

Chapter 13

Mass spectrometry and applications

Dr. Suseela Mathew

Senior Scientist

Biochemistry & Nutrition Division

Central Institute of Fisheries Technology, Cochin - 682 029

Mass spectrometry is a powerful analytical technique that is used to identify unknown compounds, to quantify known compounds, and to elucidate the structure and chemical properties of molecules. Detection of compounds can be accomplished with very minute quantities (as little as 10^{-12} to 10^{-15} moles for a compound of mass 1000 Daltons). The compounds can be identified at very low concentrations (one part in 10^{12}) in chemically complex mixtures. Mass spectrometry provides valuable information to a wide range of professionals: physicians, astronomers, and biologists, to name a few.

Initially mass spectrometers were planned to be used in the following fields:

- To detect and identify the use of steroids in athletes
- To monitor the breath of patients by anesthesiologists during surgery
- To determine the composition of molecular species found in space
- To determine whether honey is adulterated with corn syrup
- To locate oil deposits by measuring petroleum precursors in rock
- To monitor fermentation processes for the biotechnology industry
- To detect dioxins in contaminated fish
- To determine gene damage from environmental causes
- To establish the elemental composition of semiconductor materials

More frequently mass spectrometers are used today in the following areas:

- To identify structures of bio molecules such as carbohydrates, nucleic acids and steroids
- To sequence biopolymers such as proteins, peptides, oligosaccharides and oligo nucleotides
- To determine how drugs are used by the body ie. in drug discovery, combinatorial chemistry, pharmacokinetics and drug metabolism
- To perform forensic analyses such as conformation and quantitation of drugs of abuse
- To analyze environmental pollutants
- To determine the age and origins of specimens in geochemistry and archaeology
- To identify and quantitate compounds of complex organic mixtures
- To perform ultra sensitive multi element inorganic analyses

History

The technique of mass spectrometry had its beginnings in J.J. Thomson's vacuum tube where in the early part of the century the existence of electrons and "positive rays" was demonstrated. Thomson, the physicist, observed in his book "Rays of Positive Electricity and Their Application to Chemical Analysis" that the new technique could be used profitably by chemists to analyze chemicals. Despite this far-sighted observation, the primary application of mass spectrometry remained in the realm of physics for nearly 30 years. It was used to discover a number of isotopes, to determine the relative abundance of the isotopes, and to measure their "exact masses". These important fundamental measurements laid the foundation for later developments in diverse fields ranging from geochronology to biochemical research.

Principle

A mass spectrometer is an instrument that measures the masses of individual molecules that have been converted into ions, i.e., molecules that have been electrically charged. Since molecules are so small, it is not convenient to measure their masses. A more convenient unit is needed to measure the mass of individual molecules. This unit of mass is often referred to by chemists and biochemists as the dalton (Da), and is defined as follows: 1 Da = (1/12) of the mass of a single atom of the isotope of carbon-12 (^{12}C). This follows the accepted convention of defining the ^{12}C isotope as having exactly 12 mass units.

The introduction of a complex mixture in the inlet of a mass spectrometer always gives a complex spectrum which is difficult to interpret. We often observe a lot of peaks and the most important corresponds to the major constituent. That's why it is very difficult to identify one precise compound in the spectrum of a mixture. The principle of MS/MS method is the use of filiation relations. This method introduces a further specificity to recognize a

substance in a mixture. We can consider the mass spectrum of a mixture containing the compound A. Even if the mass spectrum contains 2 characteristic ions m_1+ and m_2+ of the compound A, we can't conclude the presence of A in the mixture. As a matter of fact, other compounds present in the mixture can also give m_1+ and m_2+ .

The presence of these 2 ions is not an efficient evidence to conclude that A is in the mixture. But if we can demonstrate that m_2+ comes from the decomposition of the ion m_1+ in the mass spectrum, we have further information which confirms the presence of A in the mixture. If we can compare the mass spectrum of the mixture and the clean up sample of A; and if we observe the same relations (same m/z and same intensity), then we can be sure that A is present. So as to prove these filiations, we should use 2 mass spectrometers in series. The first mass spectrometer (MS1) is used to select, from the primary ions, those of a particular m/z value which then pass into the fragmentation region. The ion selected by the MS1 is the parent ion or a precursor ion and can be a molecular ion or resulting from the primary fragmentation. Dissociation occurs in the fragmentation region. The daughter ions are analyzed in the second mass spectrometer (MS2). In fact, MS1 can be viewed as an ion source for MS2.

