wjpmr, 2016, 2(3), 45-52

SJIF Impact Factor: 3.535

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Review Article

ISSN 2455-3301

WJPMR

BIOANALYSIS: AN EXTENSIVE APPLICATION IN PHARMACEUTICAL INDUSTRIES

Srijita Dutta*

Asst. Professor, Department of Pharmaceutical Technology, Bengal School of Technology, Sugandha, Delhi Road, Near Chuchura Railway Station, Hooghly, West Bengal.

***Correspondence for Author: Dr. Srijita Dutta**
Asst. Professor, Department of Pharmaceutical Technology, Bengal School of Technology, Sugandha, Delhi Road, Near Chuchura Railway Station, Hooghly, West Bengal.

Article Received on 01/03/2016 **Article Revised on 20/03/2016** **Article Accepted on 10/04/2016**

ABSTRACT

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. Standards set by regulatory bodies regarding method development and validation increasingly define the boundaries between speed and quality. With this emphasis in the use of PK/toxicokinetics and the greater potencies of newer drugs, a sensitive and specific bioanalytical technique is essential. Many scientific endeavors are dependent upon accurate quantification of drugs and endogenous substances in biological samples; 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 exposureresponse (pharmacokinetics/pharmacodynamics studies). Bioanalysis also applies to drugs used for illicit purposes, forensic investigations and environmental concerns. The need for sound bioanalytical methods is well understood and appreciated in the discovery phase and during the preclinical and clinical stages of drug development. Therefore, it is generally accepted that sample preparation and method validation are required to demonstrate the performance of the method and the reliability of the analytical results. Now it is widely accepted that bioanalysis is an integral part of the pharmacokinetic/pharmacodynamic characterization of a novel chemical entity from the time of its discovery and during various stages of drug development, leading to its market authorization.

KEYWORDS: Bioanalysis, Pharmacokinetics, Toxicology, Validation.

INTRODUCTION

Bioanalysis is a sub-discipline of analytical chemistry covering the quantitative measurement of xenobiotics (drugs and their metabolites, and biological molecules or concentrations) and biotics (macromolecules, proteins, DNA, large molecule drugs, metabolites) in biological systems.

It 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. Standards set by regulatory bodies regarding method development and validation increasingly define the boundaries between speed and quality. Bioanalysis encourages the submission of any forward looking applications, including biosensors, microfluidics, miniaturized analytical devices, and new hyphenated and multi-dimensional techniques. [1]

Bioanalytical testing provides a quantitative measure of the active drugs and their metabolites in biological system for the purpose of pharmacokinetics/ pharmacodynamics study. It is performed across the value chain of drug development and plays a key role in lead optimization and moving a drug candidate along the development process. The bioanalytical testing during the drug discovery and lead optimization stage is conducted in-house by the pharmaceuticals whereas the later stages such as long term toxicity and Phase II studies are outsourced.

The growth in small molecule NCEs (New Chemical Entity) has been less than 1% per annum in contrast to that of large molecule NBE (New Biological Entity) which is about 25% from 2000 to 2010. Furthermore the sale of the small molecules is growing at a CAGR of 3.9% and that of large molecules at a CAGR of 10.1% from 2009 to 2015. This growth in the number of molecule development and sales for large molecules indicate that the therapeutics coming into the market over the next 5-10 years would be consisting 30-50% of biologics. Bioanalytical testing of these large molecules

has been a challenging task for the pharmaceutical industry as the traditional techniques used for classical drugs are not adaptable to the large molecules having high molecular weight and complex structure. This has led into the advancement of techniques such as Ligand binding assays (LBA), Maldi-TOF-MS, size exclusion affinity chromatography, HRMS etc. that can be used for large molecule bioanalytical testing. The CROs are addressing the industry need by adding these services through mergers/ acquisition or expansion which can be leveraged by the pharmaceuticals.

ECONOMIC ASPECTS OF LARGE MOLECULE DRUG DEVELOPMENT

The time and funds required to discover and develop a new drug is around 10 years and requires approximately $1 billion, and the average number of large molecules being approved a year is 9 compared to small molecules which is 23 a year, hence it is very crucial to evaluate the candidates critically at the early stages of the drug development. The failure of drugs at various stages of the development and the expiry of patents mainly for the existing biologics has led to a recession in the Pharma R&D as the expected Biologic market value that will be off patent in the next 5 years is around $54 billion. Therefore the pharmaceuticals are in a spree to reduce their in- house capabilities and fixed costs and bioanalytical testing soaring the list of large spend by pharmaceuticals, the need for sound bioanalytical testing aiding as a critical tool in the drug discovery and development has to be understood well.[2]

