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Review on Liquid Chromatography-Mass Spectroscopy Overview

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Abstract: The Liquid Chromatography-Mass Spectrometry (LC-MS) is a powerful analytical method with very high sensitivity and specificity. LC-MS is mixture of Liquid Chromatography (LC) and Mass Spectrometry (MS). With the Liquid Chromatography (LC) the separation of additives may be completed and then the sample eluents from LC are transferred into Mass Spectrometry (MS) wherein the detection, identity and determination of masses of components can be achieved in presence of other components. LC-MS is used in determination, of pharmaceutical drug materials, intermediates and its related compounds for quantitative and qualitative reason. LC-MS is used most significantly in in-vitro dissolution, bio-equivalence, bioavailability and metabolite studies. Also LC-MS is utilized in simple studies, agrochemical, forensic laboratories and food industries.

Keywords: High performance Liquid Chromatography, Mass Spectroscopy, Recent Advances of LC-MS.

INTRODUCTION

Liquid chromatography-Mass spectrometry (LC-MS also known as High performance liquid chromatography-Mass spectrometry (HPLC-MS). LC-MS it is a combination Technique which is developed in 1980s.It is a method of analysis that combines sensitive mass spectral detection with high resolution chromatographic separation .[1] In LC-MS mass spectrometry improves in sample structure elucidation and elemental composition determination. Mass spectrometry in LC-MS helps to determine the essential composition and structural elucidation of a sample.[2] there are a number of other clinical operations of LC- MS, and the technique is more generally applicable than GC- MS owing to the broader range of natural atoms that can be analysed and the higher use of LC separations in clinical laboratories. The reasons for choosing LC- MS over LC with conventional detectors are basically the same as with GC- MS, namely high particularity and the capability to handle complex mixtures. Operations of

electrospray MS were reviewed in The Clinical Biochemist Reviews in 2003. [3] The current review focuses on the principles of LC- MS, practical considerations in setting up LC- MS assays and reviews some of the major applications in clinical biochemistry, concentrating on small atom operations. [4]

This bicycle technique can be used to analyze biochemical, natural, and inorganic compounds usually found in complex samples of environmental and biological origin. Therefore, LC-MS may be applied in an extensive range of sectors which includes biotechnology, environment monitoring, food processing, and pharmaceutical, agrochemical, and cosmetic industries. Novel substances and new instrumental configurations are beneath study to enhance the performance of the different ion Sources. Protection risks may be identified at the early levels through non targeted monitoring technologies. Furthermore, the variety of fragmentation strategies that can be Combined in new instrumentation overall enhances work in the omics fields, particularly proteomics and metabolomics. Although MS-based totally methods are getting step by step greater effective, reliable, and easily available, the primary drawbacks are still related to sample complexity and preparation, mass accuracy, often requiring the usage of high resolution mass spectrometry (HRMS) to guarantee the univocal identification of the targeted compounds, and the need of high-throughput and screening analyses when a great number of samples have to be analyzed.[5]

Instrumentation

Liquid chromatography-mass spectrometry (LC-MS)

The liquid chromatography and mass spectrometry is a combination technique in which used to separation power of HPLC with detection of mass spectrometry (MS) The different parts of LC-MS instrument are listed as Follows.

a) Liquid Chromatography (LC)

b) Mass Spectrometry (MS)

