Chapter

Analytical, Bioanalytical, Stability-Indicating Methods: Key Part of Regulatory Submissions

Mahesh Mukund Deshpande

Abstract

According to the International Conference for Harmonization (ICH), the validation and verification data must be included in the Electronic Common Technical Document. The validated analytical procedure gets automatically Food and drug approved (FDA) if it is part of New drug application (NDA), Abbreviated new drug application (ANDA) or Biologic license application (BLA). The analytical, bioanalytical and stability-indicating methods are essential part of all above said regulatory submissions. There are certain ways to generate these analytical methods like U.S. pharmacopeia/National Formulary which are Food and drug approved. The validated analytical method can also be submitted by any researcher or agency which can gets the food and drug approval. It is necessary that the methods which are Food and drug approved can only be applied to the various drugs and drugs products. In the current chapter, the meaning and requirements of analytical methods, procedures, acceptance criteria and evaluation of stability indicating methods, need, recommendations for bioanalytical methods are discussed in detail. The analytical techniques like HPTLC, HPLC, Spectrophotometry and Hyphenated techniques are also discussed as these are playing important role in validation of these methods.

Keywords: noncompendial and compendial analytical methods, forced degradation analytical techniques, bioanalytical methods, analytical techniques

1. Introduction

The United States of America (USA), Europe and Japan were developed the Common Technical Document (CTD) which should be implement while applying for registration of pharmaceutical product for human use. For the development of these guidelines International conference for Harmonization (ICH) plays important role and currently these are becoming the part of ICH guidelines [1]. The identity, strength, quality, purity and potency are important points of Investigational new drug application (IND), New drug application (NDA), Abbreviated new drug application (ANDA). The related analytical methods of drug substance and product should be included in NDA and ANDA. A Complete description, total manufacturing process including analytical procedures should

ensure compliance with standards and potency should be part of Biologics license application (BLA). It is must to meet all standards of guidelines provided for analytical procedures. All these parameters should be suitable for their purpose wherever applicable. Detail Analytical procedures including detail validation parameters are the important part of Electronic Common Technical Document Specification as per International conference of Harmonization (ICH). The analytical procedure is Food and Drug Approved (FDA) if it a part of Approved NDA, ANDA or BLA. These methods can be generated from FDA recognized sources like U. S. Pharmacopeia/National Formulary (USP/NF) or if anyone submits validated procedure that will be accepted by FDA. The only validation or verification data of FDA approved methods of new products are considered for applications to various drug products (**Figure 1**).

Every manufacturer must generate large amount of corrected data for safety and efficacy of drug for commercial viewpoint. As it is mandatory to follow Current Good Manufacturing practices (cGMPs) for manufacturing purpose, likewise each analytical activity must follow Good Analytical Practices.

Method Validation, calibrated instrument, and training are three important tasks of Good analytical practices (GAPs). Commercially available dosage form is an outcome of several steps which are systematically carried out during product development. It is very important that all steps should be carried in systematic manner to ensure complete drug development stage. In recent years there is special focus on efficiency and efficacy of drug product and for this clinical study is most important task but apart from this there are various behind the scene activities are associated with drug development process without which pharmaceutical drug development is not possible. Among these behind the scene activities Method Development and Validation has its own uniqueness to ensure the drug development.

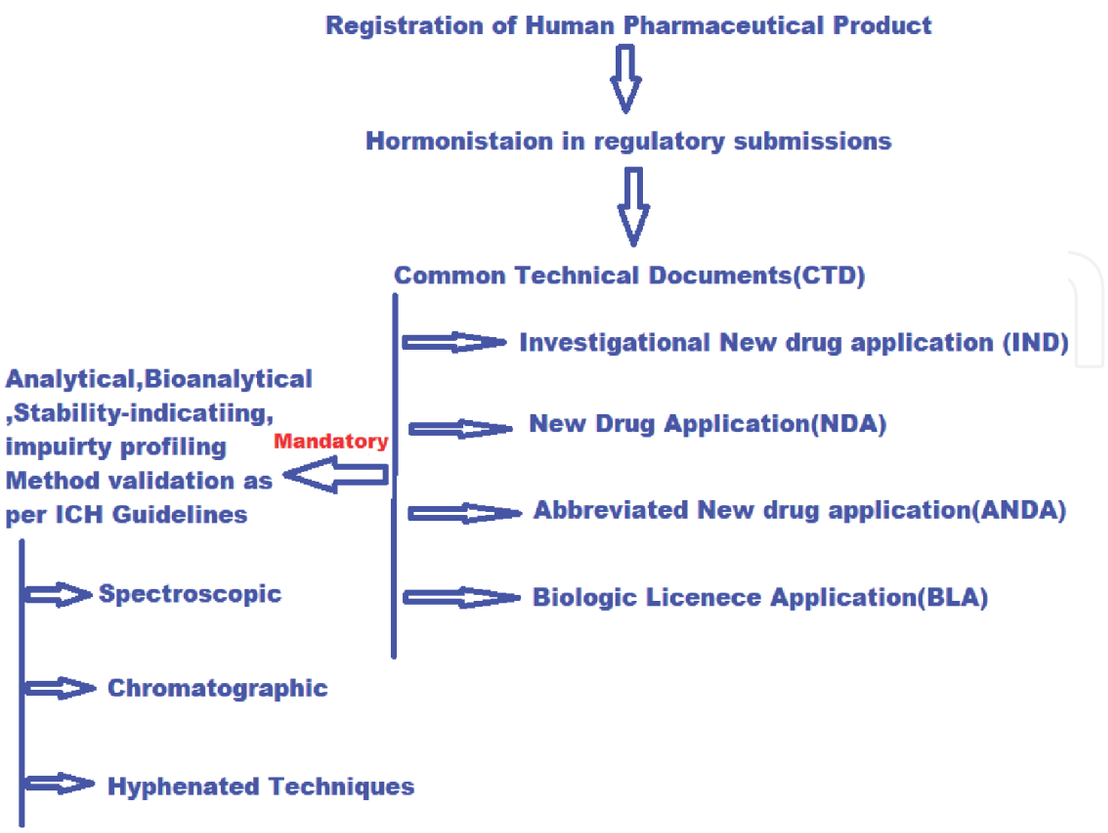

Figure 1.
Flow chart showing essential requirements of registration of pharmaceutical product.

