IJBPAS, October, 2022, 11(10): 4557-4566

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

REVIEW ON PRINCIPLES, INSTRUMENTATION AND APPLICATIONS OF LC – MS IN PHARMACEUTICAL ANALYSIS

MINIMOL M, M. VIJEY AANANDHI AND P. SHANMUGASUNDARAM*

Department of Pharmaceutical Chemistry and Analysis, Vels Institute of Science Technology and Advanced Studies (VISTAS), Chennai-600 117, Tamilnadu, INDIA

***Corresponding Author: Dr. P.Shanmugasundaram; E Mail: samsimahe@gmail.com**

Received 20th Jan. 2022; Revised 24th March 2022; Accepted 13th April 2022; Available online 1st Oct. 2022

https://doi.org/10.31032/IJBPAS/2022/11.10.6456

ABSTRACT

LC – MS: Liquid chromatography – Mass spectroscopy, a rapidly growing as well asthe most chosen option in Pharmaceutical Analysis. The discovery of LC- MS is a milestone in biomedical research and in 1980s; the first LC-MS was presented. LC – MS (Liquid chromatography – Mass spectrometry) system is aunion of Liquid Chromatography (LC); High Performance Liquid Chromatography (HPLC) together with Mass Spectrometry. The LC – MS assumes crucial part in many fields. This review essentially centers on the principle, instrumentation and applications of LC-MS method. LC-MS isutilized in variousssteps of drug development counting Identification and stability screening of metabolite, Peptide Mapping, Identification of impurity, Mapping of glycoprotein, and Screening for bio affinity. LC-MS is also outstanding in numerous spaces, countingforensic toxicology and therapeutic drug monitoring (TDM).

Keywords: LC-MS, HPLC, MS, Ionization Source, Mass Analyzer, Mrtabolomics

INTRODUCTION

The combination of Liquid chromatographic analysis with Mass - spectroscopy leads to significant development in pharmaceutical analysis. The method LC-MS were the best delicate procedure utilized in investigation studies in drugs **[1]**. Coupling of Mass spectroscopy with chromatographic methods are continuously alluring due to its high

specificity and sensitivity of Mass spectroscopy when compared to other chromatographic detectors **[2]**. Mass spectrometry (MS) and Liquid chromatography (LC) are analytical approaches plays crucial part in their particular fields, finding solutions for different analytical issues. The approaches of these two methods are entirely unique. Liquid chromatography utilizes high pressure for the effective partition of analytes and Mass spectroscopy is a sophisticated detector for chromatography **[3]**.

BASIC PRINCIPLE INVOLVED IN LC-MS

High Performance Liquid Chromatography (HPLC):

The basic principle of HPLC is Adsorption. In the method HPLC, using eluent with high pressure, sample is pass across the column loaded with stationary phase. In LC-MS, Reversed Phase Liquid Chromatography (RP-LC) is used for introducing the samples into the MS **[4]**.

Mass spectrometry (MS):

In MS, it estimates mass to charge (m/z) proportions of charged components. Sample components are ionized to produce ions (charged particles). These formed ions were isolated based on mass-to-charge proportion using electromagnetic fields and are identified. Then the signals are handled into mass spectra **[4]**.

LC–MS:

Important elements present in LC–MS structure are Autosampler, HPLC, Ionization source and Mass Spectrometer are illustrated in fig 1. All these elements were controlled by computer system. At the point when interface HPLC with the MS, some restrictions are arises on the eluent and flow rate. Mixture of water with methanol or acetonitrile isutilized for the purpose ofeluent in typical reversed phased HPLC system that connected to MS. Modifiers for the mobile phase are utilized to work on the chromatography of the analytes. But in many situations the modifiers have to be volatile in nature. Formic acid and Ammonium acetate or acetic acid is some examples of mobile phase modifiers that are commonly used **[5]**.

