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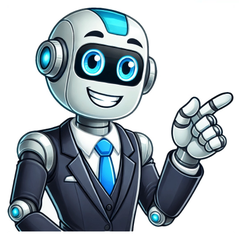

Post Views: 6,879 Analytical method validation in Pharma is a critical process in the development and implementation of methods used for data analysis in various scientific disciplines, including pharmaceuticals, environmental monitoring, food and beverage testing, and clinical diagnostics. This process ensures that the analytical method validation in pharma are appropriate, reliable, and capable of providing accurate and reproducible results within specific conditions. The process involves several key components and steps, each designed to assess different characteristics of the method. Here are the main components of Analytical Method Validation: 1. Specificity 2. Linearity 3. Accuracy 4. Precision 5. Detection Limit (LOD) and Quantitation Limit (LOQ) 6. Robustness 7. System Suitability Testing Validation of an analytical method follows a specific protocol that includes defining the application, scope, and specifications of the method; designing validation studies; conducting experiments; and analyzing and interpreting the results. This rigorous process ensures that the method is suited for its intended purpose and can produce reliable, consistent results critical for decision-making processes in regulatory compliance, quality control, and research development. Analytical method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Analytical monitoring of a pharmaceutical product is necessary to ensure its efficacy throughout all phases of its shelf life; such monitoring is in accordance with the specifications elaborated during product development. Method validation ensures that the selective method will give reproducible, reliable and consistent results adequate for the intended purpose, it is, therefore, necessary to define precisely both the conditions in which the procedure is to be used and the purpose for which it is intended. The objective of this validation study is to; • Ensure and justify, through extensive testing, that the precision and consistency are in accordance with already established Acceptance Criteria. • Assess the effect of variables (within already set operating limits) on the testing method • Identify and solve the problem(s), if any, encountered during testing. • Establish confidence on the existing testing process. • Find ways and means to increase productivity and improve quality. • Assure that complete process is under control. The Scope of this document is i. To describe the work required for the Validation of the Test Method for the determination of Ciprofloxacin, This method is routinely used at the time of manufacturing and during stability studies. ii. This Validation Protocol also describes the analytical parameters to be used for the validation of the test method. iii. The method (SOP No.________) be used for the determination of Ciprofloxacin in is also explained in this protocol along with the acceptance criteria. iv. This Protocol also defines the facilities, responsibilities and equipment, apparatus, glassware, material and documents that are used for the validation studies. v. In this validation protocol, the analytical results are evaluated by the application of statistical techniques and presented by means of graphical techniques. vi. This method validation protocol applies to all test methods performed for release or stability evaluation of all strengths of Ciprofloxacin Tablets. 4.0 Periodic Revalidation: In case there is no change or modification in the Validated Method, the Revalidation will be performed after every 5 years. Any modification or changes in the Validated Test Method (SOP No.________) being used for the determination of Ciprofloxacin should be controlled and will be entered into the Change Control Form (Form No.________) in accordance with the change control procedure (SOP No.________). Provide Justification for proposing a test method. The proposed procedure should also be validated according to this Validation Protocol. 6.0 Validation Parameters: Following analytical parameters are to be considered 6.1 Calibration of Spectrophotometer: i. Calibration of Wavelength a. Turn the main power "ON" and both lamps. b. The instrument will confirm the filter automatically. c. Set the wavelength from 200 – 400 nm. d. Set the wavelength from wavelength counter. e. Fill the cell with blank and check the blank reading. f. Wash them all with distilled water g. Fill it with the $K_2Cr_2O_7$ solution. h. Note the absorbance from screen Wavelength (nm) Absorbance 235 nm (minima/valley) 0.748 (0.740 – 0.756) 257 nm (maxima) 0.865 (0.856 – 0.894) 313 nm (minima/valley) 0.292 (0.289 – 0.295) 350 nm (maxima) 0.640 (0.634 – 0.646) If the instrument shows absorbance at the above-mentioned wavelength with limits, then the calibration has completed, otherwise, it has to recalibrate. ii. Calibration of Stray light: • Take 1.2 g of Potassium chloride previously dried at 105 °C for two hours into the 100 ml volumetric flask. • Add water to dissolve and make up to volume with water. • Check the absorbance of a 1.2 % w/v solution of potassium chloride at a path-length of 1cm. • The absorbance should be greater than 2.0 at about 200nm when compared with water as reference liquid. iii. Calibration of Resonance Power: • To prepare 0.02 % v/v Toluene, take 0.02 ml of Toluene into the 100 ml volumetric flask. Add Hexane to volume. • Record the spectrum of a 0.02 % v/v solution of Toluene in Hexane. • The ratio of the absorbance at the maximum at about 269 nm to that at the minimum at about 266 nm is not less than 1.5 Set of parameters and criteria there off to ensure that the system is working properly. System suitability performed during entire validation of this method, by preparing five dilutions of the same concentration of Ciprofloxacin Working Standard and shows the results of system suitability by the application of statistical techniques i.e. Mean, Standard Deviation and Relative Standard Deviation (%). 6.3 Selection of Solvent: Prepare 50µg/ml of stock solution in four portions by dissolving 5 mg of Ciprofloxacin in 100 ml of phosphate buffer having pH of 2.5 and pH 7.4 respectively, in 0.1N HCl and in Distilled water. Then prepare Standard solution of various concentrations (1, 2, 3, 4, 5µg/ml) by dilution from each stock solution. Found λmax of Ciprofloxacin in each media by scanning them over the UV range of 190nm to 400nm. Plot concentration vs. absorbance and prepare standard curve by scanning the standard solutions of Ciprofloxacin at the respective wavelength (λmax) of different solvents. 6.4 Calibration Curve (Linearity): A calibration curve is a general method for determining the concentration of a substance in an unknown sample by comparing to a set of standard samples of known concentration. A calibration curve is simply a graph where concentration is plotted along the x-axis and absorbance is plotted along the y-axis. After making several Calibration Standards at different concentrations. After running each one on the instrument and getting the absorbance, the points are then plotted on the graph. The points are then connected with a line. That line represents the calibration curve. i. Prepare solution of Ciprofloxacin Working Standard at concentration 0.01 mg./ ml. in different media i.e. 0.1 N HCl, Distilled Water, Phosphate Buffer pH 2.5 and Phosphate Buffer pH 7.4 and scan over the UV range 190 to 400 nm to determine the λmax. ii. Prepare 5 dilutions of Ciprofloxacin Working Standard at conc. i.e. 1, 2, 3, 4 & 5 µg./ ml. iii. Scan all the 5 solutions of the different concentration of all the 4 media at the determined λmax using the same media as a blank iv. Subtract the reading of blank from the reading of the solution to get the correct value. v. Draw the Calibration Curve. 6.5 Limit of Detection (LOD): The lowest amount of analyte in the sample which can be detected but not necessarily quantitated. Analysis of sample with a known concentration of the analyte and by establishing minimum concentration at which analyte can be reliably detected. LOD may also be calculated based on the standard deviation (SD) of the response and the slope of the calibration curve (S) at levels approximating the LOD according to the formula: LOD =3.3(SD/S). The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines. S = Slop of Calibration Curve SD = Standard Deviation of response 6.6 Limit of Quantitation (LOQ): The lowest amount of an analyte in a sample which can be quantitatively determined with a suitable precision and accuracy. Analysis of sample with a known concentration of the analyte and by establishing minimum concentration at which analyte can be reliably quantitated. LOQ may also be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOQ according to the formula: LOQ =10(SD/S). The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines. S = Slop of Calibration Curve SD = Standard Deviation of response 6.7 Accuracy (% Recovery): The difference between theoretical added amount and the practically achieved amount is called accuracy of the analytical method. Accuracy was determined at 5 different level 50%, 75%, 100%, 125% and 150% of the target concentration in triplicate. i. Prepare Placebo and draw 6 sample of the same weight ii. Prepare three (3) replicate for following levels. • Prepare three samples without addition iii. Analyze all the samples and calculate the % recovery • % age recovery should be 98 to 102 % • The coefficient of determination (r2) should be greater than 0.9998. • The % RSD of the assay or recovery values should not be greater than 2.0%. Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision may also be explained by the terms, Intermediate Precision and Repeatability. 