WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 12, 693-711. Review Article ISSN 2277– 7105

A REVIEW ON LC- MS TECHNIQUE AND IT'S APPLICATION

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Article Received on
04 August 2021,

Revised on 24 August 2021,
Accepted on 14 Sept. 2021

DOI: 10.20959/wjpr202112-21775

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ABSTACT

Chromatography is a separation technique used to separate the respective compound from a mixture using a stationary Phase and mobile phase. Introduction of chromatography is a millstone event in biomedical research. Chromatographic separation is based on the basis of adsorption, partition, ion exchange, molecular exclusion, affinity and Chirality. Liquid Chromatography/Mass spectroscopic analysis (LC/MS) is quick developing and it's the well-liked appliance of liquid chromatographers. Liquid chromatography-mass spectrometry (LC-MS/MS) is a method that uses liquid chromatography (or HPLC) with the mass spectrometry. The Liquid Chromatography-Mass Spectrometry (LC-MS) is an effective analytical method with very high sensitivity and specific. Mixture of chromatography with spectrometry is initial announced in 1967 and initial LC-MS system was introduced in 1980s. It has been continuously used in development of drug at many different stages including Metabolic assurance Screening, Metabolite Identification as well as In Vivo Drug Screening, Impurity Identification, Peptide tracing, Glycoprotein Mapping, Natural Products Dereplication, Bio-affinity Screening. LC-MS is now applied successfully to daily analysis in many fields, consisting therapeutic drug monitoring (TDM), clinical and forensic toxicology as well as adultrantrate control. This advancement in LCMS was initially and still is sustain by the demand for extra powerful analytical and bio-analytical methods that can accurately and precisely differentiate target analytes from huge complexity mixtures in a conscious and selective way. With recent advances in instrumentation, the use of liquid chromatography

(LC) and mass spectrometry (MS) has become an effective two-dimensional (2D) hyphenated technology.

KEYWORDS: Chromatography, Liquid Chromatography/Mass Spectroscopy (LC/MS), Liquid Chromatography (HPLC), Mass Spectroscopy (MS).

INTRODUCTION

- Chromatography[1]

Chromatography could be a separation technique to separate the individual compound from a mixture using a two phase, one is stationary and second is mobile phase. The chromatography word obtained from Greek, 'Chroma' suggests that colour and 'graphein' mean writing, thus the word chromatography suggests that 'colour writing'. Chromatography was Initial developed by the Russian botanist 'Mikhail Tswett' in 1903; he separated coloured arrange pigments through carbonate column.

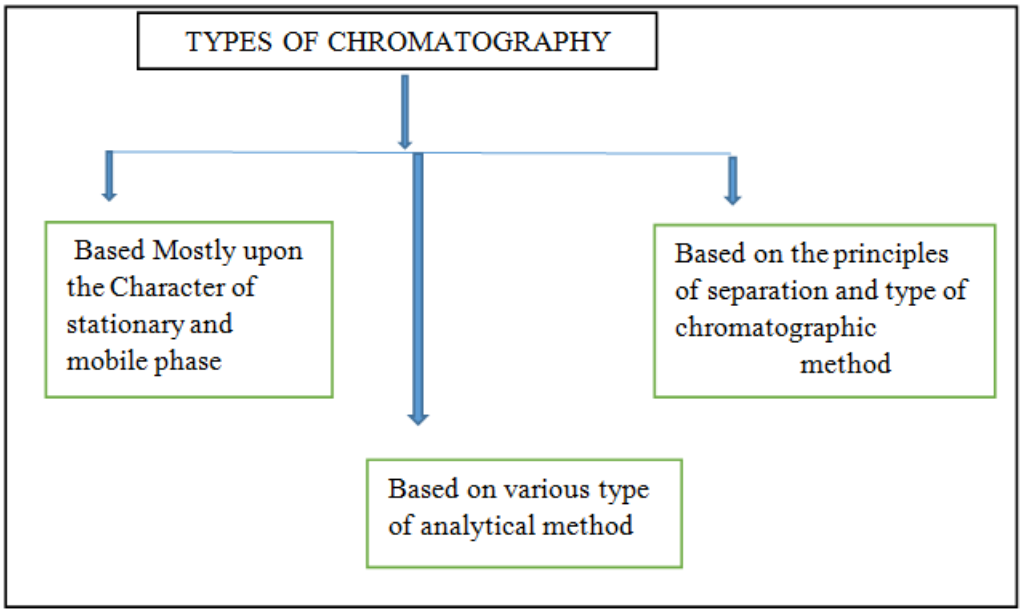

Figure 1: Types of chromatography.

1. Based mostly upon the character 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, Column chromatography)

2. Based on the principles of Separation and Type of chromatographic technique

- **Adsorption chromatography:** The mobile i.e. liquid or gaseous phase is Adsorbed into the surface of a stable solid phase. The separation of the Compound relies on affinity towards stationary phase. The compounds That have additional affinity with stationary phase will be going to be Eluted slowly and compounds with less affinity with stationary phase are go to be eluted quickly. Eg. Chromatography, TLC, HPLC and LC-MS.

