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A SHORT REVIEW ON BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION

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ABSTRACT

This paper's review examines the practices relevant to pharmaceutical preparation within biological matrices and aims to deliver practical tactics for the analysis of selectivity, specificity, detection limits, low quantitation thresholds, linearity, range, accuracy, precision, recovery, stability, resilience, and robustness of liquid chromatography techniques to assist pharmacokinetic (PK), toxicokinetic, bioavailability, and bioequivalence assessments. Bioanalysis, utilized for the quantitative assessment of pharmaceuticals and their metabolites in biological fluids, holds a pivotal position in the appraisal and elucidation of bioequivalence, PK, and toxicokinetic assessments. Developing precise and nuanced methods for quantitatively assessing medications and their breakdown products is vital for successfully conducting both preclinical and clinical pharmacology studies, including biopharmaceutical research.

KEYWORDS: Bioanalytical method, Plasma, validation, ICH Guidelines.

INTRODUCTION

1.1 Bioanalytical Techniques

Bioanalytical techniques, employed for the quantitative determination of drugs and their metabolites in biological fluids and creates a specific procedure to enable a coalesce of interest to be identified and at the same time to be quantified in a matrix. A coalesce is measured by several procedures. The choice of analytical procedures involves many considerations, such as: concentration levels, chemical properties of the analyte, specimen matrix, cost of the analysis, experimental speed, quantitative or qualitative measurement, required precision and necessary equipment[2]. Bioanalytical method validation comprises all criteria determining data quality, such as selectivity, accuracy, precision, recovery, sensitivity, and stability.[1,2]

1.2 Drug Analysis in Various Biological Media

Blood, urine, and faeces are the most commonly acquired samples for biopharmaceutical analysis, especially if the drug or metabolite is poorly absorbed or substantially eliminated in the bile. Saliva, breath, and tissue are examples of other media that can be used. The nature of the investigation heavily influences the selection of sampling media. In a clinical pharmacokinetic investigation, for example, medication levels necessitate the use of blood, urine, and saliva. A bioavailability study may necessitate drug level data in blood and/or urine, but a drug identification or drug addiction concern may only necessitate one type of biological sample.[2,5]

The nature of the drug investigation heavily influences the selection of sample media. In a clinical pharmacokinetic study, for example, medication levels necessitate the use of blood, urine, and perhaps saliva. Bioavailability research may necessitate medication level measurements in blood or urine. The steps involved in estimating medicines in biological fluid are sample collection, sample treatment, separation of the compound of interest from the matrix, and analysis.

Bioanalysis can determine the therapeutic efficacy of a specific medicine. Bioanalysis is important in the pharmaceutical industry. The following steps are involved in bioanalysis.[6,8]

- Biological fluid selection and collection
- Sample preparation -Analyte extraction from biological matrix.
- Analyte detection is accomplished through a variety of approaches.

1.3 Methods of extraction of Drugs in Biological Matrix

The desired analyte should be extracted from the biological fluid after it has been selected. This phase in

the bioanalytical approach is more crucial since sample preparation can be done using several extraction methods. The preparation of the sample takes time and should be done carefully due to its importance. If the biological matrix is liquid, such as blood, plasma, or urine, liquid-liquid extraction is employed; if it is solid, liquid-solid extraction is utilized.

The following are the most well-known and widely utilized extraction methods

1. Protein precipitation method.
2. Liquid-liquid extraction method. (LLE)
3. Solid-phase extraction method. (SPE)
4. Filtration.

Table 1.1: Different types of extracting methods from biological Fluids.

S.No	Types
1.	Dilution followed by injection
2.	Solid Phase extraction [SPE]
3.	Protein precipitation [PE]
4.	Filtration
5.	Liquid-liquid extraction [LLE]
6.	By using equilibrium dialysis or ultra filtration protein extraction.
7.	Restricted access media.
8.	Solid-supported liquid-liquid extraction [SS-LLE].
9.	Monolithic columns.

