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Bioanalysis in drug discovery and development

Abstract

Recent years have witnessed the introduction of several high-quality review articles into the literature covering various scientific and technical aspects of bioanalysis. 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.

Key words: Bioanalytical, method validation, metabolism, pharmacokinetics, toxicokinetic

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INTRODUCTION

The discovery and development of a new drug costs around $1 billion and it may take approximately 10 years for the drug to reach the marketplace. [1] Drug discovery and development is the process of generating compounds and evaluating all their properties to determine the feasibility of selecting one novel chemical entity (NCE) to become a safe and efficacious drug. Strategies in the drug discovery and drug development processes are undergoing radical change. For example, the contribution of pharmacokinetics (PK) to both processes is increasing.[2,3] Furthermore, toxicokinetics has now become established as an essential part of toxicity testing.[4,5] With this emphasis in the use of PK/toxicokinetics and the greater potencies of newer drugs, a sensitive and specific bioanalytical technique is essential.

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.[6-9] 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.[10-14] Additionally, multiple analytical procedures are available for prescription medicines (Rx) and/or generic products.[15-23] Bioanalytical data generated in discovery and pre-clinical programs are a valuable guide to early clinical programs. Plasma concentration–response data from these programs can be compared with those obtained in man. Such comparisons are particularly valuable during the phase one-initial dose escalation study. To maximize this, it is our practice to generate PK data between each dose increase.[24]

BIOANALYSIS

Bioanalysis is a term generally used to describe the quantitative measurement of a compound (drug) or their metabolite in biological fluids, primarily blood, plasma, serum, urine or tissue extracts.[25] A bioanalytical method consists of two main components

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Sample preparation: 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.[26] Most bioanalytical assays have a sample preparation step to remove the proteins from the sample. Protein precipitation, liquid–liquid extraction and solid phase extraction (SPE) are routinely used.[27]

Detection of the compound: The detector of choice is a mass spectrometer.[26] 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.[28] 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.[29]

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,[30] 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).[31-33] 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.[34]

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).

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).[35]

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.[36]

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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.[30] 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.[35,36] The duration of these time intervals is not defined. Within/intraday, - assay, -run and -batch are commonly used to express the repeatability. Expressions for reproducibility of the analytical method are between interday, -assay, -run and -batch. The expressions intra/within-day and inter/between-day precision are not preferred because a set of measurements could take longer than 24 hours or multiple sets could be analyzed within the same day.[37]

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.[37] The LOD is also defined as the lowest concentration that can be distinguished from the background noise with a certain degree of confidence. There is an overall agreement that the LOD should represent the smallest detectable amount or concentration of the analyte of interest.

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.

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". The concentration range of the calibration curve should at least span those concentrations expected to be measured in the study samples. If the total range cannot be described by a single calibration curve, two calibration ranges can be validated. It should be kept in mind that the accuracy and precision of the method will be negatively affected at the extremes of the range by extensively expanding the range beyond necessity. 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.[38] 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.

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.[30]

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.

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.

Stability

It is the chemical stability of an analyte in a given matrix under specific conditions for given time intervals.[30] 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.[39] 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). The ICH guidelines are summarized in Table 1.

The drug research can be divided functionally into two stages: discovery/design and development [Figure 1].

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Table 1: US FDA guidelines for bioanalytical method validation