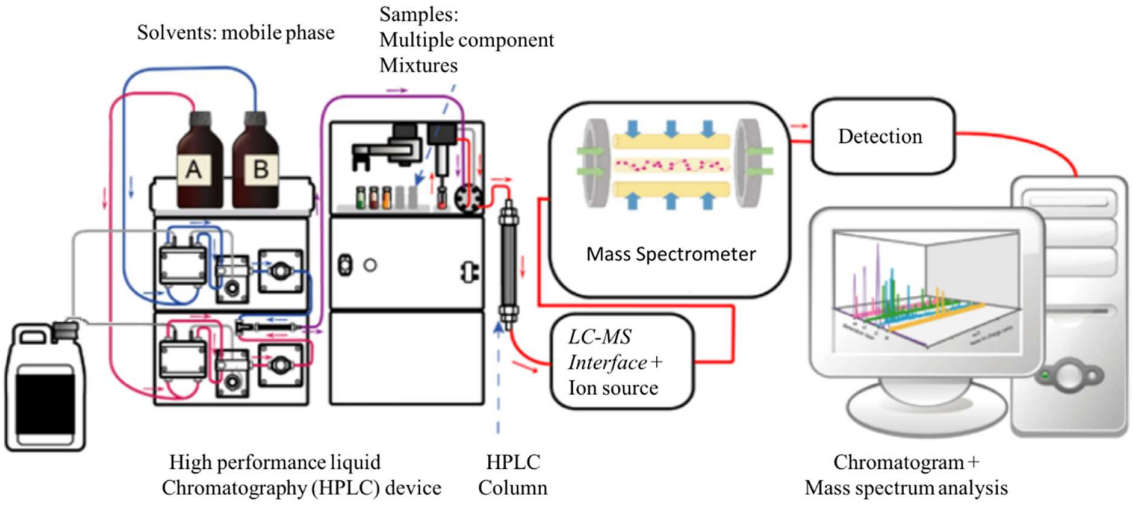

Figure 1: LC-MS

a) Liquid Chromatography (HPLC)

Liquid Chromatography (HPLC) high performance liquid chromatography it is based on separation of substances or impurities from a mixture compounds can be carried out by using liquid mobile and solid stationary phase. There are different types of chromatography like Normal phase chromatography, Reverse phase chromatography, adsorption chromatography, ion-exchange chromatography, size exclusion chromatography, chromatography, chiral separation, affinity chromatography by using different packing of columns with high efficiency small amount of complex mixture can be separated. The components of HPLC are listed below: different packing of columns with high efficiency small amount of complex mixture can be separated. The components of HPLC are listed below

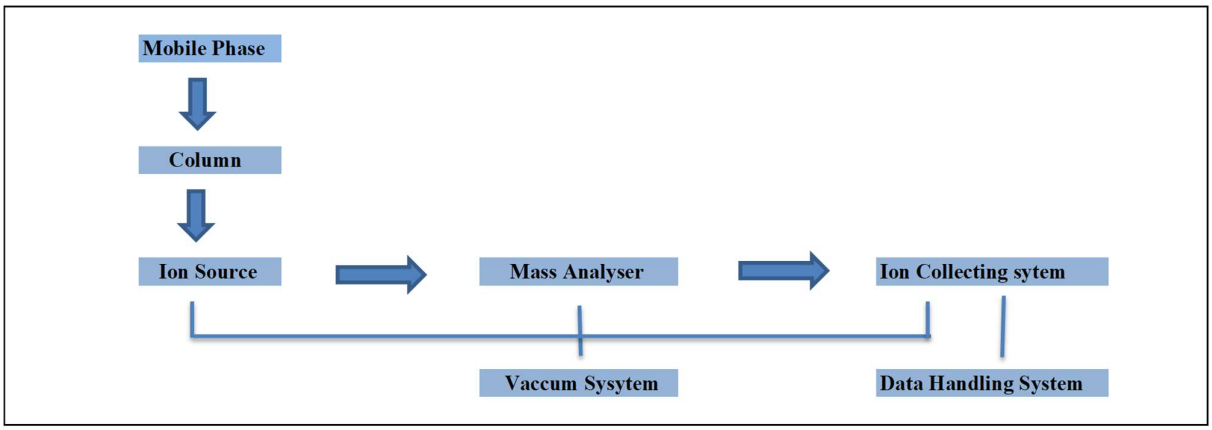

Figure 2: Schematic Block Diagram of LC-MS System

1) Pump:

Pump it consists of material which is inert towards solvents or any mixed composition of aqueous buffer and organic solvents. It delivers high volume of mobile phase up to 10mL/ min. There are three major types of pumps are used i.e. reciprocating pump, Syringe pumps and pneumatic pressure pumps.

2) Sample Injector:

Sample injector it's used to introduce sample volume into the chromatographic system. Generally sample volume from 1| 1L to 100| 1L can be injected. The injection volume can be increase by injector circle up to 2mL volume. There are two major types of injectors used i.e. Automatic injectors and mass-produced injectors. Automatic injectors are more comfortable and user friendly and are more accurate and precise as compare to automatic injectors.

3) Column:

Columns it's stationary phase which consists of silica material in combination with carbon chain. Generally the column length used is about 50 mm to 300 mm. The columns used in HPLC are consists of Octadecyl (C18), Octyl (C8), Cyano, Amino, Phenyl packing's. The columns are used on the base of nature of compounds to be separated

4) Detector:

Detectors and recorder the detectors is most important part of HPLC. There are different types of detectors used are UV-Visible detectors, PDA detectors, Refractive index (RI) detectors, Electrochemical detector, luminescence detectors and conductivity detectors. The signal received from detector can be recorded as peak and separate data can be stored in a software.

