Journal of Chromatography B, 1043 (2017) 3–11

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Bioanalytical method development and validation: Critical concepts and strategies

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ARTICLE INFO

Article history:
Received 16 June 2016
Received in revised form
17 September 2016
Accepted 19 September 2016
Available online 20 September 2016

Keywords:
Bioanalysis
Bioanalytical method development
Sample preparation
Liquid chromatography
Tandem mass spectrometry
Method validation
GLP

ABSTRACT

Bioanalysis is an essential part in drug discovery and development. Bioanalysis is related to the analysis of analytes (drugs, metabolites, biomarkers) in biological samples and it involves several steps from sample collection to sample analysis and data reporting. The first step is sample collection from clinical or preclinical studies; then sending the samples to laboratory for analysis. Second step is sample clean-up (sample preparation) and it is very important step in bioanalysis. In order to reach reliable results, a robust and stable sample preparation method should be applied. The role of sample preparation is to remove interferences from sample matrix and improve analytical system performance. Sample preparation is often labor intensive and time consuming. Last step is the sample analysis and detection. For separation and detection, liquid chromatography-tandem mass spectrometry (LC–MS/MS) is method of choice in bioanalytical laboratories. This is due to high selectivity and high sensitivity of the LC–MS/MS technique. In addition the information about the analyte chemical structure and chemical properties is important to be known before the start of bioanalytical work.

This review provides an overview of bioanalytical method development and validation. The main principles of method validation will be discussed. In this review GLP and regulated bioanalysis are described. Commonly used sample preparation techniques will be presented. In addition the role of LC–MS/MS in modern bioanalysis will be discussed. In the present review we have our focus on bioanalysis of small molecules.

1. Introduction

Bioanalysis has an important role in drug development. Today bioanalysis is an essential part in toxicological evaluation and in pharmacokinetic and pharmacodynamics studies during drug development. Bioanalytical method development is one of the bottle necks for drug development. Additionally bioanalytical method validation is a crucial for the quantitative determination of various types of analytes in biological matrices. The bioanalysis procedure includes sampling, sample preparation, analysis, calibration and data evaluation and reporting (Fig. 1). In modern bioanalysis a good sample preparation and a hyphenated instrumentation are required. In pharmaceutical research companies the development of comprehensive bioanalytical methods is very important during the process of drug discovery and development. In addition the method validation has an important role in regulatory bioanalysis to ensure the quality of the applied method. Bioanalytical method validation is very important for supporting of new drug applications or biologics license applications.

The present review provides critical points to consider when carrying out bioanalytical method development and validation. Until now, different publications have been published with method validation trends [1–3] but still lack of concise and comprehensive review for method validation providing specific requirements for conducting GLP nonclinical and clinical study sample analysis. In this paper required information for bioanalytical method development and method validation according to GLP guidelines will be presented.

2. Bioanalysis concept

Bioanalysis is covering the identification and quantification of analytes in biological samples (blood, plasma, serum, saliva, urine, feces, skin, hair, organ tissue). Bioanalysis is not only measuring of small molecules such as drugs and metabolites but also to iden-

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http://dx.doi.org/10.1016/j.jchromb.2016.09.028
1570-0232/

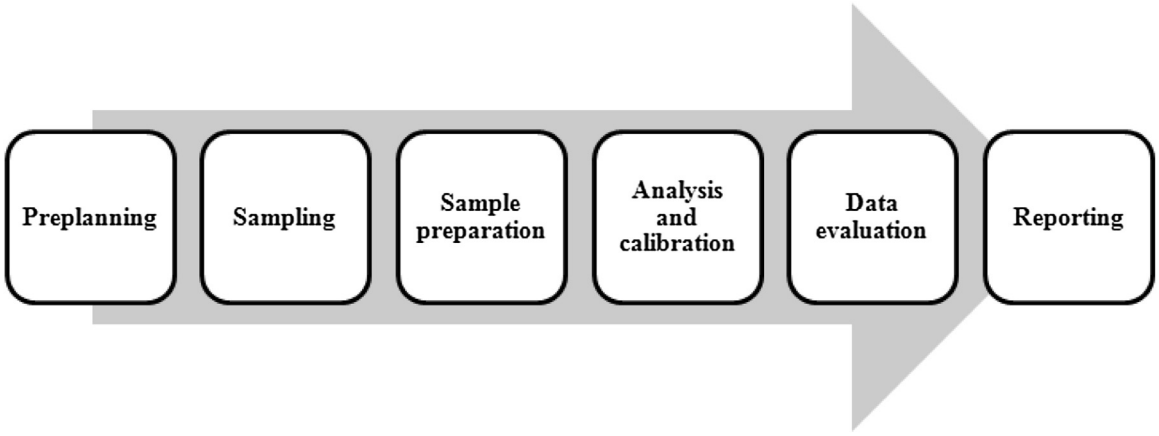

Fig. 1. Schematic diagram of bioanalytical workflow.

