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BIOANALYTICAL TECHNIQUES IN PHARMACEUTICALS – AN OVERVIEW

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ABSTRACT

The development of bioanalytic techniques brought continuous discipline where the future holds many exciting opportunities for continuous development. To obtain a limited amount of the drug and its metabolites is the major impact of bioanalysis on the pharmaceutical industry. The aim is to develop pharmacokinetics, toxicokinetic, bioequivalence and exposure response as pharmacokinetic /pharmacodynamic studies. Various bioanalytical methods were performed in bioanalytical studies such as hyphenated techniques, chromatographic techniques, and ligand binding assays. This review emphasizes the role of bioanalytical techniques and hyphenated instruments in assessing the bioanalysis of the drugs.

KEYWORDS: Bioanalyst, Hypenated technique, Spectroscopy, Chromatography.

INTRODUCTION

The field of bioanalysis has grown significantly from early studies on drug use using many simple and advanced techniques, and in today's Bioanalyst it is ready to face modern challenges. A bioanalytical method is a set of processes involved in the collection, processing, storage, and analysis of a biological matrix of chemical compounds. Bioanalytical methodology (BMV) is the process used to determine whether a quantitative analysis method is suitable for chemical applications. Bioanalysis includes a moderate Xenobiotic dose of drugs such as their metabolites, and organic molecules in non-natural or concentrated molecules and Biotics such as macromolecules, proteins, DNA, large molecule drugs, metabolites in the biological system. Bioanalysis is an ongoing discipline in which the future

holds many exciting opportunities to further improve sensitivity, specificity, accuracy, efficiency, assay performance, data quality, data management and processing, cost analysis and environmental impact. The major impact of bioanalysis in the pharmaceutical industry is to obtain a limited dose of the drug or its metabolites for the study of pharmacokinetics, toxicokinetics, bioequivalence and exposure responses as pharmacokinetic / pharmacodynamic studies. The focus of bioanalysis in the pharmaceutical industry provides a balanced measure of the active drug and / or its metabolites (s) for the purpose of pharmacokinetics, toxicokinetics, bioequivalence and exposure-response (pharmacokinetics / pharmacodynamics studies) Reliability of findings analysis is of paramount importance to forensic and clinical toxicology, as is the need for accurate interpretation of toxicological findings. Unsatisfactory results may not only be challenged in court, but may also lead to improper legal consequences for the defendant or improper treatment of the patient. Over the past decade, similar discussions have been taking place in the highly relevant field of pharmacokinetic studies (PK) for the registration of medicines.

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 commonly used in bioanalytical studies include.

Hyphenated techniques

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

Chromatographic methods

- HPLC (high performance liquid chromatography)

Gas chromatography

LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY(LC-MS/MS)

Liquid Chromatography along with mass spectroscopy techniques are used in Bioanalytical liquid chromatography. LC-MS is usually utilized in laboratories for the quantitative and chemical analysis of drug substances and biological samples. LC-MS has contended a major role in analysis and interpretation of bioavailability, bioequivalence and pharmacokinetic knowledge. Through LC-MS biological samples are determined throughout all phases of technique development of a drug in analysis 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 analysed. 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

C18, C8 are the most popular reversed phase packing's and widely used in reversed phase. category. In addition to these C4, C2 and phenyl bonded are available. The reversed phase sorbents of the phase usually consist of the preparation of organic solvent (e.g., methanol) followed by aqueous solvent (e.g., water).

Normal Phase Chromatography: Normal phase packing's contains silica, amino acid and alumina. Normal phase packing usually requires conditioning with non-polar solvent and elution is carried with the polar solvents. Compounds with basic pH groups are retained by silica. However, polar compounds are retained on the silica surface and in this case amino can be used.