Mass Spectrometry:

Mass spectrometry it is uses for the analysis of charged gas molecular (ions) according to the exact molecular weight and molecular format of organic compound. Below listed are the different components of Mass spectrometers as follows.

Ion Sources:

a) Electrospray Ionization (ESI)

b) Atmospheric Pressure Chemical Ionization (APCI)

c) Atmospheric Pressure Photoionization (APPI)

a) Electrospray Ionization (ESI):

ESI generates ions by first drawing and spraying sample solutions at the tip of a capillary tube, where a high voltage of about ± 3 to 5 kV is applied. This generates a fine mist of charged droplets with the same polarity as the applied voltage. To accommodate a larger LC flow rate, the nebulizer and heating gas flows from outside the capillary to speed up the solvent evaporation process. As this process continues, the electric field on the droplet surface increases. When the mutual repulsive force of the charges exceeds the liquid surface tension (i.e. repulsion), fission occurs. It is thought that as this evaporation and fission cycle is repeated, the droplets eventually become small enough that the sample ions are liberated into the gas phase (based on the ion evaporation model). A schematic representation of the generation and desolvation processes in ESI for positively charged ions are illustrated in. Similarly, negatively charged ion are generated by applying a negative voltage on the ESI probe.

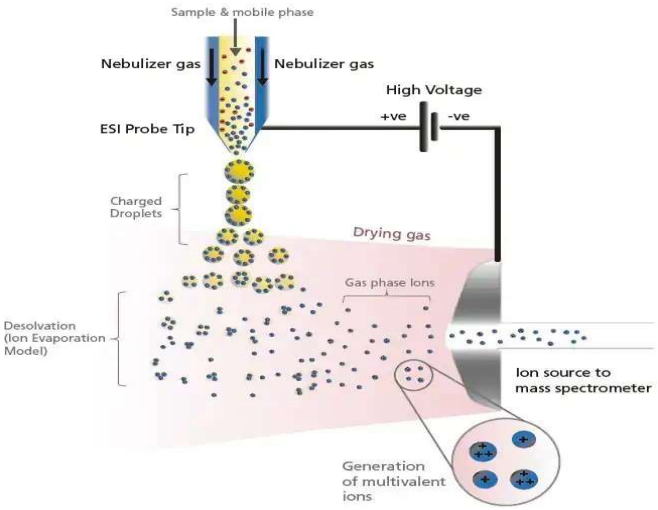

Figure 3: Schematic of the ionization and desolvation processes in ESI positive (+) mode

b) Atmospheric Pressure Chemical Ionization (APCI):

The other API technique is APCI, which is a type of chemical ionization. Though the interface design is analogous to ESI, the ionization principle differs, making it more suitable for low- and medium- polarity composites (non-polar particles). As illustrated in APCI vaporizes the solvent and sample particles by spraying the sample solution into a heater (about 400 °C) using a gas, similar as N2. Solvent particles are ionized by the nimbus discharge needle to induce stable response ions. Protons are transferred between these stable response ions and sample particles (ion- molecule reaction) and this leads to ionization. These ion- molecule responses are known to involve several patterns, similar as proton transfer reactions and electrophilic addition reactions. Unlike ESI, APCI involves an advanced energy process and doesn't have the tendency to form multiply- charged ion (MNH) n. accordingly, it's generally used for analyzing largely fat-soluble composites or compounds that don't ionize in solution.

