Gas Chromatography-Mass Spectrometry

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Additional information is available at the end of the chapter

1. Introduction

Gas chromatography (GC) is a widely applied technique in many branches of science and technology. For over half a century, GC has played a fundamental role in determining how many components and in what proportion they exist in a mixture. However, the ability to establish the nature and chemical structure of these separated and quantified compounds is ambiguous and reduced, and requires a spectroscopic detection system. The most used, is the mass spectrometric detector (MSD), which allows obtaining the "fingerprint" of the molecule, i.e., its mass spectrum. Mass spectra provide information on the molecular weight, elemental composition, if a high resolution mass spectrometer is used, functional groups present, and, in some cases, the geometry and spatial isomerism of the molecule.

2. Gas chromatography

In a gas chromatographic system, the sample to be analyzed may be a liquid solution or a collection of molecules adsorbed on a surface, e.g., the solid-phase microextraction (SPME) system. During the transfer into the GC, the sample is volatilized by rapid exposure to a zone kept at relatively high temperature (200-300°C) and mixed with a stream of carrier gas (Ar, He, N_2, or H_2). The resulting gaseous mixture enters the separation section, a chromatographic column, which in its current version is a fused-silica tubular capillary coated internally with a thin polymer film. Upon their displacement through the column, analyte molecules are partitioned between the gas carrier stream (mobile phase) and the polymer coating (stationary phase), to an extent which depends mainly on their chemical structure. At the end of the separation section, the molecules reach a detection system in which a specific physical property (thermal conductivity) or a physico-chemical process (ionization in a flame, electron capture) gives rise to an electric signal which is proportional to the amount of molecules of the same

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identity. A data system permits to process these data to produce a graph of the variation of this detector signal with time (chromatogram). Thus, four principal sections are distinguishable in the chromatograph: introduction (injector), separation (chromatographic column), detection, and data handling units. Each section has its own function and its responsibility for the quality of the analysis and the results obtained. The injection system, for example, should ideally transfer the sample to the column quantitatively, without discrimination on molecular weights or volatility, and without chemical alteration (decomposition or isomerization). It is a critical step, especially for quantitative analysis. For correct GC operation, among other conditions, this gateway to the column should remain unpolluted, clean, inert, and leak-free. The main requirement for an analyte in GC is that it should be volatile enough to be present in detectable amounts in the mobile phase. Substances with low vapor pressure will not enter the chromatographic column, will accumulate at the injection system, and may eventually clog its conduits. Very polar, thermolabile, ionic and high-molecular weight compounds are not compatible with regular GC analysis. Depending on the molecular structure of the analyte and the functional groups available, it is possible in some cases to obtain a chemical derivative which has a higher vapor pressure and is therefore more amenable to GC analysis.

One of the most important characteristics of the chromatographic column is its resolution, or the ability to separate components with very similar distribution constants between the mobile and stationary phases (K_D). Chromatographic resolution is a function of many operational parameters. Among them, the nature of the stationary phase, mobile phase, temperature, the size of the column, that is, its length (L), inner diameter (ID) and the thickness of the stationary phase (d_f). As the number of components in the mixture increases, and the structural similarity between its components grows (isomerism), longer columns are required for complete compound separation. Alternatively, for the same purpose, one can employ smaller internal diameter columns. Obviously, increasing the length of the column markedly increases the analysis time. So, as the analysis of polyaromatic hydrocarbons (PAHs) or controlled drugs is regularly accomplished using 30 m long columns, the separation of hydrocarbons in gasoline requires longer, 100 m columns.

2.1. Sample preparation for GC analysis

Sample preparation for GC analysis involves techniques which preferentially isolate volatile and semi-volatile substances and prevent the presence of ionic or high molecular weight species in the mixture to be injected into the GC. These techniques can be divided roughly into three major groups: Distillation, Extraction, and Headspace Methods. Additionally, there are some *sui generis* methods which combine techniques from two different groups, for example, SDE, Simultaneous Distillation – Solvent Extraction [1]. Distillation techniques exploit differences (must be large) in physicochemical properties (volatility or vapor pressure). When applied to vegetal material or to other solid matrix which contains volatile compounds, the distillation can be performed in different ways: (1) Steam distillation (SD), (2) hydrodistillation (HD), or (3) Water-Steam distillation. The resulting extracts or distillates are volatile mixtures suitable to GC or GC-MS analysis, which may only require a drying step (addition of sodium sulfate) prior to injection into the chromatograph. Some distillation processes are designed to

isolate particular substances or fractions at reduced pressure (vacuum) in special columns (molecular distillation) and many operate at industrial scale. However, in general, these techniques are not suitable for studying and isolating compounds at trace levels.

In order to improve extraction efficiency and substantially reduce distillation times, micro-wave radiation has been actively used in the past 30 years as heat source [2,3]. Microwave-assisted hydrodistillation (MWHD) is a common example of a lab-scale technique for essential oil and other volatile mixture isolation which requires about ¼ of the time employed when conventional heating is used in HD. Microwave-assisted extraction is used mostly for the extraction of solid samples (plant material, soil, tissue, etc.), with water or an organic solvent as the extracting agent [4].

The second family of techniques includes various methods of extraction where the analyte isolation is based on differences in their solubilities in solvent(s) or in the adsorption or absorption on a material such as a microporous solid (activated carbon, silica gel, alumina, molecular sieves, etc.) or a porous polymer (PDMS, Tenax, Porapak, Chromosorb, synthetic resins, etc.).

Liquid-liquid extraction (LLE) in continuous or batch mode is the most used solubility-based extraction method for liquid samples. Extraction selectivity is achieved by a proper choice of solvent (polarity, boiling point, dielectric constant, hydrogen bonding capacity, availability, accessibility, cost, etc.). The extract obtained must often undergo clean-up and concentration processes. However, LLE poses a number of technical problems, among others, emulsion formation, high cost, long extraction times, automation difficulties, toxic solvent disposal, possibility of cross contamination, etc. Despite these difficulties, the number of published works that involve the use of LLE grew during the last decade [5]. Solid-phase extraction (SPE) has emerged during the last 4 decades as an alternative to LLE [6]. It substantially reduces the use of solvents and combines extraction, clean-up and target analyte concentration into a single step. In addition, the method includes the possibility of automation. Another solubility-based extraction technique employs a solvent at temperature and pressure above its critical point. Supercritical fluid extraction (SFE) requires an initially high investment in equipment, but has been used increasingly in areas in which traces of the extraction agent are not desired (natural products, environmental, food, forensic, and in many industrial applications). In this regard, carbon dioxide is the most common fluid employed because when the extract is returned to standard temperature and pressure conditions, CO_2 separates completely in a spontaneous manner because it is a permanent gas under this conditions. SFE selectivity is achieved by varying the operating conditions (temperature and pressure), which modify the supercritical fluid density and in turn, the solubility of the analyte [7]. The use of co-solvents (ethanol, acetone) is another option, particularly, to enhance the extraction of polar substances. For GC-MS analysis, the SFE extract often requires additional cleaning steps to remove fats or pig-ments, waxes or other high-molecular weight compounds (Figure 1).

Soxhlet extraction is a solubility-based extraction method for solid samples. It´s one of the most traditional extraction methods, used in the analysis of soil, polymers, natural products, etc., in areas such as biochemistry, agricultural sciences, biology, or environmental research. This exhaustive extraction affords mixtures which require clean-up to remove heavy components

and other interferences. Long experimental times and high solvent consumption in Soxhlet extraction have led to its replacement in many applications by most efficient methods, such as Accelerated Solvent Extraction (ASE) [8]. In ASE, the use of higher temperature and pressure permits to attain higher selectivity while significantly reducing the time and the amount of solvent required. Extraction, concentration and clean-up are performed in a single step, and the equipment may be coupled in line with GC-MS.
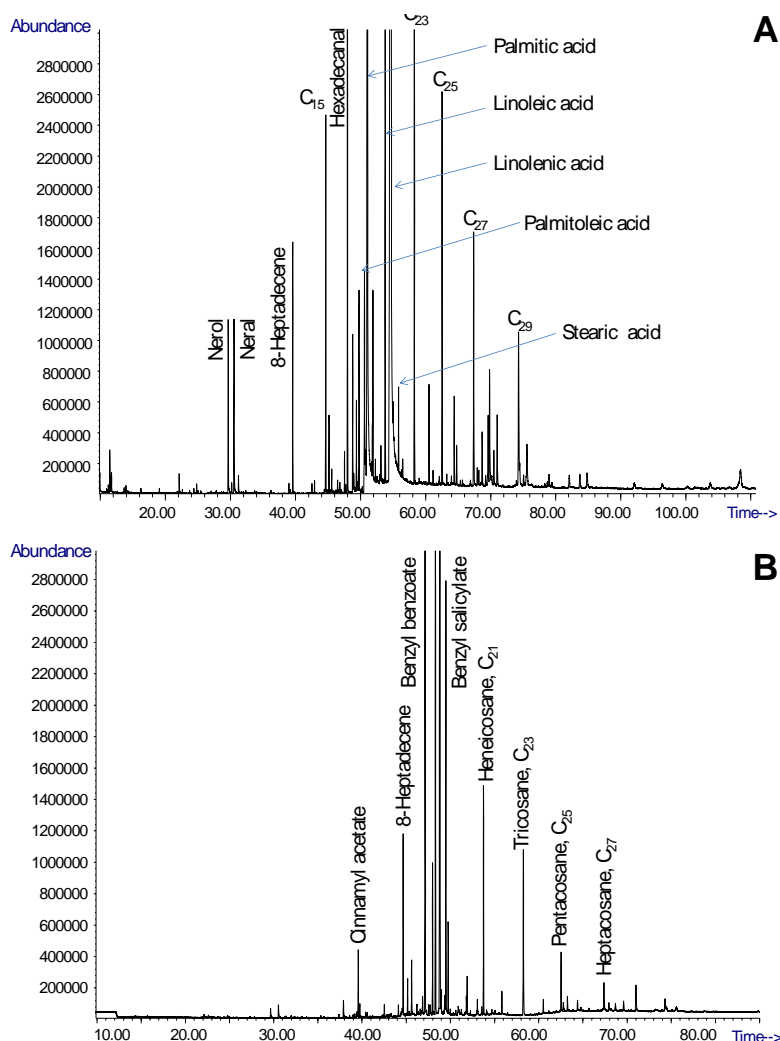

Figure 1. Total ion currents (TICs, chromatograms) of the coffee fresh flower extracts obtained by (A) extraction with hexane (without clean-up) and (B) extraction with supercritical CO_2 followed by clean-up and fat elimination. GC-MS (Electron impact, 70 eV), DB-5MS nonpolar capillary column (60 m x 0.25 mm x 0.25 µm). Split 1:30.

Solid-phase microextraction (SPME) constitutes a fundamental development in sample preparation techniques. In about 20 years of growth, this technique has become the dominant approach to sample preparation for the analysis of volatile substances in biological, environ-mental, forensic (arson, explosives residue, analysis of drugs in body fluids and tissues) studies, food quality control, or the monitoring of different processes *in vitro* and *in vivo* (breath analysis, fermentation processes, microbiological, pharmacokinetics, etc.) [9,10]. In SPME, the extraction occurs by the mass transfer of analytes from the sample to a polymer coating over a fused-silica fiber. This transfer continues until the chemical potential of each substance in all phases is the same. The fiber is subsequently inserted at the injection port of the chromatograph for direct admission of the analytes into the chromatographic system. SPME sampling with the polymer-coated fiber may take place by exposing it to the headspace above the sample (liquid or solid), or by immersion of the fiber in the sample (liquid), directly, or within a protective membrane. The SPME sampling process is amenable to automation and operation coupled to a GC. One of the most interesting applications of SPME is the target-analyte extraction accompanied by its derivatization on the fiber, which has been previously loaded with a reagent (Figure 2) [11].

The use of the vapor phase above the sample (headspace) as a means for performing extraction processes has great advantages from the analytical point of view, because it eliminates many interferences, mostly the solvent peak, which can impede the detection of volatile compounds. In the study of natural products, direct headspace sampling has allowed the study of the volatile fraction around whole plants or parts thereof (Figure 3) [12]. Classical approaches to monitor the headspace above a sample may be divided into static and dynamic versions. The volatile fractions thus collected are suitable for GC- MS analysis, as they are free of interference from nonvolatile very polar (ionic) or high - molecular weight matrix components. Static headspace consists of the direct transfer of a headspace volume to the GC injection port. This is the sampling method regularly used in the analysis of BTEX (benzene, toluene, ethylbenzene, and xylenes) in water, quantification of trihalomethanes in drinking water, ethanol, volatile compounds in paints, determination of residual solvents in polymers and pharmaceuticals, hydrocarbons in soil, analysis of beverages and perfumes, among many other applications [13]. When the target-analyte concentration in the sample is very low (ppt, ppb), enrichment (pre-concentration) is required, and in these cases, dynamic headspace is the preferred sampling mode. Basically, by purging the vapor phase with an inert gas, the analytes are collected in a solvent or are "frozen " (cryo-trapping) or are ad(b)sorpted in a sorbent (purge and trap, P & T), and later on are recovered by the action of temperature (thermal desorption) [14] or are eluted with solvent.