Now it is widely accepted that bioanalysis is an integral part of the pharmacokinetic/pharmacodynamic characterization of a novel chemical entity from the time of its discovery and during various stages of drug development, leading to its market authorization. In this compilation, the important bioanalytical parameters and its application to drug discovery and development approaches are discussed, which will help in the development of safe and more efficacious drugs with reduced development time and cost. It is intended to give some general thoughts in this area which will form basis of a general framework as to how one would approach bioanalysis from inception (i.e., discovery of a lead molecule) and progressing through various stages of drug development. [3]

The emergence of the field of bioanalysis as a critical tool during the process of drug discovery and development is well understood and globally accepted.[4-8] Over the past few decades, a plethora of assays has been continuously developed for NCEs to support various stages of discovery and development, including assays for important metabolites.[9-12] Additionally, multiple analytical procedures are available for prescription medicines (Rx) and/or generic products.[13-21] Bioanalytical data generated in discovery and pre-clinical programs are a valuable guide to early clinical programs.

Bioanalytical methods employed for the quantitative determination of drugs and their metabolites in biological matrix (plasma, urine, saliva, serum etc) play a significant role in evaluation and interpretation of bioavailability, bioequivalence and pharmacokinetic data.[22] Chromatographic methods such as Gas Chromatography (GC), Liquid Chromatography Mass Spectrometry (LC-MS) etc. are commonly used in laboratories for the qualitative and quantitative analysis of drug substances and biological samples throughout all phases of method development of a drug in research and quality control. Further, method validation is carried out to ensure that the method developed is accurate, specific, reproducible and rugged over the specified range in which an analyte is analyzed.[23] 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.[24] 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.[25] 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.[26, 27] The fundamental bioanalytical method validation parameters include precision and accuracy, sensitivity, reproducibility, recovery and stability. However, the stability of the method can be determined by several methods including freeze and thaw method, short-term temperature stability study, long term stability and bench top stability.

A bioanalytical method consists of two main component Sample preparations

Sample preparation is a technique used to clean up a sample before analysis and/or to concentrate a sample to improve its detection. When samples are biological fluids such as plasma, serum or urine, this technique is described as bioanalytical sample preparation. The determination of drug concentrations in biological fluids yields the data used to understand the time course of drug action, or PK, in animals and man and is an essential component of the drug discovery and development process.[28] Most bioanalytical assays have a sample preparation step to remove the proteins from the sample. Protein precipitation, liquidliquid extraction and solid phase extraction (SPE) are routinely used. [29]

Detection of the compound: The detector of choice is a mass spectrometer.[28] Currently, the principle technique used in quantitative bioanalysis is high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) using either electrospray ionization (ESI) or atmospheric pressure chemical

ionization (APCI) techniques.[30] The triple quadrupole (QqQ) mass spectrometer (MS), when operated in the selected reaction monitoring (SRM) mode, offers a unique combination of sensitivity, specificity and dynamic range. Consequently, the QqQ MS has become the instrument of choice for quantitation within the pharmaceutical industry. Since ESI and APCI can be operated at flow rates as high as 1 and 2 mL/min, respectively, most of the convenience columns (e.g., C18, C8, C4, phenyl, cyanopropyl) are compatible. Recent technological advances have made 1.7 µm particle size packing material available. Coupling with high pressure pump and high-speed acquisition MS, ultra-high pressure liquid chromatography (UPLC) offers unique high-throughput and resolving power to obtain maximum chromatographic performance and superior assay sensitivity. [31]

Before a bioanalytical method can be implemented for routine use, it is widely recognized that it must first be validated to demonstrate that it is suitable for its intended purpose. A GLP (Good Laboratory Practices) validated bioanalytical method is needed to support all development studies (e.g., toxicology studies and human clinical trials). According to the Food and Drug Administration (FDA) GLP guidance, [32] there is a general agreement that at least the following validation parameters should be evaluated for quantitative procedures: selectivity, calibration model, stability, accuracy (bias, precision) and limit of quantification. Additional parameters which might have to be evaluated include limit of detection (LOD), recovery, reproducibility and ruggedness (robustness).[33,35] Validation involves documenting, through the use of specific laboratory investigations, that the performance characteristics of the method are suitable and reliable for the intended analytical applications. The acceptability of analytical data corresponds directly to the criteria used to validate the method. [36]

In early stages of drug development, it is usually not necessary to perform all of the various validation studies. Many researchers focus on specificity, linearity and precision studies for drugs in preclinical through Phase II (preliminary efficacy) stages. The remaining studies penetrating validation are performed when the drug reaches the Phase II (efficacy) stage of development and has a higher probability of becoming a marketed product. Presently, Guidelines for pharmaceutical methods in United States pharmacopoeia (USP), International Conference on Harmonization (ICH) and FDA provide a framework for regulatory submission must include study on such fundamental parameters.

