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A REVIEW ON BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION AND STEPS FOR BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION

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ABSTRACT
A Bioanalytical method meansthe quantitative and qualitative analysis of drugs in biological matrices. Bioanalytical method should be useful to studies in area of human clinical and non-clinical study. Bioanalytical method plays an important role in estimation and analysis of bioavailability-bioequivalence, pharmacokinetic and toxicokinetic studies. A Bioanalytical method has three essential Component method development, method validation and sample analysis. Every step in the method must be studied to decide which Environment, matrix or procedural variable can interfere with the estimation of analyte in the matrix from the time of set up to time of analysis. various steps for development of bioanalytical method. Linearity, accuracy, precision, selectivity, sensitivity, reproducibility, Matrix effect, dilution integrity and stability are some of the Validation parameters. In this present review article includes some points regarding Introduction on bioanalytical method development and method validation, steps for how to method develop, Beneficial to Quality assurance to determine safety and efficacy of drug and its metabolites.

KEYWORDS: Step for bioanalysis, How to method develop, Bioanalyticaltechniques (PPT, LLE, SPE), Estimation of drug in biological matrix.

INTRODUCTION[1,8]
Bioanalytical method shares specifically to determine the concentration of drug or its metabolites or both in biological matrixes such as plasma, Blood, serum, urine, etc.

Blood:-Blood is a body fluid that supplies necessary substance to the body's cells. Elements of the blood are RBC, WBC, and Platelets. Plasma: -Blood plasma is the yellow liquid component of the blood, in which the blood cell in whole blood would normally be suspended. Serum: - Blood serum is blood plasma without fibrinogen or other clotting factor. Urine:- the fluid Produce by the kidneys to remove waste products, excess water and other substance from the body. A Bioanalytical method meansthe quantitative and qualitative analysis of drugs in biological matrices. Bioanalytical method development means to Creating a simplest Procedure that use for Quantitative measurement of analytes in biological matrix is reliable and reproducible for intended use. Bioanalytical Method Validation includes all the Process that demonstrates that a Particular method used for Quantitative Measurement of analyte in given Biological matrix is reliable and reproducible for intended use. Various Techniques use in bioanalytical method**. 1.**HYPHENATED TECHNIQUES in this **LC**–MS (liquid chromatography–mass spectrometry), GC–MS (gas chromatography–mass spectrometry), LC–DAD (liquid chromatography–diode array detection), and CE–MS (capillary electrophoresis–mass spectrometry).**2.** CHROMATOGRAPHIC METHODS in this GC (gas chromatography), UPLC (Ultra performance liquid chromatography), Supercritical fluid chromatography.**3.** ELECTROPHORESIS, **4.** LIGAND BINDING ASSAYS in this ELISA (Enzyme-linked immunosorbent assay), MIA (magnetic immunoassay), RIA (radioimmunoassay).**5.** MASS SPECTROMETRY.6.NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY. Various Methods Used For Sample Preparation Is As Follow- Solid Phase Extraction (SPE), Liquid-Liquid Extraction (LLE), Protein Precipitation (PPT), Hybrid Extraction Technique. The Various Validation Parameters Are:-The Various Validation Parameters Are:-1.Accuracy, 2.Precision, 3.Selectivity, 4.Sensitivity, 5. Recovery, 6.Matrix Factor, 7. Calibration curve, 8. Stability, I Short term stability, II Long term stability, III Freeze Thaw stability, IV Stock solution stability. Significance of bioanalytical method is in bioavailability, bioequivalence, toxicology, clinical and non-clinical study.

OBJECTIVE

The aim for validating a Bioanalytical process is to determine the performance and reliability of a method. Validated bioanalytical method used to support registration of new drug. Bioanalytical method is a documenting Process that use for specific laboratory investigations, that validated method have characteristics are suitable and reliable for the intended analytical application.

NEED OF BIO-ANALYTICAL METHOD

Bioanalytical methods use for Investigate the pharmacokinetic of new drug candidates, Compare pharmacokinetic profiles of different formulations, monitor drug levels to establish the appropriate dose or frequency of administration, for fast and reliable measurement of the compounds in biological matrices.

