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Bioanalysis by LC-MS/MS: A review

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Abstract
Liquid chromatography-mass spectrometry (LC-MS/MS) is a analytical technique which is used with High Performance liquid chromatography (HPLC) and the mass spectrometry. LC-MS/MS is commonly used technique in analytical laboratories for the qualitative and quantitative Determination of drug substances and biological samples. LC-MS has played a vital role in the evaluation and interpretation of bioavailability-bioequivalence and pharmacokinetic data. This review article reviews the recent Advancement in sample preparation, LC-MS/MS analysis of biological samples with LC-MS method development, validation, optimization, and different types of extraction procedures with different types of cartridges used in sample processing. In this review, we have described the Solid-phase extraction technique which is commonly used for sample preparation and reduces the matrix effect in LC-MS/MS analysis.

Keywords: bioanalysis, LS-MS/MS, bioavailability, matrix effect

1. Introduction
Bioanalysis determines the quantitative determination of drugs and their metabolites in the biological matrix. This plays a significant role in the evaluation and interpretation of bioavailability, bioequivalence, and pharmacokinetic data [1]. Chromatographic methods such as High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS) are commonly used in laboratories for the qualitative and quantitative analysis of drug components and biological samples. For the determination of this kind of substances method development and method validation is carried out. In the part of method development, procedure is set according to regulatory authorities and method validation ensure that the developed method is accurate, specific, reproducible and rugged, Over the specific range in which an analyte is analyzed. This all procedure from method development to bioanalysis of biological matrix by LC-MS, guidelines were applied through regulatory authorities [2]. Method validation for bioanalysis includes all of the procedures that demonstrate a particular method developed and used for the quantitative measurement of analytes [3]. The bioanalytical method validation parameters meet the requirements for the intended bioanalytical application. The parameters including precision and accuracy, sensitivity reproducibility, recovery, and stability. The selection of an appropriate sample extraction method, solvent system, and Colum for the LC-MS system gives accurate results [4].

2. Bioavailability and Bioequivalence
Bioavailability and bioequivalence studies were done according to regulatory bodies to ensure that the studied new drug entity has been equivalence to reference products. Clinically, bioequivalence and bioavailability focus on the release of a drug substance from its dosage and absorption in humans and animals during the clinical phase. For the determination of bioavailability of drug substance, between two products such as a reference product and new drug entity or generic drugs, pharmacokinetic studies are carried out. For this study, dosage to be applied to healthy patients [5]. The measurement of plasma concentration is done using LC-MS and the data which is obtained during analysis, used to determine various pharmacokinetic parameters such as the area under the curve (AUC) and the peak plasma concentration of a particular drug (Cmax). Bioequivalence studies are a vital part of registering a new drug entity. These studies measure the bioequivalence or bioavailability of two or more formulation of the same active ingredient [6].

3. Sample Processing
Biological samples which contain the analyte are usually blood, plasma, urine, serum. Blood is usually collected from human subjects with different time intervals after does is applied. Blood is obtained with an anticoagulant like EDTA, heparin. Plasma is obtained by centrifugation. During the sample preparation, Various types of cleaning procedures were done involving solvent extraction and chromatography for the effectively separate the drug components from biological material. There are various sample preparation techniques used, e.g. protein precipitation (denaturation), liquid-liquid extraction, and solid-phase extraction [7].

3.1. Protein Precipitation technique
In this technique, biological products or biomolecules are precipitate using ammonium salt. For the precipitation of biological samples for bioanalysis, trichloroacetic acid, perchloric acid, methanol, Acetonitrile, etc. are used.
Protein denaturation or precipitation is important because of the presence of proteins, lipids, salts, and other endogenous material in the sample. It causes the deterioration of LC-MS/MS columns and also interferes with the assay.

3.2. Liquid-Liquid Extraction
This technique is based on the principles of differential solubility and partitioning equilibrium of analyte molecules between aqueous and the organic phases. An immiscible

organic solvent is used for the separation of phases.

3.3. Solid Phase Extraction

Solid-phase extraction (SPE) is used for the isolation of a wide variety of biological matrices. The separation depends on the affinity towards the stationary phase. An analyte which is loaded with the stationary phase can then be eluted from the solid-phase extraction cartridge with the appropriate solvent. Solid-phase extraction cartridge is usually a reversed-phase or an ion exchange resin [8].

There are different types of Solid Phase Extraction Cartridges.

HLB Cartridge, which is made by a specific ratio of two monomers hydrophilic N-vinylpyrrolidone and lipophilic divinylbenzene and available in various particle sizes such as (60µm, 30µm, 15µm, etc.)

MCX Cartridge is a mixed-mode cation exchange polymeric sorbent. It achieves higher selectivity and sensitivity for extracting basic compounds with cation-exchange groups.

MAX Cartridge, it is usually designed to silica-based mixed-mode SPE sorbents. This cartridge has a mixed-mode anion-Exchange and stable from pH 0 to 14.

WCX Cartridge, is usually designed to provide sample preparation for strong bases and quaternary amines and stable from pH 0 to 14.