6.8.1. Intermediate Precision: Intermediate precision refers to variations within a laboratory as with, different instruments, by different analysts, and so forth. • Prepare ten replicate samples solutions from the same composite sample according to the analytical method by three different analysts keeping the other condition same. • Analyze the samples according to procedure by the three analysts on the same day • Calculate the assay results for each sample. • Assay Limit 97 % to 103 % • The coefficient of determination (r2) should be greater than 0.9998. • The % RSD of the assay or recovery values should not be greater than 2.0%. Repeatability refers to variations within a laboratory as with different days, different equipment etc. keeping the other conditions same. • Prepare ten replicate samples solutions from the same composite sample according to the analytical method by one analyst keeping the other condition same. • Analyze the samples according to procedure by the one analyst on ten different days • Calculate the assay results for each sample. • Assay Limit 97 % to 103 % • The coefficient of determination (r2) should be greater than 0.9998. •The % RSD of the assay or recovery values should not be greater than 2.0%. When the procedure is carried out in different laboratories using different equipment, reagents by using the sample from the same homogenous batch in each case. Evaluate the results obtained from the variability of the analyst–to–analyst, day-to-day, laboratory-to-laboratory, equipment-to-equipment etc., this analytical data will provide information about the reproducibility of the test procedure. • Prepare ten replicate samples solutions from the same composite sample according to the analytical method by one analyst keeping the other condition same. • Analyze the samples according to procedure by one analyst on the same day • Arrange for the testing of the sample from the same batch using the same analytical procedure from any other laboratory. • Assay Limit 97 % to 103 % • The coefficient of determination (r2) should be greater than 0.9998. • The % RSD of the assay or recovery values should not be greater than 2.0%. Robustness is the measure of the capacity of an analytical method to remain unaffected by small but deliberate variation in the procedure. It provides an indication about the variability of the method during normal laboratory conditions. • Prepare ten replicate samples solutions from the same assay composite sample according to the analytical method by one analyst keeping the other condition same. • Prepare another ten replicate samples solutions from same composite sample according to the analytical method by one analyst with a minor deviation i.e. 15 minutes sonication instead of 30 minutes stirring, keeping the other condition same. • Analyze the samples according to procedure by the one analysts on the same day • Calculate the assay results for each sample. • Assay Limit 97 % to 103 % • The coefficient of determination (r2) should be greater than 0.9998. • The % RSD of the assay or recovery values should not be greater than 2.0%. Degradation information obtained from stress studies (e.g., products of acid and base hydrolysis, thermal degradation, photolysis and oxidation) for the drug substance and for the active ingredient in the drug product. Procedure (Thermal Degradation): • Prepare triplicate samples solutions from the same assay composite sample according to the analytical method by one analyst at room temperature • Prepare triplicate samples solutions, and standard solution according to the analytical method by one analyst and subjected to heat treatment at 30 °C, 40 °C, 50 °C, 60 °C & 70 °C for one hour • Analyze the all samples according to procedure by the one analysts on the same day Acceptance Criteria: (Not for all samples) • Assay Limit 97 % to 103 % • The coefficient of determination (r2) should be greater than 0.9998. • The % RSD of the assay or recovery values should not be greater than 2.0%. 7.0 Statistical Techniques: Mean is the sum of individual values divided by the total number of individual values. Mean is denoted by x̄ and is calculated by the formula The standard deviation is a measure of how widely values are dispersed from the average value (the mean). The statistic used as a measure of the dispersion or variation in a distribution, equal to the square root of the arithmetic mean of the squares of the deviations from the arithmetic mean. iii. Relative Standard Deviation: Standard Deviation divided by the and multiplied by 100 iv. Coefficient Correlation A measure of the interdependence of two random variables that range in value from –1 to +1, indicating perfect negative correlation at –1, the absence of correlation at zero, and perfect positive correlation at +1. Also called coefficient of correlation. S = Slop of Calibration Curve SD = Standard Deviation of response vi. Limit of Quantitation: S = Slop of Calibration Curve SD = Standard Deviation of response Determination of Ciprofloxacin HCl SOP No. ________________ Weigh and transfer 58.2 mg. Working Standard (Ciprofloxacin HCl) equivalents to 50 mg. Ciprofloxacin into 100 ml. volumetric flask add 10 ml. of 0.1 N HCl and shake to dissolve make the volume up to the mark with 0.1 N HCl and mix thoroughly. Filter the solution through Whatman filter paper; discard the first 10 ml. of the filtrate. Pipette out 1 ml. of the filtered solution to a 100 ml. of volumetric flask and make the volume up to 100 ml. with 0.1 N HCl and mix thoroughly. Weigh 20 tablets and determine the average weight Powder 20 tablets and mix thoroughly, weigh and transfer a quantity of powder equivalent to 250 mg. of Ciprofloxacin into 500 ml. volumetric flask adds 100 ml. of 0.1 N HCl allow stirrer using magnetic stirrer for 30 minutes and make the volume up to the mark with 0.1 N HCl and mix thoroughly. Pipette out 1 ml. of the resulting solution to a 100 ml. of volumetric flask and make the volume up to 100 ml. with 0.1 N HCl and mix thoroughly. It consists of 0.1 N HCl. Operate the Spectrophotometer according to SOP and measure the absorbance of the sample and standard preparation at λmax 276 nm of Spectrophotometer using blank preparation for adjusting the instrument. Percentage = Absorbance of sample × P x 100 Absorbance of standard × 100 Where; P = Potency of working standard (As is basis) Determined amount = Percentage × Labeled amount Preparation of phosphate buffer of pH 2.5 The buffer solution was prepared by adding 16.3308 g. Potassium Dihydrogen Phosphate (KH_2PO_4) and 3.4836 g Dipotassium Hydrogen Phosphate (K_2HPO_4) in 200 ml distilled water. pH of the solution was adjusted to 2.5 by Phosphoric Acid (H_3PO_4) Preparation of phosphate buffer of pH 7.4 The buffer solution was prepared by adding 2.7218 g. Potassium Dihydrogen Phosphate (KH_2PO_4) and 8.709 g. Dipotassium Hydrogen Phosphate (K_2HPO_4) in 200 ml distilled water. pH of the solution was adjusted to 7.4 by Sodium Hydroxide (NaOH). Preparation of 0.1N HCl acid: 0.83 ml of 37% pure concentrated HCl was taken in a 100 ml volumetric flask. The volume was made up of distilled water and the content is thoroughly mixed it by shaking. 10.0 Facilities/Responsible Personnel/Equipment/Material Documentation Validation of Test Method (SOP No.________) being used for the Determination of Ciprofloxacin HCl in 250, 500 and 750 mg. Tablets will be performed in Chemical Lab. at ________________ (Company Name and Address). 10.2 Identification of Responsible Personnel: 10.3 Identification of Documentation Document Title SOP/QF Number Validation Master Plan Method Validation Protocol (SOP) Method Validation Protocol (QF) Method Validation Report (QF) Testing Method Change Control Procedure (SOP) UV- Spectrophotometer Analytical Balance 10.4 Machine/ Equipment Machine/ Equipment Qualification Status Qualification No. UV. Visible Spectrophotometer Qualified IQ: ________ OQ: ________ PQ: ________ Digital Balance Qualified IQ: ________ OQ: ________ PQ: ________ 10.5 Identification of Apparatus/Glassware Apparatus/Glassware Volumetric Flask Pipette M. Fakhir Khaleeq QA/ QC Manager SWISS Pharmaceutical (Pvt.) Ltd.Karachi, Pakistan E-mail: mfakhir@yahoo.com Validation is the most important activity in the pharmaceutical industry. There are multiple types of validation in the pharmaceutical, such as Analytical Method Validation, Process Validation, Cleaning Validation, Equipment Validation, HVAC System Validation, Facility Validation, and Computer System Validation. In every industry validation is plays an important role to produce results in a consistent manner. The validation procedure authorizes documentary evidences that a particular process/method/activity will consistently produce a product with the expected result (predetermined requirements). Any food and drug regulatory agency around the world does not only require products that meet its specifications, but they also require processes, procedures, intermediate inspection stages, and testing adoptions that are designed to produce similar, reproducible, consistent results that satisfy the regulatory and security requirements of the product being manufactured. The process of validation allows for the development of such procedures. This is to ensure that the food and drug products are of a higher standard. The definition of processes validation is to gather and evaluate data through the design phase and to the final stage of production, in order to establish scientific evidence that a process is able to consistently produce quality products. A good validation system should ensure that every phase, every process, and every change has been adequately evaluated before implementation. Testing a sample of a final product does not equate to conclusive evidence that all of the products in a batch meet the specification. Let's go over different types of validation and their phase in the pharmaceutical industry. In this section, we will discuss different types of validation in more detail. Analytical validation is designed to verify that the selected analytical method is capable of providing reliable results that are appropriate for the intended purpose. Different parameters are considered as part of the analytical method validation process. These are as follows: AccuracyPrecisionReproducibilityRepeatabilitySpecificationLinearityRangeLimit of DetectionLimit of Quantitation Cleaning validation is a documented setup that provides a high degree of assurance that a particular system/piece of equipment is cleaned to a predetermined standard and within acceptable limits. Pharmaceutical products