1.3.1 Protein precipitation[9]

The interaction of the precipitation reagent with protein groups provides the basis for protein precipitation. Soluble proteins typically have a hydrophobic core surrounded by a hydrophilic surface containing non-intermolecular bound ionic groups. Organic solvents disrupt protein intramolecular hydrophobic interactions. When a volume of solvent (usually acetonitrile) is added to serum, the proteins precipitate, leaving the analyte of interest in the solvent, which can then be injected directly or dried down and reconstituted in a smaller volume to concentration before injection. While this is the quickest and easiest way for sample preparation, it is also the most likely to produce ion suppression concerns, particularly in ESI, where coelution of endogenous chemicals such as lipids, phospholipids, and fatty acids affects the ESI droplet desolvation process.

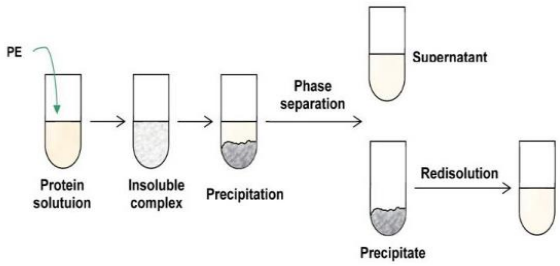

Figure 1.1: Protein precipitation technique.

1.3.2 Liquid-Liquid Extraction[10]

Liquid extraction is a typical technique for extracting analytes from liquid matrices. The analyte is isolated from interferences by partitioning or distributing the sample between two immiscible liquids or phases. LLE typically has one aqueous phase (generally the denser or heavier phase) and one organic solvent phase (often the lighter phase). The polar aqueous phase is preferred by hydrophilic chemicals, whereas the organic solvent is preferred by hydrophobic ones. The two phases used in this strategy should be inseparable. By partitioning the sample between these two immiscible liquids or phases, it is particularly useful for separating analytes from interferences.

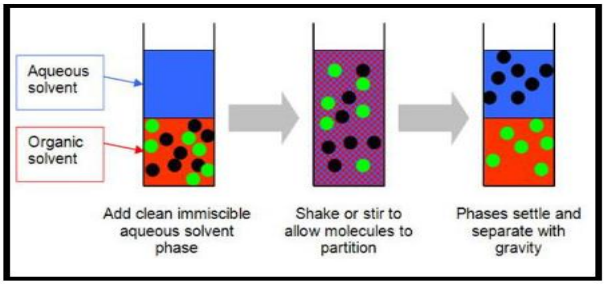

Figure 1.2: Liquid- Liquid Extraction.

1.3.3 Solid Phase Extraction[11]

SPE is a sample preparation technique in which chemicals that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture based on their physical and chemical properties. To isolate analytes of interest from a wide range of biological matrices, solid phase extraction can be utilized. SPE separates a mixture into desirable and undesired components by utilizing the affinity of solutes dissolved or suspended in a liquid for a solid through which the sample is passed. As a result, the stationary phase retains either the desired analytes of interest or the undesired contaminants in the sample. Depending on whether it contains analytes or contaminants; the part that passes through the stationary phase is collected or discarded. If the required analytes are present in the part retained on the stationary phase, they can be removed from the stationary phase for collection in a subsequent step in which the stationary phase is washed with a suitable eluent. The solid-phase extraction method similarly has two phases, one solid and one liquid.

The analyte is held on the solid phase as the sample passes through, followed by analyte elution using a suitable solvent. The solid phase in this case is a plastic disposable column or cartridge packed with sorbent-like reversed phase material (C-18silica) bonded to a hydrocarbon phase. SPE is a solid-liquid phase separation of analytes from biological samples that requires the selective transfer of a liquid and solid state. The differential interaction with a solid phase sorbent material physically separates the analyte from the biological matrix. These sorbents, which are typically packaged in disposable cartridges or discs, can be polar, non-polar, or ionic depending on the experimental needs.