Selectivity (specificity)	Analyses of blank samples of the appropriate biological matrix (plasma, urine or other matrix) should be obtained from at least six sources. Each blank should be tested for interference and selectivity should be ensured at LLOQ
Accuracy	Should be measured using a minimum of six determinations per concentration. A minimum of three concentrations in range of expected concentrations is recommended for determination of accuracy. The mean should be ±15% of the actual value except at LLOQ, where it should not deviate by ±20%. This deviation of mean from the true values serves as the measure of accuracy
Precision	Should be measured using a minimum of five determinations per concentrations. A minimum of three concentrations in the range of expected concentrations is recommended. The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV) except for the LLOQ, where it should not exceed 20% of the CV
Recovery	Recovery experiments should be performed at three concentrations (low, medium and high) with unextracted standards that represent 100% recovery
Calibration curve	Should consist of a blank sample (matrix sample processed without internal standard), a zero sample (matrix sample processed with internal standard) and six to eight non-zero samples covering the expected range, including LLOQ
LLOQ	Analyte response should be five times the response compared to blank response. Analyte peak should be identifiable, discrete and reproducible with a precision of 20% and an accuracy of 80–120%
Freeze–thaw stability	Analyte stability should be determined after three freeze–thaw cycles. At least three aliquots at each of the low and high concentrations should be stored at the intended storage temperature for 24 hours and thawed at room temperature. When completely thawed, refreeze again for 12–24 hours under same conditions. This cycle should be repeated two more times, then analyze on third cycle. Standard deviation of error should be <15%. If the analyte is unstable, freeze at –70°C for three freeze–thaw cycles
Short-term stability	Three aliquots of each of the low and high concentrations should be thawed at room temperature and kept at this temperature for 4–24 hours and analyzed. % Deviation should be <15%
Long-term stability	At least three aliquots of each of low and high concentrations at same conditions as study samples. Analyze on three separate occasions. Storage time should exceed the time between the date of first sample collection and the date of last sample analysis
Stock-solution stability	Stability of stock solutions of drug and the internal standard should be evaluated at room temperature for at least 6 hours. % Deviation should be <15%
Quality control (QC) samples	QC samples in duplicates at three concentration levels (one near the 3× LLOQ, one in mid-range, one close to high end) should be incorporated at each assay run. At least four out of every six should be within 15% of respective nominal value. Two of six may be outside 15% but not both at the same concentration. Minimum number QCs should be at least 5% of total number of unknown samples or six total QCs, whichever is greater

Lower limit of quantification

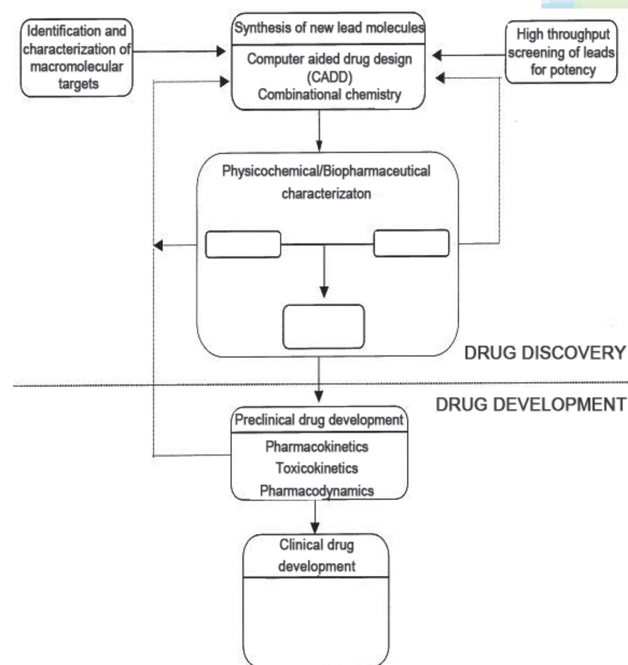

Figure 1: Different stages of discovery/design and development

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

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of development. Additionally, it becomes necessary to quantify active metabolite(s) in both animals and humans.[40]

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.[41] 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). Using automated techniques, ultrahigh throughput can be obtained by the most advanced laboratories and tens of thousands of compounds can be screened in one day. In the secondary screening stage, physiochemical properties such as solubility, lipophilicity and stability are determined by using octanol–water partition coefficient and pK_a. These measurements are useful in predicting protein binding, tissue distribution and absorption in gastrointestinal tract[42] 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.[43-46] 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.[47-50] These important tests are collectively referred to as ADME characteristics (Absorption, Distribution, Metabolism and Elimination).[26] Figure 2 shows an illustration of a possible scenario of bioanalysis in discovering drugs which are active *in vitro* and improving these by modification of the chemical structure optimized for *in vivo* activity.[51]

