WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 1, 745-768. Review Article ISSN 2277– 7105

A REVIEW ON ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

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Article Received on
13 November 2020,

Revised on 04 Dec. 2020,
Accepted on 25 Dec. 2020

DOI: 10.20959/wjpr20211-19519

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ABSTRACT

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. Describesthe analytical method development, optimization and validation and provide examples of successful method development and validation. In HPLC, the text presant an overview of FDA, ICH regulatory guidelines complian with validation requirements for regulatory agencies, and method validation criteria stipulated by the use pharmacopeia FDA and ICH. The analytical method validation is essential for analytical method development and tested extensively for specificity, linearity, accuracy, precision, range, detection limit, quantization limit, and robustness. Analytical method development and validation allows to confirm that an accurate and reliable potency measurement of a pharmaceutical preparation can be performed.

KEYWORDS: Analytical method development and validation, guidlines, regulatory, accurate and reliable potency measurement.

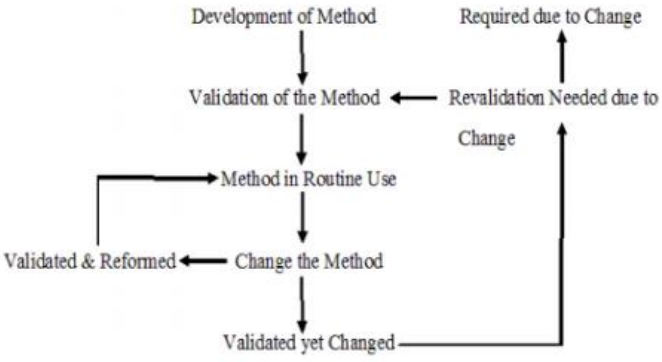
INTRODUCTION

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to

judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. It is the process of defining an analytical requirement, and confirms that the method under consideration has performance capabilities consistent with what the application requires. Use of equipment that is within specification, working correctly and adequately calibrated is fundamental to the method validation process. Likewise the operator carrying out the studies must be competent in the analysis under study and have sufficient knowledge of the method/analysis to draw conclusions from the observations as the validation work proceeds. Quite often method validation evolves from method development and so the two activities are often closely tied, with the validation study employing the techniques and steps in the analysis as defined by the method development. Analytical methods need to be validated or revalidated.

ANALYTICAL METHOD VALIDATION

Validation should now not be implicit one after the other from the development of a technique. therefore whole procedure of analytical method development and validation can be taken into consideration in an entirety as represented inside the general scheme. The method's performance traits should be based totally on the proposed use of the technique. those consist of analyte, its predicted attention, sample matrix, viable inquisitive materials, regulatory requirement, application (qualitative/quantitative), necessity for robustness, detection and quantization restrict, accuracy and precision expectation, distinctive types of system and the places in which the technique can be run, capacity requirements for analyst, and so on. earlier than an device is used to validate a way, its overall performance have to be validated. but nonetheless after technique improvement it wishes to be proven as in step with requirement which gives certain level of confidence for its supposed use To investigate the presence of either pharmacopoeial or non-pharmacopoeial product novel techniques are developed to reduce the value besides time for higher precision and strength.



Analyst before the development of new technologies, do not forget below mention criteria

1. Is this technique possesses the needful sensitivity?
2. Is this method sufficiently selective for direct use without interference by means of the opposite element within the sample?
3. Is the accuracy and precision doable with this technique?
4. Are the reagents and equipment required on this method available or obtained at a reasonable price.
5. Is the time requires to perform this technique applicable.

When Should Methods Be Validated

A method should be validated when it is necessary to verify that its performance parameters are adequate for use for a particular analytical problem. For example: Method just developed. Demonstration of the equivalence between two methods, e.g. a new method and a standard. This ensures that particular validation terminology together with the statistics used is interpreted in a manner consistent within the relevant sector.

Basic Criteria For New Method Development For Drug Analysis

- The drug or drug combination may not be official in any pharmacopoeias.
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations. Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients.
- Analytical methods for the quantitation of the drug in biological fluids may not be available. Analytical methods for a drug in combination with other drugs may not be available.
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliab

Types of Analytical Procedures To Be Validated

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests;

- Quantitative tests for impurities' content;

- Limit tests for the control of impurities;
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Although there are many other analytical procedures, such as dissolution testing for drug products or particle size determination for drug substance, these have not been addressed in the initial text on validation of analytical procedures. Validation of these additional analytical procedures is equally important to those listed here in and may be addressed in subsequent documents.

