CHAPTER 6

Mass Spectrometry

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6.1 INTRODUCTION

Mass spectrometry is a technique which complements infrared, ultraviolet, and nuclear magnetic resonance spectroscopy and gas chromatography. It gives information about the structural groups which make up the molecule. The spectrum relates the apparent mass of a fragment of the molecule to its relative intensity based on the strongest peak in the spectrum (Fig. 6.1).

In 1886, Goldstein[9] observed streams of luminous gas in back of a perforated cathode in a discharge tube. The luminosity was due to the passage

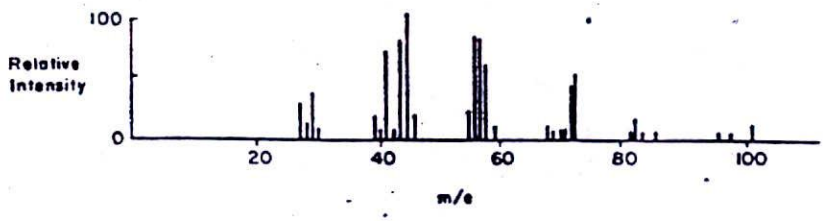


FIGURE 6.1: Idealized mass spectrum.

of ionized particles in the gas. It was found that these particles were positively charged and that they could be deflected by electric or magnetic fields. Since they could be deflected by electric and magnetic fields, it was possible to determine velocity and the charge to mass ratio (e/m) for the particles in the stream.

An early method of positive ray analysis was J. J. Thomson's parabola method.[23] Electric and magnetic fields were aligned parallel to the ray, causing deflections perpendicular to one another (Fig. 6.2). The relationships between the magnetic and electric fields and the ionized particle are basic to mass spectroscopy.

If a uniform electric field R_E is applied to a single positively charged particle moving downward perpendicularly to the plane of the paper toward point 0, (Fig. 6.3), the deflection of the particle can be to the right or left along the x axis, depending on the polarity of the field. The distance the particle is deflected ob is given by:

$$x = k_1 \frac{R_E e}{m V^2} \tag{6.1}$$

where k_1 is a constant depending on the apparatus, e is the charge, m is the mass, and V is the velocity of the particle. Equation (6.1) may be rearranged to give:

$$V^2 = \frac{k_1 R_E e}{mx} \tag{6.2}$$

Now with the electric field off, if the magnetic field (R_H) is applied to deflect

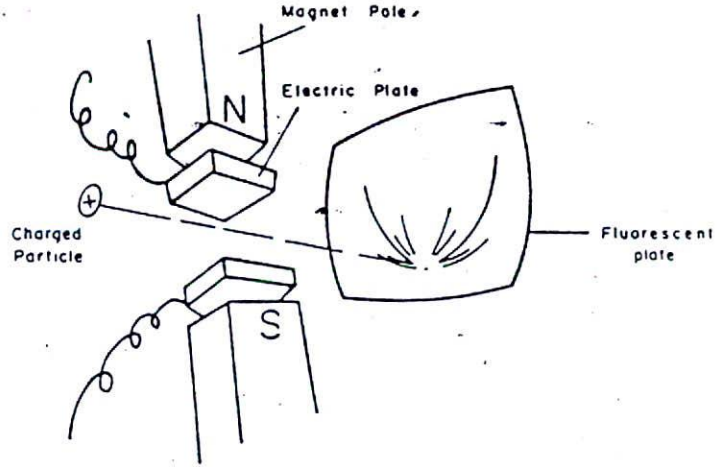


FIGURE 6.2: Positive ray apparatus.

the particle along the y axis to a, the deflection oa is given by:

$$y = \frac{k_2 R_H e}{mV} \tag{6.3}$$

where k_2 also depends on the apparatus. Again rearranging and squaring,

$$V^2 = \left(\frac{k_2 R_H e}{my}\right)^2 \tag{6.4}$$

When both fields are applied simultaneously, the particle is deflected to c or d. Under these conditions, $V_1^2 = V_2^2$, so

$$\frac{k_1 R_E e}{mx} = \frac{k_2^2 R_H^2 e^2}{m^2 y^2}$$

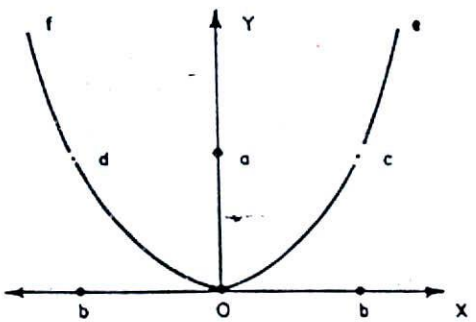


FIGURE 6.3: Path of ionized particle in electric and magnetic fields.

Rearranging,

$$\frac{y^2}{x} = \frac{k_2^2 R_H^2 e^2 m}{k_1 R_E e m^2} = \frac{k_3 R_H^2 e}{R_E m} \tag{6.5}$$

If now we have a *beam* of particles with the same e/m ratio and hold R_H and R_E constant, then y^2/x is a constant, which is the equation of a parabola. If the particles all had the same velocity, they would appear as a spot on the parabola. Since a real beam of ionized particles has a range of velocities, a definite parabola is obtained (Fig. 6.3). Thomson used a photographic plate

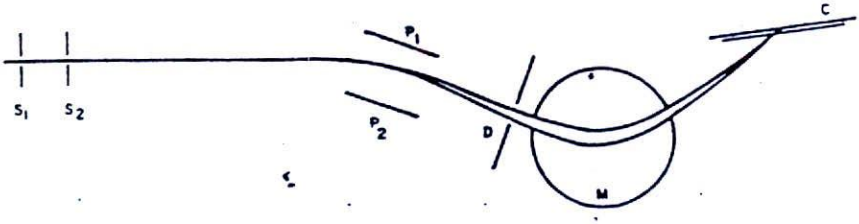


FIGURE 6.4: Aston's mass spectrograph.

to record the positive rays and measure the constants. When the discharge tube contained hydrogen, the e/m value was found to be the same as that found for the hydrogen ion in electrolysis. At the same time, other parabolas were observed at e/m 1/2, 1/12, 1/16, 1/28, and 1/44 which were interpreted as due to hydrogen molecule, carbon atom, oxygen, carbon monoxide, and carbon dioxide. Parabolas were found for any gas introduced into the discharge. When neon ($m = 20$) was studied, a faint line at $m = 22$ was also observed. This was the $m = 22$ isotope of neon. Its discovery marked our first awareness of isotopes.

