Applications of Liquid Chromatography-Mass Spectrometry

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Abstract

It is a sophisticated analytical technology with very high sensitivity and specificity, the Liquid Chromatography-Mass Spectrometry. Combining Liquid Chromatography (LC) with spectroscopy is known as LC-MS (MS). It is possible to separate components using the Liquid Chromatography (LC) technique, which is followed by the transfer of the samples to Mass Spectrometry (MS) where the detection, identification and determination of masses may be done in the presence of other elements. Quantitative and qualitative analysis of pharmaceutical drug ingredients, intermediates, and related chemicals is accomplished using LC-MS. in-vitro dissolution, bio-equivalence, bioavailability, and metabolite investigations are the most common uses of LC-MS. It is also utilised in fundamental research, agriculture, forensics and the food and beverage sectors. LC-MS Instrumentation and uses of the LC-MS method are briefly reviewed in this article.

Keywords

High-Performance Liquid Chromatography (HPLC); Liquid Chromatography Mass Spectrometry (LC-MS)

1. Introduction

Liquid Chromatography-Mass Spectrometry (LC-MS)

HPLC is one of the most often utilised analytical techniques in the pharmaceutical business for determining and quantifying pharmacological compounds and their associated chemicals. HPLC is widely utilised in the pharmaceutical, chemical, and pesticide sectors because of its great repeatability and precision. As the name suggests, the LC-MS method is a combination of Liquid Chromatography and Mass Spectrometry (LC-MS) (MS). HPLC (LC) uses a chromatographic column to separate mixture components. For the most part, the divided components can't be recognised only by LC. Identification of new and recognised chemicals as well as structural elucidation are all possible using mass spectrometry. An individual component's unique mass spectrum is not enough to identify a complex mixture; instead, each component's mass spectrum overlaps with each other. Connecting LC with a mass spectrometer is a tough task (MS). The liquid eluents are transferred from LC to MS through an interface. An increasing number of research [1] employ LC-MS in order to better understand drug solubility, bioavailability, bioequivalence and pharmacodynamics. In fundamental research, pharmaceutical, agrochemical, culinary, and other sectors, preparative LC-MS systems may be utilised for mass-directed purification of particular compounds from such mixtures [2,3].

2. Instrumentation

Liquidchromatography-mass spectrometry (LC-MS)

It's a combination of HPLC and Mass Spectrometry known as LC-MS, and it's utilised to get the separation power of HPLC and the detection power of Mass Spectrometry (MS). Figure 1 depicts the LC-MS schematic block diagram. The pieces of an LC-MS apparatus are shown in the following table.

Liquid Chromatography, to start with (LC)

Analysis of liquids using HPLC and mass spectrometry

The Liquid Chromatography (HPLC) Liquid mobile and solid stationary phases are used to separate components of mixtures in high-performance liquid chromatography Reversed Phase Chromatography (RPC), Ion

Exchange Chromatography (IELC), Chiral separation and affinity liquid chromatography are all examples of distinct chromatography techniques [3]. With the aid of

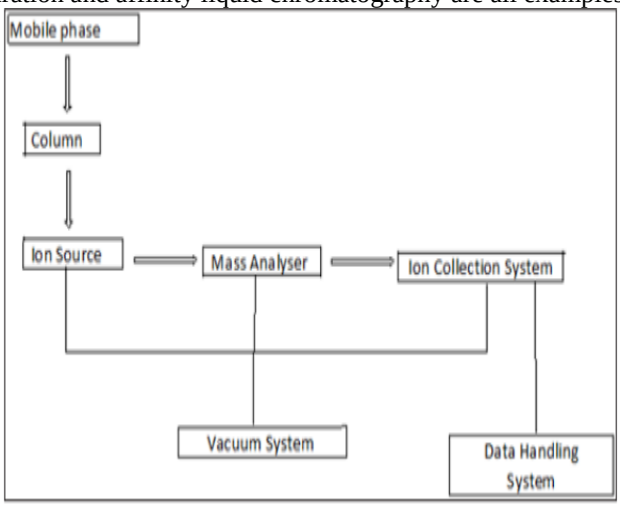

Figure 1: Schematic Block Diagram of LC-MS System.

