IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 2, August 2023

# A Detailed Examination of the Validation of the Analytical Method

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**Abstract:** *Reliability of the results obtained from analytical techniques for identifying the characteristics of materials connected to drugs is vital. They might be the starting point for decisions on how to provide the drug to patients. Research and manufacturing of medications both need the validation of analytical techniques to ensure that they are appropriate for their intended usage. To comply with GMP rules, pharmaceutical firms must create a comprehensive validation policy that describes the validation procedure. The purpose of this validation is to show that processes pertaining to pharmaceutical research, production, manufacturing, and analytical testing can be completed in an effective and standardized manner. This review article offers guidelines for carrying out validation aspects of the analytical approach utilized in pharmaceutical analysis*

**Keywords:** Reliability

## I. INTRODUCTION

The study of isolating, measuring, and characterizing the chemical constituents of materials—both natural and artificial—that consist of one or more compounds or elements is known as analytical chemistry. There are two main categories in analytical chemistry: quantitative analysis, which finds the amount of a certain element or compound in the sample, and qualitative analysis, which finds the chemical components in the sample.

Pharmaceutical analysis [1-3] is crucial for quality control and assurance when looking at pharmaceutical formulations and bulk drugs. The pharmaceutical and medical sectors are expanding quickly [4], and the demand for creative and well-organized analytical techniques is being driven by the production of medications both locally and abroad. As a result, the development of analytical techniques has become paramount in analysis.

Scientific and useful analytical techniques have advanced as a result of the development of analytical instruments. Thanks to developments in analytical method development and analytical apparatus, analysis has become more precise and accurate while taking less time and money [5]. A governing organization's fundamental criteria are produced as a consequence of the creation and approval of analysis-related procedures for usage with residual solvents, drug products, associated chemicals, active pharmaceutical components, and excipients[6].

The development of analytical methods eventually leads to the creation of official test techniques[7]. Consequently, these methods were used by quality control laboratories to confirm the legitimacy, potency, purity, safety, and functionality of the drug. Regulatory agencies give analytical manufacturing processes top emphasis. Regulatory agencies need the applicant to show control over the whole drug development process via the application of validated analytical methods in order to approve a medicine[8].

Every day, new and inventive pharmaceuticals are introduced onto the market. Therefore, it is crucial to create new processes and introduce them to ensure their quality. These are the requirements that contemporary pharmaceutical analysis must meet.

The least amount of time and maximum efficiency are required for the analysis.

Pharmacopoeia standards must be followed for the accuracy of the analysis.

The chosen strategy has to be precise and targeted.


DOI: 10.48175/568

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## II. TYPICAL INSTRUMENTAL TECHNIQUES

The four types of drug estimation methodologies are biological, physicochemical, chemical, and physical. Of these, physicochemical and physical processes are often used; most physical techniques pertinent to analysis include examining the different physical properties of an item. They consist of solubility, color, turbidity or transparency, density or specific gravity (for liquids), melting and freezing points, and moisture content. Physicochemical approaches are used to analyze the physical phenomena resulting from chemical reactions[9, 10]. Optical techniques like refractive, polarimetric, emission spectrophotometry, and turbidometry; electrochemical techniques like potentiometry, amperometry, and polarography; and chromatographic techniques like paper, column, thin layer, gas liquid chromatography, and high performance liquid chromatography are generally favored in the field of physicochemical techniques [13, 14]. Nuclear reaction-based methods like Nuclear Magnetic Resonance gained popularity. One well-known and useful tool is the GC-MS combination. Among the chemical methods are volumetric and gravimetric procedures, which mainly depend on complex formation, acid-base, and redox processes. When the sensitivity at the mg level is sufficient and the interferences are negligible, titrations in complexometry and non-aqueous fluids have been extensively utilized in pharmaceutical analysis. The modern techniques (HPLC, UPLC, GLC, GC-MS/MS, LC-NMR, and Liquid Chromatography-Mass Spectrometry), which are very sensitive, accurate, and need relatively few samples for analysis, are among the alternatives for testing utilizing cutting-edge technology.

