Novel Approaches in Drug Designing & Development
ISSN: 2575-8942

Juniper Publishers
key to the Researchers

Prospective
Volume 6 Issue 2 - August 2021
DOI: 10.19080/NAPDD.2021.06.555681

Nov Appro Drug Des Dev

Insights of LC-MS in Drug Discovery, Drug Development and Modern Analysis

Shibam Das, Divya Shikha and Rohit Bhatia*

Department of Pharmaceutical Analysis, ISF College of Pharmacy Moga, India

Submission: August 12, 2021; **Published:** August 24, 2021

***Corresponding author:** Rohit Bhatia, Department of Pharmaceutical Analysis, ISF College of Pharmacy Moga, Punjab-142001, India

Keywords: Drug discovery; Metabolites; Toxicity; Chromatography; Bioanalysis

Abbreviations: LC–MS: Liquid Chromatography and Mass Spectrometry; NCEs: New Chemical Entities; PK: Pharmacokinetic; API: Atmospheric Pressure Ionization; SRM: Source and Operated in Selected Reaction Monitoring

Perspective

Drug discovery and development is the most critical and tedious process in the modern age. It involves invention, discovery, synthesis or repurposing of potent therapeutic candidates. Various division of science along with researchers, scientists, industrial experts and academicians throughout the globe are continuously working towards designing or developing newer molecules with improved potency and reduces toxicity. Bioanalysis is promising branch of science which has been proved to provide significant tools utilized in drug discovery process. The term bioanalysis usually defined as quantification of biotics (macromolecules, proteins, DNA, big molecule drugs, metabolites) and xenobiotics (chemically manufactured or naturally extracted drug candidates and genetically generated biological molecules and their metabolites or post-translationally modified products) in biological systems, for instance blood, plasma, urine, serum or extracts of tissue [1,2].

Liquid chromatography is an analytical separation technique that is used to separate the analyte of interest from mixture. Liquid chromatography uses liquid as mobile phase and solid stationary phase to separate molecules of interest efficiently. Mass spectroscopy is used to measure the molecular mass of the interest sample. It determines the mass of the sample molecule by quantifying mass to charge ratio of its ion [3]. The combination of liquid chromatography and mass spectrometry (LC–MS) is a powerful tool due to its selectivity, low sample volume requirements, sensitivity and speed which has multiple of applications. Innovative and successful research efforts in the past years on the design of a potential interface connection between LC and MS have made LC compatible with MS [3]. To create a novel lead candidate with desirable therapeutic properties such as efficacy, bioavailability, and low toxicity is the goal of drug discovery for preclinical assessment [4]. The process of drug discovery includes three primary analysis activities: target identification; lead identification; and lead optimization [3].

Preclinical development or studies mainly aims to measure safe dose for human and to determine the potential toxicity of the product. LC/MS-based analysis operations are mainly concerned with the detection of contaminants, degradants, and metabolites [3]. The clinical development stages consist three distinct phases that are phase I (experimental treatment on a small group of often healthy people 20 to 80 to evaluate its safety and side effects and to find the correct drug dosage), phase II (uses more people around 100 to 300, the emphasis in Phase II is on effectiveness), phase III (collection of more information regarding safety, potency and studying different doses and different population), phase IV (once the drug passes phase III, FDA approves their use) [3]. Once the clinical development studies are completed manufacturing process starts where the drugs are packages and distributed across the state. All the guidelines are followed during manufacturing process along with those periodic inspections during progress.

LC-MS Support to Drug Discovery Processes

In vitro ADME profiling of new chemical entities (NCEs) is commonly used in drug development to rank order NCEs and better understand their liabilities. Drug metabolism and pharmacokinetic (PK) characteristics of NCEs must be evaluated and optimised for success in drug research and development. High throughput LC-MS/MS employing triple quadrupole mass

spectrometers with an atmospheric pressure ionization (API) source and operated in selected reaction monitoring (SRM) mode has evolved as an enabling tool for quantitative bioanalysis in drug development [5].

In vitro drug metabolism is one of the earliest screening methods in drug development, where experiments for oxidation, reduction, and glucuronide conjugation (phase-I & phase-II metabolism) are done in different species (rat, dog, monkey, and human) and the metabolite produced is examined [5]. The structure of many metabolites is difficult to deduce, especially when complicated rearrangement results in a unique metabolite and/or the generation of metabolites with isobaric molecular ions [6,7]. Recent advancements in LC-MS/MS technology enable for the identification of metabolites with great sensitivity up to femtomole levels. Mass spectrometry is an appropriate detector for identifying the structure of metabolites, whereas liquid chromatography (LC) with a diode array detector is commonly used to separate mixtures of metabolites and pharmaceuticals. The development of triple quadrupole mass spectrometers (tandem mass spectrometers) in combination with liquid chromatography (LC) has had a considerable influence on metabolite identification studies. A triple quadrupole ion trap mass spectrometer combined with LC is one of the best tools for characterising metabolite structures [5].

