

A Review: Analytical Method Development and Validation

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ABSTRACT

Development and validation of analytical method play an essential role in the discovery, development and manufacturing of pharmaceuticals. Every year, number of drugs entered into the market; hence it is mandatory to develop newer analytical methods for such drugs. After the development, it becomes necessary to validate the new analytical method. Method development is the process which proves that the analytical method is acceptable for use. Validation of analytical method gives information about various stages and parameters like accuracy, precision, linearity, Limit Of Detection, Limit Of Quantification, specificity, range and ro-

bustness. Validation should be done as per regulatory guidelines such as ICH guidelines. This article was prepared with an aim to review analytical method development and validation.

Keywords: Analytical method, Spectroscopy, UV-VIS spectroscopy, Chromatography, HPLC, Method development, Validation

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INTRODUCTION

Analytical chemistry is a branch of chemistry which deals with identification of components (qualitative) and determination of quantity of components (quantitative) of substances or samples or mixture. There are two types of analysis, one is qualitative analysis and another one is quantitative analysis. In qualitative analysis, there is identification of components or analyte of mixture or sample is carried out. In quantitative analysis, there is determination of amount of components or analyte of mixture or sample is carried out (Kenkel J, 2003). Analytical data is required not only in chemistry but also in other sciences like biology, zoology, arts such as painting and sculpture, archaeology, space exploration and clinical diagnosis. Important areas of application of analytical chemistry are quality control in manufacturing industries, monitoring and control of pollutants, clinical and biological studies, geological assays, fundamental and applied research (Kissinger PT, 2002).

ANALYTICAL METHOD

Analytical method includes use of a specified technique and detailed-stepwise instructions which are used in qualitative, quantitative or structural analysis of a sample for one or more analytes (Kissinger PT, 2002).

Analytical methods are mainly classified into two types: Classical methods and Instrumental methods (Figure 1). A method in which the signal is proportional to the absolute amount of analyte is called classical method. A method in which the signal is proportional to the analytes concentration is called instrumental method (Harvey D, 2000).

Classical methods are divided into 3 main types are: a) Separation of analyte, b) Qualitative analysis and c) Quantitative analysis. Separation of analyte includes extraction, distillation, precipitation and filtration. Qualitative analysis includes boiling point, freezing point, colour, odour, density, reactivity and refractive index. Quantitative analysis includes gravimetric analysis and volumetric analysis.

Instrumental methods are divided into four main types are: a) spectroscopic methods, b) electrochemical methods, c) chromatographic methods and d) other techniques.

Spectroscopic methods include ultraviolet-visible spectroscopy, infrared spectroscopy, Raman spectroscopy, atomic absorption spectroscopy and atomic emission spectroscopy, x-ray spectroscopy and nuclear magnetic spectroscopy.

Electrochemical methods include Potentiometry, Coulometry and Voltametry.

Chromatographic methods include column chromatography, paper chromatography, thin layer chromatography, high performance liquid chromatography, gas chromatography and modern methods (LC-MS, GC-MS, LC-MS-MS, GC-MS-MS, LC-NMR and GC-NMR).

Other techniques include x-ray methods, radioactivity, mass spectrometry, optical methods (Refractometer, optical rotation) and thermal methods (Thermogravimetry, differential thermal analysis and differential scanning calorimetry) (Ravisankar P, *et al.*, 2015; Jeffery GH, 1989).

INTRODUCTION TO SPECTROSCOPY

Spectroscopy is the study of interaction of electromagnetic radiation with matter. These interactions involve absorption and emission of radiation (energy) by the matter. Spectroscopy are of two types, absorption spectroscopy and emission spectroscopy. The study of electromagnetic radiation absorbed by the sample, in the form of spectra is called absorption spectroscopy (UV-visible, IR, NMR, microwave and Radiowave spectroscopy). The study of electromagnetic radiation emitted by the sample, in the form of spectra is called emission spectroscopy (flame photometry and fluorimetry). Spectroscopy is useful for the study of atomic and molecular structure and used in the analysis of a wide range of samples. Atomic spectroscopy is the study of interaction of electromagnetic radiation with atoms, changes in energy takes place at atomic level