Steps in LC-MS/MS Method Development

For effective method proper knowledge about the sample is needed. The information necessary about analyte like[5]

• Concentration range of compounds
• Stability of drugs
• Sample Solubility
• Molecular weights of the compound
• Number of compounds present

Method Optimization

During the stage of optimization, initial sets of conditions that involved during method development are developed and amplified according to resolution and its peak shape, the plate calculates asymmetry, strength, elution time, detection limits, measurement limit, and total calculation of a specific interest analyst. Method optimization can follow any of the two most common methods such as manual or computer-driven. The manual method involves variation of one test at a time, while holding everything else consistently or constant, and recording the change in response. The variables may include stationary phase composition, flow rates mobile and temperature etc.[6]

Mode of Separation Technique

As most of the pharmaceutical compounds are polar in nature so therefore reverse phase chromatography is usually tested first in which non-polar stationary phase is used. The mobile category consists of water or buffer and the organic phase i.e., (acetonitrile or methanol). Thus, polar compounds begin to elute first and non-polar compounds are stored longer. n-octadecyl (RP-18), n-octyl (RP-8), ethyl (RP-2), phenyl, cyano, diol and hydrophobic polymers are the stationary phases used in reverse phase chromatography. It is the first choice of many samples; mainly neutral or non-ionized compounds dissolved in water-organic compounds. The normal phase is tried if the reverse phase fails where the sample is retained strongly with 100% acetonitrile as a mobile phase.

Selection of Stationary Phase/Column

Before selecting a column it is necessary to understand the properties of the column packaging material. pH above 8 and polymeric particles tends to dissolve in silica, for example, polystyrene or poly methacrylate are used to separate bases, which can withstand a very strong basic mobile phase. Silica particles have higher concentrations of silanol, -SiOH is used for the chemical bonding of stationary phases in response to salinization with chlorosilanes. A half portion of the silanol groups are chemically bonded and the rest are capped with tri methyl silyl groups to render as inert. The commonly used non-polar bonded phases (for chromatography of the reverse phase) are C18 and C8 and C18 is the most popular (known as ODS for octadecylsilane); The C8 is intermediate in hydrophobicity, whereas the C18 is not polar. Phenyl groups are also applicable [R = (CH_2) 3 C_6H_5.

Selection of Mobile Phase

The main criterion for selecting and optimising the mobile phase is to obtain a complete separation of all impurities and degradants from each other from the top of the analyte peak. The parameters that need to be considered when 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) quickly became a popular tool for liquid chromatography. It is a powerful analytical method that combines the solvent power of liquid chromatography with the specification of mass spectrometry detection. Liquid chromatography separates the sample components and the introduced to the mass spectroscopy. Mass spectrometry creates and detects charged ions. LC-MS data can be used to provide information about molecular weight, composition, identification, number of specific sample components. Mass spectrometry is a process that can be used for large samples such as a biomolecule; their molecular weight can be measured with an accuracy of 0.01% of the total molecular weight of the sample. Structural data can also be generated using a specific type of mass spectrometers that are commonly used by many analyzers, which is also known as tandem mass spectrometers. This can be achieved by separating the sample inside the instruments and analyzing the products produced.[8]

Mass Spectrometry

Ionization source, analyzer and detector are the three-fundament part of Mass Spectrometry.

Sample Introduction

Samples can be injected directly into the ionization source or may undergo some form of chromatography at the ionization source. This method usually involves the LC-MS process in which the mass spectrometer is directly integrated (HPLC) or (GC).

Methods of Sample Ionization

Many ionization methods are available each with its own advantages and disadvantages. The ionization method used depends on the type of sample being investigated and the mass spectrometer available. Ionization methods come in many forms 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 serves as a main filter)
2. Q2 (acts as a collision cell where selected ions are divided into fragments)
3. Q3- The resulting fragments are analyzed by a third quadrupole.

Detection and Recording of Sample ions

The detector detects the current ion, amplifies it, and the signal is transferred to a data system where it is recorded in form of mass spectra. The m/z values of ions are set against their potential to indicate the amount of material in the sample, the molecular mass of each element, and the relative quantity of the various substances in the sample. Various types of detectors are provided to suit the type of analyzer and the most widely used are photomultiplier, electron multiplier and micro-channel plate detectors.

Figure 1: LC/MS/MS Instrument.[23]

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) is a method that combines the elements of gas-liquid chromatography along with mass spectrometry to identify various substances within a test sample. Drug discovery, fire research, environmental analysis, explosives investigation, and identification of unknown samples are the applications of GC-MS. GC-MS can also be used for airport security to find luggage or on people. In addition, it can identify trace elements in objects that were thought to be scattered beyond identification. GC-MS has been widely described as the "gold standard" for forensic substance detection because it is used to perform specific tests. A particular test certainly indicates the actual presence of substance in a given sample. Non-specific tests only indicate that substances falls into the category of substances. While non-specfic test can statistically enhance the identity of the substance, this can lead to false positive identification.

