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Bioanalytical Method Development and Validation: A Review

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ABSTRACT

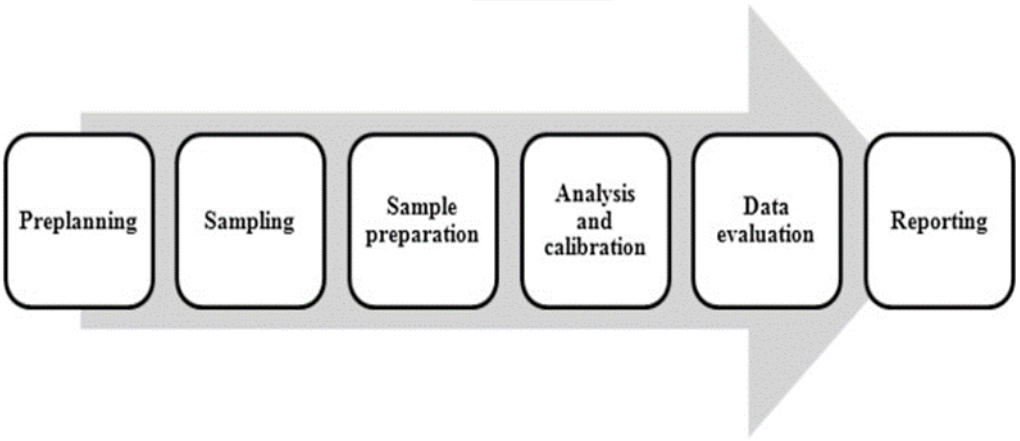
Bioanalytical method used for the analysis of analytes and their metabolite, endogenous compound and biomarker from biological fluid. In this review article, a brief discussion on the development and validation of bioanalytical methods. Bioanalytical method is the process of determining whether a quantitative analytical method is appropriate for biomedical applications. Contains all processes to be displayed Quantitative measurement of analytes in specific biological samples, such as blood plasma, serum or urine. The procedure is clinically and non-clinically toxicokinetic and Pharmacokinetic studies to evaluate drug efficacy and safety. Bioanalytical method that evaluates traits *in vivo* Bioavailability, bioequivalence and drug interactions. This article focuses on different extract Techniques such as liquid-liquid extraction, solid-phase extraction and protein precipitation which play an important role in sample preparation and detection by RP HPLC and Continuous evaluation of the most important methods of validation of bioanalytical methods. From Development process to ensure the quality and reliability of bioanalytical sample development methods Various validation parameters such as accuracy, precision, reproducibility and stability are performed By the Food and Drug Administration (FDA).

INTRODUCTION

Steps included in the collection; Biological data processing, storage and analysis, Bioassays are a series of a sample of the compound. Bioanalysis Method validation (BMV) is establishing an appropriate quantitative method for biochemical applications. Reassurances to the quality of the method and its reliability comes from performing a minimum series of experiments of validation and obtaining satisfactory results.

In May 2018, the Center for Drug Evaluation and Research (CDER), the Food and Drug Administration, the Center for Veterinary Medicine (CVM), and the U.S. Department of Health and Human Services published industry guidelines for the development and validation of bioanalytical methods.

Advances in analytical methods and validation play an important role in drug discovery, improvement and manufacturing. The primary focus of analytical measurement is to obtain consistent, accurate and rational knowledge. To achieve this goal, proven analytics strategies play a major role. Results from validation technique can be used to select the standard, authenticity, and uniformity of analytical results.



NEED OF BIOANALYTICAL METHOD VALIDATION

- It is essential to used well-characterized and fully validated Bioanalytical methods to yield reliable results that can be satisfactorily interpreted.

- It is recognized that Bioanalytical methods and techniques are constantly undergoing changes and improvements; they are at the cutting edge of the technology.

- It is also important to emphasize that each Bioanalytical technique has its own characteristics, which will vary from analyte to analyte, specific validation criteria may need to be developed for each analyte.

- Moreover, the appropriateness of the technique may also be influenced by the ultimate objective of the study. When sample analysis for a given study is conducted at more than one site, it is necessary to validate the Bioanalytical method(s) at each site and provide appropriate validation information for different sites to establish inter-laboratory reliability.

