An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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ARTICLE INFO

Key Words

Linagliptin, Methanol, UV spectrophotometry, Development, Validation, ICH guidelines

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

ABSTRACT

Liquid Chromatography /Mass Spectrometry (LC/MS) is fast developing and it is the preferred tool of liquid chromatography. Liquid chromatography-mass spectrometry (LC-MS/MS) is a technique that uses liquid chromatography (or HPLC) with Mass spectrometry. Analytical chemistry is a technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry. (LC-MS/MS) is mainly used for the qualitative and quantitative analysis of drugs, their metabolites in various biological samples.

INTRODUCTION

Liquid Chromatography/Mass Spectrometry (LC/MS) is the advanced highly preferred tool of liquid chromatography along with the detection of Mass spectroscopy. It is a powerful analytical technique that combines the resolving power of liquid chromatography with the detection capacity of mass spectrometry [1-3]. Liquid chromatography (LC) separates the sample components and then introduces them to the mass spectrometer (MS). The MS creates and detects charged ions. The LC/MS data may be used to provide information about the molecular weight, structure, identity, and quantity of specific sample components.

LC-MS INSTRUMENTATION

Chromatography: Chromatography is a separation technique to separate the individual compound from a mixture using a stationary and mobile phase. The chromatography word obtained from Greek, 'chroma' means colour and 'graphy' mean writing, hence the word chromatography means 'colour writing'.

Types of chromatography

1. Based upon the nature of stationary and mobile phase

- Gas-solid chromatography
- Gas-liquid chromatography

Solid-liquid chromatography (column chromatography), Thin Layer Chromatography [TLC], High Performance Liquid Chromatography [HPLC], Liquid Chromatography-Mass Spectrometry [LC-MS]) Liquid-liquid chromatography (paper partition chromatography)

2. Based on the principles of separation and type of chromatographic method

• **Adsorption chromatography:** The mobile (liquid or gaseous) phase is adsorbed into the surface of a stationary solid phase. The separation of the compound is based on an affinity towards the stationary phase. The compounds which have more affinity with the stationary phase will be eluted slowly and compounds with less affinity with the stationary phase will be eluted fast.

• **Partition chromatography:** Separation of compounds is based on the partition of a solute between two solvents. In this form of chromatography a liquid stationary phase, which is immiscible with the mobile phase, is adsorbed to the surface of the solid adsorbent.

Liquid Chromatography-Mass Spectrometry

Liquid chromatography-mass spectrometry (LC-MS or HPLC-MS) is an analytical technique that combines the physical separation abilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry. LC-MS is a powerful technique used for many applications that have very high sensitivity and selectivity.

Principle of LC-MS: The typical LC-MS system is a combination of HPLC with MS using an interface (ionization source). In HPLC, the sample is forced by a liquid at high pressure (the mobile part) through a column that's filled with a stationary phase typically composed of on an irregular basis or spherically formed particles chosen or derivative to accomplish particular styles of the separations. [4] The sample is separated by LC, and the separated sample species are sprayed into an atmospheric pressure ion source, where they are converted into ions in the gas phase. The mass analyzer is then used to sort ions according to their mass to charge ratio and detector counts the ions emerging from the mass analyzer and may also amplify the signal generated from each ion.

Requirement of LC: Usually, LC used in LC-MS is HPLC. The principle of separation in HPLC is normal phase mode or reverse-phase mode of adsorption. Normal phase constricts with polar stationary phase with non-polar solvent/mobile phase and reverse-phase constricts with non-polar stationary phase with polar solvent/mobile phase.

HPLC Instrumentation [5-9]: Mainly HPLC instrument contains a pump, mixing unit (solvent degassing system), the injector (manual/auto), guard column, analytical columns, detectors, recorder, and integrators.

Detectors used in HPLC:

1. UV detector
2. Florescence
3. Electrochemical detector
4. Mass spectrometric

Requirements of LC-MS instrumentation: Mainly the LC-MS contains liquid chromatography assembly, ion generation unit/ ionization source, mass analyzer, and mass spectrometric data acquisition.