STEPS FOR BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION[6]

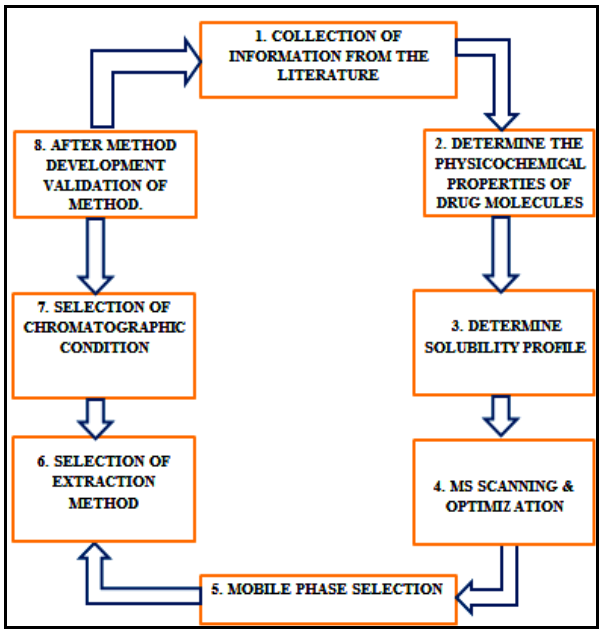

Figure: Steps for Bioanalytical Method Development and Validation.

1. COLLECTION OF INFORMATION FROM THE LITERATURE

First to collect all the information from the literature. literature Sourcelike Mark Index, Bioanalytical Abstract, Therapeutics of the Drug, Medline, Drug bank, Pubchem, Online Data base Various Regulatory agencies Information on Reference Formulation or any other Reliable source. From the Literature Collect Information's Like Chemical Name, Synonym, Molecular Formula, Molecular Weight, IUPAC Name, colour of the Drug, from the molecular structure gate idea about which type of bond present in molecule.

2. DETERMINE THE PHYSICOCHEMICAL PROPERTIES OF DRUG MOLECULE

Physiochemical Properties of an analyte of interest such as Molecular Weight, Solubility, Structure, dissociation Constant (Pka) And Melting Point, Which Helps In Selection of Suitable Extraction Method, Pharmacokinetic Parameter of the drug Cmax, ADME of the drug.

3. DETERMINE SOLUBILITY PROFILE

Solubility: - solubility is defined as, "the number of grams of substance which will dissolved in 100 grams of the solvents at a stated temperature". Solute:-the substance which is dissolved in solvent is called Solute. Solvent:-the substance in which solute is dissolved called solvent. Very soluble means 1 gm of solute substance will require less than 1 ml of solvent. Solubility of solid substance in solvent mainly depends strongly on the temperature and slightly pressure. the solubility increase with increase in temperature.[9]

Table: Type of Solubility.

TYPE OF SOLUBILITY	APPROXIMATE VOLUME OF SOLVENT IN "ml" per gram of solute at 20 to 30 0C Temp.
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10—30
Sparingly soluble	From 30—100
Slightly soluble	From 100—1000
Very slightly soluble	From 1000– 10,000
Insoluble	More then 10,000

Determination of Lower and Upper Limit of Quantification

The lowest concentration of an analyte in a sample that can be quantitatively determined with an acceptable precision and accuracy is usually 5 times of the Cmax value. After calculating ULOQ and LLOQ value we have prepare standard stock solution from which solutions of different concentration are prepared.

Selection of drug volume to be spiked
The volume of the analyte of interest to be depends upon the volume of plasma spiked. Analyte concentration is normally 5% of the spiked plasma volume. For example, if spiked plasma volume is 500µl, so the volume of analyte to be added will be 25µl**.**

Sample Preparation
Sample preparation technique is used to clean up a sample by removing endogenous material as well as to concentrate a sample before analysis to exclude errors in its detection.

4. M.S. SCANNING & OPTIMIZATION
In Bioanalytical method development various hybrid techniques are used that mention in introduction. In ion polarity mode polarity of Performed Molecule is express by Polarity of the drug molecule in solution. If the molecule is acidic in nature they form negative ions (n-1) in solution and if drug molecule is basic in nature they form Positive ions (n+1) in solution. In this n= molecular weight of the drug molecule. The most common scan modes are Mass Spectrometer Scan mode: Q_1MSScanand Q_3MS Scan. MS/Ms scan Modes: Product, Parent and Natural lose. Data Dependent Scan mode: Full scan mode, (SIM)Selected Iron Monitoring and (MRM) Multiple Reaction Monitoring.

5. MOBILE PHASE SELECTION
For the selection of mobile phase firstly we have to consider pka, pH value, polarity of drug and nature of column. If drug is polar, one concept is there that polar loves polar and we want grater solubility of drug in mobile phase for fast separation so we select solvent as a mobile phase that having same polarity as drug and that will reduce retention time. Column: if drugs polar then we have to select non-polar column because drug and Mobile phase are same in nature they on Retention time of the Drug. PH: following formula is used.