VALIDATION PARAMETERS

There is a general agreement that at least the following validation parameters should be evaluated for quantitative procedures: selectivity, calibration model, stability, accuracy (bias, precision) and limit of quantification. Additional parameters which might have to be evaluated include LOD, recovery, reproducibility and ruggedness (robustness).

1. Specificity/selectivity

A method is specific if it produces a response for only one single analyte. Since it is almost impossible to develop a chromatographic assay for a drug in a biological matrix that will respond to only the compound of interest, the term selectivity is more appropriate. The selectivity of a method is its ability to produce a response for the target analyte which is distinguishable from all other responses (e.g., endogenous compounds such as protein, amino acids, fatty acids, etc).[37]

2. Accuracy

Accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. This is sometimes termed as trueness. The two most commonly used ways to determine the accuracy or method bias of an analytical method are (i) analyzing control samples spiked with analyte and (ii) by comparison of the analytical method with a reference method.[38]

3. Precision

It is the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogenous volume of biological matrix.[32]

There are various parts to precision, such as repeatability, intermediate precision, and reproducibility (ruggedness). Repeatability means how the method performs in one lab and on one instrument, within a given day. Intermediate precision refers to how the method performs, both qualitatively and quantitatively, within one lab, but now from instrument-to-instrument and from day-to-day. Finally, reproducibility refers to how that method performs from lab-to-lab, from day to-day, from analyst-to-analyst, and from instrument-to instrument, again in both qualitative and quantitative terms.[37-38]

4. Detection Limit

The LOD is the lowest concentration of analyte in the sample that can be detected but not quantified under the stated experimental conditions.[39] There is an overall agreement that the LOD should represent the smallest detectable amount or concentration of the analyte of interest.

5. Quantitation Limit

The quantitation limit of individual analytical procedures is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

6. Linearity

According to the ICH definition, the linearity of an analytical procedure is its ability (within a given range)

to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. If the total range cannot be described by a single calibration curve, two calibration ranges can be validated. Correlation coefficients were most widely used to test linearity. Although the correlation coefficient is of benefit for demonstrating a high degree of relationship between concentration and response data, it is of little value in establishing linearity.[40] Therefore, by assessing an acceptable high correlation coefficient alone the linearity is not guaranteed and further tests on linearity are necessary, for example, a lack-of-fit test.

7. Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.[32]

8. Robustness

It is the measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal usage.

9. Extraction recovery

It can be calculated by comparison of the analyte response after sample workup with the response of a solution containing the analyte at the theoretical maximum concentration. Therefore, absolute recoveries can usually not be determined if the sample workup includes a derivatization step, as the derivatives are usually not available as reference substances.

10. Stability

It is the chemical stability of an analyte in a given matrix under specific conditions for given time intervals.[32] The aim of a stability test is to detect any degradation of the analyte(s) of interest during the entire period of sample collection, processing, storing, preparing, and analysis.[41] All but long-term stability studies can be performed during the validation of the analytical method. Long-term stability studies might not be complete for several years after clinical trials begin. The condition under which the stability is determined is largely dependent on the nature of the analyte, the biological matrix, and the anticipated time period of storage (before analysis).

DRUG DISCOVERY/DESIGN

Initially, in the discovery stage, the aim of bioanalysis could be merely to provide reasonable values of either concentrations and/or exposure which would be used to form a scientific basis for lead series identification and/or discrimination amongst several lead candidates. Therefore, the aim of the analyst at this stage should be to develop a simple, rapid assay with significant throughput to act as a great screening tool for reporting some predefined parameters of several lead contenders across all the various chemical scaffolds.

