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ANALYTICAL METHOD VALIDATION–A REVIEW

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ABSTRACT

Modern analytical techniques employ a range of techniques that vary from simple qualitative chemical test to the use of most sophisticated and expensive computer controlled instruments. This instrumentation provides the lower limit of detection required to assure the safety. In pharmaceutical industry the chemical composition of raw material, intermediates and finished products quality need to be monitored to ensure satisfactory consistency. Modern analytical techniques employ a range of techniques that vary from simple qualitative chemical test to the use of most sophisticated and expensive computer controlled instruments. The process of analytical method validation should demonstrate that the method is fit for its purpose. The validation should follow a plan that includes the scope of the method, the method performance characteristics and acceptance limits.

KEYWORDS: Robustness, Ruggedness, Gravimetric, Thermogravimetric.

INTRODUCTION[1,7]

Analytical chemistry is basically concerned with the determination of the chemical composition of matter however, identification of substance, the elucidation of its structure and quantitative analysis of its composition are the aspects covered by modern analytical techniques. The qualitative and quantitative analysis can be done by various analytical methods. Various analytical techniques can be revised and some of them give accurate result, example Spectrophotometric method. Modern analytical techniques employ a range of techniques that vary from simple qualitative chemical test to the use of most sophisticated and expensive computer controlled instruments. Analytical instrumentation plays an important role in the production and evaluation of new products. This instrumentation provides the lower limit of detection required to assure the safety. In pharmaceutical industry the chemical composition of raw material, intermediates and finished products quality need to

be monitored to ensure satisfactory consistency. A drug may be defined as a substance meant for diagnosis, cure, mitigation, prevention or altering any structure or any function of the body or animals. The quality of a drug is the degree of possession of all those characteristics designed and manufactured into it, which contributes in the performance of the intended function, when the drug is used as directed.

STAGES INVOLVED IN ANALYTICAL PROCEDURES

Definition of the problem: Analytical information and level of accuracy required, Cost, timing, availability of laboratory instruments and facilities.

1. Choice of technique and method: Selection of the best technique for the required analysis such as chromatography, infrared spectrometry, titrimetry, thermogravimetry.
2. Sampling: Selection of a small sample of the material to be analyzed. Where this is heterogeneous, special procedures need to be used to ensure that a genuinely reprenstative sample is obtained.
3. Sample pre-treatment or conditioning: Conversion of the sample in to a form suitable for detecting or measuring the level of the analyte (s) by the selected method. This may involve dissolving it, converting the analyte(s) in to a specific chemical form or separating the analyte from other components of the sample that could interfere with the detection.
4. Qualitative analysis: Tests on the sample under specified and controlled conditions. Tests on reference materials for comparison. Interpretation of the tests.
5. Quantitative analysis: Preparations of the standards containing known amount of the analyte(s) or pure reagents to be reacted with the analyte. Measurement of the instrumental response to each sample under the same condition as for the standard. All measurements must be replicated to improve the reliability of the data.
6. Preparation of report or certificate of analysis: This should include the summary of the analytical procedure, the results and their statistical assessment and details of any problems encountered at any stage of analysis.
7. Review of the original sample: The results need to be discussed with regard to their significance and their relevance in solving the original problem.

SELECTION OF APPROPRIATE ANALYTICAL METHOD

1. The purpose of the analysis, the required timescale and any cost constraints.
2. The level of analyte expected and the detection limit required.

3. The nature of the sample, the amount available and the necessary sample preparation procedure.
4. The accuracy required for a quantitative analysis.
5. The availability of reference materials, standards, chemicals, solvents, instrumentation and any special facilities.
6. Possible interference with the detection or quantitative measurement of the analyte and possible need for sample clean up to avoid matrix interference.
7. The degree of selectivity available-methods may be selective for a small number of analytes or specific for only one.
8. Quality control and safety factors.

