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Chapter

Mass Spectrometry and Its Importance for the Analysis and Discovery of Active Molecules in Natural Products

Paco Noriega, Gabriela Gortaire and Edison Osorio

Abstract

Mass spectrometry is one of the best techniques for analyzing the structure of a molecule. It usually provides information about the molecular weight of a substance, and it can present atomic mass units and up to ten thousandths of atomic mass units depending on the accuracy of the mass analyzer. In addition, it provides information on the positive ions formed in the ionization process, which is linked to the chemical structure of the molecule and the nature of the bonds. This technique is widely used for analyzing compounds from natural products. The development of the technique combined with the use of software and databases has been remarkable in recent years, improving the ionization processes and the ion analysis. Since natural products generally constitute a mixture of a complex quantity of components, mechanisms have been developed for coupling to chromatographic techniques of various kinds. This review aims to show how mass spectrometry has contributed to the qualitative quality control in natural products, as well as in the finding of new metabolites of industrial interest.

Keywords: Mass spectrometry, Natural Products, GC/MS, HPLC/MS, new metabolites

1. Introduction

Mass spectrometry is an analytical technique whose purpose is discovering new molecules, determining quantities of known components and determining structural and chemical properties of a molecule.

The detection capability in mass spectrometry is very small, of about 10^{-12} grams and its application field is multifaceted, being used in industries such as: chemical, pharmaceutical, biotechnology, food, among others. It is frequently used in environmental and medical sciences, and in molecular biology.

Some of its most common uses are related to:

Performing doping tests in athletes [1].
Locating petroleum reservoirs through the use of precursors in the rocks [2].
Controlling fermentation of products in biotechnology processes [3].
Determining genetic damages [4].

Determining the presence of contaminants in food [5].
Identifying the structure of biomolecules, such as nucleic acids [4].
Analyzing the biodegradation of medications [6].
Establishing the age of geochemical and archeological samples [7].

The origin of mass spectrometry goes back to the experiments by J. J. Thompson, which evidenced, on one side, the presence of electrons, and on the other side, the presence of positive radiation, when energy falls into a vacuum tube to which a difference in electric potential was applied [8]. Thompson remarked the importance that this new technique might have in the field of chemical analysis and described it in his book "Rays of Positive Electricity and Their Application to Chemical Analysis" [9]; however, despite this interesting possibility of use, mass spectrometry was relegated to the field of experiments in physics. It was not until the 1940s, that the first analytical mass spectrometers started to be developed.

At present, Mass Spectrometry and Nuclear Magnetic Resonance, are the most complete and widespread techniques in educational and research labs around the world, regarding the study and discovery of organic molecules. In the field of natural products many of the studies about secondary metabolites have been validated in a mass spectrometer, since it is a very complete technique for the identification and control of this type of substances.

2. Mass spectrometry, fundamentals and instrumentation

A mass spectrometer is an instrument with the capability of measuring the mass of a molecule after it has been ionized. Due to the extremely small mass of a molecule expressed in grams or kilograms, it is more convenient to measure its molecular mass, expressed as mols; for example, the mass of a hydrogen atom is 1.66×10^{-24} grams, but its mol is approximately 1 gram, or if it is desired in Daltons, considering that this unit is equivalent to 1/12 of the mass of an isotope carbon-12.

The spectrometry does not directly measure the mass of an isotope, but rather its mass-to-charge ratio of the ions that are formed (m/z), where z is the charge, most of the ions formed in the mass spectrometry have a value of charge of $z = 1$.

The mass spectrometers are constituted by various components, namely: 1) system for introducing the sample, 2) ionization source, 3) mass analyzer, 4) detection system and 5) data analysis system. Components 2, 3 and 4 should be necessarily subject to a vacuum system; a summarized scheme of the instrument may be seen in **Figure 1**.

The parts that diversify and create the different instrumentation variants are the ionization source and the mass analyzer.