As will become clear in what follows, a mass spectrometer does not actually measure the molecular mass directly, but rather the mass-to-charge ratio of the ions formed from the molecules. In many cases, the ions encountered in mass spectrometry have just one charge ($z=1$) so the m/z value is numerically equal to the molecular (ionic) mass in Da. Mass spectrometrists often speak loosely of the "mass of an ion" when they really mean the m/z ratio, but this convenient way of speaking is useful only for the case of singly-charged ions. A mass spectrum is a graph of ion intensity as a function of mass-to-charge ratio. The most intense peak in the spectrum is termed the base peak and all others are reported relative to its intensity. The peaks themselves are typically very sharp, and are often simply represented as vertical lines. The highest molecular weight peak observed in a spectrum will typically represent the parent molecule, minus an electron, and is termed the molecular ion (M^+). Generally, small peaks are also observed above the calculated molecular weight due to the natural isotopic abundance of ^{13}C , ^2H , etc. Many molecules with especially labile protons do not display molecular ions; an example of this is alcohols, where the highest molecular weight peak occurs at m/e one less than the molecular ion ($m-1$). Fragments can be identified by their mass-to-charge ratio, but it is often more informative to identify them by the mass which has been lost. That is, loss of a methyl group will generate a peak at $m-15$; loss of an ethyl, $m-29$, etc.

Mass spectrometer- Components

The common component of a mass spectrometer is given in Figure 1. The different components of the mass spectrometer are: Inlet system, Ion source, Mass analyzer and Detector. Samples can be introduced to the mass spectrometer directly via. solids probe, or in the case of mixtures, by the intermediary of chromatography devices (e.g. Gas

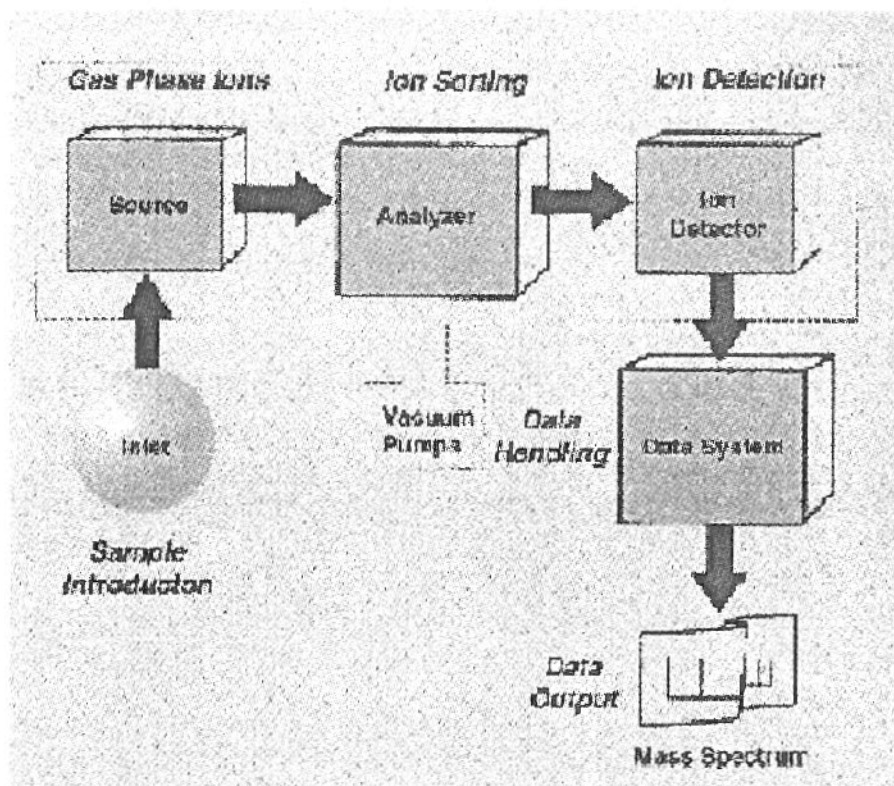


Fig 1. Components of a Mass Spectrometer

chromatography, Liquid chromatography, Capillary electrophoresis, etc). Once in the source, sample molecules are subjected to ionization. Ions formed in the source (molecular and fragment ions) acquire some kinetic energy and leave the source. A calibrated analyzer then analyzes the passing ions as function of their mass to charge ratios. Different kind of analyzer(s) can be used viz Magnetic, Quadrupole, Ion trap, Fourier Transform, Time of Flight, etc. The ion beam exiting the analyzer assembly is then detected and the signal is registered.

