Review Article

Bioanalytical Method Validation: A Comprehensive Review

P. Ravi Sankar*, A. Sai Geethika, G. Rachana, P. Srinivasa Babu, J. Bhargavi
Vignan Pharmacy College, Vadlamudi, Guntur – (A.P) India.
***Corresponding author's E-mail:** banuman35@gmail.com

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ABSTRACT

Measurement of drug concentrations in biological matrices (such as serum, plasma, faces, skin, blood, urine, saliva and other organ tissues) is an important aspect of medicinal product development. Such data may be required to support applications for new actives substances and generics as well as variations to authorised drug products. The results of animal toxicokinetic studies and of clinical trials, including bioequivalence studies are used to make critical decisions supporting the safety and efficacy of a medicinal drug substance or product. This guideline provides recommendations for the validation of bioanalytical methods applied to measure drug concentrations in biological matrices obtained in animal toxicokinetic studies and all phases of clinical trials. Furthermore, this guideline will describe when partial validation or cross validation should be carried out in addition to the full validation of an analytical method. This comprehensive review paper describes the introduction, bioanalytical work flow, method development steps, types of extractions, types of method validation, validation parameters, and stability in matrix, micro extraction techniques, and examples of drugs using different sample techniques.

Keywords: Bioanalytical method development, validation parameters, documentation, application, biological matrices, pharmacokinetic studies.

INTRODUCTION

Bio analysis[1] is defined as Quantification of analyte / metabolites in human biological matrix (Blood, Plasma, Serum, Urine, faeces, skin, saliva and other organ tissues) by using chromatographic[2-10] devices, eg. HPLC, LC-MS, GC-MS etc.. Bio analysis is not only measuring of small molecules such as drugs and metabolites but also to identify large molecules such as proteins and peptides. Bioanalysis is well established in pharmaceutical companies to support drug discovery and drug development. Bioanalysis is also important in many research areas such as Forensic analysis, doping control and identification of biomarkers for diagnostic of many diseases.

Bioanalysis has an important role in drug development.

- ✓ Toxicological evaluation,
- ✓ Pharmacokinetic studies[11-12]
- ✓ Pharmacodynamics studies.

Bio analytical method validation(BMV)[13-22] plays is a crucial for the quantitative determination of various types of analytes in biological matrices and physiological matrices, and the methods could be applied to studies in areas of human clinical pharmacology and nonhuman studies. It play a significant role in the evaluation and interpretation of bioequivalence, pharmacokinetic (PK), and Toxicokinetic studies.

The Bioanalysis procedure includes preplanning, sampling, sample preparation, analysis, calibration and data evaluation and reporting. In modern Bioanalysis a good sample preparation and a hyphenated instrumentation are required.

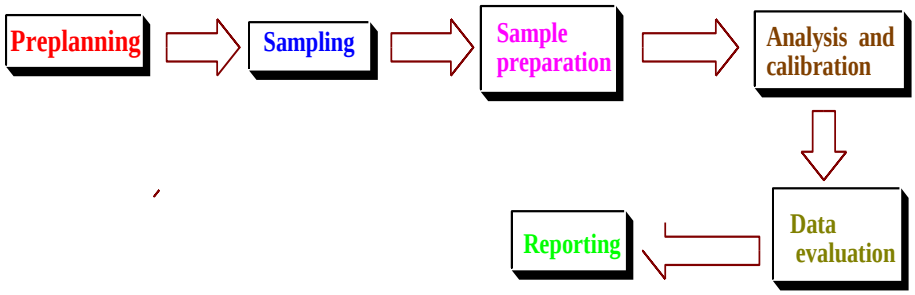


Figure 1: Bioanalytical work flow

Requirements: 1) Authenticated source for Biological Matrix. 2) Reference or working Standards.3) Solvents and Chemicals. 4) Chromatographic Devices- Instruments, Columns. 5) Well trained Man Power 6) Literature.