2. Noncompendial analytical method validation

The objective and plan of work should be clearly defined prior to start the work. This data is based on scientific findings from the method development and Optimization. The validation of results should be obtained by approved protocol. Then sponsor must follow cGMP's which includes detail procedure, validation characteristics and acceptance criteria by the use of qualified instrumentation. All the protocols of drug substance and product analytes in respective matrices should be prepared and followed. All the results of validation studies, application should be included.

3. Compendial analytical procedures

The analytical procedure official in pharmacopeias, are cross checked for implementation stage and its suitability should be checked. The verification protocol should include details of data which explains suitable analytical procedure official in USP/NF for drug product or drug substance.

The following points are to be included in the verification protocol

1. The compendia method which should be verified with acceptance criteria's.

2. All parameters related to each aspect of method that is reagent, equipment, validation characteristics that is specificity, Limit of quantitation (LOQ), Precision, accuracy, should be included in validation are covered by procedure and extent of verification. There is no need to include robustness study for compendia assays if there is no deviation. For a BLA, if the methods are already specified in FDA regulations, there is must to take pre-approval from FDA to change in analytical method.

3.1 Statistical analysis

The statistical analysis is important work after finishing the method development and validation. The statistical values of validation are compared with the predetermined acceptance criteria.

The statistical parameters used are based on proper principle and required for evaluation of parameter. The methods like analysis of variance (ANOVA) for analysis of regression analysis, (R^2-Correlation coefficient) to measure the linearity are applied for studying validation characteristics. In case of observed data is not distributed then it is transformed normal distribution or distribution free approach. By using validates software or independent verification for correctness the data can be analyzed.

3.2 System suitability requirement for potency assay

Before starting actual analysis of standard sample, it is necessary to check whether system is working properly or not. This important task can be completed by analyzing system suitability. In this all integral system that is equipment, electronics, analytical operations and samples are evaluated. These system suitability parameters are depending on method or procedure under validation.

System suitability can be evaluated according to following points:

1. The system suitability measures the performance of given system of samples on a given day. 2. The variable parameters like chromatographic columns, column aging, mobile-phase variations, changes in instrumentation are checked whether they are working properly or not.

2. It is nothing but the part of method validation. The experience and information obtained at the time of method development, which is helpful to determine system suitability of final method.

3. At every time when system is used for performing the assay there is necessary to use system suitability test. For longer period if it is continuously in use then there is need to reevaluate the system suitability at proper intervals.

4. The system suitability means criteria and parameters obtained collectively which can explain the system are working properly [2].

The important aspects of pharmaceutical development program are analytical method development, validation method transfer but it is fact that they are less considered in sense of total contribution in development process, time and economy. At the time of drug development phases, all the analytical method related activities are interrelated. In early development stages they are related and occur one after another in coming phases of development. During drug development process the changes may require to be performed in current methods and these changes in methods again requires validation or method transfer treatment.

If one's objective of method development and validation is achieved, then it can prove that the laboratory facilities are accurate and fit for further development process that is one can say optimized. Method validation is the "process of demonstrating that analytical procedures are suitable for their intended use." Both method validation and methods transfer have important share in drug development and further changes in methods. To generate supportive data during manufacturing and quality control, these methods provides a valuable data by comparing with specifications including all types stability study, Safety, characterization and drug performance can obtain with these supportive methods.

Method development is the simultaneous process as the gradual development of drug product continues. The system suitability parameters are set of tests to checks the proper working of the system. After performing robustness with proper statistical data collection one can set the criteria for final system suitability of the method.

These methods focus on active pharmaceutical ingredient (API) behavior. As the knowledge about API and drug product goes on progress the analytical methods become more refined. The important aspects of analytical method are that should be robust, simple and meeting the regulatory guidelines. Various trial an error experiments are to be carried out to develop the method. The performance criterion's to be finalized before the final validation of method. Forced degradation study which is integral part of stability-indicating method and system suitability tests are one of the key points of method development and final validation [3].

3.3 Impurity profiling

The International Conference for Harmonization (ICH) guidances are available that are related to the qualification of impurities in new drug substances that are produced by chemical synthesis. These impurities can be addressed in two perspectives that are chemistry aspects and safety aspects. The chemistry aspects explain the identification and classification of impurities, the various analytical procedures and setting of specifications. In the safety aspects explains the qualification of impurities which are not addressed in clinical trials. In this aspect the threshold limits are defined. This ICH guidance classifies impurities in three classes as Organic, Inorganic and Solvent. Each class of impurities should be properly reported, with all aspects, developed during synthesis, storage of the new drug product. The

analytical procedure including validation reports related to impurities should be properly reported [4]. In concern with the above discussed guidelines related to qualification of impurities in new drug substances produced by chemical synthesis, impurities which are classified as degradation product developed with the reaction with excipients or container closure system may termed as reaction products. All the observed degradants during manufacturing and stability study should be reported. All the data related to their identification, specification, analytical procedures for quantification, their limits of detection and quantification should be reported [5].

4. Stability-indicating method

To ensure safety, efficacy and quality of drug product there are need of stability indicating methods. The Food and Drug Administration (FDA) defines the stability indicating method as a validated analytical procedure that accurate and precisely measure active ingredients (drug substance or drug product) free from process impurities, excipients and degradation products [6].

To obtain forced degraded samples for assessing selectivity of method, method development and method validation are three important steps of stability indicating methods.

4.1 Importance of forced degradation

As per guidance document available for stability indicating method does not contain any explanation about extent up to which the degradation should be carried out. There are no certain guidelines regarding stress degradation. Therefore, it is always necessary to keep all experimental conditions of degradation with more reality and deliberate degradation.

The main purpose of forced degradation is to obtain stability of drug. It should provide information about route of degradation and utility towards the stability indicating [6].