The initial method of analysis developed during the discovery phase of the molecule, with some modifications, may sometimes serve as a method of choice to begin with as the NCE enters the preclinical development stage. Since the complexity of development generally tends to increase as the lead candidate enters the toxicological and clinical phase of testing, it naturally calls for improved methods of analytical quantization, improvement in selectivity and specificity, and employment of sound and rugged validation tools to enable estimation of PK parameters that would also aid in the decision-making of the drug molecule's advancement in the clinic in addition to safety and tolerability data gathered at all phases of development. Additionally, it becomes necessary to quantify active metabolite(s) in both animals and humans.[42]

Drug discovery/design consists of identification and characterization of new targets (enzymes or receptors), synthesis of new lead molecules, screening of new lead molecules for in vitro and/or in vivo biological activities, and physicochemical characterization of leads.[43] For discovery, the priority is to examine a large number of compounds and determine which pharmacologically active compounds are most suitable for drug development. In practice, when a compound is obtained which has the required biological activity, a number of analogues or chemically similar compounds will be synthesized and tested to optimize the preferred characteristics of the compound (a process known as lead optimization). In the secondary screening stage, physiochemical properties such as solubility, lipophilicity and stability are determined by using octanolwater partition coefficient and pKa. These measurements are useful in predicting protein binding, tissue distribution and absorption in gastrointestinal tract.[44]

In parallel studies, information is learned on a drug molecule's absorption, distribution (including an estimate of protein binding), metabolism and elimination by sampling from dosed laboratory animals (called in vivo testing) and from working cells and/or tissues removed from a living organism (called in vitro testing since the cells are outside a living animal). For in vivo characterization of PK and bioavailability, it is necessary to administer the drug to selected animal species both intravenously and by the intended route of administration (usually oral). Whole blood samples are collected over a predetermined time course after dosing, and the drug is quantified in the harvested plasma by a suitable bioanalytical method. The use of in vitro drug metabolism approaches for the prediction of various in vivo PK characteristics is widely practiced in the pharmaceutical industry.[45,48] In particular, in vitro metabolic stability assessment using hepatic subcellular fractions to predict in vivo hepatic clearance is employed

as part of the initial screening of candidates in a lead optimization program. This is because the liver is the main organ involved in the metabolism of xenobiotics, the process by which most drugs are cleared from the body. The correlation between in vivo hepatic clearance values and the intrinsic clearance values determined from liver microsomal incubation experiments is also well documented.[49,52] These important tests are collectively referred to as ADME characteristics (Absorption, Distribution, Metabolism and Elimination).[28]

ADME/PK screening is usually taken to mean in vitro systems for studying absorption and metabolism. However, in vivo studies still provide the definitive assessment of overall drug disposition, and progress has been made in overcoming some of the constraints associated with this approach. Previously, drug metabolism studies were performed at a late stage of drug development process and very often not until the phase of clinical studies. Therefore, inadequate metabolism and PK parameters were the major reason of failure for NCEs.[53]

DRUG DEVELOPMENT

It focuses on evaluation of safety/toxicity and efficacy of new drug molecules. However, majority of the drug molecules fail in subsequent drug development program because the efficacy and safety are not governed by its PD characteristics alone. It also depends to a large degree on the biopharmaceutical (e.g., solubility, stability, permeability and first pass effect) and PK (clearance rate, biological half-life, extent of protein binding and volume of distribution) properties of the drug, since these properties control the rate and the extent to which the drug can reach its site of action, i.e., biophase.[54] Some data on reasons for withdrawal of candidate drugs from development have been published by the Center for Medicines Research.[55]

Preclinical Stage

Once a chemical is identified as a new drug candidate, extensive preclinical analyses must be completed before the drug can be tested in humans.[56] The main goals of preclinical studies (also named nonclinical studies) are to determine a product's ultimate safety profile. Each class of product may undergo different types of preclinical research. For instance, drugs may undergo pharmacodynamics (PD), PK, ADME, and toxicity testing through animal testing. During preclinical investigation, validation should be formalized and mandated as per the required norm. The validation should address as many parameters as possible which are relevant, to obtain unambiguous analytical data [the list could include accuracy, precision, specificity, selectivity, linearity range, lower limit of quantification (LLQ), upper limit of quantification (ULQ), dilution effect, stability or extraction recovery]. Since the data gathered during this stage, especially PK and toxicokinetic properties of the NCE, would become part of the initial investigational new drug and clinical trial (IND/CTA) filings in several regions, the adherence to certain rigid validation parameters and protocols becomes of paramount importance. [57]

In the pharmaceutical industry, the term toxicokinetics is generally used to describe the PK performed at the dose levels used in the toxicological risk assessment of drugs. The aims of the toxicokinetic evaluation are:

- To define the relationship between systemic exposure to test compound and the administered dose,
- To provide information on potential dose- and time-dependenciesin the kinetics,
- To determine the effect of age on the PK in animals, provide clearer delineation when there are sex-related differences, determine whether there are any changes in kinetics in pregnancy (during reproductive toxicology studies) and also provide greater detail on inter species comparisons.