Table.4. Data elements required for assay validation

Type of analytical procedure / Characteristics	Identification	Testing for impurities: Quantitat.	Testing for impurities: Limit	ASSAY - dissolution (measurement only) - content/potency
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Interm. Precision	-	+ (1)	-	+ (1)
Specificity (2)	+	+	+	+
Detection Limit	-	- (3)	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this characteristic is not normally evaluated

+ signifies that this characteristic is normally evaluated

(1) in cases where reproducibility (see glossary) has been performed, intermediate precision is not needed

(2) lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s) or may be needed in some cases.

A brief description of the types of tests considered in this document is provided below

- Identification tests are intended to ensure the identity of an analyte in a sample.
- This is normally achieved by comparison of a property of the sample (e.g., spectrum, chromatographic behavior, chemical reactivity, etc) to that of a reference standard;
- Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test;

- Assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance. The same validation characteristics may also apply to assays associated with other analytical procedures (e.g., dissolution).

METHOD VALIDATION

Definition

Analytical method validation is "A Documented evidence, which provides a high degree of assurance that a specific process will consistently produce, a product meeting its pre-determined specifications and quality attributes.

STEPS IN METHOD VALIDATION

1) Prepare a validation protocol or operating procedure for the validation.
2) Define the application, scope, and purpose of the method.
3) Define the performance parameters and acceptance criteria.
4) Define validation experiments.
5) Verify relevant performance characteristics of equipment.
6) Qualify materials, e.g. standards and reagents.
7) Perform revalidation experiments.
8) Adjust method parameters or acceptance criteria if necessary.
9) Perform full internal and external validation experiments.
10) Define SOPs for executing the method in routine.
11) Define criteria for re-validation.
12) Define types and frequency of system suitability tests and Analytical Quality Control Checks (AQC) for routine.
13) Document validation experiment and results in the validation.

Strategy For The Validation of Methods

The validity of a specific method should be demonstrated in laboratory experiments using samples or standards that are similar to unknown samples analyzed routinely. The preparation and execution should follow a validation protocol, preferably written in a step-by-step instruction format. This proposed procedure assumes that the instrument has been selected and the method has been developed. It meets criteria such as ease of use; ability to be automated and to be controlled by computer systems; costs per analysis; sample throughput; turnaround time; and environmental, health and safety requirements.

Analytical Method Development

Successful acceptance of the validation parameters and performance criteria, by all parties involved, requires the cooperative efforts of several departments, including analytical development, QC, regulatory affairs and the individuals requiring the analytical data. The operating procedure or the Validation Master Plan (VMP) should clearly define the roles and responsibilities of each department involved in the validation of analytical methods. The scope of the method and its validation criteria should be defined early in the proces Various steps are involved in the development of an analytical method are as follows:

1. Purpose of Analytical Method Development

In the pharmaceutical industries, analytical method development gives very important information on the potency of a drug, the drug's bioavailability, the drug's stability and also its effects. In the very first step, the purpose of conducting any analytical method development is established.[6]

2. Highlighting of Steps

In the second step of Analytical Method Development, the steps involved in the development are recorded in a laboratory book or online database.

3. Analyte standard characterization

In this step, both the biological and chemical properties (such as solubility, optical isomerism, etc.) in addition to the physical properties of the analyte are collected. After that, the standard analyte is equal to 100% purity is obtained and stored according to its specific requirements (refrigerator, desiccators and freezer).When multiple components are to be analysed in the sample matrix, the number of components is noted, data is assembled and the availability of standards for each one is determined. Only those techniques (spectroscopic, MS, GC, HPLC etc.) that are compatible with sample stability are considered.

4. Requirement of The Method

Requirement for the method development of the analysis are done and recorded. All the materials, reagents and instruments are procured that are required for the analysis of the sample. The required LOD, LOQ, Specificity, linearity,range, accuracy and precision are defined.