## III. ANALYTICAL METHOD DEVELOPMENT[15-18]

When approved procedures for the examination of innovative items are unavailable, new approaches are being developed. Novel approaches are developed to analyze existing pharmaceutical or non-pharmacopoeial items in order to reduce costs while enhancing accuracy and durability. These processes are optimized and verified by trial runs. Alternative strategies are proposed and put into practice to replace the present technique in the comparative laboratory data, taking into account all relevant advantages and downsides.

The objective of creating analytical techniques

Drug analysis reveals the identity, characteristics, and concentration of medications in combinations, such as dosage forms and biological fluids. The main applications of analytical methods in drug development and manufacturing are to provide information about potency (which can be directly related to the requirement for a known dose), impurity (related to the safety profile of the drug), bioavailability (which includes crucial drug characteristics like crystal form, drug uniformity, and drug release), stability (which indicates degradation products), and the effect of manufacturing parameters to ensure consistent production of drug products[19].

By using a series of methods designed to stop and eradicate errors at different stages of the production process, quality control aims to analyze and identify a real and accurate product. The choice to release or dispose of a product is determined by one or more categories of control measures. The ability to provide a clear, analytical process for a range of complex formulations is among the most crucial subjects. The pharmaceutical industry's fast expansion and continuous global medication manufacturing have led to a rapid rise in the demand for novel analytical procedures. Consequently, the main analytical task in a quality control laboratory is now designing analytical methodologies.

The following factors have contributed to the development of novel drug analysis methods:

when a medication or combination of medications is not included in the pharmacopoeias as official.

when the current drug lacks a decorous analytical procedure that is mentioned in the literature due to patent constraints.

when the formulation excipients' involvement makes it impossible to use analytical methods for the drug's formulation.

It is found that the analyte in body fluids cannot be quantified using analytical methods.

It's likely that expensive solvents and reagents are required by the analytical techniques used today. Furthermore, time-consuming extraction and separation procedures can be necessary.

Protocols for the creation of the technique

The relevant documentation is submitted in accordance with the development procedure. Any data necessary for these inquiries must be stored in an electronic database or lab notebook.

Analyte standard characterization

All available data about the analyte's physico-chemical properties, including optical isomerism and solubility, as well as its structure, are compiled.

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The analyte standard is obtained, which is about 100% pure. The freezer, desiccators, and refrigerator must be arranged for optimal storage.

The sample matrix correctly indicates the number of components, shows the findings, and approximates the accessibility of standards while studying several components.

Techniques like spectroscopy, HPLC, GC, MS, and so on are considered when paired with sample stability.

Procedure details

The requirements of the analytical method must be stated in order to develop the analytical figures of merit, such as linearity, selectivity, range, accuracy, precision, detection limits, etc.

Look through the literature and earlier techniques.

The automatic computerized literature search provided by Chemical Abstracts Service is highly handy. With reference to relevant books, journals, USP/NF, AOAC, and ASTM publications, all information pertaining to the drug's literature is examined for physico-chemical characteristics, synthesis, solubility, and acceptable analytical procedures.

Choosing a method

Because adjustments have been made when required, methodology has evolved as a result of the proper application of the information contained in the literature. Occasionally, obtaining additional equipment is required to develop, modify, replicate, and confirm existing procedures for analytes and samples.

if none of the methods already in use for analysis of the analyte under investigation can be used.

preliminary investigation and setup of the instruments

By setting up the appropriate instruments, the equipment's installation, operation, and performance certification are verified in accordance with the standard operating procedures of the laboratory.

Improvement

One parameter is changed at a time during optimization, and a collection of conditions is separated before using a trial-and-error approach. The previously indicated activity has to be finished with a strict, methodical approach, paying close attention to each step, and recording any dead ends.

keeping track of important analytical metrics

The actual determined analytical figures of merit—linearity, quantitation and detection limits, analysis time, expense, sample preparation, etc.—are also documented.

Evaluation of the development procedure using real samples

The sample solution should be used to totally and clearly identify the drug's peak interest, regardless of any other matrix components.

Quantitative sample analysis demonstration and determination of the genuine sample recovery %

The proportion of actual, spiked reference drug recovered into a sample matrix free of analytes is estimated. The optimization for recovery repeatability (average ± standard deviation) across samples must be shown. As of now, a complete recovery is not necessary since the results can be reliably predicted with a high degree of accuracy.