Pump:

Any mixture of aqueous buffering solution and organic solvents is inert to this substance. High-volume mobile phase delivery of up to 10mL/min is possible. Reciprocating pumps, syringe pumps, and constant pressure pumps are the most common kinds of pumps.

Sample injector:In the chromatographic system, it is utilised for introducing sample volume. Samples may be injected with volumes ranging from 1 to 100 mL. The injector loop allows for an increase in injection volume of up to 2mL. There are two main kinds of injectors, namely Automatic and Manual. In comparison to manual injectors, automatic injectors are more convenient, user-friendly, and accurate [3].

Columns: Carbon chain and silica material make up the stationary phase. Column lengths typically range from 50 mm to 300 mm. HPLC columns are made up of Octadecyl (C18), Octyl (C8), Cyano, Amino, and Phenyl packing's, which are utilised in HPLC. Based on the kind of chemical being separated, the columns are chosen. [4]

d. Detectors and recorder: This is the most critical component of HPLC. UV-Visible detectors, PDA detectors, RI detectors, electrochemical detectors, fluorescence detectors, and conductivity detectors are a few of the many kinds of detectors in use. The peak of the signal from the detector may be captured and saved in a computer programme.

Massspectrometry

Ionic species associated to the investigation's analyte are measured using mass-to-charge ratio mass spectrometry, an analytical method. As well as providing structural information, MS may be utilised to identify an analyte's molecular mass and elemental makeup [5]. The ionisation source and interfaces are both critical components in LC-MS. Here are the many parts of a mass spectrometer as follows:

a. Ionization Sources and Interfaces
b. Mass Analysers

Ionization/Ion Source and Interfaces:

It is used to separate mixtures of liquid components, such as methanol, acetonitrile, or water. The mass spectrometer's ion source receives the liquid sample, which contains a complex combination of ions. Because the ion source is located in a very small space, it requires a high level of vacuum. It is difficult to mass vaporise liquid droplets without losing the combination of components because of the pressure difference. Because of this, interfaces are employed to fix the issue. Listed below are some of the most frequent mass spectrometer interfaces.

Direct liquid Introduction (DLI): Direct Liquid Introduction (DLI) uses vaporised solvent as a chemical ionisation and reagent gas to ionise the sample prior to introduction. The normal and reverse phase solvent systems have both been used in this research.. Methanol/water and acetonitrile/water mixtures up to 60% water are utilised as reverse phase solvents. When heated, capillaries may get obstructed by the salts in the buffer.

A method known as "direct liquid introduction" Direct Liquid Introduction (DLI) uses vaporised solvent as a chemical ionisation and reagent gas to ionise the sample prior to introduction. It has been utilised with both the normal and reverse phase solvent system. Most of the reverse phase solvents utilised are water/acetonitrile and water/acetonitrile up to 60% water. Buffers containing salts are generally prohibited because they increase the risk of capillaries plugging when heated.

Atmospheric-Pressure Ionization (API):

Nebulisation, evaporation, and ionisation are the three primary processes of atmospheric-pressure ionisation (API). Atmospheric pressure ionisation and Electrospray Ionization (ESI) are the two primary API methods (APCI). Small droplets are created in Atmospheric Pressure Ionization (API) by passing a stream of liquid (solvent) and the sample via a capillary tube. As a result of the ionisation process, a higher or lower percentage of droplets have an electric charge that is either positive or negative. Solvent is evaporated in a huge heating chamber. Droplets become smaller and smaller as the solvent evaporates. Molecules and ions collide in the process. The resultant ions were then fed into the mass analyzer by capillary [2,8]. Analytes of intermediate molecular weights may be analysed using the Atmospheric-pressure ionisation (API) method.

Electrospray Ionization (ESI):

The Electrospray Ionization (ESI) invented by Fenn and his colleagues is the most useful ion source. To conduct an Electrospray Ionization (ESI) experiment, a liquid sample is pushed through an electrode-coated capillary tube at a high positive or negative electric potential of 3-5kV[1]. These droplets are created because of this.

