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Review Article

Liquid Chromatography-Mass Spectrometry: A Review

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Abstract

The analytical technique known as liquid chromatography-mass spectrometry is incredibly precise and sensitive. It's a really powerful tool. Detection, identification, and mass determination of components in the presence of additional components are carried out by mass spectrometry using sample eluents from liquid chromatography and mass spectrometry. Liquid chromatography is used to identify pharmaceutical medication components, intermediates, and related compounds for both quantitative and qualitative applications. Liquid chromatography is mostly used in-vitro dissolution, bioequivalence, bioavailability, and metabolite research. Liquid chromatography mass spectrometry is also used in forensic labs, agrochemical firms, fundamental research, and the food industry. Applications, instrumentation, and the liquid chromatography-mass spectrometry principle are covered in this review.

Keywords: Liquid Chromatography, Mass Spectrometry, High Performance Liquid Chromatography, Bioequivalence, Metabolite research.

Introduction

High-performance liquid chromatography (HPLC) is a commonly used analytical technique in the pharmaceutical industry for the identification and quantification of pharmacological substances and their associated molecules. Because HPLC is so precise and repeatable, it is widely used in the chemical, pharmaceutical, and pesticide industries.

Mass spectrometry (MS) and liquid chromatography (LC) are combined in the hyphenated analytical method known as liquid chromatography-mass spectrometry (LC-MS). Chromatographic columns in HPLC are used to separate the constituent parts of mixtures. In general, LC cannot positively identify the separated components on their own. Furthermore, mass spectrometry is used to determine the structures of both known and unknown compounds. Identifying mixes with mass spectrometry alone is not helpful since a mass spectrum mixture is essentially a complex of overlapping spectra from separated individual components. It is difficult to connect liquid chromatography (LC) and mass spectrometry (MS). An interface is used to transmit liquid eluents from LC to MS. LC-MS shown in Figure 1 is more frequently used in studies pertaining to pharmacodynamics, bioavailability, bioequivalence, and dissolution.[1]

With preparative LC-MS systems, certain chemicals can be rapidly and mass-directedly purified from such combinations. This is beneficial for fundamental research as well as the food, pharmaceutical, and agrochemical industries. [2,3]

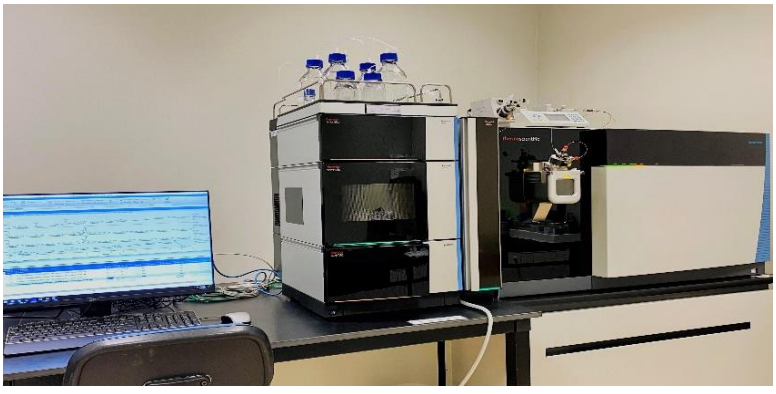

Figure 1: Liquid Chromatography - Mass Spectrometry (LC-MS).[4]

Basic principle of LC-MS

The basic principle of mass spectrometry (MS) is to use any suitable technique to create ions from inorganic or organic substances. Then, the ions are separated based on their mass-to-charge ratio (m/z), and their corresponding m/z and abundances are used to identify the ions both quantitatively and qualitatively. The analyte can be thermally ionized by exposure to strong photons, electrons, or ions, or by an electric field. One ionized atom, molecule, or one of their partners or fragments could comprise the ions. [5]

In the past, HPLC techniques were divided into two subclasses that each supported the stationary phases and the polarity of the mobile component that was necessary for them. Using pure or pH-adjusted water organic mixtures, such as water - acetonitrile and water - methanol, as stationary parts, reversed part liquid natural process (RP-LC) methods employ octadecylsilyl (C18) and similar organic-modified particles. In processes referred to as the "traditional phase liquid natural process," clean or mixed organic mixes (NP-LC) are combined with materials like colloid, which serves as the stationary component. RP-LC is most frequently used in the LC-MS apparatus since it suggests putting samples into the MS.[1]

