Basics of Mass Spectrometry

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Abstract. This article summarizes the basic principles of mass spectrometry instrumentation with special emphasis in sample introduction methods, ionization techniques and mass analyzers used in the different mass spectrometry techniques.

1. Introduction

Mass spectrometry (MS) is a powerful analytical technique [1, 2]. Over the past decades, mass spectrometry has undergone tremendous technological improvements that have made it an essential analytical tool in chemistry, biochemistry, pharmacy and medicine. Mass spectrometry is employed to analyze combinatorial libraries, sequence biomolecules, structural elucidation of unknowns, environmental and forensic analysis, quality control of drugs, flavours and polymers. Mass spectrometry has both qualitative and quantitative uses.

To achieve these goals, a mass spectrometer ionizes the chemical compounds to generate charged molecules or molecule fragments and measures their mass-to-charge ratios (m/z).

A mass spectrometer [3] consists of an ion source (ionization), a mass analyzer (separation of ions) and a detector (detects and measures the number of ions formed). Mass analyzer, detector and some ion sources operate under high- vacuum conditions in order to allow ions to reach the detector without colliding with other gaseous molecules or atoms (see Fig. 1). Sample molecules are introduced into the instrument through a sample inlet.

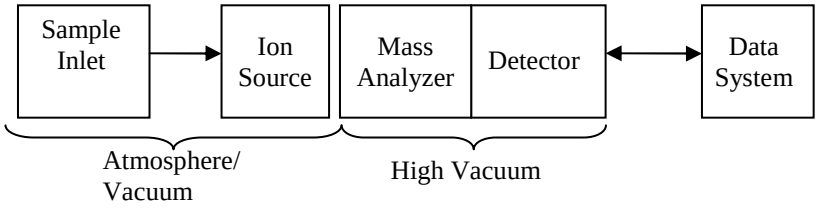

Figure 1. Schematic diagram of the mass spectrometry technique

Results are presented as a *Mass Spectrum:* a two-dimensional representation of signal intensity (abundance of ionic species) (ordinate) versus m/z (abscissa). The most intense peak of a mass spectrum is called *base peak*. In most representations of mass spectral data, the intensity of the base peak is normalized to 100% of relative intensity.

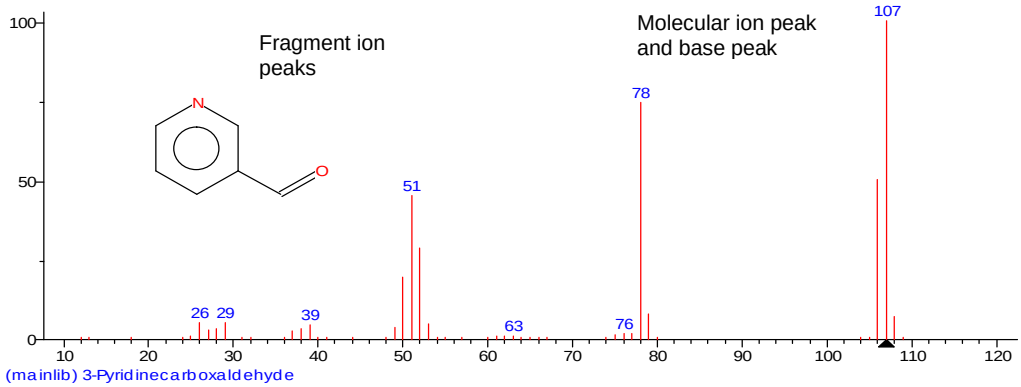

Figure 2. Typical mass spectrum of a 3-Pyridinecarboxaldehyde

Ionization of compounds can be achieved by several mechanisms in the source: ionization of a neutral molecule through electron ejection, electron capture, protonation, cationization, deprotonation, transfer of a charged molecule to the gas phase. Ionic species obtained correspond to whole molecule or fragments.

Mass spectrometry is used with direct insertion probes, but also in tandem with gas chromatography (GC-MS), liquid chromatography (LC-MS) or other separation techniques, fitted with different kind of ionization sources. The basis of these combined techniques is reviewed in this contribution. Examples of applications will be given elsewhere in this Handbook.