Instrumentation

The GC-MS consisting's of two major building blocks: a gas chromatograph and a mass spectrometer. The gas chromatograph uses a capillary column based on column size (length, width, film size) and phase structures. The differences in the chemical composition between the different molecules in the mixture of compound will separate the molecules as the sample travels the length of the column. The molecules are stored by the column and then elute (emerge) from the column at different times (called the retention time), and this allows the mass spectrometer to capture, ionize, accelerate, and detect ion molecules separately. Mass spectrometer does this by breaking each molecule into ionized fragments and identify the fraction.[8]

GC-MS schematic

These two components, used together, allow a fine degree of substance identification rather than a distinct use. If we use gas chromatography or mass spectroscopy alone, then it is impossible to make accurate identification of specific molecule. The process of mass spectrometry usually requires a pure sample while gas chromatography using a traditional detector (e.g. Flame ionization detector) cannot distinguish between multiple molecules that may take the same amount of time to move in a column (e.g. with the same retention time), resulting in two or more molecules that co-elute.[9] Sometimes two different molecules may have the same pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the error possibilities.

Purge and trap GC-MS

A purge and trap (P&T) concentrator system is used to introduce samples, for the analysis of volatile compounds. The target analytes are extracted and mixed with water and then placed in a air-tight chamber. Inert gas such as Nitrogen (N2) is released/bubbled into the water; this is known as purging. Head space above the water, the volatile compounds are moved and through pressure gradient it is drawn (caused by the introduction of a purge gas) out of the chamber. Volatile compounds are drawn along with a heated line onto a 'trap'. The trap is a column of adsorbent substances in the ambient temperature that holds the compounds by returning them to the liquid phase. The trap is heated and the sample compounds are inserted in the GC-MS column via interface volatiles, which is a separate entry system. P&T GCMS is well suited for volatile organic chemicals.

Types of mass spectrometer detectors

Quadrupole mass spectrometer is the most common mass spectrometer (MS) associated with gas chromatograph (GC).One of the most common detectors is the ion trap mass spectrometer. In addition, one can obtain a magnetic sector mass spectrometer, but these devices are more expensive and bulkier and are rarely found in high-service laboratories. Other detectors may be encountered as a flight time (TOF), tandem quadrupoles.[9]

Ionization

As the molecules travel the length of the column, they pass through the transmission line and enter into mass spectrometer they are ionised by different methods with a typically a single method being used at any given time. Once the sample is separated it will be detected, usually with an electron multiplier diode, which converts the ionized mass fragments into an electrical signal.

Electron ionization: In electron ionization (EI) molecules enter MS (the source is quadrupole or ion traps itself in the MS ion trap) where they are fitted with free electrons released/emmited from the filament, not unlike a filament one would get in a standard light bulb. Electrons bombard the molecules, causing the molecule to split in a characteristic and productive way. This method of "hard ionization" leads to the formation of many fragments of low mass to charge ratio(m/z). Hard ionization is considered by spectrometrists as the use of molecular electron bombardment, while "soft ionization" is charge by molecular collisions with the introduced gas. The pattern of molecular fragmentation depends on the electron energy used in the system, usually 70 eV (electron Volts).[10]

Analysis

Mass spectrometer is used in one of two ways: full scanning or Selected Ion Monitoring (SIM). The standard GC-MS device is capable of performing both functions individually or in combination, depending on the configuration of the specific device. The main goal of instrument analysis is to measure the amount of substance. This is done by comparing the relative concentrations between the atomic masses in the spectrum produced. Two types of analysis are possible, comparative and real. Comparative analysis actually compares a given spectrum to a spectrum library to see if its characteristics are present in a specific sample in the library. Another method of analysis measures peaks in relation to another. In this way, the highest peak is given a value of 100%, and some peaks are given equal amounts. All values above 3% are assigned.[25] The total mass of an unknown compound is usually indicated by the height of the parent (parent peak). The isotope pattern in the spectrum, which different from elements that have many isotopes, can also be used to identify the various substances present.