A classic system requires the formation of ions in gaseous phase; however, the latest instrumentation advances have generated methodologies that enable introducing molecules in liquid phase or even in solid phase. The process for generating the mass spectrum is:

1. Production of ions and fragmentation

2. Separation of the ions according to their mass-to-charge ratio

3. Detection

The ion production, also known as ionization, occurs in different manners. The classic one is by means of the interaction of an electric current (electronic

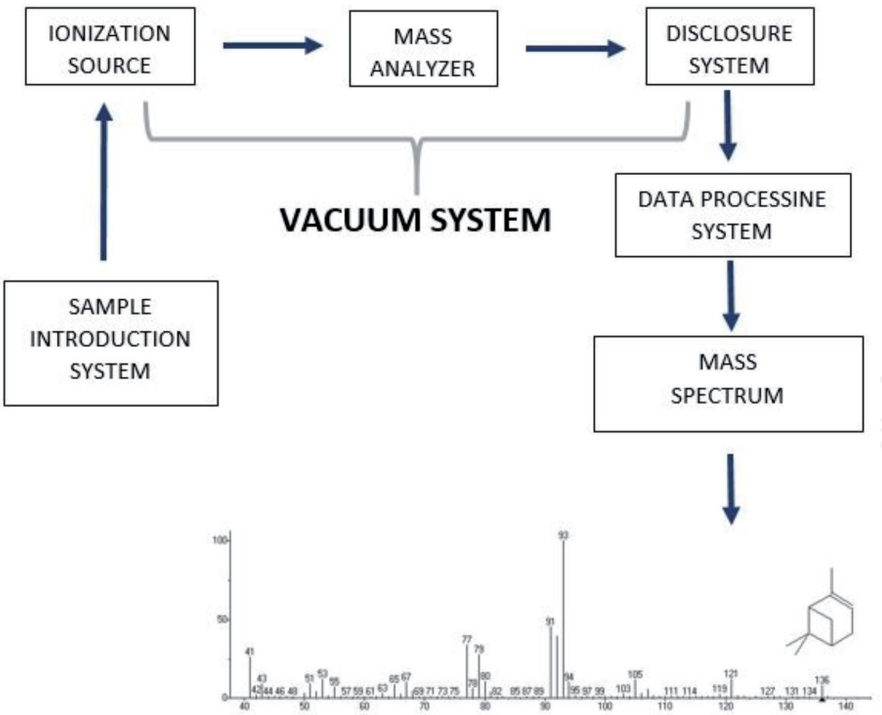

Figure 1.
Scheme of a mass spectrometer.

ionization) with a substance in vapor phase. The energy is considered as high and has been standardized at 70 eV, which is greater than the energy of the bonds of any molecule.

Another method is chemical ionization (Cl) where the rupture is produced due to the incidence of a gaseous substance with an extra proton, for example CH_5^+, on substances in vapor phase. Chemical ionization is less energetic than electronic ionization and produces less fragmentation.

Other types of ionization of recent development are:

(Fast Atom Bombardment, FAB): impact of atoms at high velocity on a sample dissolved in a liquid matrix.

(Secondary Ion Mass Spectrometry, SIMS): impact of ions at high velocity on a thin film of sample deposited on a metal substrate, or dissolved in a liquid matrix (Liquid SIMS).

(Plasma Desorption, PD): impact of fragments of nuclear fission, for example, of the ^{252}Cf on a solid sample deposited on a metal foil.

(Matrix Assisted Laser Desorption Ionization, MALDI): impact of high energy photons on a sample enclosed in an organic solid matrix.

(Field Desorption, FD): imposition of a strong electric field on a sample deposited on a special metal probe.

(Electrospray Ionization, ESI): formation of charged liquid particles which are emitted by desorption or desolvation.

The purpose of the mass analyzers is to separate the ions according to their mass-to-charge ratio. The mass analyzers have different features, namely:

Magnetic sector mass spectrometry. They deviate the trajectory of the ions in circular trajectories that depend on the momentum/charge ratio.

Quadrupole. Consists of 4 poles or bars arranged parallelly, the separation of the ions is the result of the application of a combination of continuous (DC) and alternating at a radiofrequency (RF) electric fields.

Ion trap. The ion trap operates similarly to the quadrupole, with the difference that it may hold and store the ions inside the trap.

Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR). The ions are trapped electrostatically in a cubic cell inside a constant magnetic field.

Time off flight (TOF). They separate ions according to the time employed to travel a particular distance; an ion of smaller mass will have a larger velocity, based on equation $Ec = mv^2/2$ that relates kinetic energy with mass and velocity.

Figure 2 shows the ionization sources, as well as the diverse analyzers which will finally determine the types of instruments that are found in the market.

