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Applications Of Liquid Chromatography-Mass Spectrometry (LC-MS) In Drug Metabolism Studies

Dr Puneet Sudan[1*], Mr. Pravin Nilkanth Bendle[2], Dr. J. Lokeshwari[3], Dr. Salam Himika Devi[4], Dr. Kanu Shil[5], Dr Navadha Bhatt[6]

[1*]*Professor, Chandigarh Pharmacy College, Chandigarh Group of Colleges, Jhanjeri-Mohali 140307, Email: puneetsudan22@gmail.com*
[2]*Assistant Professor, N.G. Acharya and D.K. Marathe College Chembur, Mumbai, Maharashtra-400071, Email: pravinbendle31@gmail.com, 0000-0002-3259-0477*
[3]*Associate Professor, Vels Medical College and Hospital, VISTAS Periyapalaya, Thiruvallur- 601102, Email: lokeshwari@gmail.com*
[4]*Guest Faculty, Department of Zoology, Manipur University, Email: himikasalam6@gmail.com 0000-0002-0702-7818*
[5]*Assistant Professor, Faculty of Paramedical Sciences, Assam Downtown University, Guwahati-781026, Assam, Email: kanuhptu@gmail.com*
[6]*Assistant Professor, Department of Chemistry, Graphic Era Hill University, Dehradun, Email: Joshinavadha@gmail.com*

***Corresponding Author:** Dr Puneet Sudan*
Professor, Chandigarh Pharmacy College, Chandigarh Group of Colleges, Jhanjeri-Mohali 140307, Email: puneetsudan22@gmail.com

Abstract

With a view of getting necessary information on the metabolism of drugs and their assorted pharmacokinetics aspects, liquid chromatography-mass spectrometry has turned into an integral and very important technology. This review is a good one in the sense that it covers LC-MS applications in drug discovery, preclinical stages, clinical trials, and postmarketing surveillance. In the subsequent article, an account of LC-MS principles and their application as well as techniques of liquid chromatography and mass spectrometry about the identification and quantification of different metabolites and view of metabolic pathways are described. Improvements in LC-MS techniques including, high-resolution mass spectrometer (HRMS) and LC coupled with a tandem mass spectrometer (LC-MS/MS) have improved the methods of identifying metabolites through elements of sensitivity, accuracy, and efficiency. The update also presents such fresh developments as the hybrid systems on LC-MS-NMR hybrids and the application of radiolabeled compounds for distinguishing between metabolites. Furthermore, the use of LC-MS as well as the coupling of LC-MS with bioinformatics and machine learning for quick and detailed metabolite identification is also highlighted in the article. Therefore, through the analysis of such novel achievements, the review also highlights how LC-MS is instrumental in the manipulation and enhancement of drugs' efficacy while monitoring the potential toxicity of drugs through the investigation of their metabolites and pharmacokinetic properties.

Keywords: Liquid Chromatography-Mass Spectrometry (LC-MS), Pharmacokinetics, High-Resolution Mass Spectrometry (HRMS), Biotransformations, Proteomics, Metabolomics

1. Introduction

LC-MS has emerged as one of the most significant analytical technologies employed in drugmetabolism and pharmacokinetic (DMPK) research fields in the last twenty years. During drug development, new chemical entities may go through multiple stages of drug discovery and development, and during this process, important information related to metabolism, metabolites, enzymatic biotransformations, and stability regarding the drug