5. Review of Literature and Existing Methodology

Literature survey and prior methods: All the data of literature related to the drug are reviewed for its physical and chemical properties, manufacturing, solubility and applicable analytical ways with reference to relevant books, journals, United States pharmacopeia/national formulary (USP/NF), association of official agricultural chemists (AOAC) and American society for testing and materials (ASTM) publications. Chemical abstracts service (CAS) automated computerized literature searches are convenient.

6. Choosing An Analytical Method

By using the information obtained from the literature during the literature review, a specific methodology is modified to cater for accurate output and also because methods change with the requirements of the analyte. If there is no previous method in the literature being reviewed regarding the analyte, the procedure goes on uninterrupted.

7. Instrumental Setup and Initial Studies

Required instruments for the analytical methodology development are set up within the laboratory. Installation qualification, operational qualification and performance qualification of instrumentation using laboratory standard operating procedures (SOP's) are verified. They are usually universal and standardized for ease of use in any laboratory set up.

8. Optimization of Method

During optimization one parameter is modified at a time and set of conditions are isolated, rather than using a trial and error approach. Optimization of an analytical method is done in reference to a systematic and procedural plan while making sure to critically follow all the documented steps.

9. Evaluation of Analytical Figures of Merit

Documentation of the analytical figures of benefit set upon is completed. These analytical figures of merit include limit of quantitation (LOQ), limit of detection (LOD), linearity, time per analysis, cost, sample preparation.

10. The Evaluation of Method Development Actual Sample

The specimen solution needs to prompt specific, complete recognition of the peak interest of the medication other than all different matrix part.

10. Estimation of Percent Recovery of Real Samples and Demonstration of Quantitative Sample Analysis

Percentage recovery of spiked, actual standard medication into a sample grid which includes no analyte is evaluated. Optimization to reproducibility of recuperation from test to test must have appeared. It is not always essential to get 100% restoration so far as the outcome are reproducible to perceive with high degree of assurance.[2,4] Need of analytical method development and validation: The need of the analytical method development and validation emerged because of international competition, maintaining the quality of product in high business and moral reasons. Various International regulative Agencies have set the standard and fixed the protocol to match the reference for granting approval, authentication and registration.

Standards Are

1) United States Food and Drug Administration (US FDA).
2) Current Good Manufacturing Practice (cGMP) regulations.
3) Good Laboratory Practice (GLP) regulations.
4) The Pharmaceutical Inspection Cooperation Scheme's (PIC/S).
5) Pharmaceutical Inspection Cooperation Scheme (PIC/S).
6) The International Conference for Harmonization (ICH).
7) Quality Manual ISO/IEC 17025 issued by International. Organization for Standardization.

8) World Health Organization (Who).

When some changes are created in the validated nonstandard methods, the influence of such changes must be documented and a new validation method should be carried out. If standard methods are available for a particular sample take a look at, the most recent edition should be used.

Validation

Validation is the process of establishing documentation evidence demonstrating that a procedure, process, or activity carried out in testing and then production maintainethe desired level of compliance at all stages.

Circumstances

- Completely new procedure. Latest equipment.
- Procedure and equipment which have been adjusted to suit altered needs and,

- Procedure where the finished result test is a poor and undependable marker of product quality Important stages in validation. The action identifying with validation studies can be categorized.

MAINLY INTO THREE STAGES

Stage 1

This includes pre-validation qualification stage which covers all exercises identifying with product studies and improvement, formulation pilot batch testing, scale-up research, exchange of innovation to business scale groups, setting up stability conditions, and managing of in-process, finished pharmaceutical formulations, qualification of equipment, master documents, and process limit.

Stage 2

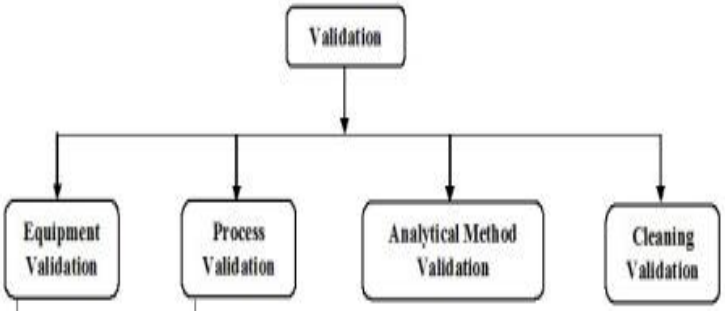
This involves process validation phase. It is intended to check that every installed limit of the vital process parameter is substantial and that satisfactory products can be created even below the worst situations.