The analyser and detector of the mass spectrometer, and often the ionisation source too, are maintained under high vacuum to give the ions a reasonable chance of travelling from one end of the instrument to the other without any hindrance from air molecules. The entire operation of the mass spectrometer, and often the sample introduction process also, is under complete data system control on modern mass spectrometers.

Methods of Sample Ionisation

Many ionisation methods are available and each has its own advantages and disadvantages.

Ionization Methods

Electron Ionization and Chemical Ionization

Electron ionization (EI) is widely used in mass spectrometry for relatively volatile samples that are insensitive to heat and have relatively low molecular weight. The spectra, usually containing many fragmentation peaks, are useful for structural characterization and identification. Small impurities in the sample are easy to detect. Chemical Ionization (CI) is used to enhance the abundance of the molecular ion. For both ionization methods, the molecular weight range is 50 to 800 Da. In rare cases it is possible to analyze samples of higher molecular weight. A diagrammatic representation of electron ionization is given in Figure 2.

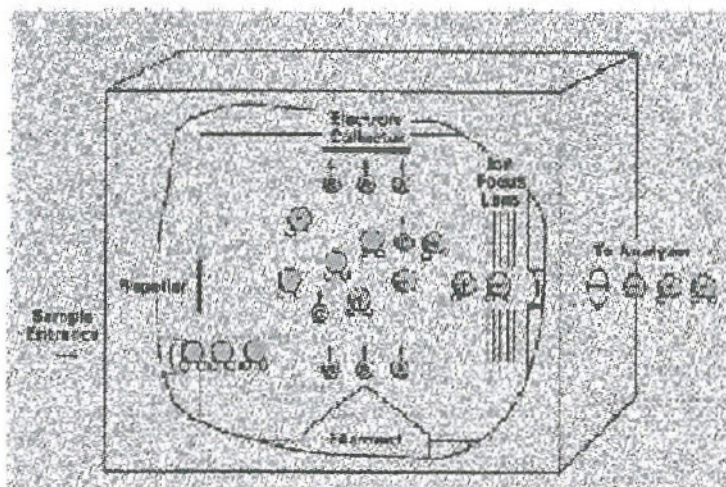


Fig. 2. Electron Ionization source: Ions are generated by bombarding gaseous molecules with a beam of high energy electron.

Fast Atom Bombardment Ionization

Fast atom bombardment ionization is a softer ionization method than EI. The spectrum often contains peaks from the matrix, which is necessary for ionization, a few fragments and a peak for a protonated or deprotonated sample molecule. FAB is used to obtain the molecular weight of sensitive, non-volatile compounds. The method is prone to suppression effects by small impurities. The molecular weight range is 100 to 4000 Da.

MALDI-TOF

Matrix-assisted laser desorption (MALDI) is used to determine the molecular weight of peptides, proteins, oligonucleotides, and other compounds of biological origin as well as of small synthetic polymers. The amount of sample needed is very low (pmoles or less). The analysis can be performed in the linear mode (high mass, low resolution) up to a molecular

weight of m/z 300,000 (in rare cases) or reflectron mode (lower mass, higher resolution) up to a molecular weight of 10,000.

Electrospray Ionization (ESI)

Electrospray ionization allows production of molecular ions directly from samples in solution. It can be used for small and large molecular-weight biopolymers (peptides, proteins, carbohydrates, and DNA fragments), and lipids. Unlike MALDI, which is pulsed, it is a continuous ionization method that is suitable for using as an interface with HPLC or capillary electrophoresis. Multiple charged ions are usually produced. ESI should be considered a complement to MALDI. Electrospray Ionisation (ESI) is one of the Atmospheric Pressure Ionisation (API) techniques and is well-suited to the analysis of polar molecules ranging from less than 100 Da to more than 1,000,000 Da in molecular weight.