For acidic drug
PH = pKa + log (ionized/unionized)
= pKa + log(100/1) = pKa+ 2
For basic drug
PH = pKa + log (unionized/ionized)
= pKa + log (1/100) = pKa- 2

6. SELECTION OF EXTRACTION METHOD
In the method develop pH, pKa, solubility, Ionization of the compound are main component in optimization of Extraction method. Solubilityof the compound Depends up on Polar(Hydrophilic), non-Polar (Hydrophobic) nature of Solute and solvent. Polarity of the solvent determine by Dipole Moment of a compound. Polarity of compound gives idea about which type of solute dissolve in which type of solvent. Polar dissolve in polar and Non-polar dissolve in non-polar. Mainly Three Extraction Method is available for extraction of analyte from biological Matrix.

1. PPT
Protein Precipitation methodthis extraction technique mainly depend on a Protein binding. Generally > 85% Protein binding of drug analyte with Plasma Protein then 1st prime gives to PPT Method For optimization of extraction Method. Main advantage of this method is reducing Steps of procedure and less time require. Main drawback of this method is less Recovery obtain.

2. LLE
Liquid-Liquid Extraction (Solvent Extraction, Partitioning) is generally based on a Solubility of a compound in two Different Immiscible Liquid Phase (Water and Organic Phase). Selection of the LLE Method based on log P value (P= [organic concentration]/[aqueous concentration]). If log P value >2 it means solubility of analyte is more in organic phase then go for LLE method and if < 2 It means Solubility is more in aqueous phasethen go for SPE Method. Compare to PPT Method LLE Method gives good recovery.

3. SPE
Solid phase extraction is based on absorption of analyte in to sorbent, SPE Extract the analyte from biological fluid. SPE Method have 5 steps1conditioning step, 2.washing(Equilibrium) step, 3.Loading of the sample,4. washing step, 5.Elution step. Compare to this all method SPE give more recovery but SPE have long process step. type of SPE 1.reverse phase, Normal Phase, Ion exchange, Anion-Cations Exchange.

7. SELECTION OF CHROMATOGRAPHIC CONDITIONS
In optimization of chromatographic condition optimize this all parameters: Mobile Phase, Column, Flow Rate, Retention Time, Run Time, Injection Volume, Column Oven Temperature, Pressure Range, Purging Time etc. this all Parameters first done on aqueous vial(analyte dissolve in respected solvent). mobile phase, column, flow rate, retention time optimize in different ratio and different solvent of mobile phase by modifying this all parameter optimize mobile phase, column and all parameter on aqueous phase. After optimization satisfactory chromatography obtain then go for trials on Blank biological matrix and take idea about matrix gives interference effect on retention time or not then Prepare set of Un-extracted (aqueous) and extracted (spiked)sample of linearity rang and checkany interference on retention time of analyte. After optimization of all parameter pre-method validation perform to check all the validation parameter give the result is in the range of acceptance criteria.

8. AFTER METHOD DEVELOPMENT VALIDATION OF METHOD
Bioanalytical method development means to Creating a simplest Procedure that use for Quantitative measurement of analytes in biological matrix is reliable and reproducible for intended use. Method development means to optimize extraction, chromatographic, and

Spectrometric Detector condition to achieve the Desired Quantitation Outcome. A common, Comprehensive, vast, Systematized & scientific Approach is required to Develop Rugged, Reproducible & Error-Resistance Bioanalytical Method. From the 1 to 7 stepgenerate simplest method for qualitative and quantitative estimation of analyte in biological matrix then method validation is done. Bioanalytical Method Validation include all the Process that Demonstrate that a Particular method used for Quantitative Measurement of analyte In given Biological matrix are reliable and reproducible for intended use.

THE VARIOUS VALIDATION PARAMETERS ARE[10,11,12]

1.Accuracy, 2.Precision, 3.selectivity, 4.sensitivity, 5. Recovery, 6. Matrix Factor, 7.Calibration curve, 8.Stability, I Short term stability, IILong term stability, III Freeze Thaw stability, IVStock stability. Guidelines for bioanalytical USFDA: - United states food and Drug Administration (guidance for Industry), EMA: - European Medicines Agency(Guideline on Bioanalytical methodvalidation), ANVISA: Agencia Nacional de Vigilancia Sanitaria(Guideline for validation of analytical and Bioanalytical method).

SUMMARY AND CONCLUSION

Bioanalytical method and related pharmacokinetic, toxicokinetic andbioequivalent data mainly utilized in pharmaceutical research and development as well as drug discovery and development process. Bioanalytical method must be validated Process that Demonstrate that a Particular method used for Quantitative Measurement of analyte in given Biological matrix are reliable and reproducible for intended use. And this review article represents steps that indicate how bioanalytical method is developed.

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