***Corresponding Author:** Dr Puneet Sudan
*Email- puneetsudan22@gmail.com

molecule may be needed for the decision-making process in safety, efficacy and pipeline management (Smith & Obach, 2006). Compared to traditional tools such as liver microsomes and hepatocytes, LC-MS enables the qualitative and quantitative identification of the drug metabolites generated in models closer to the in vivo conditions such as the liver slices and plated hepatocytes (Di & Obach, 2015). This is closer to in vivo metabolic conditions and perhaps that is why its effects are different. The most frequently employed systems in DMPK studies are quadrupole time-of-flight (QTOF) and triple quadrupole (QqQ) LC-MS, which display high sensitivity and allow the acquisition of full scan MS and MS/MS data (Xie et al., 2012). In the recent past, LC-MS has achieved increased speed, increased resolution, enhanced mass accuracy, and better analysis, the high-resolution MS can determine metabolites that differ by 0.001 Da while high mass accuracy has a variation of not more than 5 ppm which aids in determining the molecular formula of the compound (Zhang et al., 2012). Bioanalytical LC-MS assays for the determination of drugs and metabolites in biological fluids have been preferred over HPLC with UV detection due to its higher selectivity and pg/mL sensitivity suitable for PK studies in ADME and TOF-MS-based clinical research in small animals and clinical samples (Xu et al., 2007). Validations of these methods are strictly complied with to assess accuracy and precision, selectivity, recovery, and stability as per the regulatory standards (Kaza et al., 2019). The improvements in the sample preparation techniques, as well as the evolution of chromatography, have enhanced LC-MS regarding pharmacokinetic analyses (Zhang et al., 2012).

In metabolism studies, the LC-MS in combination with offline NMR spectroscopy has been the preferred method for the identification and characterization of metabolite. Recent developments in new hybrid LC-NMR-MS systems have made a much more efficient online analysis possible (Planz et al., 2016), while the use of MS-NMR and LC-MS-NMR allows the identification of less abundant metabolites within a shorter period. It also becomes easier to differentiate between drugs and their metabolites using radiolabeled or stable isotope-labeled compounds Mutlib (2008); LC-MS takes an important role in detecting labeled drug metabolites. There is a way to receive the definitive human ADME data based on the in vivo studies using accelerator MS with the LC-MS to microdose 14C-drugs, and this will give such critical data to select candidates without the pharmacological effects (Burt et al., 2016).

2. Principles and Techniques of LC-MS

2.1 Liquid Chromatography (LC) Used in Drug Metabolism

HPLC or UPLC stands for High-Performance Liquid Chromatography and Ultra-Performance Liquid Chromatography respectively, these are instrumental analytical techniques that are used in the separation of compounds in a sample for drug metabolism. HPLC on the other hand can work at pressures of up to 400 bar with 3-5µm particle size columns while UPLC on the other hand employs sub 2µm particles and operates at pressures of up to 1000 bar to allow a faster, efficient, and high-resolution separation that is suitable for high throughput screening (Swartz, 2005). The primary advantages of UPLC include enhanced separation, sensitivity, and productivity in comparison to standard HPLC. Two techniques apply the differential adsorption of analytes on the silica support in a chromatographic column, by using both, mobile and stationary phases (Snyder et al., 2011). For complex samples, replacing the concentration of the mobile phase at some point is used to enhance separation by gradient elution. The stationary phase is responsible for the selection of the column chemistry, as well as the elution condition that offers appropriate separation of the analyte from the mobile phase.

Table 1: Comparison of HPLC and UPLC Techniques

Parameter	HPLC	UPLC
Particle Size	3-5 µm	< 2 µm
Operating Pressure	Up to 400 bar	Up to 1000 bar
Separation Efficiency	Moderate	High
Speed	Moderate	Fast
Sensitivity	Moderate	High
Throughput	Standard	High

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2.2 Mass Spectrometry (MS)

MS is rather effective when it comes to the identification and determination of the concentration of metabolites of the drug of interest by determining the m/z ratio. There are three main types of mass analyzers used: quadrupoles, which utilize oscillating electric fields for ion separation; time-of-flight (TOF) instruments, which offer m/z from the amount of time it takes certain ions to travel a specific distance; and ion traps, which use three-dimensional electric and magnetic fields to confine and further analyze ions (March 1997; Glish & Burinsky, 2008; Li et al., 2007). Some of the common ionization techniques is Electrospray ionization (ESI): This technique is suitable for polar and large molecules, particularly drug metabolites when combined with liquid chromatography (Horning et al., 1977) Atmospheric pressure chemical ionization (APCI): This method is ideal for compounds with low polarity and thermally labile compound (Fenn et al., 1989). MS has been improved a lot by the developments of mass analyzers and ionization sources to characterize drug metabolites in their properties and structures more comprehensively.