The FDA has issued guidance document on drug interaction studies [8]. For the first time, transporter-mediated medication interactions are listed in regulatory guidelines. Screening compounds has become extremely frequent for their inhibition and induction capabilities. The introduction of fast, selective, and sensitive LC-MS tests has made screening drugs for their CYP inhibition potential much easier. Walsky and Obach used stable isotope-labeled internal standards to design and validate high-pressure liquid chromatography-tandem mass spectrometry analytical techniques. Because of its excellent sensitivity and selectivity, this analytical method allowed for the use of very low microsomal protein incubation doses (0.01–0.2mg/ml) [5].

The oral route of medication administration is the preferred route for several reasons, including simplicity of administration and higher patient compliance. As a result, developing medicines that can be absorbed successfully via the gut epithelium is critical [5]. The Caco-2 cell monolayer experiment has become a common in vitro technique for investigating intestinal absorption [9]. The use of radio-labeled chemicals in Caco-2 tests is no longer necessary thanks to the development of LC-MS/MS. Furthermore, mass spectrometry allows many chemicals to be measured at the same time, decreasing the number of incubations and improving the experiment's throughput. The metabolism of substances by Caco-2 cells may also be studied using LC-MS and LC-MS/MS [5]. For protein binding assays, pharmaceutical scientists are now utilising HPLC and LC-MS/MS using a Human serum albumin column [5].

LC-MS Support to Preclinical Development Studies

In vitro studies in preclinical setting PK studies are used to assess the efficacy of drug candidates in animals. Bioanalytical assays can be used to assess drug absorption and elimination. Toxicokinetics and tissue distribution studies focus on how a drug is distributed in various tissues, such as the blood, kidney, lung, spleen, skeletal muscles, heart, adipose tissue, liver, brain, gut, stomach, testes, and ovary [5]. LC-MS is utilized in the preclinical development stage for impurity, degradant, and metabolite detection [3].

In a single dosage bioavailability investigation, LC-MS/MS was also employed. Marathe et al. [10] employed LC-MS/MS for sample analysis in a study of preclinical pharmacokinetics and in vitro metabolism of BMS 690514, a strong inhibitor of EGFR and VEGFR2. Cassette dosing is a method of administering many chemicals to a single animal at the same time, and LC-MS/MS is used to analyse the samples in cassette dosing. According to a number of studies published, excretion mass balance studies combined with LC MS/MS have provided crucial information on the metabolism and distribution of medications including gemopatrilat, dasatinib, and PF-04971729 (Pfizer molecule) in animals and people [5].

LC-MS Support to Clinical Studies

The application of mass spectrometry has progressed significantly, both in routine analysis and for research facilities [11]. Liquid chromatography combined with tandem mass spectrometry (LC/MS/MS) technologies is developing as a supplementary technique to established clinical research. The high-throughput and specificity of LC/MS/MS is contributing remarkable benefits to clinical diagnostic laboratories that conducts regular analysis. The advancements of LC, API, and MS/MS have resulted in the development of a variety of clinical diagnostic tests. MS is frequently used to analyse steroid hormones. Differential steroid analysis is very important for the diagnosis and treatment of complicated endocrine diseases such as primary hyperaldosteronism, adrenal insufficiency, congenital adrenal hyperplasia, Cushing's syndrome, and gonadal dysfunction. The analysis of multi-steroid has only been made practicable by using multidimentional LC-MS/MS. ESI-LC/MS/MS with a linear ion trap was used to identify thirteen distinct steroids in protein precipitated serum [12]. Blood spots may now be examined by MS thanks to MALDI ionisation. The example where this technique is used clinically is during the newborn screening for phenyl ketonuria, which is a congenital disorder in which phenylalanine hydroxylase is dysfunctional [13].

Role of LC-MS in Biomarker Detection

Biomarkers are molecules that found in blood and these indicate the physiological or pathological conditions of the body that can be detected. Biomarkers have number of functions during drug developmental process. Since there is need to qualify protein

How to cite this article: Shibam D, Divya S, Rohit B. Insights of LC-MS in Drug Discovery, Drug Development and Modern Analysis. Nov Appro Drug Des Dev. 2021; 6(2): 555681. DOI: 10.19080/NAPDD.2021.06.555681

biomarker, the use of LC-MS/MS has gained a lot of attention for protein quantification [14]. To decrease total analysis time, assess the quantitative performance and repeatability of nano-LC-MALDI analysis in biomarker discovery, and analyse the robustness of biomarker selection, Benkali et al. [15] employed dual decoupling between nano-LC, MS, and MS/MS for urinary biomarker detection, since these techniques reduces the overall analysis time. Protein profiling can also be done with ion exchange chromatography or chromato focusing to separate proteins based on their charge and a Reversed-Phase Liquid Chromatography-Electrospray Ionization Mass Spectrometer to separate proteins based on their hydrophobicities using Two-dimensional Liquid Chromatography Mass Spectrometry (2-DLC) [16]. Gerber et al. [17] utilised LC/MS/MS with SRM detection to measure the native and phosphorylated forms of a tryptic fragment generated from a protein extracted from cell lysate by sodium dodecyl sulphate polyacrylamide gel electrophoresis in order to quantify target proteins.