Instrumentation

Liquid chromatography - Mass spectrometry (LC-MS)

High-resolution chromatographic separation is combined with focused and sensitive mass spectrum detection in the analytical technique known as LC-MS. Combining LC with MS is a significant achievement in the history of chromatography. Mass spectroscopy helps provide structural clarity and helps identify the constituent elements of a sample in LC-MS. It's a highly sensitive and selective approach that works well in a variety of settings. It is widely used in pharmaceutical pharmacokinetic studies and is most frequently applied in the field of bioanalysis. Furthermore, pharmacognosy, especially molecular pharmacognosy, uses LC-MS to identify the constituents of different phenotypic cloning procedures.[6]

The Liquid Chromatography-Mass Spectrometry (LC-MS) technique combines the advantages of mass spectrometry (MS) with liquid chromatography (HPLC) for separation and mass spectrometry for detection. Figure 2 below displays the LC-MS schematic block diagram. The following is a list of the many components of the LC-MS instrument.\

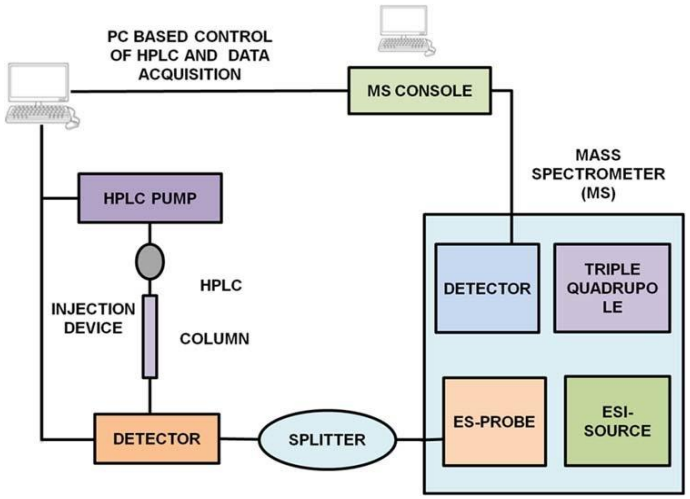

Figure 2: Schematic Diagram of LC-MS System.[7]

Liquid chromatography: HPLC (High Performance Liquid Chromatography): This type of chromatography is characterized by its ability to separate mixture components through the use of a solid stationary phase and a liquid mobile phase. There are diverse categories of chemical analysis, such as affinity liquid chromatography, reverse phase chromatography, ion-exchange liquid chromatography, chiral separation, and normal phase liquid chromatography.

Through the use of a tiny quantity of complex mixtures can be separated using several column packing techniques that have high efficiency. Below is a list of HPLC's constituent parts.

a. Pump: It is made up of materials that are inert to solvents or any combination of organic solvents and an aqueous buffer. Up to 10 mL/min of high mobile phase volume is delivered by it. Syringe pumps, constant-pressure pumps, and reciprocating pumps are the three main types of pumps that are employed.

b. Sample Injector: A sample volume is introduced into the chromatographic system using it. Typically, one can inject a sample volume ranging from 1µL to 100µL. Up to a 2 mL volume, the injector loop can be used to enhance the injection volume. Automatic and manual injectors are the two main types of injectors that are employed. Compared to manual injectors, automatic injectors are more precise, accurate, and comfortable to use. [3]

c. Columns: It is a stationary phase made up of carbon chains combined with silica material. Typically, columns with lengths ranging from 50 to 300 mm are employed. Octadecyl (C18), Octyl (C8), Cyano, Amino, and Phenyl packings are the columns used in HPLC. Depending on the type of compound that needs to be separated, different columns are used.[8]

d. Detectors and recorder: The most crucial component of the HPLC is the detector. There are several types of detectors that are employed, including conductivity, UV-visible, PDA, electrochemical, refractive index (RI), and fluorescent detectors. The detector's signal can be recorded as a peak, and the corresponding data can be saved in software.