- **Partition chromatography:** Separation of compounds relies on partition of a solute between two solvents. During this chromatography a liquid stationary phase, that is immiscible with the Mobile phase, is adsorbable to the surface of the solid adsorbent. Chromatography is carried out as term for adsorption column Chromatography. Partition of element of sample between the mobile Phase (sample) and stationary phase (liquid on solid support) retards. The extraction of some parts of sample which supplies the premise for Separation.

- **Ion exchange chromatography:** The principle concerned for this technique is reversible exchange of ions, between the ions present in the solution (mobile phase) and ion exchange resin (stationary solid Phase). This method may be any classified as cationic exchange Chromatography and anionic exchange chromatography.

- **Molecular exclusion chromatography:** It's otherwise known as as gel Permeation or gel filtration. This technique is employed to separate the proteins, peptides and oligonucleotides on the basis of size. The Column is packed with inert porous spheres (column media). Once a Mixture of different sized molecules passed through the column, the smaller molecules will enter the pores of the spheres (column media) and will take more time for elution. On opposite hand, the larger Molecules could not enter the pores of the spheres and can be eluted quicker.

- **Affinity chromatography:** This methodology is most selective and used to separate the antibodies, proteins and enzymes from the biological Matrix. It's promoted biological interactions among two molecules, like Enzyme and substrate, receptor and ligand, or antibody and antigen When a mobile phase consist of mixture of proteins/

Antibodies/enzymes are reach through the stationary phase, only the Specific protein binds to its respective ligand in the stationary phase. This protein next can be extracted by changing the ionic strength or Ph.

- **Chiral chromatography:** This kind of chromatography commonly Used to separate visual isomers (leva and dextrose forms) of the Molecules.

3. Based on various type of analytical method

- Capillary electrophoresis
- Chromatography with conventional detectors
- Gas chromatography (GC)
- Liquid chromatography
- Super critical fluid chromatography
- Hyphenated techniques (Mass-spectrometry)
- GC-MS and GC-MS/MS
- LC-MS and LC-MS/MS
- Supercritical Fluid Chromatography-MS (SFC-MS), Capillary Zone Electrophoresis-MS (CZE-MS)

Liquid chromatography

The liquid chromatography (LC) or The High Performance Liquid chromatography (HPLC) is one of most common analytical technique employed in pharmaceutical industry for determination and quantification of drug substances and its connected substances. Due to high reproducibility and accuracy, HPLC is habitually employed in pharmaceutical, chemical and pesticide industries.[2]

There are chromatography like normal phase liquid chromatography, Reversed phase chromatography, Ion-exchange liquid chromatography chiral separation and affinity liquid chromatography. By using various packing of columns with high efficiency small amount of complex mixture can be separated.[2]

- **Instrument of HPLC[2]**

The components of high pressure liquid chromatograph are as follow.

1) Solvent reservoir and degassing system
2) Pumps

3) Precolumns
4) Sample injection system
5) Columns
6) Temperature controller (Thermostat)
7) Detectors

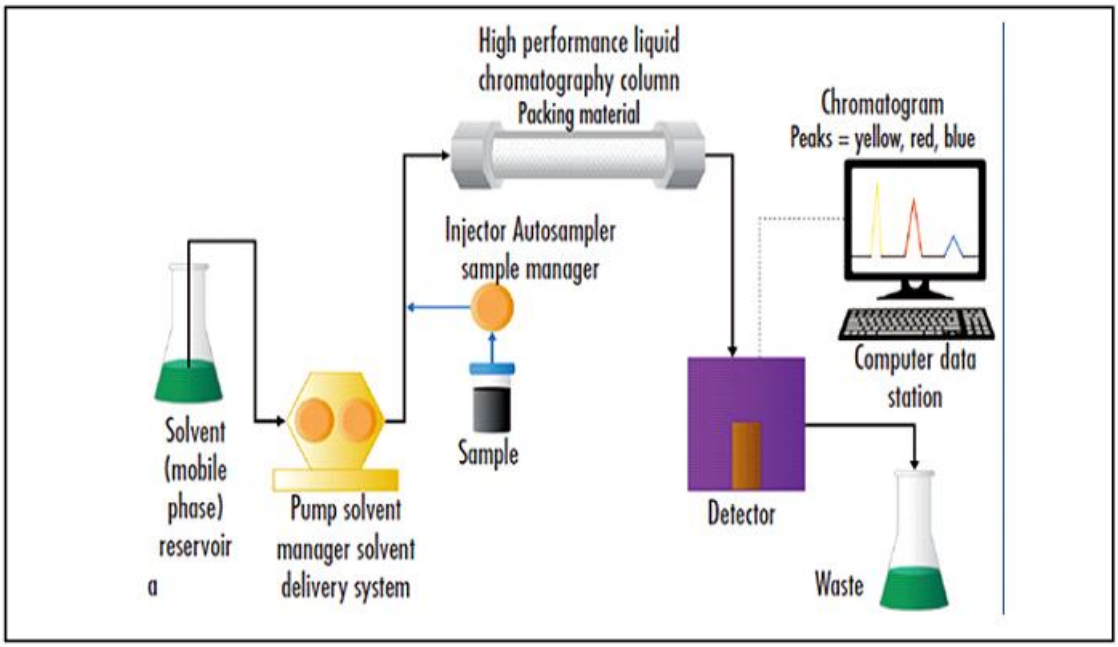

Figure 2: Instrument of liquid chromatography.