2. From sample to mass spectrometer

2.1. Sample introduction in GC-MS

When an analysis of a sample is to be performed by GC-MS, it is necessary to plan some previous steps. Often, if the sample is introduced as it is, no information is obtained, and, in some cases, the apparatus can be damaged. In a gas chromatograph, the sample is introduced in an injector where the gases or vapours resulting from the sample are normally split and part of them are introduced into the chromatographic column by means of a carrier gas. As a result the effluent enters continuously in the ionization source. There are other injection configurations such as splitless (where there is no split of the vapour gases before the column), on column and other specific for large volumes.

The first condition that analytes have to fulfill is to be volatile and thermostable. Some small molecules are volatile and, as a result, no preparation is needed if a convenient column to analyse them is used. But many molecules are not volatile enough, especially if they are polar. Therefore, chemical strategies must be planned to improve their volatility. There are many derivatization reactions for gas chromatography described in the literature, the most used among them, are methylation and silylation of polar functional groups such as –OH and $–NH_2$.

Different injection ports are available to inject different kind of samples.

- Direct injection; liquid or gas samples can be injected into the injection port using a syringe. Packed columns can analyze some hundred micrograms of analytes meanwhile capillary columns can only be charged with less than ten micrograms.
- Head-space injector is a common system used to analyse gases and vapours in equilibrium with liquid or solid samples (studies of residual solvents, contaminants, etc). The sample is introduced in a vial, stopped with a septum, closed hermetically, and placed on a thermostatic block at a determined temperature. After an equilibration time with the inner atmosphere, an aliquot of that vapour phase is injected into the column.
- Cooled injection devices are very versatile. In the past, they were designed for the analysis of very volatile molecules, but today combinations of cooled and heated areas (liquid N_2, decompression of CO_2, peltiers and electrical resistances) give to this devices a wide applications range. They allow venting the solvent, avoiding its entrance in the column, and focusing the analytes in bands of its chromatographic analysis. A simple injector of this kind is used in mineral analysis to trap CO_2 to be analyzed by isotope ratio mass spectrometry (IRMS).
- Pyrolysis of the sample is useful in some applications, such as in oil and polymer studies. The sample is introduced in a high temperature oven and a ramp of temperature is activated (50 – 1000 ºC). During the ramp heating, the sample decomposes and the fragments of the molecules resulting from the pyrolysis are analysed.
- Elemental analyzers fitted with GC are also used to introduce the gases resulting from the combustion or pyrolysis of a sample into a mass spectrometer. N_2, CO_2, H_2O and SO_2 yielded by combustion, or CO and H_2 coming from pyrolysis of a sample are analysed in a mass spectrometer, usually to study the isotopic composition of the elements N, C, H, O and S in the analyte.
- Fibers and other absorbents or adsorbents are also used as a trap for specific substances in liquid or gaseous samples (water, atmosphere…). The material in which the analytes are adsorbed is introduced in the injector and heated to desorb the analytes, which are then separated and analyzed in the mass spectrometer.

2.2. Ionization Sources for GC-MS

2.2.1. Electron Impact Ionization

In a vacuum region, a current passes through a metallic filament, chosen as the source of electrons. An electrostatic field is used to detach electrons from the heated filament, which are focused so as to form a beam of electrons that will impact into the analyte area. This area is the end of the

column or the cup of a direct introduction probe, in its way to the counter electrode. As a result of electron impact (EI), the analyte molecules are ionized and fragmented. Using electric fields and lens, the resulting ions are driven into the spectrometer where they are analyzed. Usually, a wide range of molecular fragments is obtained, increasing in number when the energy of the electron beam increases. Usually only positive ions are analyzed. Extensive EI ionization spectra databases are available in the literature.

2.2.2. Chemical Ionization

While EI is a very strong form of ionization, chemical ionization (CI) is very gentle. Usually EI results in a great number of fragments of the molecule and often the molecular ion signal cannot be observed. In CI, the number of signals is less than in EI and, as a result, the pseudomolecular ion yields a high signal. A reagent gas is ionized in the ion source by electron impact, and the resulting ions are accelerated and they impact against the analyte molecules. The obtained fingerprint depends on the energy, pressure, temperature, purity, and nature of the gases used to ionize. Methane and ammonia are the most common used gases. The mechanisms of ion formation are: proton transfer, exchange of charge, formation of adducts and abstraction of an anion. In each experiment, the identification of the ions must be studied.