2.3 Coupling LC with MS (Interface Techniques)

The connection between liquid chromatography and mass spectrometry depends on the transportation of individual analytes into the mass spectrometer at an optimal rate. The most frequently used ionization methods are known as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). ESI is more popular because it can ionize almost all types of compounds and is capable of producing a large number of ions from the liquid phase and analyte before it is analyzed by mass (Fenn et al., 1989). APCI ionizes less polar compounds that have been vaporized at atmospheric pressure before analyte mass spectrometry, and, therefore, is beneficial in some instances (Horning et al., 1977). It allows compounds in the liquid phase to be ionized and vaporized to be introduced into the mass spectrometer.

2.4 Optimization of LC-MS Parameters for Drug Metabolism Studies

To maximize the resolution of analytes in drug metabolism samples, careful chromatographic conditions such as choice of stationary phase, pH of mobile phase, and gradient conditions have to be optimized (Snyder et al., 2011). Other technical factors that have a further impact on the ionization and fragmentation of target drug molecules and their metabolites are also significant in mass spectrometry such as ion source temperature, capillary voltage, and collision energy. Therefore, a rigorous validation of the developed LC-MS method that will confirm the accuracy, precision, sensitivity, and specificity is indeed important if quantitative and qualitative analysis of drugs and their metabolites in the biological matrices is to be achieved effectively (Shah et al., 1992). In conclusion, careful tuning and cross-checking of the LC-MS data analysis parameters is a crucial factor in achieving measurable, reproducible outcomes in drug compound metabolite investigations.

3. Applications of Liquid Chromatography-Mass Spectrometry (LC-MS) in Drug Metabolism Studies

3.1 Identification of Metabolites

Liquid chromatography-mass spectrometry (LC-MS) is an analytical technique that has wide applicability in drug metabolism studies; it integrates the separation ability of LC with the sensitivity feature of MS (Gowda and Djukovic, 2014; Prakash et al., 2007). LC-MS is particularly effective in providing high specificity to distinguish phase I and II drug metabolites. Additionally, tandem mass spectrometry (LC-MS/MS) conducts additional rounds of mass analysis to fragment metabolites into structurally significant ions. Therefore, the structure of the metabolites can be inferred from the fragmentation pattern (Zhang et al., 2019; Stresser et al., 2014). It also helps in the analysis of the changes that drugs undergo in the body and in the identification of metabolites that could be pharmacologically or toxicologically active. In sum, the LC-MS methods offer qualitative data on the drug biotransformation routes and metabolite identification as the key issues in the drug development process.

3.2 Quantification of Metabolites

HPLC is an obligatory method for describing drug metabolism and measuring metabolic reactions in the organism. LC-MS enables the identification of metabolizing enzymes, more specifically, cytochrome P450 and uridine diphosphate glucuronosyltransferases (Rendic & Guengerich, 2012). Moreover, LC-MS helps in the determination of significant kinetic parameters such as the affinity constant (Km) and the maximal rate (Vmax) thus shedding light on the efficiency of metabolic enzymes (Li et al., 2013). LC-MS makes pathway reconstruction possible in only 100 words together with the ability to study and understand drug metabolizing enzymes and enzyme kinetics.

3.3 Metabolic Pathway Elucidation

Some of these techniques include selected reaction monitoring (SRM) and multiple reaction monitoring

(MRM) whereby identification and quantification of drug metabolites in biological matrices can be achieved through liquid chromatography-mass spectrometry (LC-MS) even at trace levels to give relevant information on drug metabolism and disposition (Niessen, 2003; Korfmacher, 2005). It should be noted that to obtain quantitative and reproducible data on metabolite concentrations, sensitive calibration procedures including the use of internal standards, and calibration curves must be employed. Quantification limits call for mass spectrometer sensitivity, chromatographic separation, and biological matrix complexity (Xu et al., 2007). As techniques that allow accurate quantitation of analytes at trace concentration levels, SRM and MRM extend the reach of quantitative LC-MS for PK and toxicological applications of drug candidates. Therefore, it is very important to pay much attention to the calibration and setting of the quantification limits grounded on the quantitative and qualitative characteristics of the analytical procedure as well as biological properties.

