labile biomolecules, including polypeptides and small proteins.

Ionization from the liquid state, followed by evaporation/desolvation of charged droplets, includes techniques such as ion spray, thermospray (TSP) and electrospray ionization (ESI). These methods differed mainly in the manner in which ionization was initiated. Multiple charged molecular ions could be formed under ESI, facilitating the measurement of high molecular masses, even on conventional instruments (i.e. those with a mass range up to 2000 or 4000 The).

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* Dalton (Da) is the unit of mass (also known as the mass unit, u) and is 1/12 of the mass of C (defined as 12.000000).

More efficient pumping systems were required to cope with the increased gas volumes generated by vaporizing liquids.

Separation techniques such as liquid chromatography and capillary electrophoresis coupled to mass spectrometry (LC/MS and CE/MS respectively) have extended the advantages first associated with the analysis of volatile compounds by GC/MS to compounds of low volatility and high molecular weight. Tandem mass spectrometry (MS/MS) collision-induced dissociation (CID), focal-plane array detectors, ion traps and hybrid instruments are providing a high sensitivity structure elucidation facility for nonvolatile compounds similar to that provided by EIMS of volatiles. Recently, laser desorption (LD), and especially matrix assisted laser desorption ionization (MALDI), combined with time-of-flight (ToF) mass analysis has extended the practical application for biomolecules. These techniques require minimum ionization energy (minimum energy of excitation for an atom or molecule) to remove an electron in order to produce a positively charged ion.

Fourier transform mass spectrometry (FTMS), also known as Fourier transform ion cyclotron resonance (FT-CR) mass spectrometry is only slowly entering into the commercial area.

Historical development in mass spectrometry

19th century

1886 Eugen Goldstein observed canal rays.

1898 Wilhelm Wien demonstrated that canal rays can be deflected using strong electric and magnetic fields. He showed that the mass-to-charge ratio of the particles has opposite polarity and is much larger compared to the electron. He also realized that the particle mass is similar to the one of hydrogen particles.

1898 J.J. Thomson measured the mass-to-charge ratio of electrons.

20th century

1901 Walter Kaufmann used a mass spectrometer to measure the relativistic mass increase of electrons.

1905 JJ Thomson began his study of positive rays.

1906 Thomson was awarded the Nobel Prize in Physics “in recognition of the great merits of his theoretical and experimental investigations on the conduction of electricity by gases”.

1913 Thomson was able to separate particles of different mass-to-charge ratios. He separates the ^{20}Ne and the ^{22}Ne isotopes, and he correctly identifies the m/z = 11 signal as a doubly charged ^{22}Ne particle.

1919 Francis Aston constructed the first velocity focusing mass spectrograph with mass resolving power of 130.

1922 Aston was awarded the Nobel Prize in chemistry “for his discovery, by means of his mass spectrograph, of isotopes, in a large number of non-radioactive elements, and for his enunciation of the whole-number rule”.

1931 Ernest O. Lawrence invented the cyclotron.

1934 Josef Mattauch and Richard Herzog developed the double-focusing mass spectrograph.

1936 Arthur J. Dempster developed the spark ionization source.

1937 Aston constructed a mass spectrograph with resolving power of 2000.

1939 Lawrence received the Nobel Prize in Physics for the cyclotron.

1942 Lawrence developed the Calutron for uranium isotope separation.

1946 William Stephens presented the concept of a time-of-flight mass spectrometer.

1956 Fred McLafferty proposed a hydrogen transfer reaction that is known as the McLafferty rearrangement.

1959 Researchers at Dow Chemicals developed an interface to couple gas chromatograph to a mass spectrometer.

1966 FH Field and MSB Munson developed chemical ionization.

1968 Malcolm Dole developed electrospray ionization.

1969 HD Beckey developed field desorption.
1974 Comisarow and Marshall developed Fourier Transform Ion Cyclotron Resonance Mass Spectrometry.
1976 Ronald MacFarlane and co-workers developed plasma desorption mass spectrometry.
1984 John Bennett Fenn and co-workers used electrospray technique to ionize biomolecules.
1985 Franz Hillenkamp, Michael Karas and co-workers described and coined the term matrix-assisted laser desorption ionization (MALDI).
1987 Koichi Tanaka used the "ultra fine metal plus liquid matrix method" to ionize intact proteins.
1989 Wolfgang Paul received the Nobel Prize in Physics "for the development of the ion trap technique".
1999 Alexander Makarov presented the Orbitrap mass spectrometer.