The results of all types of analysis can be distinguished in two ways

QUALITATIVE ANALYSIS

To refer identity of the product, i.e., it yields useful clues from which the molecular or atomic species, the structural features or the functional groups in the sample can be identified.

QUANTITATIVE ANALYSIS

To refer the purity of the product, i.e., the results are in the form of numerical data corresponding to the concentration of analytes. In both the analysis, the required information is obtained by measuring a physical property that is characteristically related to the component of interest (the analyte). This instrumentation provides the lower limit of detection required to assure the safety. In pharmaceutical industry the chemical composition of raw material, intermediates and finished products quality need to be monitored to ensure satisfactory consistency.

ANALYTICAL TECHNIQUES

TECHNIQUES	**APPLICATIONS**
SPECTROSCOPY	
Atomic Absorption And Emission Spectroscopy**(AAS/AES)**	To analyse alkali and alkaline earth metals in dilute solution, natural liquids, and extracts at trace levels.
Ultraviolet-Visible Spectroscopy **(UV/Vis)**	To analyse molecular (organic) and ionic species capable of absorbing at UV or Visible wavelengths in dilute solutions.
Fourier Transform Infrared Spectroscopy **(FT-IR)**	To analyse only molecular compounds (organic compounds, natural products, polymers, etc.).
Fourier Transform Raman Spectroscopy **(FT-Raman)**	To analyse molecular (organic) compounds which are not responding well in the IR region and hence, it is an alternate to IR.
Nuclear Magnetic Resonance	To identify and characterize the organic and inorganic

Spectroscopy **(NMR)**	compounds.
Microwave Spectroscopy	To analyse simple gaseous molecules in Far IR region, to study their stereo chemistry.
Electron Spin Resonance Spectroscopy **(ESR)**	To study the formation and life time of the free radicals formed in organic reactions and also finds applications in biological works.
Molecular Fluorescence Spectroscopy	To study the molecular and ionic compounds in dilute solutions capable of giving fluorescence, fiinds applications in vitamin analysis.
CHROMATOGRAPHY	
High Performance Liquid Chromatography **(HPLC)**	To separate and analyse complex mixtures or solutions which include liquids and solids of both organic and inorganic origins.
Gas Chromatography **(GC)**	To separate and analyse mixtures of volatile organic compounds, solvent extracts and gases.
THERMAL ANALYSIS	
Thermo gravimetric Analysis **(TGA)**	To study the mass changes of materials like polymers, glasses, ceramics, etc., such as evaporation, decomposition gas absorption, de-sorption, dehydration, etc.
Thermo mechanical Analysis **(TMA)**	To study the expansion coefficient of composite and laminate materials.
Differential Thermal Analysis **(DTA)**	To study the exothermic and endothermic behavior of clay materials, ceramics, ores, etc.
Differential Scanning Calorimetric **(DSC)**	To study the glass transition temperature, curing process of the thermo set polymers and heat of melting of thermoplastic polymers.
X-RAY TECHNIQUE	
X-Ray Fluorescence **(XRF)** Spectrometry and X-Ray Photoemission Spectrometry **(XPS)**	To identify the elements and their valence states present in the surface of the materials.
X-Ray Diffractometry **(XRD)**	To study the crystalline properties of solid substances.
MICROSCOPY	
Scanning Electron Microscopy **(SEM)**	To study the topography, electronic structure and compositions of metals, ceramics, polymers, composites and biological materials.
Transmission Electron Microscopy **(TEM)**	To study the local structures, morphology, and dispersion of multicomponent polymers, cross sections and crystallizations of metallic alloys, semiconductors, microstructure of composites, etc.
Scanning Probe Microscopy **(SPM)**	To study the hardness and topography of materials like ceramics, polymers, composites, etc., on a nano-scale range.
ELECTRO-CHEMICAL TECHNIQUES	
Polarography	To study and determine metals, metal complexes and organic compounds in trace levels.
Capillary Electrophoresis **(CE)**	To study and characterize biologically active compounds like proteins, amino acids and other bio-molecules.
MISCELLANEOUS TECHNIQUES	
Total Organic Carbon	To monitor pollutants in environmental studies by