Ionization source: The most common ionization sources are Electron spray ionization (ESI), Atmospheric pressure chemical ionization (APCI), Atmospheric pressure Photoionization (APPI) and Matrix-assisted laser desorption/ionization (MALDI). Apart from this Electron impact (EI) and chemical ionization (CI) or negative chemical ionization are also used as ionization sources in MS.

Electron spray ionization (ESI): ESI as an important interface in LC/MS. It is used to analyze polar molecules that make preformed ions in solution by transforming ions in solution to ions in the gas phase. The ionization of heat-labile and high molecular weight compounds such as proteins and peptides can be done by this technique. ESI can be effectively used as an interface for HPLC. ESI resembles LC/MS interface such as TSP and ion evaporation. [10-24]

Atmospheric pressure chemical ionization (APCI): In APCI ions are produced at atmospheric pressure by the reaction between

analyte molecule and reagent gas.

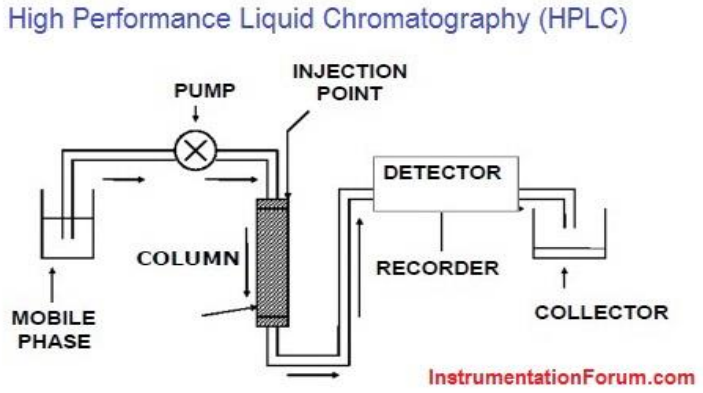

Figure 1: INSTRUMENTATION OF HPLC

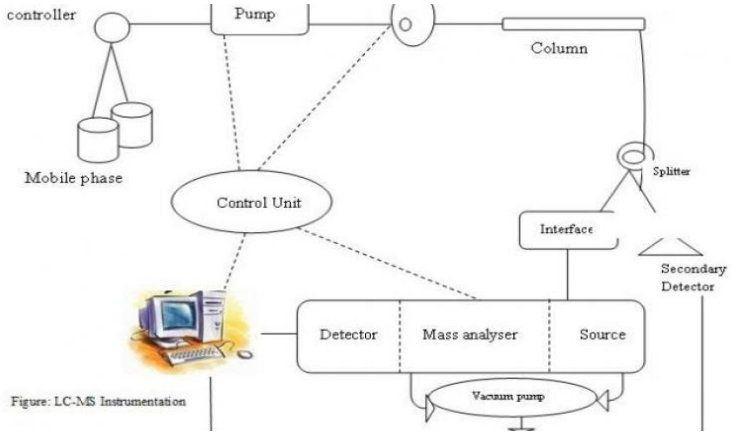

Figure 2: INSTRUMENTATION OF LC-MS

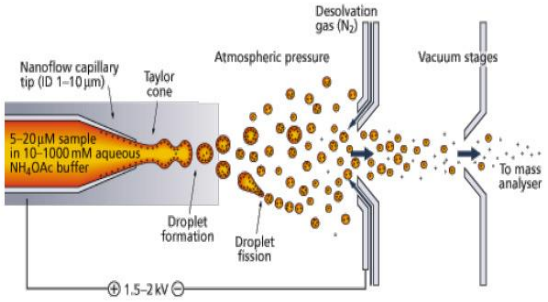

Figure 3: Electron spray ionization

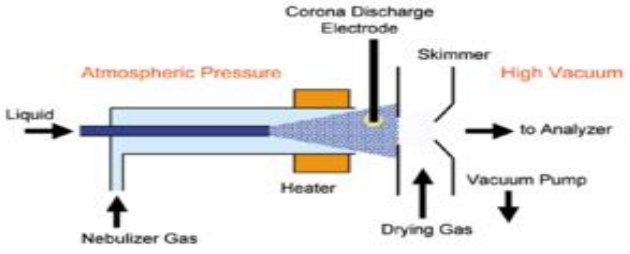

Figure 4: Atmospheric pressure chemical ionization (APCI)