Mass spectrometry: An analytical method called mass spectrometry measures the mass-to-charge ratio of ionic species that are connected to the analyte that is being studied. Analytes can be thoroughly structurally elucidated as well as have their molecular mass and elemental content determined using mass spectrometry (MS).[9]

i. Ionization Sources and Interfaces

ii. Mass Analysers

Ionization/Ion Source and Interfaces: The liquid chromatography method separates liquid mixtures, most commonly consisting of methanol, acetonitrile, and water. This mixture-containing liquid is poured into the mass spectrometer's ion source. Given that the ion source is highly vacuumed, it is challenging to mass evaporate the liquid droplets without losing the component mixture because of the pressure difference. Interfaces are thus utilized to address this issue. The following is a description of the many interface types that are frequently seen in mass spectrometers.

a. Direct liquid Introduction (DLI): Direct Liquid Introduction (DLI), ionization is often achieved by vaporizing the solvent to produce a chemical reagent gas and ionization. Solvent systems in both the normal and reverse phases have been employed. Methanol/water and acetonitrile/water mixtures up to 60% water are the reverse-phase solvents that are used. Salt-containing buffers are generally prohibited because they increase the risk of capillary plugging during heating. Thermal energy and liquid flow rate are combined to operate Direct Liquid Introduction (DLI). Only a restricted flow rate of the liquid enters the contact. Analyte ions generated with the aid of thermal energy were subsequently introduced into the ion source via a pinhole diaphragm or capillary inlet.[10,11]

b. Atmospheric-Pressure Ionization (API): Three main phases make up atmospheric pressure ionization (API): nebulization, evaporation, and ionization. The two primary methods of atmospheric pressure ionization (APCI) and electrospray ionization (ESI) are known as API. In atmospheric pressure ionization (API), a mist of tiny droplets is created when a stream of liquid (solvent) carrying a sample is pushed through a thin capillary tube and nebulized in a huge chamber. There is an ionization process, and a certain percentage of droplets have an excess of either a positive or negative electrical charge. Solvent evaporation occurs in a huge heating chamber. The solvent disappears from the droplets, causing them to get progressively smaller. The ions and molecules collide with one another. After that, the produced ions entered the mass analyzer via a capillary. [12,13]

c. Electrospray Ionization (ESI): Fenn and his colleagues invented the most useful ion source, called Electrospray Ionization (ESI). The liquid sample used in electrospray ionization (ESI) was run through a stain-resistant steel capillary tube that was kept at a high positive or negative electric potential (approximately 3-5 kV) [1].

This leads to the formation of charged droplets at the capillary tip, which vaporize later. The solvent evaporation causes the droplets to shrink and increase in surface charge. The highly charged droplets collide until they transform into gas-phase ions. These gas-phase ions enter the low-pressure area of the ion source via the capillary sampling opening.[14]

The main benefit of ESI is that it increases the amount of charge in ions by one to three when the molecule is 1000 DA or higher. As a result, the m/z ratio is consistently less than 2000. The molecular weight of peptides, proteins, biological samples, polymers, nucleotides, sugars, and organometallics can be determined using LC-MS with electrospray ionization (ESI) shown in Figure 3. Additionally, biological research and medical analysis regularly employ it.[15]

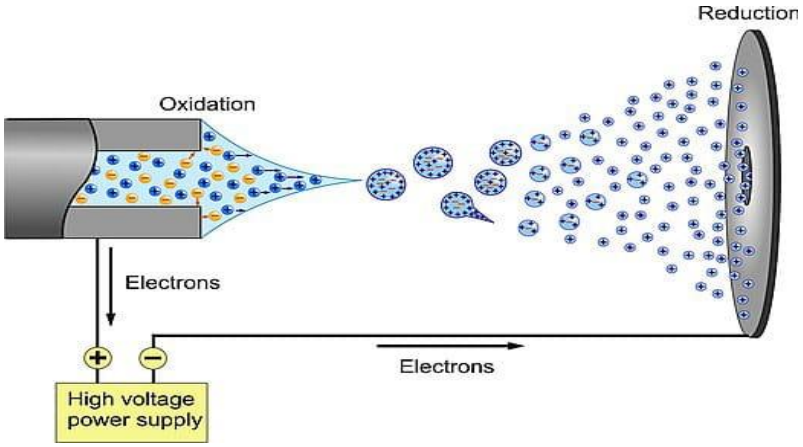

Figure 3: Electrospray ionization source.[16]

d. Atmospheric Pressure Chemical Ionization (APCI): The two main processes in the Atmospheric Pressure Chemical Ionization process are the analyte evaporation/desolvation and the charged transfer reaction in the vapor phase, which produces the vapor phase ions.