Sorption-based sampling techniques, which involve aspects of both static and dynamic headspace, have been developed in the past 2-3 decades. The great development experienced by the SPME technique has served to identify, through its many applications, some areas for improvement. This has generated a collection of new methods of micro-scale sampling, that achieve better concentration of the analytes of interest and in several cases, the use of a larger volume of sorbent phase (polymer, normally) permits the collection of larger amounts of analyte than SPME. In the inside needle capillary adsorption trap (INCAT), a polymer layer
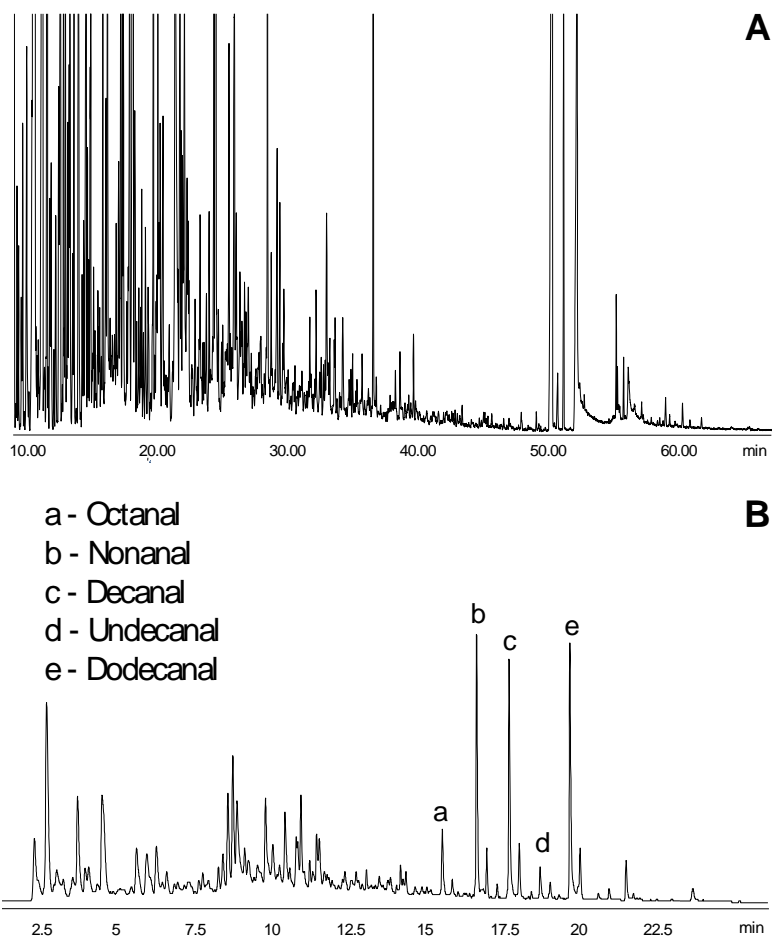
Figure 2. Total ion current (TIC, chromatogram) of the coffee roasted beans volatile fraction isolated by (A) P&T (purging with nitrogen and trapping with dichloromethane). GC-MS (Electron impact, 70 eV), DB-5MS nonpolar capillary column (60 m x 0.25 mm x 0.25 μm), split 1:30; and (B) Headspace-SPME with simultaneous aldehyde on-fiber derivatization with pentafluorophenylhydrazine (PFPH). Temperature: 50°C and exposition time: 30 min. GC-ECD, DB-5MS nonpolar capillary column (30 m x 0.25 mm x 0.25 μm). Split 1:30.

coats the inside of the needle of a syringe, or the inside is filled with activated carbon [15]. Headspace solid-phase dynamic extraction (HS-SPDE) is a sampling technique in which the needle of a hermetic gas sampling syringe is coated with absorbent polymer (PDMS) on which the analytes are deposited when the syringe is actuated repeatedly over the sample [16]. The number of suction and discharge cycles determines the amount of accumulated analyte. Stir bar solid-phase extraction (SBSE) and tape sorption extraction (STE) are additional examples of sampling techniques using higher absorbent phase volume. In the first one, the magnetic stir bar is coated with absorbent polymer. Upon exposure to the solution containing the
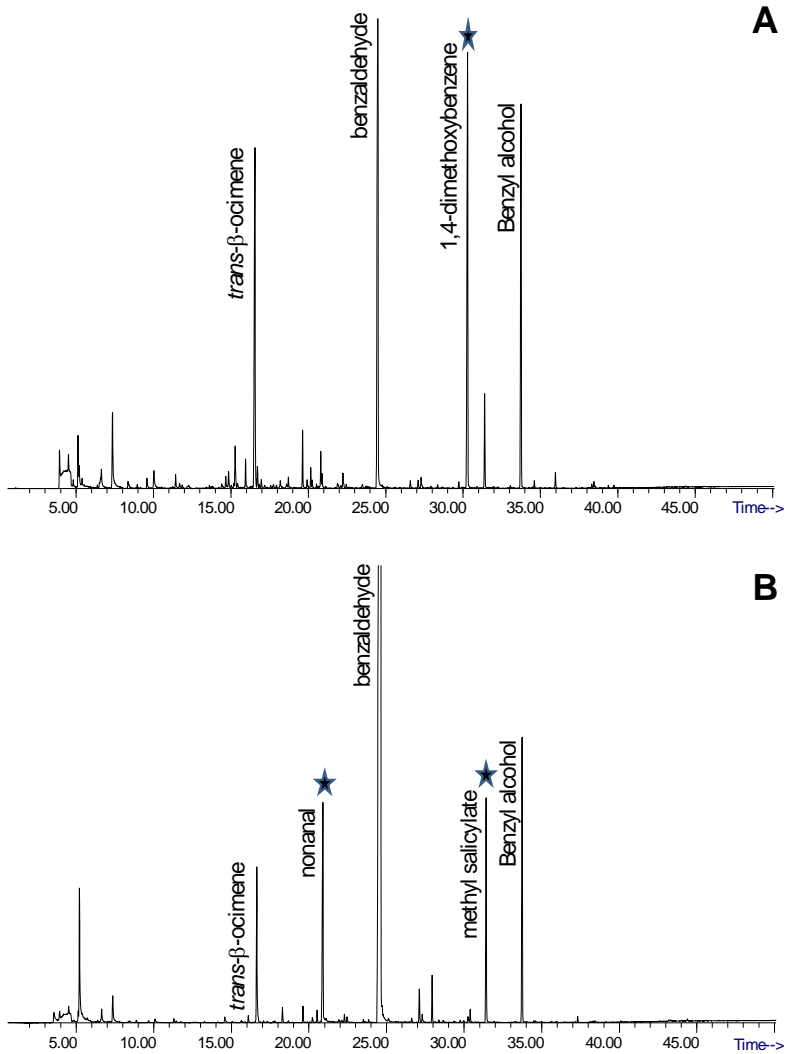
Figure 3. Total ion currents (TICs, chromatograms) of the passion fruit flowers (*Passiflora edulis*) volatile fractions obtained by headspace- SPME from different flower parts: (A) male stamens and (B) female carpels. Notice the composition differences which are very important in the pollination processes. CAR/PDMS-fibre coating. Headspace-SPME exposition time: 30 min. GC-MS (Electron impact, 70 eV), DB-5MS nonpolar capillary column (60 m x 0.25 mm x 0.25 µm). Splitless injection mode.

analytes of interest, the stir bar is subjected to thermal desorption for transfer of the analytes to the chromatograph [17]. In STE, a thin strip of PDMS is placed for a fixed time in direct contact with the plant surface, the skin, or the object to be sampled. Analytes are subsequently transferred to the chromatograph by thermal desorption [18].

2.2. Sample introduction systems

Another absolutely essential parameter to determine how many components in a mixture are detected is the injection mode of the mixture into the GC. The main objective of an injection system, or simply the injector or the injection port, is to transfer the sample to the chromatographic column in a rapid and quantitatively reproducible manner. Ideally, this critical step in GC, has to meet a number of conditions. These include: (1) The sample must enter the column in a chromatographic band as thin as possible, to reduce peak broadening; (2) the percentage composition or relationship among the components of the mixture analyzed should be the same before and after injection; that is, there should be no discrimination by molecular weight, or volatility at the injection port; (3) during their transfer to the column, components should not react with each other or undergo chemical change, e.g., isomerization, hydrolysis, polymerization, etc. or become adsorbed onto the injector body. The sample must be transferred quantitatively without alterations. The sample injection must be precise, accurate, reproducible and predictable, quantitative and without discrimination of any kind. The injection mode is selected according to the following criteria: (1) type of chromatographic column (packed column, mega–bore, capillary), (2) the chemical nature of the analytes (molecular weight, volatility, polarity, temperature stability, reactivity, etc.) and (3) the purpose of the analysis (qualitative or quantitative, to detect traces or, conversely, to measure high concentrations of some components, etc.).

Here are some considerations on the most common injection ports for capillary columns. The "universal" injector does not exist. For capillary columns two main types of injectors are available: vaporization and on-column (direct injection into the column without prior sample volatilization) injectors. There are also devices that can be connected in line with the GC injection port, for example, headspace sampler, pyrolysis, a purge and trap system, a thermal desorption system, a switching valve to transfer gas samples, etc. The injection of the sample can occur either manually or via an autosampler. In the vaporization injector, the sample (1 – 2 μL) crosses a septum to enter a glass cylinder which acts as the liner of a chamber which is heated independently. Upon exposure to the high liner temperature (> 200 °C), the sample vaporizes quickly and the resulting gaseous mixture is swept towards the column by the carrier gas. Conventional vaporization injectors can operate in two primary modes, i.e., split (sample is divided at the column inlet) and splitless (without division of the sample) [19]. In a standard split/splitless injection port, the gas flow may transport all of the injected sample to the column (splitless mode) or only a fraction of it (split mode). Concentrated samples are injected in the split mode so that only a small aliquot enters the column (Figure 4). On the other hand, when analyzing trace level compounds, for example, with environmental or forensic samples, complete transfer of the sample to the column, without division, is required (splitless mode). This mode involves slowly transferring (ca. 1 mL/min) the sample to the column through the hot injection port, which may lead to thermal decomposition and/or isomerization of some components of the mixture. A special pulsed splitless mode has been devised to enable the analysis of thermally labile compounds at trace levels, which is performed by increasing pressure on the top of the column during the injection, while the sample is transferred to the column. In this case, the residence time of the sample in the hot region (250-300 °C) of the

injection port is dramatically reduced and so is the probability of the thermal decomposition of some components (Figure 5). A mode of complete sample transfer to the column, which avoids component discrimination by their volatility or molecular weight, is the on-column injection, i.e., applying the sample directly to the column head. Very detailed work on the design and application of various methods of sample injection to GC equipment has been developed by Dr. Konrad Grob [19,20].
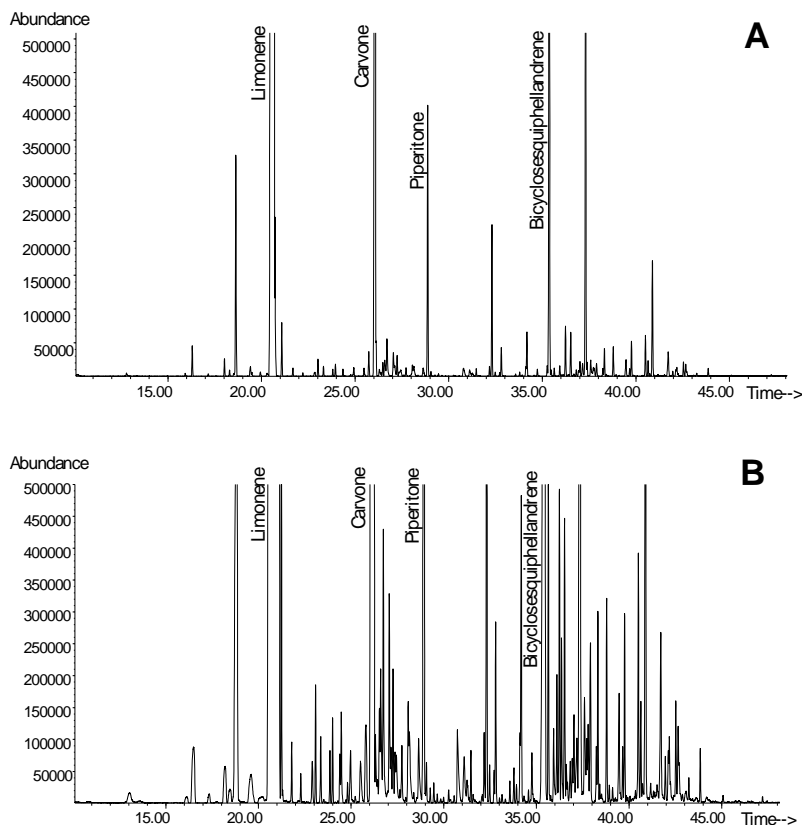

Figure 4. Total ion currents (TICs, chromatograms) of the *Lippia alba* (Verbenaceae family) essential oil obtained by hydrodistillation. A. 1:30 Split injection (ratio, 1:30, Inj. vol.1 μL) and B. Splitless injection mode (Inj. vol.1 μL). Almost twice the number of compounds were registered in the chromatogram obtained by splitless injection (ca. 90) in comparison to those registered in split-mode injection (>0,05%). GC-MS (Electron impact, 70 eV), DB-1MS nonpolar capillary column (60 m x 0.25 mm x 0.25 μm).