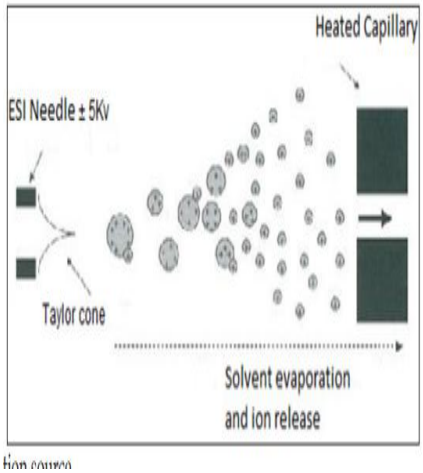

Figure 2: Electrospray ionization source

Atmospheric Pressure Chemical Ionization (APCI):

Evaporation and desolvation of analytes are two of the primary processes in the Atmospheric Pressure Chemical Ionization (APCI), which generates the vapour phase ions. Atmospheric Pressure Chemical Ionization (APCI) involves the use of a tiny capillary tube to nebulize liquid (solvent) containing a sample into a huge chamber under pressure. The evaporation of solvent occurs at atmospheric pressure in a huge heating chamber, and tiny droplets are formed. The ionisation has occurred.. Typically, ionisation occurs between 250 and 400 degrees Celsius. Chemical processes subsequently transfer the ions' charges to molecules. The mass analyzer's capillary aperture receives the resultant ions. Analytes with intermediate molecular weights, such as less polar and nonpolar analytes, are well-suited to this method [11].

Thermo spray and Plasma spray Ionization (TSPI):

The Thermo spray is utilised for both liquid intake and ionisation. It is a variation of thermospray that uses plasma. Thermo spray employs a capillary tube that is heated, resulting in the evaporation of the solvent in the sample solution. Electrified droplets are now in existence. Droplets become smaller and smaller as the solvent evaporates. Electric charge density on droplet surfaces rises with increasing concentration. Following this, the mass spectrometer uses an electrostatic voltage mechanism to analyse the ions. It is not possible to increase the quantity of ions in plasma spray by using corona discharge or plasma, however the ions produced in thermo spray may be. The neutral molecules become increasingly ionised as a result of the electric discharge. This improvement raises the molecule's ionisation potential. When it comes to clinical and medical diagnostics, plasma sprays are the most sensitive and commonly employed.

f. Atmospheric pressure photo Ionization (APPI):

Photons are utilised to excite and ionise molecules in Atmospheric pressure photo Ionization (APPI). Excitation and ionisation of analyte from eluent are the two primary processes in the atmospheric pressure photoionization (APPI). By using Atmospheric Pressure Photo Ionization (APPI), the eluent from LC may be converted to gas, similar to the process known as Atmospheric Pressure Chemical Ionization (APCI). For photon production, the APPI relies on a Kr lamp. High-intensity photons from the Kr lamp are used to excite and ionise atoms and molecules. In order to reduce ionisation of analytes, the range of energy is chosen. They are delivered to the mass analyzer (m/z) by a small capillary aperture. Analytes that are difficult to ionise by Electrospray Ionization or Atmospheric Pressure Chemical Ionization might benefit from this method [13,14].

g. Particle Beam Ionization:

The particle beam interface created by Browner and his colleagues allows the solvent and solute to be separated with the least amount of solute loss possible. This procedure is similar in nature to Thermo spray (TSP), Atmospheric pressure chemical ionisation (APCI) and Electrospray ionisation (ESI). The eluent, which is a liquid removed from HPLC or LC, is fed into a small tube. Using helium gas, a high-velocity spray of liquid droplets is created in the liquid being injected. The drops move through a heating chamber, where the solvent evaporates and the liquid droplets become smaller until they're hardly visible. As a particle beam, the sprayed liquid droplets emerge from the heating chamber. It then goes through an ionisation chamber, comparable to Electro spray Ionization and Atmospheric Pressure Chemical Ionization [2]. [3]

h. Continuous Flow Fast Atom Bombardment (FAB):

Interfacing using the FAB is a straightforward, high-sensitivity method that is easy to learn. Fast atoms like Argon(Ar) or xenon are used to attack the liquid target in FAB. An ultra-thin metal plate/probe is coated with glycerol before the sample is placed on top. As soon as this probe is introduced, it is bombarded with high-speed atoms that ionise the samples and send them to the mass analyzer (m/z). Molecules that are big and thermally unstable are best suited for the FAB. The surfactants and proteins that it is used for [16,17]