A liquid-containing sample is nebulized through a thin capillary tube and nebulized into a huge chamber in the atmospheric pressure chemical ionization process shown in Figure 4. Small droplets are created when the solvent evaporates at atmospheric pressure in a large heating chamber. There is ionization. Ionization typically occurs between 250 and 400 °C. The charges are subsequently transferred from the ions to the molecules via chemical processes. The mass analyzer's capillary aperture allows the resultant ions to pass through. For less polar and non-polar analytes with modest molecular weights, it is commonly employed.[17]

Chemical ionization at atmospheric pressure is used in MS analysis of samples pyrolyzed under controlled conditions. Analysis of moderately polar, non-labile materials can be done using online LC-MS using a heated pneumatic nebulizer interface in conjunction with APCI. Samples and biopolymers that are highly polar, thermolabile, and ionic require electrospray ionization at atmospheric pressure in addition to on-line separation by LC or CE. Atmospheric pressure ionization's formerly sluggish progress has been hastened by the ability to determine the molecular mass of proteins, nucleic acids, and other polymers via electrospray ionization.[18]

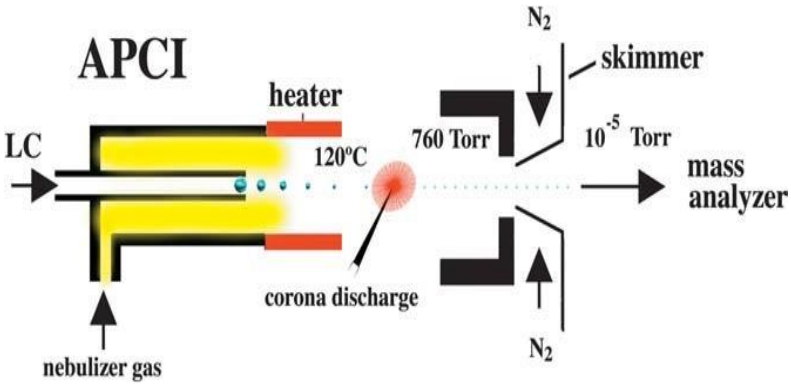

Figure 4: Atmospheric pressure chemical ionization.[19]

e. Thermo spray and Plasma spray Ionization (TSPI): Thermo spray serves as both an ionization source and a liquid intake device. A variation on thermal spray is plasma spray.

Thermo spray involves passing a liquid sample solution through a heated capillary tube, causing the solvent to evaporate. The droplets that are charged form. The droplets get smaller and smaller as the solvent evaporates. On the surface of droplets, the density of electric charge rises. After that, the ions are sent into a mass spectrometer that uses an electrostatic voltage system.[13]

Ions are not produced by the plasma spray per se; instead, they are produced in the thermal spray. The quantity of ions can be enhanced by plasma or corona discharge. The neutral molecules become increasingly ionized as a result of the electric discharge. This improvement causes the molecule to ionize more. Because of its increased sensitivity, the plasma spray technique is frequently employed in clinical and medical analyses.[20]

f. Atmospheric pressure photo Ionization (APPI): Using photons to excite and ionize the molecules is known as atmospheric pressure photoionization, or APPI. Analyte ionization from eluent and excitation are the two primary processes in atmospheric pressure photoionization (APPI).

The eluent from LC vaporizes into gaseous phase, much as atmospheric pressure chemical ionization (APCI) in atmospheric pressure photo ionization. The APPI generates photons using a Kr lamp. High intensity photons produced by a Kr lamp are used to excite and ionize molecules. In order to reduce analyte ionization, the energy range is chosen. After that, the ionized analytes are put into the mass spectrometer (m/z) through a capillary aperture.