Method Development Steps

1. Literature search for drugs.
2. Physicochemical properties of the compound.
3. Dose & Cmax of the compound.
4. Selection of chromatographic device.
5. Reference standard preparation.
6. Selection of Internal Standard.
7. Tuning of the compound.
8. Optimization of chromatographic parameters.
9. Optimization of Extraction procedure.
10. Sample storage.

1) Physicochemical properties of the compound

a) Solubility b) pKa C) molecular weight d) molarity e) pH . f) Log P

2) Dose & Cmax of the compound

a)These two are required to find out the required LLOQ level and to fix the required Linearity range.

b)As per regulatory guidelines

c)LLOQ should be 5 half life of the Cmax

d)ULOQ should be 2 – 2.5 times of the Cmax

3) Selection of chromatographic device

a)Selection of Chromatographic device is depends on the required sensitivity.

b)Sensitivity ranged from sub pg/mL to µg/mL level

4) Selection of Internal Standard

Internal standard should preferably labeled compound if not Structurally similar or pKa similar

5) Tuning of the compound

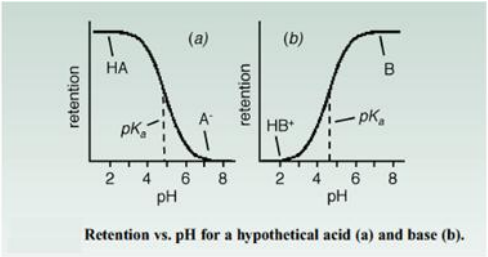
a)Source dependent Parameters-Curtain gas, nebulizing gas, Sheet gas, Source Voltage etc

b)Compound Dependent Parameter-Declustering Potential, entrance potential, collision Energy, Exit Potential etc.

6) Optimization of chromatographic parameters:

- Mobile Phase, Mobile Phase Ratio, Column, Flow rate, Temperature, Injection volume, Carry Over

OPTIMIZATION OF MOBILE PHASE pH



Retention vs. pH for a hypothetical acid (a) and base (b).

Figure 1: It is the representation of graph between Retention vs PH for a hypothetical acid (a) and base(b)

OPTIMIZATION OF MOBILE PHASE pH:

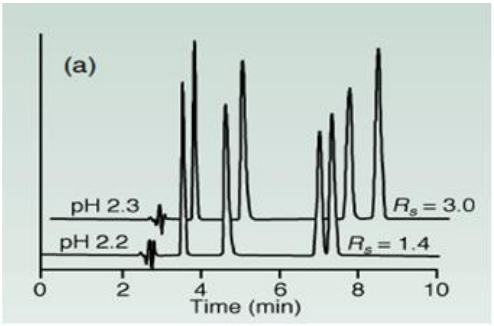


Figure 2: Representation of graph between Time (min) vs Basic analytes.

The effect of small changes in mobile phase pH on separation.

a) Basic analytes: p-anisidine, m-toluidine, 4-chloroaniline, 3-aminobenzonitrile (in retention order); 27:73 methanol/phosphate buffer.

7) Optimization of Extraction procedure

Types of Extractions

1) Protein Precipitation (PPT), 2)Liquid-Liquid Extraction (LLE), 3)Solid Phase Extraction (SPE), 4)Hybrid Extraction.

1) **LIQUID-LIQUID EXTRACTION**

Liquid liquid extraction is the direct extraction of the biological material with a water-immiscible solvent. An aqueous sample (e.g., plasma, urine) and an immiscible organic solvent are mixed to remove the analyte into the organic phase for injection into an analytical system.

- Provide good recovery and clean sample
- Used for the extraction of basic and acidic drugs from biological samples
- An efficient method especially to eliminate salts. Time consuming,Not suitable for extraction of several analytes with different polarity,Evaporation step is often required prior to analysis.

The analyte is isolated by partitiong between the organic phase and the aqueous phase. The distribution ratio is

effected by a number of factors Choice of extracting solvent, PH of aquephase, relative lipophilicity (or) hydrophobicity of the analyte; ex: teritary-butylmethyl ether, dichloro- methane, hexane, diethyl ether etc.