Analyzer **(TOC)**	determining the carbon contents of the trace compounds.
Elemental Analyzer **(CHN/S)**	To estimate percentage compositions of elements like carbon, hydrogen, nitrogen and sulphur present in newly synthesized organic compounds, pharmaceuticals, etc.
Polarimetry	To analyse and quantitative optically active compounds like sugar.
Circular Dichroism **(CD)** and Optical Rotatory Dispersion **(ORD)**	To get the structural information of optically active Compounds like, amino acids, proteins, etc.
Vibrational Circular Dichroism **(VCD)** and Vibrational Linear Dichroism **(VLD)**	Same as above but in the IR region. VLD measurement is employed to study the molecular orientations of thin polymer films.
Mass Spectrometry **(MS)**	To identify the organic compounds. Often used as detectors with HPLC and GC.
Laser Light Scattering System **(LLIS)**	In the study of macromolecules like polymers, gels, proteins,etc., for determining molecular mass & size and their associations.

ANALYTICAL PARAMETERS USED IN ASSAY VALIDATION AS PER ICH GUIDELINES[8-12]

The process of analytical method validation should demonstrate that the method is fit for its purpose. The validation should follow a plan that includes the scope of the method, the method performance characteristics and acceptance limits. Parameters usually examined in the validation process are limits of detection and quantification, accuracy, precision, selectivity/specificity, linearity, range and ruggedness. A validation report should be generated with all experimental conditions and the complete statistics. If standard methods are used, it should be verified that the scope of the method and validation data, for example, sample matrix, linearity, range and detection limits Comply with the laboratory (s) analyses requirements, otherwise the validation of the standard method should be repeated using the laboratory(s) own.

KEY PARAMETERS OF THE ANALYTICAL METHOD VALIDATION

It is important for one to understand the parameters or characteristics involved in the validation process. The various performance parameters, which are addressed in a validation exercise, are grouped as follows.

(1) Accuracy

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. Accuracy may often express as percent recovery by the assay of a known amount of analyte added. Accuracy may be determined by applying the method to samples or mixtures of excipients to which known amount of analyte have been added both above and below the normal levels expected in the samples. Accuracy is then calculated from the test results as the percentage of the analyte recovered by the assay. Dosage form assays commonly provide accuracy within 3-5 % of the true value. The ICH documents recommend that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e. three concentrations and three replicated of each concentration).

(2) Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. This is usually expressed as the standard deviation or the relative standard deviation (coefficient of variation). Precision is a measure of the degree of reproducibility or of the repeatability of the analytical method under normal operating circumstances. Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study, different occasions, different laboratories, different batch of reagents, different analysts and different equipments.

Determination of Repeatability

Repeatability can be defined as the precision of the procedure when repeated by same analyst under the same operating conditions (same reagents, equipments, settings and laboratory) over a short interval of time. It is normally expected that at least six replicates be carried out and a table showing each individual result provided from which the mean, standard deviation and co-efficient of variation should be calculated for set of n value. The RSD values are important for showing degree of variation expected when the analytical procedure is repeated several time in a standard situation. (RSD below 1% for built drugs, RSD below 2 % for assays in finished product).

Determination of reproducibility

Reproducibility means the precision of the procedure when it is carried out under different conditions-usually in different laboratories-on separate, putatively identical samples taken from the same homogenous batch of material. Comparisons of results obtained by different analysts, by the use of different equipments, or by carrying out the analysis at different times can also provide valuable information.

(3) Linearity and range

The linearity of an analytical method is its ability to elicit test results that are directly (or by a well defined mathematical transformation) proportional to the analyte concentration in samples within a given range. Linearity usually expressed in terms of the variance around the slope of regression line Calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analytes. The linear range of delectability that obeys Beer's law is dependent on the compound analyzed and the detector used. The working sample concentration and samples tested for accuracy should be in the linear range. The claim that the method is linear is to be justified with additional mention of zero intercept by processing data by linear least square regression. Data is processed by linear least square regression declaring the regression co-efficient and b of the linear equation y = ax + b together with the correlation coefficient of determination r^2. For the method to be linear the r^2 value should be close to1. The range of an analytical method is the interval between the upper and lower levels of the analyte (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written.