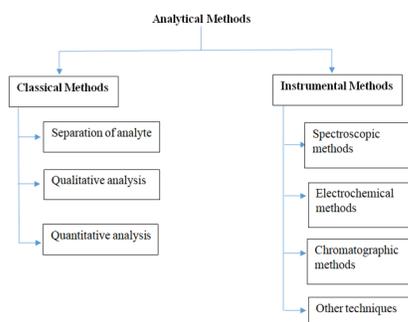


Figure 1: Classification of Analytical Methods

(e.g. atomic absorption spectroscopy and flame photometry). Molecular spectroscopy is the study of interaction of electromagnetic radiation with molecules, changes in energy takes place at molecular level (e.g. ultraviolet and infrared spectroscopy) (Chatwal GR and Anand SK, 2002).

UV-VIS spectroscopy

In UV-visible spectroscopy, the amount of light absorbed at each wavelength of UV and visible region of electromagnetic spectrum is measured. This absorption spectroscopy uses electromagnetic radiations between 200 nm to 800 nm and is divided into the ultraviolet (UV, 200-400 nm) and visible (VIS, 400-800 nm) regions (Kumar S, 2006).

The principle of UV-Visible spectroscopy is based on the absorption of ultraviolet light or visible light by sample or chemical substance which results in the production of different spectra. When a molecule absorbs UV radiation, the electron present in that molecule undergo excitation, this causes transition of electron within a molecule from a lower level to a higher electronic energy level and the ultraviolet emission spectra arise from the reverse type of transition. Most commonly used solvents in UV spectroscopy are water, methanol, ethanol, ether, chloroform, carbon tetrachloride, cyclohexane and dichloroethane. Applications of UV spectroscopy are detection of functional groups, detection of conjugation, detection of geometrical isomers and detection of impurities (Chatwal GR and Anand SK, 2002).

Instrumentation of UV-Visible spectroscopy

A. Radiation sources: Most commonly used radiation sources are tungsten lamp, hydrogen discharge lamp, deuterium lamp, xenon discharge lamp and mercury arc (Figure 2).

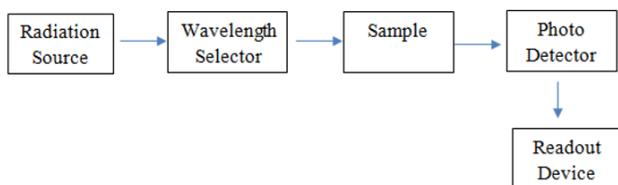


Figure 2: UV-Visible spectroscopy

B. Wavelength selector: The monochromator is used to disperse the radiation according to the wavelength. The basic elements of a monochromator are an entrance slit, a dispersing element and an exit slit.

C. Sample cell: In UV-Visible spectroscopy sample containers are used to hold liquid sample are called as cells or cuvettes. Cuvettes are made from quartz.

D. Photo detector: Most commonly used detectors in UV spectrophotometer are barrier layer cell, photocell and photomultiplier tube.

E. Readout device: The output from the detector is suitably amplified and then displayed on a readout device (Chatwal GR and Anand SK, 2002).

INTRODUCTION TO CHROMATOGRAPHY

Chromatography is a physicochemical method for separation of mixture of compounds. Chromatography is a method of separation of mixture of compounds into individual components between two phases, a stationary phase and a mobile phase (Luxminarayan L, et al., 2017).

Chromatography is classified as follows:

1. Based on interaction of solute to stationary phase
 - Adsorption chromatography
 - Partition chromatography

- Ion exchange chromatography
 - Molecular exclusion chromatography
2. Based on chromatographic bed shape
 - Column chromatography
 - Planar chromatography
 - Paper chromatography
 - Thin layer chromatography
 - Displacement chromatography
 3. Techniques by physical state of mobile phase
 - Gas chromatography
 - Liquid chromatography
 - Affinity chromatography (Luxminarayan L, et al., 2017).

HPLC

HPLC stands for high performance liquid chromatography or high-pressure liquid chromatography. HPLC can separate, identify and quantify the compounds present in any sample which can be dissolved in liquid (Chawla G and Chaudhary KK, 2019).