- **Solvent reservoir:** High Pressure Liquid Chromatography makes use of a single solvent or a mixture of solvent as a mobile phase, which is contained in a reservoir. The modern HPLC equipment have two or more reservoir from which different solvent can be introduced into a chamber at the rates which can be varied so as to adjust the polarity of the mobile phase.

- **Degassing system:** degassing of these solvent should be done so as to remove air or oxygen dissolve in them, which if present in the mobile phase, can rupture the packing of the column or can produce unwanted peaks in the chromatography. Degassing of the mobile phase can be done by four method:

1. By filtration under vacuum
2. By distillation of the mobile phase
3. By ultra-sonication
4. By sparging an inert gas of low solubility

- **Pumps:** It consists of material which is inert towards solvents or any mixed composition of aqueous buffer and organic solvents. It convey high volume of mobile phase up to 10mL/min. The high pressure generated by HPLC pumps doesn't constitute an explosion hazard, because liquids are not terribly compressible. There are three types of pumps are use in HPLC. They are described below:

1. Reciprocating pumps
2. Syring or displacement type of pumps
3. Pneumatic or constant pressure pumps

- **Sample injector:** It is wont to introduce sample volume into the chromatographically system. Generally sample volume from 1µL to 100µL are often injected. The injection volume are often increase by injector loop up to 2mL volume. There are two major styles of injectors used i.e. Automatic injectors and Manual injectors. Automatic injectors are unit more well off and user friendly and are more accurate and precise as compare to manual injectors.[2]

- **Columns:** It is stady phase which consists of silica material in mixure with carbon chain. Commonly the column breadth used is about 50mm - 300mm. The columns used in High Liquid Performance Chromatography consists of Octadecyl (C18), Octyl (C8), Cyano, Amino, Phenyl packing's. On the basis of nature of fusion to be separated by using the columns.[2]

- **Detectors and Recorder:** The detectors is most important part of HPLC .There are various types of detectors used are UV-Visible detectors, PDA detectors, Refractive index (RI) detectors, Electrochemical detector, Fluorescence detectors and conductivity detectors. The signal accepted from detector are often recorded as peak and respective data can be stored in a software.

➢ **Process**

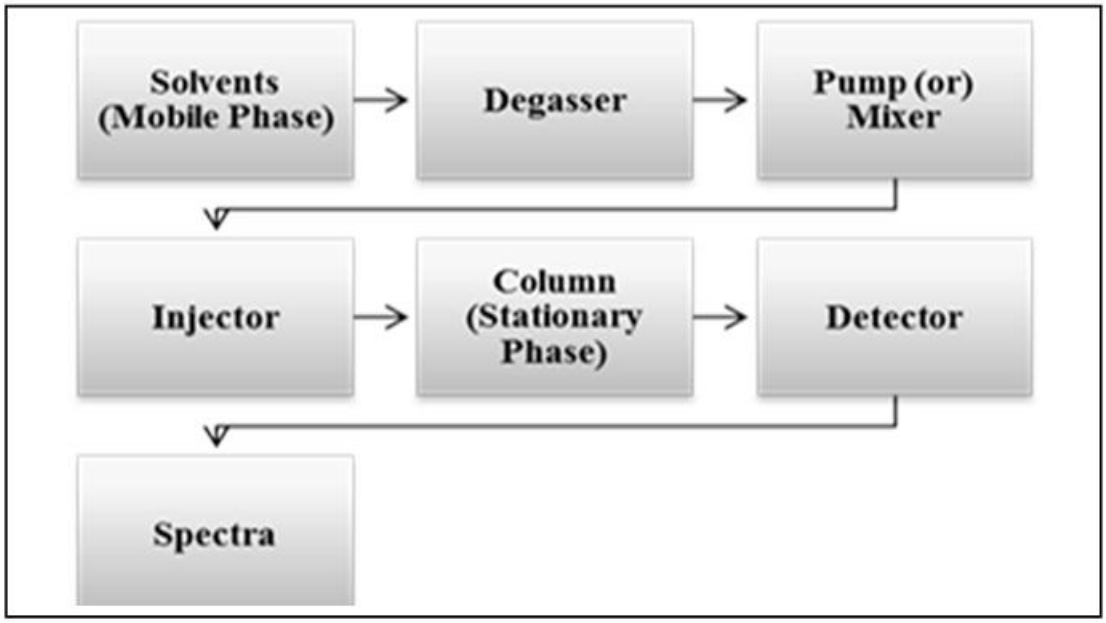

Figure 3: Process step of liquid chromatography (LC) OR HPLC.