The most common ionization sources utilized in interfacing HPLC mobile phase together with MS were Atmospheric Pressure Chemical Ionization (APCI) and Electrospray Ionization (ESI). ESI and APCI are additionally alluded to as pressure ionization sources because ionization happens at atmospheric pressure. Blend of excessive voltage and thermal energy is utilized for ESI and APCI for ionization to deliver the ions for examination by the Mass

Spectrometry. Utilization of high voltage field (3–5 kV) in ESI results in column effluent nebulization that produces charged droplets and is focussed towards the mass analyzer. The size of these droplets decreases as droplets come to the passage way towards the mass analyzer. When these drops become more modest, each ionappears in Ion evaporation process. Then at that point, these particles are separated by MS. Within the APCI, the column eluant is vapourized by heat and a corona discharge was utilized to convert dissolvable atoms into ions, at that point, it generates the analyte ions by chemical ionization methods **[6]**. Atmospheric Pressure Photoionization (APPI) is utilized to convert the column mobile phase into vapours and ionization done by UV lamp which generates protons **[7, 8]**.

DIFFERENT TYPES OF MASS SPECTROMETERS:

Different mass spectrometer utilized in interfacing of HPLC. They are Time-Of Flight (TOF) - mass spectrometer, Single quadrupole mass spectrometer, Triple Quadrupole MS–MS system, Ion-trap mass spectrometer. Single Quadrupole Mass Spectrometer that develops mass spectrum for chromatographic peak which is eluted from liquid chromatographic column. TOF Mass Spectrometer that develops elevated mass resolution spectrums of every particle which is examined. Triple Quadrupole MS–MS device mainly utilized for bioanalytical assays and also can be used for detection of metabolites. The Ion-trap Mass Spectrometer that provides MSn data which is crucial for structural elucidation assay. There are other mass spectrometers, including hybrid systems having extraordinary capacities. Hybrid mass spectrometers join both essential kinds of mass spectrometers. An illustration of hybrid mass spectrometer is 'Q-TOF' MS–MS that is combination of Quadrupole mass spectrometer and TOF mass spectrometer **[5, 9]**.

INSTRUMENTATION OF LC-MS [4]

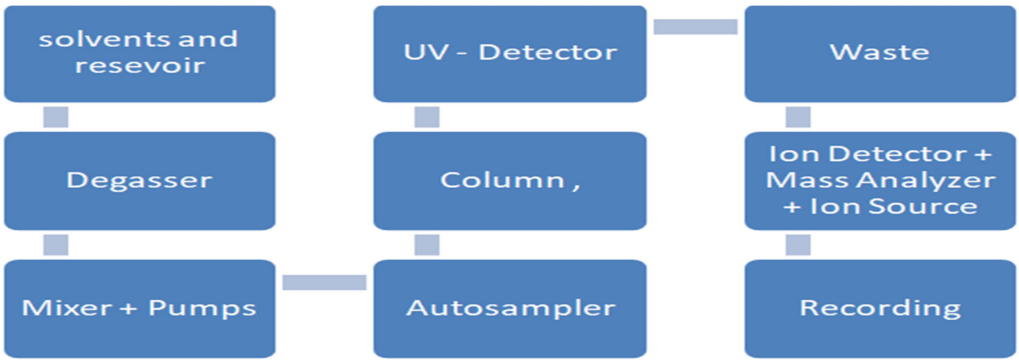

Figure 1: Diagrammatic representation of LC-MS

Ionization source:

Commonly utilized ionization sources are ESI, APCI, MALDI, Electron impact (EI) and Chemical ionization (CI) or negative chemical ionization **[10]**.

Electrospray ionization (ESI):

Electrospray is delivered with the use of solid electric field in a liquid going inside the capillary tube of weak flux and this generates charged huge drops. Huge drops are directly exposed to solvent evaporation. Increment in charge density because of vaporization of solvent makes coulombic repulsion in order tosurvive the surface tension of liquid and that cause the release of ions from droplets. Sensitivity for detection is in between ten to eight microliter and requires huge bulk to expand sensitiveness in identification. Sample with large mass, non-volatile nature and liquids were ionized by ESI. Drawbacks of ESI are less fragmentation, less sensitivity and instable source **[11]**.