The main principle of liquid chromatography is adsorption. It is a chromatographic technique in which mobile phase is liquid. Sample is in the form of liquid solution. Sample is injected into a column of a porous material (stationary phase) and a liquid phase (mobile phase). Sample move through the column with mobile phase by high pressure delivered by a pump. Sample components travel according to their affinity towards the stationary phase. The component which has more affinity towards the stationary phase travels slower. The component which has less affinity towards the stationary phase travels faster. The components are separated from each other (Vidushi Y and Meenakshi B, 2017). The most common solvents used for HPLC are n-hexane, methylene chloride, chloroform, methyl-t-butyl ether, Tetrahydrofuran (THF), Isopropanol (IPA), Acetonitrile (MeCN or CAN), Methanol (MeOH) and water (McPolin O, 2009). Fundamental chromatographic parameters are efficiency (number of theoretical plates), retention factor, selectivity, resolution and pressure (Ravisankar P, et al., 2015). Applications of HPLC are chemical separation, purification and identification. Other applications of HPLC include pharmaceutical applications, environmental applications, forensics, clinical, food and flavour (Figure 3) (Malviya R, et al., 2010).

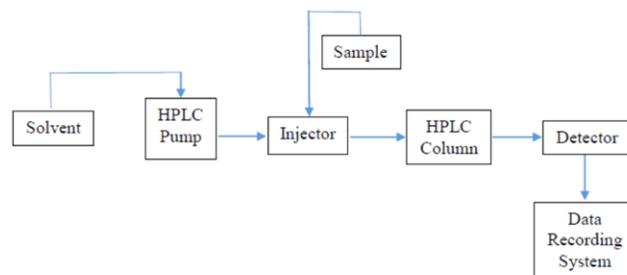


Figure 3: HPLC system

Instrumentation of HPLC

Components of the HPLC system:

- A. Solvent reservoir, mixing system and degassing system
- B. High pressure pump
- C. Sample injector
- D. Column
- E. Detector

F. Data recording system

1. Solvent reservoir, mixing system and degassing system: Solvent reservoir stores the solvent (mobile phase). These are glass or stainless-steel containers. The most common type of solvent reservoir is glass bottle (Jena AK, 2011). In addition to delivery of mobile phase, the pump must mix solvents with high accuracy and high precision. Two types of mixing unit are low pressure mixing and high pressure mixing (Agilent Technologies, 2016). Degassing system removes entrapped air bubbles from the solvent. Degassing is done by degasser techniques are ultra-sonication and filtration (Jena AK, 2011).

2. High pressure pump: The role of pump is to force a liquid and give a specific flow rate. Flow rate is expressed in millilitres per minute (ml/min). Normal flow rate is 1-2 ml/min. Pump pressure range is 6000-9000 psi (400-600 bar) (Chawla G and Chaudhary KK, 2019). Commonly used pump types are constant pressure pump, syringe type pump and reciprocating piston pump (Hamilton RJ, Sewell PA, 1982).

3. Sample injector: The liquid sample is introduced into the mobile phase by sample injector. Sample valve come between the pump and the column (Jena AK, 2011). An injector (auto sampler) is able to inject the sample into the continuous flowing mobile phase stream that carries the sample into the HPLC column. Typical sample volumes are 5-20 microliters (µl) (Chawla G and Chaudhary KK, 2019). Two types of injector are manual injector and automatic injector (Hamilton RJ, Sewell PA, 1982).

4. Column: Column is a place where actual separation of components takes place. Column is made up of stainless steel. It is 5-25 cm long and 2-4.6 cm internal diameter (Chawla G and Chaudhary KK, 2019).

5. Detector: The detector can detect the individual component that elute from the column and convert the data into an electrical signal (Chawla G and Chaudhary KK, 2019). Types of detector used are of two types, specific detectors and bulk property detectors. Specific detectors include UV-VIS detector, photo diode array detector, fluorescence detector and mass spectrometric detector. Bulk property detectors include refractive index detector, electrochemical detector and light scattering detector (Hamilton RJ, Sewell PA, 1982).

6. Data recording system: The output is recorded as a series of peaks and the area under the peak can be calculated automatically by the computer linked to the display (Malviya R, *et al.*, 2010).

Analytical method development

Analytical method development is the activity of selecting an accurate assay procedure to find out the composition of a formulation. Development of analytical method is the process which is used to prove that an analytical method is suitable for use in laboratory. Analytical methods must be used inside GMP and GLP environments and should be developed by using the given protocols and acceptance criteria in the ICH guidelines Q2 (R1) (Chauhan A, *et al.*, 2015).