Forced degradation can be able to judge excipients or non-drug substances. It also provides information useful for structure elucidation of degradation products. Importantly it gives data related to thermal, hydrolysis, and oxidation and photo degradation behavior of drug substance and drug product. It is very important to know about chemical behavior of drug product and drug substance in formulation development, manufacturing and packaging. The data helps in quality improvement [7].

4.2 Acceptance criteria for forced degradation

There are vast discussions among the various scientists that what should be the limits of stress testing? Generally, values in between 5–20% are proper and acceptable for chromatographic assays. The acceptable stability limit for small molecules are 90% of label claim and generally employed by pharmaceutical scientist that is 10% degradation is optimum for use in analytical validation. There are some experiments in which very little or no degradants are obtained due to exceptional stability of molecule under study in such case accelerated storage 40^0 c for 6 months should be carried out. If positive result is obtained, then the stability of drug is noted. But overstressing the drug substance may produce false results [7].

According to the recent recommendations of Food and Drug administration (FDA) and ICH guidelines, the stability indicating property of analytical method can be obtained by carrying out forced degradation study. The pathway

of decomposition from API, solution and formulation also be determined. The structural information, their characterization and isolation of major degradants are the important part of the new drug approval (NDA). The use of forced degradation study is primary to understand molecular chemistry of drugs, its stability indicating properties and its degradation products and their pathways. In most of the cases the Hydrolysis, Oxidation, Photolysis, Racemization, and Decarboxylation are the type of reactions that are responsible for decomposition of most the drugs. However, the regulatory guidelines do not define the procedures to carry out degradation study. Therefore, there are various approaches to carry out forced degradation study [8].

4.3 Stress conditions

In pharmaceutical industry thermal, hydrolysis, oxidation and photo degradation are generally employed. If one must serve the purpose of degradation, the expected degradation should be achieved. The optimum percentage of degradation should be obtained in all types of conditions or in minimum of one according to FDA guidelines. If no degradation is achieved, then in that case, all reports related degradation experiment carried out should be produced. It is important fact to obtain the degradation as per expected level. The degradation in between 5 and 20% is recommended [9].

4.4 Hydrolysis

By using acid and base the hydrolysis studies are performed. Generally, a chemical reaction is carried out with water to obtain decomposed analyte. The wide pH range that is from 2 to 12 is used for acid and base, which is to be used for hydrolysis purpose. For acid hydrolysis generally Hydrochloric acid (HCl) or Sulfuric acid (H_2SO_4) is used and for base hydrolysis Sodium or Potassium hydroxide is used. According to stability of molecule, the concentration of acid or base is decided. One can used more than one stress conditions to obtain desired degradation. If the desired degradation is not achieved at room temperature, then higher temperature is used. After the degradation process completed the degraded samples are neutralized by same acid or base so as it can easily injected in HPLC column without any harm to silica stationary phase. For water insoluble samples alcoholic acid or base are used for obtaining degradation [9].

4.5 Oxidation

To carry out oxidation degradation of drug substance or drug product, generally hydrogen peroxide is used. Apart from hydrogen peroxide metal ions, oxygen and radical initiators can also be employed. The oxidizing agent, its quantity requirement, properties are depending on the drug substance under study. If hydrogen peroxide is used as a degradant, in that case combination of stress should not be employed. If elevated temperature is used in case of Hydrogen Peroxide, it leads to hydrolysis instead of oxidation because in Hydrogen Peroxide O-O are not stable and they may decompose at ambient temperature also. Due to heating, these bonds break faster and oxidation occurs. Sodium metabisulfite solution is used for neutralization of oxidation degraded samples [9].

4.6 Heat

Active pharmaceutical ingredients, Dosage form with or without humidity can be undergoing thermal degradation. The sample is exposed to heat. (weather there is

humidity or absence of humidity as mentioned earlier) In case of liquid, humidity is completely avoided. While applying stress to liquid samples especially for injections, oral solutions, and syrups as further diluting the samples, the precaution should be taken because these types of samples may loss water and concentration of actual sample. By obtaining multiple time results, the detail information about primary and secondary degradation can collected. If any molecule is so stable that it cannot generate degradation, in such situation the energy analogous to the accelerated stress condition is to be applied to express efforts taken for obtaining degradation [9].

4.7 Photo stability

The exposure to light is one of the important degradation steps to obtain degradation caused by light. This degradation is evaluated by obtaining any unacceptable change due to light. The recommendations related to photo stability are described in ICH guidelines Q1B.The UV–VIS light exposure with not less than 1.2 million hours to achieve degradation of sample. The samples are preferably exposed to cool white fluorescent light and near ultraviolet lamp. The natural light can be used, if specific instrument is not available, but there will be intensity problem as it is varying with time, weather conditions, pollution etc. due to which natural light becomes not suitable for degradation [9].

4.8 Evaluation of results

After generating forced degradation samples obtained by accelerated stress conditions, their evaluation is the important task. For evaluation purpose, each sample should be studied individually. The Chromatographic techniques including High performance liquid chromatography (HPLC), Ultra-performance liquid chromatography (UPLC), UHPLC and Capillary Electrophoresis are commonly used techniques for this important task. The most important work is development and validation of stability-indicating method which can be able to separate every degradation product from each other and from drug. Therefore, peak purity is important in sense of selectivity determinations of the method. One more important parameter is sensitivity, which can be helpful to asses' impurities at lower level. There are chances that the impurity peak may get depressed at the time of method development which is co-eluted. Many times, it may happen that two unknown impurity get merged due to which false results are appear for stability, therefore it is important to implement such analytical method that can have capacity to resolve each unknown impurity and that is helpful to control out of specification results [9] (**Figure 2**).