In most mass spectrometry analyzers, with the exception of the FT-ICR, ions are detected after the separation, transforming the collision energy of the ions on the detector, in order to produce in it further emission of electron and photon ions that are opportunely measured in charge or light detectors.

A mass spectrometer of recent development is the orbitrap, which is a modification of the ionic trap; in this the ions are injected tangentially in an electric field, and they remain turning around a central electrode, highlighting a high mass resolution of them [10].

2.1 The mass spectrum

A mass spectrum consists of a diagram of ionic abundance as a function of its mass-to-charge ratio. The mass spectra are reported as simple histograms, such as the one seen in **Figure 3**.

In this example all the ions are positively charged; it is observed the molecular ion at a value m/z of 32, a majority ion at m/z 31 due to the loss of the H from the OH group, and an ion at m/z 15 which is due to the loss of the hydroxyl (OH).

Depending on the ionization source and the energy employed, spectra will be available with more or less positive fragments aside from the information about the molecular weight of the molecule.

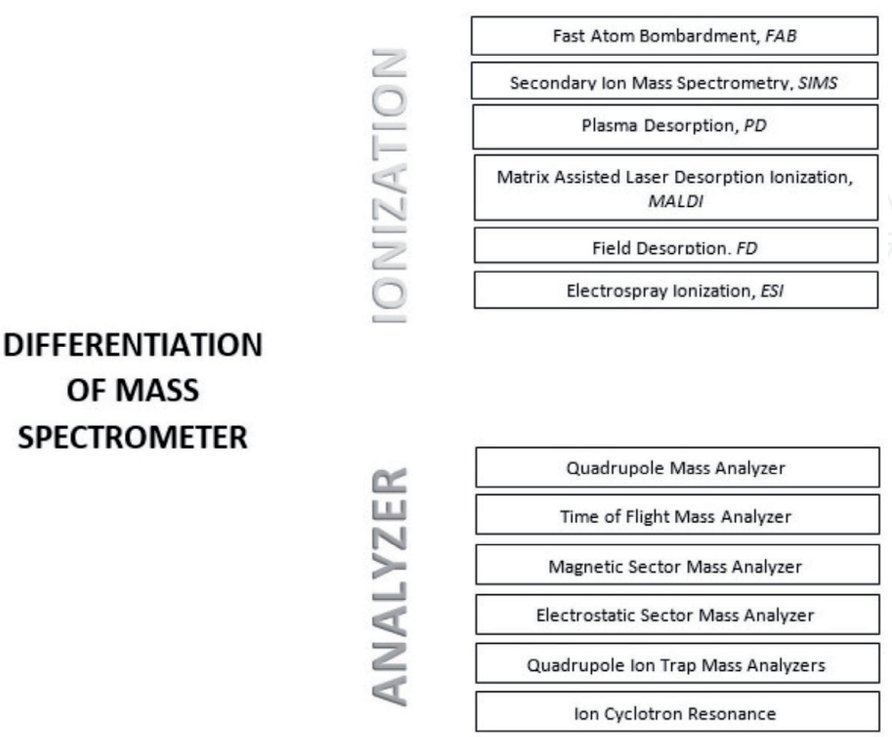

Figure 2.
Ionization sources and analyzers in mass spectrometry.

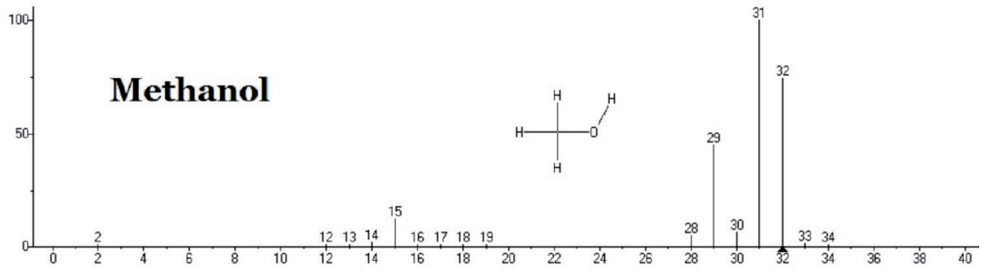

Figure 3.
Mass spectrum of the methanol.