However, the overall aim in conducting toxicokinetics during safety studies is to extrapolate the risk assessment from the toxicity test species to humans.[28] Whilst preliminary PK and toxicokinetic data are obtained in drug development in preclinical species, the definitive kinetics is obtained in drug development by conducting single dose experiments in preclinical species and in humans. These data are essential in defining the dosage regimen in man and ensuring that the therapeutic benefit is maximized.[58- 63]

Clinical Stage

Clinical trials are used to judge the safety and efficacy of new drug therapies in humans. Drug development comprises of four clinical phases: Phase I, II, III and IV. Each phase constitutes an important juncture, or decision point, in the drug's development cycle. A drug can be terminated at any phase for any valid reason. As the molecule advances into clinical development, the developed assay for human sample analyses (plasma, serum or urine matrix) needs to be more rugged, robust and be able to withstand the test of time during this the longest phase of clinical development.[64-67]

CONCLUSION

Reliable data obtained from selective, sensitive and reproducible analysis of a drug and its metabolites in biological samples is a fundamental and crucial part of every successful drug development program. Indeed, the same principles apply to many areas such as forensic science, toxicology and sports doping testing. The field of bioanalysis has matured significantly from early studies in drug metabolism using simple colorimetry. With the proliferation of sophisticated hyphenated techniques linking advanced separations with mass spectrometry and NMR as detection systems, automation and robotics, today's bioanalyst is well equipped to deal with the modern challenges of analyzing xenobiotics in biological matrices much faster and with a higher level of confidence. Furthermore, bioanalysts are now

involved with the discovery, measurement and qualification of pharmacogenomic profiles and biomarkers and, subsequently, the development of diagnostic kits to individualize patient characterization and treatment.

In todays highly competitive global drug development arena, it is more important than ever that the modern bioanalytical laboratory is optimized for speed and success. The content is uniquely targeted to those working on the analysis of drugs and metabolites in biological matrices. This is, primarily, bioanalysts working in pharmaceutical research and development, clinical laboratories, clinical toxicologists, forensic toxicologists and sports doping analysts.

The need for sound bioanalytical methods is well understood and appreciated in the discovery phase and during the preclinical and clinical stages of drug development. Therefore, it is generally accepted that sample preparation and method validation are required to demonstrate the performance of the method and the reliability of the analytical results. For bioanalytical methods, sample preparation techniques, the essential validation parameters with their guidelines and application of validation work in drug discovery and development phase have been discussed here.