3.4 Pharmacokinetic Studies

High-Performance Liquid Chromatography equipped with Mass Spectrometry (LC-MS) plays a significant role in pharmacokinetic analysis because of the high accuracy of the drug concentration measurements in the biological fluids (Kumari Rayala et al., 2022). This system of analysis allows the determination of the drugs and particularly the metabolites in the biological fluids such as plasma, urine, and tissue samples and therefore is useful for the study of the absorption, distribution, metabolism, and excretion (ADME) profile of drugs (Rezende et al., 2013). One such use is tracking the drug concentration change over a period to establish crucial dynamic pharmacokinetic variables that aid in understanding drug effectiveness, safety, and schedules (Beccaria and Cabooter, 2020). For instance, LC-MS delivers quantitative data on peak plasma concentration and half-life, as well as AUC which is crucial for pharmacokinetic modeling and performance assessment. In summary, the high sensitivity and quantitative nature of LC-MS position it as a key tool in determining the definitive ADME and PK profiles of drugs.

3.5 Toxicology and Safety Assessment

LC-MS is one of the most important and widely used tools in drug safety evaluation. It makes it possible to identify reactive metabolites that may cause adverse reactions or toxicity that may be due to drug-induced liver injury or other unpleasant effects such as carcinogenicity or teratogenicity (Grillo, 2015). LC-MS helps in differentiation as well as quantitation of these metabolites that may aid in the assessment of the safety of drugs. Also, LC-MS is suitable for identifying biomarkers that reflect the action of toxic effects or negative impact of the drug on the body (Amacher, 2010). Biomarkers to indicate a possible safety concern from the commencement or to design safer drugs can be recognized from the proteomic or metabolomic alterations that occur due to a drug. In conclusion, LC-MS is particularly important for identifying reactive metabolites that cause toxicity and for identifying biomarkers that are used for monitoring drug safety to determine the overall safety of new drugs.

Table 2: Summary of LC-MS Applications in Drug Metabolism

Application	Description	Reference
Identification of Metabolites	Qualitative analysis and structural elucidation of drug metabolites	Gowda & Djukovic, 2014
Quantification of Metabolites	Measurement of metabolite concentrations using calibration strategies	Rendic & Guengerich, 2012
Metabolic Pathway Elucidation	Mapping metabolic pathways and identifying enzymes involved	Niessen, 2003; Korfmacher, 2005
Pharmacokinetic Studies	Determining ADME profiles and pharmacokinetic parameters of drugs	Kumari Rayala et al., 2022
Toxicology and Safety Assessment	Identifying reactive metabolites and biomarkers for drug safety evaluation	Grillo, 2015

4. Advances in LC-MS Technologies for Drug Metabolism

LC-HR/MS is instrumental in metabolism research because its mass accuracy and sensitivity toward metabolites are very good (Zheng et al., 2022). The high resolution helps in unambiguous metabolite identification and determination of structures of metabolites to decipher biochemical pathways more

efficiently, particularly for new or low-abundance analytes. MS/MS gives copious structural data from product ion spectra for determining functional groups and chemical characteristics of metabolites (Wang et al., 2022); it helps in the elucidation of unknown structures and paths (Qi et al., 2021). Innovations such as the use of UHPLC, hybrid techniques, and powerful mass analyzers have improved the separation efficiency, speed, sensitivity, and resolution. The combination of ion mobility with mass spectrometry, also known as ion mobility-MS coupling, or IM-MS, enhances the ability to separate complex mixtures. In the past few years, bioinformatics and data analysis techniques have evolved to facilitate the analysis of more extensive datasets, which are required to fully delineate metabolites. The new approach of machine learning predictions of metabolite properties and interaction (Chi et al. 2024) helps in the identification of the transformation as well as the accumulation of knowledge of the process. Thus, the improved LC-MS equipment and highly refined data analysis are adding value to drug metabolism research – from discovering previously concealed metabolites to tracing the metabolic pathways and determining the pharmacokinetic parameters. The integrated workflows can make metabolite identification and description more efficient and complete.