2.2 Introducing the sample in the mass spectrometer

High purity solid samples may be directly placed in a probe inside the instrument, in which it occurs the evaporation of the sample that has been introduced in the vacuum system. Gaseous or liquid samples require special systems for feeding the regulated flow.

When the sample to be analyzed is a complex mixture of compounds, chromatography equipment may be coupled to the mass spectrometer, such as gas chromatography (GC) or high-performance liquid chromatography (HPLC). The GC/MS systems were developed in the 60s, because the samples that enter to chromatographic column are already in gaseous phase and this facilitates its introduction in the mass spectrometer; an instrument of this type is observed in **Figure 4**. The coupling with liquid chromatography did not occur until the 80s, due to difficulty of producing an operational vacuum system.

At present, the development of GC/MS and LC/MS systems provide a great variety of instruments that facilitate the separation and analysis work, both for quantifying as well as for discovering new structures, with the field of natural products being one of the most benefited from these latest advances.

2.3 MS-MS analysis

The coupling of two MS–MS mass analysis states is useful for analyzing compounds in complex mixtures and for determining the structure of unknown molecules.

Figure 4.
Gas chromatography equipment coupled to mass spectrometer. Life Sciences Laboratory, Salesian Polytechnic University.

The MS–MS evaluation offers the possibility of analyzing the ions formed in a further fragmentation of those ions formed in the first test.

If the first ionization technique offers the possibility of having various fragments in a highly purified sample, the second ionization offers the possibility of acquiring valuable structural information, and through it achieve a high possibility of determining the structure of a new molecule.

3. Mass spectrometry and natural products

The high development achieved in the last hundred years by mass spectrometry with a great variability of techniques and instruments, makes possible that basically all molecules that are part of natural products may be analyzed, both qualitatively and quantitatively [11, 12], **Figure 5** shows various spectra of natural substances obtained by electronic ionization. The extracts coming from biological matrices with natural products are generally a mixture of various compounds, and thus it is very common the use of GC or LC coupled systems [13, 14].

New techniques such as Electrospray Ionization, have increased the number of possible biomolecules to be analyzed, including those of high molecular weights [15]. Similarly, the use of powerful mass analyzers makes it possible to analyze molecular ions or fractionated ions with an extremely efficient resolution [16].

3.1 Essential oils

Essential oils are metabolites of volatile nature; therefore, studies of chemical composition are relatively simple, generally achieving percentages above 90% when investigating the molecules that compose these natural products [17, 18].

The combination of evaluations of mass spectra and retention indices provide information that may be verified in specific databases for this type of compounds