Different configurations of the liner offer efficiency and uniformity of the mixing process. A specific liner configuration is recommended for each type of injection, information which can be found in the catalogs of different manufacturers of these accessories. The inlet temperature should be high enough to ensure complete evaporation of all analytes, but not so high that
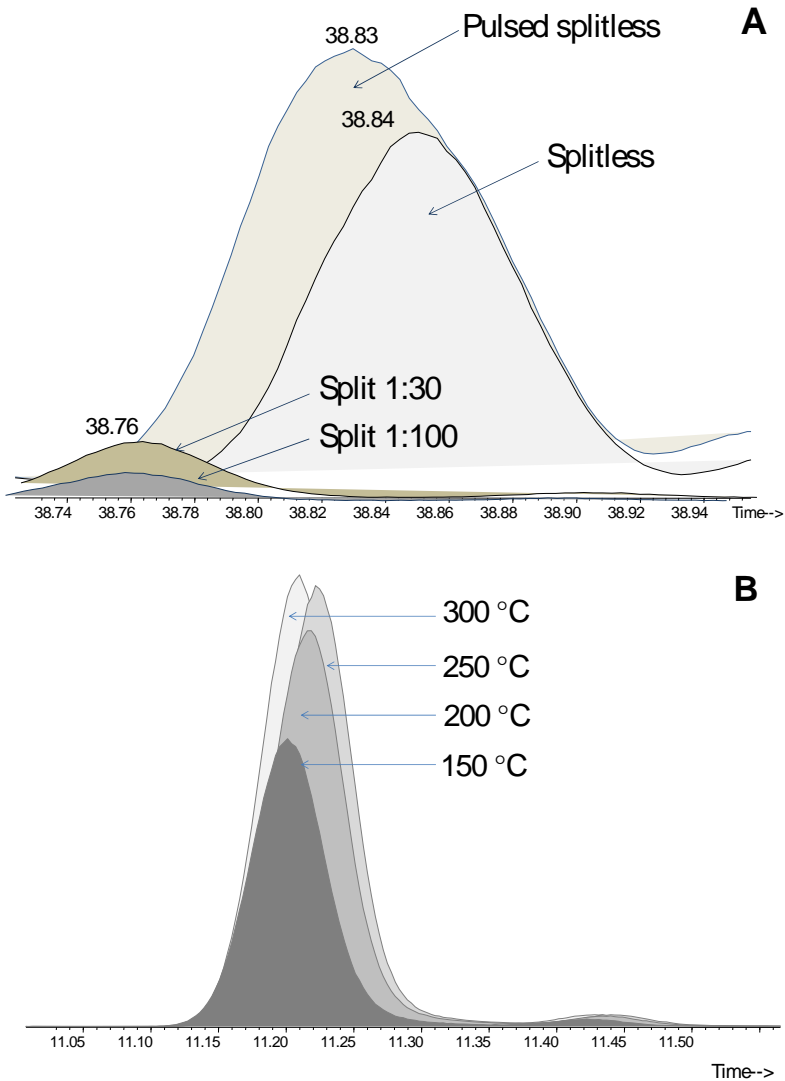
Figure 5. Chromatographic peak of ethyl benzoate obtained by GC-MS (Electron impact, 70 eV), on the DB-5MS non-polar capillary column (60 m x 0.25 mm x 0.25 μm). The sample was injected in different modes: split, splitless and pulsed splitless. Notice the retention times shift and the change of chromatographic areas. B. The influence of injection port temperature on the chromatographic peak area of limonene (monoterpene, $C_{10}H_{16}$). DB-5MS column (60 m x 0.25 mm x 0.25 μm). 1:30 Split ratio.

causes thermal decomposition. Too low temperatures will produce incomplete volatilization, a loss of analytes and a slow entry of volatilized substances to the column, causing chromatographic peak broadening. A potential problem is the systematic contamination of the injector

and the development of a "memory effect". More commonly, the temperatures used in conventional vaporizing injectors range from 200 to 320 °C, the temperature of 250 °C being quite common in the practice of GC [21-24]. It is important to note that when the liquid sample is vaporized, it expands many times with respect to its initial volume. Expansion volumes depend on: (1) the type of solvent, (2) inlet temperature and (3) inlet pressure of the carrier gas at the top of the column [23]. Before injection, all these factors must be taken into account, i.e., what amount of sample to be injected, in which solvent and under what conditions (injection temperature and inlet pressure in the column). The flashback, i.e., the "return" of the sample vapor by the purge gas line, may occur when the expanded volume of the injected sample volume exceeds that of the liner (ca. 0.5- 1 mL). Excess vapors can escape and pressure is created not only by the carrier gas line (flashback), but also by the septum purge line; the more volatile components of the sample can be "lost" and this will affect the accuracy and precision of the GC analysis.

The injection port is a critical site in any chromatographic system, since it may be the cause of contamination (high noise) and the source of so-called ghost peaks, whose origin may be related to recondensation of the less volatile substances in the bottom of the septum or the carrier gas transfer line from flashback problems [24]. Injection efficiency, optimum speed and consistency of the mixture entering the column will determine the width of the chromato- graphic peak, its symmetry, and, ultimately, efficient and reproducible separation and quantification. When choosing the injection technique, the following variables are chosen and optimized: (1) the injection mode (split, splitless, on-column or programmed temperature vaporizer, PTV), (2) the volume of the sample to be injected, (3) injector temperature, (4) the type of liner (shape, volume, packaging), (5) the initial temperature of the column (more critical for injection in splitless mode), (6) the form of injection, manually or automatically, (7) the rate at which the septum is pierced or the needle is removed from the injection port (important parameters for decreasing analyte discrimination based on volatility), (8) the solvent and the number of washes of the syringe with different solvents, (9) the type of septum (material, density, thermal stability, bleeding), for instance, the conventional septum or Merlin Micro- seal™, which is highly recommended for GC-MS operations.

2.3. Separation procedure

The chromatographic column is the heart of the GC. There are two types of columns, packed and open tubular, often called capillaries (when the ID is equal to or less than 0.32 mm). In packed columns, solid particles (silica, alumina, molecular sieves, porous synthetic polymers, etc.) serve as support or are embedded in a liquid (squalene, polyethylene glycol, etc.), which acts as a stationary phase as means of separating components within the GC. Because the gas solubility in liquids is very low, solid phases are particularly suitable for the separation of gaseous components, such as permanent gases, natural and refinery gas, volatile aldehydes and alcohols, car exhaust, etc. The use of packed columns is fairly limited today, because open tubular columns have much higher separation efficiencies. Current gas analysis uses porous layer open tubular columns (PLOT), a type of capillary columns, where analyte adsorption is combined with the high separation efficiency provided by capillary columns [21].

Column efficiency is expressed by the so-called number of theoretical plates, N, or by means of the height equivalent to a theoretical plate, HETP [22]. The higher is the number of N and lower the value of HETP, the higher the resolution (efficiency) of the column. As indicated by its name, open tubular columns have an opening, an internal space, which allows the carrier gas to move freely without having to penetrate or pass through, with strength, the porous support. This latter process, the tortuous pass, accompanied by the phenomenon of multiple paths, is typical for packed columns and is the main cause of their much lower efficiency compared with open tubular columns [22]. The GC column selection process should take into account the following parameters and properties: (1) phase type (nature, polarity), (2) dimensions (length, L, and inner diameter, ID) and (3) phase thickness. Stationary phase polarity is chosen according to the polarity of the analytes of the mixture, following the general principle of "like dissolves like". But often, it is difficult to find a "universal" stationary phase, since in many samples both polar and nonpolar compounds can be found, with diverse volatility (Figure 6). In nonpolar columns (polydimethylsiloxanes), analyte elution happens according to their volatility, i.e., in increasing order of their boiling points. An example is the technique of simulated distillation for the analysis of petroleum fractions [25]. In polar columns (e.g., polyethyleneglycols), the predominant factors for analyte separation are its polarity (the dipole moment, μ) and the strength of its interaction with the stationary phase (dipole, hydrogen bonding). Wall-coated open tubular columns (WCOT) are the most commonly used in capillary GC and their stationary phases can be divided, roughly, into two great classes: (1) substituted siloxane-type polymers with a wide range of polarities and (2) poly(ethyleneglycol) (PEG), which are highly polar. The polysiloxanes may possess different substituents which provide to them some polarizability. The polysiloxane phases range from nonpolar 100% poly(dimethylsiloxane) slightly polar 5-95% -phenyl poly(methylsiloxane), to polar and strongly polar phases containing trifluoropropyl and cyanopropyl substituents, respectively. Modern GC columns have cross-linked and immobilized (chemically bonded to the column wall) polymer stationary phases. This provides uniformity, chemical and thermal stability, and reproducibility of results during extended use. Chiral stationary phases for separating optical isomers give rise to a separate family of columns. Among these, good performance has been achieved by the presence of cyclodextrins within the stationary phase. Enantiomers appear in the chromatogram with different retention times due to the different strength of their association with the cyclodextrins when these adducts involve the non-polar cyclodextrin´s inner cavity [26].

Capillary columns are divided according to their length, L, into: (1) short (5-15 m), (2) medium (20-30 m) and (3) large (50-100 m). Perhaps the length L = 30 m is the most common column used for many analyses (drugs, pesticides, polyaromatic hydrocarbons, etc.) [21,22]. With increasing column length the number of theoretical plates (N) and resolution also increase, but the duration of the chromatographic run is greatly increased. Long columns are required for the analysis of complex, multicomponent (> 50 substances) samples, for example, gasoline, perfumes, essential oils, aromas, VOCs, etc. [22,23], or when there are isomers in the mixture which have similar boiling points or dipole moments and, consequently, their distribution constants, K_D, are very close. The longer the columns are, the greater the pressure drop across them (the pressure difference between inlet and outlet of the column). Also, due to their greater
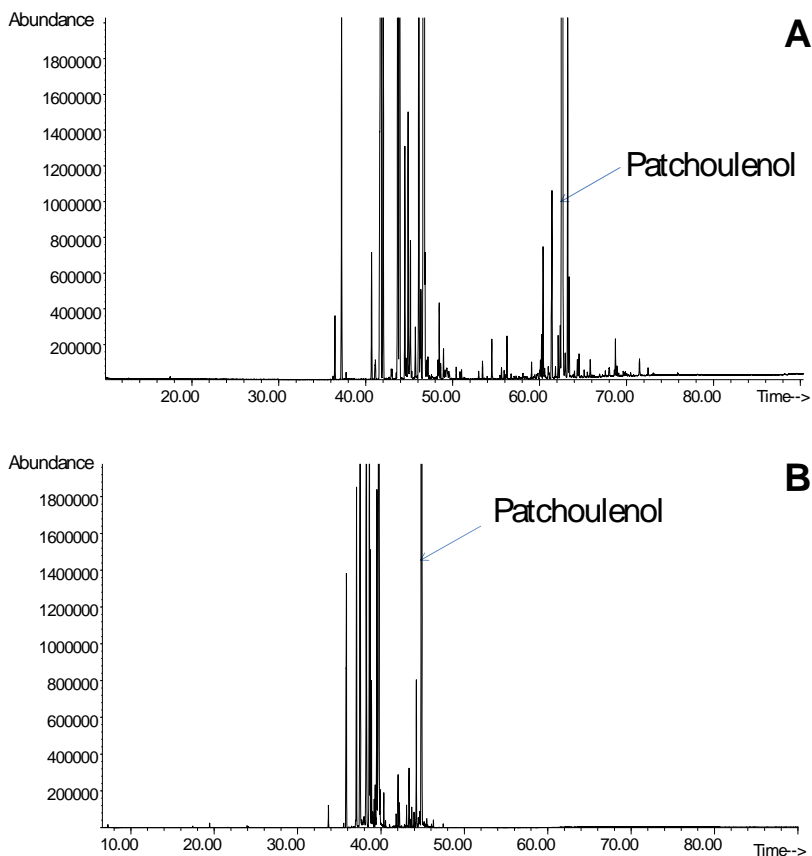
Figure 6. Comparison of the total ion currents (TICs, chromatograms) of the patchouli (*Pogostemon cablin*) essential oil obtained by hydrodistillation. The complex mixture of sesquiterpene hydrocarbons and their oxygenated derivatives (alcohols and aldehydes) was analyzed in two capillary columns of the same dimensions (60 m x 0.25 mm x 0.25 m), but different stationary phases: (A) DB-WAX and (B) DB-5MS. GC-MS (Electron impact, 70 eV), Split 1:30. The same oven temperature program was used with both columns: 50 °C (5 min), 4 °C min^{-1} to 250 °C (15 min).