- The bioanalytical technique has its own characteristics, which will vary from analyte to analyte, specific validation criteria may need to be developed for each analyst.

- The bioanalytical methods yield to reliable and satisfactorily result interpreted.

- Validation involves documenting, through the utilization of specific laboratory investigations, that the performance characteristics of the technique that are suitable and reliable for the intended analytical applications.

SEPRATION AND DETECTION BY RP-HPLC

High performance liquid chromatography (HPLC) is a powerful bioanalytical technique. HPLC columns have a large selective range and thus HPLC has been applied to separate many drugs and metabolites in different matrices. The main detector type used in HPLC is UV-vis. HPLC-UV has been used to separate and measure a variety of analytes and matrices. UV detector is economical but can only be used for high analyte concentration (micrometer range), the most useful detector for small sample volume and low concentration level (nm, PM) is mass spectrometry.

VARIOUS ACTIVITIES ARE CARRIED OUT IN THE DEVELOPMENT OF THE BIOANALYTICAL METHOD. THE STEPS INVOLVED IN DEVELOPING THE METHOD USING RP-HPLC ARE AS FOLLOWS:

Documentary research on drugs.

Define analytical and optimization techniques

Setting benchmarks

Selection and optimization of the extraction method

Improvement of the HPLC method

INSTRUMENTATION FOR BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION (12)

Gas chromatography, high pressure liquid chromatography, Mass spectrometry (MS) combining LC and GC Procedures such as LC-MS, LC-MS-MS, GC-MS, GC-MSMS Used for quantitative analysis. For quantization Traditional low-molecular-weight drugs in the biotechnology field. Fluid mass changed dramatically. Methods based on spectroscopy, especially LC-MS and LCMS-MS. The 90s were awesome Advances in Mass Spectrometry, development of new interfaces, ionization and detection Technology. This allows for rapid improvements. Hyphenation and Heavy Commercial Use Mass spectrometry-based assay. This method replaced the traditional HPLC, GC and GC-MS assays.

METHOD DEVELOPMENT

Steps

Method development is a complex process with several steps 8, which are as follows:

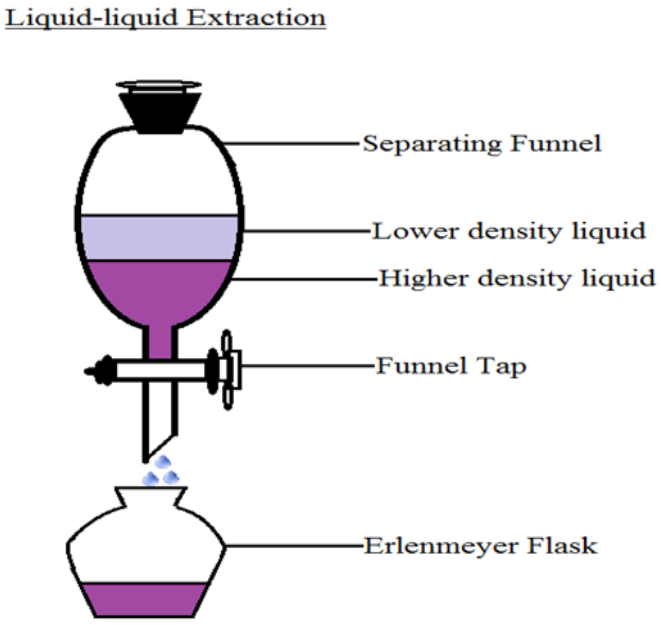
- **Literature search and drug information.**
- **Selection of the initial conditions of the method**
- **Verification of analytical methods in aqueous standards**
- **Development and optimization of sample processing methodology**
- **Verification of the analytical method in the biological matrix**
- **Pre-validation**
- **Validation of biological analysis method**
- **Good laboratory performance.**

TYPES OF BIOANALYTICAL METHOD DEVELOPMENT

1. Liquid liquid extraction
2. Solid phase extraction
3. Protein precipitation
4. Solid phase microextration (SPME)
5. Matrix solid phase dispersion (MSPD)
6. Supercritical fluid extraction

1. LIQUID LIQUID EXTRACTION (13,14,15)

It's based on that Principle of differential resolution and distribution. Analytical balance between aqueous and the organic phases generally preclude extraction from one phase to another and distribution of analyte particles between two immiscible phases. Post extraction analysis separating the organic layer from the aqueous phase; evaporate in the presence of gaseous nitrogen to obtain Dry form of the sample. Today's traditional LLE is replaced with advanced and superior technology Liquid phase microextraction (LPME), drop of liquid, etc. Phase Microextraction (DLPME) and support Membrane extraction (SME).