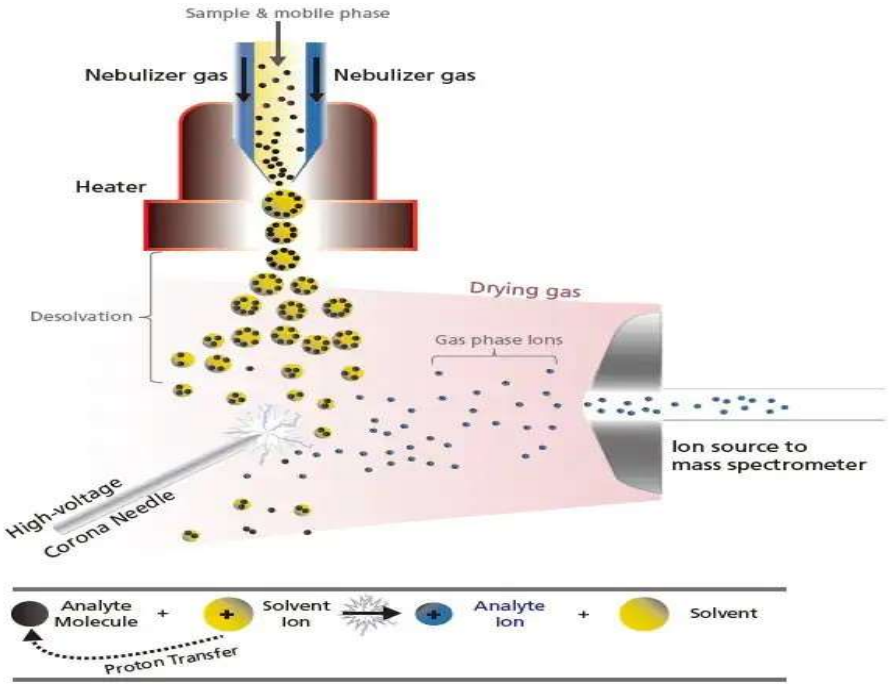

Figure 4: Schematic of the ion-molecular reaction (e.g. proton transfer) in APCI.

c) Atmospheric Pressure Photoionization (APPI):

APPI ionizes analytes by irradiation of short- wavelength vacuum ultraviolet (VUV) light. The interface design in APPI is very much the same as APCI, only with the exchange of the high- voltage nimbus discharge needle to the VUV lamp. Also, the nebulizer and heater are used to produce droplets and to vaporize the solvent. Upon VUV light irradiation, the analytes absorb a photon and get electronically excited. However, the analyte ion that was ionized due to the photons may receive a proton from the hydrogen in the solvent, to come a protonated cation, if the analyses' ionization energy is lower than the photon energy. This ionization technique achieves good sensitivity ionization with low to moderate polarity compounds.[6]

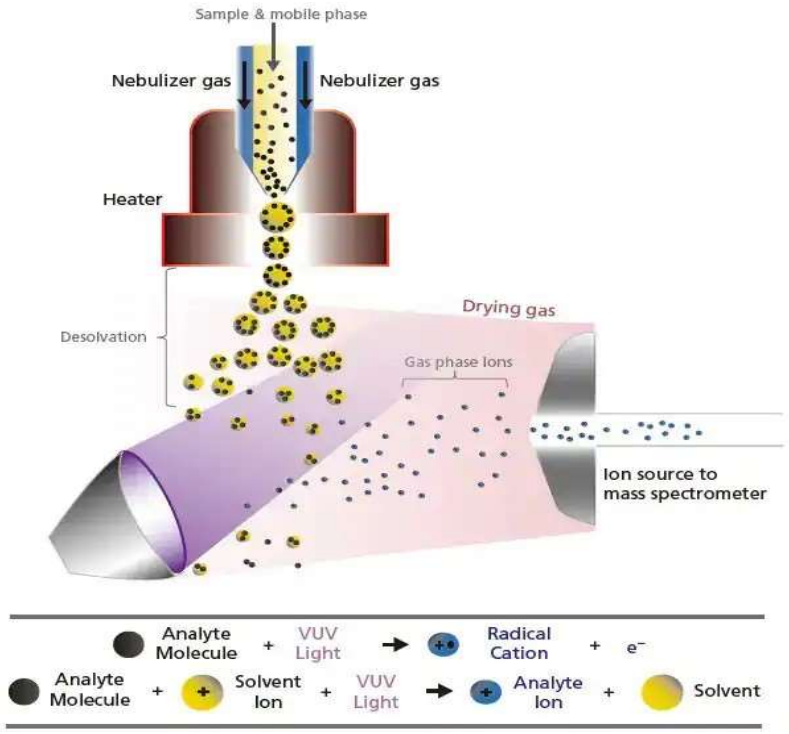

Figure 5: Schematic of the ionization process by UV light in atmospheric pressure photoionization (APPI).

Application

Molecular Weight Determination:

One of the key applications of API electrospray and APCI LC/MS systems is in determining molecular weights. API-electrospray LC/MS can be used to rapidly determine the molecular weight of a protein. Although pure proteins can be done by infusion, this recombinant protein required chromatography

Pharmaceutical Applications:

It is common used in Rapid Chromatography of Benzodiazepines the information available in a mass spectrum allows some compounds to be separated even though they are chromatographically unresolved.