This method works well for non-polar analytes that are extremely challenging to ionize using atmospheric pressure chemical ionization and electrospray ionization.[21,22]

g. Particle Beam Ionization: In order to separate the solvent from the solute with the least amount of solute loss possible, Browner and his colleagues created the particle beam interface. Nebulization and evaporation share similarities with atmospheric pressure chemical ionization (APCI), thermospray (TSP), and electrospray ionization (ESI) processes.[23]

Eluent is delivered through a small tube into this liquid that has been separated from the HPLC or LC. Helium gas is injected into the liquid, causing a high-velocity spray of liquid droplets to form. The heating chamber is where the liquid droplets from the nebulizer get smaller and smaller as the solvent starts to evaporate. A beam of particles known as the spray of liquid droplets emerges from the heating chamber. After that, the beam goes through an ionization chamber in a manner akin to atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI).[12]

h. Continuous Flow Fast Atom Bombardment (FAB): The FAB is a straightforward, highly sensitive interface approach. FAB involves the bombardment of a liquid target with fast atoms like xenon or argon (Ar). After dispersing the material onto a thin-layer metal plate or probe, it is dissolved in glycerol. After inserting the probe into the mass spectrometer, a fast-moving atom beam bombards it, ionizing the samples before they enter the mass analyzer (m/z). Large, thermally unstable molecules are treated with FAB. It is used with proteins and surfactants.[24,25]

Mass Analyser: Following ionization, the ions are moved to a mass analyzer, where they are separated based on their mass-to-charge (m/z) ratio. The mass analyzer is typically used to measure the object's reaction, speed, time, and rate.

a. Quadrupole

b. Time of flight

c. Ion trap

d. FTICR (Fourier transfer ion cyclotron resonance)

a. Quadrupole Mass Analyser: The most practical and widely used mass analyzer is this one. It is made up of two plains with parallel rods that are positioned between a detector and an ion source. The mass analyzer, or the ion separation process based on m/z in time or space.[10]

Four hyperbolic or cylindrical rods arranged in a radial array parallel to one another make up the linear quadrupole mass analyzer. An oscillating radio frequency alternating current (RF) voltage is superimposed on opposite rods that are charged in a +ve or -ve direct current (DC) potential.[26]

When DC and RF are combined and applied to the rods, the ions of a specific m/z have stable paths and are sent in the direction of the detector. Conversely, ions with an unstable mass and energy are released onto the rods.

The ions that were added to the quadrupole using a modest accelerating potential imply. As the ions pass through the quadruple filter, they oscillate in a plane that is perpendicular to the rod length.

Consequently, by providing DC and RF power at a consistent ratio, ions carrying m/z will flow in the direction of the detector. The ratio of the DC to RF potential determines the resolution. The quadrupole can scan at up to 1000 m/z and is often operated at less than 4000 m/z. Because of the unit mass resolution, mass accuracy is rarely greater than 0.1 m/z.[27]

Typically, the RF values fall between one and two MHZ. There's a possibility of a 1000V DC voltage and a 6000V maximum RF voltage. Figure 5 below displays the quadruple mass analyzer schematic diagram.

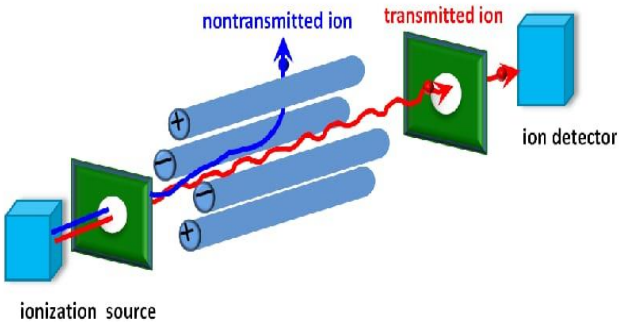

Figure 5: Diagram of Quadrupole Mass analyser.[28]

b. Time of flight analyser (TFA): The moment the most reliable application of flight is in a wide range of ion sources and inlet systems. There is no magnetic field here, so electrostatic maintenance and calibration are straightforward and uncomplicated. After being removed from the source, the ions are exposed to an accelerating voltage. The mass of the ion and its charge determine how long the drift or flight will take to complete.[29]

For singularly charged ions (z = 1, m/z = w), the duration required to arrive at the mass of the ions determines the detector. Lighter ions will strike the detector first when they trend in that direction.[26]

All of the ions are simultaneously scanned and identified. It is possible to employ extremely fast mass range scanning for very large m/z values. Figure 6 below displays the Time Flight mass analyzer schematic diagram.