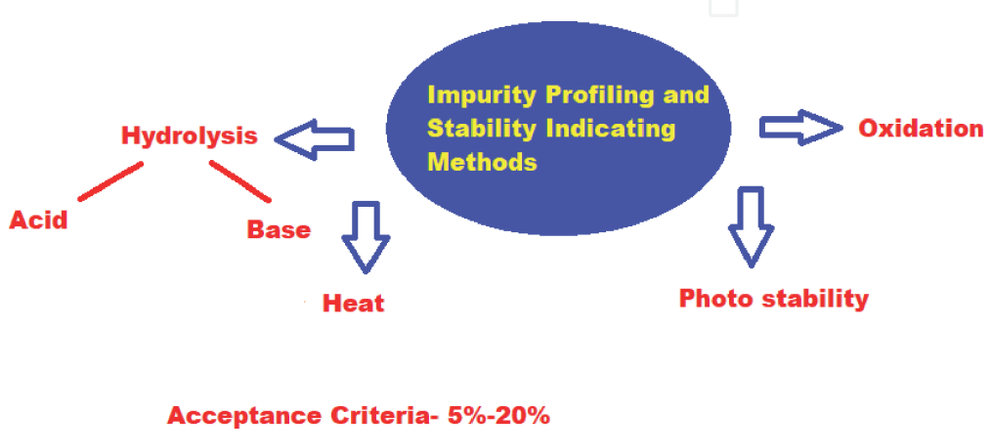

Figure 2.
Important parameters and acceptance criteria for impurity profiling and stability indicating methods.

5. Bioanalytical method development and validation

There were various regulatory agencies had done serious efforts to regulate bioanalytical method development and validation. Almost from last three decades there were large progresses in this area. The various regulatory agencies that were worked can be listed as US FDA, American association of pharmaceutical scientists (AAPS), Health protection Branch HPB, Association of analytical chemists (AOAC), Center for Veterinary medicine (CVM), U. S. Department of Health and Human Services Food and drug Administration, Center for Drug Evaluation and Research (CDER), European Medicine Agency (EMA), China Food and Drug Administration (CFDA), European Bioanalytical forum (EBF), Global CRO Council (GCC), The Brazilian health regulatory agency (ANVISA, Brazil). To regulate and harmonize bioanalytical method development and validation first workshop was held in 3–5 December 1990, report of which was published in pharmaceutical research and in other journals. On basis of the reports of this workshop, the FDA was issued draft guidance on bioanalytical method development and validation in January 1999.The second FDA guidance was published in May 2001 on the basis of workshop which was held in January 2000.The recommendations for bioanalytical method development and validation for macromolecules was published in 2006. The recommendations for regulation and harmonization of bioanalytical methods were again refreshed in 2006. In 2010, a draft guidance was published by EMA for development and validation of bioanalytical methods. As per above discussion this can be concluded that there were serious efforts carried out to regulate bioanalytical method development and validation by the various abovementioned regulatory agencies.

6. Need of bioanalytical method development and validation

The various manufacturers are applying for Investigational new drug application (IND), New drug application (NDA), Abbreviated new drug application (ANDA) to FDA. There was harmonization in this process related to human clinical pharmacology, bioavailability (BA) and Bioequivalence (BE), pharmacokinetic evaluation (PK), non-human pharmacology and toxicology studies and preclinical studies, which should be included in abovementioned applications. To obtain the data related to abovementioned requirements, there is need of development and validation of bioanalytical methods in biological matrices such as blood, serum, plasma or urine [10].

The most recent FDA guidance document on bioanalytical method development and validation was released in May 2018. Before this there was a guidance documents in 2001 and its revision in 2013 were released. The overall previous recommendations remain same, only the following points are revised.

- The validation criteria for dilution and carryover
- There was clarification on the number of Quality control (QC) samples and replicates
- There will be no acceptance criteria for QCs for accuracy and precision
- The QCs should have to cover the sample concentration range
- The LLOQ should be evaluated for interference for each run

- There will be the further acceptance criteria within the different batches
- The internal standard (IS) and the drift should be monitored

In this document following clarity regarding Ligand Binding Assay (LBA) was added

- The accuracy and precision runs
- The control of each sample should be included with clear definition
- The consistency in standard calibrator preparations

The significant change in final document, the incurred sample reanalysis section was added which includes endogenous compounds, biomarkers, diagnostic kits, bridging data and dried blood spots [11]. The guidance documents (M10) on Bioanalytical Method Development and Validation was released by International Council for Harmonization of technical requirements for pharmaceuticals for Human Use (ICH), in February 2019. Simultaneously, American association of Pharmaceutical scientists (AAPS), European bioanalysis forum (EBF), Japan Bioanalysis forum (JBF), China Bioanalysis forum (CBF) were organized a workshop of industry, academia, and health authorities to discuss this draft guidance. The objective of these discussions was the M10 guidelines which are for Bioanalytical Method Development and validation which are part of regulatory submissions. This guidance document explains the validation of Bioanalytical Methods form, which the concentration of analyte is determined from biological fluids. The concentration was obtained from pivotal nonclinical pharmacokinetic studies which are useful for taking the decisions over the regulatory submission including all phases of clinical trials [12].

6.1 Key principles of bioanalytical method validation and establishment

- Accuracy, precision, selectivity, sensitivity, reproducibility and stability are the fundamental parameters that ensure the acceptability of bioanalytical method.
- There should be specific protocol, study plan, report or SOP for bioanalytical method development and validation.
- How the analyte is being get affected by environmental, matrix, or procedural variables? Every step, including time of collection of matrix and overall investigation time, should be clarified.
- The physiological nature of samples gives variable matrix. When there are Liquid Chromatography-Mass spectrometry-Mass Spectrometry (LC-MS-MS) based procedures, then protocol should be designed to avoid matrix effect, matrix may change during method validation.
- It is necessary to validate bioanalytical method for the intended use or application.
- There should be written method validation report to claim the results.

- The same biological matrix as the matrix in the intended samples should be used for validation purposes. It is necessary in case of limited availability of matrix like bone marrow.

- The stability at the time of matrix during collection and storage should be assessed before analysis.

- The stability of analyte in matrix from dosed subjects should be finalized in case of potentially labile metabolites.

- The parameters like accuracy, precision, reproducibility, response function, and selectivity of method for endogenous substances, metabolites, and known degradation products should be set for biological matrix.

- In case of selectivity the evidence should be produced that substance being quantified is the intended analyte.