Biochemical Application:

It is commonly used to determine Detection of Glycosylation in a Tryptic Map of a 60 KD Protein in biochemical Proteins are sometimes chemically or enzymatically digested to identify the components. The digestion mixture is then chromatographed to produce a peptide map. Shows two chromatograms for a 60,000 Dalton glycoprotein from a mass spectrometer and a UV detector

Food Applications:

Aflatoxins are toxic metabolites produced by certain fungi in foods. The total ion chromatogram for four aflatoxins; each could be uniquely identified by their mass spectra

Current Advances of LC-MS

Analysis of metabolites in body fluids by LC–MS

LC – MS/ MS became increasingly important for the analysis of complex human body fluids during the last two decades LC – MS/ MS is being adopted in clinical laboratories for the quantitative analysis of a number of analytes from physiological matrices. Still, a number of preanalytical guidelines and requirements have to be considered in the validation process.[8]

Lipids

The mass spectrometric profiling of lipid pathways and networks is challenging due to the different chemical structures of the different lipid classes. A huge number of LC – MS/ MS applications in the field of lipidomics was published during the last decade. Maximum of them are applied in the field of biomarker search. Only many lipid species (steroids, bile acids) are measured in clinical routine laboratories by LC – MS/ MS. A summary of newly developed LC – MS/ MS methods is provided in. During the last years, lipidomic studies in body fluids were dominated by the analysis of phospholipids with its two main classes, the glycerophospholipids and the sphingomye. Phosphatidylcholines, lysophosphatidylcholines and sphingomyelins can be monitored by a precursor ion scan of m/ z 184(positive ion mode), whereas phosphatidylinositol, phosphatidylglycerol, phosphatidylserine and phosphatidic acid can be profiled by a precursor ion scan of m/ z negative ion mode.[9]

Carbohydrates

Carbohydrate metabolism includes the pentose, fructose, and galactose pathways, the gluconeogenesis and finally the glycogen storage processes. Up to now, non on-targeted comprehensive metabolomics study investigating the entire carbohydrate metabolome has been published. Several general targeted approaches were published for the quantitative analysis of intermediates of the pentose phosphate pathway. These LC – MS methods are based on either Group Specific Internal Standard Technology labelling or the separation on ion- brace- loaded C (18) HPLC columns. Reference values for dihydroxyacetone phosphate, d- ribose 5- phosphate, the two sum parameter d- ribulose 5- phosphate/ d- xylulose 5- phosphate and d- fructose 6- phosphate/ d- glucose 6- phosphate, and d- sedoheptulose 7- phosphate were established for dried blood spots, fibroblasts and lymphoblast .[10]

Vitamins, organic acids, intermediate energy metabolites LC – MS/ MS

Has emerged as a particularly versatile and informative approach for the establishment of methods suitable for the simultaneous analysis of the tricarboxylic acid cycle. Two LC – MS/ MS methods for the profiling of intermediate energy metabolites

Are available for haemodialysis or atmospheric aerosols but not for serum or plasma. Vitamin B(2) and B(6) species, neopterin, cotinine, tryptophan metabolites, folates and folate catabolites involved in the transfer of one- carbon units, or cofactors for the applicable enzymes were measured by LC – MS/ MS.[11]

LC-MS Based Approaches for Food Analysis

The demand for safe and high Quality foods has significantly increased in current years. Food safety and satisfactory have become of more importance, and the governments of many nations have increased the quantity of relevant legislation and demands for food authentication. In consequence, the development of more robust, efficient, cost-effective, and powerful analytical methodologies is continuously needed in order to face these requirements. MS is one of the most suitable techniques because it is featured by excellent high specificity, sensitivity, and throughput. MS has been widely used in food safety and quality evaluation, and current advances in MS can provide quicker and more accurate methods capable of provide better qualitative and quantitative results. Additionally, coupling mass analyzers with separation techniques, such as liquid chromatography (LC-MS) and gas chromatography (GC-MS), have significantly improved food analysis for screening, identification, structural characterization, and quantitation purposes.