A. ASTON'S MASS SPECTROGRAPH

A more sensitive type of equipment was developed by Aston.[1] While the magnetic and electric fields in Thomson's positive-ray apparatus produced deflections in planes at right angles, in Aston's arrangement (Fig. 6.4) they were in the same plane, but in opposite directions. The positively charged particles first pass through two very narrow parallel slits S_1 and S_2 and the resulting fine beam is spread out by means of an electric field applied across plates P_1 and P_2. A section of the beam emerges through another small slit at D and then passes through a magnetic field M. This field is so arranged as to bend the rays in the opposite direction to that of the electric field, and bring them to a sharp focus on the photographic collector C.

Aston did not determine the absolute value of e/m from his mass spectrographic data. He took the value of 16.000 for the oxygen isotope $m = 16$

and evaluated all other masses in terms of this. Since there is a significant amount of other oxygen isotopes present in normal oxygen, this led to a small but observable difference between chemical atomic weights based on atmospheric oxygen, a mixture, as 16.000. This difference was resolved in 1961 by an international congress, which took the mass of carbon as 12.000 and compared masses from that basis.

B. DIRECTION FOCUSING

A different principle for separating the fragments was used by Dempster.[3] The positive particles were accelerated by passage through an electric field. When a high potential was used, the particles had uniform kinetic energy. If V is the accelerating potential and e the charge on the particle, the energy is Ve. Kinetic energy of the particle, $1/2\ mv^2$, where m and v are mass and mass and velocity, respectively, is then equal to Ve.

$$Ve = 1/2\ mv^2 \tag{6.6}$$

A thin beam of accelerated ions was directed through a narrow slit into a magnetic field which bends the path of the beam into a semicircular path. By means of a second slit, the beam was directed onto a plate connected to a device for measuring ion currents.

The radius of curvature r of the path of a charged particle moving in a magnetic field H is determined by:

$$e/m = \frac{v}{Hr} \tag{6.7}$$

So, combining Eq. (6.6) and (6.7)

$$e/m = \frac{2V}{H^2r^2} \tag{6.8}$$

Only particles with a definite value of r can pass the second slit and register on the electrometer. The e/m values for the particles having a path of this radius is determined by the accelerating potential V and the strength of the magnetic field H.

Recent developments in the design of mass spectrometers utilize this double focus to extend their usefulness. The ions formed initially are brought to a focus at a slit designed to pass ions with only a particular *mass*. These then are further separated in the magnetic field into beams with a definite mass-to-charge ratio. This leads to extremely high resolution and enables the operator to use a scale linear with respect to mass. The linear mass scale enables chemists to interpret spectra directly in terms of mass (m/e), which is much more readily visualized than the e/m scale which was used to investigate the basic phenomena. Figure 6.5 gives the basic configuration of a modern mass spectrograph.

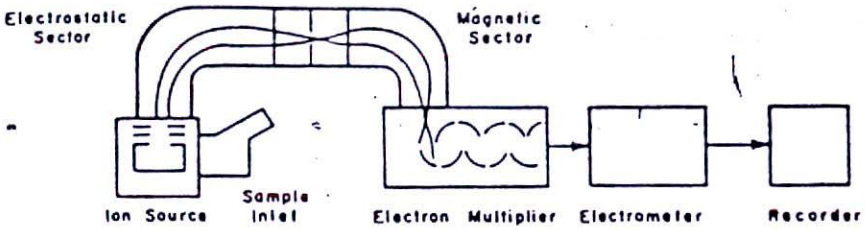


FIGURE 6.5: Modern mass spectrograph (block diagram).

6.2 INSTRUMENTATION

The major function of the mass spectrometer is to provide means to dissociate the target molecule and ionize the fragments, to separate the ionized fragments according to their mass, and to provide a detector to measure the relative abundance of each ion type according to its *m/e* ratio.

A. ION FORMATION

In the ionization chamber, the molecules of the sample are exposed to bombardment with energetic electrons. Below the ionizing potential of the molecule, usually about 10 V, there is no response. At the ionizing potential, an electron is removed from the molecule to form the parent ion, which is the positively charged molecular ion. At still higher applied potentials, enough energy is supplied that one or more bonds in the molecule are broken. The fragments may be sufficiently stable that they proceed through to the detector, or they may react with other fragments or may decompose. The actual mechanism of ion formation is not well understood; much danger of misinformation arises by unwarily accepting the presence of a fragment in a spectrum on the basis of its mass number.

In some spectra, peaks are present which can best be interpreted as if the molecule had lost *two* electrons. The peak appears at exactly *half* the mass of the fragment.

When the ion fragment decomposes in flight, the resulting metastable ions have less than full kinetic energy, so the apparent mass is less than the true mass. The apparent mass M_a is related to the masses of the original ion M_0 and the daughter ion M_d by the equation

$$M_a = \frac{(M_d)^2}{M_0}$$

One fragment of the decomposed particle retains the negative charge. It is possible to modify instruments to study this negative ion, but intensities are usually low, so most work has been done with the positive ion. Certain halogenated compounds are satisfactorily studied as negative ions.

The chief limitation of mass spectrometer is the necessity for having the sample in the vapor phase. Heating the injection port is normal procedure, but this frequently brings about thermal degradation of the compound. Large molecules can only be studied if information can be gained from the more volatile breakdown products.