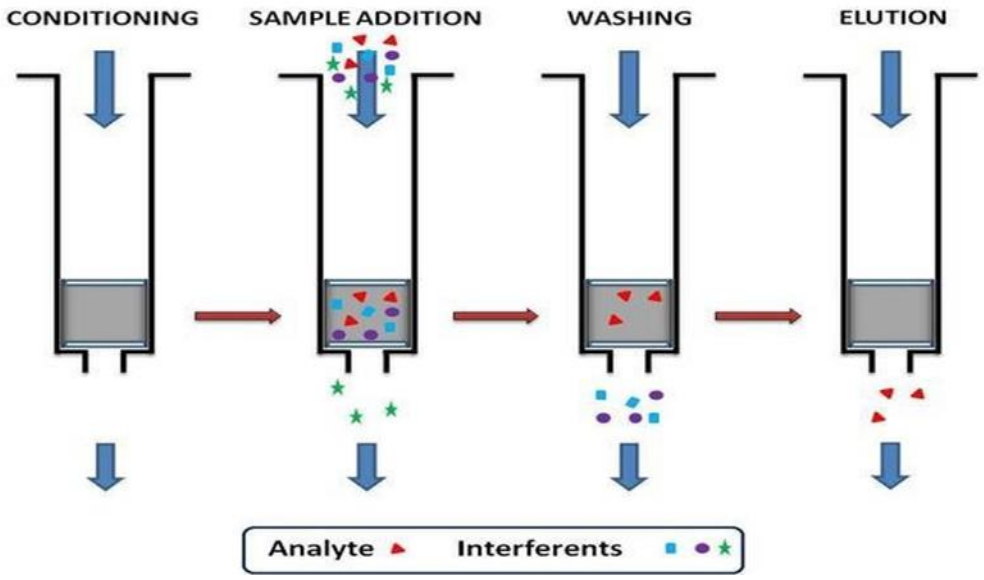


2. SOLID PHASE MICROEXTRACTION (SPME) (16,17)

This is a simple, solvent-free method where sampling, extraction, concentration and introduction of the sample in one one step without solvent. SPME consists of fused silica fiber Coated with polyacrylate, polydimethylsiloxane, carbowax or is placed in contact with another modified bonded phase sample and is exposed to steam, it can also be placed in a gaseous sample stream to separate the analyte. Analysis of concentrates on a wide range of investment materials. Finally, the fibers are transferred to the analysis tools Like Gas Chromatography (GC) and Mass Spectrometry/GC (GC/MS) for target separation and quantification analyze it using a syringe. For routine analysis of volatile and semi-volatile compounds, SPME can be Jack. Direct introduction of SPME combined with high performance liquid chromatography (HPLC) and HPLC-MS are slightly volatile or thermally unstable compounds not suitable for GC or GC-MS.

3. SOLID PHASE EXTRACTION (16,18,)

In SPE, the liquid and solid state. The main advantage of SPE is elimination of impurities present in the analysis help increase method sensitivity. Remove fine dust analysis is the main output of SPE. In SPE, many typically 12-24 samples with low amounts of solvent with automation being the main contributor. In SPE sample recovery is higher. Disposable small pole or use the cartridge to split.