2.3. Sample introduction in LC-MS

The preparation of the sample is very important before attempting its analysis by LC-MS. It is important to choose a suitable solvent in order to get the maximum ionization efficiency of the analyte, because using a mass spectrometer we will only analyze ions: positive or negative. The simplest case is an aqueous solution of an ionic salt: it yields solvated anions and cations in solution. But many substances are not ionic and then ionization is not possible or only in a very small degree.

The election of the solvent depends on both the nature of the analyte and the ionization source of the spectrometer. When the sample is introduced via direct infusion, the solvent and the liquid carrier are usually the same. In this case, a syringe is used to push the liquid solution to the interface. If we use a liquid chromatograph, the composition of the mobile phase is usually different from the solvent of the sample and, in addition, it can vary with time (gradient). In general, the solvents used in LC-MS must be volatile or vaporizable, free of crystallizations or other solid formations when they are vaporized to avoid obstruction of the capillary entrance to the mass spectrometer.

2.4. Ionization sources for LC-MS

2.4.1. Electrospray Ionization

Nowadays the most universal ion source in High Performance LC-MS is electrospray ionization (ESI). In fact, we would say nitrogen- assisted electrospray, usually named by its acronym ESI. The liquid that carries the analytes is pushed to a nebulizer installed into a chamber at atmospheric pressure. The chamber is placed in front of a cone that has a capillary orifice, allowing the entrance of the ions in the mass spectrometer. There is a potential difference between the cone and the spray tip, that is, an electrostatic field. The polarity of this field determines the ions to be analyzed. In addition, the chamber is heated in order to evaporate the solvent. In these conditions, the ions that are in solution are both desolvated and attracted to the entrance of the spectrometer.

2.4.2. Atmospheric Pressure Chemical Ionisation

Atmospheric pressure chemical ionization (APCI) sources can be considered as a variant of ESI sources, in which a discharge needle is added in the chamber. Between the needle and ground, a high difference of potential is established, which creates a corona discharge that ionizes molecules present in the spray (mainly from the solvent). In ESI sources, the ions must be present in the sprayed solution, while in APCI sources, the ions formed are accelerated in the electrostatic field originating at the tip of the cone. In this pathway, the formed ions impact on the neutral analyte

molecules and transfer the electrical charge to them. As a result, the analyte molecules become ions, which are "visible" for the mass spectrometer.

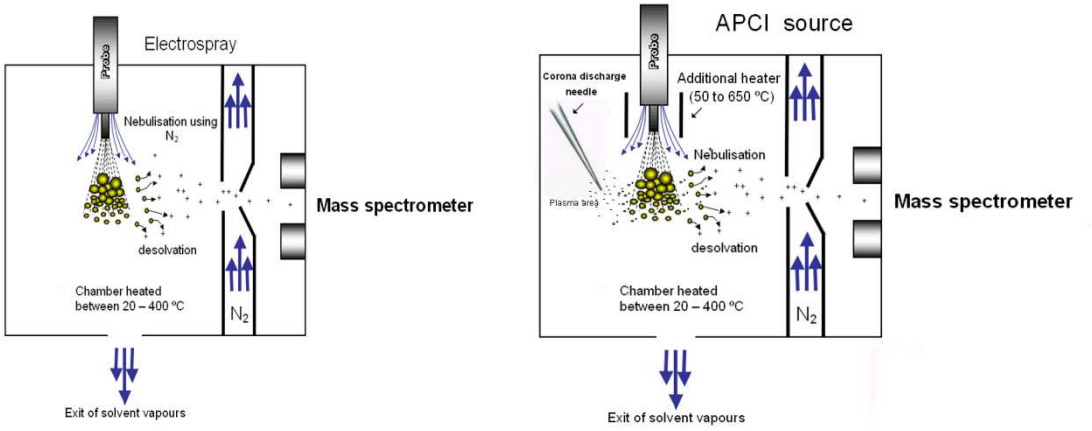

Figure 3. Schematic representation of ESI and APCI sources

2.4.3. Atmospheric Pressure Photoionization

In atmospheric pressure photoionization (APPI) sources, the ionization of the solvent molecules, which will ionize the analyte molecules by charge transfer, is produced by an intense source of ultraviolet light that illuminates the spray.