B. ION SEPARATION

The positively charged molecular fragment is subjected to an accelerating potential and passed through collimating slits into a magnetic field, which

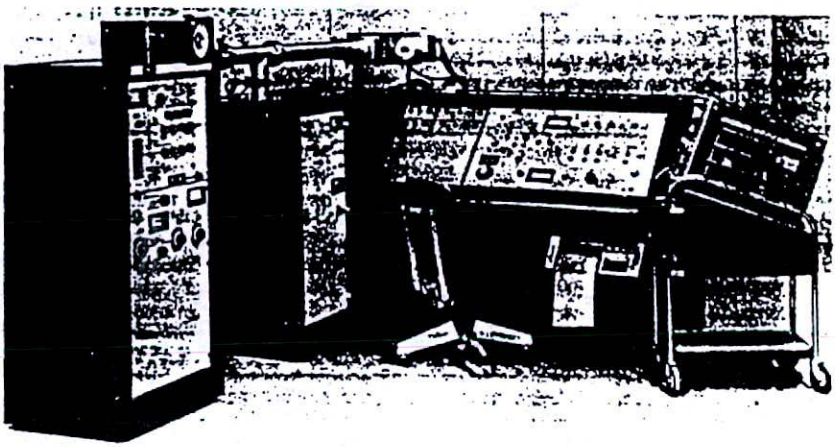


PLATE 6.I: Bendix time-of-flight mass spectrometer. Courtesy Bendix Corporation.

sorts the fragments into groups with the same *m/e* ratio. With high resolution instruments, species of the same mass, but differing in elemental composition and therefore in fractional mass, can be resolved. A precision of 1 in 100,000 is claimed.

A time-of-flight instrument resolves the ion beam by measuring the flight time of the ions of each mass. A pulse of ions is formed by bombarding the sample molecules with a short burst of ionizing electrons. In rapid sequence the ionizing electrons are turned off and an accelerating pulse pulls the ion into high speed acceleration, which then passes into a field-free "drift" tube. All ions receive the same kinetic energy, and so those with heavier mass move more slowly. The ions are collected by an electron multiplier with the extremely fast response needed to record a complete spectrum in 100 μsec. The simplest instrument to serve as a recording system is an oscilloscope (see Plate 6.I), which is very useful in monitoring the fast-changing effluent from a gas chromatograph.

C. ION DETECTION[23]

Photographic detection was used originally, but development is tedious, and the precision of densitometry is not great. An electrometer or electron multiplier is nearly always used with modern instruments. Electronic amplification of the ion current allows an oscilloscope, a pen and ink recorder, or a digital computer to be used. High resolution instruments again use photographic detection, but the resolution rather than the intensity is the feature of interest.

The instrument must cover a mass range at least beyond the molecular weight of the compound to be investigated, and the resolving power should be such that the species differing by 1 mass unit in the region of the molecular weight can be distinguished. The advantages of mass spectrometry are that a very small sample is needed—not more than a few micrograms—and the accuracy of analyses is within a few tenths of 1%.

6.3 INTERPRETATION OF SPECTRA

Mass spectrometry can give information at several levels of complexity. The evaluation of rearrangements and bond fissions that have caused each peak in a spectrum is a concentrated effort in advanced research. Such information is then applied to elucidation of the structure of complex molecules. Mass spectral data are used to determine relative quantities of known materials in a mixture. This ability has wide applications in industrial work. The gross pattern can give qualitative identification of known substances, and is an excellent check for absence of a suspected material. Both infrared and ultraviolet examination are often inadequate for this purpose. Gas chromatography is an excellent technique for separating compounds, but a single peak does not mean the compound is pure and so is unreliable for identification of a material. When gas chromatography and mass spectrometry are combined, a powerful analytical tool results.

Now realize that ions in a mass spectrograph can appear through several processes. *First*, a single electron can be removed with no other bond being affected. This gives a positive ion with mass equal to the molecular weight, referred to as the *parent* ion. While some researchers use the symbol P, this peak is referred to here as the M peak. The strongest peak in the spectrum is referred to as the base peak and intensities of all other peaks are expressed as per cent of the base (Fig. 6.6).

Second, a single bond can be split:

$$R - CH_3 - R + CH_3^+$$

This type of cleavage gives rise to most of the principle fragments. More usually when the energy is high enough to disrupt *one* bond, the molecule

will split in several places and the pattern must be interpreted in terms of the possible fragments. Relative quantities of these ions indicate the strength of the bonds uniting them to the rest of the molecule.

Third, the incident energy can cause cleavage with rearrangement of a hydrogen atom or larger group.

Fourth, two electrons may be lost, giving rise to an ion of apparent *m/e* one-half the true value. These four effects can explain most of the major peaks in a spectrum.

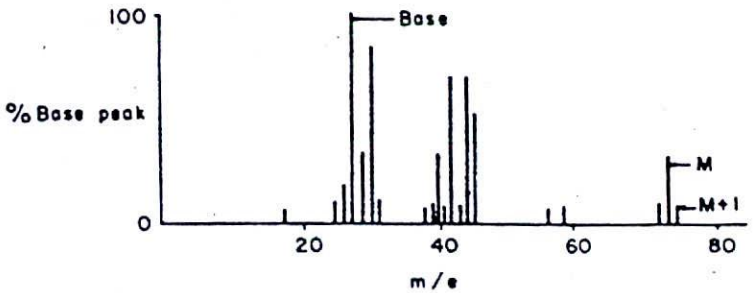


FIGURE 6.6: Idealized mass spectrum.

The greatest aid to qualitative analysis is a large library of mass spectra which have been coded for information retrieval. Most laboratories have collected spectra of the compounds and compound types in which they are interested. Study of the available patterns yields many generalizations regarding the particular compound under study.[17] Libraries of spectra of common materials are also commercially available.