thermal inertia, the heating rate of these columns must be slower (2-6 °C min^{-1}), in order to achieve reproducible change in temperature. The analysis of polyaromatic hydrocarbons (PAHs) can be successfully accomplished in 30 m-long columns, whereas the separation of hydrocarbons in gasoline requires longer 100 m columns. The main drawbacks of long columns are extended analysis time and higher cost, but very high sensitivity and resolution can be achieved. A compromise has to be found among resolution, sensitivity and time (cost) of analysis. A significant alternative to increase the resolution of the column and shorten analysis time is to reduce the column internal diameter, which in capillary columns most commonly is 0.25 or 0.32 mm. When the column length is doubled, the resolution is also doubled, but when ID is reduced by half, the resolution increases almost fourfold. Decreasing I.D. leads to a marked reduction in analysis time. This led to the development of a new branch in GC, named

fast chromatography, which has found very interesting applications [27-29]. The columns employed are thin or ultra-thin (0.1 or 0.05 mm), but shorter (1-5 m) than in conventional GC [29]. The "fast" columns also require a detector with very fast response, particularly in the case of comprehensive GCxGC; in the fast -GC-MS configuration, the most effective mass analyzer is the time-of-flight (TOF) [30]. The main shortcoming of fast GC is its low sample capacity, which for many analyses, for example, environmental or forensic, can become a serious limitation. Inlet pressures in the overhead GC column increase greatly as the ID is reduced. For example, 30 m columns (100 °C) with IDs of 0.32 and 0.10 mm require carrier gas (helium) pressures of 9.5 and 114 psi, respectively. Roughly, the resolution of the column increases with its length and with the decrease of ID and phase thickness, while the analysis time increases with the rise of the column ID and of the thickness of the stationary phase and, above all, with the length of the column. The main tasks after correctly selecting the column should focus on the installation of the column and its conditioning, maintenance and precautions to be taken during operation. The latter is related to the care of the GC column, which includes not exceeding the permitted temperature limits, preventing the entry of oxygen, water, mineral salts, acids or bases or high molecular weight compounds (interference), maintaining a hermetically sealed GC system, devoid of leakage. With time, gaskets, seals, traps (for moisture, hydrocarbons or oxygen) wear out or saturate and should be replaced.

For capillary columns, the most recommended carrier gases are helium or hydrogen; an average linear velocity of 25-35 cm/s allows obtaining the required separation efficiency. Since the gas viscosity varies with temperature, the average linear velocity of the carrier gas will decrease during the chromatographic run as oven temperature increases. For substances with high distribution constants (K_D), this reduction in the flow rate of the carrier gas impinge upon the shape of their chromatographic peaks (asymmetry, tailing) and increases the retention times and the overall analysis time. Hence, special devices have been introduced, the electronic pressure controls, which, among other functions, allow maintaining constant flow in the column during the chromatographic run so that carrier gas pressure input to the system is changed with increasing oven temperature, in a synchronized, electronically governed manner.

2.4. Multidimensional and comprehensive separation systems

Essential oils, fragrant liquids consisting of volatile secondary metabolites isolated by distillation of aromatic plants are multicomponent mixtures, which may contain up to 300 substances [31]. The essential oil is an excellent model for gas chromatography, as its constituents possess different functional groups; among them are hydrocarbons, alcohols, aldehydes, ethers and esters, acids, and others. Most of these substances belong to the family of terpenoids (C10 and C15), but also include phenolic derivatives. The concentration of the constituents in the essential oils can vary over a wide range, from μg/kg or mg/kg to g/kg. Many components of the oils have isomers. This leads to two problems in reaching the analytical goals, namely: (1) total separation of all components, and (2) the correct assignment of their structures. For some substances present in the essential oils very close retention times are observed, at least in one of the columns (non-polar or polar); in addition, some constituents possess very similar

mass spectra. Complete separation of the components of essential oils can be achieved with complete gas chromatography (comprehensive GCxGC), which has been applied very successfully to such mixtures [32]. Multidimensional chromatography allows separating in a second column the peaks of partially or completely co-eluted substances, through the operation of "heart -cutting" using switching pneumatic valves (nowadays micro-fluidics technology) between the two columns and diverting part of the effluent from the first to the second column. This technique has played an important role in the development of methods for complex mixtures separation. Multidimensional chromatography requires at least two detectors and some configurations can have three columns in the same or in separate chromatographic ovens.

Along with this and other conventional approaches, nowadays there is a modern solution, although relatively inaccessible to many laboratories, due to its cost, for the separation of multicomponent mixtures. It is called complete, total, or more commonly, comprehensive chromatography, GCxGC. It uses two columns connected by a modulator. In contrast to conventional multi-dimensional gas chromatography, GC x GC requires a single detector and both columns can be in the same or in two separate ovens. There are different types of modulators, e.g., thermal rotary modulator (sweeper), "jet" cryogenic system, valve modulator, and longitudinal cryogenic modulator, among others [32]. The eluent from the first column is split into very small slices by means of the modulator, which transfers them one after another, in a row, from the first into the second column. The first column (1D) is a conventional column, with length of 25 or 30 m, and the second column (2D) is a fast chromatography, a very short micro-bore column. Stationary phases in both columns are "orthogonal", i.e., if the first one is non-polar, the second column is polar, and vice versa. Modulation time, i.e., the transfer of a tiny portion of the first column eluent to the second, must be very short and commensurate, but not longer, with the elution time of a component in the second column. Thus, the latter is very short and very thin and allows separating the components in a few seconds. Since the second column is connected to the detector (MSD, FID or μ-ECD), it should have high reading and signal processing speeds. In most cases, the time-of-flight mass detector (TOF) is the best, although still very expensive option [32].

2.5. Overview of GC detectors

The detection system differentiates the analyte molecules from the mobile phase, which is transparent to the detector. Detector response (signal) is based on measuring a physical property of the flow of analyte molecules (ionic current, thermal conductivity, fluorescence, refractive index, photon emission, electron capture, etc.). The signal should be proportional to the amount of analyte that emerges from the column, thus establishing an interdependent relationship and carrying out a quantitative analysis, which is an essential part of a chromatographic determination and leads to the answer on how many components and in what proportion they are present in a mixture. Common detectors used in gas chromatography are classified into (1) universal detectors, e.g., thermal conductivity detector (TCD), mass selective detector (MSD) operated in full scan mode, or infrared detector; (2) selective detectors, e.g., nitrogen-phosphorus detector (NPD), electron capture detector (ECD) or flame photometric

detector (FPD), among others, and (3) specific detectors, e.g., MSD operated in selected ion monitoring (SIM) mode, thermal energy analyzer (TEA), or atomic emission detector (AED). The flame ionization detector (FID) could be considered "near- universal" since only water and permanent gases are transparent. There is no well-defined boundary between the specific and selective detectors [33]. Specific detectors are actually highly selective detection systems, which can obtain a response to one particular compound, an analyte or target of interest present in a complex mixture. For example, in a urine sample, detect and quantify testosterone, which is one of the analytes to be determined quantitatively for doping control in sports. The selectivity of a detector response is related to a substance that has a specific atom, for example, nitrogen, phosphorus (NPD) or sulfur (FPD) or a functional group, e.g., electronegative group(s) (ECD) or unsaturated groups and aromatic rings (PID, photoionization detector) or the substances, which have a common structural fragment, for example, a phenyl, benzyl or an acyl radical, etc. (MSD operated in SIM mode; Figure 7).
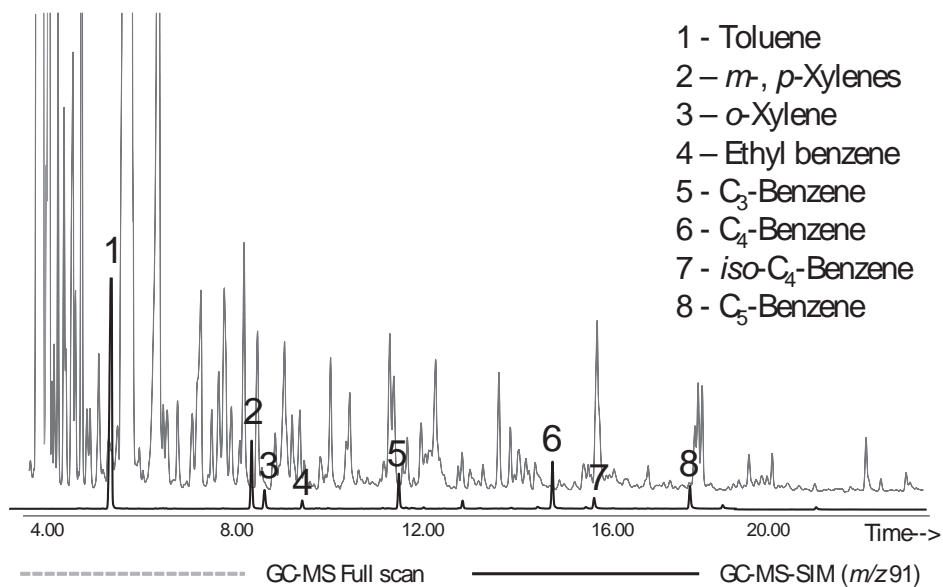

1 - Toluene
2 – m-, p-Xylenes
3 – o-Xylene
4 – Ethyl benzene
5 - C_3-Benzene
6 - C_4-Benzene
7 - iso-C_4-Benzene
8 - C_5-Benzene

GC-MS Full scan   GC-MS-SIM (m/z 91)

Figure 7. Selective mass-spectrometry detection of alkyl benzenes present in the volatile fraction isolated by P&T from roasted coffee beans. GC-MS-SIM (Electron impact, 70 eV, selected monitoring of m/z 91 ions, characteristic for alkyl benzenes). DB-5MS nonpolar capillary column (60 m x 0.25 mm x 0.25 μm). Split ratio 1:30.

All detectors are distinguished by their sensitivity, minimum detection and quantitation levels (in the case of MSD, also by the minimum identification level), linearity, sensitivity to changes in gas flow, temperature or pressure. The sensors have different noise levels, volumes, are distinguished by a higher or lower sensitivity and sophistication, for simplicity in operation, cost, and other typical features to be taken into account when choosing and operating a GC detector. Because the volumes of GC detectors are larger than the

capillary column attached to them, the dead volume formed could generate chromatographic peak broadening and affect resolution. This difference in volumes is offset by an auxiliary gas (make-up), usually nitrogen, whose flow is 20-40 mL/min, depending on the detector type. High make-up flows lead to more symmetrical and thin chromatographic peaks, but compromise sensitivity. The trend in modern GC instruments is to reduce the volume of the detector. Today, there are micro versions of several detectors, for example, μ-ECD or μ-TCD. Most of the GC detectors are destructive detectors. These include, for example, ionization detectors (FID, NPD, FPD, MSD), whose response is sensitive to the change in mass of the analyte, while the response of non-destructive detectors (TCD, infrared detector, ECD, PID) is sensitive to the change of the analyte concentration. That is, when operating the TCD or ECD it is important to maintain the flow of gases (carrier gas and make-up or auxiliary) constant. Nondestructive detectors can precede in series a destructive detector; e.g., in tandem TCD followed by FID or NPD.

The major limitation posed by conventional detectors (FID, TCD, ECD, NPD) is the ambiguity to uniquely identify an unknown substance. Absolute retention times (t_R) or relative to an internal standard (t_{RR}) and certified standards, are used for the presumptive identification of a compound. This is a screening analysis. Confirmatory identification of a compound in a complex mixture, analyzed by GC, necessarily requires obtaining the "fingerprint", i.e., spectrum, of the compound. The mass spectrum (MS) has information about a unique combination of charged fragments (ions) generated during the dissociation or fragmentation of the previously ionized molecule. The complementarity of chromatographic analysis (screening) with confirmatory spectral data is achieved using the combination of GC-MSD. A GC-MSD analysis provides information on (1) retention times, (2) chromatographic areas and (3) mass spectra of each component in a mixture, obtained by its ionization with electron impact (EI) at 70 eV. The value of 70 eV was established as a standard to obtain the MS, which are also part of the databases and commercial spectral libraries, because for most organic compounds, the highest ionization efficiency and reproducibility are achieved with bombarding electrons of this energy. The ionization energy (potential) of organic molecules varies from 7 to 14 eV. During the collision with electrons of 70 eV, energy is transferred to the molecules to ionize them and form the molecular ion, M^+, but also to produce fragmentation of those excited M^{+*} ions, which possess sufficient internal energy to break a chemical bond. The spectra obtained with lower energy electrons (15-20 eV) are called low voltage spectra, but they are rarely used in the GC- MSD method, because they often cannot achieve the sensitivity required for the analysis.

