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General Instrumentation of LC-MS Technique and their Recent Applications

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Abstract:- This review summarizes the advancement in liquid chromatography-mass spectrometry (LC-MS). Liquid Chromatography and Mass Spectrometer is a hyphenated technique of separation of compounds. over the past years, the combination 0f high-performance liquid chromatography and mass spectrometry (LC-MS) has been used which has a remarkable effect on drug research and development. LC-MS played a vital role in the assessment and interpretation of bioavailability and bioequivalence. This article reviews the principle of LCMS analysis using liquid chromatography and mass spectrometry. Mass spectrometry comprises 3 important parts that are ionization source, mass analyzer, and detector.

Keywords:- *spectrometry; bioequivalence; chromatography; mass analyzer; bioavailability.*

I. INTRODUCTION

Gas chromatography, column chromatography, liquid chromatography-mass spectrometry are some Chromatographic techniques that are preferentially used in analytical laboratories for the quantitative (estimation) and qualitative(identification) analysis of pharmaceutical products and biological analytes at all stages of a drug's development in investigation and quality control [1].

LC-MS is a coupling technique that is consists of liquid chromatography and mass spectrometry which merges the separation strength of Liquid Chromatography with the recognition power of mass spectrometry. LC-MS, will ionize many compounds and separate them according to their m/e- (mass/charge) ratio) in presence of many ionization techniques like atmospheric pressure chemical ionization (APCI) electrospray ionization (ESI) [2].

II. PRINCIPAL

LC is a comprehensive method of analysis, which is widely used for the isolation of compounds from the super complex mixture of compounds and also utilizes the principle of LC-MS (liquid chromatography and mass spectrometry). Present-time HPLC (high-performance liquid chromatography) for liquid chromatography is regularly utilized. The analysis of lower volatility and higher polarity chemical compounds in the wide mass range without derivatization is promoted by High-Performance Liquid Chromatography. It is difficult in Liquid Chromatography to ensure certain chemicals are peaked even if there is only one chemical present in the sample. So that it is inevitable to add Mass Spectrometry, which gives contribution towards the molecular weight of all chemicals in the peak, so to recognize them it can be used. Mass Spectrometry (MS) depends on the identification of ions that are going through a vacuum. Mass spectroscopy not only provides the mass of the compounds but also provide fragmentation pattern and the presence of isotopes in analyte compounds. Mass spectrum results in valuable information about the structure, molecular weight of the compound, purity, and identity of the choice of a compound [3].

III. PARTS OF LC-MS SYSTEM

A. A chromatographic column

There are two phases are present in a chromatographic column that is named as mobile phase and stationary phase in which stationary phase contains a porous solid and in mobile phase liquid is used. Compounds present in the mobile phase are moved through the stationary phase and get separated. Compounds are separated into columns according to their separation properties such as affinity towards mobile phase, which result in elution at various points. Two Liquid chromatographic methods are generally used in proteomics, Strong cation exchange chromatography (SCX)- In this method separation is based on charge. Reverse-phase chromatography (RV)- In this method separation of compounds is based on hydrophobicity.

B. An ionization source

Ionization source used to convert separated compounds into ions. Mostly two typical ionization techniques are used that are following:

- (ESI)Electrospray ionization
- (MALDI)Matrix-assisted laser desorption/ionization.

C. Mass analyzer

In mass analyzer ionized masses of eluted compounds are taken and get separated based on their mass to charge ratio. In mass spectrometers there are some different types of mass analyzers are used:

- Ion trap
- Fourier transform ion cyclotron resonance (FTICR)
- Time-of-flight (TOF)
- Quadrupole-
- (ESI) Electrospray ionization
- (MALDI) Matrix-assisted laser desorption/ionization.

D. Detector

To record the relative abundance of ions at different mass /charge (m/z) locations to record the relative abundance of ions detectors are used [4].

IV. LC-MS/MS ANALYSIS

The LC-MS/MS system consists of a Nexera X2 high-performance liquid chromatography system and an LCMS-8060 triple quadrupole and Talanta 222 (2021) 121625 3 mass spectrometers (Kyoto, Shimadzu Corporation, Japan).