(4) Limit of Detection and limit of Quantitation

Limit of detection (LOD)

The limit of detection is the parameter of limit tests. It is the lowest level of analytes that can be detected, but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions. The limit test thus merely substantiates that the analytes concentration is above or below a certain level. The determination of the limit of detection of instrumental procedures is carried out by determining the signal-to-noise ratio by comparing test results from the samples with known concentration of analytes with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted. The signal-to-noise ratio is determined by dividing the base peak by the standard deviation of all data points below a

set threshold. Limit of detection is calculated by taking the concentration of the peak of interest divided by three times the signal-to-noise ratio. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (S_a) which may be related to LOD and the slope of the calibration curve, b, by

LOD = 3 S_a / b

Limit of quantification (LOQ)

Limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices such as impurities in bulk drugs and degradation products in finished pharmaceuticals. The limit of quantitation is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied. It is measured by analyzing samples containing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision are attainable. The standard deviation multiplied by a factor (usually 10) provides an estimate of the limit of quantitation. In many cases, the limit of quantitation is approximately twice the limit of detection.

LOQ = 10 S_a / b

(5) Selectivity and Specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. Selectivity may be expressed in terms of the bias of the assay results obtained when the procedure is applied to the analytes in the presence of expected levels of other components, compared the results obtained when the procedure is applied to the analyte in the presence of expected levels of other components, compared to the results obtained on the same analytes without added substances. When the other components are all known and available, selectivity may be determined by comparing the test results obtained on the analytes with and without the addition of the potentially interfering materials.

(6) Robustness and Ruggedness

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its

reliability during normal usage. The determination of robustness requires that methods characteristic are assessed when one or more operating parameter varied.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory. Ruggedness is normally expressed as the lack of the influence on the test results of operational and environmental variables of the analytical method. For the determination of ruggedness, the degree of reproducibility of test result is determined as function of the assay variable. This reproducibility may be compared to the precision of the assay under normal condition to obtain a measure of the ruggedness of the analytical method.

(7) Stability and System suitability tests

Stability of the sample, standard and reagents is required for a reasonable time to generate reproducible and reliable results. For example, 24 hour stability is desired for solutions and reagents that need to be prepared for each analysis. System suitability test provide the added assurance that on a specific occasion the method is giving, accurate and precise results. System suitability test are run every time a method is used either before or during analysis. The results of each system suitability test are compared with defined acceptance criteria and if they pass, the method is deemed satisfactory on that occasion. The nature of the test and the acceptance criteria will be based upon data generated during method development optimization and validation experiments.

Data elements required For Assay Validation

There are various analytical methods used for the examination of pharmaceutical materials. Not all the characteristics referred above will need to be considered in all cases. Analytical methods may be broadly classified as per WHO (World Health Organisation) as follows:

Class A Tests designed to establish identity, whether of bulk drug substances or of a particular ingredient in a finished dosage form.

Class B Methods designed to detect and quantitative impurities in a bulk drug substance or finished dosage form.

Class C Methods used to determine quantitatively the concentration of a bulk drug substance or of a major ingredient in a finished dosage form.

Class D Methods used to assess the characteristic of finished dosage forms, such as dissolution profiles and content uniformity.