Stage 3

It is also called as the validation maintenance stage, it requires constant review of all procedure related archives, including validation of the review reports, to guarantee that there have been. no modifications, departure, failures, and alteration to the production procedure and that all standard operating procedures (SOPs), involving change control procedures, had been observed. At this phase, the approval team involving people representing all essential departments also guarantees that there have been no modifications/deviations that ought to have brought about requalification and revalidation.

TYPES OF VALIDATION

Validation is classified into following types



EQUIPMENT VALIDATION

The key concept of validation is to give a high degree of reported confirmation that the equipment and the procedure conform to the written guidelines. The degree (or intensity) is dictated by the complexity of the device or system. The validation should give the essential data and test methods required to give that the device and technique meet determined prerequisites.

Equipment Validation includes the following

INSTALLATION QUALIFICATION (IQ)

IQ guarantees all crucial processing, packaging system, and ancillary items are in compliance with the installation. It checks that the equipment has been established or installed as per the manufacturer's suggestion in a systematic way and positioned in surrounding appropriate for its meant purpose. Installation qualification points include.

- Equipment layout character that is the material of construction cleanability and many others.
- Installation situations like wiring, functionality, utility and so forth.
- Calibration, preventative protection, cleansing plans.
- Safety characteristics.
- Supplier documentation, prints, illustrations, and hand operated.
- Software documentation.
- Enlist the spare components.
- Environment-related conditions like clean room necessities, humidity, and temperature.

OPERATIONAL QUALIFICATION (OQ)

OQ performed to give a high level of degree of affirmation that the equipment works as proposed. OQ concerns consist of:

- Process control limits like temperature, time, stress, line velocity, set up conditions, and so on.
- Software parameters.
- Crude material details.
- Process operating methods.
- Material managing necessities.
- Process change control.
- Short-term balance and capability of the technique.

- The use of statistically valid procedures inclusive of screening examinations to optimize the technique can be utilized throughout this stage.

PERFORMANCE QUALIFICATION (PQ)

PQ checks that the device is repeatable and it is uniformly producing a quality item. PQ concern consists of:

- True product, procedure parameters, and process set up in OQ.
- Adequacy of the product.
- Guarantee of technique ability as built up in OQ.
- Process repeatability, prolonged process stability.

Process Validation

The process validation is a component of the coherent prerequisites of a quality management system. Process Validation is the most essential and perceived parameters of current good manufacturing practices. The objective of a quality system is to produce items that are matched with their proposed use uniformly.

 Process validation is reported evidence which gives a high level of affirmation that a particular procedure will produce a product meeting its determined prerequisites.

Prospective Validation

It is described as the well-known reported program that a device does what it indicated to do based on pre-planned protocols. This validation is normally performed previously for distribution both of a newer item or item made under a revised production process. In this validation, the protocol is accomplished before the procedure is placed into industrial use.

- Short depiction of the procedure.
- Equipment/facilities list is to be utilized involving observing/recording equipment) collectively with its calibration status.
- Finished dosage forms for discharge.
- List of analytical techniques, as suitable.
- Proposed in-process controls with specification criteria. Additional testing to be completed, with specification limits and analytical approval, as suitable.
- Sampling design, Techniques for recording and assessing outcomes.
- Functions and obligations.
- Proposed timetable.

Concurrent Validation

It is same as prospective validation with the exception of the working firm, will offer the product at the time of qualification runs, to the society at its market cost, and furthermore like retrospective validation. This type of validation includes in-process observing of vital processing steps and product checking out. This helps to produce and reported proof to demonstrate that the manufacturing technique is in a condition of control.

- In remarkable conditions, it might be acceptable not to finish the validation program before routine manufacturing begins.
- The choice to complete simultaneous approval must be supported, archived and accepted by authorized personnel.
- Documentation prerequisites for simultaneous validation are similar as designated for prospective validation.

Retrospective Validation

It is characterized by the established reported confirmation that a system does what it implies to do on the audit and investigation of historical data. This is accomplished by the survey of the ancient manufacturing testing information to demonstrate that the procedure has always remained in control.

- Batches are produced for a definite duration (last 10 successive batches).The number of lots discharged every year.
- Batch size/strength/producer/year/period.
- Master manufacturing/packaging files.
- Current particulars for active ingredients/finished materials.
- List of process deviations, corrective actions, and modification to production archives.
- Data for stability study for a few batches.