Atmospheric pressure chemical ionization (APCI):

Eluent is nebulized by nitrogen gas which is then vaporized with heat of high temperature greater than 400°C. Then, at that point, the subsequent vapor is exposed to a corona discharge electrode for makes ions. This is the Principle of this technique **[12]**.

Matrix-assisted laser desorption/ionization (MALDI):

MALDI methods are utilized to huge and potentially unstable molecules like polymers, peptide, dendrimers, protein and fullerenes. MALDI includes implanting analyte in matrixes that ingests energy and afterwards nitrogen UV laser of 337 nanometers aregiven to the matrix in vacuum to generate ions of analyte. The significant purpose of matrix; to assimilate photons energy, to reduces intermolecular force as well as aggregations of analyte molecules **[13]**.

The mass spectrometer analyzer:

Analyzers are the important partin mass spectrometer which isolates the ionized molecules dependent on mass to charge ratio and also output them to the detector and later changed over to a digital output.

Quadrupole Mass analyzer:

Quadrupole and triple quadrupole were broadly employed since it is simple as well as it covers different mass range (10 - 4000 AMU). Quadrupole provides great linearity, good resolution, mass accuracy (0.1 - 0.2 AMU), scanning speed (5000 AMU / Second). The working includes; Electric fields were utilized for isolates ion depends upon the mass- to -charge proportion during these ions were passes through central axis of 4 parallel equally distant pole and ions were

isolated by quadrupole depends upon their steadiness of directions in the fluctuating electric field given to the rods. Quadrupole, contain 2sets metallic rods and opposing rod pair is associate done by oneby electrically. Voltage of RF is developed within 1set of rod and the other. Then an immediate voltage current is superposed on radio frequency voltage. Also ions were passes downs through quadrupole in middle of the rod. Ionhaving specific massto-charge ratio get reaches the indicator having a given proportion of voltages and the remaining ions follow shaky directions and get collide with rods. That allows the screening of an ion and permits the driver to filter for specified of m/z-values with constantly changing the using voltage **[5, 9]**.

Ion trap analyzer:

Ion trap analyzer or Quadrupole ion trap analyser (QIT) is mostly utilized in GC – MS rather than LC – MS. The main working theory involved in this analyzer is trapping of ionby an instrument containing a ring electrode as well as two end cap electrodes. Applied DC and RF fields are used to manipulating these ions. The specific amplitude of the given voltages helps intrapping ions of determined mass to charge ratio in examining equipment and non chosen ions were exit the trap **[14]**.

Time of Flight (TOF) analyzer:

Time of Flight analyzer is used in single MS systems. The Time of Flight is connected to quadrupole or withother TOF orwith an Ion Trap (QIT/TOF) in MS/MS configuration. In Time of Flight analyzer, ions are accelerated with high speedutilizing electric field presented in drift tube of equipment and these speedup ions were identified by sensor **[15]**.

Magnetic Sector Mass Analyser:

In this analyzer, Ions were accelerated within flight tube using magnetic field and these ions were isolated by mass- to -charge ratio. When mobile charges go in for the magnetic field, charges are diverted to an angular movement of a specific radius that is perpendicular with given magnetic field. The ions present within the magnetic field experiences 2 forces that are equal because of the presence of centripetal force and magnetic field.

Fourier transform-ion cyclotron resonance (FT-ICR):

When Ions enters the chamber, it gets caught in circular orbits by magnetic and electric fields. The ions produce a time dependent current with a Radio-frequency (RF) electrical field. That current ischanged over into orbital frequencies by Fourier transforms. Fourier transform – ioncyclotron

resonance mass analyzers can perform various stages of MS and also they have wide mass range and FT-ICR have excellent mass resolution **[16, 17]**.

Detectors:

Detectors mainly utilized in MS are Dynolyte photomultiplier, Electron multipliers and Micro channel plates. Electron multipliers dynode converts negative or positive ions into electrons and it is intensified and detected. Detectors are mainly utilized in ion trap instrument and squadrupole. Dynolyte photomultipliers that changes the charged ionto electron and electrons get stick to phosphor. Itrelease photon, and then the photon are allowed to collide with PMT for record signals. Microchannel Plate (MCP) has exponentially low response and high sensitivity so it is commonly employed in TOF spectrometers **[18]**.