21st century

2002 John Bennett Fenn and Koichi Tanaka were awarded one-quarter of the Nobel Prize in chemistry each "for the development of soft desorption ionisation methods ... for mass spectrometric analyses of biological macromolecules".

APPLICATIONS OF MASS SPECTROMETRY

1. Identification of unknown compounds

From mass spectra of any compound, we can get the information about:

- Molecular weight
- Structural characteristics
- Elemental composition of molecular ion and fragment ions

2. Monitoring of chemical reactions

During the last several decades, mass spectrometry (MS) has rapidly developed as a practical technique that can be used to monitor chemical reactions and investigate reaction mechanisms (Fig. 1.3). The real-time discovery of intermediates and products provides critical information regarding the reaction mechanism, which can facilitate the optimization of reaction conditions. With appropriate ionization methods, most chemical compounds can be ionized and detected with MS hybrid systems. Reactions can be monitored either by observing the disappearance of the reactants or appearance of the products in the mass spectrum.

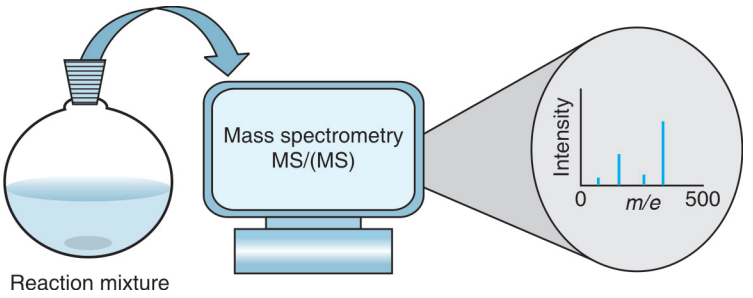

Fig. 1.3: Monitoring of chemical reactions

3. Determination of sequence of amino acids in peptide chain

There are three different types of bonds that can fragment along the amino acid backbone: the **NH–CH**, **CH–CO**, and **CO–NH** bonds (Fig. 1.4). Each bond breakage gives rise to two species, one neutral and the other one charged, and only the charged species is monitored by the mass spectrometer. The charge can stay on either of the two fragments depending on the chemistry and relative proton affinity of the two species. Hence, there are six possible fragment ions for