Mass spectroscopy

MS is an analytical technique for determining the mass-to-charge ratio of charged particles. It's used to figure out particle masses, determine the elemental content of a sample or molecule, and deduce the chemical structures of molecules like peptides and other chemical compounds. MS works by ionizing chemical substances to produce charged molecules or molecule fragments, which are then weighed and their mass-to-charge ratios calculated. in a very classic MS procedure, a sample is loaded in to the MS instrument and undergoes vaporization The components of the sample are ionized by one of a mixture of methods (e.g., by impacting them with an electron beam), which results in the formation of charged particles (ions). Electromagnetic fields separate the ions in an analyser based on their mass-to-charge ratio. The ions are typically detected using a quantitative approach. Mass spectra are created from the ion signal.[3]

The technique has both qualitative and quantitative uses. These have identifying unknown compounds, determine the atom composition of elements in a molecule, and verify the structure of a compound by observing its fragmentation. Another uses embrace quantify the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry. Mass Spectroscopy is currently in very simple use in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds.

- **Instrument**[3]

MS instruments consist of three modules.

1. **An ion source:** Which might convert gas phase sample molecules into ions.
2. A **mass analyzer:** Sorts the ions by their masses by applying electromagnetic fields.
3. A **detector:** A detector is a device that calculates the abundances of each ion present by measuring the prize of an indication capacity.

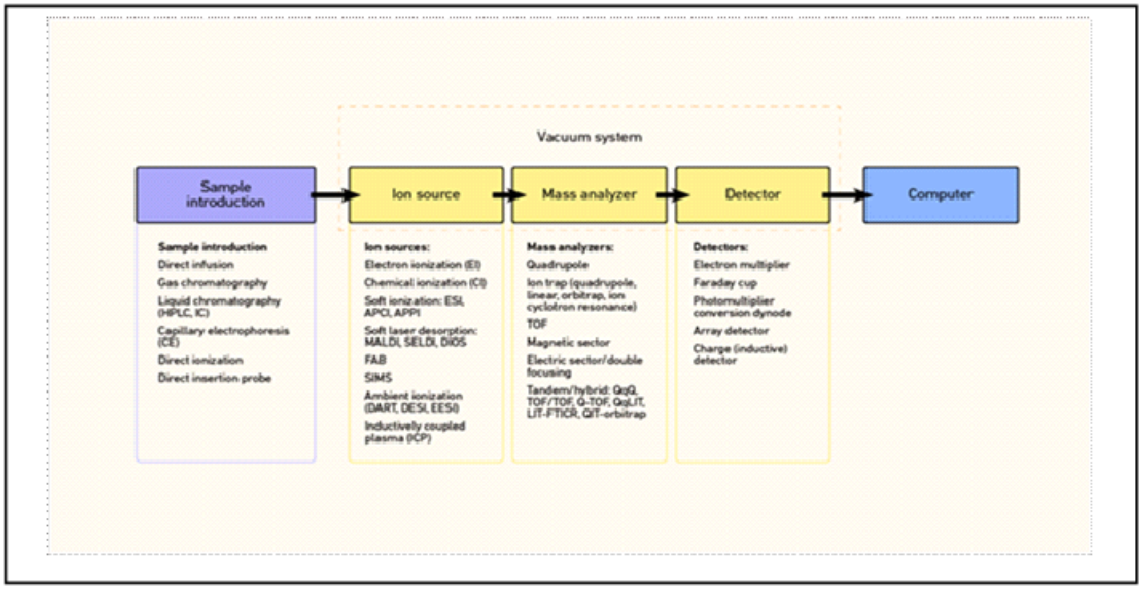

Figure 4: Instrument of mass spectroscopy.