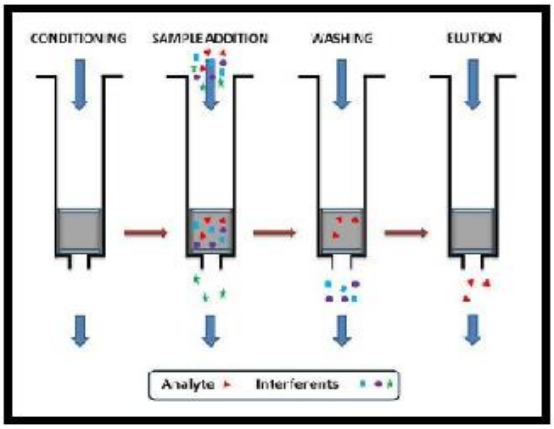

Figure 1.3: Solid Phase Extraction.

1.4 Types of Bioanalytical Method Validation[12]

1.4.1 Full validation

The full validation is an establishment of all validation parameters to apply to sample analysis for the bioanalytical method for each analyte. It is important

- When developing and implementing a bioanalytical method for the first time
- For a new drug entity
- When metabolites are added to an existing assay for quantification then Full
- Validation is done.

1.4.2 Partial validation

These are modifications to bioanalytical methods for which full validation is not necessary.

Modifications done for bioanalytical methods such as, small changes like change in species with matrix (from rat plasma to mouse plasma), change in matrix with in a species (from human urine to human plasma) change in laboratories or analysts, instruments, change in sampling process procedures, change in analytical method like changing detector.

1.4.3 Cross validation

In these two bioanalytical methods are compared. The "reference" method which is original one is compared with the revised one "comparator". This is done where two bioanalytical methods are compared and from that same data is prepared for study. This is done in two ways.

Spiked matrix samples and subjected samples validation done at sane site or done at different sites i.e. different laboratories or by using different techniques in same laboratory.

1.5 Parameters involved bio analytical validation as per ICH M10[17]

1. Accuracy
2. Precision
3. Robustness
4. Limit of quantification
5. Limit of detection
6. Ruggedness
7. Linearity
8. Stability
9. Matrix effect.

Table 1.2: Bioanalytical Method Validation Parameters.

Parameters	Definition	Method	Acceptance criteria
Accuracy	Exactness of expository system communicates the closeness of understanding between the esteem which is acknowledged either as a traditional genuine esteem or acknowledged reference esteem and the esteem found. This is some of the time named trueness[2].	Measured using a minimum of 5 determinations per concentration and a minimum of 3 concentrations in the range of expected concentration is recommended	Mean value should be within 15% . nominal value(or)LLOQ (lower limit of quantification) should not deviate by more than 20%.
Precision	The accuracy of a systematic strategy communicates the closeness of understanding (level of disseminate) between the arrangement of estimations got from various inspecting of the same homogeneous example under the recommended conditions[3].	Measured using a minimum of 5 determinations per concentration and at minimum of 3 concentrations	precision should not exceed 15%. %CV (co-efficient variation) (or)LLOQ, should not exceed 20% .
Robustness	The vigor of a logical strategy is a measure of its ability to stay unaffected by little, yet consider varieties in technique parameters and gives a sign of its unwavering quality amid ordinary use.		
Limit of quantification (LOQ)	The quantitation uttermost compasses of an individual consistent methodology is portrayed as the most negligible measure of analyte in an illustration, which can be quantitatively chosen with fitting precision and accuracy[1].		The analyte response of should be precision maximum 20% and accuracy of 80-120%.
Limit Of detection(LOD)	The discovery utmost reaches of an individual explanatory technique is the most minimal measure of analyte in an example, which can be recognized yet not really quantitated under expressed exploratory conditions		
Ruggedness	Ruggedness is a measure for the susceptibility of a method to small changes that might occur during routine analysis like small changes of pH values, mobile phase composition, temperature[5].		
Linearity	The linearity of an investigative strategy is its capacity to acquire test comes about, which are straight forwardly relative to the focus of analyte in the example. The scope of investigative method is the interm between the upper and lower fixation of analyte in the example for which it has been exhibited that	Should consist of a blank sample, a zero sample, and 6–8 non-zero samples covering the expected range, including LLOQ.	The LLOQ should be at least 10% of the expected maximum concentration. ULOQ(upper limit of quantification) should be at least 2 times the expected C_{max} value. Regression coefficient (R2) > 0.98 .