LC – MS/ MS based metabolomics in clinical applications

In clinical diagnostics quantitative targeted analysis by atmospheric pressure ionization/ quadrupole tandem mass spectrometry paved the way of metabolome examinations into clinical routine application. The first routine application of metabolite screening by tandem mass spectrometry in the clinical laboratory was introduced by Millington and Chace in the 1990s. The screening of amino acids and acylcarnitines for the detection of inborn errors in amino acid and fatty acid metabolism enhanced the panel of screened diseases included in new-born screening programs worldwide. About 30 metabolic parameters of amino acid and adipose acid metabolism were introduced into the screening panel for the detection of inherited metabolic diseases. This was the firstmulti-parametric metabolic approach, which proved to be a precious and effective preventative diagnostic strategy.[12]

Inherited metabolic diseases

A novel concept for analysis of metabolic pathways by LC – MS/ MS was introduced for the new-born screening of lysosomal storehouse conditions. Then, metabolic rates of specific enzyme substrates were analysed simultaneously, which enables the new-born screening of Morbus Gaucher, Morbus Pompe, Morbus Fabry, Morbus Niemann- Pick A/ B and Morbus Krabbe.[13]

Cancer studies

Cancer cells are known to produce a unique metabolic fingerprint. Thus, the identification of cancer specific biomarkers might lead to earlier detection of cancer development. Numerous NMR- based studies were performed to profile metabolites by comparing cancer patients with healthy controls. Tumours, in general, feel to be characterized by elevated phospholipids (e.g. choline- containing phospholipids). Particularly cross-sectional metabolome studies based on LC – MS/ MS were performed for the following cancer types prostate, breast, ovarian, colorectal, renal cell and kidney cancer. A potential part for sarcosine in prostate cancer progression was shown. A summary of metabolite

biomarkers which have been identified in cross-sectional LC – MS/ MS based cancer studies are summarized.[14]

Diabetes and coronary heart disease

Identification of high- risk individualities for type 2 diabetes (T2D) and cardiovascular disease (CVD) is currently assessed by global threat factors like age, sex, metabolic and lifestyle variables. In the last decade, numerous genomic, transcriptomic, proteomic and metabolomics studies have been performed in T2D and CVD patients searching for biomarkers with higher predictive values. However, most studies described cross-sectional differences in metabolite levels between cases and controls. In serum and plasma, differences in carbohydrates (glucose, mannose, and deoxyhexose), ketone bodies, phosphatidylcholines and free adipose acids have been shown to be associated with insulin resistance. Especially the branched chain amino acid metabolism was identified to be associated with severe insulin resistance in fat people. The analysis of branched chain and aromatic amino acids in 201 serum samples from individualities, developing T2D during the last 12 years of follow- up in the Framingham offspring study, identified leucine, isoleucine, valine, phenylalanine, and tyrosine as biomarkers for T2D risk prediction.

LC- MS/ MS analysis of biogenic amines

Recent publications show that the introduction of LC- MS/ MS methods in special clinical chemistry labs has important implications for the analysis of biogenic amines and their different metabolites such as catecholamine's and indoles. Catecholamine and metabolites the improved analytical performance with respect to conventional HPLC and immunoassays is exemplified in the measurement of plasma free metanephrines for the diagnosis of pheochromocytoma. The first assays of plasma free metanephrines made use of HPLC with electrochemical detection. This necessitated the use of extensive sample clean-up and long chromatographic run times. The introduction of mass spectrometry led to more specific methods with simpler although manual sample clean- up. However, this method, which utilized solid phase extraction (SPE), was unable to reach the sufficient analytical sensitivity to reach the required detection limit. In addition, long chromatographic run times were needed to achieve separation of the metanephrines and potential interferences. The introduction of online solid phase extraction coupled to LC- MS/ MS (XLC- MS/ MS) shortened chromatographic run times and allowed automation of sample preparation. In addition, particularity was increased by application of weak cation exchange SPE with sample preconcentration due to on- line elution into the LC- MS/ MS and hydrophilic interaction chromatography (HILIC). The application of HILIC resulted in fast chromatography and small peaks with good signal- to- noise rate, although short dwell times were required. Particularity was also increased by the use of additional qualifiers measured in the tandem mass spectrometer[16]