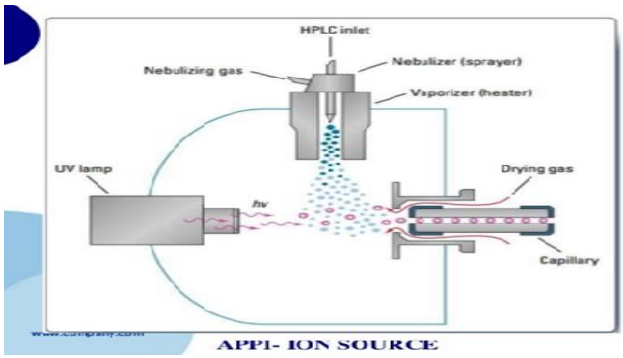

Figure 5: Atmospheric pressure Photo ionization

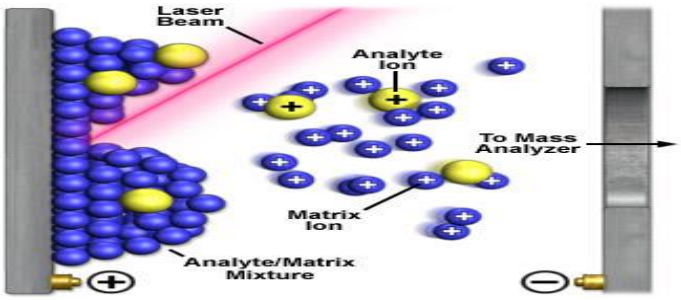

Figure 6: Matrix-assisted laser desorption/ionization

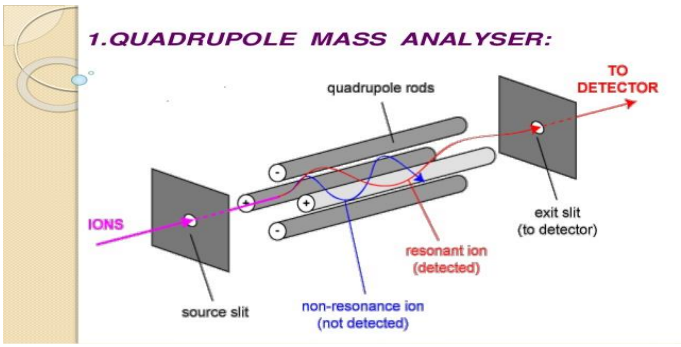

Figure 6: Quadrupole Mass analyzer

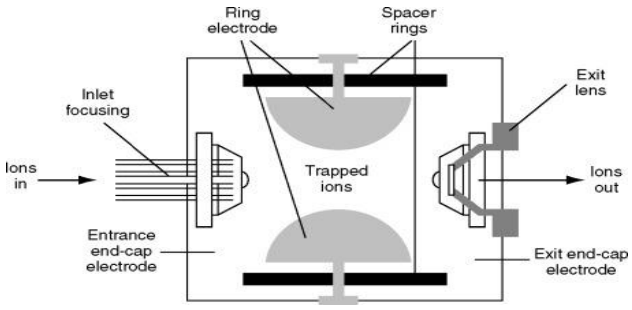

Figure 7: Ion trap analyzer

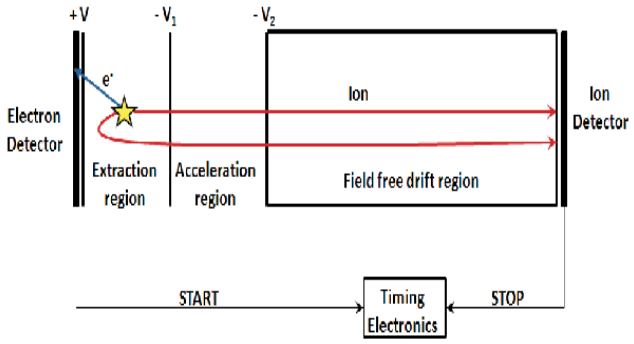

Figure 8: Time of Flight (TOF) analyzer

The ionization of solvent molecules is initiated by corona discharge at the tip of the corona needle. The LC eluent is introduced into a heated pneumatic nebulizer [8-9] where the liquid is nebulized pneumatically into a heated tube, allowing the droplets to collide