The requirements for method development are as follows:

- 1) Qualified analysts
- 2) Instruments-qualified and calibrated
- 3) Documented methods
- 4) Reliable reference standards
- 5) Sample selection and integrity
- 6) Change control (Chauhan A, *et al.*, 2015)

Analytical method development is useful for:

- 1) New process and reactions
- 2) New molecule development
- 3) Active ingredients (Macro analysis)

- 4) Residues (Micro analysis)
- 5) Impurity profiling
- 6) Degradation studies
- 7) Herbal products (Chauhan A, *et al.*, 2015)

Steps involved in method development:

1) Standard analyte characterization:

- All the known information about analyte and its structure is collected for example physical and chemical properties.
- The standard analyte with 100% purity is received. Proper storage condition is arranged such as freezer, refrigerator and desiccators.
- Estimation of multiple components from the sample matrix are analyzed, the number of components are considered, data is compiled and the availability of standards is determined for each component.
- Those methods (Spectroscopic, HPLC, GC, MS, etc.) are considered only, which are suitable with sample stability (Ravisankar P, *et al.*, 2014).

2) Method requirements: Requirement of analytical methodology is necessary to establish the analytical figures of advantage such as linearity, precision, accuracy, Limit Of Detection, Limit Of Quantification, specificity, selectivity and range etc. are marked (Ravisankar P, *et al.*, 2014).

3) Literature survey and prior methodology: All types of information (Physical properties, chemical properties, solubility, manufacturing, related analytical methods etc.) regarding the analyte are obtained by doing literature survey by referencing books, journals, pharmacopoeias etc. Chemical Abstract Service (CAS) automated computerized literature searches are also helpful for literature survey (Ravisankar P, *et al.*, 2014).

4) Selecting a method: The methodology is developed by using the information obtained from the literature. The method is being revised where necessary. Few times, there is a need to include extra instrumentation to reproduce, modify, validate or improve available methods for samples and analytes.

If there is no any established method for analyte in the literature, then such compounds are searched which are identical in chemical properties and structure of analyte (Ravisankar P, *et al.*, 2014).

5) Proper instrumental arrangement and initial studies: The necessary equipment must be set up. Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ) are verified by using Standard Operating Procedures (SOP's). Every time new things (e.g. solvents, filters and gases) are used. For example, method development is never initiated with previously used HPLC column. The analyte solution, standard solutions of known concentrations and solvents are prepared. It is necessary to begin with a genuine, known standard instead a complex sample matrix. If the sample is very close to the standard (active drug), after that it is probable to begin work with the actual sample (Ravisankar P, *et al.*, 2014).

6) Optimization: A single parameter during optimization is changed at a time and the set of terms is different, instead of using a trial and error approach. There is work has been done from the systematic plan and each case is documented in a lab notebook (Ravisankar P, *et al.*, 2014).

7) Proper documentation of analytical figures of merits: The initially determined analytical figures of merit are Limit Of Detection (LOD), Limit Of Quantification (LOQ), linearity, evaluation time, expenses, sample preparation etc. are documented (Ravisankar P, *et al.*, 2014).

8) Evaluation of method development along with actual samples: The prepared solution for analyte needs to be specific, absolute identification of the peak interest of the medicament apart from all the dif-

ferent matrix parts (Ravisankar P, *et al.*, 2014).

9) Determination of percentage recovery of actual sample and demonstration of quantitative sample analysis: The percent recovery of spiked, genuine standard analyte into a sample matrix that do not have analyte is estimated. Ability to reproduce recovery from sample to sample has been optimized. If the results are reproducible then it is not required to obtain 100% recovery. The verification of validity of analytical method is done only by laboratory study. Therefore, documentation of such successful studies is a basic requirement to determine a method is satisfactory for its desired application (Ravisankar P, *et al.*, 2014).

VALIDATION

Validation is a concept developed in the United States in 1978. The concept of validation has been broadened over the years to achieve many activities like from analytical methods used to control quality of drug substances and drug products up to computerized systems for clinical trials, process control or labelling. Validation is best seen as a necessary and prime part of cGMP.

