Bioanalytical Techniques – An Overview

Syed Saida Begum*, B. Sai Sushmaa, S.Vijayaraja
Department of Pharmaceutical Analysis
Sree Vidyanikethan College of Pharmacy, Tirupati,
Chittoor (Dt), Andhra Pradesh.
msg2saida@gmail.com

ABSTRACT

The development of the bioanalytical techniques brought a progressive discipline for which the future holds many exciting opportunities to further improvement. The main impact of bionalysis in the pharmaceutical industry is to obtain a quantitative measure of the drug and its metabolites. The purpose is to perform the pharmacokinetics, toxicokinetics, bioequivalence and exposure response like pharmacokinetic / pharmacodynamic studies. Various bioanalytical techniques are performed in bioanalytical studies such as hyphenated techniques, chromatographic techniques, and ligand binding assays. This review extensively highlights the role of bioanalytical techniques and hyphenated instruments in assessing the bioanalysis of the drugs.

Keywords: Bioanalysis, pharmacokinetics, Bioequivalence, Chromatography

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 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)

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.

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 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

Instrumentation:

The GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties. The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off of) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detect the fragment [8].

GC-MS schematic:

These two components, used together, allow a much finer degree of substance identification than used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame ionization detector) cannot differentiate between multiple molecules that happen to take the same amount of time to travel through the column (i.e. have the same retention time), which results in two or more molecules that co-elute9. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error.

Purge and trap GC-MS

For the analysis of volatile compounds a purge and trap (P&T) concentrator system may be used to introduce samples. The target analytes are extracted and mixed with water and introduced into an air tight chamber. An inert gas such as Nitrogen (N2) is bubbled through the water; this is known as purging. The volatile compounds move into the headspace above the water and are drawn along a pressure gradient (caused by the introduction of the purge gas) out of the chamber. The volatile compounds are drawn along a heated line onto a 'trap'. The trap is a column of adsorbent material at ambient temperature that holds the compounds by returning them to the liquid phase. The trap is then heated and the sample compounds are introduced to the GC-MS column via a volatiles interface, which is a split inlet system. P&T GCMS is particularly suited to volatile organic compounds.

Types of mass spectrometer detectors

The most common type of mass spectrometer (MS) associated with a gas chromatograph (GC) is the quadrupole mass spectrometer. Another relatively common detector is the ion trap mass spectrometer. Additionally one may find a magnetic sector mass spectrometer, however these particular instruments are expensive and bulky and not typically found in high throughput service laboratories. Other detectors may be encountered such as time of flight (TOF), tandem quadrupoles [9].

Ionization

After the molecules travel the length of the column, pass through the transfer line and enter into the mass spectrometer they are ionized by various methods with typically only one method being used at any given time12. Once the sample is fragmented it will then be detected, usually by an electron multiplier diode, which essentially turns the ionized mass fragment into an electrical signal that is then detected. Electron ionization: In the electron ionization (EI) the molecules enter into the MS (the source is a quadrupole or the ion trap itself in an ion trap MS) where they are bombarded with free electrons emitted from a filament, not unlike the filament one would find in a standard light bulb. The electrons bombard the molecules, causing the molecule to fragment in a characteristic and reproducible way. This "hard ionization" technique results in the creation of more fragments of low mass to charge ratio (m/z). Hard ionization is considered by mass spectrometrists as the employ of molecular electron bombardment, whereas "soft ionization" is charge by molecular collision with an introduced gas. The molecular fragmentation pattern is dependent upon the electron energy applied to the system, typically 70 eV (electron Volts) [10].

Analysis

A mass spectrometer is typically utilized in one of two ways: full scan or selected ion monitoring (SIM). The typical GC-MS instrument is capable of performing both functions either individually or concomitantly, depending on the setup of the particular instrument. The primary goal of instrument analysis is to quantify an amount of substance. This is done by comparing the relative concentrations among the atomic masses in the generated spectrum. Two kinds of analysis are possible, comparative and original. Comparative analysis essentially compares the given spectrum to a spectrum library to see if its characteristics are present for some sample in the library. Another method of analysis measures the peaks in relation to one another. In this method, the tallest peak is assigned 100% of the value, and the other peaks being assigned proportionate values. All values above 3% are assigned[25]. The total mass of the unknown compound is normally indicated by the parent peak. The isotope pattern in the spectrum, which is unique

for elements that have many isotopes, can also be used to identify the various elements present.