are contaminated by a variety of materials including lubricants, airborne materials, preparation residues, and microbes. Therefore, an appropriate cleaning procedure plays an important role in preventing contamination and cross-contamination. A process validation shows documented proof, which can provide a higher level of assurance a product will meet all the program's specified quality characteristics and specifications consistently. Validation of the process also assures repeatability and decreases manufacturing errors, resulting in a higher level of output of a predetermined quality. Process validation can be classified into four types based on the stage where the process is being evaluated: Prospective ValidationConcurrent ValidationRetrospective ValidationRevalidation Equipment or instrument validation is a common process for every industrial instrument or equipment. There are three main phases for equipment validation in pharma and are as follows: Pre-validationProcess validationPost validation maintenance Generally, there are 4 types of equipment validation and are cover under pre-validation and process validation phases. The 4 types of equipment validation are: Design Qualification (DQ)Installation Qualification (IQ)Operational Qualification (OQ)Performance Qualification (PQ) 1. Pre-validation of equipment: The pre-validation is the first phase of equipment validation. Pre-validation includes Design Qualification (DQ) and Installation Qualification (IQ). Design Qualification consists of; User Requirement Specification (URS), Functional Specification, Operational Specification, Vendor qualification, Factory Acceptance Test (FAT), and Installation consists of Site Acceptance Test (SAT), Installation of hardware and software. 2. Process validation of Equipment: The next phase of validation is Process validation. Process validation includes Operational Qualification (OQ), Performance Qualification (PQ), and Revalidation. Operational Qualification consists of; Functional Tests, Security tests, Performance validation consist of the ongoing performance of the equipment, and specified application testing. Change in material, changes in equipment, change in packing material, change in the manufacturing process are part of revalidation. These all are types of validation in pharmaceutical industry. Also read: Clinical Trial in Human medicines as per EU What is Clinical Trial as per FDA Clinical Data Management Interview Questions And Answers HPLC Interview Question and Answers Gas Chromatography Interview questions and answers Karl Fischer Titration Interview Question and Answer Dissolution Interview Question and Answer What Is Change Control In pharma? CAPA Process in Pharmaceutical Management System Interview Question and answer on Polarimeter For interview preparation refer YT channel: Pharmabeej Validation in Pharmaceutical Industry Analytical validation is a vital process in the pharmaceutical industry, ensuring the methods used for drug analysis are accurate, reliable, and consistent. By validating analytical methods, pharmaceutical companies guarantee the quality, safety, and efficacy of their products, aligning with global regulatory requirements. This article explores what analytical validation is, why it is essential, and its key components. What is Analytical Validation? Analytical validation confirms that an analytical method can consistently deliver accurate and reproducible results. Whether it is for identifying impurities, determining potency, or verifying stability, analytical validation is critical to every stage of drug development and manufacturing. Why is Analytical Validation Important? 1. Regulatory Compliance Regulatory authorities like the FDA, EMA, and WHO mandate analytical validation to ensure pharmaceutical products meet stringent quality standards. Compliance with guidelines such as ICH Q2 (R1) is essential for approval and market entry. 2. Ensures Product Quality Analytical validation guarantees the quality of each drug batch, minimizing deviations and ensuring consistency across production cycles. 3. Accurate Dosage Delivery Validated methods ensure that the correct dosage of active pharmaceutical ingredients (APIs) is present in the final product, protecting patients from underdosing or overdosing risks. 4. Impurity Detection A validated analytical method identifies and quantifies impurities, ensuring the safety of the drug product. 5. Product Stability Assessment Stability studies rely on validated methods to determine a drug's shelf life, storage conditions, and expiry date. 6. Cost Efficiency Investing in method validation reduces the risk of batch failures, recalls, and additional testing, saving time and resources. Parts of Analytical Validation Analytical validation involves several critical components, each serving a unique purpose in assessing a method's reliability and applicability. These include: Specificity: Identifying the target analyte without interference from impurities or other components. Linearity: Ensuring results are proportional to the analyte concentration within a specified range. Accuracy: Delivering results close to the true or standard value. Precision: Consistently reproducible results under the same or varied conditions. Robustness and Ruggedness: Reliability under minor variations in method parameters or testing environments. LOD and LOQ: Establishing the method's sensitivity to detect and quantify small amounts of analyte. For detailed explanation , check out our previous article on parts of analytical validation. Table of contentAnalytical method development and validation play a unique role at each stage of pharmaceutical development in managing quality, safety and efficacy. However, both require knowledge as well as expertise, which is why I decided to share my skill-based knowledge on this topic, In this article, you will learn about analytical method development and validation, their advantages, disadvantages, elements, quality attributes and differences between AMD and AMV with FAQs.Image created in CANVAAnalytical method development is the innovative process of optimizing procedures for the analysis of pharmaceutical substances. It falls under the pharmaceutical research and development domain to ensure that pharmaceuticals meet regulatory standards for quality, safety and efficacy.Related Topic: HPLC Method Development Steps For PharmaceuticalsValidation is the process of confirming that an analytical method is suitable for its intended purpose and produces reliable, reproducible results under specified conditions outlined in the method. This ensures that the method, once developed, meets specific regulatory requirements and can be used consistently for routine analyses.Related Topics:The following are the main elements of the Analytical method development:Related Topic: Pharmaceuticals Structural Elucidation: How to Make StrategyThe following are the main elements of the Analytical method development:Approved Method or Monograph: It is required to prepare an analytical method validation protocol.Analytical Method validation protocol: It is required to perform an analytical method validation study.Analytical Method validation report: It contains validation study work.Method validation parameters: Depending upon the criticality of the method all or some of the following parameters can be performed during method validation:Specificity: The specificity parameter is used to establish that there is no interference between an adjacent impurity peak or an impurity peak with the main peak or a blank peak with the main peak or an impurity peak.Accuracy and Recovery: The closeness of the measured value to the true value (the reference or accepted standard).Precision: The degree of reproducibility of results under normal operating conditions, typically evaluated by assessing repeatability and intermediate precision.Linearity: The ability of the method to give results that are directly proportional to the concentration of analyte over a specified range.Range: The interval between the upper and lower concentration limits within which the method can provide accurate and precise results.RRF or Relative response factor: It is calculated from linearityDL or Detection Limit: The smallest amount of analyte that can be reliably detected but not necessarily quantified.QL or Quantification Limit: The lowest concentration of analyte that can be reliably quantified with acceptable precision and accuracy.Robustness: The method's capacity to remain unaffected by small variations in operational conditionsStability of Solutions: The Stability of Solutions is performed to avoid any failure during analysis in sequenceRelated Topic: What is the difference between validation and verificationThe following main quality attributes play a vital role in Analytical Method Development and Validation:ResponsibilitiesGLP (Good laboratory practice)GMP (Good manufacturing practice)SOP (Standard operating procedure)Data integrityCAPA (corrective and preventive action)OOS (out of specification)OOT (out of trend)Technology transfer (TT)Regulatory complianceAudit management &QBD (Quality by design) approachThe following are the responsibilities of different professionals/ departments:Analytical Professional/Validation Specialists: They are responsible to perform analytical method development and validation studiesQuality Control (QC) Professional: QC professionals are involved in method validation in reproducibility tests. They are not involved in Analytical method developmentRegulatory Affairs Personnel: They coordinate with various regulatory agencies such as FDA, EMA and provide answers to any queries related to AMD or AMV.Method development and method validation intrinsically linked to GLP (Good Laboratory Practice), GMP (Good Manufacturing Practice), Data Integrity, SOP, CAPA (Corrective and Preventive Action), OOS (Out of Specification), OOT (Out of Trend) It has happened.GLP (Good Laboratory Practice): GLP ensures that laboratory processes, including method development, are properly documented, validated, and performed with high standards of quality and integrity. GLP governs analytical research and chemical research activities.GMP (Good Manufacturing Practice): GMP guidelines require that the methods used in the analysis of pharmaceuticals be thoroughly validated.Data Integrity: Method development and validation activities must comply with stringent data integrity principles (e.g., ALCOA-Attributable, Legible, Contemporaneous, Original, and Accurate). This ensures that the data generated during these processes is accurate, complete, and traceable.CAPA (Corrective and Preventive Actions): During method development and validation, any observed issues or deviations must be investigated and addressed using CAPA. If a method does not perform as expected or shows inconsistencies, CAPA procedures are implemented to correct the problem and prevent future occurrences.OOS (Out of Specification): OOS refers to results that fall outside predefined acceptance criteria. It is only related to validation and not to development.Analytical method development and validation provide the technical basis to ensure that transferred technology produces consistently high-quality products, complies with regulatory standards, and minimizes the risks associated with transfer between sites or teams.The analytical method development and validation are helpful in technology transfer in the following ways:Ensuring Consistency and Reproducibility:Method Development: Developing robust analytical methods ensures that the same results can be obtained regardless of where the testing is performed. This is especially important when transferring technology across different sites or between different teams.Validation: The validation of these methods demonstrates that they consistently produce accurate results within specified limits, ensuring that the quality of the product is maintained even when produced in new locations or scaled-up processes.Regulatory Compliance:Regulatory agencies (such as the FDA, EMA, etc.) require validated analytical methods for product testing, quality control, and stability studies. Proper validation ensures that the methods comply with these regulations, facilitating smooth technology transfer by reducing the risk of regulatory issues during inspections or audits.Risk Mitigation:Analytical methods provide critical data on the quality attributes of the product, such as purity, assay, degradation products, and impurities. During technology transfer, having validated methods ensures that the new production process or manufacturing site will maintain product quality, thus minimizing the risks of batch failures or product recalls.Reproducibility Across Different Stages of Production:Analytical method development and validation ensure that these methods are capable of maintaining the quality at different production stagesData IntegrityValidated analytical methods ensure the integrity of the data generated throughout the technology transfer process.Training and Knowledge TransferThe analytical methods that have been developed and validated are often critical tools for training personnel at the receiving site. By transferring both the method itself and the rationale behind its validation, organizations can ensure that the new team has the necessary knowledge to maintain consistent product quality.The analytical method development and validation plays a vital role in the submission, approval, and ongoing compliance of pharmaceuticals such as:Ensures Consistency and Quality Control: It controls the quality of the pharmaceuticals such as assay, purity, impurity profile, and stability.Supports Regulatory Compliance: Regulatory authorities require that all analytical methods used in the testing of pharmaceuticals must be validated according to the approved protocolDemonstrates the Drug's Safety and Efficacy: It plays a vital role in maintaining safety and efficacy of the pharmaceuticals.Enables Stability Testing: Validated stability indicating methods (SIMs) are used to test the stability of pharmaceuticals over time, ensuring that they maintain their efficacy and safety during storage. Stability studies, supported by SIMs, are required for regulatory submissions.Improves Traceability and Documentation: A well-documented validation process provides a clear, reproducible method for analyzing the drug. This documentation is crucial for the DMF, as it offers regulatory agencies detailed evidence that the product can consistently meet quality standards.Facilitates Communication with Regulatory Authorities: The inclusion of comprehensive method development and validation data helps in addressing regulatory questions or concerns during the review process, providing a clear understanding of how the drug is tested and ensuring all regulatory requirements are met.Analytical verification plays an important role in audit inspection in pharmaceutical industries. Method validation review is required in almost all audits.Analytical Method Development and Validation plays a vital role in DMF filing and answering the deficiency letter queries.The QBD approach helps analytical method development and validation by providing scientific rationale in the understanding of critical parameters of the method, reducing variability, and ensuring reliability and compliance. This structured RFT (right first time) approach not only improves the efficiency of method development and validation but also reduces risk.The QbD approach helps in analytical method development and validation in the following ways:Understanding the Analytical Target Profile (ATP): It outlines the purpose of the method and the critical parameters that must be controlled to achieve the desired results. This focus helps avoid trial-and-error during method development and guides the optimization of method parameters.Identification of Critical Quality Attributes (CQAs): In QbD, the Critical Quality Attributes (CQAs) are identified early and ensures that the method delivers reliable and consistent results under various conditions.Risk Assessment and Control of Variables: It is very helpful in identifying the critical variables which may lead to failure of AMV or AMD.Design of Experiments (DoE): QbD helps in using design of experiments (DOE) to systematically explore the effect of various factors on method performance. DOE helps in identifying the optimal combination of conditions (e.g., temperature, pH, mobile phase composition) for reliable method development. It is also helpful in finalising the validation protocol and in performing robustness test.Analytical Method Transfer: QbD helps in Analytical method transfer from the Analytical laboratory to QC (quality control).Cost-effectiveness: QbD helps in reducing Analytical method development and validation costRegulatory Compliance: QbD provides more robust rationale for decisions made during method development, allowing for more streamlined regulatory submissions, approvals, and complianceThe following are the main differences between Analytical Method Development and ValidationElementsAMDAMVDefinitionAMD is an innovative work and it is performed to establish analytical conditions to perform analysisAMV is a skill-based work and it is performed to establish the reliability of the developed analytical methodSelection of appropriate techniquesRequired (e.g., HPLC, GC, spectrometry)Not required. Established technique is usedOptimizing conditions Required(e.g., mobile phase, temperature, detector settings)Not required.STP/MonographNot neededEither STP or Monograph is required to perform AMVProtocolNot neededApproved protocol is needed to perform validationLiterature reportVery helpful for AMDNot neededSamplesAMD may be performed using pure or impure samplesRepresentative samples with valid COAs are required to perform AMVStandards/ImpuritiesAMD may be performed using individual sample or contaminated /impure sample or standardStandards/Impurities (as mentioned in the monograph) with valid COAs are required to perform AMVValidation parametersOnly selected parameters are performed to find out the criticality of the method and avoid any surprise/ failure during complete validationAll parameters (as defined in the validation protocol) are performed to find out the criticality of the method and avoid any surprise/ failure during complete validationReportDevelopment report or verification repot or mini-validation report is preparedComplete validation report is preparedApproval processAnalytical project coordinator and Analytical headAnalytical/Validation project coordinator, Analytical /Validation head, QC head and QA headGLP/GMP requirementPerformed under GLP environmentPerformed under GLP and GMP environmentData integrityMust be maintainedMust be maintainedGuidelinesNot essentialRequiredSOPNot essentialRequiredLIRNot requiredRequiredQBDPlay a vital role in designing the development experimentPlay a vital role in designing the validation protocolAnalytical Method Development and Validation are very helpful in:Ensuring Product Quality, Safety and Efficacy:Analytical Method Development: Method must be able to provide specific, accurate, precise and reproducible resultsValidation: Validation ensures that the analytical method is suitable for the intended purposeSupporting Pharmaceuticals DevelopmentDuring Pharmaceutical development, analytical methods are used to characterize, identify and quantify the pharmaceuticalsRegulatory ComplianceRegulatory agencies such as FDA, and EMA require that pharmaceutical products meet quality standards. Analytical methods must be developed and validated to comply with regulatory guidelines..Validation provides documented evidence that the method is reliable. If the method is not validated as per the guidelines then many deficiency letter related questions may arise.Ensuring ConsistencyAnalytical methods ensure consistency in production batches during QC analysisControlling Impurities in PharmaceuticalsBe it purchasing the ingredients, reaction mix, intermediates and the final API method is used at every stage to monitor the qualityStability StudiesStability indicating method plays a vital role about formation of degradation impurities during stability studiesBatch Release and Quality ControlIn quality control (QC), analytical methods are used to test and release or reject raw