Solid Phase Extraction (SPE)

Solid phase extraction is carried out by using a six step process.

Condition and equilibrium, sample pre-treatment, sample loading, washing, drying, elution, Highly efficient, Cost-effective, High-reproducibility, Advantageous such as separation and concentrating of trace analytes, Nature and amount of the sorbent, Loaded sample volume (Enough recovery), Composition and volume of the washing and elution solutions are effective,a sorbent of 50–200 mg is used as cartridge to separate the required analytes from a complex matrix.

1. Reverse phase SPE
2. Normal phase SPE
3. Ion exchange SPE

REVERSE PHASE SPE

Polar sample matrix includes nonpolar analytes using nonpolar sorbent.

Sorbents used: Bonded silica (C_4, C_8, C_{18} and Phenyl, with 40 µm particle size and 60 Å pore size) and polymer sorbent as polystyrene, Retention mechanism is based on Hydrophobic interactions between analytes and nonpolar sorbent materials., less selective, polar sample matrix includes nonpolar analytes using nonpolar sorbent.

NORMAL PHASE SPE

Polar analytes in nonpolar matrices using polar sorbents.

Sorbents used: Silica with polar functional groups (Si-CN, Si-NH_2, Si-Diol and pure silica). Retention mechanism is based on hydrogen bonding between analytes and sorbent.

ION EXCHANGE SPE

Most selective method for charged analytes .ANIONIC ANALYTES (ACIDIC DRUGS)**:** Isolated with quaternary amine bonded silica or Si-NH_2 as anion exchange. CATIONIC ANALYTES (BASIC DRUGS) **:**Si- Strong cation exchange (propyl sulfonic acid bonded) Si- weak cation exchange (Carboxy propyl phase).

PROTEIN PRECIPITATION (PPT)

Appropriate for plasma or blood samples especially at high analyte concentration Induced by the addition of: A miscible organic solvent (acetonitrile, acetone or methanol), Salt (aluminium chloride), Metal ions (zinc sulphate), Changing the sample pH to alter the nature of the solution (Trichloro acetic acid, Perchloric acid, Meta phosphoric acid and Tungstic acid. Different sample techniques are shown in the Table 1.

Table 1: Comparison of Sample Techniques

Parameter	PPT	LLE	SPE
Workability	Less	More	More
Selectivity	Bad	Good	Very good
Ion suppression	High	Low	Low
Automation	Low	Low	High
Analyte suitability	Hydrophilic	Lipophilic	Hydrophilic &Lipophilic
Cost	Low	High	High

Hybrid extraction Technique

Selective extraction of Analyte by using the combination of two or more extraction techniques" E.g. PPT and SPE or PPT and LLE. Hybrid extraction is intended for Improve Specificity, Improve detection limits, Improve recovery.

Method Validation

Method validation is a process to demonstrate that a method will successfully meet or exceed the minimum standards recommended in the guidelines.

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.

Types of method validation

1. Full Validation

2. Partial validation

3. Cross validation

Full Validation

- Full validation is important when developing and implementing a Bioanalytical method for the first time.
- Full validation is important for a new drug entity.
- A full validation of the revised assay is important if metabolites are added to an existing assay for quantification.

Parameters to be Validate:[23-28]

1)Specificity 2) Auto sampler Carry Ove 3) Sensitivity 4) Precision & Accuracy 5)Recovery 6) Matrix Factor 7) Ruggedness 8) Stabilities 9) linearity

SPECIFICITY/ SELECTIVITY

Specificity is for identification of analyte (s) or metabolite (s) or matrix components interferences at their respective RT's on Biological Matrix. Selectivity is evaluated by injecting extracted blank plasma and comparing with the response of extracted LLOQ samples processed with internal standard.

Procedure

6 Lots of intended anti-coagulant, 2 Lots of Heamolized, 2 Lots of Lipemic

Acceptance criteria

Should less than 20 % of the LLOQ response for analyte (s),Should be less than 5 % of the internal standard response.