TYPES

1. **Reversed phase**
2. **Normal phase**
3. **Ion exchange**

ADVANTAGES OF SPE/ LLE (19)

1. Analysis is fully extracted.

2. Highly efficient interference isolation analytics and low consumption organic solvent.

3. Easy to collect total analysis part.

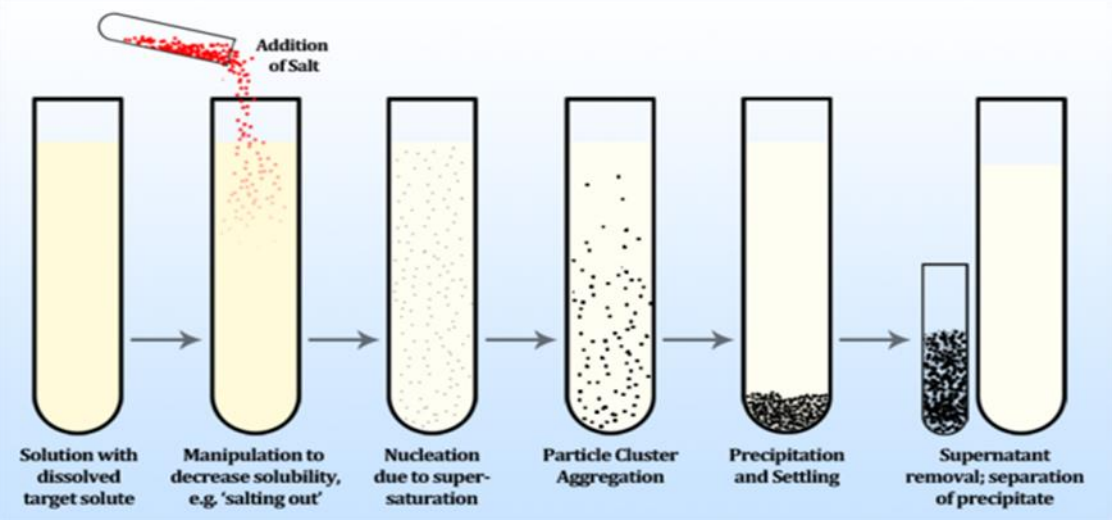
4. Convenient procedures manually remove particles easily.

5. Easily automated.

4. PROTEIN PRECIPITATION (20)

A way to eliminate overlapping of arrays. This can be obtained by denaturation and precipitation. Widely used precipitators such as Trichloroacetic acid and perchloric acid. Various organic solvents such as methanol, acetonitrile, acetone, and ethanol are excellent choices for plasma protein removal. Compatible with high-performance liquid chromatography (HPLC) mobile phases**.**

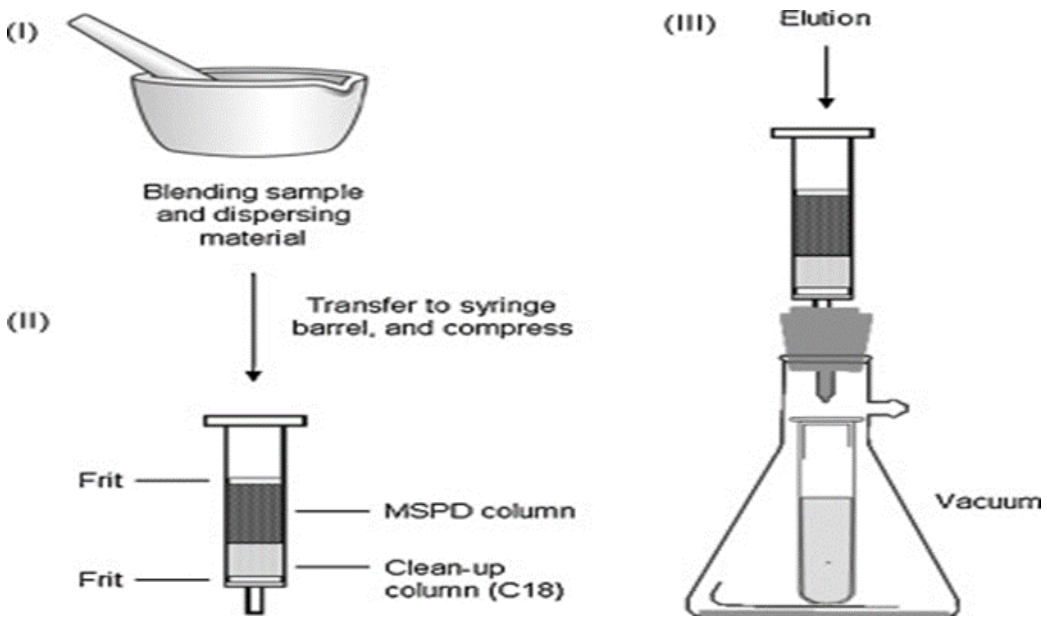
5. MATRIX SOLID PHASE DISPERSION (MSPD) (16, 21)