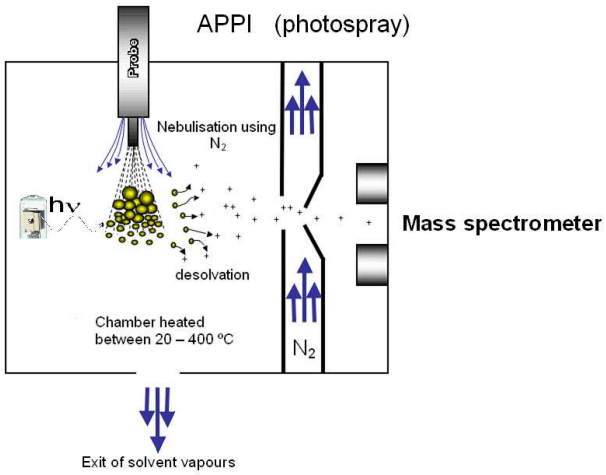

Figure 4. Schematic representation of an APPI source

2.4.4. Plasma torch

When elemental analysis is the goal using a mass spectrometer, a plasma torch is the ionization source of choice. The resulting technique is referred to as inductively-coupled plasma mass spectrometry (ICP-MS). Usually three concentric flows of argon are used in this source: in the centre a nebulizer is used to spray the analytical solution, a second flow of argon is used in a corona to generate the plasma inside a strong radiofrequency field, and finally a third argon flow protects the instrument parts from the high temperatures reached in the plasma region (up to 10,000 K). When a nebulized sample enters into the plasma region, almost all the molecules break down in their atomic components and many of the atoms lose one electron becoming single charged ions. Aided by an electrostatic field and often by the aspiration of a vacuum pump, the created ions follow a beam toward the tip of the sampling cone, where a capillary hole allows their entrance into

the mass spectrometer for mass analysis. The ICP-MS coupling results in a powerful elemental analyzer, very sensitive and capable to cover almost all the elements of the periodic table. For many metals and non metals, it reaches parts per trillion of the element in the analytical solution.

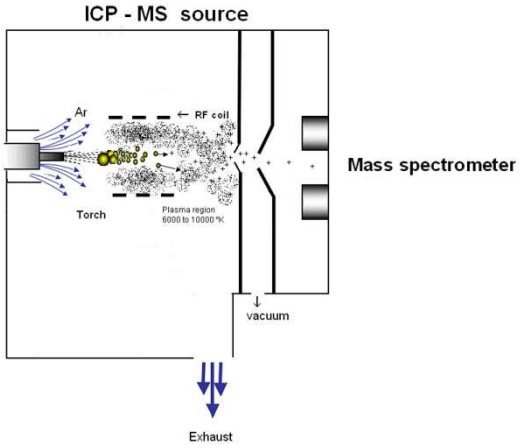

Figure 5. Schematic representation of an ICP-MS source

2.4.5. Matrix Assisted Laser Desorption/Ionization

Matrix-assisted laser desorption/ionization (MALDI) is an ionization technique mainly used with time-of-flight (TOF) mass spectrometers. Samples are prepared as solid solutions, where the solvent is a substance, named matrix, able to be ionized by a laser beam. When the solid solution is irradiated with a pulsed laser beam (in the ultraviolet range) the heat evaporates small quantities of the solid and a small part is ionized mainly by charge transfer. An electrical field attracts the ions into the spectrometer.

Polymers and biopolymers are the main substances analyzed by using this technique, but it can also be used for the analysis of many organic molecules, inorganic complexes and minerals. Its characteristic is the softness, and usually only single charged ions are obtained. When combined with the high resolution of a TOF analyzer, results in a very good tool to determine molecular weights with high accuracy.