The coupled technique GC-MSD is the most used tandem combination in instrumental analytical chemistry and is applied to investigations in forensic, environmental, natural products, foods, flavors and many other areas [34]. Among the chromatographic spectroscopic detectors (AED, IR or MSD), useful for the determination of the chemical structure or elemental composition of analytes, the mass spectrometer is the most widely used today thanks to its sensitivity, operability and, above all, to a large amount of *sui generis* structural information that it can provide [35].

2.6. Mass selective detector

A mass spectrometer attached to the gas chromatograph is often referred to as "mass detector" or "mass selective detector" (MSD). The MSD consists of an ionization chamber which in the large majority of situations uses electron ionization or electron impact (El) to provide the energy to the analytes which could fragment and generate the ions to be detected. Chemical ionization (Cl) of positive ions or negative ions, is a complementary, mild ionization method. CI is used as an alternative when no molecular ions are registered in the mass spectra obtained by El, since fragmentation is excessively intense, due to the high lability of molecular ions, M$^{+\bullet}$ (extremely short lifetime, $< 10^{-6}$ s) [36]. Ions formed in the ionization chamber are removed therefrom by a series of electrodes that collimate (focus) and accelerate and direct them to a mass analyzer. The potential energy created by the accelerating field (E) is converted into kinetic energy. The analyzer separates ions according to their m/z ratio. Several types of mass analyzers may be used in the GC-MS equipment. Quadrupole (Q) and ion trap (lT) are the most common; in recent years, there has been an increase in the use of time-of-flight analyzers (TOF); lately, resonance ion-cyclotron analyzers with Fourier transform (FT-ICR) and orbitrap have become commercially available. The use of magnetic deflection mass analyzers is much less frequent [34,37,38].

The charm –from an analytical standpoint-, of a mass selective detector, is its ability to operate in three modes of data acquisition, namely: universal, selective and specific. When a scan is complete (the MSD detector functions as a universal detector), the mass spectra obtained for all mixture components are the basis for recognizing or identifying them. The mass scanning rate depends on the type of analyzer used (quadrupole, ion trap, time-of-flight or magnetic sector) and the mass range to cover. The mass range for the entire sweep is established in accordance with the nature of the sample, that is, the range of molecular weights of its components. The low end of the scanned mass range for aliphatic compounds, alcohols, amines, etc., can be set to m/z 30-40 (lower masses are not recommended, as they correspond to background signals); the minimum mass for aromatics can be set to m/z 50. The upper end of the scan range should correspond to the molecular weight of the heaviest substance present in the mixture, plus 40 - 50 units. If the mass range is chosen poorly, for example, if for the GC-MSD analysis of low molecular weight, highly volatile compounds (molecular weight less than 150 Da), a very wide mass range is set, e.g., m/z 30-550, the number of spectra obtained per unit of time will be small, which affects the quality, reproducibility and reliability of the analytical data [34-36]. On the other hand, high scan speeds demand fast response from the accompanying hardware, to avoid loss of information.

Selected ion monitoring, SIM, is the selective mode of operation of the MSD. Instead of scanning a mass range, only a discrete set of m/z values is monitored. This permits to obtain a much higher signal accumulation per unit time, which means that the sensitivity is greater (30-100 times) than when full scan is used. GC-MS-SIM analysis of an extract or a complex mixture permits the selective detection of homologous molecules, isomers, or structural derivatives, when the selected ion is a common feature in their mass spectra. However, the detection becomes specific, highly selective, when the monitored ions constitute a unique combination which is found only in the mass spectrum of the target analyte.

Besides its use in performing quantitative analysis with higher selectivity and sensitivity, the SIM mode helps to reduce the possibilities of false positives in substance identification. Presumptive identifications are based solely on matching retention times of target analytes and standard compounds, but this result should be confirmed by GC-MSD-SIM, when compounds of interest are in the mixture at trace levels [39].

To setup a GC-MSD analysis in SIM mode, generally three characteristic "diagnostic" ions are chosen (one for quantification and other ones as "qualifiers"). The ion selection criteria attend the following recommendations: 1) the ion´s abundance should be higher than 30%; 2) the mass of the selected ion should be high, preferably, because low-mass ions are common to many substances; 3) the selected ion should be structurally representative of the molecule. For example, it could be a molecular ion, or a genetically-related fragment; 4) the selected ion should not coincide with those from background (m/z 17, 18, 28, 32, 40, 43, 44), stationary phase bleeding (*e.g.,, m/z* 73, 147, 207, 281, 355), thermal degradation of the septum, or plasticizers (m/z 149). The partial ion currents of the selected ions are measured in a retention time window in which the standard substance elutes (e.g., $t_R \pm 0.5$ min). Then the sample is analyzed under the same GC-MS-SIM operational conditions used with the standard substance. When there are several substances of interest, the same procedure is applied to each one and the selected ions are scanned only within the retention time window of its corresponding standard substance. The identity of the target analyte in the sample can be confirmed only when the following 3 criteria are satisfied: 1) the retention times of the analyte and the standard substance match; 2) the S/N ratio is higher than 1:3 – 1:10; 3) the intensity ratios of the selected ions are the same in the spectra of the analyte and of the standard substance. If the retention times are the same (a necessary, but not sufficient condition), but the intensity ratios of the selected ions in the spectra of the target compound and of the standard do not match, that is, are not identical, differ by more than 15%, the situation of a " false positive" is observed, i.e., the alleged presence of the target analyte in the sample is not confirmed [40].

3. Gas chromatography-mass spectrometry

The need to unequivocally identify the components of complex mixtures was the motivation for the development of different instrumental coupling techniques (tandem), including the widely and successfully used (with volatilizable substances), gas chromatography coupled with mass spectrometry (MS). GC-MS is an extremely favorable, synergistic union, as the compounds susceptible to be analyzed by GC (low-molecular weight, medium or low polarity, in ppb-ppm concentration) are also compatible with the MS requirements. Besides both analyses proceed in the same aggregation state (vapor phase). The only "conflict" (short-term and already resolved) between GC and MS, were the different working pressures, i.e., atmospheric at the GC column exit and low (10^{-5} - 10^{-6} Torr) in the ionization chamber, respectively. This drawback was overcome by technically introducing an efficient vacuum pump (turbomolecular and gas-jet pumps) and, above all due to the introduction of gas chromatography capillary columns (internal diameter 0.18 to 0.32 mm id, traditionally used in GC- MS), which are inserted directly into the ionization chamber of a mass detector [34,40].

3.1. Ionization modes

The essence of a mass spectrometric method revolves around the process of ionization of the molecule, with or without subsequent cleavage or fragmentation. In most cases the ionization of the molecule is dissociative. Its mechanisms can be various, e.g., subtraction or addition of an electron, protonation or deprotonation, nucleophilic, electrophilic addition or subtraction and cluster formation, among some other processes leading to ion formation. The ionization of a molecule is an energy consuming process, which can be supplied by accelerated or thermal electrons (electron impact or electron capture), by photons (photoionization, corona discharge, laser beam), by atoms or ions accelerated by a high electrostatic field gradient or thermal impact, among other mechanisms. A rather large number of methods have been developed to transfer energy for the ionization process, to thermolabile, high- or low-molecular weight, polar or non-polar molecules, in the gas phase (electron impact, EI, chemical ionization, CI, photoionization, PI, field ionization, FI) or in the condensed phase (field desorption, FD, laser desorption, LD, fast atom bombardment, FAB, plasma desorption, PD, secondary ion mass spectrometry, SIMS, matrix-assisted laser desorption ionization, MALDI) [36].

The ionization of neutral molecules is followed by fragmentation, or dissociative ionization, whose product ions can be separated with varying degrees of accuracy, depending on the "spectroscopic balance", i.e, analyzer used. Obtaining a mass spectrum is a *sui generis* energy balance which involves energies ranging from electronic excitation of a molecule (e.g., ultraviolet-visible spectroscopy) up to its atomization (absorption or atomic emission). The ineludible first step is to ionize the molecule (removing or adding an electron, or a proton), which informs about its molecular mass or exact elemental composition when the resulting molecular ion is detected. On the other hand, it is necessary that some of the ionized molecules dissociate or fragment, which reveals what constituent groups comprise the molecule and how they are combined. Electron ionization (EI) is the oldest technique for organic molecule ionization; it is the most widespread and the one with the largest number of applications, for example, GC-MS. It is not so accurate to call it "electron impact", since the size and energy of an electron, compared to an organic molecule of 150-500 Da, does not really allow "impacting" it, such as a ping-pong ball little impact would cause to a rhino. The interaction takes place between the bombarding electron and an electron belonging to the molecule [34-37, 40].

The interaction of an accelerated electron with a molecule leads to the excitation of molecular electrons and a molecular ion, $M^{+\bullet}$, will be formed if the imparted energy permits it, that is, if it is enough to remove an electron from the neutral molecule in the vapor phase. The formed ion's molecular mass is "numerically" equal to that of the molecule because in comparison, an electron mass is negligible. Electrons are excellent agents for ionizing organic molecules. First, because it is easy to obtain them: just pass an electric current through a tungsten or rhenium wire (filament or cathode). Second, their energy is adjustable with the voltage applied between cathode (thermo-electron emitter) and anode (ground connection), where the average standard energy -worldwide accepted convention- is 70 eV. For most organic molecules, the maximum ionization efficiency is already reached with bombarding electrons with energy of 50-60 eV. Standard mass spectra are taken at 70 eV because they so achieve the highest repeatability and reproducibility. Mass spectra libraries are also formed with the spectra obtained by electron

ionization energy of 70 eV. All this facilitates the comparison of the spectra taken on different spectrometers with those of databases and other instruments. The bombarding electron energy of 70 eV far exceeds that required to ionize organic molecules. The ionization energies (or ionization potential, IP) for organic molecules lie in the range of 6 to 13 eV, and depend on their molecular structure. For example, to ionize the cyclohexane molecule, 9.9.eV of energy are required; for benzene 9.2 eV, toluene 8.8 eV, pyridine 8.2 eV, and naphthalene 8.1 eV [41,42].

The ionization energy of an organic molecule decreases with the presence therein of π electrons (unsaturated bonds, aromatic ring) or heteroatoms (N, O, S) and with this the ionization cross section of the molecule increases. Small molecules such as H_2O, HCN, CO or CO_2 have quite high ionization energies, namely, 12.6, 13.9, 14.0, and 13.3 eV, respectively (and also their heats of formation are large). When organic molecules are ionized with the loss of 18, 27, 28 or 44 mass units, these small molecular species are practically never recorded as ions in their mass spectra, precisely by having ionization potentials higher than those of any complementary fragment ions, e.g., $(M - H_2O)^{+\bullet}$, $(M - HCN)^{+\bullet}$, $(M - CO)^{+\bullet}$ or $(M - CO_2)^{+\bullet}$. This is also a reflection of the Stevenson-Audier rule [43,44], which states that for a fragmentation process (usually referred to simple bond breaking, but also applied to some simple rearrangement processes) the positive charge is localized on the fragment ion that possesses lower ionization potential. If the molecular ion is not recorded in the mass spectra of volatile analytes and thermally stable substances (alcohols, esters, aliphatic, branched hydrocarbons, etc.), this can be explained by its high lability and very short lifetime ($<10^{-6}$ s), and by the absence in the molecule of structural elements (double bonds, aromatic rings, heteroatoms) to enable stabilization by different mechanisms, generally, through effective charge delocalization. The molecular ion will not become distinguishable in the spectrum by reducing the bombarding electrons energy. If they are not recorded at 70 eV electron energy, they are not detected either on the so-called low voltage spectra, at lower electron energies (10 - 30 eV). This is because the sensitivity of the method falls dramatically, that is, the number of molecular ions or fragment ions decreases with the electron energy. For volatile, thermostable but labile molecules in whose mass spectra no molecular ions are recorded, "soft" ionization methods should be used, e.g., chemical ionization (CI) or in some cases, chemical derivatization turns out to be a useful solution [41].