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.[52] Nowadays, introduction of *in vitro* approaches into drug metabolism enables the characterization of the metabolic properties of drug candidates at an earlier stage in the drug development process, at early preclinical studies performed during the drug discovery phase. Recently, the major reasons for high attrition rates have instead been identified to be lack of efficacy and safety, together accounting for approximately 60% of the failures. Cassette dosing is now an established method within the pharmaceutical industry as it provides a relatively quick way of

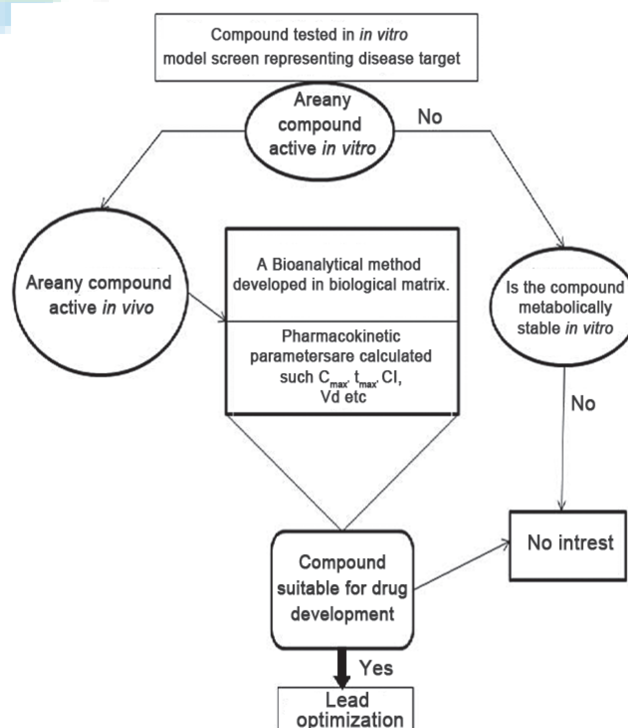

Figure 2: Possible scenario of bioanalysis in discovering drugs

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ranking compounds according to their PK properties and requires the use of fewer animals.[53,54]

A list of experiments that are commonly performed to assess the ADME characteristics of potential lead compounds in drug discovery is given in Table 2.[26]

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 [55]. Some data on reasons for withdrawal of candidate drugs from development have been published by the Center for Medicines Research[56] [Table 3].

Table 2: List of experiments to assess Absorption, Distribution, Metabolism and Elimination

Parameter examined	Typical experiments
Absorption	Caco-2 cells, MDCK cells, PgP transport
	In vivo PK profiling
Distribution	*In vitro* protein binding
	In vivo tissue distribution studies
Metabolism	Metabolic stability
	Microsomes, subcellular fractions, hepatocytes
	P450 inhibition studies
	Microsomes
	P450 induction studies
	Gene chips, multiple dosing
Elimination	Quantitation of drugs and metabolites in biological fluids

Table 3: Reasons for failure in drug development[57]

Reasons	%
Poor biopharmaceutical properties	40
Lack of efficacy	30
Toxicity	21
Commercial reasons	8

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.[57] 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. The developed assay at this stage may differ from the original assay of the discovery phase in that an internal standard addition may be used (in the event that an internal standard was not used before) to ensure reliability of the quantitation. It is a common practice in some pharmaceutical companies to incorporate a stable isotope labeled compound of the parent as an internal standard (IS) to provide extra comfort in the bioanalysis of NCEs at this stage.[58] It is especially valuable in lead optimization for studying the PK of multiple compounds administered simultaneously. Plasma levels of the drug are normally monitored to permit the calculation of PK parameters such as C_{max} [maximum plasma concentration (e.g., ng/mL)] and AUC [area under the plasma concentration–time curve

Table 4: Objectives of the four phases in clinical drug development and typical numbers of volunteers or patients involved

Phase I	Phase II	Phase III	Phase IV
Establish safe dosing range and assess PK; also called first time in man (FTIM) 20–80 males Volunteers	Demonstrate efficacy, identify side effects and assess PK 200–800 patients	Gain data on safety and effectiveness in a larger population of patients; assess PK 1000–5000 patients Several	Expand on approved claims or demonstrate new claims; examine special drug–drug interactions; assess PK A few thousand to thousand patients