The solid solution is prepared from a liquid solution of the matrix in a volatile solvent in which the sample is also dissolved. A small drop of few microliters of the solution containing the sample and the matrix is placed on a plate and the liquid solvent is allowed to evaporate (acetonitrile, methanol, aqueous trifluoroacetic acid…). The result is a small dry spot on which a laser beam is focused. The more common matrices are 2, 5-dihydroxybenzoic acid (DHB); alpha-cyano-4-hydroxycinnamic acid; 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid); ferulic acid, picolinic acid, and other molecules with aromatic rings or conjugated double bonds.

Many of the novel sources being studied nowadays are variants of those described above, while others are innovative. All these novel sources are aimed at improving sensitivity, stability and easy operation.

3. Mass analyzers

The mass analysis of ions in the gas phase is based on the interactions of ions with electric and magnetic fields. Until the 1960s, most of the mass spectrometers were dedicated to physics, organometallics and organic chemistry and were based on magnetic sectors. In these spectrometers, high-resolution conditions were generally achieved by means of an electrostatic sector. The double-focusing systems were instruments of larger dimensions and difficult to use, and were mainly used in high-level and academic environments. The development of devices based on electrodynamic fields led to the production of quadrupoles and ion traps of small dimensions and mass spectrometers became bench-top instruments. Their easiness of use, as compared to double-

focusing instruments, and the possibility of interfacing them easily with data systems moved mass spectrometry to the application labs.

The mass analyzer is the portion of the mass spectrometer where charged analyte molecules are separated based on the *m/z* ratio. In addition to the choice of ionization source, many different types of mass analyzers can be chosen. These include magnetic sector, ion trap (IT), quadrupole systems, TOF and Fourier transform mass analyzers. Each mass analyzer has its advantages and disadvantages. Here, a description of the currently most widely employed analyzers is given.

3.1. Time-of-flight

The linear TOF mass analyzer is, from the theoretical point of view, the simplest mass analyzer. It simply consists of an ion source and a detector, and between them is a region under vacuum. It is based on accelerating a group of ions towards the detector where all the ions are given the same amount of energy through an accelerating potential. Because the ions have the same energy but different mass, the lighter ions reach the detector first because of their greater velocity. As a result ions with different *m/z* ratios reach the detector at different times, which is proportional to the square root of their *m/z* value. The calibration of the time scale with respect to the *m/z* value can be easily obtained by injection of samples of known mass (standards of calibration). When compared to quadrupole (Q) and sector systems, this analyzer cannot operate in a continuous mode. The main advantages of this analyzer are its high speed and wide mass range (when analyzing high-molecular weight compounds such as polymers or biomolecules, TOF mass analyzers are often employed due to their virtually unlimited mass range).

In its hybrid configuration, the Q-TOF instrument is capable of performing MS/MS analysis. The ions of interest generated in the source, are selected by the quadrupole mass filter Q1. The collision takes place in Q2. The product ions are analysed by the TOF analyzer, and thus accurate masses of the collisionally generated product ions (as well as their precursors) can be easily obtained, allowing the determination of their elemental composition. The resolution of TOF instruments can be improved by using a reflectron. Today, resolutions up to 20.000 are achieved by commercial instruments.

With the same aim (MS/MS analysis) modern instruments with two TOF analyzers (TOF/TOF configuration) are available.

3.2. Fourier transform ion cyclotron resonance

Fourier transform ion cyclotron resonance (FTICR) is a type of mass analyzer which involves accelerating ions in a particle accelerator known as cyclotron. The charged particles move in circular paths perpendicular to a uniform magnetic field at a characteristic frequency known as the cyclotron frequency, which is dependent on the *m/z* ratio. Pulsed radiofrequency (RF) radiation is used to excite ions to paths of larger radius and this radiation causes them to move in phase, which creates an image current. A Fourier transform is then employed to obtain oscillation frequencies for ions with different masses, resulting in an accurate reading of their *m/z* ratio and high-mass resolution. The FTICR analyzer differs from other kinds of mass analyzers in that ions are not separated prior to detection. Thus, the different ions are not detected in different places as sector instruments or at different times as in TOF instruments, but all ions are detected simultaneously over a given period of time. This technique offers very high resolution (up to 100.000) that can be increased (up to 1.000.000) by increasing the magnetic field strength of the magnet or by increasing the detection duration. It is important to note that a signal is generated only by the coherent motion of an ion under ultra-high vacuum (10^{-11}-10^{-9} Torr) and that the signal has to be measured for a minimum time (from 500 ms to 1s) to provide high resolution. This could be a drawback, if compared to TOF systems, in order to couple them with ultra-performance liquid chromatography (UPLC) systems.