Auto Sampler Carry Over (ASCO):

Procedure: Diluent, ULOQ, Diluent, LLOQ

Acceptance criteria : Response of the diluent injected after ULOQ should be less than 5 % of the LLOQ.

Sensitivity

Sensitivity is defined as the lowest analyte concentration in the matrix that can be measured with acceptable accuracy and precision (i.e., LLOQ). The lower limit of quantification (LLOQ) is the lowest concentration of analyte in a sample which can be quantified reliably, with an acceptable accuracy and precision. The LLOQ is considered being the lowest calibration standard (see Accuracy and Precision). In addition, the analyte signal of the LLOQ sample should be at least 5 times the signal of a blank sample. The LLOQ should be adapted to expected concentrations and to the aim of the study. As an example, for bioequivalence studies the LLOQ should be not higher than 5 % of the Cmax, while such a low LLOQ may be not necessary for exploratory pharmacokinetic studies.

Procedure

Injecting the 6 replicates of LLOQ concentration

Acceptance criteria

% Accuracy should be within 80 to 120 %, % CV should be ± 20 %.

Precision & Accuracy (P&A)

Precision

The precision is the closeness of agreement (i.e., degree of scatter) among a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. The acceptance criteria is < 15 % CV. At LOQ, 20 % deviation is acceptable.

Accuracy

Closeness of determined value to the true value. Closeness to the nominal Value The mean value should be within ± 15 % of the theoretical value, except at LLOQ, where it should not deviate by more than ± 20 %.

Procedure

Injecting 6 replicates of at least 3 different levels of the concentrations

HQC: Near to the ULOQ.

MQC : Middle of the Calibration curve.

LQC : Less than 3 time of the LLOQ**.**

Acceptance criteria: % Accuracy should be within 85 to 115 %, except LLOQ, for LLOQ it should be 80 to 120 %**,** % CV should be ± 15 % except LLOQ, for LLOQ it should be 20 %.

Recovery

Determination of Extraction efficiency .The detector response obtained from an amount of the analyte added to and extracted from the biological matrix, compared to the detector response obtained for the true concentration of the pure authentic standard. Recovery pertains to the extraction efficiency of an analytical method within the limits of variability

Procedure

AQ. Injections: six replicates of 3 different levels (H, M, L) Post Extracted Injection: six replicates of 3 different levels (H, M, L)

Acceptance criteria

% CV of Mean Recovery from three different concentrations should be within 15 %

Matrix Effect or Matrix Factor or ISTD Normalization

Determination of Matrix ions effect on Analyte or internal standard Matrix effect studied by comparing the response of extracted samples spiked before extraction with response of the blank matrix sample to which analyte has been added at the same nominal concentration just before injection.

Procedure**:** AQ. Injections: six replicates of 3 different levels (H, M, L) Post Extracted Injection: six replicates of 3 different levels (H, M, L) with 6 different lots of Blank Matrix

Acceptance criteria: ISTD normalization should be within 15 %.

Ruggedness

Different Analyst

Different Column

Different Instrument

Procedure

One Precision & Accuracy for each change

Acceptance criteria

% Accuracy should be within 85 to 115 %, except LLOQ, for LLOQ it should be 80 to 120 %

% CV should be ± 15 % except LLOQ, for LLOQ it should be 20 %

Stability

Chemical stability of an analyte in a given matrix under specific conditions for given time intervals. Analyte change in any respect affect the chromatographic behavior which may complicate the method development.