Full Scan MS

Full scan is useful in determining unknown compounds in a sample. It provides more information than SIM when it comes to confirming or resolving compounds in a sample. During instrument method development it may be common to first analyze test solutions in full scan mode to determine the retention time and the mass fragment fingerprint before moving to a SIM instrument method. [11]

Selected ion monitoring

In selected ion monitoring (SIM) certain ion fragments are entered into the instrument method 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 is only looking at a small number of fragments (e.g. three fragments) during each scan. More scans can take place each second[12].

Gas chromatographs

Routine maintenance operations used include checks on the septum, injector liner, gas pressures and inlet filters (e.g. oxygen scrubber, moisture trap and charcoal trap), baseline signal level and background noise. Depending on the degree of usage of the instrument, it is sensible to have a routine maintenance program involving weekly change of the septum and injector liner.

Figure 2: GC/MS Instrument[24]

High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography is a form of column chromatography used frequently in bio chemistry and analytical chromatographic packing material (stationary phase),a pump that moves the mobile phase(s) through the column, and a detector that shows the retention time of the molecules, retention time varies depending on the interactions between the stationary phase, the molecules being analyzed and the solvent(s) used. Bionalytical method development is the process of creating a procedure to enable a compound of interest to be identified and quantified in a matrix. By using biological products can be measured by several methods and the choice of bioanalytical method involves several considerations of quantitative or qualitative measurement, and precision are required with necessary equipment. The bioanalytical chain describes the process of method development by biological samples includes sampling, sample preparation, separation, detection and evaluation of the results. [13]

Some General procedures for sample preparation are

liquid/liquid extraction
solid-phase extraction (SPE) and
Protein precipitation.

Liquid – Liquid extraction

It is based on the principles of differential solubility and partitioning equilibrium of analyte molecules between aqueous (the original sample) and the organic phases. Now a day's Liquid-Liquid extraction has been replaced with advanced and improved techniques like liquid phase micro extraction, single drop liquid phase micro extraction and supported membrane extraction. Liquid – Liquid extraction generally involves the extraction of a substance from one liquid phase to another liquid phase [14].

Solid Phase Extraction (SPE)

Solid phase extraction is selective method for sample preparation where the analyte is bound onto a solid support, interferences are washed off and the analyte is selectively eluted. Due to different sorbents, solid phase extraction is a very powerful technique. Further Solid phase consists of four steps they are; conditioning, sample loading, washing and elution.

Protein Precipitation

Protein precipitation is often used in routine analysis to remove proteins. Precipitation can be induced by

the addition of an organic modifier, a salt or by changing the Ph which influence the solubility of the proteins [15]. The samples are centrifuged and the supernatant can be injected into the HPLC system or be evaporated to dryness and thereafter dissolved in a suitable solvent. A concentration of the sample is then achieved. However, the protein precipitation technique is often combined with SPE to produce clean extract. Salts are other alternative to acid organic solvent precipitation. This technique is called as salt induced precipitation. As the salt concentration of a solution is increased, proteins aggregate and precipitate from the solution [16].

Conditioning

The column is activated with an organic solvent that acts as a wetting agent on the packing material and solvates the functional groups of the sorbent. Water or aqueous buffer is added to activate the column for proper adsorption mechanisms.

Sample Loading and elution

Distribution of analyte–sorbent interactions by appropriate solvent, removing as little of the remaining interferences as possible**.** Typically, sorbents used in SPE consists of 40µm diameter silica gel with approximately 60 A0 pore diameters. The most commonly used format is a syringe barrel that contains a 20µm frit at the bottom of the syringe with the sorbent material and another frit on top, referred to as packed columns. Analytes can be classified into four categories; basic, acid, neutral and amphoteric compounds. Amphoteric analytes have both basic and acid functional groups and can therefore functions as cations, anions or zwitterions, depending on pH [17].