materials, in-process samples, and APIs. Hence, the method must be reliable and validated to avoid any failureRisk MitigationAnalytical method validation helps to minimize analytical method risks and ensure the reliability of results.The following are the advantages of the Analytical method development and Validation:Method reliabilityRegulatory ComplianceConsistency in analytical resultMaintaining pharmaceuticals qualityCost EfficiencyRisk ManagementData Integrity and TraceabilityReproducibility Across LaboratorieThe following are the disadvantages of the Analytical method development and Validation:Both Analytical method development and Validation takes a lot of timeNeeds knowledge as well as expertiseCostly activitiesMethod development and validation ensures that pharmaceuticals meet required quality standards and are safe for consumer use. I hope this article has helped you understand Analytical method development and Validation. You may also want to check out other articles on my blog, such as:Analytical method development is an innovative task and is done to establish the analytical conditions to perform the analysis whereas AMV is a skill-based task and is done to establish the reliability of the analytical method developed.AMD: Analytical method developmentAMV: Analytical method validationCAPA: Corrective and preventive actionOOS: Out of specificationOOT: Out of trend Pharmaceutical analysis is an important part of the research and production of pharmaceutical goods. Method validation is an important step in assuring that the analytical method used to analyze a pharmaceutical product is trustworthy and accurate. Joachim Ermer's handbook "Method Validation in Pharmaceutical Analysis: A Guide to Best Practice" is a complete guide that gives full information on the best practices for method validation in pharmaceutical analysis. This guidebook is an excellent resource for anybody engaged in the development and manufacture of pharmaceutical goods, such as analytical chemists, quality control people, and regulatory affairs experts.Chapter 1: Introduction to Method Validation in Pharmaceutical AnalysisThe handbook's first chapter introduces the notion of technique validation in pharmaceutical analysis. The chapter discusses the significance of method validation in guaranteeing the reliability and accuracy of an analytical method used to analyze a pharmaceutical product. The chapter also covers the numerous regulatory standards that must be completed while validating a pharmaceutical analytical technique.Chapter 2: Validation ParametersChapter 2 of the handbook focuses on the various validation parameters that must be considered when validating a method for pharmaceutical analysis. The chapter discusses the importance of accuracy, precision, specificity, limit of detection, limit of quantitation, linearity, range, and robustness in method validation. The chapter also provides detailed information on how to determine these validation parameters and how to interpret the results.Chapter 3: Method DevelopmentChapter 3 of the handbook provides detailed information on the process of method development for pharmaceutical analysis. The chapter discusses the various factors that must be considered when developing a method for pharmaceutical analysis, including the nature of the analyte, the nature of the matrix, and the type of analytical instrument being used. The chapter also provides information on the various techniques that can be used to optimize a method for pharmaceutical analysis.Chapter 4: Method Validation ProtocolChapter 4 of the handbook provides detailed information on how to develop a method validation protocol for pharmaceutical analysis. The chapter discusses the various elements that must be included in a method validation protocol, including the scope of the method, the validation parameters that will be assessed, the acceptance criteria, and the statistical methods that will be used to analyze the data.Chapter 5: Method Validation StudyThe handbook's Chapter 5 describes in full how to perform a technique validation study for pharmaceutical analysis. The chapter outlines the many procedures that must be followed to validate a technique for pharmaceutical analysis, such as sample preparation, sample analysis, and result interpretation. The chapter also discusses how to document the findings of a technical validation study.Chapter 6: Method TransferChapter 6 of the handbook provides detailed information on the process of method transfer for pharmaceutical analysis. The chapter discusses the various factors that must be considered when transferring a method from one laboratory to another, including the equipment used, the personnel involved, and the analytical procedures used. The chapter also provides information on how to document the results of a method transfer study.Chapter 7: Robustness TestingThe handbook's Chapter 7 contains in-depth information on the procedure of robustness testing for pharmaceutical analysis. The chapter examines the numerous elements that might impact the performance of a pharmaceutical analysis technique, such as changes in the analytical equipment, changes in the analytical circumstances, and staff changes. The chapter also explains how to run a robustness test and how to evaluate the findings.ALSO READ: Analytical Method Validation Protocol for NystatinChapter 8: Method OptimizationChapter 8 of the handbook provides detailed information on the process of method optimization for pharmaceutical analysis. The chapter discusses the various techniques that can be used to optimize a method for pharmaceutical analysis, including the use of different analytical conditions, the use of different sample preparation techniques, and the use of different statistical methods for data analysis. The chapter also provides information on how to document the results of a method optimization study.Chapter 9: Qualification of Analytical InstrumentsThe handbook's Chapter 9 contains in-depth information on the process of certifying analytical equipment for pharmaceutical analysis. The chapter examines the different elements that must be addressed while certifying analytical equipment, such as the instrument's accuracy and precision, detection and quantification limitations, and linearity and range. The chapter also discusses how to document the findings of an instrument qualification study.Chapter 10: Stability StudiesChapter 10 of the handbook provides detailed information on the process of stability studies for pharmaceutical analysis. The chapter discusses the various factors that can affect the stability of a pharmaceutical product, including temperature, humidity, and light exposure. The chapter also provides information on how to conduct stability studies for pharmaceutical products and how to document the results.Chapter 11: Method Validation for Biotechnology ProductsChapter 11 of the handbook focuses specifically on method validation for biotechnology products. The chapter discusses the unique challenges that arise when validating methods for biotechnology products, including the complexity of the product and the potential for product variability. The chapter also provides information on how to validate methods for biotechnology products and how to document the results.Chapter 12: Analytical Quality by DesignChapter 12 of the handbook provides detailed information on the concept of analytical quality by design (AQbD). The chapter discusses the importance of AQbD in ensuring the quality of pharmaceutical products and the role of method validation in implementing AQbD. The chapter also provides information on how to implement AQbD in the development and validation of analytical methods for pharmaceutical products.The handbook "Method Validation in Pharmaceutical Analysis: A Guide to Best Practice" by Joachim Ermer is a comprehensive resource that provides detailed information on the best practices for method validation in pharmaceutical analysis. The handbook covers a wide range of topics, including validation parameters, method development, method validation protocol, method validation study, method transfer, robustness testing, method optimization, qualification of analytical instruments, stability studies, method validation for biotechnology products, and analytical quality by design. This handbook is a valuable resource for anyone involved in the development and manufacturing of pharmaceutical products, including analytical chemists, quality control personnel, and regulatory affairs professionals. By following the best practices outlined in this handbook, pharmaceutical companies can ensure the quality and reliability of their analytical methods, which is essential for the development and manufacturing of safe and effective pharmaceutical products.Method Validation in Pharmaceutical Analysis: A Guide to Best PracticeAdopting a practical approach, the authors provide a detailed interpretation of the existing regulations (GMP, ICH), while also discussing the appropriate calculations, parameters and tests. The book thus allows readers to validate the analysis of pharmaceutical compounds while complying with both the regulations as well as the industry demands for robustness and cost-effectiveness.Following an introduction to the basic parameters and tests in pharmaceutical validation, including specificity, linearity, range, precision, accuracy, detection and quantitation limits, the text focuses on a life-cycle approach to validation and the integration of validation into the whole analytical quality assurance system. The whole is rounded off with a look at future trends.With its first-hand knowledge of the industry as well as regulating bodies, this is an invaluable reference for analytical chemists, the pharmaceutical industry, pharmaceutics, QA officers, and public authorities. ALSO READ: SOP for Analytical Method Validation Standard Operating Procedure (SOP) and Guideline for Validation of Analytical Method (AMV). The objective of validation of an analytical Method (AMV) is to demonstrate that it is suitable for its intended purpose. Procedure for Analytical Method Validation (AMV) 1.0 Objective : To lay down procedure for validation of Analytical Method (AMV) to be followed in laboratory. The objective of validation of an analytical Method (AMV)is to demonstrate that it is suitable for its intended purpose. 