Recent Techniques of LC-MS

The very first step in protein characterization is molecular weight MW) determination. This can most easily be accomplished using either electrospray ionization (ESI) or matrix- assisted laser desorption

ionization (MALDI). Protein primary sequence determination is usually performed by a bottom- up method. Bottom- up this approach involves the enzymatic digestion of purified proteins or combinations of proteins into minor peptides. The peptide digests are also separated analysed by LC/ MS or LC/ MS/MS. There are two approaches to assist further protein identification. One is to subject the digested peptides (e.g. tryptic peptides) to a direct search against a genome or protein database for protein identification (peptide mass fingerprinting) in the case of purified proteins or very simple combinations of proteins. The other approach is to carry out tandem mass spectrometric(MS/ MS) analysis of the digested peptides using collision- induced dissociation(CID) to obtain fragment ions for a database search(sequence label) Amino acid specific fragment ions from cleavages of amide- bonds are ' b ' ions N- terminus) and ' y ' ions(C- terminus) under CID conditions. Several MS systems can be utilized for bottom- up LC/ MS and LC/ MS/ MS studies. These include 3D and linear ion trap , quadrupole- time- of- flight, Orbit rap, Fourier transform ion- cyclotron resonance(FTICR)- MS and other hybrid mass spectrometers linear ion trap- Orbit rap and linear ion trap- FTICR- MS. The operation principles of these MS systems have been covered extensively in the literature. Note that the high- resolution accurate mass capability of Orbit rap and FTICR- MS in both MS and MS/ MS modes further enhances the accuracy of protein identification.

LC–MS IN PHARMACOKINETICS AND BIOEQUIVALENCE STUDY OF HERBAL DRUGS

The identification and measurement of the phytoconstituents present in herbal drugs is necessary to explore the therapeutic basis and action mechanisms. After administration of an herbal medicine, it gets absorbed into the blood and generates several therapeutic activities. The bioavailability is a crucial factor to assess the efficacy of herbal medicine as it is mandatory that the active components reach the target site.[19] Therefore, the absorption and efficacy of HM is required to understand the mechanism of ADME of herbal medicine. Unfortunately, the concentrations of the compounds present in the blood are very low, and thus it is difficult to detect in vivo. In this context, the integration of MS-based technologies offers higher sensitivity and specificity for the qualitative and quantitative measurement of the metabolites. The fact that it provides extra sensitivity, specificity, and good separation in complex samples makes LC–MS/MS the ultimate tool in the determination of many types of chemical compounds, such as phytochemicals. The serum pharmacochemistry approach has been developed for the screening, identification, and quantification of bioactive components from herbal drugs to reveal their therapeutic effects after oral administration. This study mainly reflects the interaction between the body and drugs in conjunction with metabonomics technologies by using metabolic biomarkers to evaluate the therapeutic effect of HD, thus validating the therapeutic claim. In 2016, Zhang and his co-workers performed chromatographic fingerprinting and serum pharmacochemistry of Sanziguben Granule (TCM) to identify the bioactive components for a quality control. Visnagin is a furanocoumarins derivative obtained from Ammi visnaga. This plant has several pharmacological activities against kidney stones, cardiovascular diseases, and different fungal, bacterial, and viral diseases. A sensitive and highly selective LC–MS

method was developed to determine visnagin in rat plasma. An LC–MS/MS method with orthogonal Z-spray electrospray interface system was developed to determine the pharmacokinetics and oral bioavailability of curcumin and dimethoxy curcumin from Curcuma longa. In this study, several pharmacokinetic parameters, namely, AUC, $t_{\frac{1}{2}}$, C_{MAX}, and T_{MAX}, of curcumin and dimethoxy curcumin were determined. Another comparative pharmacokinetics study of quercetin, kaempferol, and isorhamnetin present in Ginkgo biloba extract was reported by Chen et al. The pharmacokinetic parameters of mangiferin (from Mangifera indica) in rat plasma with UPLC–MS/MS were calculated by Han et al. Another LC–MS-based method was developed for simultaneous estimation of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol in rat plasma. In this context, Mehta and his co-workers did an extensive review on pharmacokinetic profiles of 50 different therapeutically effective.

CONCLUSION

The LCMS is an advance analytical technique used with combination of HPLC and mass for separation power of HPLC with detection power of mass spectrometry. It is widely used in pharmaceutical industry, biological application, clinical research, food industry, chemical industry, environmental and forensic applications. Standardized micro-flow LC-MS workflows are combining High sensitivity with high robustness for rapid profiling of biological liquid, ready for the analysis of thousands of samples. For research scientist schooled in LC-MS and comfortable with system complexity, demand might include interchangeable ionization sources, precise control over means and modes of fragmentation great flexibility in software option and fast turnaround of software enhancements. For them technical innovation remain at the top of the list.

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