The sample is dissolved in a polar, volatile solvent and pumped through a narrow, stainless steel capillary (75–150 μm i.d.) at a flow rate of between 1 $\mu\text{L}/\text{min}$ and 1 mL/min . A high voltage of 3 or 4 kV is applied to the tip of the capillary, which is situated within the ionisation source of the mass spectrometer, and as a consequence of this strong electric field, the sample emerging from the tip is dispersed into an aerosol of highly charged droplets, a process that is aided by a co-axially introduced nebulising gas flowing around the outside of the capillary. This gas, usually nitrogen, helps to direct the spray emerging from the capillary tip towards the mass spectrometer. The charged droplets diminish in size by solvent evaporation, assisted by a warm flow of nitrogen known as the drying gas which passes across the front of the ionisation source. Eventually charged sample ions, free from solvent, are released from the droplets, some of which pass through a sampling cone or orifice into an intermediate vacuum region, and from there through a small aperture into the analyser of the mass spectrometer, which is held under high vacuum. The lens voltages are optimized individually for each sample.

In certain cases, the solution is placed into a metallic capillary (flow rate: 1 to 10 mL/min). An electric field is applied between the capillary's point and an electrode, and so multicharged droplets are produced and accelerated to the electrode. Different parameters of "spray" also exist.

Electrospray ionisation is known as a "soft" ionisation method as the sample is ionised by the addition or removal of a proton, with very little extra energy remaining to cause fragmentation of the sample ions. By raising the voltage applied to the sampling cone, say from 25 to 80 V, extra energy is supplied to the sample ions which can then fragment. The m/z spectrum then has extra peaks corresponding to sample fragment ions which can help in the structural elucidation of the sample. This phenomenon is known as "cone voltage" or "in-source" fragmentation and although it can provide useful information it must be remembered that it is not specific so if there are a number of components in a sample, all will fragment to give rise to an extremely complicated spectrum.

In "Thermospray Ionisation": the dissolution of the product is due to the temperature effect. The ionisation is produced by "corona" discharge. In "APCI" (Atmospheric Pressure Chemical Ionisation) an electric field is combined with a "corona" discharge. In "Particle beam ionisation" the solution under pressure forms a jet at the end of the capillary that collides with a gas flow. In Nanospray Ionisation, flow in the capillary is only 20 nL/min. Nanospray ionisation (Wilm and Mann, *Anal. Chem.*, 1996, 68, 1), is available on the Q-T of mass spectrometer is a low flow rate version of electrospray ionisation.

A small volume (1-4 μ L) of the sample dissolved in a suitable volatile solvent, at a concentration of ca. 1-10 pmol/ μ L, is transferred into a miniature sample vial. A reasonably high voltage (ca. 700-2000 V) is applied to the specially manufactured gold-plated vial resulting in sample ionisation and spraying. Desolvation is followed by ion extraction through the sampling cone, which is situated at 90° to the original flow of solute and solvent, and then through the extraction cone (another 90° turn) into the analyser for separation and analysis of the ions according to their m/z ratios, as with standard ESI-MS. The two right-angled bends in the ionisation source have led to its name of Z-spray.

The flow rate of solute and solvent using this procedure is very low, 30-1000 nL/min, and so not only is far less sample consumed than with the standard electrospray ionisation technique, but also a small volume of sample lasts for several minutes, thus enabling multiple experiments to be performed. A common application of this technique is for a protein digest mixture to be analysed to generate a list of molecular weights for the components present, and then each component is to be analyzed further by tandem mass spectrometric (MS-MS) amino acid sequencing techniques.

Electron-Capture Ionization

Electron-capture (sometimes called Negative Ion Chemical Ionization or NICI) is used for molecules containing halogens, NO_2 , CN, etc, and it usually requires that the analyte be derivatized to contain highly electron-capturing moieties (e.g., fluorine atoms or nitrobenzyl groups). Many ionisation methods are available and each has its own advantages and disadvantages. The ionisation method to be used should depend on the type of sample under investigation and the mass spectrometer available.

With most ionisation methods, there is a possibility of creating both positively and negatively charged sample ions, depending on the proton affinity of the sample. Before embarking on an analysis, the user must decide whether to detect the positively or negatively charged ions.

Double focusing mass spectrometers use a combination of magnetic and electrical fields to focus and sort ions. A common configuration for a sector instrument is the geometry shown in Figure 3, in which a magnetic "sector" follows an electric "sector". The slit acts as a filter to select for a specific m/z value. The electric sector focuses the ions with respect to differences in kinetic energy that they may have as they exit the source region. "Double focusing," this combination of "angular" or "directional" focusing and energy focusing,

provide mass resolution high enough to separate ions of the same nominal mass but different chemical formulae.