- The concentration range of analyte should be defined on standard samples including their statistical parameter which clears the standard curve.

- To define concentration and response relationship an enough sample should be analyzed. This relationship should be continuous and reproducible. For this purpose, the standard used should be from dynamic range and nature of the concentration-response relationship. Generally, six to eight concentrations excluding blank can be used to define standard curve. In case of nonlinear concentrations more standard may be recommended.

- There should be proper demonstration to show the ability to dilute samples originally above the upper limit of the standard curve by accuracy and precision parameters in the validation.

- In case of high throughput analyses like multiplexing, multicolumn and parallel systems, enough Quality control (QC) samples should be assessed to prove control of the assay. Based on the run size, the number of QC samples should be determined.

There should be proper placement of the QC samples in the run.

- There is a need to set a specific acceptance criterion for bioanalytical method to be considered as a valid method. That should be achieved for accuracy and precision for validation of QC samples over the range of standards.

6.2 Specific recommendations for bioanalytical method validation

- There should be minimum six standard points for matrix based standard curve excluding blank, which may be single or replicates and should cover the entire range of expected concentrations.

- Standard curve should explain the concentration-response relationship with appropriate weighting and statistical tests for goodness of fit.

- The Lower limit of quantitation, (LLOQ) should be measured with acceptable accuracy and precision which is the lowest concentration of the standard

curve. By using a least five samples independent of standards and its coefficient of variation, the LLOQ can be established. The LLOQ should not be confused with the limit of detection and/or the low-Quality Control (QC) samples. The upper limit of quantification will be defined by highest standard.

- The accuracy and precision should be determined by using minimum of five determinations per concentration level excluding blank samples. The average value should be within ±15% of the theoretical value. The LLOQ should be up to ±20%. The coefficient of variance of precision should not exceed 15% and for LLOQ should not exceed 20%. The methods which give the results of accuracy and precision with these above-mentioned values should be acceptable.

- There should be proper demonstration of concentration of analyte in biological matrix with which the accuracy and precision is determined. This can be performed by analyzing replicate sets of QC samples from same biological matrix. This QC sample should be representative of entire concentration range selected for standard curve. From which one concentration within LLOQ, one should be middle one that is middle QC (MQC) and last should be upper limit of standard curve that is High QC (HQC).

- All outliers should be included in reported method validation data and accuracy and precision data. The values of outliers that are determined statistically can also be reported with the calculations of accuracy and precision.

- The storage temperature stability in biological matrix should be determined for analyte. The freeze-thaw stability at minimum of three cycles of two concentrations in triplicates should be studied.

- The ambient temperature stability of analyte should be determined over the time period equal to typical sample preparation, sample handling and analytical run times.

- In case of instrument failure, reinjection reproducibility should be evaluated to determine an analytical run could be reanalyzed.

- For determination of specificity of assay method, a minimum of six concentration of same matrix should be studied. In case of hyphenated techniques like mass-spectrometry based methods, it is not important to study six independent matrices. There should not any compromise to study matrix effect to ensure precision, selectivity and sensitivity in case of Liquid Chromatography-Mass spectrometry (LC-MS) and Liquid Chromatography-Mass spectrometry-Mass Spectrometry (LC-MS-MS) based procedures. The selectivity should be evaluated throughout method development, method validation and it should be continued up to the application of method to actual study samples.

The acceptance/rejection criteria for spiked, matrix-based calibration standards and validation of QC samples should be based on theoretical concentration of analytes. For studying accuracy and precision, the specific criteria should be set in the standard concentration range [13] (**Figure 3**).

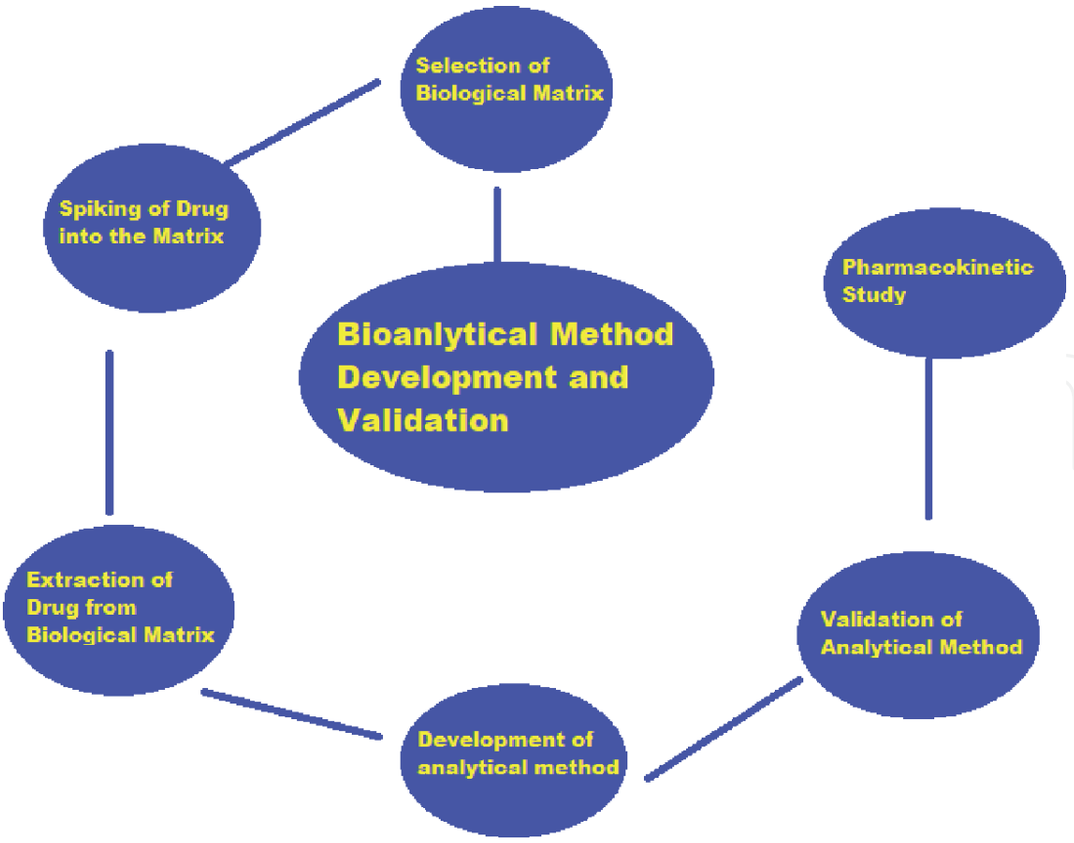

Figure 3.
Sequence showing development and validation of bioanalytical method.

