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Bioanalytical strategies and instruments: An overview

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Abstract
The advancement of the bioanalytical procedures brought a dynamic teach for which the future holds many energizing chances to encourage change. The principle effect of bionalysis in the pharmaceutical business is to acquire a quantitative measure of the medication and its metabolites. The reason for existing is to play out the pharmacokinetics, toxicokinetics, bioequivalence and presentation reaction like pharmacokinetic/pharmacodynamic thinks about. Different bioanalytical systems are performed in bioanalytical concentrates, for example, hyphenated strategies, chromatographic procedures, and ligand restricting measures. This audit broadly features the part of bioanalytical methods and hyphenated instruments in evaluating the bioanalysis of the medications.

Keywords: bioanalysis, bioequivalence, chromatography, capillary electrophoresis

Introduction
The field of bioanalysis has matured significantly from early studies in drug metabolism using many simple and advanced techniques, and in today's Bioanalyst is well equipped to deal with the modern challenges. A bioanalytical method is a set of procedures involved in the collection, processing, storage, and analysis of a biological matrix for a chemical compound. Bioanalytical method validation (BMV) is the process used to establish that a quantitative analytical method is suitable for biochemical applications. Bioanlysis covers the quantitative measurement of Xenobiotics of drugs such as their metabolites, and biological molecules in unnatural locations or concentrations and Biotics like macromolecules, proteins, DNA, large molecule drugs, metabolites in biological systems. Bioanalysis is a progressive discipline for which the future holds many exciting opportunities to further improve sensitivity, specificity, accuracy, efficiency, assay throughput, data quality, data handling and processing, analysis cost and environmental impact. The main impact of bioanalysis in the pharmaceutical industry is to obtain a quantitative measure of the drug or its metabolites for the study of pharmacokinetics, toxicokinetics, bioequivalence and exposure-response like pharmacokinetic/ pharmacodynamic studies. The focus of bioanalysis in the pharmaceutical industry is to provide a quantitative measure of the active drug and/or its metabolite(s) for the purpose of pharmacokinetics, toxicokinetics, bioequivalence and exposure–response (pharmacokinetics /pharmacodynamics studies) The reliability of analytical findings is a matter of great importance in forensic and clinical toxicology, as it is of course a prerequisite for correct interpretation of toxicological findings. Unreliable results might not only be contested in court, but could also lead to unjustified legal consequences for the defendant or to wrong treatment of the patient. In the last decade, similar discussions have been going on in the closely related field of pharmacokinetic (PK) studies for registration of pharmaceuticals.

As per Bioanalytical Method Validation (BMV) guidelines for industry, these guidelines are applied to bioanalytical methods that are used for the quantitative determination of drugs and their metabolites in biological matrices such as plasma, urine and preclinical studies [1]. Bioanalytical method validation includes all of the procedures that demonstrate that a particular method developed and used for quantitative measurement of analytes in a given biological matrix is reliable and reproducible [2]. Validation of a bioanalytical method is the process by which it is established that the performance characteristics of the method meet the requirements for the intended bioanalytical application. These performance characteristics are expressed in terms of bioanalytical method validation parameters [3, 4]. The fundamental bioanalytical method validation parameters include precision and accuracy, sensitivity.

Bioanalytical Techniques
Some techniques normally utilized in bioanalytical studies embody

Hyphenated Techniques
- LC–MS (liquid chromatography–mass spectrometry)
- GC–MS (gas chromatography–mass spectrometry)
- CE–MS (capillary electrophoresis–mass spectrometry)

Chromatographic Strategies
- HPLC (high performance liquid chromatography)
- Gas activity

Liquid Chromatography-Mass Spectrometry (LC-MS/MS)
Bioanalytical liquid chromatography-mass spectrometry is a technique that uses liquid chromatography with the mass

spectrometry. LC-MS is commonly used in laboratories for the quantitative and qualitative analysis of drug substances, drug products and biological samples. LC-MS has played a significant role in evaluation and interpretation of bioavailability, bioequivalence and pharmacokinetic data. Through LC-MS biological samples are determined throughout all phases of method development of a drug in research and quality control.

Method Development

Method of analysis are being routinely developed, improved, validated, collaboratively studied and applied. Chromatographic separations are mainly required which depend on the samples to be analyzed. The chromatographic procedure is important for the systemic approach to LC-MS/MS method development. In most cases as desired separation can be achieved easily with only a few experiments. In other cases a considerable amount of experimentation may be needed.