	the scientific technique has an appropriate level of exactness, precision and linearity.		
Stability	The stability of an analyte under various conditions are to also be studied during method validation process. The conditions used in stability experiments must reflect situations likely to be encountered during actual specimen handling and analysis[3]. **Freeze-Thaw stability:** The stability of an analyte should be determined, after complete three freeze and thaw cycles. **Short-Term-temperature stability:** The stability of an analyte in biological matrix under ambient temperature should be examined. **Long Term stability**: The stability of an analyte in the matrix should cross the time period from specimen collection until the last day of experimental work.	Performed using 3 aliquots at HQC(higher quality control) and LQC(lower quality control) at intended temperature for 24 h after 3 freeze-thaw cycles. Three aliquots of each of the HQC and LQC should be thawed at room temperature and kept for 4–24 h before analysis. Determined by storing three aliquots each of HQC and LQC under the same conditions intended for study samples, concentration of stability samples to be compared with the mean of back-calculated values of the standards from 1st day of long-term stability testing	Stability sample should be within 15%.
Matrix effect	The combined effect of all components of the sample other than the analyte on the measurement of quantity[4].	Three blank specimens from each of not less than six batches of matrix under screening to be extracted. For matrix effect MQC (middle quality control), LQC and HQC spiking dilutions are spiked and internal standard dilution above extracted blank specimens	

1.6 Stability

The stability of the analyte under diverse experimental conditions warrants thorough investigation during the process of method validation. The conditions employed in stability assessments ought to mirror scenarios that are likely to arise during the handling and analysis of actual samples. The subsequent stability conditions have been delineated by the FDA and are deemed prudent for examination.[14]

1.6.1 Stock solution stability

The stability of the stock solution necessitates evaluation at ambient temperature over a duration of six hours.

1.6.2 Short-term temperature stability

The analyte's stability within biological fluids at ambient temperature should be systematically assessed. A total of three aliquots, showing both low and high concentrations, should be preserved for no less than twenty-four hours ahead of the analysis.

1.6.4 Long-term temperature stability

The stability of the analyte within the matrix must extend beyond the interval from sample collection until the final day of analysis.

1.6.5 Freeze and thaw stability

The stability of the analyte should be quantified following three cycles of freezing and thawing. Before thawing at room temperature, three aliquots that include both low and high concentration levels must be frozen for a full twenty-four hours.

1.6.7 Post-preparative stability

The stability of the analyte throughout the various stages of the analytical process must be critically evaluated.[15,16]

1.7 Application of validated method for routine drug analysis

The assays for all samples of an analyte within a biological matrix should be completed within the designated timeframe for which stability data are accessible. Generally, biological samples may be subjected to analysis with a single determination, devoid of duplicate or replicate analyses, provided that the assay method demonstrates satisfactory variability as defined by validation data.[17] This assertion holds true for methodologies where accuracy, precision, and variability consistently remain within acceptable limits. In instances where the procedure is complex and the analyte is labile, making high precision and accuracy specifications challenging to attain, duplicate or even triplicate analyses may be warranted to facilitate a more reliable estimation of the analyte.

2. CONCLUSION

Bioanalytical methods are used to analyse an analyte in a biological matrix. Bioanalytical method validation is the process of determining the suitability of the given bioanalytical methodology for providing the required analytical data. Validation of the bioanalytical methods demonstrates and ensures that the methods used for the quantification of analyte in biological fluids are reliable, reproducible and suitable for its intended application.

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