7. Analytical techniques for method development and validation

The various analytical techniques are available for Qualitative and Quantitative analysis, which can be used in above explained types of analytical methods. The chromatographic techniques used as a separation tool and spectroscopic techniques are used for an identification and to obtain structural information. Among all these techniques High performance liquid chromatography, High performance thin layer chromatography, Spectrophotometric techniques and Hyphenated techniques are explained in brief.

7.1 High performance liquid chromatography

When we draw the attention towards the working principle of HPLC, which involves the injection of small sample (Generally in μl) into the stationary phase composed of 3–5-micron tiny particles. The injected components of the sample moved through the abovementioned stationary phase with the mobile phase which is forced through high pressure by the pump.

The HPLC technique is having advantage of High speed, Efficiency, Sensitivity and vastly superior over the simple liquid chromatography. The process of separation of components of sample involves chemical and physical interactions with the stationary phase particles. The separated components are detected at the end of column by the detector in the form of the liquid chromatogram (**Figure 4**).

The HPLC can be applied for separation of non-volatile compounds like Aspirin, Ibuprofen, Acetaminophen and then for separation of salts like Sodium Chloride, Potassium Phosphate. For the separation of proteins like Egg white and Blood

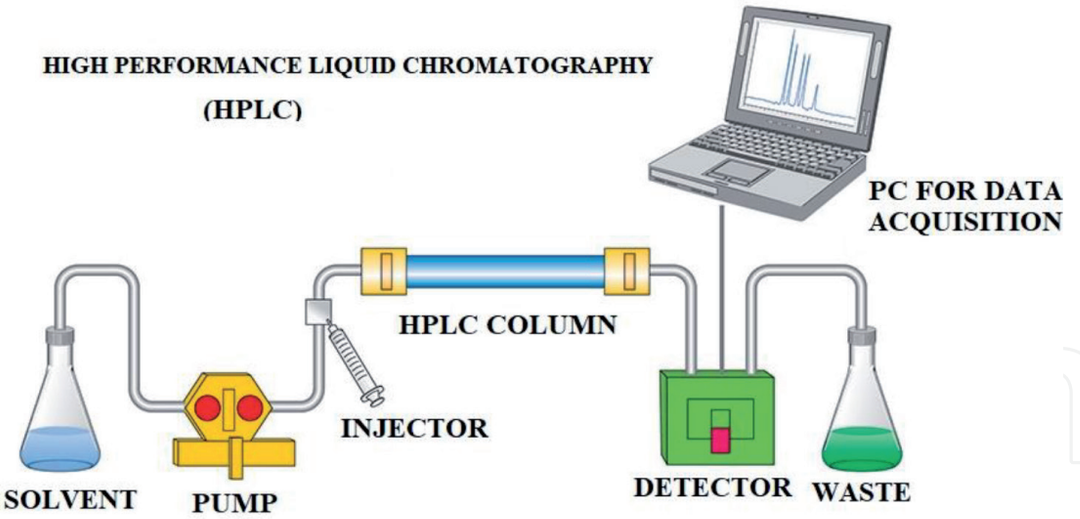

Figure 4.
Flow diagram of working principle of HPLC.

proteins. The HPLC can also be applied for separation of Organic Polymers, Heavy Hydrocarbons, Natural Products, Thermally unstable compounds and Enzymes [14].

For separation of many complex mixtures including biological samples high performance liquid chromatography is the best form of liquid chromatography (HPLC). HPLC is widely used for qualitative and quantitative analysis of different types of pharmaceuticals due to its sensitivity. By using HPLC, one can obtain individual sample with its role in that sample. In 20th century, the HPLC methods were appeared for assay of bulk drugs and later become a principal method of Pharmacopeia. The interaction among the solute molecules and stationary phase, decides the mode of chromatography. HPLC is more versatile technique as various modes are available. By using HPLC, the proper values of precision can obtain with excellent specificity of the methods. Though the specificity, precision and accuracy are obtained with HPLC methods, the system suitability parameters are first analyzed before analyzing these parameters. The more attention should also be providing for high accuracy, precision and specificity. By doing wide literature survey it was observed that HPLC is widely used technique among all chromatographic techniques. One of the reasons for this is detection system of HPLC which can able to detect every component of mixture. The UV detector is most widely used detector for HPLC. The Ultra-violet (UV) detector can analyze various wavelengths simultaneously by giving multiple wavelength programmers on HPLC software. Every component present in mixture which UV can detect that can be obtained by UV detector. A Photodiode array (PDA) detector is one of useful spectroscopic detector. By placing at the image plane of spectrophotometer various wavelength can be scanned simultaneously. For analysis of alcohols, sugars, carbohydrates, fatty acids and polymers, the refractive index detector is used as there is restriction for UV absorption of these compounds. The Refractive Index (RI) detector is one of the lowest sensitivity detectors but it can be applied for trace detection with low noise. For analyzing oxidizable and reducible substances, the electrochemical detector is implemented. In this detector the electrical output obtained by electron flow due to chemical reaction at electrode surface due to presence of above-mentioned compound is used for qualitative and quantitative analysis of these types of samples. Among various detectors available for HPLC, the most sensitive detector is fluorescence detector. The sensitivity of fluorescence detector is 10–1000 times more sensitive as compared with the UV detector. If sample contains any specific

fluorescent compound, then it can be easily detected by this detector. For estimation of pharmaceuticals especially fluorescence detector is applied. As most of pharmaceuticals are polar in nature, there analyses are carried out as reverse phase HPLC. In recent years most of the researchers used reverse phase chromatography with UV detection, due to that the results are obtaining with best reliability, analysis, repeatability and sensitivity. Generally, in pharmaceutical industry Octadecyl silyl (ODS) C18 is mostly used stationary phase. Many drugs can be easily obtained in pharmaceutical formulations and biological fluids by using HPLC. Nowadays, HPLC is one of the important tools for solving many problems in pharmaceutical industry. There are certain limitations to HPLC that high price of column, HPLC grade solvents and it is difficult to obtain long term reproducibility due to nature of column packings.