5. Case Studies and Real-world Applications

LC-MS is a popular method for the determination of metabolic profiles of drugs in the body, which helps in the identification of metabolites. This is further illustrated by the following two case studies. The first study by Peng et al., 2005 employed LC-MS for isolation and identification of imatinib, an anticancer tyrosine kinase inhibitor, in patient plasma and urine samples. The authors used the metabolomics approach to achieve the study objectives and revealed several metabolites as well as established that imatinib is mainly metabolized by CYP3A4. These findings were used to develop dosing guidelines and a discussion of possible drug interactions with imatinib, including the emphasis on CYP3A4. Another study by Hinson et al., 2010 employed LC-MS to analyze the metabolic and toxic profile of acetaminophen. To this extent, by isolating and characterizing acetaminophen metabolites including the toxic and reactive metabolite NAPQI, the study contributed towards explaining the pathways of liver injury due to acetaminophen overdose. The formation of NAPQI with glutathione was shown as evidence of detoxification whose exhaustion is pointed as the source of toxicity in cases of overdose. Collectively these examples demonstrate the versatility of LC-MS in drug metabolism studies ranging from identifying phases to understanding the mechanism of toxicity for designing specific counteragents and therapies.

6. Challenges and future directions

Liquid chromatography-mass spectrometry (LC-MS) is a versatile analytical tool that has wide application fields such as metabolomics, proteomics, and drug discovery. Nevertheless, some drawbacks are still present in the technique such as low analysis resolution which compromises the possibility of analyzation of complex biological samples, the problem of establishing high sensitivity in conjunction with selectivity, problems with determination of quantities in complex mixtures and data analysis and interpretation (Cajka & Fiehn, 2014). The biological sample matrix also causes interferences in ionization and compromises quantitativeness (Annesley, 2003). To achieve higher resolutions and sensitivities, developmental work is underway concerning ionization mechanisms and mass analyzers (Kaufmann and Mairinger, 2014). Higher automation and multiplex PCR could improve the sample throughput to accommodate more samples (Zhang et al., 2012). Possible future developments are putting LC-MS together with proteomics and metabolomics to understand drug metabolism thoroughly because proteins and metabolites respond to drugs (Peterson et al., 2012). Integrating LC-MS data into system biology platforms could also enhance understanding of cell metabolism to develop effective one-person treatment (Catherman et al., 2014). In this regard, such advances could enhance the process of drug discovery through more efficient screening, biomarker discovery, and classification of drug candidates that are early into development (Koulman et al., 2009). Greater clarity and sensitivity of metabolic profiling could also allow for more rigorous scrutiny by the regulatory bodies of metabolic pathways and interactions with drugs. Thus, further development of LC-MS technologies is expected to provide higher efficiency and selectivity in the development of new drugs, as well as more detailed regulatory studies.

7. Conclusion

Therefore, the Liquid Chromatography-Mass Spectrometry (LC-MS) based technique has greatly advanced the field of drug metabolism and pharmacokinetic studies due to its sensitiveness and specificity. This analytical technique offers vital information in the development of strategies for the identification and characterization of metabolites, determination of metabolic routes, and estimation of kinetic parameters of the drug. New developments have been made on LC-MS for example high-

resolution mass spectrometry coupled with enhanced data analysis techniques which has improved the ability of LC-MS to resolve intricate metabolic profiles and enhance pharmacokinetic parameters. In addition, advances in technology-implemented LC-MS/MS, combined hygiene HPLC-NMR-MS systems and application of stable isotope-labeled compounds have also enhanced metabolite identification and quantification. They also help to contribute to the precise discovery of the metabolism of drugs and contribute to the enhancement of safer and more effective therapeutic entities. Tomorrow LC-MS will become even more closely linked to other omics strategies as well as bioinformatics, which will help to move forward in regards to drug behavior and metabolism, as well as aid in improvement in the drug discovery, development, and subsequent safety assessment.

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