SYSTEM SUITABILITYTESTING

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

The parameters that are affected by the changes in chromatographic conditions are,

- Retention (K_A)
- Resolution (R_s)
- Capacity factor ($k^{/}$)
- Selectivity (□)
- Column efficiency (N) and
- Peak asymmetry factor (A_s)

FORCED DEGRADATION STUDIES[8-20]

Forced degradation studies typically involve the exposure of representative samples of drug substance or drug product to the relevant stress conditions of light, heat, humidity, acid / base hydrolysis, and oxidation. Forced degradation plays an important role in the drug development process. It furthers understanding of the chemistry of the drug substance and drug product and facilitates the development of stability indicating analytical methodology. Usually conducted before final formulation, forced degradation uses external stresses to rapidly screen material stabilities.

Longer term storage tests are usually used to measure similar properties when final formulations are involved because of the stringent FDA regulations. These tests are generally more expensive (because of the time involved) than forced degradation which is therefore used for rapid selection and elimination tests.

As stated in ICH Q1A, forced degradation studies can be used as a predictive tool. The initial purpose of these studies is to investigate stability-related properties of an API and understand

the degradation products and pathways of the compound. They are also used to provide samples for the development of stability - indicating analytical methods for the API. The information gleaned from a forced degradation investigation can also be utilized in several other areas of development, including analytical development (methods development), formulation development (formulation choice and storage conditions), manufacturing / processing parameters (synthesis / salt selection of API and manufacture of formulations), safety / toxicological concerns (possible genotoxic degradation products), metabolism (identification of possible metabolites) and discovery (design of better or more stable APIs).

Forced degradation (stress - testing) is distinguished from a formal stability program (stability studies) in ICH Q1A. While accelerated storage is part of stability studies, forced degradation studies are expected to be more strenuous than accelerated conditions (higher temperatures and possibly higher humidity) and are expected to include oxidative and photolytic stress experiments. However, specific conditions for the testing to be done are not mentioned in the guidelines.

Extent of Degradation

The question of how much degradation is sufficient to meet the objectives of stress studies is widely discussed, especially with respect to conventional therapeutics. A degradation level of 10 to 15 % is considered adequate for validation of a chromatographic purity assay. Carr and Wahlich suggest that chromatographic methods for product-related impurities (including degradants) should be validated by spiking experiments within the range of 0 to 20 % if the expected range of impurities is 0 to 10 %. Other authors recommend less than 10 % degradation of active ingredient in the stressed samples. Reynolds and others suggested that DS (Degradation standard) spiked with a mixture of known degradation products can be used to challenge methods employed for monitoring stability of DP(Degradation product).The apparent consensus among pharmaceutical scientists is that samples degraded ~10 % are optimal for use in analytical method validation. These considerations apply to small organic pharmaceuticals for which stability is dictated by the typical pharmaceutical limit of 90 % of label claim.

No such limits for physico - chemical changes, losses of activity, or degradation during shelf life have been established for individual types or groups of biological products. In general, international and national regulations for biological products provide little guidance with respect to stability - related issues. These issues should be considered on a case-by-case basis.

As a group, biological products form a wide variety of product-related degradants under stress conditions. In cases with multiple degradation pathways, it appears to be beneficial to develop multiple product-related variants to challenge the specificity of analytical methods, even when some of degradants may be present at concentrations exceeding 10 %. Do this when accelerated stability studies do not provide clear indication of the degradation pathways. When a stress factor generates only one degradation product, for example higher molecular weight non-covalent aggregates, 10 to 15 % level of aggregation may be sufficient to challenge the specificity of such methods as size exclusion chromatography (SEC) or light scattering.

The forced degradation experiments do not necessarily result in product decomposition. The study can be stopped if no degradation is observed after DS or DP has been exposed to a stress that exceeds conditions of accelerated stability protocol. Protocols for generation of product-related degradation may differ for DS and DP due to differences in matrices and concentrations. For example, sugar additives often present in DP are known to stabilize proteins vis-a-vis denaturing conditions.