Calibration curv

The response of the instrument with regard to the concentration of analyte should be known, and should be evaluated over a specified concentration range. The calibration standards should be prepared in the same matrix as the matrix of the intended study samples by spiking the blank matrix with known concentrations of the analyte. There should be one calibration curve for each analyte studied in the method validation and for each analytical run. Ideally, before carrying out the validation of the analytical method it should be known what concentration range is expected. This range should be covered by the calibration curve range, defined by the LLOQ being the lowest calibration standard and the upper limit of quantification (ULOQ), being the highest calibration standard. The range should be established to allow adequate description of the pharmacokinetics of the analyte of interest. A minimum of six calibration concentration levels should be used, in addition to the blank sample (processed matrix sample without analyte and without IS) and a zero sample (processed matrix with IS). Each calibration standard can be analysed in replicate. A relationship which can simply and adequately describe the response of the instrument with regard to the concentration of analyte should be applied. The blank and zero samples should not be taken into consideration to calculate the calibration curve parameters. The calibration curve parameters should be reported (slope and intercept in case of linear fit). In addition, the back calculated concentrations of the calibration standards should be presented together with the calculated mean accuracy values (see definition of Accuracy below). All the available (or acceptable) curves obtained during validation, with a minimum of 3 should be reported. The back calculated concentrations of the calibration standards should be within ± 15 % of the nominal value, except for the LLOQ for which it should be within ± 20 %. At least 75 % of the calibration standards, with a minimum of six calibration standard levels, must fulfill this criterion. In case replicates are used, the criteria (within ± 15 % or ± 20 % for LLOQ) should also be fulfilled for at least 50 % of the calibration standards tested per concentration level. In case a calibration standard does not comply with these criteria, this calibration standard sample should be rejected, and the calibration curve without this calibration standard should be re-evaluated, including regression analysis. In case all replicates of the LLOQ or the ULOQ calibration standard are rejected then the batch should be rejected from the validation, the possible source of the failure be determined and the method revised (if necessary). If the next validation batch also fails, then the method should be revised before restarting validation. Although the calibration curve should preferably be prepared using freshly spiked samples, it is allowed to use previously prepared and stored calibration samples, if supported by appropriate stability data.

Stability in Matrix

Bench Top Stability (BT), Freeze & Thaw Stability (FT), Long Term Stability (LT), Wet Extract Stability (WE), Auto sampler Stability (ASS), Blood Stability (BS), Analyte and IS stock stability in solvent, Short Term Stability in matrix, In-injector stability in matrix.

Stability in Aqueous Solution

Long term & Short term Stock Solution Stability

Long term & short term working solution stability

Acceptance Criteria:

For matrix stabilities: Mean stability should be with 85 to 115 %

For aqueous stabilities : Mean stability should be with 90 to 110

Concomitant medication effect

Determination of concomitant medications (i.e. effect of OTC drugs) effect on intended Analyte.

Procedure:

Addition of Cmax concentration of Concomitant drug in HQC and LQC concentration.

Acceptance Criteria:

Accuracy : Should be within 85 to 115 %

Precision : Should be ± 15 %

Partial Validation

Partial validations are modifications of already validated Bioanalytical methods and the modifications do not affect all validation parameters. Typical modifications include, but are not limited to:

- Analytical procedure transfers between laboratories
- Change in analytical methodology (e.g., change in detection systems, chromatographic conditions).
- Change in anticoagulant in harvesting biological fluid
- Change in counter ion of the same anti coagulant
- Change in matrix within species
- Change in sample processing procedures
- Change in species within matrix
- Change in relevant concentration range
- Changes in analytical instruments and/or software platforms

Cross Validation

Cross validation will performed for the following conditions

- Two or more analytical procedures are used to generate data within the same study or across different studies.
- Data are generated using different analytical techniques (e.g., LC-MS/MS versus LC-UV) in a single study.
- Sample analyses within a single study are conducted at more than one site.
- When sample analyses within a single study are conducted at more than one contract laboratory

Microextraction Techniques

- To achieve high throughput sample preparation
- To handle small sample volumes and to decrease solvents

- Solid phase microextraction (spme)
- Stir bar sorptive extraction (sbse)
- Microextraction by packed sorbent (meps)
- Packed tips
- **Solid Phase Microextraction (SPME)**
- 1989 -Accepted as a powerful solvent-free extraction technique.
- A fused silica fiber is coated with a stationary phase.
- Equilibrium is established by exposing fiber with the analyte in matrix.
- Direct extraction of analytes with immersion of the fiber into the sample.