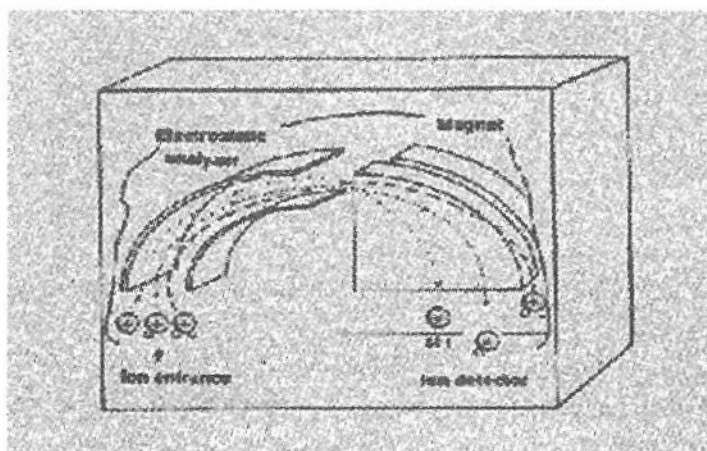


Fig. 3. Electrostatic and magnetic sectors in a double focusing arrangement. The slit is the directional and energy focus point

Detection and Recording of Sample Ions

The detector monitors the ion current, amplifies it and the signal is then transmitted to the data system where it is recorded in the form of mass spectra. The m/z values of the ions are plotted against their intensities to show the number of components in the sample, the molecular weight of each component, and the relative abundance of the various components in the sample.

Detectors

The purpose of detector is to translate the ion arrival into an electric signal measured by the electronic system of the mass spectrometer. Two different classical types of detectors are present.

Electrons Multiplying

The principal sorts of electron multiplying are the "channeltron" and the "micro channel plate". The principle is based on the impact of an ion on a surface composed by half conductors. This impact creates electrons which are accelerated to another surface where they also create other electrons. Those electrons are recovered and the number of them is proportional to the signal's intensity.

Photodetectors

Electrons are created in the same way and interact with a phosphorescent surface which generates photons. Those photons are also recovered and the number of them is proportional to the signal' intensity.

Mass Analysers

The main function of the mass analyser is to separate, or resolve, the ions formed in the ionisation source of the mass spectrometer according to their mass-to-charge (m/z) ratios. There are a number of mass analysers currently available, the better known include quadrupoles, time-of-flight (TOF) analysers, magnetic sectors, and both Fourier transform and quadrupole ion traps.

These mass analysers have different features, including the m/z range that can be covered, the mass accuracy, and the achievable resolution. The compatibility of different analysers with different ionisation methods varies. For example, all of the analysers listed above can be used in conjunction with electrospray ionisation, whereas MALDI is not usually coupled to a quadrupole analyser.

Tandem (MS-MS) mass spectrometers are instruments that have more than one analyser and so can be used for structural and sequencing studies. A tandem mass spectrometer can be thought of as two mass spectrometers in series connected by a chamber that can break a molecule into pieces perhaps like a puzzle. This chamber is known as a collision cell. A tandem mass spectrometer is often abbreviated as Tandem MS or MS/MS. A sample is "sorted" and "weighed" in the first mass spectrometer, then broken into pieces in the collision cell, and a piece or pieces sorted and weighed in the second mass spectrometer.

Two, three and four analysers have all been incorporated into commercially available tandem instruments, and the analysers do not necessarily have to be of the same type, in which case the instrument is a hybrid one. More popular tandem mass spectrometers include those of the quadrupole-quadrupole, magnetic sector-quadrupole, and more recently, the quadrupole-time-of-flight geometries.

Gas Chromatography-Mass Spectrometry

This permits separation of complex mixtures into single components before ionization and mass analysis. This is particularly useful when analyzing relatively low levels of target compounds derived from complex biological matrices. The target analyte must be relatively volatile or must be susceptible to conversion to a volatile derivative to permit GC separation. In general, the derivatized analyte should have a MW of less than 1000 Da in cases where GC-MS can be successfully applied. In special cases, derivatized analytes with MW 1000-2000 Da can be investigated. The ionization methods that can be used are EI and CI in positive and negative modes.

LC-MS

Liquid Chromatography - Mass Spectrometry allows separation of complex mixtures of non-volatile compounds before introduction to the mass spectrometer. It is used extensively for compounds that have a high molecular weight or are too sensitive to heat to be analyzed by GC. The most common ionization methods that are interfaced to LC are ESI and Atmospheric Chemical Ionization (APCI) in positive and negative-ion modes.