Figure 6: Time of flight mass.[30]

c. Ion's trap mass analyser: High resolution, high sensitivity, and various product ion scan capabilities are features of the Ion Trap Mass Analyzer. As an ion trap, a quadrupole trap operates in three dimensions. A quadrupole field is delivered to a cylindrical ring electrode in this setup. End-capped electrodes make up the other two.[31]

While one end cap electrode has several holes or apertures that allow ions to be transferred to a detector, the other has a single, tiny central aperture that allows electrons or ions to be delivered into the trap. To stabilize the ion trajectories, there is a bath gas of helium in the trap. The ions and bath gas filled with helium collide. As a result, the ions' mobility raises the analyzer's trapping efficiency. The mass spectrum is produced by expelling the ions from the trap according to their mass-to-charge (m/z) values.[32]

d. Fourier Transfer Ion Cyclotron Resonance (FT-ICR): The primary mass spectrometer is the Fourier transfer ion cyclotron resonance which is shown in Figure 8. After leaving the ionization source, the ions are sorted based on their m/z ratio in a mass spectrometer. The ions that enter the chamber are imprisoned in circles. Both the magnetic and electric fields accelerate the ions.

The ions become excited as a result, producing a time-dependent current. The mass-to-charge (m/z) ratios of the trapped ions caused them to split.

Detectors

One crucial component of a mass spectrometer is the detector, which generates current in direct proportion to the number of ions that strike it. After the ions are created and exit the analyzer, they must be found and converted into a signal. The types of detectors that are frequently used are described below.

Point Ion Collectors Detectors

In this, the mass spectrometer's ion collectors are positioned at a fixed point. Every ion is concentrated on the detector, which is positioned in a single spot. The data can be recorded along with the arrival of ions through the electric current flow. The number of ions that reach the point ion detector determines how much electric current flows there.

Array Detector

A group of point collectors arranged in a plane is called an array detector. In an array detector, the ions arrive at a spot or across a plane. Using a point ion collector, the ions with mass-to-charge (m/z) values are separated and recorded along a plane. In an array detector, spatially distinct ions having a mass range are concurrently detected.[33,34]

Applications

Application of LC-MS in Doping Test:

The 4-Methyl-2-hexaneamine doping agent can be detected in urine using the LC/ESI-MS in positive mode. When analysing the urine samples, an internal standard of tuaminoheptane is added. The unknown substance is thought to be 4-methyl-2-hexaneamine, an analog found in nutritional supplements and presumed main amine. The standard, 4-methyl-2-hexaneamine, has two unresolved peaks at RT 3.43 and 3.78 minutes that are the same as those of the unidentified molecule.[35]

Other applications:

In pharmacokinetics

Drug metabolism, excretion, and absorption are all studied using LC-MS. For the quantitative and structural elucidation of pharmaceuticals and their metabolites in biological samples (plasma, urine, saliva, serum, etc.), bioanalytical procedures are employed.[36]

In Bioavailability and Bioequivalence study

Comparative bioequivalence studies that analyze medications or metabolites quantitatively in the biological matrix, pharmacodynamics, clinical trials, and in vitro dissolution tests.[37,38]

In Determination of Molecular Weight:

The molecular weights of known and unknown chemicals are determined using LC-MS. It offers details on the molecular weight, composition, identity, and amount of sample constituents. The molecular masses of proteins, nucleic acids, polymers, and peptides can be determined using LC-MS.