OR

- Utilizing headspace to extract volatile compounds from fiber surface which are partitioned between gaseous and liquid phases.

To be Optimised

- ✓ Temperature ,pH ,Salt concentration ,Stirring rate, Equilibrium constant and equilibration time

Disadvantages

Short fiber lifetime, Low sensitivity of analytes in complex matrices

Stir Bar Sorptive Extraction (SBSE)

1999 by Sandra et al.

A magnetic stirring bar of quartz coated with polydimethylsiloxane (PDMS) is used.

Compared to SPME the SBSE has more coated polymer (> 50 times) and so higher extraction efficiency is obtained. Cost effective technique.

Microextraction By Packed Sorbent (MEPS)

2004 - Novel, simple, fast, cost-effective, readily automated.

The sorbent is fitted in a liquid handling micro syringe as a plug with low-void-volume.

When the sample is drawn through the syringe, the analytes are adsorbed onto the solid phase.

The sorbent is then washed with water and lastly the analytes were absorbed by suitable solvents

Packed Tips

Packed sorbents (silica and monolithic based sorbents, imprinted polymers) in a single tip or 96-tips (monolithic).

Great efficiency and selectivity in bioanalysis.

The extraction steps and process are almost similar to SPE and MEPS.

Monolithic packed 96-tips is a clean, fast, high-throughput and semi-automated sample preparation and can handle small sample volumes. Different sorts of drugs using different samples techniques are represented in table 2.

Table 2: Examples of pharmaceuticals using different sample techniques:

Drug	matrix	LC column	Sample preparation	LLOQ
Amisulpride	Human plasma	Polar RP (75x4.6 mm,4µm)	LLE	2.0 ng mL^{-1}
Acebutolol and Metoprolol	Human plasma	Zorbax SB-C_{18} (50X2.1mm,3µm)	MEPS	1.0µg L^{-1}
Antimalarials drug candidate (LAFIS 01)	Rat plasma	Luna C_{18} (50X2mm,5µm)	PPT	10 ngmL^{-1}
Antibiotics	Human plasma	Caltrex Resorcinearene (125X2mm,5µm)	SPE	1.0ng mL^{-1}
Anabolic and Corticosteroids	Human urine	C8,C-18 RP (50x2.1mm)	LLE,SPE	0.1-2.0ng mL^{-1}
Busulphan	Human plasma	Hypersil gold (100x2.1mm,3µm)	MEPS	5µg mL^{-1}

Burpropion and its metabolites	Human plasma	AGP column(100x2.0mm,5µm)	PPT	0.5-2.0ngmL^{-1}
Cyclophosphamide	Human plasma	ZorbaxSB-C_8 (50x2.1mm,3µm)	MEPS	0.5µg mL^{-1}
Cefdinir	Human plasma	RP18 Waters (50x2.1mm,3µm)	PPT	5.0ngmL^{-1}
Fluoxetine	Human plasma	Ascentis express C_{18}(75x4.6mm,2.7µm)	SPE	2.0ngmL^{-1}
Illicit drugs	Human saliva	PFP Kinetex (100x2.1mm,2.6µm)	MEPS	0.5-30ngmL^{-1}
Immuno suppressive	Blood, plasma	CP ChromSpher (20x3mm,3µm)	PPT	2.0nM
Ketamine	Human hair	Zorbax Eclipse Plus C_{18} (50x2.1mm,1.8µm)	LLE	100 pgg^{-1}
Lidocaine and metabolites	Human urine and plasma	YMC basic 9150x3.0mm,3µm)	SPE UF	1.6-5.0nM
Midodrine	Human plasma	RP C_{18} column(50x4.6mm,5µm)	LLE	0.5ng mL-^{1}
Metoprolol(enantiomers)	Human plasma, saliva	Chiral cellulose-SB (150x4.6mm,5µm)	MEPS	2.5ng mL^{-1}
Nicotine and its metabolites	Human blood	Accucore Phenyl Hexyl (100x3mm,2.6µm)	SPE	15 ngmL^{-1}
0pioids, Morphine, ,Codeine,Oxycodone,fentanyl	Human blood	Raptor Biphenyl (50 x2.1mm,2.7µm	SPE	1.0 ngmL^{-1} (fentanyl:0.1ng)
Propranolol, diclofenac	Human hair	Luna-C_{18} (50x2.1mm,5µm)	SPE	1.0 ngmL^{-1}
Roscovitine and olomoucine	Human plasma	Zorbax SB-C_8 (50x2.1mm,3µm)	MEPS	0.5,1.0 µgmL^{-1}
Teicoplanin	Human plasma	HS-C_{18} (75x3mm,3µm)	Dilution with water and direct injection	1.0 µgmL^{-1}
Ziprasidone	Human plasma	Symmetry-C_8 (150 x2.1mm,5µm)	LLE	0.1ngmL^{-1}
35 psychotropic drugs and metabolites	Human hair	Atlantis T3(150x2.1mm,3µm)	LLE	0.5-5.0 Pgg^{-1}