IRMS

Isotope Ratio MS is capable of very precise determination of $^{13}\text{C}/^{12}\text{C}$ ratios. It is exploited principally in examining trace enrichment of ^{13}C in small molecular-weight analytes (e.g. protein-derived amino acids) after biosynthetic incorporation of a ^{13}C -labeled precursor.

Fourier Transform Mass Spectrometry (FTMS)

The basis for FTMS is an ion trap (Penning cell) that allows ions formed by EI, CI, MALDI, and ESI to be accumulated and stored for time periods as long as minutes. During this time, reactions of the ions with neutral molecules can be followed. The method has the highest resolving power in mass spectrometry, a high upper mass limit, high sensitivity, non-destructive detection, and high accuracy for mass measurement. Because it uses Fourier transform detection, signal averaging and simultaneous wide-mass detection are possible.

Matrix Assisted Laser Desorption Ionisation

Matrix Assisted Laser Desorption Ionisation (MALDI) deals well with thermolabile, non-volatile organic compounds especially those of high molecular weight and is used successfully in biochemical areas for the analysis of proteins, peptides, glycoproteins, oligosaccharides, and oligonucleotides. It is relatively straight-forward to use and reasonably tolerant to buffers and other additives. The mass accuracy depends on the type and performance of the analyser of the mass spectrometer, but most modern instruments should be capable of measuring masses to within 0.01% of the molecular weight of the sample, at least up to ca. 40,000 Da.

MALDI is based on the bombardment of sample molecules with a laser light to bring about sample ionisation. The sample is pre-mixed with a highly absorbing matrix compound for the most consistent and reliable results, and a low concentration of sample to matrix works best. The matrix transforms the laser energy into excitation energy for the sample, which leads to sputtering of analyte and matrix ions from the surface of the mixture. In this way energy transfer is efficient and also the analyte molecules are spared excessive direct energy that may otherwise cause decomposition. Most commercially available MALDI mass spectrometers now have a pulsed nitrogen laser of wavelength 337 nm. The different ion trajectories in a TOF spectrometer is illustrated in Figure 4.

Selected/Multiple Reaction Monitoring

Both of the analysers are static in this case as user-selected specific ions are transmitted through the first analyser and user-selected specific fragments arising from these ions are measured by the second analyser.

The compound under scrutiny must be known and have been well-characterised previously before this type of experiment is undertaken. This methodology is used to confirm unambiguously the presence of a compound in a matrix e.g. drug testing with blood or urine samples. It is not only a highly specific method but also has very high sensitivity.

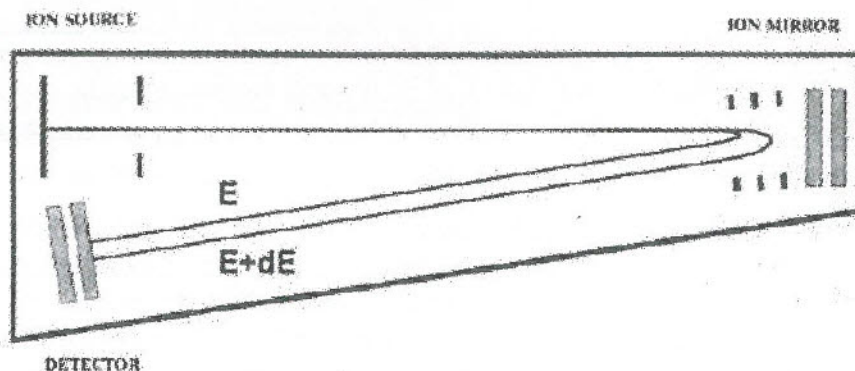


Fig. 4. Ion trajectories in a reflectron time-of-flight mass spectrometer, where E is the ion energy and dE the difference in ion energy of two ions

Sector Mass Spectrometers

Sector mass spectrometers are double focusing instruments and they use a magnet (B) with electric sector (E). When the electric sector takes place between the source and the magnet, the geometry is normal (EB) and when it is between the magnet and the detector, the geometry is inverse (BE)). If we study the EB system: the ions issued from source are placed into electric sector where they are accelerated by a potential voltage. Then, they are placed into a magnetic sector where the magnetic field is perpendicular to the direction of their movement. So, the ions describe an arc of the circle. In practical, scanning the magnetic sectors allows to focus the ion fragments on the slit of the analyser. The analysers with electric and magnetic sectors have a good resolution but are too big and expensive.