The Liquid Chromatography-Mass spectrometry (LC-MS) is wide choice for quality control and quality assurance in various stages in pharmaceutical industry. The LC-MS can be easily applied for assay of many drugs and pharmaceuticals also applied for analysis of impurities and degradation products. The most hyphenated technique like Liquid Chromatography-Mass spectrometry-Mass spectrometry (LC-MS-MS) is also available for above mentioned work [15].

7.2 High performance thin layer chromatography (HPTLC)

The advancement of Thin layer chromatography (TLC) is the HPTLC that is, High performance thin layer chromatography which an instrumental semi or automatic form of TLC. It is fast working, sensitive and can be able to analyze wide range of samples.

The HPTLC is advantageous to handle a sample with short analysis time for analysis of even complex samples including crude drugs. As automation with the instrument it can be able to analyze entire chromatogram with many parameters without any interruption. The samples can be analyzed simultaneously or independently with standard that shows the reliability of technique. The HPTLC is equipped with high performance adsorbent layers having refined uniform particles, approx. 5 microns in diameter. All processes of experimentation including method development, optimization of various parameters and documentation are performed with standardized methods. The HPTLC can be applied for both qualitative and quantitative analysis of mixtures, as the technique is automated, the quantitative mode is more optimized as compared with the TLC. Also, it can be used for the assay of the compound (**Figure 5**).

The advantages of the HPTLC are as follows:

- Colored samples can be easily separated
- Many samples can be assessed easily on single plate which reduces the cost and time.
- Two-dimensional mode is possible
- Visualizing agent used which are Specific and Sensitive for detection purpose
- Other evaluation technique can be implemented for different samples with different light absorption characteristics.
- Radio labeled compounds can be monitored and microbial activity can be assessed.
- No regeneration and cleanup are required as the technique is disposable.

Figure 5.
Diagram showing components of HPTLC.

Development of plate and evaluation of plate are separate processes therefore both can perform as per time available differently [15].

The important advantages of the HPTLC are fast, inexpensive method of analysis. It can prove over high-performance liquid column chromatography when it is performed by skilled person for quantitative analysis. The qualitative and quantitative analysis by the HPTLC with automated sample application and densitometric scanning shows very sensitive and reliable results. The HPTLC has important advantage of providing chromatographic fingerprints which can be stored as an electronic image [16].

7.3 UV-spectrophotometric methods

For the quantification of components present in solution, the UV absorption spectroscopy works on the principle of Beer-Lambert law [17] (**Figure 6**).

According to Beer-Lambert law,

$$A = \log I_0 / I = \varepsilon.c.l$$

where A = Absorbance.
I_0 = intensity of incident light.
I = intensity of emergent light.
ε = molar absorptivity.

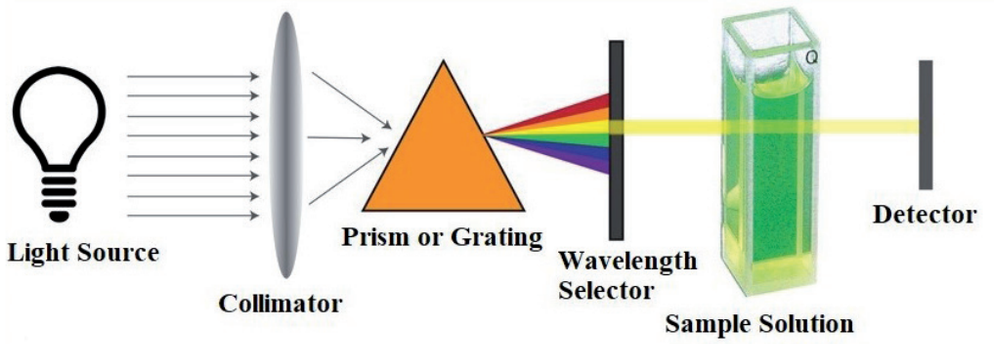

Figure 6.
Flow diagram of working principle of UV spectrophotometer.

c = molar concentration of solute.
I = length of sample cell.

The natural Ultra-Violet (UV) absorption methods and chemical reactions spectrophotometric methods are having importance in pharmacopeia. In these methods quantitative data of reflection or transmission by the analyte as function of wavelength is measured. The method is based on the fact, that functional group of analyte absorbs UV radiation at specific wavelength in a solvent system. The λmax is the term used for maximum absorption of wavelength which is independent of concentration. These methods require less time and less labor consumption. The method also gives best precision. The UV-Visible methods are applied for multicomponent analysis of samples [15].

7.4 Brief introduction of other spectroscopic techniques

The Near Infrared spectroscopy (NIRS) is one of the spectroscopic techniques that can be applied for multicomponent analysis of all types of samples and having advantage of non-destructive technique. For the purpose of raw material testing, quality control of finished product and to monitor the process, the NIR spectroscopy plays important role in recent years. The great advantage of NIR is there no requirement of sample pre-treatment, the use of fiber optic probes and both the chemical and physical parameters can be obtained in single spectrum [15].

The Nuclear Magnetic Resonance (NMR) spectroscopy is one of the advantageous techniques over UV and IR spectroscopy that it can detect the intermediate products like ions, reaction complexes, solvents of chemical reaction. The NMR spectroscopy provides unique information on the structure of intermediate due to which there is no need to restore various hypotheses to explain the mechanism of the process [18]. The mass spectrometry is one of the outstanding techniques in all type's spectroscopies due to its sensitivity, detection limits and its wide range of application. It is widely applied in biochemical problems like proteome, metabolone, drug discovery and metabolism. This technique can also be applied for pollution control, food control, forensic science and natural product or process monitoring. It can also be applied in atomic physics, reaction physics, reaction kinetics, inorganic chemical analysis, ion-molecule reactions and determination of thermodynamic parameters [19].