Full Scan MS

Full scanning helps determine unknown compounds in the sample. It provides more details than SIM when it comes to verifying or resolving compounds in a sample. It may be common to start analyzing test solutions in full scanning mode to determine the retention time during instrumental method development and fragment fingerprint before moving to the SIM instrumental method.[11]

Selected ion monitoring

Certain ion fragments are entered in the instrument method in selected ion monitoring (SIM) and only those mass fragments are detected by the mass spectrometer. The advantages of SIM are that the detection limit is lower since the instrument only looks at a small number of fragments (e.g., three fragments) during each scan. Multiple scanning is possible every second.[12]

Gas chromatographs

Checks on the septum, injector liner, gas pressure and inlet filters (e.g., oxygen scruber, misture trap and coalcoal trap), basic signal level and background noise are included in Routine maintenance operations. Depending on the usage of the instrument , it makes sense to have a repair system(routine maintenance program) that includes weekly septum replacement and injector liner.

Figure 2: GC/MS Instrument[24]

High Performance Liquid Chromatography (HPLC)

High pressure liquid chromatography is a type of column chromatography used in bio-chemistry and analysis of chromatographic packaging materials (stationary phase), a pump that moves the mobile phase through column, and the detector showing the retention time of molecules. The retention time varies depending on interaction between the stationary phase, the molecule analyzed and solvent (s) used. The development of a Bioanalytical approach to the technique of building a process to allow a compound of interest to be identified and calculated in the matrix. By using biological products, it can be measured in a number of ways and the choice of biological testing involves several considerations of quantitative or qualitative measurement, and clarification is required with the necessary equipment. A bioanalytical series describes the process of method development by biological samples including sampling, sample preparation, classification, detection and test results.[13]

Some General procedures for sample preparation are

1.liquid/liquid extraction

2.Solid-phase extraction (SPE) and

3.Protein precipitation.

Liquid – Liquid extraction

Based on the principles of differential solubility and the equilibrium distribution of analyte molecules between aqueous (original sample) and organic phases. Now Liquid-Liquid extraction have been replaced with advanced techniques such as liquid phase micro extraction, single drop liquid phase micro extraction and supported membrane extraction.

Liquid - Liquid extraction usually involves the extraction of an substance from one phase of liquid to another liquid phase.[14]

Solid Phase Extraction (SPE)

Solid phase extraction (SPE) is the preferred method of sample preparation in which the analyte is tied to the solid support, the interferences are washed and the analyte is selectively eluted. Due to the different sorbents, SPE is a very powerful method.Solid phase has four steps namely; preparation, sample loading, washing and elution.

Protein Precipitation

Protein precipitation is often used for routine analysis to remove protein. Precipitation can be done by adding an organic modifier, salt or by changing the Ph which influences the solubility of proteins.[15] Samples are centrifuged and the supernatant can be injected into the HPLC system and then dissolved in the appropriate solvent, the concentration of the sample is achieved. However, the protein precipitation process is often combined with SPE to produce pure extract. Salt is another way to reduce acid organic solvent precipitation. This process is called salt induced precipitation. As the salt concentration increases, proteins aggregates and precipitate from the solution.[16]

Conditioning

The column activates with organic solvent that acts as a wetting agent in packaging materials and solvates the functional groups of the sorbent. Water or aqueous buffer is added to activate the column for appropriate adsorption processes.

Sample Loading and elution

Distribution of the analyte-sorbent interaction with the appropriate solvent, eliminates as little of the remaining interferences as possible. Typically, the sorbents used in SPE contain 40µm silica gel with a diameter of 60 A0 pore. The most widely used format is a syringe barrel containing a 20µm frit at the bottom of the syringe containing sorbent material and another frit on top, called packed-column. Analytics can be divided into four categories; basic, acid, neutral and amphoteric compounds.Amphoteric compounds have basic and acidic activity and can therefore act as cations, anions or zwitterions, depending on the pH.[17]

Figure 3: HPLC Instrument[25]

Gas Chromatography

The term Gas Chromatography (GC) is used for all methods in which the mobile phase is gaseous. However, the stationary phase can be solid (adsorption chromatography) or liquid that carries solids (separation or absorption chromatography). The sample can be gas or liquid but, however, it is injected in the column as gas or vapour. The result of this setup is that the gas chromatography is suitable for the separation of the heat stable compounds i.e., the chemicals that can evaporate without decomposition. In GC the sample is injected into a continuous flow of eluent via an injector. The sample is then washed in a column by the eluent where the separation occurs and finally the components enter the detector accordingly in the order in which the signal value of their concentration is generated. The whole system is computer controlled. Gas used, the source of eluent i.e., mobile phase can be a high pressure cylinder or gas generator. The most widely used gases in the chromatography lab include He, Ar, N_2 and H_2. As GC is a high performance analytical method it is necessary to use very high purity gases.