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Table 5: Bioanalytical framework in drug discovery and development: Key considerations

Stage	Key objectives for bioanalysis	Suggested validation work with comments
Discovery	Screen leads for microsomal stability, *in vivo* PK screen; preliminary *in vitro* cytochrome P450 inhibitory liability screens, caco-2 cell permeability	• Selectivity and specificity (no endogenous interference; lack of interference from other coadministered agents, if cassette dosing and/or cassette analysis is pursued) • Preliminary range specification for quantitation • Number of standards (n = 3–5): Although duplicate standards are recommended, the analyst should make the judgment call if single standards are adequate • Reasonable accuracy and precision • Early read on chromatographic and detection conditions
Preclinical: Stage 1	Characterization of PK disposition in rodents/dogs/monkeys (single dose, multiple dose, absolute bioavailability); route-dependent PK disposition; formulation screening for toxicology work; definitive *in vitro* drug–drug interaction liability; *in vivo* drug–drug interaction potential, toxicokinetics (and/or exposure levels) in toxicology species	• Complete assessment of accuracy and precision (3–5 validation runs): To cover intra- and inter-day variability assessments in both %bias and precision • Confirmation of selectivity and specificity • Defined range of standard calibration curve • Fixing of LLQ and ULQ of the assay • Assessment of dilution effect on accuracy and precision • Finalization of extraction scheme, chromatographic and detection conditions • Extraction recovery assessment using acceptable methods (should cover the entire range); also, IS recovery should be assessed • Stability experiments to cover various scenarios such as freeze–thaw, bench top and processed sample stability, frozen conditions • In order to facilitate PK and toxicokinetic work in animals, it is suggested to have a frozen stability window in plasma/serum (or urine) established for 6–8 weeks Cross validation: Protocols may be developed to measure drug levels in a second species and/or a second matrix using a previous fully validated method Partial validation: As deemed appropriate, specific analytical protocols may be developed to support this. This would aid in extending the analytical range for a very specific application
Preclinical: Stage 2	Characterization of PK disposition of parent and metabolite in pharmacological and toxicological species; characterization of *in vivo* drug–drug interaction studies	Approaches to validation plan as described under Clinical stage 1 and stage 2 can be followed and modified as deemed necessary
Clinical: Stage 1	Characterization of PK disposition in healthy human subjects – single doses, multiple doses; relative bioavailability assessment (solution/suspension vs. solid dosage form); food effect potential; characterization of PK disposition in targeted patient population; delineation of PK in special populations (pediatric, geriatric, renal impairment, hepatic dysfunction)	• Similar to preclinical stage 1 scheme, all elements need to be covered • The fixing of LLQ (i.e., determination of lowest sensitivity) for the assay may be based on anticipated concentrations following the lowest dose in humans • Throughput of the assay should be optimized to obtain rapid analytical data to support the next dose escalation stage • The window for sample stability under frozen conditions needs to be for at least 6 months • Unlike preclinical, a contingency plan to develop a second analytical laboratory to validate and run the assay may be considered • Verification of robustness/ruggedness of the assay needs to be established (covering both laboratory-to-laboratory variability as well as the analyst's transfer of the assay)
Clinical: Stage 2	Characterization of the PK disposition of the parent and its metabolites in plasma, urine and serum	• The assay developed in clinical stage 1 may be used to support these activities. Since one or more metabolites are introduced, it is imperative that the assay be validated to support the quantitation of such metabolites • Separate assay procedure may be developed for the metabolites or the parent and metabolites may be assessed in a single run (to be followed, validation is required) • Validation includes the following: linearity, LLQ, ULQ, precision, selectivity, specificity and stability assessment of metabolites
Clinical: Stage 3	Evaluate the drug–drug interaction potential with agents commonly prescribed for such patient population; drug–drug interaction potential with agents having narrow safety window (digoxin, warfarin, etc.)	• Sponsor has two options in developing and validating the assays for these activities • Option 1: Methods may be developed wherein the NCE and potential metabolites are simultaneously quantified with the coadministered agent (without and/or with appropriate metabolites) • Option 2: Separate methods may be developed for the coadministered agents, while earlier developed assays for NCE and metabolites (stages 1 and 2) may be used for quantitation of parent and metabolites as the need arises • It is recommended that separate methods be developed for the coadministered agents by the sponsor because of the applicability of the assay for future • NCEs without having to necessarily validate it again (if option 1 is used) • Finalization of chromatographic detection conditions • Validation includes the following: linearity, LLQ, ULQ, accuracy, precision, selectivity, specificity and stability assessment of metabolites