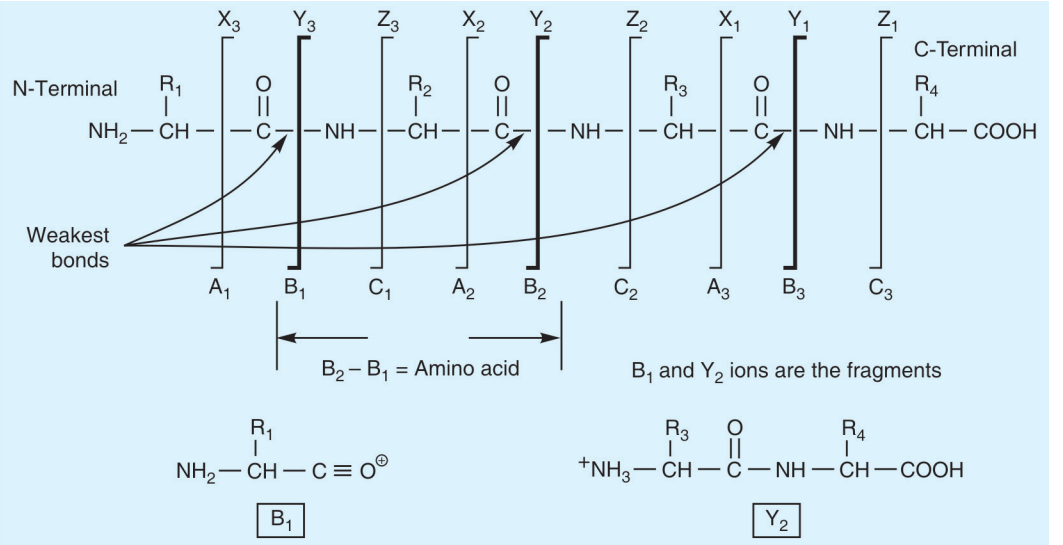

Fig. 1.4: Sequence of amino acids in peptide chain

each amino acid residue and these are labelled as in the diagram, with the **A**, **B**, and **C ions** having the charge retained on the **N-terminal fragment**, and the **X**, **Y**, and **Z ions** having the charge retained on the **C-terminal fragment**. The most common cleavage sites are at the CO–NH bonds which give rise to the B and/or the Y ions. The mass difference between two adjacent B ions, or Y ions, is indicative of a particular amino acid residue. Software are also available to determine the peptide structure (example: SEQUEST).

4. Determination of oligosaccharide structure

Structural elucidation of complex carbohydrates requires determination of monosaccharide composition, sequence, branching pattern, glycosidic linkages, and anomeric configuration (Fig. 1.5). One of the efficient methods for the derivatization of oligosaccharides, wherein the oligosaccharide is efficiently ligated to a basic aminooxyacetyl peptide by oxime formation. The resulting glycopeptide yields much higher sensitivity in matrix-assisted laser desorption/ionization mass spectrometry than does the underivatized oligosaccharide.

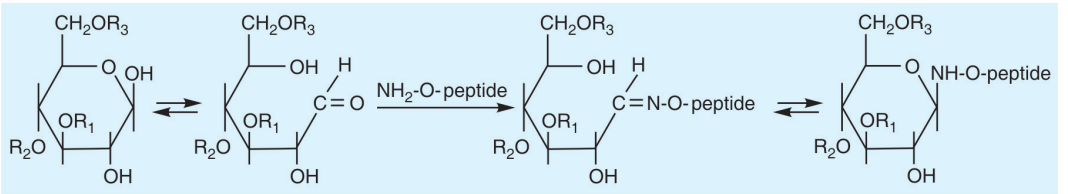

Fig. 1.5: Sequence of oligosaccharide in carbohydrate

5. Mass spectrometry in pesticides analysis

- Pesticides are indispensable chemicals and poisonous to mankind—adversely affects nerve functioning, direct exposure can cause eye problems like blurring of vision, reddening, retardation in fetal growth, etc.
- Residual analysis in food, water and environment samples is of paramount importance from viewpoint of preventive medicine.

- Frequently used pesticides are organophosphorous compounds (OP) and carbamate derivatives.
- GC-MS can determine meconium, cypermethrin, malathion, cyfluthin, etc. in the concentration of 0.01–4.15 µg/g.
- For example, in the mass spectrometry, peak at *m/e* 157 and 125 (Fig. 1.6) is focussed to detect the presence of malathion.

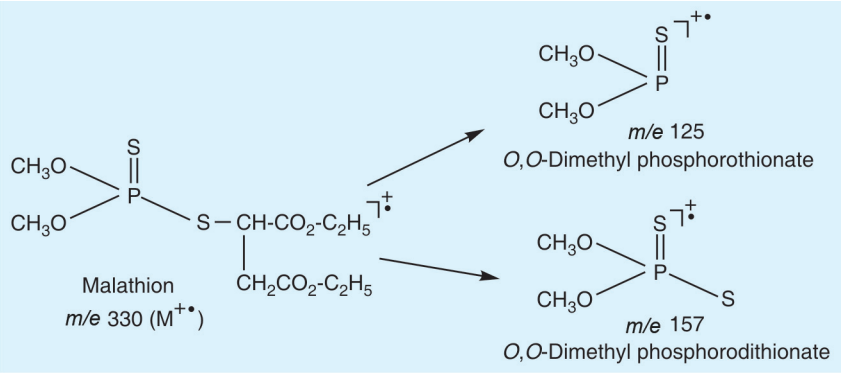

Fig. 1.6: Pesticide analysis by mass spectrometry

6. Mass spectrometry in determination of drug of abuse

Cocaine is a carboxylic acid ester that is rapidly hydrolyzed *in vivo* to the free acid benzoyl ecgonine (BEG) and detection of BEG is used to monitor cocaine abuse.