- **Process**

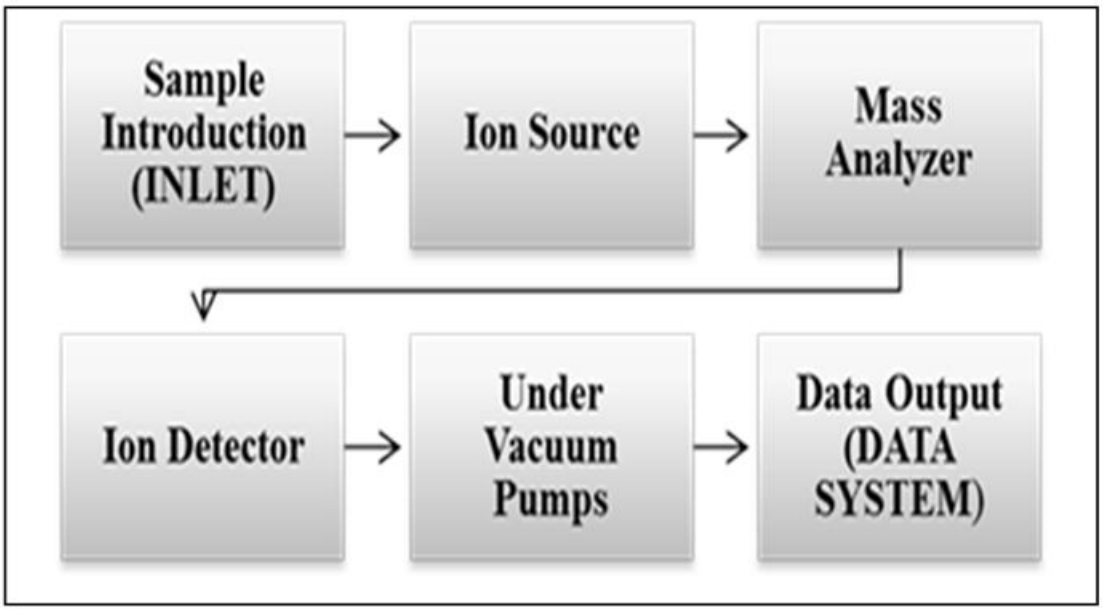

Figure 5: Process steps of mass spectroscopy.

LC-MS Technique

The spectrum is obtained using the LC-MS approach, which uses LC as a separation system and MS as a detection system. When the LC and MS operate together, they can perform multistage MS to guess the structure of the molecule, allowing for more accurate qualitative and quantitative analysis.[4]

Combination of LC with MS is a great development in the history of chromatography (1980s). MS in LC-MS helps to determine the elemental composition and structural elucidation of a sample.

Liquid chromatography-mass spectrometry (LC-MS or HPLC-MS) is an analytical technique that combines liquid chromatography's (or HPLC's) physical separation capabilities with mass spectrometry's mass analysis capabilities. LC-MS is a versatile technology with good sensitivity and selectivity that may be employed in a variety of applications. It is commonly used in pharmacokinetic studies of pharmaceuticals and is the most frequently used technique in the field of bioanalysis.[3]

- **Principle of LC –MS**

A typical LC-MS system combines HPLC and MS via an interface. The sample is separated using LC, and the separated sample species are sprayed into an ion source operating at atmospheric pressure, where they are transformed into ions in the gas phase. The mass analyzer sorts the ions based on their mass to charge ratio, and the detector counts and amplifies the ions ascending from the mass analyzer.

- **Instrument of LC-MS**

The liquid chromatography assembly, ion generating unit/ionization source, mass analyzer, and mass spectrometric data collection are the main components of the LC-MS. The mass spectrometer's ionization source is interfaced with the effluent mobile phase with separated chemical from liquid chromatography.[1]

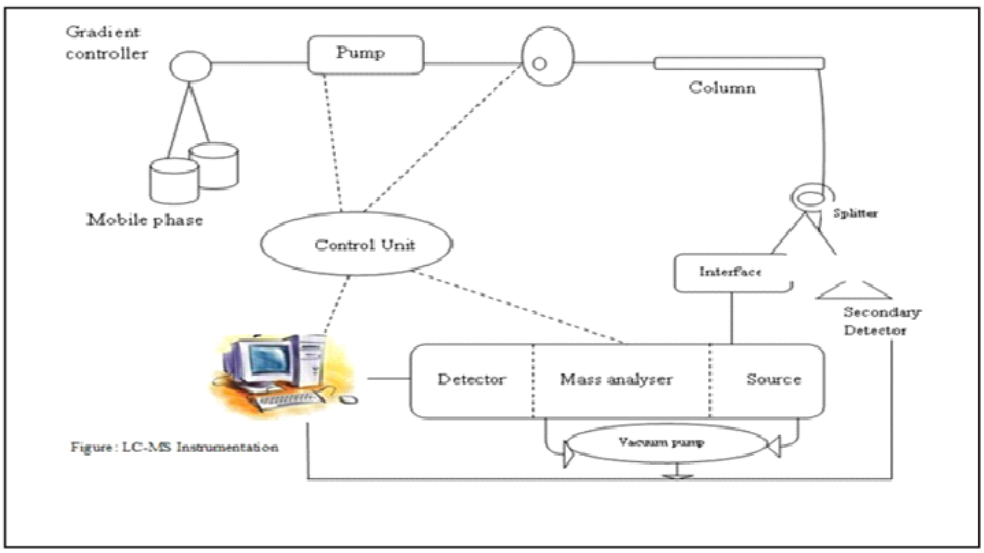

Figure 6: Instrument of LC MS Technique.