In Determination of Assay of Drug and Intermediates:

In the pharmaceutical sector, LC-MS is used to determine the assay of drug substances, drug products, intermediates, and chemicals associated with them.[3]

Environmental Applications:

LC-MS is used to detect low levels of carbaryl in food and herbicides containing phenyl urea.[39]

Molecular Pharmacognosy:

To conduct a study on ingredient difference phenotypic cloning, LCMS analyses the composition and classification of several groups of cultivated plant cells and chooses the two groups with the greatest variation in ingredient content.[40]

Characterization and Identification of Compounds Carotenoids:

Since carotenoids are not heat-stable, reversed-phase HPLC, in particular, is typically used to separate mixtures and remove contaminants rather than gas chromatography. The nuclear magnetic resonance technique is used to analyze small samples of carotenoids that were separated from biological matrices, such as human serum or tissue stop structural analysis. Therefore, only the most sensitive analytical techniques—such as mass spectrometry and liquid chromatography with photodiode-array UV/visible absorbance detection and high-performance liquid chromatography—are suitable. Combining information from tandem mass spectrometry, photodiode-array absorbance spectroscopy, mass spectrometry, and HPLC retention periods can, at the very least, validate the identity of carotenoid. Currently, moving belt, particle beam, continuous flow fast atom bombardment, electrospray, and atmospheric pressure chemical ionization (APCI) are the five LC/MS techniques that have been employed for carotenoid analysis. Of all these LC/MS interfaces, electrospray and APCI are most likely the most user-friendly and are quickly becoming the most accessible. These methods provide massive molecular ions and provide similar sensitivity (at the low p mol level).[41]

Two Dimensional (2-D) Hyphenated Technology:

With its application in a multitude of analytical and bioanalytical techniques for the analysis of proteins, amino acids, nucleic acids, carbohydrates, lipids, peptides, etc., as well as in the primary classification in the fields of genomics, lipidomics, metabolomics, proteomics, etc., LCMS has developed into a potent two-dimensional (2D) hyphenated technology. The initial preference for LCMS may have stemmed from the need for more potent analytical and bioanalytical procedures that could definitively and specifically separate the target analytes from high-complexity mixtures. Detecting various analytical and bioanalytical techniques in the past decade has made mass spectrometry play a major role in science. This hybrid class of HPLC and MS can perform both routine qualitative discovery and quantitatively directed analysis of complex mixtures. It is conceivably one of the most significant combinations of developments and separations. When combined with an MS system, it provides their LC systems with enhanced durability and accuracy, as well as better detection capabilities.[42,43]

Advantages of LC-MS

LCMS has a number of benefits over other chromatographic techniques, a few of which are as follows:

Selectivity: Mass selectivity allows for the isolation of co-eluting peaks without being limited by chromatographic resolution.

Peak assignment: In the case of complicated matrices, accurate peak assignment is ensured by generating a chemical fingerprint for the material being studied.

Molecular weight data: validating and recognizing both recognized and unidentified substances.

Information on the structure: A chemical's structure can be clarified through controlled fragmentation.

Quick technique development: Enables simple eluted analyte detection without the need to validate retention times.

Sample matrix adaptability: saves time by reducing sample preparation time.

Quantitation: With minimal instrument optimization, both quantitative and qualitative data can be collected with ease.[44]

Disadvantages:

1. Expensive

2. Not portable

3. Requires an experienced technician

4. Moderate through put.[45]

Literature Survey:

The LC-MS approach was developed by Perrenoud L. to identify 4-methyl-2-hexaneamine, a doping substance, in urine. The technique is LC-MS in positive mode with ESI. The analyte was separated on a reverse-phase C8 column using a gradient mobile phase. Single-reaction monitoring (m/z 116–57) demonstrates specificity for 4-methyl-2-hexaneamine detection [35].

Allegrand J. invented the adjustable synchrotron VUV radiation atmospheric pressure photoionization (APPI) mass spectrometry of guanine. This VUV photon source is adjustable and connected with an APPI source. Guanine was ionized through chemical processes that depended on photon energy.[21]

The most recent advancements in LC-MS for pharmaceutical analysis are discussed by Chang-Kee L. This article discusses a number of approaches and their interactions, including air chemical ionization, light ionization, and electrospray. A brief discussion has been held regarding the use of LC-MS in drug development, in vitro and in vivo drug metabolism, and the identification and characterization of impurities in pharmaceutical analysis.[1]

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