3. Literature Survey:

By using LC-MS technology, Perrenoud L. has developed a technique for detecting 4-methyl-2-hexaneamine in urine. The technique used is LC-MS in positive ESI mode. Gradient mobile phase on a reverse phase C8 column separated the analyte. For 4-methyl 2-hexaneamine, the single reaction monitoring (m/z 116-57) is very specific. Tunable synchrotron VUV radiation from Allegrand J enabled mass spectrometry of guanine to be developed. There are two sources of VUV photons, one of which is APPI, in this system. It was via chemical processes that the ionisation of guanine was achieved [13]. Analyzing anthocyanins from purple maize cobs using LC-MS was pioneered by Pascual-Teresa Sd. By using LC-MS, the nine anthocyanins have been discovered and separated. The anthocyanins in purple corn cob were identified using fragmentation patterns in MS spectra, which were obtained by a combination of liquid chromatography, diode array spectrometry, and mass spectrometry [30]. Wang Y has developed an LC-MS technique for analysing total resveratrol in grape juice, cranberry juice, and wine. Reverse phase HPLC and APCI mass spectrometric detection with positive ions were used to analyse the samples. It has been found in grape juice, cranberry juice, and wine with concentrations ranging from 1.56nmol/g, 1.07nmol/g, and 8.63 to 24.84 mol/L [31]. This article by Chang-Kee L discusses the most recent advances in LC-MS for pharmaceutical testing. In this section, techniques such as light ionisation, electrospray, atmospheric chemical ionisation, and the interfaces between these methods are described. Here, LC-MS is used to identify contaminants in pharmaceutical analysis, as well as to study drug metabolism in vitro and vivo [1]. Nishikawa M reported on the use of LC-MS in forensic toxicology for the detection of surfactants. Anionic and positive surfactants are identified as M- ions in the negative mode and as M+ ions in the positive mode, whereas non-ionic surfactants are [M+H]+ ions or [M+NH4]+ ions in the positive mode for the analysis of anionic, cationic, and non-ionic surfactants. There is a recovery range of 65.8 percent to 124 percent for anionic, cationic, and non-ionic surfactants. Hernando MD, used LC-MS to measure the concentration of pharmaceutical residues in both natural and treated water. samples such as effluent, influent, and tap water are tested for contaminants. In order to detect medications such as Ibuprofen, Ketoprofen, and Diclofenac at a trace level, SPE with liquid chromatography tandem mass spectrometry (LC-MS) is used. 7.5-75 ng/L was the technique detection limit and quantitation limit. It was invented by Souverain S, who used LC-ESI-MS to determine protein precipitation in plasma for the investigation of drug cocktails. Acetonitrile (ACN), perchloric acid (PA) and trichloroacetic acid (TCA) are used for protein precipitation (PP). In less than six minutes, the LC-ESI-MS technique was developed for the simultaneous investigation of six different chemicals. ACN is utilised as PP Literature Survey depending on efficient protein precipitation methods to extract protein from human plasma and compatibility with LC-ESI-MS By using LC-MS technology, Perrenoud L. has developed a technique for detecting 4-methyl-2-hexaneamine in urine. The technique used is LC-MS in positive ESI mode. Gradient mobile phase on a reverse phase C8 column separated the analyte. For 4-methyl 2-hexaneamine, the single reaction monitoring (m/z 116-57) is very specific. Allegrand J was the brainchild of Allegrand Mass spectrometry of guanine using adjustable synchrotron VUV light at atmospheric pressure (APPI). There are two sources of VUV photons, one of which is APPI, in this system. It was via chemical processes that the ionisation of guanine was achieved [13].

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4. Conclusion

Combining HPLC's separation power with Mass spectrometry's detection capability, the LC-MS approach is called a "hyphenated technique." Pharmaceutical, chemical, food, agrochemical, environmental, and forensic applications all make extensive use of this substance. For the qualitative and quantitative analysis of pharmaceuticals and biological materials, LC-MS is used. Also, it is utilised extensively in the pharmaceutical industry for research and testing.

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