- **Process**

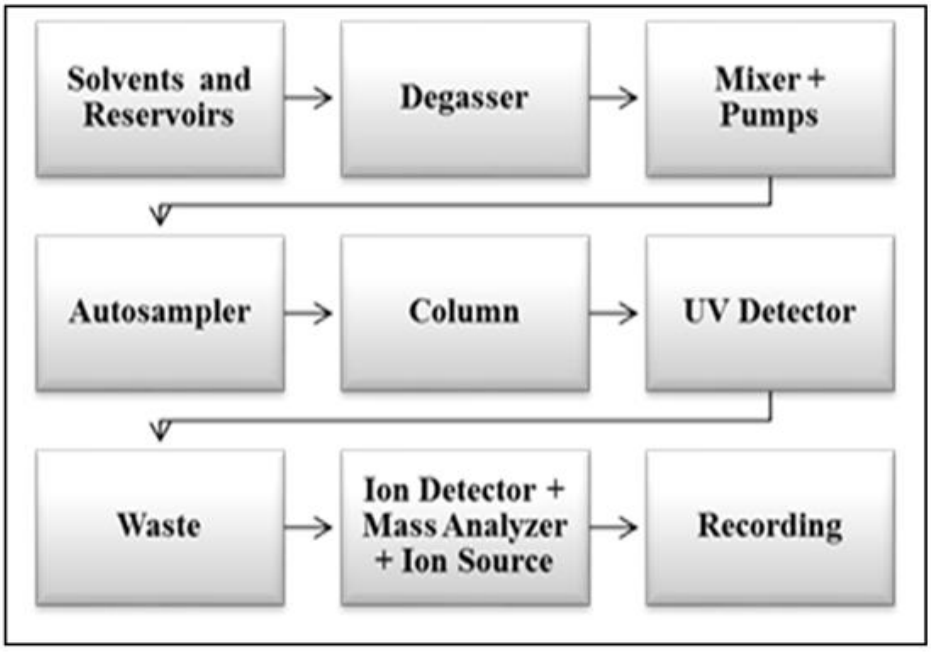

Figure 7: Process steps of LC MS technique.

- **Detector used in LC MS Technique**

There are three types of detector used in mass spectroscopy

1. Electron multipliers (Figure a)
2. Dynolyte photomultiplier (figure b)
3. Micro channel plates (Figure c)

- Electron Multipliers dual node is used to convert disciple either positive, negative ions in to electron that will be extended and detected. This will be commonly used in four of poles and ion capture instruments.
- The dynode of dynolte photomultipliers convers the charged ions in to electrons. These electrons attach to a phosphorus and through photons, and that photons are made to strike the photomultipliers to achieve multiplied signals for record.

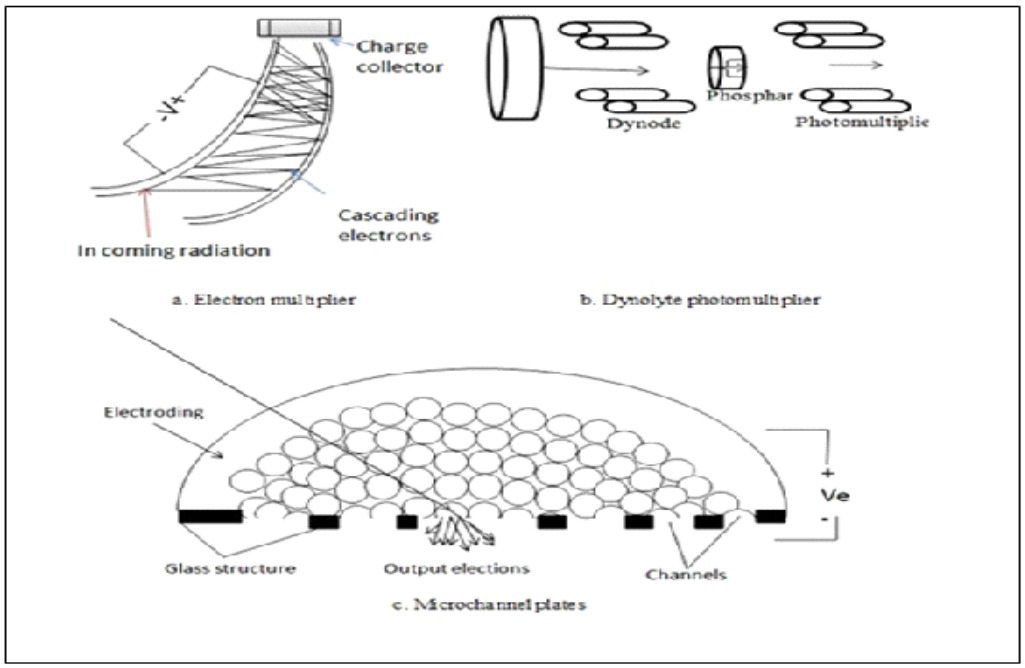

Figure 8: Detector used in LC MS Technique.