Figure.3-HPLC Instrument[25]

Gas Chromatography

GC The term Gas Chromatography (GC) is used for all methods when the mobile phase is gaseous. However, the stationary phase can be either solid (adsorption chromatography) or liquid fixed onto a solid carrier (partition or absorption chromatography). The sample can be either gas or liquid but in any case it is injected onto the column as gas or vapor. The consequence of this setup is that gas chromatography is suitable for the separation heat stable compounds i.e. chemicals which can be evaporated without decomposition. In GC the sample is injected into a continuous flow of the eluent through the injector. Then the sample is washed onto the column by the eluent where separation occur and finally components get into the detector ideally one after the other where a signal proportion to their concentration is generated. The whole system is controlled by a computer. Applied gases The source of the eluent i.e. mobile phase can be a high pressure cylinder or gas generator. The most often applied gases in a gas chromatography lab include He, Ar, N2 and H2. As GC is a high performance analytical method it is necessary to use high purity gases.

Depending on the purity of the gas (Table 1) different on-line gas purifiers should be installed, too.

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

A critical point in gas chromatography is sample inlet. It is important to introduce the sample onto the column within the shortest possible time and at the same time to have it in the gas phase In theory, gas, liquid or even solid samples can be studied by GC but because of the slow evaporation of solids the samples injected are liquids or gases almost exclusively. Gas samples are injected by means of a six-port switching valve system. These injectors contain a sample loop of calibrated volume. First the loop is filled with the sample gas by washing it with

the sample gas of 5-10 times volume This is necessary to be sure that the loop contains only the components of the sample and no air or remaining eluent gas. Upon switching, the content of the sample loop is washed into the carrier gas stream and injected to the column

Columns

The heart of the GC equipment is the column where the separation of sample components occurs. The column is placed into an oven with controlled temperature and computer controlled heating can rise its temperature up to 400-500^{0}C but can be cooled to the initiate temperature also quickly. Columns can be divided into two groups. The so called packed columns are 1-5 m length and 2-6 mm internal diameter tubes filled with the appropriate stationary phase. As it has been mentioned already, in case of a separation based on adsorption the stationary phase could be a high surface area solid such as activated charcoal, Al2O3, silica, molecular sieve or organic polymer. In case of absorption based packed column the solid material is impregnated with the stationary liquid phase and then filled into the column.

Detectors

As it has been already discussed the components of the sample are separated on the column and get into the detector one after the other where a signal proportional with their concentration is generated by means of a physical method. The most often applied detectors in GC include Thermal Conductivity Detector (TCD), Flame Ionization Detector (FID), Electron Capture Detector (ECD), Mass Spectrometry (MS) detector and Infrared Spectrophotometric (IR) detectors. Other special detectors are also available such as Photoionization Detector (PID), Flame Photometric Detector (FPD), Pulsed Flame Photometric Detector (PFPD) or Atomic Emission Detector (AED).

Figure 4: GC Instrument[26]

CAPILLARY ELECTROPHORESIS - MASS SPECTROMETRY (CE-MS)

CE-MS, combining the high efficiency and resolution power of CE, with the high selectivity and sensitivity inherent to MS, is a very attractive analytical technique. However, CE-MS coupling, mostly by means of ESI [18], was not easy to implement since a closed electrical circuit is necessary not only for the electrophoretic separation but also for an efficient ionization in the source (with CE and ESI currents in the range of mA and nA, respectively). A solution for this problem is to ground the sprayer needle in order to divert all electrical energy from the CE to the ground and build an undisturbed electrical field for ionization in the MS source. Even if the sensitivity achieved with the use of a sheath flow is generally lower compared to sheath less interfaces, the robustness of the former system is generally better and detection limits in the low femtomole range can be achieved, especially when the flow rate of the sheath liquid is reduced to 500 nL/min. The detection of the narrow CE peaks requires the use of a fast and sensitive mass spectrometer. IT and TOF systems are adequate detectors because they acquire data over a suitable mass range with rates of several spectra per second.