2.0 Scope : This SOP is applicable in quality control for validation of analytical Method (AMV). 3.0 Responsibility : To prepare the protocol and report of validation of analytical Method (AMV) and to follow the procedure during validation of analytical procedure. To review the protocol and report of validation of analytical Method (AMV). To approve the protocol and report of validation of analytical Method (AMV). Approval of the protocol and report of validation of analytical Method (AMV). 4.0 Procedure for Analytical Method Validation (AMV): Validation of analytical Method (AMV) is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications through certain standards of accuracy and reliability. Prepare validation protocol as per Annexure-I and get it approved it from authorize persons. Before starting the analytical Method validation (AMV) experiments, system suitability shall be done to determine the suitability of the chromatographic system for the analysis as per the individual method. If required theoretical plates, capacity factor, resolution factor and tailing factor shall also be calculated. When the system meets the system suitability parameters mentioned in the method, validation experiments shall be started. Bracketing standards shall be injected throughout the validation. The mean, standard deviation and relative standard deviation shall be determined as per the individual method system suitability criteria. System precision Method precision (Repeatability) Ruggedness (Intermediate Precision) Limit of Quantitation (LOQ) Filter paper selection study System suitability test (SST) The system precision is the closeness of agreement between the responses of detector. Usually expressed as the standard deviation (SD) or the relative standard deviation (RSD). Inject separately diluent (blank), standard solution (six replicates) and record the area / absorbance responses of main analyte peak/absorbance in standard solution. Calculate the % RSD of responses and retention time of main analyte peak for six replicates. For dissolution and assay, relative standard deviation for values (response) is not more than 2% and relative standard deviation for retention time is not more than 1.0%. Related substances, relative standard deviation for peak area counts is not more than 5% and relative standard deviation for retention time is not more than 1.0%. Residual solvents, relative standard deviation for peak area counts is not more than 10%. Note: Acceptance criteria can be varied depending up on the requirement of method. The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of homogeneous sample. Usually expressed as the standard deviation or the relative standard deviation. Prepare six samples of single batch and analyze as per the test Method. Calculate the % RSD for six samples. Prepare six individual sample of same batch and analyze as per test method. In case any known impurities are not present in the specimen samples. The method precision can be done by spiking the known impurities at specification level (if impurities available). Calculate the %RSD of impurities for six samples. Note (Dissolution and Assay): If multiple strengths are available methods precision can be performed on higher strength. If multiple strengths are available method precision should be performed on all strength. For dissolution method, relative standard deviation (RSD) of the six analysis shall be not more than 5% for release above 85% and not more than 10% for release below 85%. For assay method, relative standard deviation (RSD) of the six analysis shall not be more than 2%. Incase, known impurities in related substances method, relative standard deviation of six analysis shall not be more than 10 % for the impurity level greater than 1.0% and relative standard deviation shall not be more than 15% for the impurity level between 0.11% and 0.99%. This criterion is applicable for known impurities above LOQ level and for unknown impurities more than 0.1%. For known residual solvents in residual solvents method, relative standard deviation of six analysis shall be not more than 10%. Note: Acceptance criteria can be varied depending up on the requirement of method. Intermediate precision expresses within laboratory variation with different analysts of equipment within the same laboratory using same lot of drug product as specified under precision. The analysis of the same batch shall be done in six replicate analysis by using columns of the same make having different serial numbers by different analysts, by different systems on different day. The mean, standard deviation and relative standard deviation shall be calculated. For dose proportional and for pseudo proportional follow the same procedure as per method precision. Relative standard deviation (RSD) of six analysis shall be not more than 5% for release above 85%, not more than 10% for release below 85% and relative standard deviation for retention time of main analyte in standard solution should not be more than 1.0%. Overall relative standard deviation shall be not more than 5% for release above 85% and not more than 10% for release below 85% when compared with method precision results. Relative standard deviation (RSD) of the six analysis shall be not more than 2% and relative standard deviation for retention time of main analyte in standard solution should not be more than 1.0%. Overall relative standard deviation shall be not more than 2% when compared with method precision results. Relative standard deviation (RSD) of the six analysis shall be not more than 10% for the impurity level greater than 1.0% and relative standard deviation shall not be more than 15% for the impurity level between 0.11% and 0.99%. Overall relative standard deviation shall be not more than 10% for the impurity level greater than 1.0% and relative standard deviation shall not be more than 15% for the impurity level between 0.11% and 0.99% when compared with method precision results. These criteria applicable for known impurities above LOQ level and for unknown impurities more than 0.1%. Relative standard deviation (RSD) of the six analysis shall be not more than 10% and overall relative standard deviation shall be not more than 10% when compared with method precision results. Note: Acceptance criteria may be varied depending up on the requirement of method with justification. Specificity of analytical method is its ability to assess unequivocally the analyte in presence of components that may be expected to be present in the sample matrix. For assay, inject blank (diluent), placebo, identification solution (one or more active components), standard, sample solution and spike sample. Check the peak purity of main peak. For dissolution by HPLC inject blank (Dissolution media/diluent), placebo and for dissolution by UV, scan the blank, placebo solution. For related substances inject blank (diluent) and placebo in duplicate (for drug products). Prepare one sample solution spiked with known impurities at specification level, inject and check the peak purity of main peak and known impurities. Inject known impurities individually. Note: The sample can be diluted for peak purity. For residual solvents, inject blank (diluent) and placebo in duplicate (for drug products). Inject the sample solution spiked with known residual solvents at specification level and known residual solvents individually. Note: In case of multiple strengths, placebo solution can be prepared wherever placebo concentration is high. No peaks shall be eluted at the retention time of the main peak in the blank and placebo solution. Peak purity of main peak shall be passed. All the peak of blank, placebo and main analyte shall be well separated from each other in spiked sample solution. No peaks shall be eluted at the retention time of main peak in the blank (diluent) and placebo absorbance. The interference from blank and placebo shall not be more than 2% of standard absorbance. No peaks shall be eluted at the retention time of main peak and known impurities in the blank (diluent) and placebo solution. Peak purity of main peak and unknown impurities shall be passed. Observe the elution of pattern of peak elution in blank and placebo solution. Note: Acceptance criteria can be varied depending up on the requirement of method. For a stability indicating method, it is essential to perform forced degradation studies by applying appropriate accelerated stress conditions to the sample. Photolytic (Sunlight and ultraviolet light for 2-4 hours) Thermal (At 105°C or below melting point) Preparation of Humidity Chamber Prepare super saturated solution of Potassium Nitrate and keep this solution in a well closed Desiccator. Keep the sample and Placebo in the chamber. If the drug product is in dose proportionate then forced degradation study will be performed on higher strength only, but if the drug product is not in dose proportionate then force degradation study will be performed based on qualitative composition of the drug product. For assay, prepare the untreated sample solution and treated samples solution (heat, acid, base, hydrolysis, humidity and oxidation). Inject and calculate the %assay. Calculate the %difference between the untreated sample and treated samples results. Check the peak purity of main peak. For related substances, prepare the untreated sample solution and treated samples solution (light, heat, acid, base, hydrolysis and oxidation). Inject and calculate the % impurities. Calculate the %difference between the untreated sample and treated samples results. Check the peak purity of main peak. Known impurities are spiked wherever necessary. Note: For peak purity, stress sample shall be diluted, wherever required. The treated sample results shall be compared with untreated