Depending on the purity of the gas (Table No.1) different on-line gas purifiers should be installed.

Table 1: High purity gases.

V/V % purity, gas content	Quality	Total impurities
99,99 %	4.0	100 ppm (v/v)
99,995 %	4.5	50 ppm (v/v)
99,999 %	5.0	10 ppm (v/v)
99,9995 %	5.5	5 ppm (v/v)
99,9999%	6.0	1 ppm (v/v)

Sample introduction

Sample inlet is a critical point in gas chromatography. It is important to introduce the sample to the column as short as possible and at the same time to have it in the gas phase. Gas,liquid and solid samples can be studied by GC but due to slow evaporation of solids the sample injected are gases or liquid. Gas samples are injected in the form of a six-port switch system. These injections contain a moderate volume sample. First the loop is filled with sample gas by washing it with 5 to 10 times the sample gas volume. This is necessary to ensure that the loop contains only the components of the sample and no air or eluent gas remaining. When switching, the contents of the sample loop are washed in the carrier gas stream and injected into the column.

Columns

The heart of the GC is the "column" where the separation of sample components occurs. The column is placed in an oven with a controlled temperature and the computer-controlled heating can increase the temperature to 400-500^{0}C but can also be cooled to room temperature again. Columns can be divided into two groups. Packed columns are 1-5 m long and the inner diameter is about 2-6 mm with a suitable stationary phase. As already mentioned, in the case of a separation based on adsorption, stationary phase might be a high surface area solid like activated charcoal, Al_2O_3, silica, a molecular sieve or organic polymer. In the case of absorption based packing column, solid materials are placed in a stationary liquid phase column and filled into a column.

Detectors

As already discussed, the sample components are separated from the column and entered into the detector in sequence where a similar signal is obtained by physical method. The most widely used detectors in the GC include Thermal Conductivity Detector (TCD), Flame Ionization Detector (FID), Electron Capture Detector (ECD), Mass Spectrometry (MS) detector and Infrared Spectrophotometric (IR) detectors. Photoionization Detector (PID),

Flame Photometric Detector (FPD), Pulsed Flame Photometric Detector (PFPD) or Atomic Emission Detector (AED),these are the special types of detectors.

Figure 4: GC Instrument[26]

CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY (CE-MS)

CE-MS, combining high efficiency with CE resolution capabilities, and the high selectivity and sensitivity associated with MS, is a very attractive analytical method. However, the combination of CE-MS, especially with ESI[18], was not easy to use because the closed electrical- circuit requires not only electrophoretic separation but also effective ionization of the source (with CE ESI currents in the range of mA and nA, respectively). The solution to this problem is to ground the spray needle to turn all the electrical energy from the CE to the ground and to create an uninterrupted electric ionization field for the MS source. Although the sensitivity obtained with the use of sheath flow is generally low compared to shealth less interfaces, the stiffness of the anterior system is generally better and the limits of access to the lower femtomole range can be obtained, especially when sheath liquid flow rate is reduced to 500 nL / min. The detection of narrow CE peaks requires the use of a large, fast and sensitive mass spectrometer. IT and TOF systems are adequate detectors because they receive data at the right mass range at multiple spectra values per second.

CE-MS for bioanalysis of drugs

Many recent reviews include the use of CE-MS drug analysis, some of which provide fragments, when found, that ionic species enter source and IT, triple quadrupole or TOF mass spectrometers.[19] This part of the review is devoted to the analysis of drugs in biological fluids.