BEG itself has poor vapor phase properties because of the polar, ionizable carboxylate moiety, and it is therefore converted to a carboxylic acid ester, in this example, the pentafluoropropyl (PFP) ester, before GC/MS analysis. The molecular ion of PFP-BEG is *m/e* 421, and two characteristic fragment ions of *m/e* 316 and *m/e* 300 that arise from cleavages about an oxygen atom of the ester (Fig. 1.7). A ring-labeled [$^2H_3$]-BEG internal standard is added before extraction, processed along with the target analyte, and also derivatized (d_3-PFP-BEG). The ions in the mass spectrum of d_3-PFP-BEG analogous to those in the spectrum of PFP-BEG are *m/e* 424, 319, and 303, and in each case retain all three [^{2}H] atoms. GC/MS is then performed with selected ion monitoring of the ion pairs *m/e* 316 and 319, *m/e* 300 and 303, and *m/e* 421 and 424.

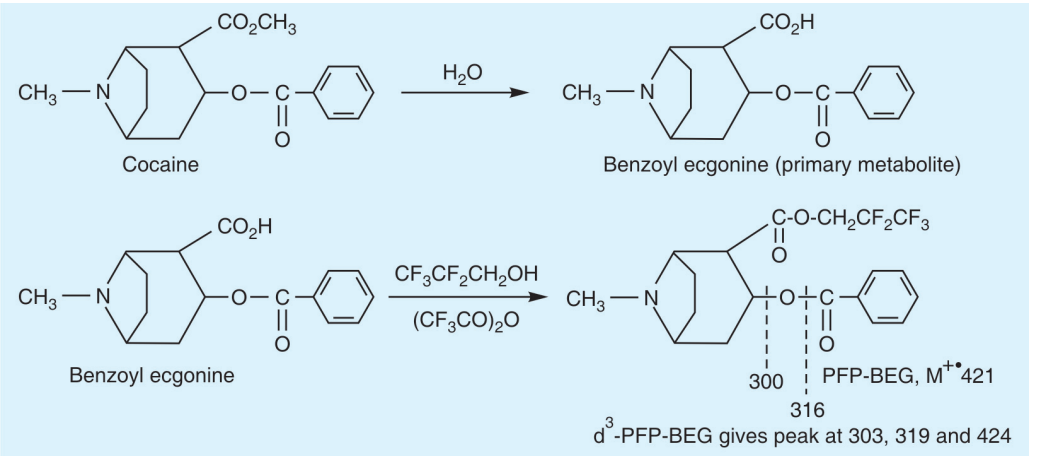

Fig. 1.7: Analysis of cocaine by mass spectrometry

7. Determination of adulteration of honey

Bee honey is a unique sweetening agent that can be used by humans without processing, and it provides significant nutritious and medical benefits. Because of its nutritional and medicinal value, honey continues to be a popular food. However, honey can easily be adulterated with various cheaper sweeteners, such as refined cane sugar, beet sugar, high fructose corn syrup and maltose syrup, resulting in higher commercial profits.

Isotopic ratio mass spectrometry (IRMS) is helpful in determination of adulteration.

Plants with the Calvin-Benson photosynthetic cycle (C3) (example: Beet, wheat) have $^{13}C/^{12}C = \delta‰$ values from –21‰ to –32‰ and plants with the Hatch-Slack photosynthetic cycle (C4) (example: Corn, sugarcane) have values from –12‰ to –19‰ of $^{13}C/^{12}C = \delta‰$; C4 plants have high ^{13}C when compared to C3 plants (Calvin and Bassham, 1962, Hatch and Slack, 1979 and Hatch et al., 1967). Honey that has $\delta^{13}C$ values less negative than –23.5‰ is considered suspect.