Analytical Method Validation

Validation of an analytical approach is established through laboratory research, that the execution attributes of the procedure meet the requirements for the proposed scientific application. Validation is required for any new or altered procedure to verify.

Method validation is a reported program that offers with that the processing system will give a high level of affirmation to meet its predicated acceptance basis.

It consists of mainly five different steps which are as follows

Qualification of the system

System qualifications permit to check that the instrument is appropriate for the planned investigation, the materials are appropriate to be used in analytical judgments, the analysts have the correct instruction, capabilities, and foregoing documentation such as analytical inclusive of analytical approaches, proper authorized protocol with pre-set up standards have been reviewed. On the off chance that the general qualifications of a device are overlooked, and trouble arises, the source of the issue will be hard to recognize.

Sampling

Sampling assists in the choice of a representative part of the fabric which is along these lines subjected to evaluation. The selection of a suitable sampling technique is of significant importance since it gives assurances that the sample chose is really illustrative of the material as a whole for the purpose of important statistical inferences. Inside the statistical literature, there is a considerable collection of work on sampling techniques, anyway the relative expenses and time engaged with every technique ought to be assessed ahead of time.

Preparation of Sample

Preparation of the sample is a key component to effective method validation. It has been mentioned that sample planning represents 60 to 80% of the work action and working expenses in an investigative lab. The literature on the preparation of the sample is enough and properly documented. In any case, the investigator ought to recall that the choice of a particular preparation technique relies upon concentrations of analytes, sample matrix, size of the sample and the instrumental method.

Analysis of Sample

The evaluation is associated with the instrument utilized to extract qualitative or quantitative data from the samples with an adequate vulnerability level. The investigation could be predictable, in a great sense, as the device has 3 interconnected fundamental components, namely input, converter, and output. The input and output are assigned by the letters x and y, and they represent the concentration and response individually. The selection of a specific analysis depends on many considerations, for example, the chemical properties of the analytical species, the concentration of the analytes in the sample, sample matrix, speed, cost, and so forth.

Assessment of Data

The essential reason behind information assessment is to outline and pick up knowledge into a specific informational index by utilizing numerical and statistical techniques. Data assessment permits extracting valuable data and reaching inferences about the inputs and outputs, and in particular about the validation procedure in general.

Cleaning Validation

Cleaning validation is a reported proof with a high level of confirmation that can uniformly clean a system or equipment to already determined and specification criteria. Cleaning approval is a reported procedure that demonstrates the efficacy and consistency in cleaning pharmaceutical production equipment. The goal of cleaning approval is to check the viability of the cleaning system for the expulsion of product deposits, degradants, additives, excipients, or cleaning agents and in a the control of potential microbial contamination.

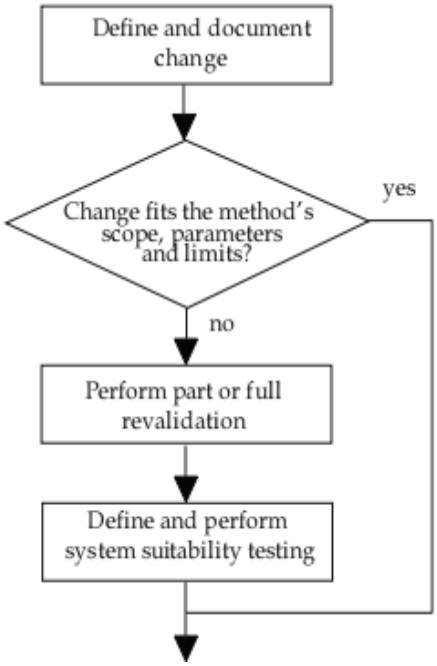
Cleaning Validation Protocol

- The goal of the validation procedure.
- Obligations regarding performing and endorsing the validation study, Equipment details.
- The interval between the end of production and the start of the cleaning techniques.
- Cleaning methods to be utilized for every product, each manufacturing device or each piece of equipment.
- The quantity of the cleaning cycle to be performed continuously.
- Routine checking equipment.
- Sampling techniques, including the basis for why a specific sampling technique is utilized.
- Clearly defined sampling areas.
- Information on recovery studies, where suitable.
- Analytical techniques including LOD and LOQ.
- The acceptance criteria, along with including the method of reasoning for setting specified limits.