In the solid matrix diffusion process, solid matrices are used for sample preparation. It is useful because the sample required is less than 1 g and the solvent is low, which is why it is also called microscale extraction method.

Almost 98% of solvent usage is reduced and sample turnaround time is 90%.

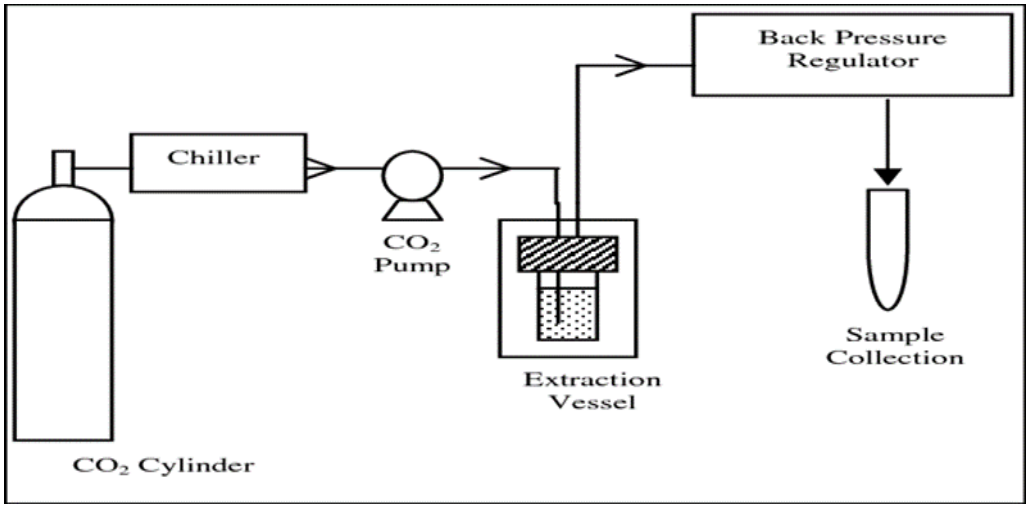
This method has recently been applied, using acidic alumina, to remove organic compounds. Two important aspects of this method are the length of the analysis and the limit of determination (LOD).



4. SUPERCRITICAL FLUID EXTRACTION (16, 22, 23)

Supercritical fluid extraction is commonly used to remove non-polar to moderately polar analytes from matrices. Regulations require that it be an alternative to organic solvents, and it also has environmental benefits. Supercritical fluids have the same density as liquids, but viscosity and diffusivity are gaseous and liquid values.

SFE is usually completed in 10-60 minutes. The solvent power of supercritical fluids can change with pressure changes and to a lesser extent with temperature. Many supercritical liquids are gases at ambient conditions. This simplifies analyte recovery compared to body fluids**.**



METHOD VALIDATION (24)

A validation process is a process used to check/prove that a developed research method is suitable for its intended use, and that it provides reliable and accurate data for particular research. Common parameters to confirm are; including selectivity, accuracy, precision, linearity and range, limit of detection, limit of measurement, recovery, strength and stability.

VALIDATION ASSURES THAT (25)

1. The test results are reliable, consistent and also there is reproducibility experience.

2. It also provides assurance in the process suitable for the intended purpose.

3. It provides assurance of product quality and system.

4. Understanding and promotion of products by International companies are possible.

5. For registration requirements for medicinal product or formulation.

TYPES OF VALIDATION

Depending upon the method used

1. **Full validation**
2. **Partial validation**
3. **Cross validation**

1. FULL VALIDATION

- Complete validation is essential when developing and applying a bioanalytical method for the first time.
- Full accreditation is essential for new hospitals.
- Full validation of the revised assay is essential if metabolites are added to existing assays for quantification.