sample and the difference shall be not more than 15%. Peak purity of main peak shall be passed. Degrade to the extent up to 15% is performed to evaluate the interference. Peak purity for main peak shall be required. Mass Balance determination: Mass balance has to be verified by demonstrating that the decrease the concentration of the substance exposed to stress conditions corresponds to an equivalent increased amount of degradation products. For all degraded samples, both assay and related substances have to be tested. If for some reason the official assay test cannot be performed, the decrease of the main peak signal in the related substances test could be evaluated and to justify mass balance. The following data should be reported both for stressed and unstressed samples (control). Amount of individual degraded substances found, total amount of degraded substances found, assay and some of assay and degraded substances. An example is provided in the below table: Impurity 1 (% w/w) Impurity 2 (% w/w) Total (% w/w) Assay Total Impurities + Assay Control 0.1 0.1 0.2 99.8 100.0 Stressed Sample 5.0 4.0 9.0 90.5 99.5 The mass balance should be in the range of 95 % – 105 % of the control sample. However, if the decrease in the assay value due to degradation is less than 5%, tighter criteria may be more appropriate. Mass balance is confirmed when the concentration of the parent drug found in the stressed sample is consistent with the amount of degraded compounds formed after the stress test. The following examples are intended to represent potential real case scenarios: Impurity 1 (% w/w) Total (% w/w) Assay Total Impurities + Assay Comment Control 0.1 0.2 99.8 100.0 Stressed sample 1 8.0 9.0 90.5 99.5 Good mass balance Stressed Sample 2 0.1 0.3 55.0 55.3 No Mass Balance (Some degradation products are not detected) Stressed sample 3 10.0 15.0 95 110 No Mass Balance (Some degradation products are overestimated) Mass balance cannot be accurately evaluated during early method development. However, the balance may be a useful tool to ensure that there is no significant degradation products unaccounted. In case mass balance is not achieved, the degradation should be scientifically evaluated and justified. Its ability (within a given range) to obtain test results which are directly proportional to the concentration levels shall be prepared. For assay, prepare and inject the standard solution in the range of 80% to 120% concentration level and Calculate the correlation coefficient “r” by calculation of a regression line by the least square method. Also determine the Residual sum of square. For content uniformity, prepare and inject the standard solution in the range of 70% to 130% concentration level and Calculate the correlation coefficient “r” by calculation of a regression line by the least square method. Also determine the Residual sum of squares. For dissolution testing, ± 20% over the specified range (e.g., if the acceptance criteria for a controlled- release product cover a region from 30% , after 1 hour, and up to 90%, after 24 hours, the validated range would be 10% to 110% of the label claim. For related substances, prepare and inject the known impurities solution and standard solution in the range of LOQ to 200% concentration level calculate the correlation coefficient “r” by calculation of a regression line by the least square method and calculate the response factor for known impurities by using the below given formula: $\text{Slope of active substance}$ Response Factor $= \frac{\text{Slope of impurity}}{\text{Slope of impurity}}$ Relative Response Factor (RRF) $= \frac{\text{Slope of active substance}}{\text{Slope of active substance}}$ Also determine residual sum of squares For residual solvents, prepare a solution of known residual solvents and standard solution in the range of LOQ to 150% concentration level, Inject and calculate the correlation coefficient “r” by calculation of a regression line by the least square method. Also determine the residual sum of squares. The value of correlation coefficient “r” for dissolution and assay shall be not less than 0.999, for related substances and residual solvents not less than 0.99. Note: Acceptance criteria can be varied depending up on the requirement of method. The range of an analytical Method is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical Method has a suitable level of precision, accuracy and linearity. The range is normally expressed in the same units as test results (e.g. percent, parts per million) obtained by the analytical method. For assay, prepare the sample solution by spiking the drug substance to the placebo at about 70%, 100% and 130% of test concentration level in triplicate in each level and calculate the RSD for recovery obtained at each level separately and overall RSD. Incase, Dissolution prepare the sample solution by spiking the drug substance to the placebo at about ± 20% specified range in triplicate in each level and calculate the % overall average recovery. For related substances, prepare the sample solution without spiking known impurities in triplicates and inject, prepare the sample solution in triplicate by spiking with known impurities at LOQ level to 150% of specification limit (as per shelf life specification limit) and calculate the % overall average recovery for known impurities. For residual solvents, prepare the sample solution without spiking known residual solvents in triplicate and inject, Prepare the sample solution in triplicate by spiking with known residual solvents at LOQ level to 150% of specification limit and calculate the % overall average recovery for known residual solvents. Note: Accuracy experiment for API to be inferred from the experiment data of specificity, linearity and precision. % recovery of the drug at each level shall be between 98% and 102% and RSD shall be not more than 2%. The overall average shall be between 98% to 102% with RSD of not more than 2%. % recovery of the drug at each spiking level shall be between 95% and105% and RSD shall be not more than 5%. The overall average recovery shall be between 95% to 105% with RSD of not more than 5%. Note: For less soluble drugs “In cases of poor drug solubility, if feasible, the stock solution may be prepared by dissolving the drug substance in a small amount of organic solvent and diluting to the final concentration with diluent”. The recovery of known impurities of the drug at each spiking level shall be between 85% and 115% and RSD from replicate analysis shall be not more than 10%. The overall average recovery shall be between 85% and 115% with RSD of not more than 10%. For residual solvents, the recovery of known residual solvents of the drug at each spiking level shall be between 85% and 115% and RSD from replicate analysis shall be not more than 10%. The overall average recovery shall be between 85% and 115% with RSD of not more than 10%. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantitate under the stated experimental conditions. The limit of detection is usually expressed as the concentration of analyte e.g. percentage, ppm, ppb etc. Based on Signal-to-Noise Ratio Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio 3:1 is acceptable for estimating the detection limit. Based on the Standard Deviation of the Response and the Slope For related substances and residual solvents prepare and inject the known impurities solutions and standard solution in the range of LOD to 200% of specification level and calculate the limit of detection by using below formula. $\text{LOD} = \frac{3.3 \times \sigma}{S}$ Where, σ = the standard deviation of the response. S = the slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte. The estimation of σ may be carried out based on the calibration curve. A specific calibration curve shall be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression line may be used as the standard deviation (σ). Prepare the LOD solution using blank/placebo spiked with known impurities or known residual solvents at determined LOD level and inject in six replicates. Calculate % RSD for six replicates of known impurities. % RSD for six replicates responses of known impurities or known residual solvent shall be not more than 30. It is the lowest concentration of analyte in a sample that can be quantitate with acceptable precision under the stated experimental condition. The limit of quantification is usually expressed as the concentration of analyte. e.g. percentage, ppm , ppb etc. Based on Signal-to-Noise Ratio Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1. For related substances and residual solvents prepare and inject the known impurities solutions and standard solution in the range of LOQ to 200% specification level calculate the limit of quantification by using below formula add perform the precision at LOQ analysis and calculate the %RSD. Calculate the limit of Quantitation by below formula: $\text{LOQ} = \frac{10 \times \sigma}{S}$ Where, σ = the standard deviation of the response. S = the slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte. The estimation of σ may be carried out based on the calibration curve. A specific calibration curve shall be studied using samples containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression line may be used as the standard deviation (σ). Prepare the LOQ solution using blank/placebo spiked with known impurities or known residual solvents at determined LOQ level and inject in six replicates. Calculate % RSD for six replicates of known impurities. % RSD for six replicates responses of known impurities or known residual solvent shall be not more than 10. Note: Acceptance criteria can be varied depending up on the requirement of method. It is essential when validating an analytical method to confirm that the analyte has adequate stability in both the standard and sample solution during analytical measurement stages of the testing. For dissolution, prepare the standard solution and perform the dissolution on one tablet or capsule as per the test method. Analyze the standard solution and sample solution at the different time intervals and calculate the % difference for the result. For assay, prepare the standard solution and sample solution as per the test method, Analyze the standard solution at the different time intervals and calculate the % difference for the result. For related substances, prepare the standard solution