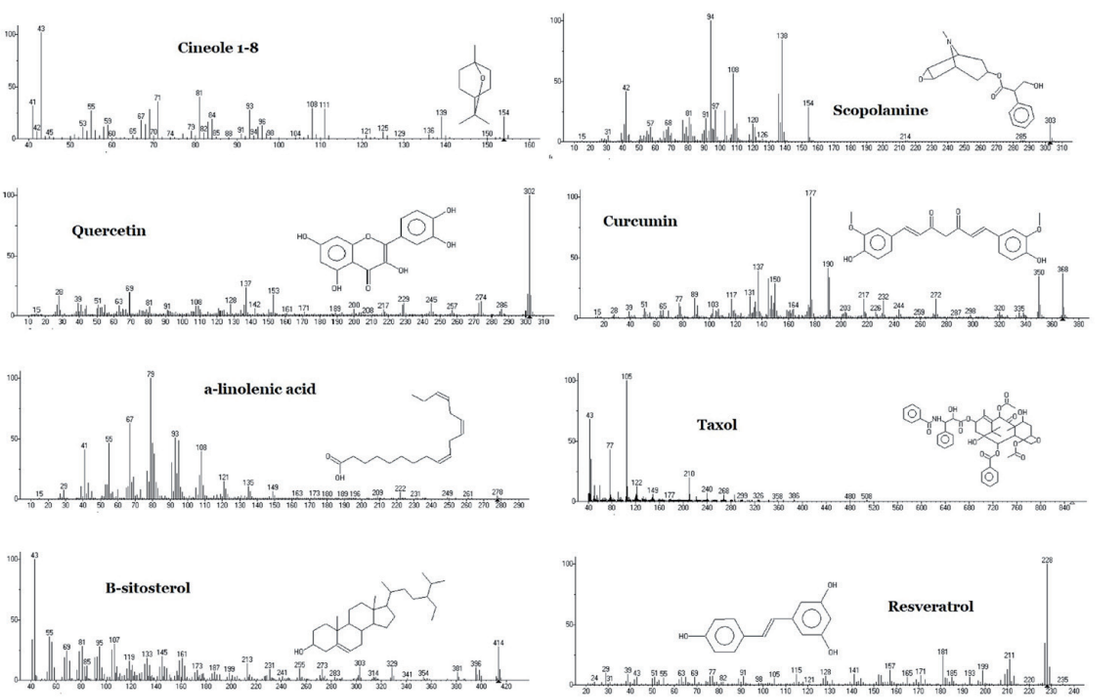

Figure 5.
Spectra of natural molecules obtained by electronic ionization.

[19]. Further studies of GC/MS in molecules separated in TLC, may reveal specific biological properties such as antibacterial or antioxidant [20].

3.2 Fatty acids

Various plant species contain saturated and unsaturated fatty acids, studies are generally carried out by GC/MS, although the compounds of high molecular weight are non-volatile, this is solved employing chemical derivatization methodologies, forming methyl or ethyl esters [21, 22]. Numerous species of nutritional and pharmaceutical interest such as *Plukenetia volubilis* [23], *Borrago officinalis* [24], and fish oil [25], are analyzed using this methodology.

3.3 Aromas and flavors

The aromas and flavors in fruits and vegetables are fundamental for distinguishing their taste features, given their volatility the GC/MS equipment is the ideal for understanding the chemistry of this group of substances. Many of these molecules are very volatile and therefore are not removable in vapor stream, for introducing them in the chromatographic system they are previously extracted with non-polar solvents [26] or may be directly injected with head space introduction systems [27, 28].

3.4 Phenols and polyphenols

Few phenolic compounds may be directly analyzed in an GC/MS system, generally those structures of low molecular weight [29].

Most of the phenolic and polyphenolic compounds are non-volatile and have two analytic paths for their structures to be determined, the first is through chemical derivatization, using the mechanism of sylitation, which make them volatile [30–31].

The second is through instruments that couple HPLC to mass spectrometry, which has made that a large part of the tests are carried out directly, after their separation in one column. The advantage of the electrospray ionization technique is the possibility of ionizing molecules that lack of volatility [32, 33].

The use of mass analyzers of resolution greater than the quadrupole, such as the TOF or the orbitrap, has resulted in values of m/z that reach more precise levels, which has resulted in greater confidence in the identification of a substance [34–36].

3.5 Alkaloids

The alkaloids are active ingredients whose structural feature is to have nitrogen in their structure, many of these molecules have a significant biological activity. Some alkaloids may be directly analyzed in GC/MS equipment, such as nicotine and other present in tobacco [37], caffeine and xanthine alkaloids [38, 39], and others whose volatility enables its separation in gas chromatography such as tropane alkaloids [40].

The most frequently used method in the test of these metabolites is the LC/MS in its diverse variants, such as the LC/MS [41], LC/MS–MS [42], some with a greater mass resolution such as the HPLC-TOF-MS [43]. The use of powerful mass analyzers such as Orbitrap, may lead to the discovery of new structures of this nature [44].

3.6 Cannabinols

The cannabinols constitute a family of natural products of about 70 compounds, of which the most important are the THC and the CBD [45]. The discovery of the endocannabinoid 1 and endocannabinoid 2 systems, 4 decades ago, awakened the medicinal interest of these substances [46]. These compounds have a high solubility in non-polar solvents and are volatile at the injection temperatures in a gas chromatography equipment, consequently a qualification and quantification of them occur in a very good manner in GC/MS systems [47, 48]. The LC/MS is also useful in the analysis of these substances [49–50].

4. Conclusions

Practically all the natural products may be analyzed by means of mass spectrometry, its high sensitivity and detailed structural information, make it an essential tool in research and product development labs.

Equipment with analyzers that have high resolutions can provide us with extremely exact values of molecular ions, and thus being able to differentiate the nature of the molecules.

At present there is a great variety of equipment coupled to LC or GC separation systems, which is ideal for the natural extracts that are generally a mixture of substances of diverse nature. Similarly, the combination of ionization and analyzer techniques, has been able to provide an instrumental variability that currently convert it in a technique highly appreciated for the discovery of new natural structures.

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