Bond Elute Plexa, it has a work on non-polar retention mechanism. It gives clean extracts which minimizes matix interference and also reduces ion suppression. For Proper Characterization of Sample, choosing the right SPE product is essential. Selection of Suitable Conditioning, Washing, and Elution Solvents during sample processing is also important. For the greater strength of the normal phase, the more polar solvent is used and for the reversed phase, the less polar solvent is used. For ion-exchange packing, strength is related to pH and Ionic or salt concentration. Buffers are mainly used to maintain ionic concentration [09, 10].

4. LC-MS/MS Method Development and Optimization:

The method of analysis is being routinely developed, validated, and applied. Required chromatographic separations are dependent on the samples to be analyzed. The knowledge of chromatographic procedure is must be a systematic approach to LC-MS/MS method development and validation [11]. For the development of a suitable method, the information collected from the literature about the physicochemical properties of drug molecules is necessary. It has also include Determination of solubility profile, Mobile phase selection, Selection of extraction and chromatographic method.

For the LC-MS/MS Method Development proper Knowledge about the Sample is necessary for an effective method development. Information of analyte like Molecular weights of compound, Sample Solubility, Drug Stability and concentration range of compounds in samples of Interest is necessary for optimization [12].

During the optimization stage, the initial sets of conditions were evolved during the improvement of method development. Various parameters were optimized during method development including mode of separation, selection of stationary phase or column and selection of mobile phase [13].

4.1. Mode of Separation Technique

Since most of the pharmaceutical compounds are polar in Nature and because of this reverse phase chromatography is normally tried.

4.2. Selection of Stationary Phase or Column

For the selection of column, it is necessary to understand the properties of column packing material. The most commonly used non-polar bonded phases are C18 and C8. C18 is particularly useful for separation of non-polar analyte. C18 and C8 provide good separations for a variety of protein and polypeptide analytes.

4.3. Selection of Mobile Phase

The parameters which essential to be considered while selecting and optimizing the mobile phase are buffer, pH of the buffer and mobile phase composition.

4.4. Optimization of Buffer

Buffer play an important role in deciding the peak symmetries and separations. Some are most commonly used buffers are phosphate buffer, phosphoric acid buffer and acetate buffer [14].

5. Mass Spectrometric Detection

LC-MS/MS is the fast and powerful analytical technique. It combines the resolving power of liquid chromatography with the detection specificity of mass spectrometry. Liquid chromatography separates the sample components according to mass to charge ratio and then introduced them to the mass spectrometry. The LC-MS data provide the information about molecules weight, structure, identification and quantity of specific sample components. Mass spectrometers are divided into three fundamental Parts. Namely, the ionization source, analyzer and detector [15].

5.1. Sample Injection

The samples can be directly injected to the ionization Source. Sample injection is done when mass spectrometer is coupled directly to HPLC.

5.2. Ionization

There are different ionization methods are available. Which are Atmospheric pressure chemical ionization (APCI), Electron spray ionization (ESI), Fast atom bombardment (FAB), and Matrix-assisted laser desorption ionization (MALDI).

Analyzers like Tandem (MS-MS) Mass Spectrometers are used. It gives the structural information of a compound by fragmenting specific sample ions. The process is happening in quadrupole which acts as a collision cell and identifying the resulting fragment ions that are produced inside the mass spectrometer. The most common Analyzers are used is quadrupole – quadrupole and quadrupole - time-of-flight.

5.3. Detector

The detector detects the ion current, amplifies it, and transmitted signals to the data system where it is recorded in the form of mass spectra. The mass to charge ratio of ions is plotted against their intensities. It shows the number of components in the sample and the molecular mass of each component. There are various types of detectors are used as a mass analyzer [16].

Matrix suppression effect is also reduced sensitivity when

the analyte signal is suppressed. LC-MS methods in Electron spray ionization and Atmospheric pressure chemical ionization is affect by matrix effects. the endogenous phospholipids and proteins found in plasma is also matrix source that for LC-MS bioanalysis. The use of proper and selective sample extraction techniques such as Liquid-Liquid Extraction and Solid Phase Extraction can minimize or reduce the matrix effect. The importance of solid phase extraction is eliminating matrix effects and improves analytical system performance.

Reduces Ion Suppression by solid phase extraction Contribute to increase the MS response, reducing lowest amount of an analyte in a sample. It can be quantitatively determined with suitable precision and accuracy [17, 18].

6. Conclusion

In The conclusion, new concepts and recent progress made in several areas including sample preparation, separation and how to reduce matrix effect for better LC-MS/MS efficiency were discussed in this review. LC-MS/MS is a reliable and better choice for bioanalysis of small molecules. The concepts covered in this review article which can be used to enhance LC-MS/MS bioanalytical method development. matrix effect caused due to the presence of unwanted analytes or other interfering substances in the sample. The development in laboratory techniques like sample processing and usage of more efficient extraction techniques drive to more accurate and precise results in the bioanalytical field.

7. References

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