and sample solution spiked with known impurities at the specification level as per the test method. Analyze the standard solution and sample solution at the different time intervals and calculate the % cumulative RSD of peak area for known impurities and main peak. For dissolution, the difference in results shall not be more than 2. Incase assay, the difference in results shall not be more than 2 for drug products and shall not be more than 1 for drug substance. For related substances, Cumulative % RSD of peak area for known impurities and main peak shall not be more than 10. Note: Acceptance criteria can be varied depending up on the requirement of method. If acceptance criteria are not met then a time limit is set within which the analysis is to be completed. This assumes that the precaution have been taken to minimize degradation. For analyses where a small degree of degradation in unavoidable, automation of the assay ensures that exactly the same degree of degradation occurs with each sample and standard. The method shall show reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions, mention the same in test method. Under the variable conditions in method parameters and system suitability parameters shall be established to ensure that the validity of the analytical Method and the conditions shall be suitability controlled or a precautionary statement shall be included in the procedure. Following deliberate variations shall be done in case of dissolution, assay and related substances: pH of mobile phase (± 0.2 units) Column oven temperature (± 5°C) Mobile phase composition (organic composition) (up to ±10% absolute) RPM (for dissolution) (±5RPM) System suitability parameters shall be performed as per the test method for each deliberate variation. For dissolution, prepare the standard solution and perform the dissolution on three tablets or capsules by deliberate variations made in the method for each condition as mentioned in protocol and analyze. Calculate the results and compare with method precision results. For assay, prepare the standard solution and sample solution in triplicate as per the test method by deliberate variations made in the method for each condition as mentioned in protocol and analyze. Calculate the RRT’s for known impurities and compare with RRT’s from specificity experiment. For Related substances, prepare the standard solution and sample solution spiked with known impurities at the specification level as per the test method by deliberate variations made in the method for each condition as mentioned in protocol and analyze. Calculate the RRT’s for known impurities and compare with RRT’s from specificity experiment. For residual solvent, prepare the standard solution and sample solution spiked with known residual solvents at the specification level per the test method by deliberate variation made in the method for each condition as mentioned in protocol and analyze. Calculate the RRT for known residual solvents and compare with RRT from specificity experiment. For dissolution, overall % RSD shall be not more than 5 with of the method precision data for individual experiments. For assay, overall % RSD shall be not more than 2 with of the method precision data for individual experiments. Related substance and residual solvent, method shall meet the system suitability criteria. To meet acceptance criteria, robustness parameters can be changed as per method requirement. For Assay/Dissolution/UOD: Prepare sample solution in triplicate as per test method. A portion of sample solution shall be centrifuged and other portion of sample solution shall be filtered with filters (e.g. PVDF/Nylon). Inject all the samples into HPLC as per method. Calculate the % Assay or % drug release difference for each sample and calculate the % difference between centrifuged vs. filtered samples. Prepare sample solution (spiked sample solution). A portion of sample solution shall be centrifuged and other portion of sample solution shall be filtered with filters (e.g. PVDF/Nylon). Inject all the samples into HPLC as per method. Calculate the % impurity for each sample and calculate the % impurity difference between centrifuged vs. filtered samples. Difference shall not be more than 2 % for Assay. Difference shall not be more than 5% for dissolution. If the impurity is less than 0.1%, no comparison shall be made. For known impurities and total impurities % difference shall be less than ±10%. System suitability tests are based on concept that the equipment, electronics, analytical operations and sample to be analyzed, Test of System suitability provide the added assurance that on specific occasion the method is given accurate and precise results. System suitability test shall be run before an experiment is initiated and whenever there is change in the environment analysis. The nature of the test and acceptance criteria shall be based upon the data generated during method development, optimization and validation experiments. System suitability parameters, which are generally required to be monitored including number of theoretical plates (efficiency of the column), internal precision (repeatability), reproducibility, tailing factor (peak asymmetry), resolution, relative retention time and capacity factor depending upon the requirement of analytical method being validated. Some other parameters may be included in system suitability test, shall be justified in validation report. Each experiment conducted as part of method validation exercise should have a corresponding system suitability test. After completion of validation experiment as part of protocol, prepare the report for the same as per annexure II. Summary – Analytical Method Validation (AMV): Analytical Performance Characteristics Assay Related Substances Dissolution Residual Solvents Accuracy √ √ * √ Precision Applicable √ Applicable √ Specificity √ √ * Applicable Linearity Applicable √ * √ Robustness √ Applicable * √ Forced Degradation √ √ – – Detection Limit – √ – √ Quantitation Limit – Applicable – √ Solution Stability √ √ * – √: Applicable – : Not applicable *: Required, depending on the nature of the specific test. If analytical method of dissolution is by HPLC, precision shall be done. If analytical method of dissolution is by UV, accuracy, precision, specificity, linearity, robustness and solution stability shall be covered. Note: The above states will be protocol bound with respective projects. Compendial methods are accepted as the official method. However suitability of the method shall be checked from in-house product under actual conditions of use. General tests and assays, which are already established, may also be validated to verify their accuracy when used for new drug products. Revalidation may be required when there are changes in the drug product e.g. new source or synthesis of drug substance, product strength, new excipients, change in level of excipients, change in manufacturing process etc. Evaluate the parameters of the validation depending on the change. Revalidation of the method is required whenever significant changes in method are done, sample solution, chromatographic system components and chromatographic conditions. The parameters of the validation shall be evaluated depending on the change. Verification of validated analytical method shall be done at-least once in three years as per approved protocol. The verification process for compendial test procedures is the assessment of whether the procedure can be used for its intended purpose, under the actual conditions of use for a specified drugs substance and/or drug product matrix. Compendial analytical Method are not required to validate these procedures, when first used in their laboratories, but documented evidence of suitability should be established under actual conditions of use. In the United States, these requirements is established in 21CFR 211.194(9) (2) of the current GMP regulations which state that the “Suitability of all testing methods used shall be verified under actual conditions of use”. Complete validation of a compendial method is not required to verify the suitability of a procedure under actual condition of use. Analytical performance characteristics used in method verification of pharmacopoeial methods shall be (but not limited to) Specificity, Precision, Linearity and Range, System Suitability and Stability in Analytical Solutions. Annexure-I and Annexure-II shall be used for the preparation of method verification protocol and report. Terminology “Validation” shall be replaced with “Verification” 5.0 REFERENCES : ICH guidelines – Q2 (R1) “Validation of Analytical Procedure : Text And Methodology” USP 38 chapter validation of compendial procedures. USP 38 chapter verification of compendial procedures. 6.0 GLOSSARY : SOP : Standard Operating Procedure AD : Analytical Development R & D : Research and development LOQ : Limit of Quantitation RSD : Relative standard deviation RPM : Rotation per minute LOD : Limit of detection SST : System suitability test SD : Standard Deviation 7.0 ANNEXURES: Annexure I: Template for Preparation of Analytical Method Validation (AMV) Protocol Sr. No. Subject Page No. 1. Protocol Approval 2. Objective 3. Scope 4. Responsibility of validation team 5. Product profile 6. Methodology 7. Incident/Deviation 8. Summary 9. Revision history Annexure II: Template for Preparation of Analytical Method Validation (AMV) Report APPROVAL: Prepared By: Functional Area Name Designation Signature/Date Quality Control Reviewed By: Functional Area Name Designation Signature/Date Quality Assurance Head Quality Control Approved By: Functional Area Name Designation Signature/Date Head QA Sr. No. Subject 1 Approval 2 Introduction 3 Objective 4 Summary Table 5 Methodology 6 Equipment & Materials 7 Validated parameters 8 Summary of system suitability 9 Incident/Deviation 10 Final Conclusion 11 Annexure 12 Revision History 1. INTRODUCTION : 2. OBJECTIVE : 3. SUMMARY TABLE : 4. METHODOLOGY : 5. EQUIPMENT & MATERIALS : 6. VALIDATED PARAMETERS : 7. SUMMARY OF SYSTEM SUITABILITY : 8. INCIDENT/DEVIATION : 9. FINAL CONCLUSION : 10. ANNEXURE : 11. REVISION HISTORY June 3, 2020 April 29, 2020 May 16, 2020