- **Advantages of LC-MS**

There are various advantages of LCMS over other chromatographic methods of which few are as follows,

- **Selectivity:** Mass selectivity can be used to isolate co-eluting peaks that are not confined by chromatographic resolution.
- **Peak assignment:** A molecular fingerprint for the compound under study is generated, ensuring correct peak assignment in the presence of complex matrices
- **Molecular weight information:** Confirmation and identification of both known and unknown compounds.
- **Structural information:** Controlled fragmentation enables structural elucidation of a chemical.
- **Rapid method development:** Provides easy identification of eluted analytes without retention time validation.

- **Sample matrix adaptability:** Decreases sample preparation time and hence saves time.
- **Quantitation:** Quantitative and qualitative data can be obtained easily with limited instrument optimization.

- **Disadvantages of LC-MS**
- Higher Operational cost
- More limited sample throughput
- Less favorable concentration sensitivity

Difference of liquid chromatography, Mass Spectroscopy and LC –MS

	Liquid chromatography	Mass spectroscopy	Lc-ms technique
Deffination	It is an analytical chromatographic Technique that is useful for separating ions or molecules that are dissolved in a solvent by using liquid mobile phase and solid stationary Phase.	It is an instrumental technique in which sample is converted to speedy moving anode ions by electron bombarding and charged particles are separate according to their masses.	It is hyphenated analytical technique which is mixture of liquid chromatography (lc) and mass spectrometry (ms) which is used with separation power of hplc with detection power of mass spectrometry (ms).separation and quantitation of components can be done.
Principle	In this, substances will move with the mobile phase at various rate depending upon their partition or adsorption.	In this, the components can be converted into gaseous phase ions and separates the ions in time or space according to mass to charge ratios and measures the quantity of ions of each mass to charge ratio.	In this, the separated components from lc can be transferred into mass spectrometer with the interfaces. Separation and determination of relative atomic masses can be performed simultaneously.
Uses	This technique is used for chemistry and biochemistry research analyzing complex mixtures	Elucidation of the structure of the organic and biological molecule	Used in pharmacokinetic-tics, bioavailability and Bioequivalence studies
	Used to quantify and separation of drug substances and drug products	Determination of molecular mass of peptides, proteins etc.	Used in metabolite studies, forensic studies

	It is used in pharmaceutical, agrochemical and pesticide industries	Monitoring gases in patients breathe during surgery.	For determination of assays of drug substances.

Various drug determine by the lc MS technique

Sr. no.	Drugs	technique
1	Proteomics analysis of different stages of non-small-cell lung cancer	Lc-ms/ms technique
2	Hydroxychloroquine, its two metabolites, and azithromycin in edta-treated human plasma	Lc-ms/ms method
3	Cantharidin in biological specimens and application to postmortem interval estimation in cantharidin poisoning	Liquid chromatography-tandem mass spectrometry (lc-ms/ms)
4	Potential antioxidant constituents from zanthoxylum zanthoxyloides leaves	Liquid chromatography-mass spectrometry
5	Tigecycline in critically ill patients	Liquid chromatography-tandem mass spectrometry
6	Molecular structure of melatonin after co-60 gamma irradiation	Liquid chromatography-mass spectrometry
7	Usnic acid in cladonia uncialis	Lc–ms/ms technique
8	6 zearalenones in animal feed	An immune affinity chromatography and lc-ms/ ms method
9	Asenapine in presence of its inactive metabolites in human plasma	Lc-ms/ms technique
10	Dexmethylphenidate from human plasma using liquid-liquid extraction	High performance liquid chromatography mass spectrometric method
11	Quantification of simotinib in human plasma	Uplc–ms/ms assay
12	Azithromycin in human plasma	A liquid chromatography–mass spectrometric method
13	Fluconazole, itraconazole, hydroxyitraconazole, posaconazole, voriconazole, voriconazole-n-oxide, anidulafungin, and caspofungin	Multiplex ultra-performance liquid chromatography–tandem mass spectrometry method
14	Acebutolo	Lc and lc–ms/ms technique
15	Dapagliflozin and saxagliptin in human plasma	lc-esi-ms/ms method