The word validation means evaluation of validity or the act of proving effectiveness. Validation is a team work involving people from different branches of plants.

Method validation is a “process of establishing documented evidence” that provides a high level of guarantee that the product (equipment) will meet the requirements of the desired analytical applications (Lavanya G, *et al.*, 2013).

Importance of validation

- Assurance of quality
- Minimal batch failure
- Reduction in rejections
- Improved efficiency and productivity
- Increased output
- Reduced testing in process and in finished goods (Lavanya G, *et al.*, 2013).

Types of validation

There are four types of validation:

- 1) Equipment validation
 - a. Design Qualification
 - b. Installation Qualification
 - c. Operational Qualification
 - d. Performance Qualification
- 2) Process validation
 - a. Prospective validation
 - b. Retrospective validation
 - c. Concurrent validation
 - d. Revalidation
- 3) Analytical method validation
- 4) Cleaning validation (Lavanya G, *et al.*, 2013)

Types of analytical procedures to be validated

- Identification tests
- Quantitative tests for impurities content
- Limit tests for the control of impurities
- Quantitative tests of the active moiety in samples of drug (Lavanya G, *et al.*, 2013)

Steps in method validation

- 1) Develop a validation protocol, an operating procedure or a validation master plan for the validation.
- 2) Define the scope, purpose and applications of the method.
- 3) Define the performance parameters and its acceptance criteria.
- 4) Define validation experiments.
- 5) Verify related performance characteristics of equipment.
- 6) Qualify materials, ex. Standards and reagent.
- 7) Perform pre-validation experiments.
- 8) Adjust method parameters or/and acceptance criteria if required.
- 9) Perform full internal (and external) validation experiments.
- 10) Develop SOPs for implementing the method in the routine.
- 11) Define criteria for revalidation.
- 12) Define type and frequency of system suitability tests and/or Analytical Quality Control (AQC) checks for the routine.
- 13) Document validation experiments and results in the validation (Lavanya G, *et al.*, 2013).

Parameters (components) of method validation

- 1) Accuracy
- 2) Precision
- 3) Linearity
- 4) Limit of detection
- 5) Limit of quantitation
- 6) Specificity
- 7) Range
- 8) Robustness

1) Accuracy: Accuracy is defined as the closeness of the test results to the true value.

2) Precision: Precision is defined as the measurement of closeness of agreement for multiple measurements on the same sample.

The precision is expressed as the relative standard deviation.

$$\%RSD = \text{Standard deviation}/\text{Mean} \times 100$$

3) Linearity: Linearity is the ability of analytical procedure to obtain a response that is directly proportional to concentration (amount) of analyte in the sample.

Linearity is expressed as the confidence limit around the slope of the regression line.

4) Limit Of Detection (LOD): LOD is defined as lowest amount (concentration) of analyte in a sample that can be detected or identified, not quantified. LOD is expressed as a concentration at a specified signal: noise ratio, usually 3:1.

$$LOD = 3.3 \times S/SD$$

5) Limit Of Quantitation (LOQ): LOQ is defined as lowest amount (concentration) of analyte in a sample that can be quantified. For LOQ, ICH has recommended a signal: noise ratio 10:1.

$$LOQ = 10 \times S/SD$$

6) Specificity: Specificity is defined as the ability of an analytical method to measure the analyte clearly in the presence of other components.

This definition has following implications:

- a. Identification
- b. Purity tests
- c. Assay

7) **Range:** The range of the method is the interval between upper level and lower level of analyte that have been determined with acceptable accuracy, precision and linearity. It is determined on either a linear or nonlinear response curve and expressed in the same unit as the test results are expressed.

8) **Robustness:** Robustness is defined as the measurement of capacity of analytical procedure to remain unaffected by small variations in method parameters (Vidushi Y and Meenakshi B, 2017).

CONCLUSION

This article gives an idea that how to develop a method, what is validation, importance of validation, types of validation, how to perform validation process and its parameters to prove that the method is suitable for its intended use. The primary objectives of development of analytical methods are for identification, purification and eventually to qualification any necessary drug etc. The development of analytical methods helps in understanding the critical process parameters and to reduce their effects on precision and accuracy. Validation is a necessary technique in the Pharma sector and that used to ensure that quality work is done in the process which supports the development of medicine and products.

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