The following conditions are required for LC-MS analysis:

S. No.	Properties	Conditions
1.	Stationary phase	Penta fluorophenyl propyl
2.	Mobile phase	formic acid in acetonitrile solution 0.1%(v/v), formic acid in water 0.1%(v/v)
3.	Injection volume	3 µL
4.	Concentration	95% (15–20 minute) 95% (11–15 minute) 35% (5–11 minute) 25% (2–5 minute) 0% (0–2 minute) 0% (20.1–30 minute)
5.	Block heater temperature	400 ◦C
6.	Column (Particle size)	3 µm
7.	Column temperature	40 ◦C
8.	Drying gas flow	10 liter/minute
9.	Nebulizer flow	3.0 L/minute
10.	Interface temperature	300 ◦C
11.	Flow rate	0.25 mL/minute
12.	Heating gas flow	10 L/minute

Table 1: Requirements for Analysis

Other MASS parameters were identified by autotuning. The data obtained through Traverse Mass was analyzed for peak picking following the specifications. Several situations, together with fragment voltage of each of the metabolites and the collision energy, were automatically set with the help of laboratory solutions and standard solutions to monitor the multiple reaction monitoring (MRM) [5]. (LC-MS/MS in MRM or SIR mode) is the technology which confirms structural information of the interest of a compound and its categorical identification. The demerits of the LC-MS/MS method are the less availability of laboratories having the human and technical resources to perform various measuring operations and the costly analysis of compounds [6].

V. LIQUID CHROMATOGRAPHY

In history, countless techniques have been described for identifying unnatural colors with analytical methods which are ranging from thin-layer chromatography (TLC) to high-pressure liquid chromatography coupled to Ultra-violet spectroscopy (HPLC–UV).

In present times, additional modern techniques are coupled with analytical techniques for the identification of food dyes, some techniques are as follows:

- HPLC– ESI-MS/MS (High-pressure liquid chromatography-electrospray ionization tandem mass spectrometry).
- HPLC– APCI–MS/MS (High-pressure liquid chromatography–atmospheric-pressure chemical ionization–tandem mass spectrometry).
- HPLC– DAD–MS/MS (High-pressure liquid chromatography–diode array detector–tandem mass spectrometry).
- CZE (Capillary zone electrophoresis)

Nowadays, HPLC (High-performance liquid chromatography) coupled with MASS (tandem Mass spectroscopy) is often preferred over the older less sensitive, and less selective more pre-established techniques [7].

In addition to suitable sample preparation, adequate particularity of the LC-MS analysis often requires good chromatographic performance. The selection of a Liquid chromatographic method depends on the specificity of the mass spectrometric (MASS) method and also on the complexity of the sample matrix [8].

VI. INSTRUMENTATION

LC-MS has been widely reviewed in past years, focusing on instrumental aspects and applications.

On joining the LC and MS two most powerful analytical techniques, three following major difficulties are found

- The unwanted ionization of thermolabile or non-volatile sample of interest.
- Incompatibility with the solvent composition is a result of the persistent consumption of non-volatile mobile phase additives in Liquid chromatography separation development.
- Incompatibility with the apparent flow rate is indicated that the need to introduce 1 ml/min of liquid discharge from a traditional Liquid chromatography column into the high vacuum of the mass spectrometer.

LC and LC-MS are particularly the most effective analytical techniques for the identification of non-volatile analytes. LC-MS is more reliable and effective because of the introduction of powerful soft ionization techniques like fast atom bombardment (FAB), thermospray and electrospray ionization (ESI), and more recently matrix-assisted laser desorption ionization (MALDI). In conclusion, in LC-MS research the ionization of thermolabile or volatile

compounds is no longer considered a problem. And for the incompatibility in flow rate, different types of technological solutions were investigated [9].

A. Ionization Source

There are various types of ionization techniques such that APCI (atmospheric pressure chemical ionization) and ESI (Electrospray ionization) are used for the analysis of analytes in LC/MS and LC/MS/MS. Some monitoring modes and ionization modes are generally employed in which monitoring modes contains SIM (selected ion monitoring), SRM (selected reaction monitoring) and ionization modes comprises of negative ion (NI) and positive ion (PI) [10]. ESI and APCI ionization techniques of LC-MS are generally used for food-sample analysis.

B. APCI

In comparison to ESI (electrospray ionization), APCI is a more energetic source since effluent is nebulized by a coaxial nitrogen stream and quickly evaporated using high temperatures (350–500C). When working with thermolabile substances these high temperatures should be considered. For weakly basic compounds APCI is very sensitive [11].