Practical considerations for robust and sensitive CE-MS coupling

CE has several advantages over HPLC, namely rapid development, low sample and solvent use, rapid separation and efficient separation and, in the particular field of chiral separation, the use of expensive stationary chiral phases is not required. However, problems with ruggedness in on-line combination of CE with MS often limit their use for quantitative purposes. Among other things, this can explained by variability in migration times (MTs), due to EOF and / or the lack of thermo stating, the part of the capillary that connects the CE equipment to the MS source. In addition, some parameters of the electro spray, the most common CE interface, must be carefully adjusted to obtain stable CE-ESI-MS conditions, namely sheath liquid composition and flow rate, nebulizing gas pressure, and capillary outlet position.

Composition and flow rate of the sheath liquid

Although the composition of the sheath liquid depends largely on the analyst studied, other rules may be set. An aqueous solution containing 50- 80% of moderate polar organic solvent is required to achieve stable spray formation, due to reduced surface tension. On the other hand, the complete content of organic solvent provides a superior response to many organic analytics, due to more efficient desolvation of compound and better spray stability.

Nebulizing gas pressure

In the ESI interface, a nebulizing gas pressure, used to aid the formation of droplets and to obtain a stable spray, consistency between sensitivity and spray stability. It is known that the use of nebulizing gas raises reduced pressure in the capillary outlet. Therefore, the separation efficiency and resolution of MTs can be reduced, due to the hydrodynamic flow generated in the capillary CE. This decrease in the separation efficiency and MTs with an increase in nebulizing gas pressure is reflected in the work. It has also been reported that nebulizing gas pressure affects the sensitivity of the detection. Indeed, with a very high pressure value, the S/N ratio was 20% lower than the lowest value due to the high noise level while the amount of the drug improved with an increase in nebulizing gas pressure.[20]

Capillary outlet position

To carry out the reproducible CE-MS analysis, it is necessary to have a well-defined process for inserting a new capillary. In the literature, a number of strategies have been reported. Recently, Ohnesorge et al described a new procedure, based on observations of the strong effect of carrying neostigmine.[21] After capillary replacement, a buffer analysis, instead of a

sample, was performed. The signal from the effect of the neostigmine carryover is adjusted to a very high level, selected as the reference level for the strength of subsequent capillary changes, assuming that the intensity of the bearing signal remains the same.

Sample preparation

Most applications dealing with drug bioanalysis in CE-MS focused on serum and urine samples, and it can be used in other biological fluids, such as hair, cerebrospinal fluid (CSF), etc. Protein the important component of plasma penetrates the capillary wall and therefore adversely affects separation efficiency, resolution and MT. Urine contains inorganic ions as well as other endogenous compounds, such as urea, which can also interfere with electrophoretic analysis. In addition, the high ionic power of the urine is not desirable to sample staking, therefore, a higher peak expansion can be observed. Among the sample preparation techniques, SPE and extraction / liquid extraction are effective cleaning procedures that can be used to increase analyte concentration. However, these complex sample preparation processes are often tedious and time consuming.[22]

Figure 5: CE/MS instrument[27]

CONCLUSION

This review aims to focus on the role of various bioanalytical approaches in pharmaceuticals and provides a comprehensive study of the literature on bioanalytical methods and instruments in drug analysis. This review also highlights the recent advances in bioanalytic techniques. Among all the bioanalytical methods, the LC-MS/MS method is widely used and a number of research literature has been reported by the LC-MS/MS method because of its sensitivity and accuracy.