Procedure for method development

Collect the physicochemical properties of drug molecules from the literature.

- Determine solubility profile
- MS scanning and optimization
- Mobile phase selection
- Selection of extraction method and optimization
- Selection of chromatographic method (based on solubility study, retention of compound)

Reversed phase chromatography

Reversed phase packing's such as C18, C8 are the most popular and most widely used for reversed phase. In addition to these C4, C2 and phenyl bonded are also available. Reversed phase sorbents generally involves conditioning with an organic solvent (e.g. methanol) followed by an aqueous solvent (e.g. water).

Normal phase chromatography

Normal phase packing's include silica, amino and alumina. Normal phase packing generally requires conditioning with a non polar solvent and elution is carried with polar solvents. Compounds which are with basic pH functional groups are retained by silica. However, polar compounds are irreversibly retained on a silica surface and in this case amino may be used.

Steps in LC-MS/MS method development

Proper knowledge about the sample is necessary for an effective method development. Some information regarding the analyte is necessary like [5]

- Number of compounds present
- Molecular weights of compound
- Sample Solubility
- Drug Stability
- Concentration range of compounds in samples of interest

Method Optimization

During the optimization stage, the initial sets of conditions that were evolved during the method development are improved and maximized in terms of resolution and peak shape, plate counts asymmetry, capacity, elution time, detection limits, limit of quantization, and overall ability to quantify the specific analyte of interest. Optimization of a method can follow either of two general approaches such as manual or computer driven. The manual approach includes varying one experimental variable at a time, while holding all others constant, and recording the changes in response .The variables might include flow rates, mobile or stationary phase composition, temperature etc. [6]

Mode of separation technique

Since most of the pharmaceutical compounds are polar in nature so reverse phase chromatography is normally tried first in which a non-polar stationary phase is used. The mobile phase consists of water or buffer and organic phase (acetonitrile or methanol). Hence polar compounds get eluted first and non-polar compounds are retained for a longer time. The stationary phases used in reverse phase chromatography are n-octadecyl (RP-18), n-octyl (RP-8), ethyl (RP-2), phenyl, cyano, diol and hydrophobic polymers. It is the first choice for most samples; especially neutral or un-ionized compounds that dissolve in water-organic mixtures. Normal phase is tried if reverse phase fails where the sample may be strongly retained with 100% acetonitrile as mobile phase.

Selection of stationary phase/column

Prior to selection of column it is necessary to understand the properties of column packing material. Silica tends to dissolve above pH 8 and cross-linked polymeric particles, for example, polystyrene or poly methacrylates are used for separation of bases, which can withstand strongly basic mobile phase. Silica particles have surface silanol groups, -SiOH which are used for chemical bonding of stationary phases by silanization reactions with chlorosilanes. About half of the silanol groups are chemically bonded and the rest are capped with tri methyl silyl groups to render them inert. The most commonly used non-polar bonded phases (for reversed phase chromatography) are C18 and C8 with C18 being the most popular (known as ODS for octadecylsilane); C8 is intermediate in hydrophobicity, where C18 is non polar. Phenyl groups are also useful [R= (CH2)3 C6H5

Selection of mobile phase

The main criterion in selection and optimization of mobile phase is to achieve optimum separation of all the individual impurities and degradants from each other and from the analyte peak. The parameters which need to be considered while selecting and optimizing the mobile phase are buffer, pH of the buffer and mobile phase composition [7].

Mass spectrometric detection and data system

Liquid chromatography/mass spectrometry (LC-MS) is promptly becoming the preferred tool of liquid chromatography. It is powerful analytical technique that combines the resolving power of liquid chromatography with the detection specificity of mass spectrometry. Liquid chromatography separates the sample components and then introduced them to the mass spectrometry. Mass spectrometry creates and detects charged ions. The LC-MS data may be

used to provide the information about molecules weight, structure, identification, quantity of specific sample components. Mass spectrometry is a technique that can be used for large samples such as biomolecules; their molecular mass can be measured with an accuracy of 0.01% of the total molecular mass of the sample. Structural information can also be generated by using certain type of mass spectrometers usually those which are employed with multiple analyzers which are also known as tandem mass spectrometers. This may be achieved by fragmenting the sample inside the instrument and analyzing the products generated [8].

Mass Spectrometry

Mass spectrometers are divided into three fundamental parts like ionization source, analyzer and detector.