Application

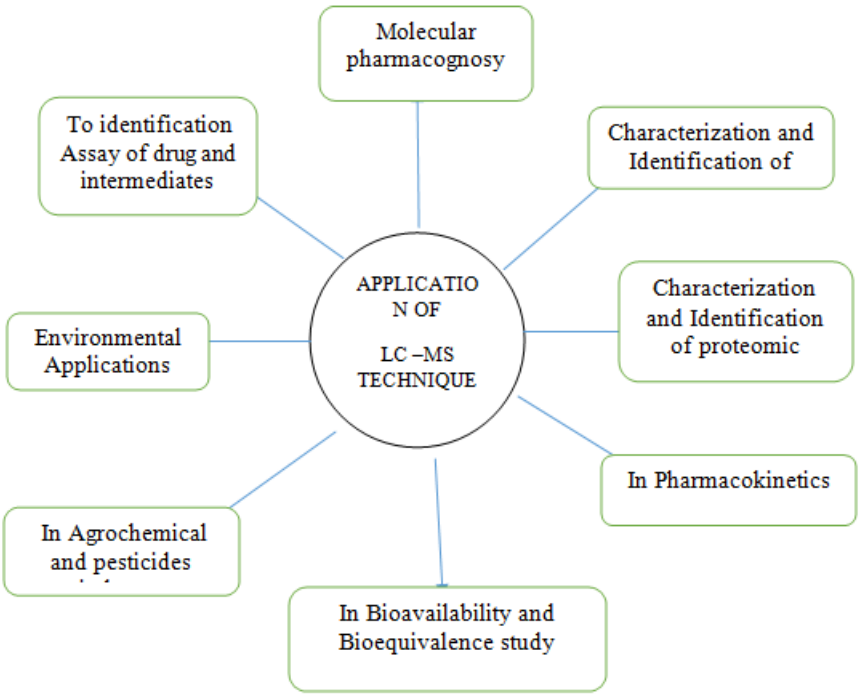

Figure 9: Various application of the LC- MS Technique.

1. **Molecular pharmacognosy:** LC MS determines the contents and classes completely different of various} teams of civilized plant cells and choose the try of teams with the most important different content of ingredient for the study ingredient distinction phenotypical biological research.[4]

2. **Characterization and Identification of carotenoids:** Because carotenoids are not thermally stable, separation of mixtures and removal of impurities is usually carried out by reversed phase HPLC (particularly HPLC) instead of GC the small samples of carotenoids which were isolated from biological matrices such as human serum or tissue prevent structural analysis by Nuclear Magnetic Resonance. Hence, only the most sensitive analytical methods are adequate such as LC-MS and HPLC with photodiode-array UV / visible absorbance detection. At the minimum level, carotenoid determination may confirmed by combining data such as HPLC retention times, photodiode-array absorbance spectroscopy, MS and tandem mass spectrometry .recent, five LC/MS techniques have been used for carotenoid analysis including moving belt, particle beam, steady flow fast atom shelling, electrospray and Atmospheric Pressure Chemical Ionization (APCI). With these LC/MS interfaces, electrospray and APCI are apparently

the simple to use and rapidly become extensively available. These models provide equal sensitivity (at the low pmol level) and produce enormous molecular.[4]

4. **Characterization and Identification of proteomic:** Liquid Chromatography / Mass Spectrometry (LC/MS) has become a powerful technology in proteomics studies in drug discovery which includes target protein characterization and the discovery of biomarkers.

A. **Glycopeptides characterization:** In present, with MS-based strategies, tandem MS fragmentation and data analysis problems provide efficient characterization of intact glycopeptides and then analysis of the peptides is done via Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS)

B. **Peptide mapping:** In earlier day's protein drugs were made from proteins refined from living organisms. Yet, they are freshly produced using recombinant technology. Insulin, interferon, and erythropoietin are some of the protein made by recombination which are available in the market.

5. **In pharmacokinetics:** LC-MS is used in the study of absorption, metabolism, and excretion of drugs. Bio analytical methods are used for quantitative and structural elucidation of drugs and its metabolites in the biological samples (plasma, urine, saliva, serum etc.)[36]

6. **In Bioavailability and Bioequivalence study:** Comparative bioequivalence studies in which quantitative determination of drugs or metabolites is measured in biological matrix, pharmacodynamics, clinical trials and In-vitro dissolution tests.

7. **In Agrochemical and Pesticides industry:** It is used in determination of different components present in the fertilizers and pesticides.

8. **Environmental applications:** LC-MS is used for detection of phenyl urea herbicides, detection of low level of carbonyl in food.

9. **In identification 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 related molecules.

Future prospective

- **Pharmacovigilance**[4]

Pharmacovigilance (PV or PhV), which is assigned to as Drug Safety. It is one of the pharmacological science that relates to the collection, detection, estimate, 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 that provides detailed profiles.

- **Organic/Inorganic hybrid nano flowers**

Analytical method of LCMS can be employed for the detection of General Nano flowers. It is helps in the development of drug delivery systems, biosensors, biocatalysts, and bio - related devices is anticipated to take multiple directions. It's envisaged that novel synthesis principles, hybrid Nano flower types, and precise mechanisms would develop. The application of Nano flowers in bio-catalysis and enzyme mimetic, tissue engineering, and the design of highly sensitive bio-sensing kits, as well as industrial bio-related devices with advanced functions, various and controllable syntheses, biocompatibility, and modifications of hybrid Nano flower structures and properties, should receive increasing attention.

SUMMARY AND CONCLUSION

The LC-MS is a hyphenated method used in combination with segregation power of HPLC with detection power of Mass spectrometry. It is often used in pharmaceutical companies, chemical, food, agrochemical industries, environmental and forensic applications. LC-MS is used for qualitative and quantitative calculation of drugs and sample of biological sources. The improvement of hyphenated techniques, high resolve mass analyzers as well as high put through segregation approached, quantitative and quantitative analysis of components of drugs and metabolites can be attain with good sensitivity.S

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