APPLICATIONS OF LC-MS

1. In Molecular Pharmacognosy: **[19]**

Determine the constituents and categories of variousgroup plant cells and study phenotypes cloning.

2. For the Characterizations and Identifications of Compounds: **[20]**

Carotenoid: LC – MS method is utilized for analysis of carotenoids counting particle beam, moving belt, continuous flow fast atom bombardment and APCI. Atmospheric pressure chemical ionization and Electrospray are LC/MS interfaces presumably the least demanding to utilize and are quickly turning into the most generally accessible technique. These methods give high sensitivity and produce enormous molecular ions.

Proteomics: Liquid chromatography – Mass spectrometry has turned into incredible innovation in proteomics studies for drug development which incorporates the discovery of biomarkers and target protein characterization.

Characterizations of glycopeptides, study of degradation products and Peptide mapping.

3. Quantitative analysis and Qualitative analysis: **[20]**

Biological samples were analyzed by quantitative bioanalysis: Preparation of samples, separation of the individual components and detection by MS - MS are the methodology of LC-MS/MS. Applications in different areas including amount estimation of biogenic amines, doping control and pharmacokinetics evaluation of immunosuppresants. Qualitative analysis and quantitative analysis of complex lipid mixtures and phytoconstituents / Plant metabolomics.

4. Use of Automated Immunoassay for Therapeutic Drug Monitoring (TDM). [22]

TDM helps in improving patient outcome who has taking drug with narrow therapuetic index. LC – MS / MS based immune assays as well as this methodsappear to be the most far and wide methodology clinical research centres.

5. Metabolomics. Metabolomics based on Mass Spectrometry has been widely utilized to acquire new bits of knowledge into human, drugs, plantsand discovery of biomarkers, food control, nutritional research and biochemistry **[23, 24]**.

Other applications include:

Determinations of molecular weight and Structural elucidation, Clinical and biochemical applications, application in Food analysis and environmental applications **[25-27]**.

CONCLUSION

LC – MS; Liquid chromatography - Mass spectrometry is apopular and best hyphenated technique having high sensitivity. The technique LC-MS is the blend ofseparating power of liquid chromatography (LC) with detecting power of mass spectrometry.It covers widescope of application in areassuch as pharmaceuticals, environmental and food analysis and industrial materials, toxicology, Pharamacovigilance.

ACKNOWLEDGEMENT

The authors are grateful to the management of Vels Institute of Science, Technology and Advanced Studies (VISTAS) for the facilities provided.

CONFLICTS OF INTEREST

The authors declare there is no conflict of interest.

FUNDING SUPPORT

The authors declare that they have no funding supports for this study.

REFERENCE

[1] Chang-Kee LIM and Gwyn LORD, Current Developments in LC-MS for Pharmaceutical Analysis, Biol. Pharm. Bull, 2002; 25(5): 547—557.

[2] James j Pitt, Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry, the Clinical Biochemist Review, 2017; 30 (1): 19-34.

[3] Achille Cappiello, Giorgio Famiglini, Filippo Mangani and Pierangela Palma, A Simple Approach for Coupling Liquid Chromatography and Electron Ionization Mass Spectrometry, Journal of the American Society for Mass Spectroscopy, 2002; 13(3): 265-273.

[4] Kumar P.R, Dinesh S.R and Rini R, LCMS- A Review and Recent Update,

World Journal Of Pharmacy And Pharmaceutical Sciences, 2016; 5(5): 377-391.

[5] Walter A. Korfmacher, Principles and applications of LC–MS in new drug discovery, Drug Discovery Today, 2005; 10(5): 1357-1367.

[6] Niessen, W.M, Progress in Liquid Chromatography-Mass Spectrometry Instrumentation and its impact on high throughput screening, Journal of Chromatography A, 2003; 1000: 413–436.