Sample Introduction

The samples can be inserted directly into the ionization source or can also undergo some type of chromatography to the ionization source. This method usually involves the LC-MS technique in which mass spectrometer is coupled directly to (HPLC) or (GC).

Methods of sample ionization

Many ionization methods are available each having its own advantages and disadvantages. The ionization method used depends on the type of sample under investigation and the mass spectrometer available. Ionization methods are of many types and include the following:

a. Atmospheric pressure chemical ionization (APCI)
b. Electro spray ionization (ESI)
c. Fast atom bombardment (FAB) and,
d. Matrix assisted laser desorption ionization (MALDI**)**

Steps of MS/MS analysis

1. Q1 (first quadrupole acts as a mass filter)
2. Q2 (Acts as a collision cell where selected ions are broken into fragments)
3. Q3- The resulting fragments are analyzed by third quadrupole.

Detection and recording of sample ions

The detector detects the ion current, amplifies it and then the signal is transmitted to the data system where it is recorded in the form of mass spectra. The m/z values of the ions are plotted against their intensities to show the number of components in the sample, the molecular mass of each component, and the relative abundance of the various components in the sample. The various types of detectors are supplied to suit the type of analyzer and the most commonly used include photomultiplier, electron multiplier and micro-channel plate detectors.

Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography–mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample1. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification2. GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a specific test. A specific test positively identifies the actual presence of a particular substance in a given sample. A non-specific test merely indicates that a substance falls into a category of substances. Although a non-specific test could statistically suggest the identity of the substance, this could lead to false positive identification.

Capillary Electrophoresis

Capillary electrophoresis is an analytical technique that separates ions based on their electrophoretic mobility with the use of an applied voltage. The electrophoretic mobility is dependent upon the charge of the molecule, the viscosity, and the atom's radius. The rate at which the particle moves is directly proportional to the applied electric field--the greater the field strength, the faster the mobility. Neutral species are not affected, only ions move with the electric field. If two ions are the same size, the one with greater charge will move the fastest. For ions of the same charge, the smaller particle has less friction and overall faster migration rate. Capillary electrophoresis is used most predominately because it gives faster results and provides high resolution separation. It is a useful technique because there is a large range of detection methods available.[1]

Introduction

Endeavors in capillary electrophoresis (CE) began as early as the late 1800's. Experiments began with the use of glass U tubes and trials of both gel and free solutions.[1] In 1930, Arnes Tiselius first showed the capability of electrophoresis in an experiment that showed the separation of proteins in free solutions.[2] His work had gone unnoticed until Hjerten introduced the use of capillaries in the 1960's. However, their establishments were not widely recognized until Jorgenson and Lukacs published papers showing the ability of capillary electrophoresis to perform separations that seemed unachievable. Employing a capillary in electrophoresis had solved some common problems in traditional electrophoresis. For example, the thin dimensions of the capillaries greatly increased the surface to volume ratio, which eliminated overheating by high voltages. The increased efficiency and the amazing separating capabilities of capillary electrophoresis spurred a growing interest among the scientific society to execute further developments in the technique.

Instrumental Setup

A typical capillary electrophoresis system consists of a high-voltage power supply, a sample introduction system, a capillary tube, a detector and an output device. Some instruments include a temperature control device to ensure reproducible results. This is because the separation of the sample depends on the electrophoretic mobility and the viscosity of the solutions decreases as the column temperature rises [3]. Each side of the high voltage power supply is connected to an electrode. These electrodes help to induce an

electric field to initiate the migration of the sample from the anode to the cathode through the capillary tube. The capillary is made of fused silica and is sometimes coated with polyimide.[3] Each side of the capillary tube is dipped in a vial containing the electrode and an electrolytic solution, or aqueous buffer. Before the sample is introduced to the column, the capillary must be flushed with the desired buffer solution. There is usually a small window near the cathodic end of the capillary which allows UV-VIS light to pass through the analyte and measure the absorbance. A photomultiplier tube is also connected at the cathodic end of the capillary, which enables the construction of a mass spectrum, providing information about the mass to charge ratio of the ionic species.

Conclusion

This survey is pointed in centering the part of different bioanalytical systems in pharmaceuticals and gives a careful writing review of the bioanalytical strategies and instruments in sedate investigation. This survey moreover features the current headways of bioanalytical systems. Among all the bioanalytical techniques, LCMSMS technique is generally utilized and tremendous number of research distributions has been accounted for by LC/MS/MS strategy due to its better affectability and accuracy.

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