One seldom begins with a sample of completely unknown origin. It may be an impurity isolated from a raw material, a single spot isolated from a thin-layer chromatogram, or a compound isolated by an organic chemist from a reaction. Any of these suggest related compounds, potentially helpful in interpretation of your sample. If this still has not solved the problem, a rough approach is to proceed as follows:

Establish the parent peak ($m/e = M$) using a low voltage spectrum and note the fragment peaks that are produced. Measure the $M + 1$ and $M + 2$ peaks carefully. These are isotopic ion peaks which can give information on the number of carbons, nitrogens, and oxygen atoms. The $M + 1$ peak is due to the 1.1 % of ^{13}C in normal carbon, plus the 0.4 % ^{15}N in normal nitrogen. Silicon and sulfur also contribute significantly to the $M + 1$ peak if they are present. The $M + 2$ peak arises from the ^{18}O isotope of oxygen, and the contribution of silica and sulfur. The halogens chlorine and bromine both have isotopes 2 mass units apart; their relative abundance, 3:1 for chlorine and 1:1 for bromine, give relative intensities for M and $M + 2$ peaks

which are quite characteristic and once observed are easily remembered (Table 6.1).

In normal spectra, the parent ion is frequently observed not at all, or in only trace amounts, so that assignment of a parent is frequently difficult. Usually interpretation is begun with a reasonable guess, which must then be justified.

Now using this information, calculate roughly the number of carbons and other atoms in the molecule. Next, examine the first major peaks lower than the parent and using background knowledge relate the difference to loss of a particular fragment. To do this more satisfactorily, some generalizations have been gathered to help.

TABLE 6.1: Natural Abundance of Heavy Isotopes

Isotope	Molecular ion	$M+1$	$M+2$
^{13}C	1	0.011	
^{15}N	1	0.004	
^{18}O	1	0.0004	0.002
^{33}S	1	0.008	0.04
^{37}Cl	1		0.33
^{79}Br	1		1

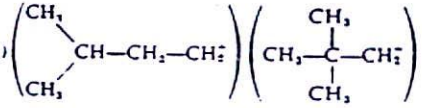
A. ALIPHATIC HYDROCARBONS[4,13,18]

Usually the most intense ion of an aliphatic hydrocarbon occurs in the C_2 to C_6 mass range. These ions appear at masses

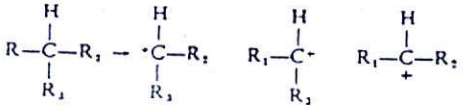
29 $(CH_3—CH_2^+)$

43 $(CH_3—CH_2—CH_2^+)(CH_3—CH^+—CH_3)$

57 $CH_3—(CH_2)_2^+$ 

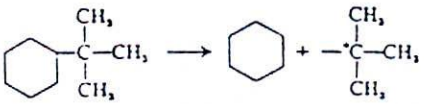
71 $(CH_3—(CH_2)_2—CH_2^+)$ 

Cleavage is easier at bonds around a substituted carbon, for example,



B. CYCLOPARAFFINS

Compounds containing cyclohexyl rings give fragments at *m/e* 83, 82, and 81, corresponding to the ring, and the ring with one and two hydrogens removed. Alkyl substituents are split off and also form a positive ion.



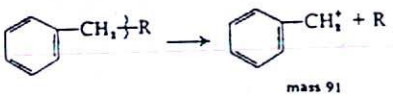
C. MONO OLEFINS

Compounds with one double bond tend to rupture at the carbon-carbon bond beta to the double bond. The fragment with the double bond usually loses the electron to become a positive ion.

$$H_2C{=}CH{-}CH_2{-}R \rightarrow H_2C{=}CH{-}CH_2^{+} + R$$

D. ALKYL BENZENES[13,15]

Meyerson has studied the dissociation in alkylbenzenes. He reported the principle ion usually results from cleavage of bonds beta to the ring. The

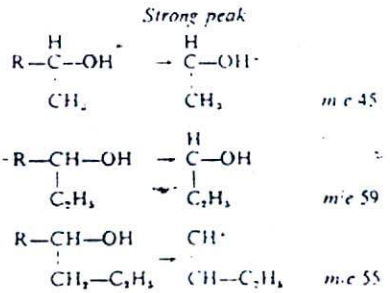


phenyl-group ion, mass 77, is found in the intensity range 1 to 14%.

E. ALIPHATIC ALCOHOLS[6]

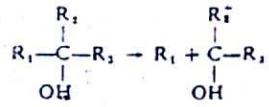
Primary alcohols R—CH_2OH cleave to give water ($M - 18$) and CH_2OH^+ ($m/e = 31$). The molecular ion R—CH_2—OH^+ is important in lower molecular weight alcohols.

Secondary alcohols can be divided depending on the location of the hydroxyl on the 2, 3, or 4 carbon atom.



The peak at *m/e* 31 is also strong.

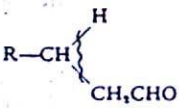
Tertiary alcohols split off the largest alkyl group to form the base peak.



F. ALDEHYDES[7]

Aldehydes give more predictable spectra than alcohols. The parent ion is found even in higher molecular weight compounds. Cleavage is at either the

α or β bond. β-Bond cleavage gives a fragment *m/e* 44 which is the base peak in straight-chain aldehydes and a fragment equal to molecular weight less 44. This is characteristic for identification work.



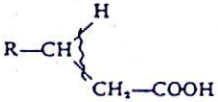
G. KETONES

A study by Sharkey et al.[19] showed most of the compounds they studied split off the larger of the alkyl groups of the ketone. The fragment (*m/e* = 43)

formed by rearrangement of ions is frequently a base peak or at least a major one.

H. ACIDS[11]

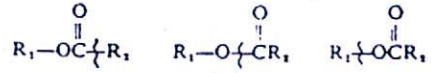
The base peak in almost all acids is attributed to a fragment (*m/e* 60) formed as follows:



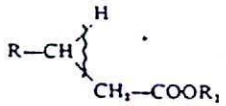
The expected COOH fragment (*m/e* 45) is also common. When strong peaks are observed at *m/e* 60 and *m/e* (M − 45), an organic acid can be expected.