7.5 Hyphenated techniques

Generally, for the qualitative and quantitative analysis of the samples, the separation technique is combined with the identification technique. In the analysis,

chromatographic and electrophoresis are used for separation or isolation of the required components. The quantitative determination or structural information of the sample under study is performed by spectrophotometry. The Hyphenated technique is nothing but the combination of both above said techniques that are separation and spectrophotometric technique. The various hyphenated techniques like Liquid chromatography-Mass spectrometry (LC-MS), Gas chromatography-Mass spectrometry, Liquid Chromatography-Nuclear Magnetic resonance (LC-NMR), Liquid chromatography-Fourier transform infra-red spectroscopy (LC-FTIR), Capillary electrophoresis-Mass spectrometry (CE-MS) are used widely for qualitative and quantitative analysis. There may be a combination of more than one separation or detection technique like Liquid chromatography-Photodiode array-Mass spectrometry (LC-PDA-MS), Liquid chromatography-Mass spectrometry-Mass spectrometry (LC-MS-MS), Liquid chromatography-Nuclear magnetic resonance-mass spectrometry (LC-NMR-MS), Liquid chromatography-photodiode array-nuclear magnetic resonance-Mass spectrometry (LC-PDA-NMR-MS) [20].

Author details

Mahesh Mukund Deshpande
Department of Pharmaceutical Chemistry, Amrutvahini College of Pharmacy, Sangamner, M.S., India

*Address all correspondence to: mahesh_deshpande11@rediffmail.com; maheshdeshpande83@gmail.com

References

[1] Jordan D. An overview of the common technical document (CTD) regulatory dossier. Medical Writing. 2014;**23**(101):101-105. DOI: 10.1179/2047480614Z.000000000207

[2] Analytical procedures and methods Validation for drugs and Biologics, Guidance or Industry, U.S. Department of Health and Human Services, Food and Drug Administration, center for Drug Evaluation and Research (CDER), Centre for Biologics Evaluation and Research (CBER); 2015. pp. 1-15

[3] Robert WL, Laurie G. The Central Role of Analytic Method Development and Validation in Pharmaceutical Development, Life Science Connect 5340 Fryling Road, Suite 300, Erie, PA 165101, 814.897.7700. pp. 1-3. Available from: www.lifescienceconnect.com

[4] ICH Topic Q3A Impurities testing guideline: impurities in new drug substances. The European agency for evaluation of Medical product, Human Medicines Evaluation Unit, CPMP/ICH/142/95. pp. 1-11

[5] ICH topic Q3B (R2) impurities in new drug product, European Medicine Agency CPMP/ICH/2738/99; June 2006. pp. 1-14

[6] Cione AP, Tonhi E, Silva P. Stability Indicating Methods. Brazil: Bioagri Laboratories; 2011. pp. 27-36. DOI: 10.5772/19940

[7] Ngwa G. Forced degradation studies, forced degradation as an integral part of HPLC stability-indicating method development. Drug Development & Delivery. 2010;**10**(5):1-4

[8] Sengupta P, Chatterjee B, Tekade RK. Current regulatory requirements and practical approaches for stability analysis of pharmaceutical products: A comprehensive review. International Journal of Pharmaceutics. 30 May 2018;**543**(1-2):328-344. DOI: 10.1016/j.ijpharm.2018.04.007

[9] Sharma MK, Murugesan M. Forced degradation study: An essential approach to develop stability indicating method. Journal of Chromatography. 2017;**801**:1-3

[10] Deshpande MM, Kasture VS, Mohan M, Chaudhari SR. Practical approach for development and validation of bioanalytical method: A review. Inventi Rapid/Impact: Pharm Analysis & Quality Assurance. 2017;**2017**(1):1-8

[11] Booth B, Stevenson L, Pillutla R, Buonarati M, Beaver C, et al. White paper on recent issues in bionalysis: FDA BMV guidance, ICH M10BMV guidance and regulatory inputs (part 2-recommendations on 2018 FDA BMV guidance, 2019 ICH M10 BMV draft guidance and regulatory agencies input on bioanalysis, biomarkers and immunogenicity). Bioanalysis. 2019;**11**(23):2099-2132

[12] Booth B, Vazvaei F, Fluhler E, Myler H, Woolf E. AAPS, workshop report on ICH M10. The AAPS Journal. 2020;**22**:10. DOI: 10.1208/s12248-019-0398-7

[13] Guidance for Industry Bioanalytical Method Validation, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Veterinary Medicine (CVM). BP; May 2001. pp. 8-11

[14] HPLC Basics. Fundamentals of liquid chromatography (HPLC). Courtesy of Agilent Technologies, Inc.; 2007

[15] Siddiqui MR, Alothman ZA, Rahman N. Analytical techniques in

pharmaceutical analysis: A review. Arabian Journal of Chemistry. 2017;**10**:S1409-S1421

[16] Srivastava MM. High-Performance Thin Layer Chromatography (HPTLC). Heidelberg Dordrecht London, New York: Springer. 2011. pp. 9-10. DOI: 10.1007/978-3-642-14025-9

[17] Stauffer E, Newman R. Other Techniques of Analysis and the Future of Fire Debris Analysis. ScienceDirect. Amsterdam: Global Business; 2008

[18] Ionin BI, Erhoy BA. Application of NMR spectroscopy in various fields of organic chemistry. In: NMR Spectroscopy in Organic Chemistry. Physical Methods in Organic Chemistry. Boston, MA: Springer; 1970

[19] Hoffmann ED, Stroobant V. Mass Spectrometry Principles and Applications. 3rd ed. Hoboken, New Jersey: John Wiley and Sons, Ltd. 2007. p. 1

[20] Patel KN, Patel JK, Patel MP, Rajput GC, Patel HA. Introduction to hyphenated techniques and their applications in pharmacy. Pharmaceutical Methods. 2010;**1**(1):2-13