8. Determination of metabolic disorders

Maple syrup urine disease (MSUD), also called branched-chain ketoaciduria, is an autosomal recessive metabolic disorder affecting branched-chain amino acids. The condition gets its name from the distinctive sweet odor of affected infants' urine, particularly prior to diagnosis, and during times of acute illness.

MSUD is a metabolic disorder caused by a deficiency of the branched-chain alpha-keto acid dehydrogenase complex (BCKDC), leading to a buildup of the branched-chain amino acids (leucine, isoleucine, and valine) and their toxic by-products (keto acids) in the blood and urine.

Newborn screening for maple syrup urine disease involves analyzing the blood of 1–2 day-old newborns through tandem mass spectrometry. The blood concentration of leucine and isoleucine is measured relative to other amino acids to determine if the newborn has a high level of branched-chain amino acids.

9. Mass spectrometry in drug metabolic studies

Mass spectrometry plays a pivotal role in drug metabolism studies, which are an integral part of drug discovery and development nowadays. Metabolite identification is helpful in understanding the metabolic fate of drug candidates and to aid lead optimization with improved metabolic stability, toxicology and efficacy profiles. The mass spectrum of buspirone (antipsychotic drug) is shown in Fig. 1.8. The peak at *m/e* 122 can be monitored to know the metabolism of buspirone.

10. Mass spectrometry in anti-doping analysis

Anti-doping analysis is a very peculiar area of forensic toxicology, aimed at detecting the abuse of prohibited substances and methods by the athletes. These analyses are carried out at an international level by 33 anti-doping laboratories accredited by the World Anti-Doping Agency (WADA), managing an overall workload of more than 220,000 samples per year. Mass spectrometry is very sensitive technique to detect the steroidal substances taken for anabolic purpose.

11. Determination of age of sample

Isotopic mass spectrometry is used to determine the isotopic ratio. Differences in mass among isotopes of an element are very small, and the less abundant isotopes of an element are typically very rare, so a very sensitive instrument is required. The most sensitive and accurate mass spectrometer for this purpose is the accelerator mass spectrometer (AMS). Some isotope ratios are used to determine the age of materials, for example, as in carbon dating. Determination of C-14 is used to determine the age of sample.