I. ESTERS[2,20]

Esters tend to dissociate at the valence bonds:



and have an intense ion due to the fragments from



J. ETHERS[11]

Symmetrical ethers undergo cleavage alpha to the oxygen atom. Mixed aliphatic ethers also cleave beta to the oxygen, losing the more highly substituted fragment.

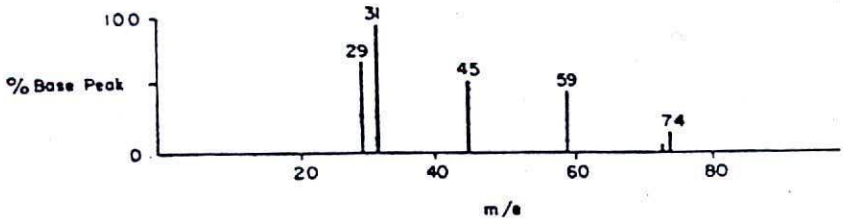
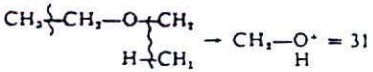


FIGURE 6.7: Major peaks of diethyl ether mass spectrum.

Peaks 74, 59, 45, and 29 in Fig. 6.7 are readily explained:

$$M = CH_3CH_2OCH_2CH_3 = 74$$
$$M - 15 = C_3H_5OCH_2^+ = 59$$
$$M - 29 = C_2H_5O^+ = 45$$
$$29 = C_2H_5^+$$

The 31 peak is most logically formed by rearrangement

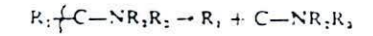


K. MERCAPTANS[13]

These compounds give mass spectra similar to alcohols. The main differances are in the relatively abundant molecular ion peak, and the existence of significant $M + 2$ due to the 4.4% of the sulfur isotope mass 34.

L. ALIPHATIC AMINES[8]

The amino group directs the fragmentation pattern so strongly that the low mass region gives most information about the molecule. The most important process is cleavage of the bond adjacent to the nitrogen.



M. PIPERIDINE RINGS

Piperidine rings are considerably more complicated than aliphatic amines because there are more possibilities for bond cleavage of molecular ions and rearrangement products. Base peak is

(*m/e* 84), but the associated peaks need careful assessment before assignment.

A study on Vitamin B_1 by Hesse et al.[12] describes the decomposition pattern

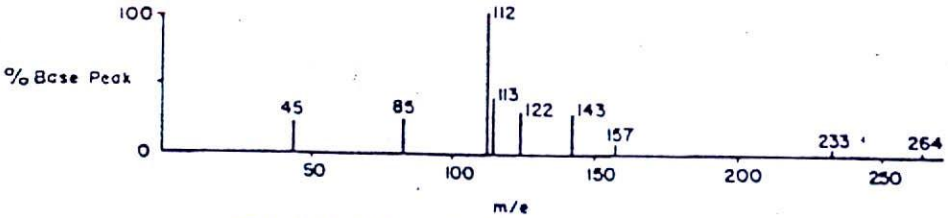
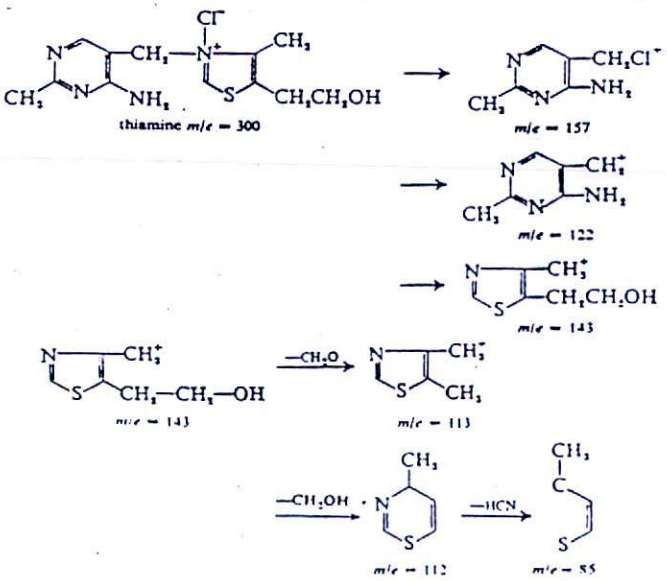


FIGURE 6.8: Major peaks of thiamine mass spectrum.

of a nitrogen-containing compound. The major peaks in the spectrum are given in Fig. 6.8.

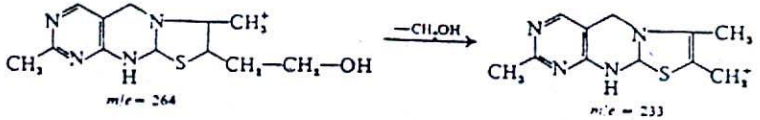
The molecule, mass 300, gives major peaks at 264, 233, 157, 143, 122, 113, 112, 85, and 45.

The obvious splits between the two rings provides three of these peaks:



Cleavage of the alcohol side chain from one of the rings gives the base peak at 112. This fragment decomposes to give the fragment with *m/e* 85 and the S=CH fragment, *m/e* 45.

To explain the peaks at 264 and 233, the authors postulated an initial cyclization to form



N. HALOGEN COMPOUNDS[22]

The presence of chlorine and bromine in a compound may be deduced from the high ratio of the corresponding heavy isotope. For a compound with one atom of chlorine, the $M + 2$ peak will be about one-third of the molecular ion. Further confirmation for chlorine is the presence of a strong doublet at masses 35 and 37, which are also in the 3:1 ratio. The $M + 2$ peak in a monobrominated compound has an intensity nearly equal to the parent (M) peak. This is because the two isotopes, masses 79 and 81, are present in equal amounts.

In general, be wary. Rearrangements often occur which obscure qualitative interpretation or mislead one into confidence in an obvious but erroneous interpretation High resolution examination of the major fragments assists greatly in avoiding this last pitfall. Also, attempting to supply a reasonable explanation of all peaks, even minor ones, will often necessitate rejection of a comfortable, obvious solution.