REFERENCES

1. http://www.futurescience.com/page/journal/bio/aims .js p
2. Sarika Khasnis. Large Molecule Bioanalysis Outsourcing opportunities & Challenges. Pharma Focus Asia Magazine.
3. Saurabh P, Preeti P, Gaurav T, and Ruchi T; Bioanalysis in drug discovery and development. Pharm Methods, 2010; 1(1): 1424.
4. Kantharaj E, Tuytelaars A, Proost PE, Ongel Z, van AH, Gillissen AH; Simultaneous measurement of drug metabolic stability and identification of metabolites using ion-trap mass spectrometry. Rapid Commun Mass Spectrom, 2003; 17: 2661.
5. Bu HZ, Magis L, Knuth K, Teitelbaum P; Highthroughput cytochrome P450 (CYP) inhibition screening via a cassette probedosing strategy; Rapid Commun Mass Spectrom, 2001; 15:,741.
6. Cai Z, Sinhababu AK, Harrelson S; Simultaneous quantitiative cassette analysis of drugs and detection of their metabolites by high performance liquid chromatogramphy/ion trap mass spectrometry. Rapid Commun Mass Spectrom, 2000; 14: 1637.
7. Scott RJ, Palmer J, Lewis I A, Pleasance S; Determination of a GW cocktail of cytochrome P450 probe substrates and their metabolites in plasma and urine using automated solid phase extraction and fast gradient liquid chromatography tandem mass spectrometry; Rapid Commun Mass Spectrom, 1999; 13: 2305.
8. Gautam N, Singh RP, Pratap R, Singh SK; Liquid chromatographic-tandem mass spectrometry assay for quantitation of a novel antidiabetic S002-853 in rat plasma and its application to pharmacokinetic study; Biomed Chromatogr, 2010; 24: 6928.
9. Satonin DK, McCulloch JD, Kuo F, Knadler MP; Development and validation of a liquid chromatography-tandem mass spectrometric method for the determination of major metabolites of duloxetine in human plasma; J Chromatogr B Analyt Technol Biomed Life Sci; 2007; 852: 5829.
10. Zeng J, Onthank D, Crane P, Unger S, Zheng N, Pasas-Farmer S; Simultaneous determination of a selective adenosine 2A agonist, BMS-068645, and its acid metabolite in human plasma by liquid chromatography-tandem mass spectrometry evaluation of the esterase inhibitor, diisopropyl fluorophosphates, in the stabilization of a labile ester containing drug; J Chromatogr B Analyt Technol Biomed Life Sci., 2007; 852: 7784.
11. He F, Bi HC, Xie ZY, Zuo Z, Li JK, Li X; Rapid determination of six metabolites from multiple cytochrome P450 probe substrates in human liver microsome by liquid chromatography/mass spectrometry: application to high throughput inhibition screening of terpenoids; Rapid Commun Mass Spectrom, 2007; 21: 63543.
12. Chawla S, Ghosh S, Sihorkar V, Nellore R, Kumar TR, Srinivas NR; High performance liquid chromatography method development and validation for simultaneous determination of five model compoundsantipyrine, metoprolol, ketoprofen, furesemide and phenol red, as a tool for the standardization of rat insitu intestinal permeability studies using timed wavelength detection; Biomed Chromatogr, 2006; 20: 34957.
13. Mercolini L, Mandrioli R, Finizio G, Boncompagni G, Raggi MA; Simultaneous HPLC determination of 14 tricyclic antidepressants and metabolites in human plasma; J Sep Sci, 2010; 33: 2330.
14. Singh RP, Sabarinath S, Singh SK, Gupta RC; A sensitive and selective liquid chromatographic tandem mass spectrometric assay for simultaneous quantification of novel trioxane antimalarials in different biomatrices using sample-pooling approach for high throughput pharmacokinetic studies; J Chromatogr B Analyt Technol Biomed Life Sci., 2008; 864: 5260.
15. Venkatesh G, Majid MI, Ramanathan S, Mansor SM, Nair NK, Croft SL; Optimization and validation of RP-HPLC-UV method with solid-phase extraction for determination of buparvaquone in human and rabbit plasma: application to pharmacokinetic study; Biomed Chromatogr, 2008; 22: 53541.
16. Navin G, Amin EL, Eugene G, Guenther H; Simultaneous Determination of Dexamethasone, Dexamethasone 21-Acetate, and Paclitaxel in a Simulated Biological Matrix by RP-HPLC: Assay Development and Validation; J Liq Chromatogr Relat Technol, 2008; 31: 147891.