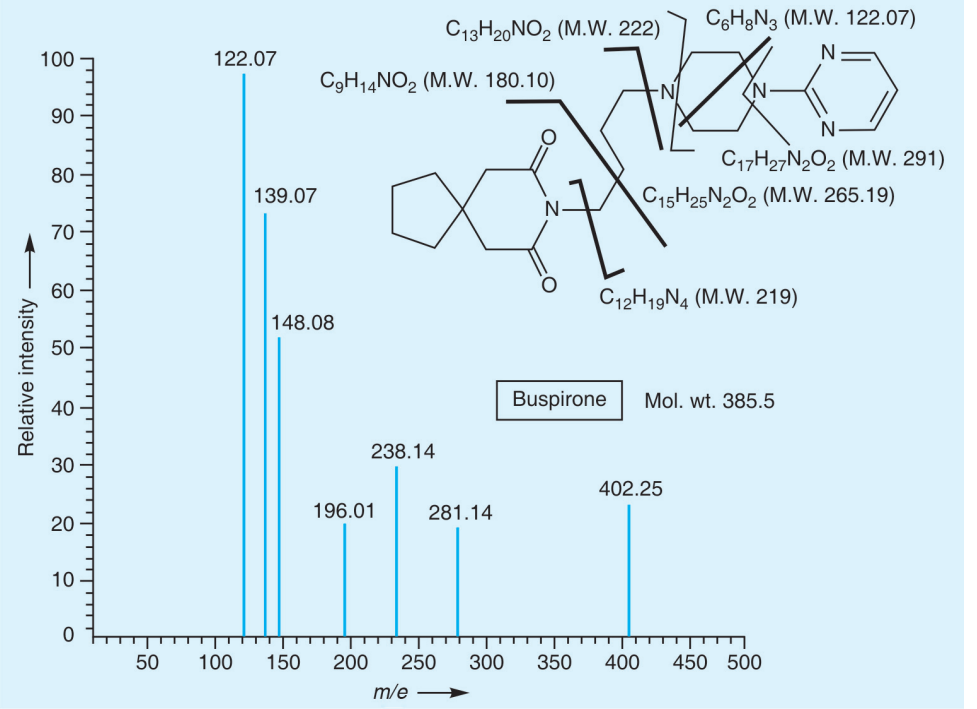

Fig. 1.8: Mass spectrum of buspirone with its metabolite

12. Monitoring of anesthesia

Anesthesia adequacy was assessed with mass-spectrometric method by monitoring the ratio of mass concentrations of end-tidal CO_2 and inhaled O_2 in every respiratory cycle during surgery. For real-time monitoring, we used a mass spectrometer with electron ionization connected to the respiratory contour of inhalation anesthesia machine. The study has demonstrated advantages of the novel method in real-time assessment of adequacy of the total intravenous anesthesia.

13. Mass spectrometry in fermentation industry

Ethanol produced by yeast fermentation, called bioethanol, accounts for approximately 95% of the ethanol production. Ethanol is produced from various kinds of substrates. The substrate used for ethanol production is chosen based on the regional availability and economical efficiency.

They monitor the composition of gas streams into and out of fermentors and bioreactors continuously, accurately and reliably. Ethanol in the vent gas is linearly related to the concentration in the fermentor broth they give a continuous monitor of the ethanol production which is particularly important for detecting the start of ethanol production and also for monitoring changes in ethanol production.

Although the molecular weight of ethanol is 46 it can be seen that the molecular ion ($CH_3CH_2OH^+$) peak at mass 46 is not the largest peak, in fact it is not even the second largest peak. The ethanol molecule tends to fragment during ionization and the largest peak is actually at mass 31 due to CH_2OH^+. Also there is considerable interference from the CO_2 in the vent gas at masses 45 and 46, due to the 13C, 17O and 18O isotopes. Therefore, we have to use mass 31 to analyze ethanol (Fig. 1.9). However, we need to consider the presence of a very large peak at mass 32 from the percentage levels of O_2 in the vent gas. We need to correct for the tail from the 32 peak if we are to make an accurate measurement of ethanol at low concentrations (ppm) at the start of ethanol production.