Nowadays, some hybrid instruments using this technology such as the Orbitrap are available. These instruments allow MS^n experiments to be performed previous to the FTMS analyzer, thus providing ultra-high resolution and accurate mass data both in precursor and in product ions.

3.3. Quadrupole mass filter

Quadrupole mass analyzers are still the most common mass analyzers in existence today. In the quadrupole mass filter, the application of a particular combination of DC and RF voltages to four parallel metal rods creates a filtering device through which only ions of a defined *m/z* value are transmitted. Changing the ratio of the voltages changes the *m/z* value of the ion that is passed through the detector. The quadrupole mass filter can also be operated in other modes, such as passing a mass range of ions through the detector. If only the rf portion of the voltage is applied to the rods, essentially all ions are passed through to the detector. Quadrupole instruments can typically resolve ions that differ by one mass unit. Hexapole and octapole ion guides have also been devised, and they operate under similar principles. Quadrupoles offer three main advantages: they tolerate relatively high pressures, they have significant mass range with the capability of analyzing up to an *m/z* of 4000 and they are relatively low cost instruments. The triple quadrupole mass spectrometer consists of three quadrupoles connected in tandem. The first and third quadrupoles act as mass filters, while the second quadrupole (non-mass filtering) serves as a collision chamber. In this way, tandem mass spectrometry can be accomplished through different MS/MS experiments: the product ion scan (where the product ions from a precursor were scanned), the precursor ion scan (where one examines all of the precursor ions capable of fragmenting to produce a particular product ion), the neutral loss scan (that involves looking at pairs of precursor and product ions that differ by a defined constant neutral loss) and the selected reaction monitoring (SRM). This one is the most sensitive mode and consists in simultaneously monitoring multiple precursor-product ion pairs.

3.4. Quadrupole Ion trap analyzer

The difference with a quadrupole is that ions, rather than passing through a quadrupole analyzer with a superimposed radio frequency field are trapped in a radio frequency field. An ion trap analyzer consists of two hyperbolic endcap electrodes and a ring electrode into a compact device that serves as mass analyzer. The motion of the ions induced by the electric field of the ions allows them to be trapped or ejected from the ion trap. In the normal mode, the radio frequency is scanned to resonantly excite and, therefore, eject ions through small holes in the endcap to the detector. As the RF is scanned to higher frequencies, higher m/z ions are excited, ejected and detected. A very useful feature in ion traps is that it is possible to isolate one ion species by ejecting the rest from the trap. The isolated ions can be fragmented by collisional activation and the fragments detected. As a result, quadrupole ion traps have been used in MS^n applications. The advantages of quadrupole ion traps include the possibility of performing LC-MS/MS in real time, their compact size and their ability to trap and accumulate ions to provide a better ion signal.

3.5. Linear Ion Trap

The linear ion trap differs from the 3D ion trap as it confines ions along the axis of a quadrupole mass analyzer using a two dimensional (2D) RF field with potentials applied to end electrodes. It has a greater dynamic range and an improved quantitative range of quantitative analysis if compared to the 3D trap.

3.6. Double-Focusing Magnetic Sector

In the double-focusing magnetic sector, ions are accelerated into a magnetic field using an electric field. A charged particle traveling through a magnetic field will travel in a circular motion with a radius that depends on the speed of the ion, the magnetic field strength and the ion's *m/z* ratio. To increase the drawback of its low resolution, an electrostatic analyzer is added to focus the ions but this technique results in a decrease in sensitivity. These are called double-sector instruments and are used with ESI, FAB and EI ionization. However, they are not widely used today mainly because of their large size and the success of TOF, quadrupole and FTMS analyzers.

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