Tandem Quadrupoles (TQMS)

Quadrupoles offer several advantages over sector instruments for analyses in which high resolution and high range mass are not required. They include the absence of high source voltage, tolerance of high pressure, ease of computer control and rapidity of scanning. TQMS are the most commonly used: it's a sequential arrangement of two mass analysing quadrupoles with a third quadrupole interposed as a gas cell.

A quadrupole is made up of four electrodes where a continued potential U and a radio-frequency potential $V \cos(\omega t)$ are applied. Each ion adopts an oscillating path whose amplitude depends on the ratio U/V and on the ratio m/z . We can stabilize or destabilize the trajectory of ions with scanning of U and V but with the ratio U/V which is always constant. Only ions which have a stable trajectory can be detected.

The first quadrupole (MS1) allows the selection of precursor ions according to m/z value. The third sector MS2 is a mass filter too for the daughter ions. In the intermediate quadrupole, there exists a high pressure. This role is to induce ionic oscillations for ions to have an important linear speed. This quadrupole is not selective according to the mass, it is

a RF-only quadrupole (alternative alimентация of radio-frequency).

Ion Trap

The principle of Ion Trap is next to that of tandem quadrupoles. The difference is that ions can be formed directly in the Trap: so, the source and the analyser are the same instrument. The excellent sensibility of the Ion Trap involves its use to detect and quantify traces. This technique is relatively new and we can find it in environmental laboratories (analysis of pesticides, micro pollutants).

Ion Cyclotron Resonance (ICR)

It is the same principle as Ion Trap. With this type of analyser, we can study ions which have a ratio m/z bigger than 5000. The pressure inside the cell is low (10⁻⁹ torr). The performances of ICR are promising but they are very expensive. A schematic representation of ion cyclotron resonance cell is given in Figure 5.

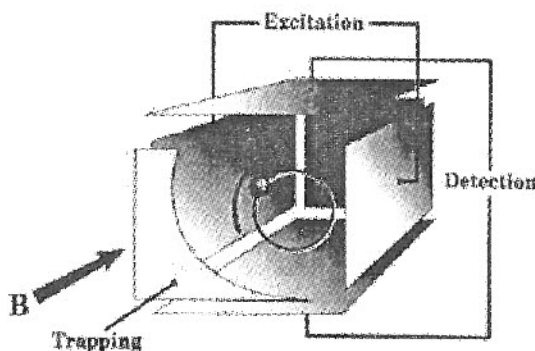


Fig. 5. Schematic representation of an ion cyclotron resonance cell

Collision Cell

The collisional activation can be divided into two categories, involving high or low energy, to which different types of collision cell are appropriate. In sector instruments, where high energy collisions are most common, the cell is usually a tight chamber of 1-3 cm length with entrance and exit slits which transmit the ion beam. Good pumping is essential to maintain a low pressure outside the cell. In some instruments, the collision cell has been electrically insulated from the mass spectrometer and can be held at a high potential to retard the ion beam and reaccelerate it on exit. This allows control of the collision energy and also reduces the kinetics energy spread of daughter ions formed.

Applications of Mass Spectrometry

a. Analysis of Food Constituents

Food aroma compounds have been studied by MSMS using an air stream to carry volatiles with an atmospheric pressure chemical ionisation source. Detection limits for daughter ion

spectra were as low as 45 mg/kg for limonene and 0,5 mg/kg for ethyl butyrate. MS/MS has been applied by Warburton (1981) for the identification of cholesterol in egg yolk and of citric acid in lemon juice. This technique has the ability to provide clean spectra. The presence of caffeine in leaf tea has been demonstrated by direct analysis of a 1 mg sample by MS/MS. Walther (1983) has reported the quantification of caffeine in beverages by obtaining parent ion spectra. With a deuterated analogue as internal standard, and recording parents of a single common fragment, the precision was better than 5%.

b. Protein Identification

Automated tandem mass spectrometry (MS/MS), coupled to online microcapillary liquid chromatography is now becoming a commonly used technique for identifying proteins. This technique typically involves digestion of a protein followed by microflow LC separation of the peptide fragments. As each peptide fragment elutes, it is automatically selected for collision induced dissociation (CID) by using powerful mass spectrometric techniques, including the real-time, automatic selection of parent ions using automated data-dependent scan functions. Protein identification is then accomplished by using computer algorithms that correlate uninterpreted protein and peptide tandem mass spectral data with sequences in protein or nucleotide databases.