REFERENCES

1. Matuszewski, B.K., Constanzer, M.L. and Chavez-Eng, C.M., 2003. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC−MS/MS. Analytical chemistry, 75(13): 3019-3030.
2. Devanshu, S., Rahul, M., Annu, G., Kishan, S. and Anroop, N., 2010. Quantitative bioanalysis by LC-MS/MS: a review. Journal of Pharmaceutical and Biomedical Sciences (JPBMS), 7(07).
3. Enke, C.G., 1997. Anal. Chem; f LC-MS Bioanalysis: Best Practices, Experimental Protocols. Anal. Chem, 69: 4885-4893.
4. Viswanathan, C.T., Bansal, S., Booth, B., DeStefano, A.J., Rose, M.J., Sailstad, J., Shah, V.P., Skelly, J.P., Swann, P.G. and Weiner, R., 2007. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharmaceutical research, 24(10): 1962-1973.
5. Causon, R., 1979. Application issues in bioanalytical method validation; sample analysis. J. Pharm. Sci, 68: 237-238.
6. Kirthi, A., Shanmugam, R., Prathyusha, M.S. and Basha, D.J., 2014. A review on bioanalytical method development and validation by RP-HPLC. Journal of global trends in pharmaceutical sciences, 5(4): 2265-2271.
7. Causon, R., 1979. Application issues in bioanalytical method validation; sample analysis. J. Pharm. Sci, 68: 237-238.
8. Amirav, A. and Granot, O., 2000. Liquid chromatography mass spectrometry with supersonic molecular beams. Journal of the American Society for Mass Spectrometry, 11(6): 587-591.
9. Iram, F., Iram, H., Arif, M., Siddiqui, A.A. and Husain, A., 2016. Bioanalytical Method Development and Validation by Hyphenated Technique (Lc-Ms/Ms). Journal of Pharmaceutical and Medicinal Chemistry, 2(1).
10. Alon, T. and Amirav, A., 2006. Isotope abundance analysis methods and software for improved sample identification with supersonic gas chromatography/mass spectrometry. Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry, 20(17): 2579-2588.
11. Adams, R.P., 2007. Identification of essential oil components by gas chromatography/mass spectrometry (Vol. 456). Carol Stream, IL: Allured publishing corporation.

12. Adlard E. R., Handley., and Alan J; Gas chromatographic techniques and applications; Sheffield Academic; IJPQA, 2001; 4(3): 2-16.
13. Eugene F., Barry., Grob., and Robert Lee; Modern practice of gas chromatography.New York; Wiley- Interscience; IJPQA, 2004; 4(3): 1-10.
14. Coates, J. and Meyers, R.A., 2000. Encyclopedia of analytical chemistry. Interpretation of infrared spectra, a practical approach. Wiley, Chichester, 10815-10837.
15. Jena, B.R., Swain, S. and Babu, S.M., 2017. An Overview of Recent Advances in Bio-Analytical Techniques. Journal of Applied Biopharmaceutics and Pharmacokinetics, 5: pp.5-10.
16. Said, R., 2010. Application of new technology LC-MS. MS for determination of therapeutic drugs, Doctoral degree thesis, Department of Medicine Division of Clinical Pharmacology Karolinska Institute, Stockholm, Sweden, 1-5.
17. Thurman, E.M. and Mills, M.S., 1998. Solid-phase extraction. New York: John Wiley & Sons, 29: 35-73.
18. Murugan, S., Pravallika, N., Sirisha, P. and Chandrakala, K., 2013. A review on bioanalytical method development and validation by using LC-MS/MS. Journal of chemical and Pharmaceutical Sciences, 6(1): 41-45.
19. Shah, V.P., 2007. The history of bioanalytical method validation and regulation: evolution of a guidance document on bioanalytical methods validation. The AAPS Journal, 9(1): E43.
20. Buick, A.R., Doig, M.V., Jeal, S.C., Land, G.S. and McDowall, R.D., 1990. Method validation in the bioanalytical laboratory. Journal of pharmaceutical and biomedical analysis, 8(8-12): 629-637.
21. Chauhan, A., Goyal, M.K. and Chauhan, P., 2014. GC-MS technique and its analytical applications in science and technology. J. Anal. Bioanal. Tech, 5(6): 222.
22. Causon, R., 1997. Validation of chromatographic methods in biomedical analysis viewpoint and discussion. Journal of Chromatography B: Biomedical Sciences and Applications, 689(1): 175-180.
23. Rozet, E., Marini, R.D., Ziemons, E., Boulanger, B. and Hubert, P., 2011. Advances in validation, risk and uncertainty assessment of bioanalytical methods. Journal of pharmaceutical and biomedical analysis, 55(4): 848-858.
24. evosciences.com/en/spectrometrie-de-masse/33-waters-xevo-tqs hclass.html.
25. azom.com/equipment-details.aspx?EquipID=902.

26. shimadzu.com/an/industry/foodbeverages/qn50420000001ua7-img/qn50420000002ffj.jpg.
27. science.sjp.ac.lk/wp-content/uploads/2012/06/GC-MS_instrument_flatform.jpg.
28. chem.agilent.com/en-US/products-services/Instruments-Systems/Automated-Electrophoresis/CE-MS-System/Pages/default.aspx.