Selection of Stress Conditions

Forced degradation is normally carried out under more severe conditions than those used for accelerated studies. The choice of stress conditions should be consistent with the product's decomposition under normal manufacturing, storage, and use conditions which are specific in each case. The ICH guidance recognizes it is impossible to provide strict degradation guidelines and allows certain freedom in selecting stress conditions for biologics. The choice of forced degradation conditions should be based on data from accelerated pharmaceutical studies and sound scientific understanding of the product's decomposition mechanism under typical use conditions. A minimal list of stress factors suggested for forced degradation studies must include acid and base hydrolysis, thermal degradation, photolysis, oxidation and may include freeze-thaw cycles and shear.

Regulatory guidance does not specify pH, temperature ranges, specific oxidizing agents, or conditions to use, the number of freeze-thaw cycles, or specific wavelengths and light intensities. The design of photolysis studies is left to the applicant's discretion although Q1B recommends that the light source should produce combined visible and ultraviolet (UV, 320 - 400 nm) outputs, and that exposure levels should be justified. Consult the appropriate regulatory authorities on a case-by-case basis to determine guidance for light-induced stress.

Degradation products arising in significant amounts during manufacture and storage should be identified, tested for, and monitored against appropriately established acceptance criteria. Examination of some degradation products generated under stress conditions may not be necessary for certain degradants if it has been demonstrated they are not formed under accelerated or long-term storage conditions.

Design your forced degradation studies to be part of impurity characterization. When identification of the impurity is not feasible, incorporate the description of unsuccessful experiments (including those conducted in stress testing studies) in the text of the application. The most frequently encountered protein variants include truncated fragments, deamidated, oxidized, isomerized, aggregated forms, and mismatched disulfide links.

Degradation pathways for proteins can be separated into two distinct classes involving chemical instability and physical instability. Chemical instability is any process that yields a new chemical entity including modification of the protein (via individual amino acid alteration), covalent bond formation, or cleavage. Physical instability refers to changes in the higher order structures (secondary and above). Non-covalent aggregation usually results from partial or full unfolding, which enhances hydrophobic interactions between protein molecules. It may also lead to denaturation, adsorption to surfaces, and precipitation. Aggregation presents a significant patient risk because protein aggregates are frequently immunogenic, therefore analytical methods employed in stability testing should detect low concentrations of aggregates. Stress the placebo in parallel with DP as a control for excipients' decomposition and to monitor the decomposition's effect on degradation pathways of active ingredients. Potential degradation pathways are extensively researched, and methods for their detection are well established.

Analytical Methods

The manufacturer should propose stability-indicating methodologies that provide assurance that changes in the identity, purity, and potency of the product will be detected. The selection of tests is product specific. Stability-indicating methods will characterize potency, purity, and biological activity. As examples, stability indicating methods may include electrophoresis, immunoelectrophoresis, Western blot, isoelec-trofocusing), high-resolution chromatography (e.g., reversed phase chromatography, SEC, gel filtration, ion exchange, and affinity chromatography), and peptide mapping.

The selected set of methods must be able to detect, separate, and quantitate all observed degradation products, although it is recognized that identification and characterization of the appropriate variants may require use of additional analytical methodologies. New analytical technologies and modifications of existing technologies are continuously being developed and should be utilized when appropriate. The list of assays challenged by stressed samples should include analytical methods employed in the stability program and those monitoring impurities.

COMMON STRESSES

There are a number of common stresses which are used to

- pH (acid / base)

  Chemical processes are often catalysed by the presence of acids and bases. The exposure of materials to these can therefore accelerate degradation reactions.
- Temperature

  In accordance to Arrhenius kinetics, increasing temperature increases the rate of any degradation process. Temperature is often used in conjunction with other stresses to increase reaction rates.
- Oxidation
- Concentration
- Light

METHODOLOGIES

Standard methodologies include:

- Wet chemistry methods
- Flow chemistry
- Calorimetry
- HPLC
- Thermogravimetric analysis

CONCLUSION

Analytical methods are necessary for development and validation department of pharmaceuticals. it require rapid development of validation. It is important for quantification of various development process .these are critical part for safe drug. It is necessary for method development of pharmaceutical dosage form. Method validation is a continuous development process. It is necessary for safer pharmaceutical dosage

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