tify large molecules such as proteins and peptides. Bioanalysis is well established in pharmaceutical companies to support drug discovery and drug development. Bioanalysis has an important role to perform the toxicokinetic (TK), pharmacokinetic (PK) and pharmacodynamics (PD) studies of new drugs. Bioanalysis is also established in clinical, preclinical and forensic toxicology laboratories. Thus bioanalysis is an important discipline in many research areas such as the development of new drugs, forensic analysis, doping control and identification of biomarkers for diagnostic of many diseases.

Bioanalysis is challenging due to the complexity of the sample matrix [4–13]. It is well known that complex matrices such as blood, plasma and urine need an intensive sample preparation prior to injection to analytical instrument. High throughput sample preparation and hyphenated analytical instruments are required in modern bioanalysis. Liquid chromatography (LC) combined with tandem mass spectrometry (MS/MS) have been used for a long time in drug bioanalysis. Method validation is most important part in regulated bioanalysis. The validation is necessary to demonstrate the bioanalytical method performance [3]. The bioanalytical workflow contains many steps from sample collection to sample analysis and data reporting (Fig. 1). Information about the chemical properties of the analyte such as stability, volatility, reactivity and polarity should be prepared before the proceeding the bioanalytical work. In addition the expectation of the analyte concentration ranges and the nature of sample matrix are important to be known.

3. Method development

Before start of bioanalytical method development there are many points to consider. These points are analyte chemical structure, pKa value, solubility properties; stability and adsorption properties (to plastic or glass). Bioanalytical method development includes two main sections, sample preparation and sample separation and detection.

Sample preparation has an important role in bioanalysis to get clean extract with high extraction efficiency. Additionally choose of detector is depending on the analyte concentration range. Moreover choose of suitable internal standard (ISTD) is an important issue in bioanalytical method development. The role of internal standard is to compensate for matrix effects and to get accurate results. The ISTD should be similar to the analyte chemical structure and chemical properties. The best ISTD for LC–MS/MS bioanalysis is stable isotopically labelled compounds [4–6]. Today LC–MS/MS is the most used instrument in bioanalysis. LC–MS/MS has replaced HPLC-UV in many clinical laboratories in high income countries.

Table 1
Bioanalytical method development and validation strategies.

Subject	Requirements/Activity
Analyte	- Chemical structure - Chemical properties - Analyte purity
Sample preparation	- Nature and property of the matrix - Matrix stability - Choose of suitable extraction method - Extraction recovery
Method validation	- Calibration curve with QC samples - Accuracy - precision - Selectivity - Matrix effect - Carry-over - Stability (short and long term)

Overview over bioanalytical method development is presented in Table 1.

In addition the analyte concentration in bioanalysis is a dynamic concept which can be determined in many investigations such as drug pharmacokinetics, drug toxicity, metabolism and absorption [3]. To have an efficient and validate method needs a stable dynamic concentration range. For nonclinical or clinical studies the concentration range of the studied analytes (high/low range) should be approximately known. This is to decide the lower and upper limits of detection and to set up a calibration curve.

4. Established extraction techniques in drug bioanalysis

Sample clean-up (sample preparation) is a primarily step before injection of complex matrices such as plasma, blood, urine and tissue into the analytical instruments. Regularly used sample preparation methods in many bioanalytical laboratories are protein precipitation (PPT), Liquid-liquid extraction (LLE), solid phase extraction (SPE). In the following sections the main sample preparation techniques will be summarized and discussed.

4.1. Liquid-liquid extraction (LLE)

Despite of huge developments in sample preparation techniques, LLE is still an attractive technique in sample preparation and it has been widely used for the preparation of aqueous and biological samples [13–24]. Basically, in LLE an aqueous sample (e.g., plasma, urine) and an immiscible organic solvent are mixed to remove the analyte into the organic phase [25] for injection into an analytical system. This method can provide good recovery and clean