6.4 MASS SPECTROSCOPY IN PHARMACY

In pharmaceutical work, it is frequently necessary to assay one component in a complex mixture. Many procedures are used to effect specificity; separations by classical techniques and chromatography, specific function analysis by colorimetry, kinetic studies, or infrared absorbance. Mass spectrometry is another technique, often very rapid, for performing these specific analyses. One example is a procedure for determination of aspirin, phenacetin, and caffeine in pharmaceutical products.[21] Many materials used in pharmacy are not readily handled by other techniques, so frequently mass spectrometry will give the only useful data. As instrumentation becomes available, more of this type of work will be reported in the pharmaceutical literature.

A. QUANTITATIVE ANALYSIS[21]

Washburn developed a general procedure for quantitative calculations with mass spectrometry where a mixture is being examined to determine quantitatively one component; all possible components must be run separately to

observe peaks that are present and to determine the ion current per unit partial pressure. We assume that contributions to each peak are additive for all components present in the mixture and expect we can find at least one peak for each compound which is unique. Where this condition is met, ion currents can be expressed using a four-component mixture as an example:

$$I_1 = i_{1a}P_a + i_{1b}P_b + i_{1c}P_c + i_{1d}P_d$$

$$I_2 = i_{2a}P_a + i_{2b}P_b + i_{2c}P_c + i_{2d}P_d$$

$$\vdots$$

$$I_m = i_{ma}P_a + i_{mb}P_b + i_{mc}P_c + i_{md}P_d$$

TABLE 6.2: Mass Spectral Data for Pure Solvents*

m/e	Cyclohexanone	Toluene	Dioxane	Acetone
28	12.5	0.5	100	1.9
43	12.2	1.8	10.9	100
55	100	—	—	0.3
58	0.1	—	23.6	27.8
91	—	100	—	—
Division/micron pressure	29	47	39	48

* Per cent of base.

where i_{ma} is current at mass m due to component a, P_a is partial pressure of component a, and I_m is the observed total ion current at mass m. One may calculate the relative peak height for a given component based on a reference peak being 1. A solution for the equations gives component peak heights. A residual peak is the value of a peak intensity after all other known compounds have been mathematically subtracted.

For example, if a solvent mixture of cyclohexanone, toluene, dioxane, and acetone is to be examined, the important peaks in the spectra of the pure solvents are given in Table 6.2. The peak at m/e 28 in a mixture of these four compounds is made up of:

$$\frac{12.5c}{100} + \frac{0.5t}{100} + \frac{100d}{100} + \frac{1.9a}{100}$$

where c, t, d, and a are the contributions of the individual solvents in the mixture. By setting up simultaneous equations equal to the number of components, the contribution of each component to the spectrum may be calculated. The contribution of the component is divided by the sensitivity (divisions/micron) to obtain the partial pressure of that component. The partial pressures, when normalized, give the molar ratios of components.

B. GAS CHROMATOGRAPHY

Joint use of a mass spectrometer and a gas chromatograph has been hailed as the ultimate analytical instrument. The gas chromatograph (see Chapter 18) is a powerful tool for effecting separations of closely related organic compounds. The mass spectrometer enables a qualitative study and identification of the highly purified fractions separated by the gas chromatograph (Plate 6.II).

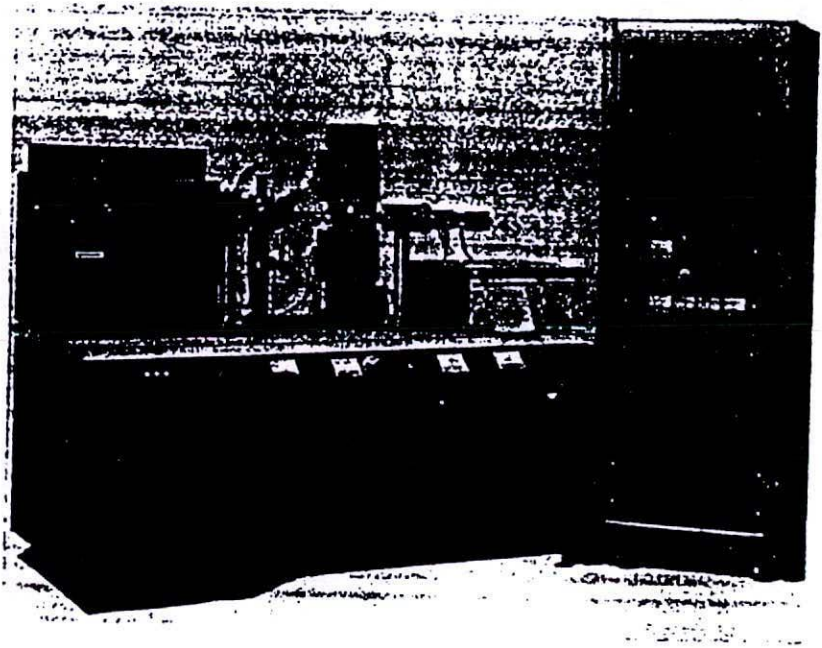


PLATE 6.II: Combined gas chromatograph-mass spectrometer. Courtesy Perkin-Elmer Corporation.

When pharmaceutical materials are studied, this combination gives both qualitative and quantitative information. A routine gas chromatographic survey demonstrated the presence of an unknown volatile material in a tank carload of specially denatured alcohol. Using mass spectrometry, the unknown was identified as diethyl ether. Knowing its identity, the concentration could then be calculated as about 10 ppm. This enabled a producer to locate a potential contaminating procedure and gave added assurance to the pharmaceutical company that it was using only high quality materials for its processing.

C. PYROLYSIS[10]

Pyrolysis of a sample followed by mass spectrometric examination of the volatile products has been used to identify many polymers and other large nonvolatile molecules. An apparatus has been described by Happ and Maier which enables examination of both volatiles and residue. This identification is important in examination of packaging materials in which pharmaceutical materials are shipped as raw materials and finished products. Infrared examination and physical tests are complementary procedures.