Bioanalysis in the Drug Discovery and Development Lifecycle

The bioanalytical process

The lead optimization / selection, confirmation, and testing process for new drug candidates is well defined as a series of activities.

Broadly, these can be split into discovery, lead optimization and preclinical development, through to clinical elevation (phases 1 to 40).

Each stage places different requirements on the bioanalytical assay used to provide information The use of LC/MS/MS assays gives the specificity, flexibility, and sensitivity to enable fast and effective decision-making at each stage.

pk and bioanalysis in drug discovery

Full pk characterization in the drug discovery phase is not required; however, in conjunction with in vitro techniques, the ability to assess the bioavailability of a compound through bioanalysis provides a good indication of suitability for advancement to development.

Some analytical priorities are:

Fast pass/fail determination of pk parameters.

Medium-sensitivity assay.

Minimum assay development.

High specificity for the compounds of interest.

pk and bioanalysis in efficacy and safety studies.

Phase I: First time in to humans.

The key requirements for this stage are that the assay must completely characterize the absorption and elimination phases of the plasma concentration-time curve. All metabolites must be fully resolved, identified, and quantified. Adverse effects of a drug (toxicokinetic, TK) are investigated and need accurate measurement of AUC and Cmax after single and multiple doses. in the way the "no-toxic-effect dose level" can be established, a key parameter when dosing in first-time-into-human and further trails. The demands placed on the bioanalytical assay are for:

- High sensitivity to ensure that the lowest effective doses can be identified.

- High sensitivity to identify and quantitate metabolites.
- Moderative throughput; sample groups are small.
- Full validation is required.

Phase II a: proof of concept

The drug compound id tested in small groups of patients to assess efficacy in treating the disease state.pk analysis is employed to assess the dose/exposure response(pk/pd).this is another key stage in deciding whether the drug should progress further through clinical trials and therefore incur the investment required.

Phase II b:

Dose ranging studies are carried out on patients to establish effective doses for phase 2 trails.

Analytical priorities include:

- High sensitivity assays.
- High specificity assays for drug compound and metabolites.
- Fast turnaround of samples.

Phase III: Long Term Studies

Large numbers of patients take part in phase3 clinical trials with the objective of showing efficacy across a wide range of populations. vast numbers of samples must be handled and analysed with a bioanalytical assay that is specific, robust, and fast.

- Assay specific to very few analytes.
- Robust to variations in matrix.
- Ability to process very large volumes of data.

CONCLUSION

Development in bioanalytical technology and the application of pharmacokinetic (PK) principles have created a synergistic partnership that plays a main, influential role in the discovery and development of novel medicines. Infact the above stated guidelines defines key elements obligatory for the validation of bioanalytical methods. The guideline focuses on the validation of the bioanalytical methods generating quantitative concentration data utilized for pharmacokinetic and toxicokinetic parameter determinations. Guidance and criteria are given on the application of these validated methods in the routine analysis of study samples from animal and human studies.

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