Atmospheric pressure Photo ionization (APPI): APPI is another LC-MS ion source/ interface for the analysis of neutral compounds that cannot be ionized using ESI. This interface is similar to the APCI ion source, but instead of a corona discharge, the ionization occurs by using photons coming from a discharge lamp. In the direct-APPI, mode singly charged analyte molecular ions are formed by absorption of a photon and ejection of an electron. In the dopant-APPI mode, an easily ionizable compound (Dopant) is added to the mobile phase or the nebulizing gas to promote a reaction of charge-exchange between the dopant molecular ion and the analyte. The ionized sample is later transferred to the mass analyzer at a high vacuum as it passes through small orifice skimmers.

Matrix-assisted laser desorption/ionization (MALDI): MALDI is an ionization technique for large and/or labile molecules such as peptides, proteins, polymers, dendrimers, and fullerenes. This technique involves embedding analytes in a matrix that absorbs energy at the wavelength of the laser. The nitrogen ultraviolet (UV) lasers are applied over the matrix in vacuum to generate ions of an analyte.

Quadrupole Mass analyzer: Quadrupole and triple quadrupole are the most widely used analyzer because it is easy to operate and it will cover wide mass range (10 to 4000 A.M.U./atomic mass unit). Quadrupole gives good linearity for quantitative work and good resolution (up to 4000), quality of mass spectra, scanning speed (5000 A.M.U per second), and mass accuracy (0.1 to 0.2 A.M.U.).

The quadrupole is composed of two pairs of metallic rods. Each opposing rod pair is connected together electrically, and a Radio Frequency (RF) voltage is applied between one pair of rods and the other. A direct current voltage is then superimposed on the RF voltage.

Ion trap analyzer: This analyzer is also known as the quadrupole ion trap analyzer (QIT). Mostly it will be used on GC/MS rather than LC/MS. The principle of the trap is to store the ions in a device (ion trap) consisting of a ring electrode and two end cap electrodes. These ions are manipulated by using applied DC and RF fields.

Time of Flight (TOF) analyzer: The time-of-flight (TOF) mass analyzer separates ions in time as they travel down a flight tube. It is a very simple mass spectrometer that uses fixed voltages and the magnetic field is not required. The major drawback of TOF instruments is its poor resolution, usually less than 500. These kinds of instruments have high transmission efficiency, very low detection limits, fast scan rates, no upper m/z limit. Recent developments in pulsed ionization technique and new instrument designs with improved resolution regained interest in TOF-MS.

Detectors: Three different kinds of detectors are used in Mass Spectrometry, i.e. Electron multipliers, Dynolyte photomultiplier, and Microchannel plates. Electron multipliers dynode is used to convert either –ve, +ve ions into electrons, that will be amplified and

detected. The dynode of Dynolyte photomultipliers converts the charged ions into electrons.

Applications of LC-MS

1. Molecular Pharmacognosy
2. Characterization and Identification of Compounds
3. Quantitative Bioanalysis of various Biological Samples
4. Qualitative and Quantitative Analysis of Complex Lipid Mixtures
5. Phytoconstituents / Plant Metabolomics
6. Automated Immunoassay in Therapeutic Drug Monitoring
7. Two Dimensional (2-D) Hyphenated Technology
8. Clinical chemistry and toxicology
9. Proteomics
10. Pharmacovigilance
11. Organic/Inorganic Hybrid Nan flowers

Pharmacovigilance: Pharmacovigilance (PV), which is referred to as Drug Safety. It is one of the pharmacological sciences which relates to the collection, detection, assessment, monitoring, and also prevention of adverse side effects with pharmaceutical products. The detection and monitoring can be done by LC-MS based disease-modifying technique which provides detailed profiles**.**

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