[7] Syage, J.A, Mechanism of [M + H](+) formation in photoionization mass spectrometry, Journal of American Society Mass Spectrometr, 2004; 15: 1521–1533.

[8] Damon B. Robb, Thomas R. Covey, Andries P. Bruins,Atmospheric pressure photoionization: an ionization method for liquid chromatography-mass spectrometry, American Chemical society, 2000; 72(15): 3653–3659.

[9] Bradley L Ackermann, Michael J Berna, Anthony T Murphy, Recent advances in use of LC/MS/MS for quantitative high-throughput bioanalytical support of drug discovery. Current Topics in Medicinal Chemistry 2, 2202; 1: 53–66.

[10] Sparkman OD. Mass Spectrometry Pitt Con, American Society for Mass Spectrometry, 2006; 17(6): 873-84.

[11] Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM, Electrospray ionization for mass spectrometry of large biomolecules, Science, 1989; 246(4926): 64-71.

[12] Van Breemen RB, Dong L, Pajkovic ND, Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry of Carotenoids, International Journal of Mass Spectrometry, 2012; 312: 163-72.

[13] Marvin LF, Roberts MA, Fay LB, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in clinical chemistry, Clinica Chimica Acta, 2003; 337(1-2): 11-21.

[14] Stafford G, Ion trap mass spectrometry: a personal perspective, Journal of the American Society for Mass Spectrometry, 2002; 13(6): 589-96.

[15] Chernushevich IV, Loboda AV, Thomson BA, An introduction to Quadrupole Time-of-Flight Mass

Spectrometry, Journal of Mass Spectrometry, 2001; 36(8): 849-65.

[16] Marshall AG, Hendrickson CL, Jackson. GS, Fourier transform ion cyclotron resonance mass spectrometry: a primer, Mass Spectrometry Review, 1998; 17(1): 1-35.

[17] Marshall AG, Hendrickson CL, Fourier transform ion cyclotron resonance detection: principles and experimental configurations, International Journal of Mass Spectrometry, 2005; 215(1-3): 59-75.

[18] Neetu SK, Ankit G, Ruchi T, Ajay B, Prashant B, A review on mass spectrometry detector, International Research Journal of Pharmacy, 2012; 3(10): 33-42.

[19] He CM1, Cheng ZH1, Chen DF, Qualitative and quantitative analysis of flavonoids in Sophoratonkinensis by LC/MS and HPLC, Chinese Journal of Natural Medicines, 2013; 11(6): 690-8.

[20] Richard B. van Breemen Liquid chromatography/mass spectrometry of carotenoids Pure 84, Applied Chemistry, 1997; 69(10): 2061-2066.

[21] Oksman-Caldentey K-M, Inz´e D, Plant cell factories in the post-genomic era: new ways to produce designer secondary metabolites, Trends Plant Science, 2004; 9(9): 433–440.

[22] Gunnar Brandhorst and Michael Oellerich, Liquid Chromatography–Tandem Mass Spectrometry or Automated Immunoassays: What Are the Future Trends in Therapeutic Drug Monitoring?, Clinical Chemistry, 2012; 58(5): 821–825.

[23] Chi Chen, Frank J. Gonzalez, Jeffrey R. Idle, LC-MS-Based Metabolomics in DruMetabolism, Drug Metabolism Reviews, 2007; 39(2-3): 581-597.

[24] Guodong Chen, Application of LC/MS to proteomics studies: current status and future prospects, Drug Discovery Today, 2009; 14(9–10) 465–471.

[25] Kaufmann B, Souverain S, Cherkaoui S, Christen P, Veuthey JL, Rapid liquid chromatography-Mass spectrometric analysis of with anolides in crude plant extracts by use of a monolithic column, Chromatographia, 2002; 200256(3-4): 137-41.

[26] Lee MS, Kerns EH, LC/MS applications in drug development,

Mass Spectrometry Review, 1999; 18(3-4): 187-279.

[27] Prakash C, Shaffer CL, Nedderman A, Analytical strategies for identifying drug metabolites, Mass Spectrometry Review, 2007; 26(3): 340-69.