This method not only provides the potential for automated high-throughput data analysis, but also imparts a high degree of specificity to database searches because the tandem mass spectrum representing every amino acid sequence displays a unique fingerprint-type pattern. Recently, the scope of this method was expanded to increase the number of proteins and peptides that can be analyzed by mass spectrometry. The technique permits the identification of 100 proteins in a single analysis using multi-dimensional chromatography with MS/MS detection using automated parent ion selection for high-throughput proteomics laboratories. This multi-dimensional liquid chromatography/MS/MS methodology precludes the isolation of individual proteins using two-dimensional gel electrophoresis and permits the rapid identification of protein mixtures.

c. Structural Studies

Despite the lack of isomer differentiation, MS/MS can provide valuable information with some type of compounds. For example, intact phosphoinositides, representing an important class of glycerophospholipids, have been the subject of study by Sherman *et al.* (1985). These compounds are heterogeneous with respect to their fatty acid composition, the nature of which determines their physical properties. FAB ionisation in the negative mode gave abundant [M-H]⁻ ions from which daughter ions were studied to obtain information about the acyl components.

In both MS/MS analysis and analysis with a triple sector instrument, the same daughter ions were observed and one fragmentation was directly indicative of the fatty acid composition. By using low energy collision (100eV) in the third field-free region of the triple sector instrument, good resolution was obtained for both parent and daughter ions.

d. Analysis of Drug Residues

MS/MS has found application in areas such as trace analysis of biological tissue, complex hazardous waste site samples, and human blood serum, as well as in drug testing. Anabolic steroids are synthetic derivatives of the male sex hormone testosterone. Although many athletic governing bodies viz. the International Olympic Committee have strict rules prohibiting use of these drugs, they are still being utilized for both human and equine performance enhancement in sporting events.

Routine testing of participants can be performed. One anabolic steroid, the presence of which has proven difficult to analyse is stanozolol [10418-03-8], $C_{21}H_{32}N_{20}$. A metabolite of the parent drug, hydroxy stanozolol, detected in equine urine eight hours after ingestion of the parent drug is actually identified, usually at very low levels. Analysis is done by LC/MS/MS which has a shortened analysis time advantage over GC/MS procedures because of the elimination of the need for a derivatization step.

e. Analysis for Contaminants

Mycotoxins (toxic secondary metabolites produced by fungi) are compounds of concern at low levels in food and animal feeds and present some difficult analytical challenges. Plattner and Bennett (1983) have applied a MS/MS instrument to a rapid detection of deoxynivalenol and zearalenone in grains. The importance of the analysis of chlorinated contaminants, and especially of polychlorodibenzodioxins (PCDD) and furans (PCDF), at low levels in environmental and biological samples has produced some of the most highly developed and validated methods of analysis.

A number of workers have investigated the use of MS/MS to contribute to this area. Pesticide residues in food are subject to regular monitoring and GC/MS is often used. MS/MS has been used for the detection of parathion (Steiner *et al.*, 1980). Using negative ionisation, the detection limit for the direct analysis of 10 mg of plant material such as lettuce is 1 mg/kg. Although the measurement of metal concentration in food is relatively routine, the identification and quantification of the organometallic compounds in which they occur is a developing field. Tandem mass spectrometry has been applied to confirm the identification of arsenobetaine and arsenocholine in fish, lobster and shrimp (Lau *et al.*, 1985).

f. Accurate Molecular Weight Measurements

For large samples such as biomolecules, molecular weights can be measured to within an accuracy of 0.01% of the total molecular weight of the sample i.e. within a 4 Daltons (Da) or atomic mass units (amu) error for a sample of 40,000 Da. This is sufficient to allow minor mass changes to be detected, e.g. the substitution of one amino acid for another, or a post-translational modification.

g. Reaction Monitoring

It can be used to monitor enzyme reactions, chemical modifications and for protein digestion.

h. Amino Acid Sequencing

It is essential for sequence confirmation, *de novo* characterisation of peptides, identification of proteins by database searching with a sequence "tag" from a proteolytic fragment. The most common usage of MS-MS in biochemical areas is the product or daughter ion scanning experiment which is particularly successful for peptide and nucleotide sequencing.

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