17. Podilsky G, Gryllaki MB, Testa B, Pannatier A; Development and Validation of an HPLC Method for the Simultaneous Monitoring of Bromazepam and Omeprazole; J Liq Chromatogr Relat Technol, 2008; 31: 87890.
18. Basha SJ, Naveed SA, Tiwari NK, Shashikumar D, Muzeeb S, Kumar TR; Concurrent determination of ezetimibe and its phase- I and II metabolites by HPLC with UV detection: Quantitative application to various in vitro metabolic stability studies and for qualitative estimation in bile; J Chromatogr B Analyt Technol Biomed Life Sci, 2007; 853: 8896.
19. Link B, Haschke M, Wenk M, Krahenbuhl S; Determination of midazolam and its hydroxyl metabolites in human plasma and oral fluid by liquid chromatography/electrospray ionization ion trap tandem mass spectrometry; Rapid Commun Mass Spectrom; 2007; 21: 1531.
20. Oostendrop RL, Beijnen JH, Schellens JH, Tellingen OV; Determination of imatinib mesylate and its main metabolite (CGP74588) in human plasma and murine specimens by ionpairing reversed-phase high-performance liquid chromatography; Biomed Chromatogr, 2007; 21: 747.
21. Keil K, Hochreitter J, DiFrancesco R, Zingman BS, Reichman RC, Fischl MA; Integration of atazanavir into an existing liquid chromatography UV method for protease inhibitors: validation and application; Ther Drug Monit., 2007; 29:103.
22. Bressolle F, Bromet P, Audran M; Validation of liquid chromatography and gas chromatographic methods application to pharmacokinetics; J. Chromatogr. B, 1996; 686: 3-10.
23. Hartmann C, McDowall RD; Validation of bioanalytical chromatographic methods. J. Pharm Biomed Anal, 1998; 17: 193-18.
24. US Food and Drug Administration, Guidance for industry- Bioanalytical method validation, Center for Drug Evaluation and Research, Rockville, MD; 2001. (Available at, http://www.fda.gov/)
25. Green MJ; A practical guide to analytical method validation; Anal. Chem; 1996; 68: 305-09.
26. Shah VP, Midha KK, Findlay J, Tonelli A, Viswanathan C; Bioanalytical method validation- a revisit with a decade of progress; Pharm. Res., 2000; 17: 1551 -57.
27. Karnes HT, Shiu G, Shah VP; Validation of bioanalytical methods; Pharm. Res., 1991; 8:221-25.
28. Wells DA; High throughput bioanalytical sample preparation methods and automation strategies; Amsterdam: Elsevier; 2002; 41.
29. Ahuja S, Rasmussen H; HPLC method development of pharmaceuticals. Chapter 11: HPLC method development for drug discovery LC-MS assays in rapid PK applications; 2007.
30. Xu X; Book Using mass spectrometry for drug metabolism studies; In: Korfmacher W, editor. Chapter 7: Fast Metabolite Screening in a Discovery Setting. 2nd ed. CRC Press; 2010.
31. Churchwell MI, Twaddle NC, Meeker LR, Doerge DR; Improving LC-MS sensitivity through increases in chromatographic performance: Comparison of UPLC-ES/MS/MS to HPLC-ES/MS/MS; J Chromatogr B Analyt Technol Biomed Life Sci., 2005; 825: 13443.
32. Guidance for Industry: Bioanalytical Method Validation. Rockville, MD: US Department of Health and Human Services, FDA, Center for Drug Evaluation and Research; 2001. Food and Drug Administration.
33. Hartmann C, Smeyers-Verbeke J, Massart DL, McDowall RD; Validation of bioanalytical chromatographic methods; J Pharm Biomed Anal, 1998; 17: 193218.
34. Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, Yacobi A; Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies; Conference report.; Eur J Drug Metab Pharmacokinet, 1991; 16: 24955.
35. Shah VP, Midha KK, Findlay JW, Hill HM, Hulse JD, McGilveray IJ; Bioanalytical method validation--a revisit with a decade of progress; Pharm Res, 2000; 17: 15517.
36. Rosing H, Man WY, Doyle E, Bult A, Beijnen JH; Bioanalytical liquid chromatographic method validation. a review of current practices and procedures; J Liq Chromatogr Relat Technol, 2000; 23: 32954.
37. Dadgar D, Burnett PE; Issues in evaluation of bioanalytical method selectivity and drug stability; J Pharm Biomed Anal., 1995; 14: 2331.
38. Karnes HT, Shiu G, Shah VP; Validation of bioanalytical methods; Pharm Res., 1991; 8: 4216.
39. Krull IS, Swartz M; Analytical method development and validation for the academic researcher; Anal Lett., 1999; 32:106780.
40. Braggio S, Barnaby RJ, Grossi P, Cugola M; A strategy for validation of bioanalytical methods; J Pharm Biomed Anal., 1996; 14: 37588.
41. Peng GW, Chiou WL; Analysis of drugs and other toxic substances in biological samples for pharmacokinetic studies; J Chromatogr, 1990; 531: 350.
42. Srinivas NR; Applicability of bioanalysis of multiple analytes in drug discovery and development: review of select case studies including assay development considerations; Biomed Chromatogr, 2006; 20: 383414.
43. Panchagnula R, Thomas NS; Biopharmaceutics and pharmacokinetics in drug research; Int J Pharm, 2000; 201: 13150.
44. Watari N, Sugiyama Y, Kaneniwa N, Hiura M; Prediction of hepatic first-pass metabolism and plasma levels following intravenous and oral administration of barbiturates in the rabbit based on quantitative structure-pharmacokinetic relationships; J Pharmacokinet Biopharm, 1988; 16: 279301.
45. Cheng KC, Li C, Liu T, Wang G, Hsieh Y, Yunsheng P; Use of Pre-Clinical In Vitro and In

Vivo Pharmacokinetics for the Selection of a Potent Hepatitis C Virus Protease Inhibitor, Boceprevir, for Clinical Development; Lett Drug Des Discov, 2009; 6: 3128.