The electron ionization occurs under reduced pressure (vacuum) of 10^{-5}-10^{-6} Torr (the mean free path is of the order of meters). It is an endothermic process accompanied by the formation of $M^{+\bullet}$ ions and their monomolecular dissociation. However, fragmentation is not a random process. The occurrence of certain fragment ions (fragmentation pattern) is a reflection of: (1) molecular structure, (2) the energy of bombarding electrons, and (3) the excess of internal energy acquired by the ionized molecule. The number of ions (ionic current) detected may be affected by pressure in the system, contamination of the ion source or by aging or deterioration of the dynode surfaces of the electron multiplier. The range of ionization methods for high molecular weight substances (e.g., proteins, nucleotides, polysaccharides, etc.), highly polar molecules (e.g., amino acids, sugars, glycosides, vitamins, pesticides etc.) or thermo-labile substances [40-42] is much larger and technically more sophisticated. Ionization of heavy or polar molecules, usually happens in parallel or immediately after desorption from the surface, where they are bidimensionally distributed as a "solution" in a very low volatile solvent.

Modifiers, polarization promoters, are added or are present as incrustations in a solid [45]. The desorption from the surface and molecular ionization occur almost simultaneously: various primary and secondary ions are generated by means of accelerated atoms (FAB), accelerated ions (SIMS), or high-energy photons (one of the versions is MALDI), which impinge on the sample holder which houses the substance in a properly prepared matrix [46].

Special methods of ionization techniques are used in tandem setups, i.e., in the coupling of liquid chromatography (LC) with mass spectrometry, LC- MS, where the liquid sample could be nebulized in one of the different interfaces available, e.g., assisted by the effect of temperature and high electrical potential; for example, thermospray (TSI) [47], electrospray (ESI) [48], or atmospheric pressure chemical ionization (APCI) [49] methods. Different mechanisms lead to the formation of ionized species, which include molecules with multiple protonation, or deprotonation, clusters, among other ions generated.

The most common technique for the ionization of "small" molecules (more than 90% of applications) is the electron impact, while the positive ion (PICI) or negative ion (NICI) chemical ionization is an important complement used when molecular ions are not recorded in the spectra obtained by EI [50]. When the molecular weight of the substance should be determined, it is clearly deduced from the CI spectra based on the mass of the protonated molecular ion, MH^+ [or deprotonated $(M-H)^-$, for NICI], along with the cluster ions, usually formed by electrophilic addition of secondary ions from reactant gases (methane, ammonia, *iso*-butane, etc.).

Both electron ionization and chemical ionization are used in the technique of gas chromatography-mass spectrometry. Unfortunately, the use of CI requires, generally, the change of the ionic volume (ionization chamber), because the residual pressures that are used in CI are much higher (up to 1 mm Hg) than those used in EI (10^{-5}-10^{-7} Torr). In fact, less than 10% of all substances in the planet can be ionized in the vapor phase by EI or CI, as the ionization process by these techniques has as major limitation the low volatility or thermal instability of many organic substances. Volatilization is a stage prior to the ionization by EI or CI; the two processes are separate both in time and space. The separation of ions in the GC-MS technique can occur with virtually all types of mass analyzers, e.g., ion traps (IT) [51], quadrupole (Q) [52], magnetic deflection analyzer [53], as well as the configurations of several tandem analyzers (e.g., QqQ, triple quadrupole [54]), and today, for the GC x GC configuration, the time-of-flight (TOF) analyzer [55].

3.2. Mass analyzers

The mass selective detectors are divided into two groups. The first group corresponds to scanning analyzers. These include sector analyzers, e.g., magnetic deflection of single or double focus (when an electrostatic analyzer is added). The magnetic sector analyzer was historically the first mass analyzer employed. However, its use in GC- MS is not common. Quadrupole analyzers are the most frequently used in tandem GC-MS systems. The second group consists of simultaneous ion transmission analyzers. These include the time-of-flight (TOF), different types of ion trap (IT) and Fourier transform mass analyzers, specifically, ion-cyclotron resonance mass spectrometers (ICR-MS), which have gained recent popularity in the

field of coupled techniques with both gas and liquid chromatography, because of its high resolution and sensitivity. The mass selective detectors are distinguished by their properties or specifications and analytical scope. The most important parameters include: (1) resolution, (2) the maximum mass that can be measured, and (3) the ion transmission. Low resolution mass analyzers (single focus magnetic deflection, quadrupoles, ion traps, linear TOF) measure nominal masses (integers) and cannot distinguish isobaric species, whereas high resolution analyzers are capable of determining exact masses (4-6 digits after the comma), a measurement that leads to the unambiguous determination of the elemental and isotopic composition. One of the most simple, classic examples, is the measurement of isobaric ions of the species CO^+, N_2^+ and $C_2H_4^+$, whose "separation" is impossible with a low resolution analyzer. It is achieved with high resolution analyzers (magnetic and electric sectors, high-resolution TOF or ICR-MS) working in the exact mass scale [55]. Of course, the gases CO, N_2 and C_2H_2 in a mixture can also be separated and quantified by GC using PLOT type columns. The mass range in which each analyzer operates is important and above all, the maximum mass that it can measure. For example, TOF analyzers have no limits to measure high mass (while the quadrupole and ion trap analyzers do); the mass range of the TOF with reflecton is virtually infinite. The mass range, resolution and accuracy that each analyzer can reach depend on its configuration, the ion separation mechanism and the degree of "monoenergizing" of ions with the same mass, i.e., the ability to decrease the dispersion in time or space, which causes signal (partial ion current) broadening. Ion transmission in mass analyzers is measured as the ratio between the ions formed in the ionization chamber and those which, after passing through the mass analyzer, reach the detector. Scanning analyzers (sector, quadrupole) have lower ion transmission values than the simultaneous transmission analyzers, i.e., TOF, orbitrap, FT-ICR-MS. The latter generally have a higher sensitivity [56].

3.3. Tandem mass spectrometry

Frequently, excessive chemical noise is observed in the ion current of extracts obtained from biological samples, food, soil, etc. This leads to a failure to achieve the required specificity and to detect and identify reliably analytes of interest, when analyzing a complex mixture with many interferences or impurities. Usually, the signal/noise (S/N) increases with the number of steps in an analytical procedure. In instrumental analysis, this is the typical case of a tandem system, for example, GC-MS or LC-MS, including multidimensional or tandem mass spectrometry (MS-MS). Equal to the cleaning of an extract, which increases the S/N in the process of final instrumental analysis, a tandem mass spectrometer includes filtering steps in its operation. For instance, in a triple quadrupole (QqQ), during the first step of ion separation, the first analyzer (MS1) executes a specific clean-up, to distinguish, in a complex mixture, the characteristic ions of the analyte; the second mass analyzer (MS2), records only signals that are characteristic of the target analyte, free from interfering signals. A device located between the two analyzers is where the selected ion can be activated, i.e. its internal energy can be increased, leading to its dissociation and the formation of fragment-ions (ion-products), which are recorded in the analyzer MS2. This device operates as a collisions cell, which cause the disassociation of stable ions selected by the first analyzer.

The classic configuration of a tandem mass spectrometer is the connection in series of MS1, collision- activated chamber and MS2, followed by a system for the detection and measurement of ionic currents. Tandem mass systems are divided into two large groups [38] depending on the types of mass analyzers involved. The first group is made up of tandem-in-time mass spectrometers. These include linear and quadrupolar ion traps, orbital traps (orbitrap) and FT-ICR-MS. In tandem-in-time instruments the ions produced in the ionization region are trapped, are isolated, fragmented and then separated according to their m/z ratio in the same physical space. The cascade of dissociation reactions of ions pre-selected and then activated and subsequently monitored, take place in the same analyzer, but occur consecutively as a function of time, thus allowing the successive record of ions which are son, grandson, grand-grandson, etc., $(MS)^n$, of the original selected ion. The second group of tandem mass (MS/MS) instruments is made up of the so-called tandem-in-space mass spectrometers. In these, at least 2 analyzers are separated in space. With these spectrometers it is possible to study not only product-ions, but also precursor ions, the reactions (transitions) between 2 related ions, or monitoring the loss of a neutral fragment. A triple quadrupole, designated as QQQ, or QqQ, belongs to this type of tandem mass spectrometers. Hybrid MS/MS configurations involve combining several analyzers of different nature or different operating principles, for example, quadrupole (Q) or ion trap (IT) with a magnetic sector analyzer (B) alone or in conjunction with electrostatic analyzer (E) or a time-of-flight (TOF) analyzer. This gives rise to different hybrid tandem equipment, e.g., EBE, EBEB, B-QI-Q2, QEB, Q-TOF, IT-TOF-TOFB, EB-TOF-TOF EBE, QBE, and other possible combinations of analyzers. Of course, the union of several analyzers greatly increases the cost of the instrument and the complexity of their operation, but, simultaneously, increases the amount and quality of analytical information obtained, the degree of reliability and specificity [55,56].

Tandem MS/MS instruments involve 2 stages of mass analysis separated by a reaction of activated or induced dissociation of ions. This happens between the mass measurement before and after the fragmentation of the ions selected in the first stage. The fragmentations are caused by collisions of the selected ions with inert gas molecules (He, Ar, Xe or N_2, at a pressure of 0.1-0.3 Pa), and by the accelerating potential of an electrostatic field applied in a collisions cell, also called cell of activated or induced collisions. When employing soft ionization methods (e.g., chemical ionization, Cl) or in the coupling of liquid chromatography with mass spectrometry with electrospray (ESI) or atmospheric pressure chemical ionization (APCI) molecular (or poliprotonated, multicharged) ions practically do not fragment, and this leads to a lack of information required for the structural elucidation of the molecule. The "energization" of the stable (non-dissociated) ions (cations), for example, molecular or quasi- molecular ion, multiprotonated species, cluster, etc., allows to forcibly induce their dissociation to subsequently extract structural information complementary to the molecular mass or elemental composition (when using high resolution mass analyzers).

Using a detection system with more than one mass analyzer such as a triple quadrupole is appropriate for the analysis of target compounds at trace level (ppt - ppb range) in complex matrices, with the presence of interferences, as in the cases of food samples, biological fluids, animal and plant tissues, soil, wastewater and other environmental samples. The MS/MS

technique is very helpful and is a valuable analytical reinforcement, required in situations where (1) there is a high chemical noise in spectra acquired in SlM mode, (2) characteristic ions coelute with isobaric impurities (same nominal mass), (3) the structure of the compound is unknown and requires additional structural information (often, it is necessary to activate molecular, quasi-molecular or protonated ions, or some stable fragment ions, to extract additional structural information through the products in which fragment-ions are dissociated), (4) the fragmentogram obtained in SIM mode requires an additional confirmatory information and finally, (5) higher sensitivity and specificity are required in the analysis (pesticide residues, petroleum biomarkers, anabolic steroids and other doping agents, etc.).

The triple quadrupole configuration provides a range of analytical experiments and modes of ionic current acquisition each of which provides certain specific information. The following describes each of the possible modes of acquisition of a QQQ instrument.

3.3.1. Full scan

This is the traditional type of ionic current acquisition, similar to that employed in spectrometers with a single quadrupole analyzer (mass filter). The first analyzer (MS1) performs a full sweep and records mass spectra of every analyte emerging from the GC or LC column, which has been ionized and fragmented into molecular ion and different ion-products. In the GC-MS technique, *ca.* 0.05-1 ng of a compound are sufficient to obtain a mass spectrum that meets quality criteria [34,41]. The intermediate quadrupole (Q2) and the mass analyzer (MS2) operate only in ion transmission mode [52,54].

3.3.2. Selected Ion Monitoring, SIM

This acquisition mode is also well known and is widely practiced in mass spectrometers with a single quadrupole. In this case, the first analyzer (MS1) allows free passage only to a small number of selected ions (usually 3), typical or characteristic of the target analyte, which is selectively sought in a complex mixture. The other two quadrupoles just transmit the ions filtered by MS1. The mass fragmentogram is built based on the partial ion currents recorded. Since each selected ion is measured for a longer period, e.g., approximately 50 ms instead of 50 μs, chemical noise is reduced (S/N is increased), lower detection levels (by a factor of 10-100) are reached, and this permits the detection of a target analyte in quantities of pg or less. SIM mode is widely used for recording both a compound of interest in a complex mixture, as well as for sensitive quantitation, but also to register groups of homologous compounds, by monitoring their characteristic ions, namely for *n*-paraffins: *m/z* 57, 71, 85, for fatty acid methyl esters: ion at *m/z* 74; for alkylbenzenes: *m/z* 91, 105, and for phthalates: *m/z* 149, 167, among other analytes of interest.