C. ESI

It is an effective method for transforming sample solution into gaseous-phase ions which is suitable for analysis by the processes of ion desorption and desolvation [12]. The efficiency of ESI depends on the composition of eluent, the mobile-phase composition for adequate chromatographic separation but sometimes to receive maximal electrospray response this is s inappropriate.

Biomolecules, more labile, ionic, and synthetic polymers are characterized by the use of ESI methods. Photons emitted by a discharge lamp (typically krypton, minor 10.6 e, and10 eV) is initiating the process of ionization in APPI. These photons ionize analyte molecules with ionization energies lower than their energy (10 eV) but typical gases and solvents used in LC-MS separation and nebulization processes are not ionized through the photons. Analyte molecules are ionized selectively Without any background interferences. Unlike ESI and APCI the ionization of analytes is based on their ionization energies rather than their proton affinities.

For the analysis of the complex sample APPI (Atmospheric pressure photo-ionization) has been proposed recently. For the analysis of polar, ionized, and high molecular weight compounds ESI ionization technique is suggested by Nunez *et al* [11]. The atmospheric pressure photoionization (APPI) is less widespread (2% of papers,) in comparison to the two above techniques ESI and APCI, which is probably elaborated by a comparable application range as for APCI.

Combining two different types of ion sources can be considered as merging the application and advantages of atmospheric pressure ionization techniques, but on the different side, their sensitivity and selectivity may be a compromise between both modes. Detection of both non-polar and polar analytes in one run is the advantage which is possible through combining two ion sources ESI/APPI or ESI/APCI, so this can elevate a large number of identified compounds for very complex mixtures [13]. With the use of 5 different types of polar pharmaceuticals, ESI showed the best result in terms of selectivity and sensitivity in comparison to the application potential of ESI, APCI, and APPI.

VII. MASS SPECTROMETRY

For TDM the most common detectors used are triple quadrupole instruments. which is comprised of two quadruples that are isolated through a collision cell. Ions of a pre-determined mass can be filtered out by this instrument and then ions are converted into a fragment in a compound-specific manner. High analytical specificity is obtained during monitoring of these fragment ions that can only come from the parent ion, but it must be remembered that the mass instrument will only separate ions with one Dalton resolution when operating at near maximum resolution. The instrument is tuned using pure solutions of analyte and the daughter ions are selected based on abundance to give the most sensitive assay [12].

A. Analyzer

In high-resolution mode, combination with LC Time-of-flight (TOF) mass spectrometers are more often used. The important merit of this instrument is that if analytical standards are not available it can help in the identification of unknown peaks in a sample.

A hybrid quadrupole time-of-flight instrument (Q-TOF) provides the most certain confirmation. This confidence is based on the combination of retention time, a mass of the quasimolecular ion selected by the quadrupole mass filter, and the complete collision-induced mass spectrum obtained by the TOF analyzer [14].

B. Working

LC-MS (Liquid Chromatography along with Mass Spectrometry) provides more absolute identification and promotes the quantitative analysis of samples.

- This instrument is composed of four vacuum stages and permits filtering from the starting, which is from the spray chamber, most solvents where never enter the capillary. Only gas dryers, Ions, and a small portion of solvents can be passed through the capillary.
- At the exit of the capillary, the skimmer does filter.
- Through the skimmer aperture ions with heavy mass and greater momentum can easily pass.
- Then the ions that can go through the skimmer are moved to the second stage of the vacuum system.
- At the second stage, using octopole the ions straight away focused to pass through on two vacuum stages.
- Ions get momentum because of atmospheric pressure through capillary sampling and this results that ions can go by the octopole.
- Then ions that are coming out from this stage, are going through two focusing lenses towards the fourth stage of vacuum systems.
- In the fourth stage of the vacuum system, ions get separated from the mass ratio to the charge with the help of a quadrupole mass analyzer.

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- The quadrupole mass analyzer has an electromagnetic field that helps in the determination of separated ions in the ratio of mass to charge that can pass by the filter at a definite time.
- At the last ions are focused on the detector. The detector records this data.