46. Liu B, Chang J, Gordon WP, Isbell J, Zhou Y, Tuntland T. Snapshot PK; A rapid in vivo preclinical screening approach; Drug Discov Today, 2008; 13: 3607.
47. Rostami-Hodjegan A, Tucker GT; Simulation and prediction of in vivo drug metabolism in human populations from in vitro data; Nat Rev Drug Discov, 2007; 6: 1408.
48. Wienkers LC, Heath TG; Predicting in vivo drug interactions from in vitro drug discovery data; Nat Rev Drug Discov, 2005; 4: 82533.
49. Naritomi Y, Terashita S, Kimura S, Suzuki A, Kagayama A, Sugiyama Y; Prediction of Human Hepatic Clearance from in Vivo Animal Experiments and in Vitro Metabolic Studies with Liver Microsomes from Animals and Humans; Drug Metab Dispos, 2001; 29: 131624.
50. Obach RS; Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: An examination of in vitro half-life approach and nonspecific binding to microsomes; Drug Metab Dispos, 1999; 27: 13509.
51. Houston JB; Utility of in vitro drug metabolism data in predicting in vivo metabolic clearance; Biochem Pharmacol, 1994; 47:146979.
52. Clarke SE, Jeffrey P; Utility of metabolic stability screening: comparison of in vitro and in vivo clearance; Xenobiotica, 2001; 31(8): 591598.
53. Evans G; A handbook of bioanalysis and drug metabolism; New York LLC: CRC Press; 2004.
54. White RE, Manitpisitkul P; Pharmacokinetic theory of cassette dosing in drug discovery Screening; Drug Metab Dispos, 2001; 29: 95766.
55. Prentis RA, Lis Y, Walker SR; Pharmaceutical innovation by the seven UK-owned pharmaceutical companies (1964-1985); Br J Clin Pharmacol, 1988; 25: 38796.
56. Joseph TD; Encyclopedia of clinical pharmacy; London; Newyork: Taylor and Francis Group, Marcel Dekker; 2003.
57. Wieling J; LC-MS-MS Experiences with Internal Standards; Chromatographia Supplement, 2002; 55.
58. Kostiainen R, Kotiaho T, Kuuranne T, Auriola S; Liquid chromatography/atmospheric pressure ionization-mass spectrometry in drug metabolism studies; J Mass Spectrom, 2003; 38: 35772.
59. Coowanitwong I, Keay SK, Natarajan K, Garimella T S, Mason C W, Grkovic D; Toxicokinetic Study of Recombinant Human Heparin-Binding Epidermal Growth Factor-Like Growth Factor (rhHBEGF) in Female Sprague Dawley Rats; Pharm Res, 2008; 25: 54250.
60. Brown AP, Carlson TC, Loi CM, Graziano MJ; Pharmacodynamic and toxicokinetic evaluation of the novel MEK inhibitor, PD0325901, in the rat following oral and intravenous administration; Cancer Chemother Pharmacol, 2007; 59: 6719.
61. Baldrick P; Toxicokinetics in preclinical evaluation; Drug Discov Today, 2003; 8: 12733.
62. Welling PG, Iglesia FA; Drug toxicokinetics; New York: Marcel and Dekker. 1993.
63. Morgan DG, Kelvin AS, Kinter LB, Fish CJ, Kerns WD, Rhodes G; The Application of Toxicokinetic Data to Dosage Selection in Toxicology Studies; Toxicol Pathol, 1994; 22: 11223.
64. De Bruijn P, Moghaddam-Helmantel IM, de Jonge MJ, Meyer T, Lam M H, Verweij J; Validated bioanalytical method for the quantification of RGB-286638, a novel multi-targeted protein kinase inhibitor, in human plasma and urine by liquid chromatography/tandem triple- quadrupole mass spectrometry; J Pharm Biomed Ana., 2009; 50: 97782.
65. Glade Bender JL, Adamson PC, Reid JM, Xu L, Baruchel S, Shaked Y; Phase I Trial and Pharmacokinetic Study of Bevacizumab in Pediatric Patients With Refractory Solid Tumors: A Children's Oncology Group Study; J Clin Oncol, 2008; 26: 399405.
66. Laurie SA, Gauthier I, Arnold A, Shepherd FA, Ellis PM, Chen E; Phase I and Pharmacokinetic Study of Daily Oral AZD2171, an Inhibitor of Vascular Endothelial Growth Factor Tyrosine Kinases, in Combination With Carboplatin and Paclitaxel in Patients With Advanced NonSmall-Cell Lung Cancer: The National Cancer Institute of Canada Clinical Trials Group; J Clin Oncol, 2008; 26: 18718.
67. Oswald S, Scheuch E, Cascorbi I, Siegmund W; LCMS/MS method to quantify the novel cholesterol lowering drug ezetimibe in human serum, urine and feces in healthy subjects genotyped for SLCO1B1; J Chromatogr B Analyt Technol Biomed Life Sci., 2006; 830: 14350.