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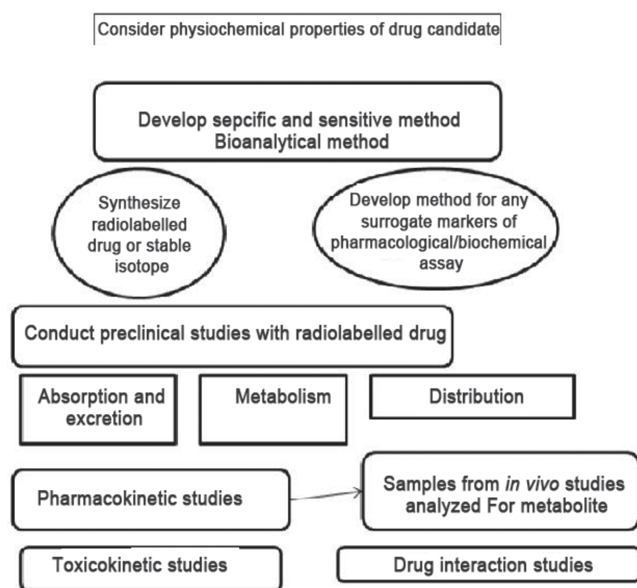

Figure 3 : Various steps of bioanalysis in the development stage of drugs

(e.g., ng h/mL)]. Distribution parameters describe the extent of drug distribution and are related to body volumes (e.g., mL or L), and time course parameters are related to time ($t_{1/2,}$ T_{max}). These PK parameters are calculated from mathematical formulae, and specific computer programs are usually used to do this (e.g., WinNonlin).[59-63] The parameters may be estimated by compartmental or non-compartmental approaches (or model-dependent and model-independent, respectively). Figure 3 shows some possible steps of bioanalysis in the development stage of drugs. However, the determination of metabolite profiles is usually performed for a limited number of lead molecules *in vivo* and *in vitro*, and in these experiments the key issues are high specificity and sensitivity rather than speed.[64]

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-dependencies in 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 interspecies comparisons.

However, the overall aim in conducting toxicokinetics during safety studies is to extrapolate the risk assessment from the toxicity test species to humans.[51] 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.[65-70]

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 [Table 4]. 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.[71-79]

The requirements and adherence to specificity, selectivity and stability will become very important. Since it is likely that patients will be concomitantly ingesting other medications, the assay has to be flexible enough to accommodate minor alterations in chromatographic conditions to circumvent the interfering peaks, if necessary.

Table 5 provides a framework of the various stages of bioanalytical assay development (discovery, preclinical and clinical), key objectives that assay would support and some suggested validation workup that may be required to achieve the end goals. Based on the presentation [Table 1], it is apparent that assays developed in the early discovery stage may find utility during the course of an NCE's progress in development.

CONCLUSION

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. The acceptance criteria should be clearly established

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in a validation plan, prior to the initiation of the validation study. The developed assay should be sufficiently rugged that it provides opportunities for minor modifications and/or ease of adoptability to suit other bioanalytical needs such as applicability to a drug–drug interaction study, toxicokinetic study as well as for characterization of the plasma levels of the metabolites. For bioanalytical liquid chromatographic methods, sample preparation techniques, the essential validation parameters with their guidelines and suggested validation work in drug discovery and development phase have been discussed here.

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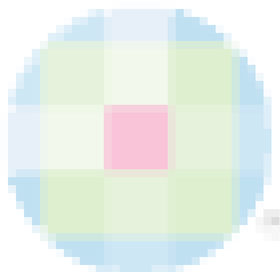

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