Revalidation

Most likely some method parameters have to be changed or adjusted during the life of the method if the method performance criteria fall outside their acceptance criteria. The question is whether such change requires revalidation. These ranges should be verified during method validation in robustness studies and should be part of the method characteristics. Arevalidation is necessary whenever a method is changed, and the new parameter lies outside

the operating range. If, for example, the operating range of the column temperature has been specified to be between 30 and 40°C, the method should be revalidated if, for whatever reason, the new operating parameter is 41°C.



Flow chart of revalidation

TRANSFERRING THE VALIDATED ROUTINE METHODS

Validated routine methods are transferred between laboratories at the same or different sites when contract laboratories offer services for routine analysis in different areas or when products are manufactured in different areas. When validated routine methods are transferred between laboratories and sites, their validated state should be maintained to ensure the same reliable results in the receiving laboratory. This means the competence of the receiving laboratory to use the method should be demonstrated through tests, for example, repeat critical method validation experiments and run samples in parallel in the transferring and receiving laboratories. The transfer should be controlled by a procedure,

The Recommended Steps Are

- Designate a project owner, Develop a transfer plan. Define transfer tests and acceptance criteria (validation experiments, sample analysis: sample type, #replicates).
- Describe rational for tests.
- Train receiving lab operators in transferring lab on equipment, method, critical parameters and troubleshooting.

- Repeat 2 critical method validation tests in routine lab. Analyze at least three samples in transferring and receiving lab.
- Document transfer results.

Validation Parameters

The main aim of method validation is to produce proof that the method will what it is supposed to do, accurately, reliable and consistent. The validation parameters as per ICH guidelines are described below:

Selectivity/Specificity

The terms selectivity and specificity are often used interchangeably, the term specific generally refers to a method that produces a response for a single analyte only, while the term selective refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other. If the response is distinguished from all other responses, the method is said to be selective. Since there are very few methods that respond to only one analyte, the term selectivity is usually more appropriate Selectivity and specificity are measures of the reliability of measurements in the presence of interferences.

Specificity was performed to determine the retention time of each drug in a mixture and in the sample. The retention time of standard drugs individually was determined, and it was found to be 3.750 min and 1.533 min for nitazoxanide and ofloxacin and retention time of both drugs in the standard mix was found to be 3.760 min for nitazoxanide and 1.542 min for ofloxacin respectively.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

Accuracy can also be described as the closeness of agreement between the value that is adopted, either as a conventional, true or accepted reference value, and the value found. The true value for accuracy assessment can be obtained in several ways. One alternative is to compare the results of the method with results from an established reference method. This approach assumes that the uncertainty of the reference method is known. Secondly, accuracy can be assessed by analyzing a sample with known concentrations (e.g., a control sample or

certified reference material) and comparing the measured value with the true value as supplied with the material.

Recovery

After extraction of the analyte from the matrix and injection into the analytical instrument, its recovery can be determined by comparing the response of the extract with the response of the reference material dissolved in a pure solvent. Because this accuracy assessment measures the effectiveness of sample preparation, care should be taken to mimic the actual sample preparation as closely as possible. If validated correctly, the recovery factor determined for different concentrations can be used to correct the final results.

4. PRECISION

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if It is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. Precision may be taken into consideration at 3 levels:

Calibration

Calibration is the most important step in bioactive compound analysis. A good Precision and accuracy can only be obtained when a good calibration procedure is adopted. In the Spectrophotometric methods, the concentration of a sample cannot be measured directly, but is determined using physical measuring quantity ‘y’ (absorbance of a solution).

4.1. Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

a) a minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each); or b) a minimum of 6 determinations at 100% of the test concentration.

From the repeatability standard deviation or or sr it is useful to calculate the ‘repeatability limit ‘r’’, which enables the analyst to decide whether the difference between duplicate analyses of a sample, determined under repeatability conditions, is significant.

It expresses the exactness below a similar operating condition over a brief interval of time and also referred as intra-assay precision. A minimum of six replicates test preparation of a similar or consistent sample ready at the 100% check.

4.2. Intermediate Precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

It expresses the exactness under inside research laboratories, in distinct days, through distinct analyst, on distinct instruments/equipment. Two different analysts each preparing six sample solutions, as per specified method.