D. ISOTOPE DILUTION[16,27]

In the complex analytical studies needed for determination of the metabolic pathway of a new drug substance, the use of radioactive isotopes has already shown its utility. One disadvantage, however, is that the radioactivity itself may affect the pathway. Another is that the half-life of the isotope may be too short to complete a lengthy study. With a mass spectrograph, it is possible to use stable isotopes and a larger range of isotopes to obtain this information.

The method requires that a sample of the compound to be determined be prepared with one element enriched in its isotope. It is assumed that the test organism cannot distinguish the labeled compound from the normal one. When the labeled compound is added to an unknown mixture, it is diluted by the normal compound. A fractionation is performed to obtain a pure sample of the component; the isotope ratio is determined on the recovered material and from the change in ratio, the amount of the compound originally present is calculated.

The weight W of component in the original mixture is given by

$$W = W_1\left(\frac{A_0 - A}{A}\right)$$

where A_0 is the concentration of the isotope in the added sample *in excess of normal*, A is the concentration of isotope in the *recovered* sample in excess of normal, and W_1 is the weight of isotope-enriched material added.

E. COMPUTER APPLICATIONS[3,25]

Studies using a high resolution, double-focusing mass spectrometer can differentiate among molecules with the same nominal molecular weight (Plate 6.III). The exact mass of the elements are sufficiently different that ion fragments $^{12}CH_3$ and $^{14}NH_1$ with exact mass, respectively, 15.023 and 15.0108, can be readily separated. Bieman and co-workers[3] have reported a technique where the exact measurements of mass are made for most of the ion fragments in a spectrum of a complex material and possible elemental structures are calculated. A computer, which is programmed to sort the

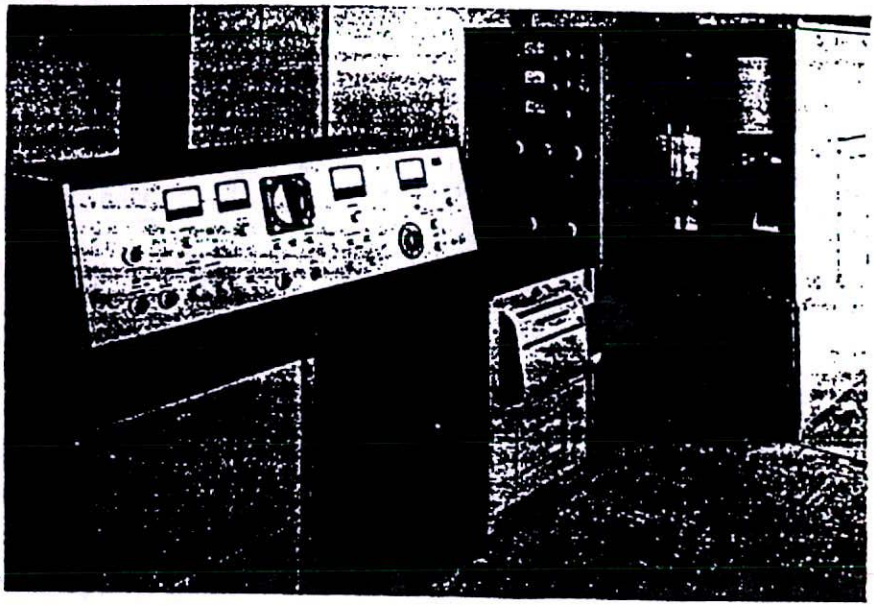


PLATE 6.III Double focusing mass spectrometer.

exact fragment weight into columns according to content of hetero atoms in ascending order of numbers of carbon and hydrogen, is used for data reduction. A very comprehensive picture of the molecular structure of even complex molecules is obtained this way (Table 6.3).

Computers also find use in the resolution of mixtures when the spectra of pure compounds are inserted. The computer can be programmed to calculate the relative amounts of the individual materials in a mixture. These calculations are otherwise very tedious and time-consuming (6.6). If the spectrum of a pharmaceutical mixture is inserted initially, the computer can watch for changes in the spectrum of subsequent samples which have been subjected to adverse conditions in stability studies.

TABLE 6.3: Exact Mass of Common Nuclides

Nuclide	Mass
^{1}H	1.007825
^{12}C	12.000000
^{13}C	13.003354
^{14}N	14.003074
^{16}O	15.994914

F. PLANT CONTROL

When production of a material is routine and the market will sustain continuous production, engineering groups are organizing automated plants where the materials going into the plant, and the products, are monitored by instruments such as mass spectrometry and gas chromatography. The data are accumulated and interpreted by massive computer installations which can be programmed to adjust the process to maintain the product within the set limits. This approach must be kept in mind by those working in the pharmaceutical industry.

PROBLEMS

P6.1. If ^{16}O as a base for atomic weight values is taken as 16.0000 and the isotopes ^{17}O and ^{18}O are present to the extent of 0.04 and 0.20%, respectively, calculate the mean atomic weight of normal oxygen on the mass spectrograph scale (16.0044). Calculate the value of ^{16}O based on $^{12}C = 12.0000$ (15.9949).

P6.2. Where major peaks appear in the spectrum at the following mass peaks, list possible structural units which may be present

$M - 15$ $(-CH_3)$

$M - 18$ $(-OH_2)$

$M - 57$ (*t*-butyl $CH_3-\overset{\overset{O}{\|}}{C}-CH_2$ CH_2CH_2CHO)

$M - 45$ ($CH_3-CH(OH)$ $-COOH$)

P6.3. Determine the number of carbon atoms in each of the compounds with the following M, $M + 1$, and $M + 2$ ratios

Compound	M	$M+1$	$M+2$	
a	48	0.51	—	(1)
b	31	1.02	—	(3)
c	58	1.4	0.26	(2)
d	27	1.5	—	(5)

P6.4. Determine whether the compound with molecular weight (310.3110) is $C_{20}H_{40}NO$ or $C_{20}H_{38}O_2$($C_{20}H_{40}NO$).