## IV. ANALYTICAL METHOD VALIDATION

To ensure that the analytical method used for a particular test meets the requirements, the analytical method validation procedure [20–24] is employed. Pharmaceutical technique validation may include support from the USP, ICH, FDA, and other recommendations. The outcomes of the validation procedure may be used to evaluate the method's quality, consistency, and reliability with reference to analytical data. Assay validation has significant importance in the pharmaceutical industry for two primary reasons: firstly, it is a fundamental part of the quality-control system; secondly, it is required for the regulation of authorized production processes.

Analytical Methods Validation Parameters

The validation of analytical procedures has been conducted in accordance with the ICH Q2 (R1) criteria[25]. Validation parameters include: 1. Appropriateness of the system

Specificity

Consistency

Correctness Precision

LEQ LOD

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Robustness
System Suitability
In the past, the pharmaceutical industry employed system suitability testing to ascertain if a chromatographic system was routinely used in pharmaceutical laboratories, where the quality of results and appropriateness for a particular analysis are the most important factors.

The system suitability testing (SST) report was evaluated using the following standards:
The number of theoretical plates, or efficiency (N).
capacity factor (K).
Comparative separation or retention (α).
Resolution (Rs).
tailing factor (T).
Relative Standard Deviation (RSD).
Theoretical efficiency/count of plates (N)
Peak dispersion degree measurement in a column is called efficiency, and it must adhere to the column's specifications. The efficiency is expressed in terms of theoretical plates. The N calculation formula is shown in the accompanying Figure. N is equal to efficiency / number of theoretical plates. Retention time of the analyte is Ve.
highest point, h =.
The Gaussian function of the half-height peak width is I 1/2.
USP method, tangential/sigma approach
Retention time (ts) or retention distance (cm) are two possible ways to describe elution volume (Ve). Peak width at base line (measured in milliliters, seconds, or centimeters) is denoted by wb. Peak height is indicated by h.
The plate number is determined by the column length. The theoretical plate number is used to calculate column efficiency. The column has to be divided into many hypothetical plates, each with a certain height and a temporal constraint on how long the analyte may stay inside of it, in accordance with plate theory. The analyte and stationary phase will instantly be in equilibrium. The following is the formula for height equivalent to theoretical plate, or HETP:
HETP = L/N, where (1) L is the length of the column. Number plate: N.
Because the previously stated capacity factor is dimensionless and independent of both the mobile phase flow rate and column dimensions—which quantify the degree of retention associated with an analyte in proportion to an unretained peak—it is sometimes referred to as a retention factor. where tR is the sample peak's retention time, tM is the unretained peak's retention time, and k' = 0 indicates that there are no chemicals in the column. K' usually has a value bigger than 2.

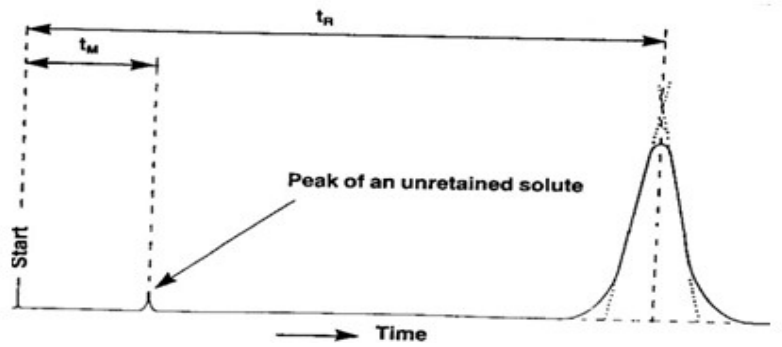


**Figure 1. Determination of capacity factor/ capacity ratio.**

Relative retention, or relative retention, is equal to the separation factor (α).
= Retention period that is computed beginning at the injection point.
+ Unretained peak time, or retention time (tR) of an inert component that was not retained by the column.
= the retention duration calculated starting at the designated reference peak injection location. (The value is 0 in the absence of a reference peak).

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Resolution (Rs)

Increasing column length, lowering particle size, raising temperature, and altering the eluent or stationary phase may all improve resolution. The ability of a column to distinguish between two medications in two different peaks or chromatographic zones is known as resolution. It may be expressed as a ratio of the apex separation between two peaks using the tangential width average of the peaks. Resolution is calculated using the method below.