Role of LC-MS in Impurity Identification

Identification and measurement of contaminants in Active Pharmaceutical Ingredients (API) is a difficult task at various stages of drug development. Impurity qualification is necessary for determining the biological safety of an individual impurity, highlighting the importance and scope of impurity identification in pharmaceutical research. During process research and safety evaluation, synthetic contaminants are of special importance. For throughput investigation of impurities, LC/MS-based methods provide a sensitive instrument for structural analysis processes. Although LC/UV is still the most popular approach for detecting and quantifying degradants, when new degradants are identified in stability or stress investigations, LC/MS is frequently employed to determine the molecular mass and MS/MS is used to give structural characterization [5,18].

In a nutshell, it can be concluded that LC-MS has a divine potential to discover and develop newer therapeutic candidates against various ailments. Also, it is a powerful technique for quantification, structural elucidation and identification of drugs/metabolites or biomarkers. Although it is associated with certain shortcomings like complex operations, high cost and special training is required for its operation.

References

1. Unger S, Li W, Flarakos J, Tse FL (2013) Roles of LC-MS bioanalysis in drug discovery, development, and therapeutic drug monitoring. Handbook of LC-MS Bioanalysis: Best Practices, Experimental Protocols and Regulations: 1-13.
2. Bhosale AA. Importance of bioanalysis in drug discovery and development: A review. Matrix 16: 17.
3. Sreekanth DN (2013) Role of LC-MS in drug discovery process.
4. Lim C-K, Lord G (2002) Current developments in LC-MS for pharmaceutical analysis. Biological and Pharmaceutical Bulletin 25(5): 547-557.
5. Solutions J (2012) Application of LC-MS in supporting PK/PD studies during drug discovery and development. Journal of Pharmacy Research 5(5): 2514-2526.
6. Lim HK, Chen J, Sensenhauser C, Cook K, Subrahmanyam V (2007) Metabolite identification by data-dependent accurate mass spectrometric analysis at resolving power of 60 000 in external calibration mode using an LTQ/Orbitrap. Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry 21(12): 1821-1832.
7. Jemal M, Ouyang Z, Zhao W, Zhu M, Wu WW (2003) A strategy for metabolite identification using triple-quadrupole mass spectrometry with enhanced resolution and accurate mass capability. Rapid communications in mass spectrometry17(24): 2732-2740.
8. Huang SM, Temple R, Throckmorton D, Lesko L (2007) Drug interaction studies: study design, data analysis, and implications for dosing and labeling. Clinical Pharmacology & Therapeutics 81(2): 298-304.
9. van Breemen RB, Li Y (2005) Caco-2 cell permeability assays to measure drug absorption. Expert opinion on drug metabolism & toxicology 1(2): 175-185.
10. Marathe P, Tang Y, Sleczka B, Rodrigues D, Gavai A, et al. (2010) Preclinical pharmacokinetics and in vitro metabolism of BMS-690514, a potent inhibitor of EGFR and VEGFR2. Journal of Pharmaceutical Sciences 99(8): 3579-3593.
11. Homer NZ (2011) Clinical mass spectrometry: a short review. Spectroscopy Europe 23(3):14.
12. Shushan B (2010) A review of clinical diagnostic applications of liquid chromatography–tandem mass spectrometry. Mass spectrometry reviews 29(6): 930-944.
13. Chace DH, Kalas TA, Naylor EW (2003) Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns. Clinical chemistry 49(11): 1797-1817.
14. Ackermann BL, Berna MJ, Eckstein JA, Ott LW, Chaudhary AK (2008) Current applications of liquid chromatography/mass spectrometry in pharmaceutical discovery after a decade of innovation. Annu Rev Anal Chem 1: 357-396.
15. Benkali K, Marquet P, Rerolle JP, Le Meur Y, Gastinel LN (2008) A new strategy for faster urinary biomarkers identification by Nano-LC-MALDI-TOF/TOF mass spectrometry. BMC genomics 9(1): 541.
16. Sahab ZJ, Semaan SM, Sang QXA (2007) Methodology and applications of disease biomarker identification in human serum. Biomarker insights 2: 21-43.
17. Gerber SA, Rush J, Stemman O, Kirschner MW, Gygi SP (2003) Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. Proceedings of the National Academy of Sciences 100(12): 6940-6945.
18. Stolarczyk EU, Kutner A (2010) Use of hyphenated LC-MS/MS technique for characterization of impurity profile of quetiapine during drug development. Acta poloniae pharmaceutica 67(6): 599-608.

How to cite this article: Shibam D, Divya S, Rohit B. Insights of LC-MS in Drug Discovery, Drug Development and Modern Analysis. Nov Appro Drug Des Dev. 2021; 6(2): 555681. DOI: 10.19080/NAPDD.2021.06.555681

DOI: 10.19080/NAPDD.2021.06.555681

How to cite this article: Shibam D, Divya S, Rohit B. Insights of LC-MS in Drug Discovery, Drug Development and Modern Analysis. Nov Appro Drug Des Dev. 2021; 6(2): 555681. DOI: 10.19080/NAPDD.2021.06.555681