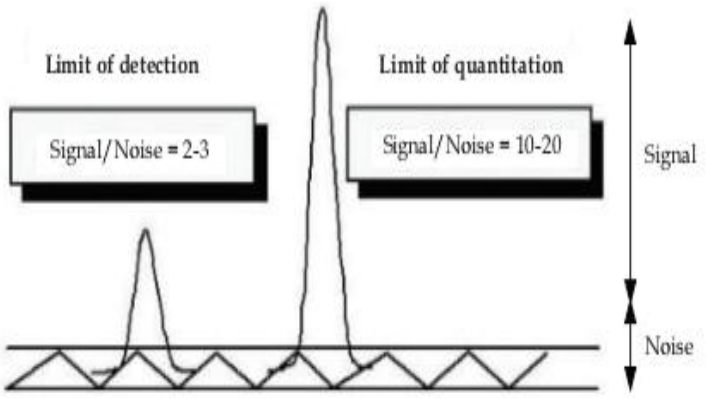
4.3. Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology). From the reproducibility standard deviation oR or sR it is useful to calculate the ‘reproducibility limit ‘R’, ‘which enables the analyst to decide wheth er the difference between duplicate analyses of a sample, determined under reproducibility conditions, is significant. These calculations can be performed directly with the built-in statistics function of the instrument, if available, or by using a pocket calculator or a PC (Personal Computer) with a suitable software pac kage (e.g. spreadsheet program).

The precision of paracetamol was checked by injecting a solution of 80 µg/ml for six times in same days, different days, and in a different time interval on the same day. The % RSD was found to be less than 3%, which showed good precision.

5. LIMT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.



Limit of detection and limit of quantitation via signal to noise

The limit of detection is frequently confused with the sensitivity of the method. The sensitivity of an analytical method is the capability of the method to discriminate small differences in concentration or mass of the test analyte.

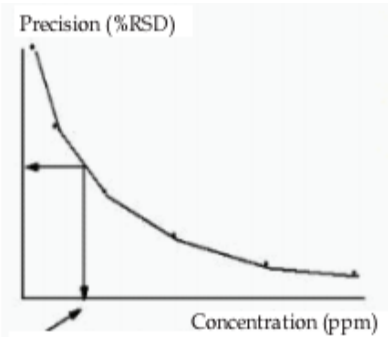
1. Visual inspection: The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

2. Standard deviation of the response based on the standard deviation of the blank: Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

6. LIMIT OF QUANTITATION

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.



The limit of quantitation is the minimum injected amount that produces quantitative measurements in the target matrix with acceptable precision in chromatography, typically requiring peak heights 10 to 20 times higher than the baseline noise. If the required precision of the method at the limit of quantitation has been specified, the EURACHEM (22) approach can be used. A number of samples with decreasing amounts of the analyte are injected six times. The calculated RSD percent of the precision is plotted against the analyte amount. The amount that corresponds to the previously defined required precision is equal to the limit of quantitation

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity may be characterized as the capacity of an analytical technique to produce outcomes which are directly related to the concentration of an analyte in the.

Standard solution of betahistine hydrochloride (BET) and prochlorperazine maleate (PRO) was taken in a 10 ml volumetric flask and diluted with 0.1 N HCL to get the final concentration in the range of 4 to 24µg/ml for BET and 3 to 18µg/ml for PRO. Prepared six times in this calibration range and absorbance determined at the respective wavelength for each drug alone.

Sensitivity

This is effectively the gradient of the response curve, i.e. the change in instrument response, which corresponds, to a change in analyte concentration. Where the response has been established as linear with respect to concentration, i.e. within the linear range of the method, and the intercept of the response curve has been determined, sensitivity is a useful parameter to calculate and use in formulae for quantitation. Sensitivity is sometimes used to refer to limit of detection but this use is not generally approved.

Range

The range of an analytical procedure 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 procedure has a suitable level of precision, accuracy and linearity.

It can be characterized as the interval amongst upper and lower quantities of analyte in the sample. Minimum of the specified range to be 80% to 120% of the test sample for the assay test.