CE-MS for bioanalysis of drugs

A number of recent reviews have covered the application of CE-MS for drug analysis, with some of them giving the fragmentations, when available, that the ionic species undergo in-source and in IT, triple quadrupole or TOF mass spectrometers [19]. This part of the review is dedicated to the analysis of drugs in biological fluids.

Practical considerations for robust and sensitive CE-MS coupling

CE has several advantages over HPLC, namely fast method development, low sample and solvent consumption, rapid and very efficient separations and, in the particular field of chiral separations, the use of expensive chiral stationary phases is not required. Nevertheless, problems of ruggedness in the on-line coupling of CE with MS often limit its application for quantitative purposes. Among others, this can be explained by variations in the migration times (MTs), due to fluctuations in the EOF and/ or the lack of thermo stating of the capillary part linking

the CE instrument to the MS source. Furthermore, some parameters of the electro spray, the most common CE interface, have to be carefully adjusted to obtain stable CE-ESI-MS conditions, namely the sheath liquid composition and flow rate, the nebulizing gas pressure, and the capillary outlet position.

Composition and flow rate of the sheath liquid

Even if the composition of the sheath liquid greatly depends on the studied analyte, some general rules can be prescribed. An aqueous solution containing 50– 80% of a moderately polar organic solvent is often required to achieve the formation of a stable spray, due to a decreased surface tension. On the other hand, a higher organic solvent content gives rise to a higher response for most organic analytes, due to a more efficient desolvation of the compound as well as a better stability of the spray.

Nebulizing gas pressure

In an ESI interface, the nebulizing gas pressure, used to assist droplet formation and to obtain a stable spray, is a compromise between sensitivity and spray stability. It is well known that the application of the nebulizing gas gives rise to a reduced pressure at the capillary outlet. Therefore, separation performance (efficiency and resolution) and MTs can be decreased, as a result of the hydrodynamic flow generated in the CE capillary. This decrease of separation performance and MTs with increasing nebulizing gas pressure is illustrated in the work. It also appeared that the nebulizing gas pressure influences the detection sensitivity. Indeed, at the highest pressure value, the S/N ratio was 20% lower than at the lowest value due to higher noise level while the abundance of drug improved with increasing nebulizing gas pressure.[20]

Capillary outlet position

To achieve reproducible CE-MS analysis, it is necessary to have a well-defined procedure to install a new capillary In the literature, several strategies have been reported. Very recently, Ohnesorge et al described a new procedure, based on the observation of a strong neostigmine carry-over effect [21]. After the replacement of the capillary, the analysis of the buffer, instead of the sample, was performed. The signal resulting from the neostigmine carryover effect was adjusted to a high intensity level, selected as reference intensity level for the following capillary changes, assuming that the intensity of the carry-over signal is constant.

Sample preparation

Most applications dealing with the bioanalysis of drugs in CE-MS were focused on serum and urine samples, although other biological fluids can be used, such as hair, cerebrospinal fluid (CSF), etc. Proteins, the main constituents of plasma, strongly adsorb onto the capillary wall and therefore adversely affect separation efficiency, resolution and MT. Urine contains inorganic ions and other endogenous compounds, such as urea, that can also interfere in the electrophoretic analysis. Moreover, the high ionic strength of urine is unfavourable to sample stacking, and therefore, peak broadening may be observed. Among sample preparation techniques, SPE and liquid/liquid extraction are efficient cleanup procedures which can also be applied to increase the analyte concentration. Nevertheless, these sophisticated sample preparation procedures are often tedious and time-consuming [22].

Figure 5: CE/MS instrument[27]

CONCLUSION

This review is aimed in focusing the role of various bioanalytical techniques in pharmaceuticals and gives a thorough literature survey of the bioanalytical methods and instruments in drug analysis. This review also highlights the recent advancements of bioanalytical techniques. Among all the bioanalytical methods, LCMSMS method is widely used and vast number of research publications has been reported by LC/MS/MS method due to its better sensitivity and precision.

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