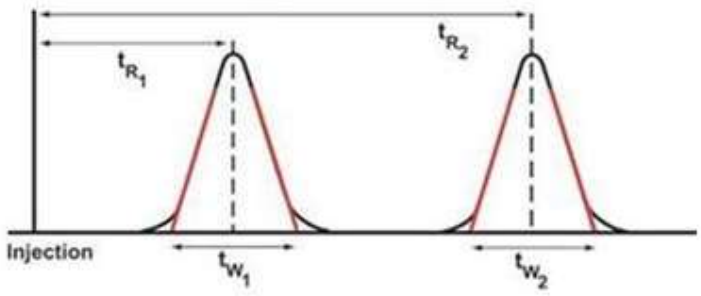


**Figure 2. Determination of resolution between two peaks.$t_{R1}$ and $t_{R2}$ are the retention times for the two peaks of components.**

tw1 and tw2 = The baseline is located between the tangents drawn to the sides of the peaks. (Drawn tangents at 0.6 times peak height). The valley that develops between the two peaks should touch the baseline if the peaks are correctly symmetric. Rs. is 1.5. Generally speaking, a resolution value of two rupees or more should be enough and preferred.

Resolution factor (R) Selectivity, efficiency, and the number of theoretical plates (N) or capacity factor all affect resolution. To separate any two peaks, you need a suitable capacity factor (ideally between 2 and 10), enough efficiency, and a sufficient number of theoretical plates (more than 2000 theoretical plates). Selectivity should also be appropriate (ideally 1.2). The necessary resolution is ≥ 1.5. In 1.5, baseline resolution is specified.

The tailing or asymmetry factor

The chromatographic peak is considered to have a Gaussian shape under ideal conditions. But in reality, there is always a departure from the normal distribution, which indicates that there is a process of migration and non-uniform distribution. Regulatory organizations like USP and EP have recommended this as a system appropriateness criterion as a result. The asymmetry factor and tailing factor are approximately equal, seldom true, and evenly distributed in most cases. Values should generally range from 1.0 to 1.5; values greater than 2 are not permitted. The peak asymmetry may be computed using the formula below.

B is the amount of time that separates the middle of the peak from the trailing edge. (10% of the maximum height) is the calculation used.

A is the distance, measured as a percentage of peak height, between the leading edge to the halfway point of the peak.

Peaks should preferably be totally symmetrical or have a Gaussian shape. The determination of the asymmetric factor and tailing is shown in Figure 1.5.

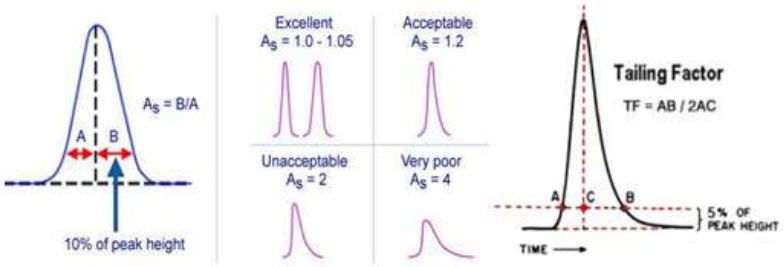


**Figure 3. Determination of tailing and asymmetric factor.**

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**Table 1. Acceptance criteria for system suitability parameters.**

| S.No | Parameter name | Acceptance criteria |
|---|---|---|
| 1 | Number of theoretical plates or Efficiency (N) | > 2000 |
| 2 | Capacity factor (K) | < 1 |
| 3 | Separation or Relative retention (α) | > 1 |
| 4 | Resolution (Rs) | > 1.5 |
| 5 | Tailing factor or Asymmetry(T) | < 2 |
| 6 | Relative Standard Deviation (RSD) | < 2 |

**Specificity**

One of the significant features of HPLC is its ability to generate signals free from interference. Specificity refers to the ability of the analytical method to differentiate and quantify the analyte in complex mixtures. An investigation of specificity is to be conducted during the determination of impurities and validationof identification tests.