P6.5. Djerassi and co-workers considered the following possible formulas for an alkaloid: $C_{45}H_{54}N_4O_8$, $C_{42}H_{48}N_4O_{81}$, $C_{42}H_{50}N_4O_7$, $C_{43}H_{50}N_4O_6$, and $C_{42}H_{46}N_4O_7$. High resolution mass spectrometry indicated the molecular weight was 718.3743. Which formula was accepted? Ans: ($C_{43}H_{50}N_4O_6$).

P6.6. Using the spectral data for the four pure solvents given in Table 6.2, calculate the contribution of each of the components to the following mass spectrum

of a mixture of cyclohexanone, toluene, dioxan, and acetone:

m/e	Intensity
28	368.3
43	300.0
55	500.6
58	126.9
91	400

Gas	*c*	*t*	*d*	*a*
28	62.5	2.0	300	3.8
43	61.0	7.2	31.8	200
91	—	400	—	—

QUESTIONS

Q6.1. Define
(a) isotope
(b) molecular weight
(c) ionization potential
(d) ion
(e) nuclide

Q6.2. Define metastable ion. Ans: If the moving ion can decompose in the region between the electrostatic accelerating and magnetic deflecting fields, the resulting ion is then deflected by the magnetic field. The *m/e* ratio of this ion, called the "metastable ion," relates the accelerate ion to the deflected ion.

Q6.3. Explain in what ways a chemist can obtain information directly from a mass spectrum.

Q6.4. How would you establish the exact mass of an unknown ion using a high resolution mass spectrometer? Ans: Run first the mass spectrum of a known material of molecular weight close to that of the unknown. Determine the difference between the known and the unknown weight.

Q6.5. Known mass 87.327 reads 39471 on a mass spectrograph. Known mass 95.318 reads 43920. Unknown mass reads 41392; what is the unknown mass?
Ans: 90.605

REFERENCES

1. F. H. Aston, *Science*, 52, 559 (1920).
2. J. H. Beynon, R. A. Saunders, and A. E. Williams, *Anal. Chem.*, 33, 221 (1961).
3. K. Bieman, *Tetrahedron Letters*, 1964, 1725.
4. R. A. Brown, *Anal. Chem.*, 23, 430 (1951).
5. A. J. Dempster, *Phys. Rev.*, 20, 631 (1922).
6. R. A. Freidel, J. L. Shultz, and A. G. Sharkey, *Anal. Chem.*, 28, 926 (1956).
7. J. A. Gilpin and F. W. McLafferty, *Anal. Chem.*, 29, 990 (1957).

8. R. S. Gohlke and F. W. McLafferty, *Anal. Chem.*, 34, 1281 (1962).
9. E. Goldstein, *Ber. Preuss. Akad. Wiss.*, 39, 691 (1886).
10. G. P. Happ and D. P. Maier, *Anal. Chem.*, 36, 1678 (1964).
11. G. P. Happ and D. W. Stewart, *J. Am. Chem. Soc.*, 74, 4404 (1952).
12. M. Hesse, N. Bild, and H. Schmid, *Helv. Chim Acta*, 50, 808 (1967).
13. I. W. Kinnear and G. L. Cook, *Anal. Chem.*, 24, 1391 (1952).
14. F. W. McLafferty, *Anal. Chem.*, 29, 1782 (1957).
15. S. Meyerson, *Appl. Spectry.*, 9, 120 (1955).
16. D. Rittenberg and G. L. Foster, *J. Biol. Chem.*, 133, 737 (1940).
17. S. M. Rock, *Anal. Chem.*, 23, 261 (1951).
18. P. N. Rylander, S. Meyerson, and H. M. Grubb, *J. Am. Chem. Soc.*, 79, 842 (1957).
19. A. G. Sharkey, J. L. Shultz, and R. A. Friedel, *Anal. Chem.*, 28, 934 (1956).
20. A. G. Sharkey, J. L. Shultz, and R. A. Friedel, *Anal. Chem.*, 31, 87 (1959).
21. A. Tatematsu and T. Goto, *J. Pharm. Soc. Japan*, 85, 624 (1965).
22. R. C. Taylor, R. A. Brown, W. S. Young, and C. G. Headington, *Anal. Chem.*, 20, 398 (1948).
23. J. J. Thomson, *Phil. Mag.*, 13, 561 (1907).
24. H. W. Washburn, H. F. Wiley, and S. M. Rock, *Ind. Eng. Chem. Anal. Ed.*, 15, 541 (1943).
25. F. A. White and T. L. Collins, *Appl. Spectry.*, 8, 169 (1964).

REVIEW ARTICLES

26. K. Biemann, *Ann. Rev. Biochem.*, 32, 755 (1963).
27. F. W. McLafferty, *Science*, 151, 641 (1966).

BOOKS

Beynon J. H., *Mass Spectrometry and Its Application to Organic Chemistry*, Van Nostrand, Princeton, N.J., 1960.

Biemann, K., *Mass Spectrometry Organic Chemical Applications*, McGraw-Hill, New York, 1962.

Budzikiewicz, H., C. Djerassi, and D. H. Williams, *Interpretation of Mass Spectra of Organic Compounds*, Holden-Day, San Francisco, 1964.

Freeman, S. K. (ed.), *Interpretive Spectroscopy*, Reinhold, New York, 1965.

Kolthoff, I. M., and P. J. Elving (eds.), *Treatise on Analytical Chemistry*, Part I, Vol. 4, Wiley (Interscience), New York, 1961.

Silverstein, R. M., and G. C. Bassler, *Spectrometric Identification of Organic Compounds*, Wiley, New York, 1963.

Willard, H. H., H. Merritt, and J. A. Dean, *Instrumental Methods of Analysis*, Van Nostrand, Princeton, N.J., 1965.