3.3.3. Product ion scan

In this acquisition mode a precursor or parent ion is selected in the first mass analyzer or filter (MS1), operating in SIM mode. The chosen parent ion (m^+) passes MS1 and is directed to the collisions cell. The Q2 quadrupole (q) operates only with applied radiofrequency (RF-mode),

which allows transmitting ions from the first analyzer to MS2. The collision gas supplied to the cell (usually, He, Ar or N_2), by means of collisions with the selected ions, provides the additional energy (ion excitation process); an applied potential in Q2 (q) permits to accelerate the ions and convert part of their kinetic energy into additional internal energy (rotational, vibrational and electronic). This potential should be optimized in order to obtain higher sensitivity (Figure 8). The increase of internal energy of the ions, that is, their "energizing" or "activation", leads to their dissociation and the formation of different fragment-ions (product ions) which are then directed to the second mass analyzer or filter (MS2). This analyzer scans the mass range smaller than the mass of the selected m^+ parent ion, since the product ions obviously weigh less than their predecessors. It should be noted, that a product-ion mass spectrum will lack the companion signals from isotopes. This acquisition mode is perhaps the most common and well known in the triple quadrupole system, and is used for various purposes and applications. It can be used in the analysis of complex mixtures or substances with impurities, without prior separation in a chromatographic column. If a soft ionization method is used, for example, positive ion chemical ionization, the mixture of ionized substances in the ion source, will produce protonated molecular ions (MH^+). The first mass analyzer MS1 selects one by one the protonated molecular ions, which are directed to the activated collision cell (q), where they dissociate; fragment ions formed from each MH^+ are separated by the MS2 analyzer (Q3) and the spectrum is recorded. In this case, the first analyzer (Q1) acts as a "chromatographic column", the second quadrupole (q) operates as an "ionization chamber", and the MS2 analyzer (Q3) fulfills the role of the mass analyzer, which makes a complete ion scan. Thus, time savings will be achieved by not using a chromatographic column and focusing the QqQ system work in the search of selected compounds of interest present in a complex mixture without prior chromatographic separation.

3.3.4. Parent ion scan

This mode of operation is used to find in a mass spectrum those (precursor) ions that can generate a given fragment (product ion). In this case, the first quadrupole (MS1) operates in the full scan mode, while the third quadrupole (MS2) operates in selected ion monitoring mode. This selected ion is the product ion (daughter ion) of interest, whose precursors are sought. Technically, this takes place as follows: in the MS2 analyzer (Q3) - only product ions with specific mass are filtered, while the first analyzer, MS1, allows passage to all ions with a mass above that of the selected product ion. These ions cross the activated collision chamber (q) where they are fragmented, resulting in, among others, the product ion of interest. MS2 operating in SIM mode is the next filter, which only daughter ions are allowed to cross. For example, in a complex mixture of 30-40 components it is of interest to detect only phthalates (plasticizer). The common ion, diagnostic for this group of compounds, is recorded at m/z 149. Then, all ions with the mass/charge ratio higher than m/z 149 pass by the first analyzer, operated in scan mode; the analyzer MS2 operates in SIM mode, filtering only ions with m/z 149, which are formed in the activated collision chamber from the precursor ions - phthalates. The mass fragmentogram finally recorded contains only chromatographic peaks corresponding to phthalates; thus the mass analyzer, QqQ operated in a precursor ion scanning becomes a selective chromatographic analyzer.
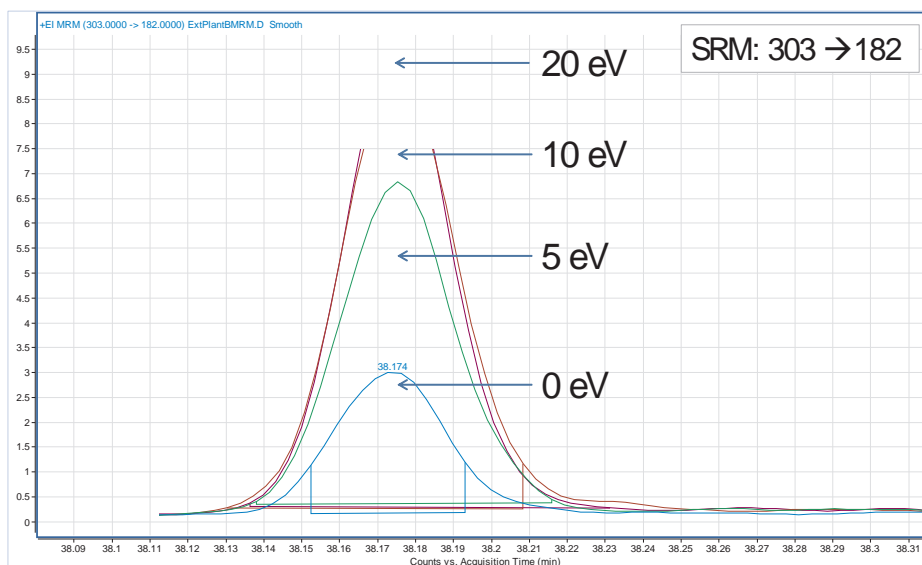
Figure 8. Single reaction monitoring (SRM) (metastable ion transition) between the molecular ion of cocaine (*m/z* 303) and one of the most abundant and characteristic ion-product (*m/z* 182), 303->182, obtained by QqQ-MS with N_2 as collision gas, and at different collision energies (potentials, 0, 5, 10, and 20 eV).

3.3.5. Constant neutral loss scan

In this QqQ operational mode, both mass analyzers, MS1 and MS2, operate in scan mode simultaneously. The scanned masses in both analyzers correspond to ions with a fixed difference, which equals the mass of the selected neutral fragment. If ions f1, f2, f3, f4, etc., pass the first analyzer (MS1), and then go through the activated collision chamber (Q2), they may experience fragmentation. The fragmentation products that will be able to pass the next analyzer (MS2) will be only those ions with the pre-established, fixed, mass difference, that is, f1-Δm, f2-Δm, f3-Δm, f4-Δm, etc. For example, if the linked scan of both analyzers corresponds to a mass difference of 28 units, if ions with *m/z* 80, 81, and 82 pass the first analyzer, MS2 will only allow free passage to ions with *m/z* 52, 53 and 54.

3.3.6. Multiple reaction monitoring, MRM

This is one of the most *sui generis*, interesting, methods of ionic current acquisition of the triple quadrupole, because it allows exploring and properly using its advantages, to turn it into a specific, highly selective and sensitive GC (or LC) mass detector. Unfortunately, when the acquisition of the ionic current is done in the SIM mode, the probability that the selected ion and a signal from the background (chemical noise) actually match is not null. This non negligible possibility of false positive or false negative results, lowers the reliability of the SIM acquisition method. In order to avoid these problems, instead of monitoring characteristic ions, the MRM experiment rather focuses on recording transitions (or transition reactions) between

ion pairs (precursor and product). Selected precursor ions (F1) are filtered in the first analyzer (MS1, SIM mode), while only ions F2, product of the transition or dissociation reaction F1 → F2 are allowed to pass the second analyzer MS2 (operating in SIM mode). Both ions must be stable and, in general, abundant in the mass spectrum of the analyte. Reaction monitoring of precursor and daughter (ion product) ions almost completely cancels the probability of coincidence of the analyte signal with one from the background and raises the value of S/N. Typically, the registration of two independent transitions along with chromatographic retention can confirm unequivocally the occurrence of an target analyte in a complex mixture (Figure 9). The use of triple quadrupole operated in MRM mode, is of particular importance for the analysis of compounds (target analytes) present at trace level in highly polluted, complex, multi interference matrices; for example, in pesticide residue analysis in foods, plants or biological or environmental samples [57]. Another important application is the analysis of biomarkers in petroleum [58].
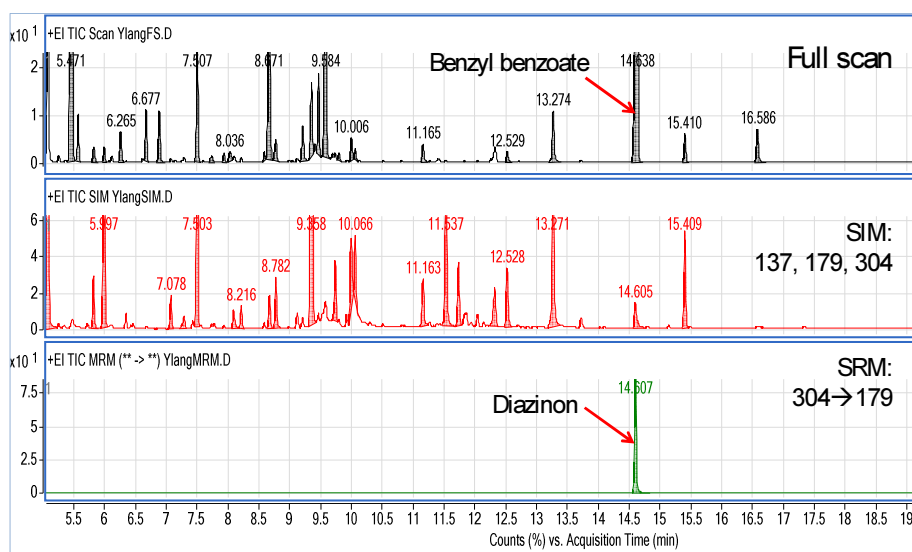

Figure 9. GC-QqQ-MS analysis of the extract obtained from ylang-ylang flowers (*Cananga odorata,* Annonaceae family). Determination of diazinon present in the extract, using different acquisition modes: full scan, SIM [characteristic ions, *m/z* 304 (M$^+$), 179 and 136] and single reaction monitoring, SRM, of the ion transition *m/z* 304 →179. Notice the highly selective and sensitive detection of diazinon (2 ppb) in the ylang-ylang extract, using QqQ-MS in the metastable ion transition mode (SRM).

4. Data analysis and interpretation

There are two basic strategies in GC- MS, for the identification of compounds. The first is the use of standard substances (certified reference material). However, not always all standards

are available, many of them are not easily accessible for a large number of analytes. The second strategy is the combination of several approaches, among which are the following: (a) retention indices (RI), in conjunction with (b) experimental mass spectra (EI, 70 eV) and (c) their comparison with those of databases of retention indices obtained in columns of orthogonal polarity (polar and nonpolar) and of standard mass spectra (EI, 70 eV). The combination of several experimental parameters and data, i.e., retention times measured in both columns and mass spectra is mandatory for the structural identification of components in a mixture. The identification can be tentative (preliminary, presumptive) or confirmatory. Confirmation (positive or unambiguous) requires, in many cases, the use of a certified standard compound. Multiple analytes from complex mixtures, however, can have similar retention times or their mass spectra seem alike or have only very small quantitative differences (ion intensities). Limonene epoxides, xylenes, and many structurally similar terpenes, are examples of this situation. However, the possibility of simultaneous coincidence of both the retention indices calculated in both columns (polar and nonpolar) and of mass spectra for two different substances, in fact, is very remote, almost unlikely. For some cases, such as those that may have legal implications, i.e., environmental, forensic cases or disputes, control of doping agents in sports competitions, it is absolutely mandatory to use certified standard substances for identification and confirmation. The analysis of an essential oil, perfume, aroma, and fragrance fractions (or any other complex mixture), in order to quantify and identify its components, done by one-dimensional chromatography, should comply with the following conditions: (1) using preferably long capillary columns (50, 60 m), (2) performing the analysis in two capillary columns with orthogonal phase (e.g., DB-1 or DB-5 and DB-WAX), (3) obtaining experimental mass spectra EI (70 eV) and doing a comparative search, preferably, on various mass spectra databases (e.g., NIST, Wiley, Adams), (4) calculating linear retention indices in two columns, polar and nonpolar, and (5) using standard compounds for further structural confirmation (Figure 10, Table 1). The combination of all these parameters allows confirmatory identification of the mixture components. In GC- MS analysis it is very important to ensure that the chro-matographic peaks are "homogeneous", as the co-elution of various substances can lead to structural misassignments.

Compound	Capillary column		
	DB-1	DB-5	DB-WAX
α-Pinene	939-942	932	1036-1038
β-Phellandrene	1025-1032	1025	1213-1216
Sabinene	972-976	969	1130

Table 1. Linear retention indices (LRI) of some monoterpenes, measured on different stationary phase columns.