Ruggedness

Ruggedness is the degree or measure of reproducibility under different situations such as in different laboratories, different analyst, different machines, environmental conditions, operators etc. In the simultaneous estimation of nitazoxanide and ofloxacin, ruggedness was performed by different analyst and in different laboratories in different days to checks for any variation in the chromatography. The % RSD for area and retention time was calculated for determination.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is characterized by the level of ability of an analytical technique, to stay similar by minute purposely change in the technique parameter. The different technique parameters which can be modified in high-performance liquid chromatography are pH, drift rate, the temperature of the column and mobile phase composition.

In the simultaneous estimation of nitazoxanide and ofloxacin, the robustness of the proposed method verified by to perform analysis under variable flow rates. The flow rate as per the developed method is 1.5 ml/min. Slight change in flow rate is 1.3 ml/min and 1.7 ml/min and chromatogram recorded. Due to a slight change in the flow rate of method shows good results and remain unaffected by that minute change.

System Suitability Parameters

System suitability test is used to check the sensitivity, resolution, and reproducibility of the chromatographic system are well for the analysis to be done. The factors mainly used in system suitability are tailing factor, a number of the theoretical plate, retention time, resolution, etc.

2. Plate Number or Number of Theoretical Plates (N)

These is a measure of the sharpness of the peaks and therefore the efficiency of the column. This can be calculated in various ways, for example the USP uses the peak width at the base and the BP uses the peak width at half the height.

1. TAILING FACTOR (T)

It is defined as the distance between the front edge of the peak to the back edge of the peak divided by twice of the front edge of the peak.

$$T = (X+Y)/2\times$$

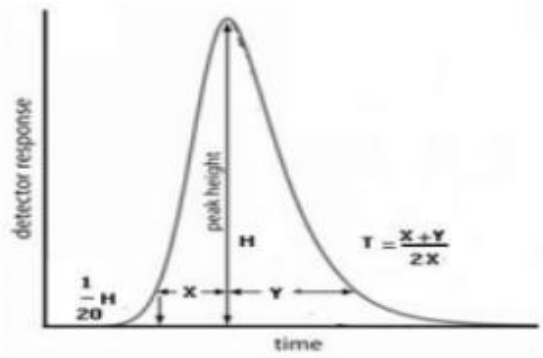


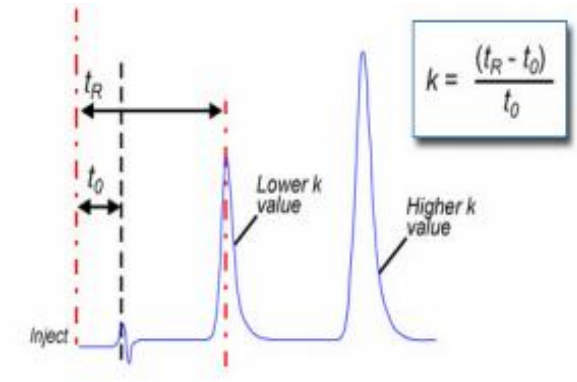
Fig. 3: Tailing factor [35]

3. CAPACITY FACTOR (K))

It can be expressed as how many times the analyte is retained with respect town retained compound. It is denoted by the symbol k. It can be calculated as:

$$K= (tR–t0)/t$$

R = retention time, 0 = dead time



Where t0

R is the retention time of the peak and t0

It is mainly utilized to examine the efficiency of the column. It can be expressed as: is the column dead time.

N = 16(tR/W)2

Retention time (tis the retention time, and W is the width at the base of peak)

4. RESOLUTION (R)

It is the measure of separation power of the complete chromatographic system. Resolution can be defined as the ratio of the distance between two peak maxima to the mean value of peak width from its baseline.

R = 2[(tR)A-(tR)B]/WA+WWhere, tBR1 and tR2 are retention time of second and first compounds, respectively.

CONCLUSION

This article gives an idea that what is validation, its types, why it is necessary, how to develop a method and how to carry out the validation procedure to demonstrate that the technique is able for its proposed reason. All validation parameters such as linearity, limit of quantitation and limit of detection, Range, specificity, robustness, ruggedness and system suitability are defined well with examples of certain drugs.

ACKNOWLEDGMENT

I am thankful to V. Swetha, Asst. Professor for the support, cooperation and guidance in searching various articles and journals for completion of this review article and thank you to School Of Pharmaceutical Sciences and Technologies, JNTU, Kakinada.

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