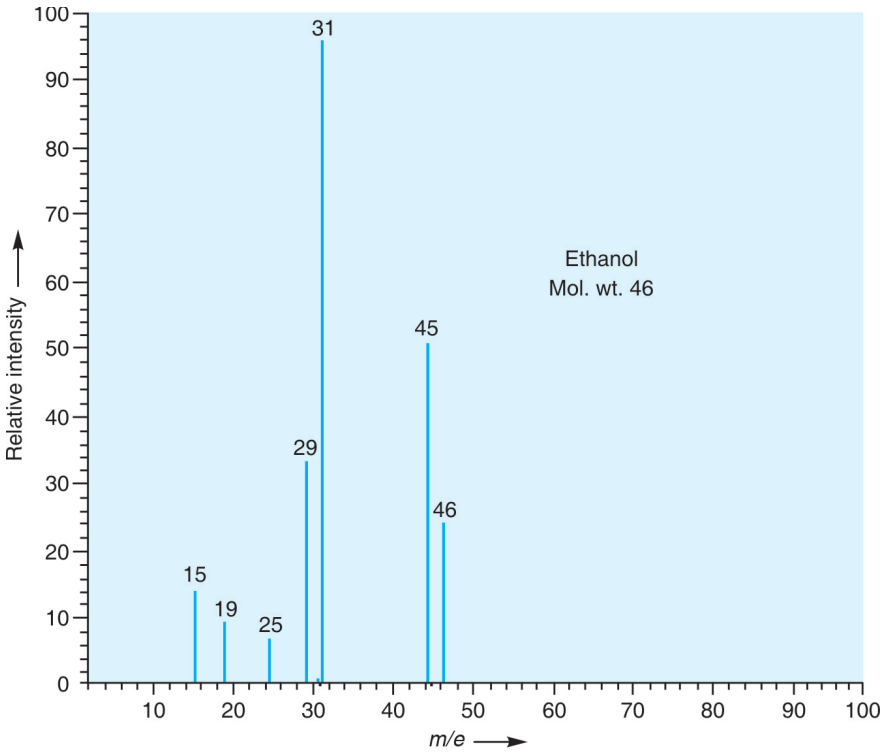

Fig. 1.9: Mass spectrum of ethanol

14. Determination of composition of crude oil (geological importance)

Crude oil is a natural multicomponent mixture. Its major part is composed of hydrocarbons (alkanes, naphthenes, and aromatics). Their content in oils ranges between 30% and 100%. The emergence of analytical techniques such as the gas chromato-mass spectrometry enabled scientists to obtain new information on the composition and structure of petroleum hydrocarbons, study in detail their homological series, and determine the distribution patterns of normal and branched alkanes, methylalkanes, and isoprenoid alkanes in oils.

15. Detection of dioxins by mass spectrometry

Dioxins are not created intentionally, but they can be produced through industrial processes, including combustion, chlorine bleaching of pulp and paper and through certain types of chemical manufacturing and processing. These are toxic, effective in low concentration and cause cancer in human beings.

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) constitute a group of polyhalogenated aromatic compounds that have become known as “dioxin” and “dioxin-like” compounds.

Gas chromatography (GC) with high resolution mass spectrometry (HRMS) is a sensitive method of detection for these compounds.

16. Mass spectrometry in environmental monitoring

Monitoring environmental pollutants is a major application of GC-MS. It is widely used in the detection of dibenzofurans, dioxins, herbicides, sulfur, pesticides, phenols, and chlorophenols in air, soil, and water.

17. Food and fragrance analysis

Aromatic compounds such as fatty acids, esters, aldehydes, alcohols, and terpenes present in food and beverages can be easily analyzed using GC-MS. The technique can also be used to detect the spoilage or contamination of food. The analysis of a wide range of oils such as lavender oil, olive oil, spearmint oil, and essential oils, perfumes, fragrances, allergens, menthol, and syrups is also possible using GC-MS.

18. Cancer diagnosis by mass spectrometry

Why to use mass spectrometry for cancer diagnosis?

The big reason is that mass spectrometry can in theory conclusively prove the presence, identify, and report the concentration of all of the small peptides and proteins in an unknown sample, such as blood. No other diagnostic protocol can do this.

Why is this important?

Cancer is often diagnosed by an abnormal amount of a particular peptide or protein in blood. These may act as **Cancer biomarkers**. These biomarkers may be (i) a mediator of the disease pathology, (ii) present at low and stable expression levels in healthy individuals and higher expression levels in patients, and (iii) simple and quick to evaluate. Such a biomarker can be assayed and linked to cancer using a defined mechanism. For example, the prostate cancer can be diagnosed by determining Zn-alpha $(\alpha)_2$ glycoprotein in serum by LC-MS/MS technique.

The mass spectrometric technique has additional advantage. Traditional cancer diagnoses are specific to one type of cancer. In theory, mass spectrometry can instead be used as a universal cancer screening.

19. Miscellaneous

Mass spectrometric techniques can be used for:

(a) Determination of gene damage due to environmental causes. DNA-damaging agents generate a plethora of products in the DNA of living organisms. The DNA damage can lead to numerous diseases including carcinogenesis. Both gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS), in single or tandem versions, have been used for the measurement of numerous DNA damage resulting products.
(b) Location of petroleum deposit by testing rock samples.
(c) Testing the purity of semiconductor material used in making microchips for computers.