4.1. Mass spectra interpretation

The large number of fragment ions (cations and cation-radicals) in the mass spectrum is due, firstly, to their formation from molecular ions which have very different excesses of internal
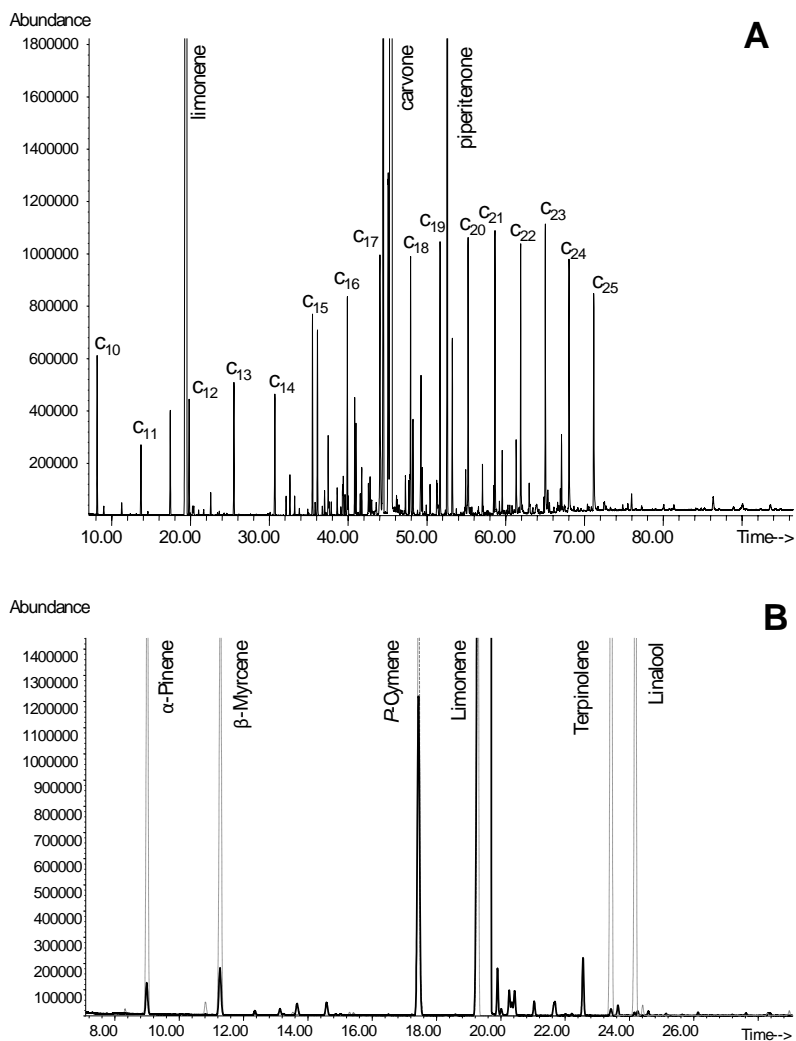
Figure 10. A. The use of *n*-hydrocarbon mixture co-injected with the sample for linear retention indices (LRI) calculation. B. Standard compounds used in the process of sample components identification.

energy. All molecular ions with internal energies lower than the potential energy of appearance of an ion fragment with the lowest formation activation energy will be recorded in the mass spectrum as not dissociated molecular ions. Their intensity in the spectrum depends on the molecular structure and, particularly, their ability to delocalize (stabilize) the positive charge, which allows the ion $M^{+\bullet}$ to exist for longer time than is required for its detection (ca. $> 10^{-5}$ s) in a mass spectrometer. One of the major limitations of the electron ionization technique lies in the fact that molecules that are ionized, should be in the vapor phase, i.e. being volatilizable

without undergoing thermal decomposition, prior to their ionization. Even so, many of the volatilizable and thermostable molecules do not exhibit molecular ions in their mass spectra, only fragment ions, something that limits obtaining information on molecular weight. In fact, less than 10 % of all existing molecules are suitable for analysis by mass spectrometry by electron ionization. The excluded cases are highly polar species (e.g., salts, amino acids), those of high-molecular weight (e.g., proteins, nucleic acids, polymers), and thermolabile compounds (e.g., sugars). In some cases, chemical derivatization of the molecule permits to increase its volatility and thermal stability and decrease its polarity.

4.2. Ion types

The user of mass spectrometry wonders at the beginning, interpreting mass spectra, why molecular ions with the same structure can generate several very different products and can be fragmented by parallel, competitive routes. The type and ratio (abundance) in a mass spectrum of daughter, parent and metastable ions is based on the following factors: (1) distribution of internal energies of molecular ions formed (also valid for fragment ions with excess energy that also dissociate afterwards); (2) activation energy of the process of fragmentation, Ea, and (3) slope of the curve of log K, where K is the dissociation constant of the parent ion through one of the possible reaction coordinates. Rearrangement (or transposition) processes are often accompanied by the formation of intense metastable ions; rearrangement products often "survive" in low voltage (10-15 eV) mass spectra, as the Ea energies for their formation, generally, are lower than those required for a simple bond rupture. As the log K curve becomes steeper, fewer metastable ions are observed in the mass spectrum corresponding to this fragmentation reaction coordinate, and particularly, most likely, this process will be of simple rupture. Accordingly, the structure of the molecule, the excess energy that it acquired during its ionization, the activation energies of possible dissociation processes and their rate, and the frequency factors involved in bond cleavage, are variables that determine the fragmentation pattern of a whole molecule and its product-ions that will be recorded in its mass spectrum. In a mass spectrum, obtained by EI, different signals are recorded, including the molecular ion (if its lifetime is greater than 10^{-6} s), fragment-ions, isotope ions, sometimes, also multicharged ion signals (such as in polyaromatic compounds). Mass spectrometers with specific configurations also detect metastable ions (products of ion fragmentation out of the ionization chamber). Each one of these ions provides structural information more or less important to the structural elucidation of the molecule. Molecular ions indicate the value of molecular mass and the elemental composition when high resolution MS is employed. Intense molecular ion signals indicate that the ionic structure can stabilize its positive charge and this normally suggests aromaticity or highly conjugated unsaturations. It is common that mass spectra of branched hydrocarbons, alcohols, and secondary or tertiary amines are devoid of the molecular ion signal. A soft ionization method, such as chemical ionization, should be used in these cases. Fragment ions contain the primary information for molecular structure elucidation. These may be cations (even electron number) or radical cations (odd electron number). They may result from simple rupture or rearrangement. Certain common fragment losses are associated with the presence of specific groups in the molecule. Alcohol molecular ions easily decay with the loss of either an OH group or an H_2O molecule, giving rise to the

(M-17)$^+$ and (M-18)$^+$ ions. Fragment ions $C_6H_5^+$ and $C_7H_7^+$ dissociate with the loss of an acetylene molecule (C_2H_2) and produce ions at m/z 51 and 65, respectively. The substance's elemental composition is derived from the relative abundance of isotopic ions. The presence of Cl, Br, S or Si can be easily determined from the isotopic peak pattern. A quality requirement of a mass spectrum is the clear distinction of isotopic ions. Multiply charged ions are found in the mass spectra of a limited class of substances, for example, those containing heteroatoms (N, S, O), aromatic or heteroaromatic rings, high unsaturation level, or when several of these elements are combined in the molecule. Their intensity is relatively low and may have fractional values. The signal at m/z 64 in the naphthalene mass spectrum (M$^+$, m/z 128) corresponds to a doubly charged molecular ion, M^{2+}. Metastable ions are formed out of the ionization chamber from ions with life time longer than a microsecond, but less than the time required to reach the detector. Their apparent mass m* (a fractional number) relates the masses of parent (m_1) and daughter (m_2) ions according to m* $= m_2^2/m_1$. Metastable ions are frequently registered in mass spectra obtained with magnetic sector analyzers. A mass spectrum of benzoic acid contains m* signals corresponding to processes of successive loss of radical OH and the CO molecule: M$^+$ → (M - OH)$^+$ and (M - OH)$^+$ → [(M - OH) - CO]$^+$. These signals confirm the genetic link between these ions. The absence of an m* ion corresponding to the direct process M$^+$ → (M – COOH)$^+$ indicates that this process does not take place.

4.3. Fragmentation patterns

The fragmentation pattern (m/z, the amount, I%, of ions that are recorded in a mass spectrum) shows how a molecule dissociates once ionized; this does not occur randomly, because it is derived from the molecular structure (nature of bonded atoms, chemical bonding strength), their spatial arrangement, ionization potential and internal energy that acquires during its collision with an electron. The fragmentation pattern is unique to each molecular structure and contains clearly detectable differences (often only quantitative), including those for the isomers (positional, geometric or stereoisomers). The mass spectrum provides information on: (1) molecular mass: if the molecular ion does not appear in the EI spectrum, it is necessary to obtain the spectrum via soft ionization, e.g., CI, but sometimes, derivatization of the molecule [35,37] can be used, in order to find its molecular weight and also to increase its volatility (that of the derivative) and the sensitivity with which it can be detected; (2) elemental composition: it is possible to know it, based on the analysis of a mass spectrum obtained by a high resolution mass spectrometer. Isotope ions or typical fragment losses (-HCN,-CO,-OH, -HS,-NH$_2$, etc.) also allow inferences about the elemental composition of the substance; the simplest case is the presence of Cl or Br atoms in the molecule; (3) functional groups in the molecule can be elucidated by noticing in the spectrum signals corresponding ions with characteristic mass, which indicate their presence, e.g., phenyl (m/z 77), benzyl (m/z 91), benzoyl, C_6H_5CO, (m/z 105), or by detecting fragments, the products of the loss of small neutral molecules, e.g., water, CO_2, CO, C_2H_2, HCN, alkyl radicals (CH_3, C_2H_5, C_3H_7, etc.); these fragment ions are related to the presence of these structural groups in the molecule; (4) spatial structure: sometimes it is possible to establish the spatial configuration based on the mass spectrum, especially when the spectra of both stereoisomers can be compared or when using low voltage mass spectra (10-20 eV) or chemical ionization, on which the differences in conformational energy (enthalpy

of formation) can be observed more clearly, based on differences in intensities of characteristic fragment ions, or in ionization potentials. Generally, dissociative ionization processes can be divided into the following two major groups: (1) single rupture reactions (homolytic or heterolytic) and (2) rearrangement reactions, which can be rearrangement of the molecular skeleton (for example, tropylium ion formation, $C_7H_7^+$) or reactions accompanied by hydrogen transpositions, for example, the classic McLafferty rearrangement [34]. The most common single rupture reactions are cation formation (for example, in hydrocarbon chains), allylic and benzylic ruptures, retro-Diels-Alder (RDA) reaction in monounsaturated cyclic systems, and acyl ion formation, among others. Generally, the energy required (activation energy, Ea, of the process) for rearrangement is lower than that required for single rupture. Single rupture processes are generally less selective than the molecular skeleton or hydrogen transpositions, and are also less susceptible to steric effects in the molecule compared to rearrangement processes. The mass spectra obtained with 70 eV or lower voltage (10-30 eV) of the same substance differ markedly. In the latter, molecular ions prevail (relative increment) and some products survive, basically, hydrogen rearrangements or transpositions, which require lower activation energy. The binding energy of atoms in the ionized molecule is in the order of 2-4 eV, whereas the ionization energy of the organic molecule is of the order of 6-13 eV. The energy of the bond involved in the dissociation is an important factor, especially when there are competitive processes to form predominantly one product versus another. The interpretation of mass spectra of organic molecules, obtained by electron ionization, is based on the study of the fragmentation pattern, which depends on molecular ion and ion-fragment dissociation mechanisms. Fragmentation schemes are not necessarily speculative, because numerous experimental verifications of fragmentation routes can be found. Different methods are included within the experimental tools to establish fragmentation schemes and mechanisms: (1) using soft (CI, FI) ionization techniques, (2) chemical derivatization, (3) isotopic labeling (e.g., with D, ^{15}N, ^{18}O) and chemical marking (introduction to the molecule of substituents that are not released during the fragmentation, e.g., F, OCH_3, etc.); (4) high resolution mass spectrometry, which allows to establish the elemental composition of each ion; (5) metastable ion study to determine the relationship between parent ions and subsidiary ions, (6) use of tandem configurations and study of collision-activated dissociation (CID, Collision-Induced Dissociation); (7) photoionization (PI) and determination of energies (potential) of ionization and ion appearance; (8) quantum mechanical calculations of formation energies and ion structures. Obviously, not all experimental techniques can be performed on a single mass spectrometer, but the whole set will provide sufficient information to correctly set the scheme or even the mechanism for the fragmentation of an organic molecule ionized by electrons. Many references [34,35,36,41,42,48,52], contain information about fragmentation of organic molecules, their mechanisms and the classification of simple rupture or transposition processes. Generally, it is considered that the energies involved during the electron ionization are very high (70 eV) and can "level" or mask thermodynamic differences generated by the spatial characteristics in isomeric molecules. However, steric factors exert pronounced effects on activated complex structure and are more relevant to the rearrangement processes. Increasing the internal energy of ion $M^{+\bullet}$ causes a decrease of rearrangement products. Decreasing the energy of bombarding electrons to 10-20 eV, can reduce the internal energy of the ions $M^{+\bullet}$ and

increase the number of rearrangement products (transpositions). Stereoisomeric effects are demonstrated most notably in the mass spectra of chemical ionization or those taken in tandem configuration instruments when collision activated reactions (CID) are used.

5. Conclusion

The association of separation and mass spectrometric techniques is a key that opens up a rich and multidimensional analytical space for the investigation of complex mixtures with high sensitivity, selectivity and specificity. The variation of chromatographic conditions and the modulation of mass spectrometric data acquisition or the use of tandem spectrometers, permit to focus the analytical methods towards different goals in these investigations. Trace-level contaminant detection, molecular structure characterization, or classification, are just a few of the many applications of gas chromatography coupled to mass spectrometry performed currently with varying degrees of resolution and sensitivity on very complex mixtures.

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