VIII. LC-MS INTERFACES

In this portion, the different economically accessible interfaces are discussed:

- *The Moving-belt interface:* It comprises an interminable Kapton ribbon that is consistently moving, and which also moves the column elute from the Liquid Column segment outlet towards the ion source of MASS.
- *In a direct liquid introduction interface:* The column elute is nebulized by liquid jet development at a little diaphragm and the successive disintegrate into small droplets.
- *In a thermospray interface:* The little droplets and a flow of vapor are produced out of a warmed vaporizer tube gram of a thermospray interface. Nebulization happens because of the interruption of fluid by the extending vapor that is produced at the wall of the tube upon evaporation of a portion of the liquid in the tube.
- *In a continuous-flow or dynamic fast atom bombardment (CF-FAB) interface:* The persistent stream, normally 5-15 ~l/min, is blended in fitting FAB network solvent. on the target, a balance between the formation of a uniform liquid film and solvent evaporation is reached. Ions are generated through the bombardment of the liquid film by fast atoms.
- *In a particle-beam interface*: Near an atmospheric-pressure desolvation chamber, the column effluent is nebulized, either pneumatically or by thermospray nebulization, atmospheric-pressure desolvation chamber is connected to a momentum separator, where analytes of the low-mass molecules are efficiently pumped away while high mass molecules are preferentially transferred to the MS ion source. The analyte molecules are transferred to a conventional ion source, where they disintegrate upon collisions at the heated source walls. The gaseous molecules that are released by the ion source are then ionized by EI or CI [9]. There is a new type of electron ionization LC-MS was developed, which uses supersonic molecular beams for LC and MS interface and as a medium for electron ionization of vibrationally cold sample molecules in a fly-through ion source.
- *Library-based identification:* Unlike with ESI and/or APCI Library, searchable EI mass spectra are provided. On sharing with Particle Beam LC-MS and 'Direct EI' This is an important useful feature, that enables automated, fast, and reliable molecular identification with sample names and structures at the isomer level.
- *Non-polar compound analysis:* Both semi-polar and non-polar compounds are analyzed by EI-LC-MS with SMB and unlike ESI or APCI based LC-MS it can analyze non-polar compounds accepting and polycyclic aromatic hydrocarbons (PAHs) and saturated hydrocarbons [15].

IX. APPLICATIONS

- Mass spectrometric techniques are very useful in the identification of hormones such as estrogens and progestogens [10].
- Liquid Chromatography-Mass spectroscopic techniques are consistently used to recognize impurities during the development and manufacturing process of pharmaceutical products and used to initiate the protective evaluation of batches that are used in clinical studies [16].
- LC-MS/MS is a more significant apparatus in therapeutic drug monitoring (TDM) as it serves expanded specificity and affectability contrasted with other analytical methods [12].
- LC-MS is widely acceptable to the examination of the scope of semi-polar compounds including numerous secondary metabolites(alkaloids, Glycosides, Terpenoids, etc) of interest for the plant researchers and nutritionists [17].
- LC-MS applications give the option in contrast to plasma testing, to keep away from blood withdrawal, plasma partition, refrigerated shipment, or, then again, if conceivable, to get better data about drug exposure in target cells [18].
- LC-MS is usually utilized in research facilities for the qualitative and quantitative examination of drug substances, drug items, and natural samples.
- LC-MS/MS has assumed a significant partin the assessment and understanding of bioavailability, bioequivalence, and pharmacokinetic information of medications [19].
- LC-MS/MS helps to identify different disorders with a single injection, which is more important in high throughput laboratories. Estimating distinctive acylcarnitines and amino acids can be utilized to recognize up to 45 different inherited disorders depending on how diseases are counted [20].
- LC-MS has turned into a significant and broadly utilized technique in the examination of metabolites inferable from its predominant specificity, affectability, and effectiveness [8].
- LC-MS is utilized for the identification of organometallic compounds present in ecological grids [21].
- LC-MS/MS is utilized for quantitative examination of glucocorticoids and energizers present in natural liquids [22].

X. CONCLUSION

HPLC along with mass spectroscopy promotes the analysis of higher polarity and lower volatility chemical compounds in the wide mass range without derivatization. For the analysis of nonvolatile analytes, LC and LC-MS are especially interesting analytical techniques. LC-MS/MS offers superior specificity and sensitivity and it is the only viable measurement technique besides immunoassay for compounds without natural chromophores. LC-MS/MS is invented as a superior technique to immunoassay because immunoassay has many problems. LC-MS is very useful for the identification of both semipolar and nonpolar compounds identification.

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