2. PARTIAL VALIDATION (26, 27, 28)

Partial validations are already validated and modified bioanalytical methods. Partial validation does not necessarily require full revalidation or other revalidation. Partial verification of accuracy and accuracy may vary within an individual examination. Modifications of the bioanalytical method that fall into this category include:

1. Bioanalytical method of transfer between laboratories or analyzers
2. Change in analytical method (e.g. change in identification systems)
3. Changing the Anticoagulant in the Collection of Biological Fluids
4. Change in sample processing procedure
5. Change in focus range
6. Evolution of tools and/or software platforms
7. Limited sample size (e.g, pediatric research)

8. Rare matrices

9. Selective demonstration of analysis in the presence of concomitant selective drugs

10. Demonstration of an analysis given defined outputs

3. CROSS VALIDATION (29)

Cross-validation is the comparison of validation parameters when using two or more bioanalytical methods to generate data within the same study or different studies. An example of cross-validation is when the original validated bioanalytical method serves as a reference and the revised bioanalytical method is compared**.**

STEPS INVOLVED IN METHOD VALIDATION (29)

1. Development of verification protocol

2. Purpose, goal and method planning

3. Performance criteria and acceptance criteria

4. Check the relevant performance characteristics of the equipment

5. Qualified material, for example standard reagent for purity, precise amount and enough stability.

6. Run pre-validation experiments

7. Changes in the development of validation methods

8. Create a standard operating procedure (SOP).

9. Validate the bioanalytical sample to ensure quality and reliability and various pharmacokinetic parameters.

10. Enter the results in the validation test and the validation report.

VALIDATION PARAMETERS

1. Accuracy

The accuracy of an analytical method describes how closely the averaged results of the method approximate the true value (concentration) of the analyte. Accuracy is determined by repeated analysis of samples containing known amounts of the analyte. Accuracy should be measured using at least 5 measurements per concentration.

2. PRECISION (30)

The precision of a bioanalytical method is a measure of random error and the degree of agreement between a series of measurements obtained by injecting a series of standards or analyzing a series of samples from different samples from a homogeneous lot is lost.

3. LINEARITY

Linearity defines the ability of the method to obtain test results that are directly proportional to the concentration of the analyte in the sample. ICH 7 guidelines recommend analyzing at least 5 concentrations to determine linearity.

4. LIMIT OF DETECTION (31)

The limit of detection (LOD) is the lowest concentration of analyte in a sample that can be detected but not measured under specified experimental conditions. It is also defined as the lowest concentration that can be distinguished from background noise with some degree of confidence.

5. LIMIT OF QUANTIFICATION

The LLOQ is the lowest level of an analyte in a sample that can be measured quantitatively with reasonable precision and accuracy. Determining the LLOQ on the basis of precision and accuracy is probably the most practical approach, where the LLOQ is defined as the lowest sample concentration that can be measured with acceptable precision and accuracy**.**

6. ROBUSTNESS

According to ICH guidelines, the robustness of an analytical method is a measure of its ability to remain unaffected by small but intentional changes in method parameters and gives an indication of its reliability during normal use.

7. RANGE

The distance between high and low concentration analytes. The range of a bioanalytical test is the concentration over a period of time over which an analyte can be measured with acceptable accuracy and precision.

8. RECOVERY

Extraction efficiency of the analytical process. Expressed as a percentage of the known amount of analyte passed through the sample extraction and processing steps of the method.

9. STABILITY

Stability is defined as the chemical stability of an analyte in a particular sample matrix under particular conditions. Stability of the analyte throughout the analysis study is a prerequisite for reliability.

Long term stability

Stock solution stability

Freeze thaw stability

Bench top stability

Processed sample stability

CONCLUSION

This article gives an idea of the development of the bioanalytical method, its requirements, guidelines, various methods and validation processes. Validation is an essential technology in pharmaceuticals and is used to ensure that quality is maintained throughout the process that supports drug production and development. This overview also describes sample preparation

of compounds/drugs in various body fluids, thus understanding and describing bioanalytical method development and validation from a fundamental perspective.

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