An ICH guideline defines specificity as ability to assess unequivocally the analyte in the presence ofother compounds that may be likely to be present. Typically these might be impurities, degradants, matrix, etc. The definition has the following implications:

**Assay:** This enables an accurate report on the strength or content of analyte in a sample in order to arrive at an accurate result.

The Range and Linearity

The degree to which a response vs. concentration calibration plot closely resembles a straight line is known as the method's linearity. One way to evaluate linearity is to do single measurements at various analyte concentrations. After that, a linear least-squares regression is used to analyze the data. The necessary linearity information is provided by the plot slope, intercept, and correlation coefficient that result.

Accuracy

Three criteria are used to classify the accuracy of an analytical procedure: closeness of agreement between a series of measurements obtained from numerous samplings of the same homogeneous sample under identical analytical circumstances.

Repeatability: accuracy using the same analyst for a short amount of time and the same operational circumstances.

Intermediate precision: many days, instruments, analysts, etc. are used to test the procedure.

Studying reproducibility among laboratories.

According to the ICH recommendations, repeatability must be appropriately verified using a minimum of six determinations at 100% of the test concentration or at least nine determinations within the procedure's defined range (e.g., three concentrations / three replicates each).

Accuracy: The degree to which the measured value closely resembles the real value is the measure of accuracy. A sample whose "true value" is known is examined using a high accuracy technique, and the measured value matches the actual value exactly. Recovery studies are usually used to illustrate and assess accuracy. Three methods exist for assessing accuracy:

contrasting with a benchmark.

Analyte recovery that was spiked into a blank matrix.

addition of the analyte as usual.

How the specific or total contaminants are to be identified should be obvious.

The detection limit

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The process of determining the minimal level at which analyte may be consistently detected under specified experimental conditions—albeit not always quantified as an exact value—involves analyzing samples with known concentrations of the analyte. The concentration of analyte (ppm) in the sample is often used to represent the detection limit.

The ICH recommends many methods for figuring out a sample's detection limit, depending on the kind of analyte, the equipment being used, and the method's applicability. The appropriate methods are

visual assessment.

ratio of signal to noise.

Variance of the answer standard.

The linearity plot's slope standard deviation.

LOD may be calculated using the formula LOD = 3.3 δ/S^(7), where δ is the standard deviation of the calibration curve intercepts.

S is the linearity plot's slope.

The quantitation limit

The lowest drug concentration in a sample that can be determined under confirmed experimental circumstances with the necessary accuracy and precision is known as the limit of quantitation.

The ICH suggests the following four techniques for estimating LOQ, which are similar to LOD. The appropriate methods are

visual assessment.

ratio of signal to noise.

Variance of the answer standard.

The linearity plot's slope standard deviation.

LOQ may be computed using the formula LOQ = 10 δ/S.

where δ is the response standard deviation.

S is the calibration curves' mean slope.

Sturdiness

An analytical method's robustness is determined by how well it can withstand slight, intentional changes to its parameters. The pH, mobile phase composition, sample temperature, flow rate, column temperature, and sample temperature are examples of changeable procedure parameters in the HPLC process.

## V. STATISTICAL TREATMENT OF ANALYTICAL DATA

The role of an analyst is to use analytical procedures to get a specified result that is as close to the real value as possible. In the absence of information about the accuracy and precision of the used approach as well as potential causes of mistake, the analyst's level of trust in his findings will be quite low. There is usually some degree of fluctuation in experimental measurements, thus definitive conclusions cannot be made. We may reject unlikely findings and accept those with a high likelihood of accuracy thanks to statistics.

The goal of making a determination is to arrive at a reliable approximation of a "true" value. When evaluating the stated criteria used to choose an analytical technique, accuracy and precision are often the first obvious points that spring to mind for application. The combination of accuracy and precision yields the error of a single determination. These are some of the most important and crucial factors that are used to evaluate the analytical processes based on the outcomes that are obtained.

Accuracy

Variance is the square of the standard deviation (S2). The coefficient of variation, or CV, is expressed as an absolute value, or RSD or % RSD. It is often expressed as a percentage and is used to compare the degree of uncertainty across several measurements with differing total magnitudes.

**Accuracy**[26]

The difference between the real or most likely value for the quantity tested and the mean „x" of the collection of findings is often referred to as accuracy. According to IUPAC, accuracy is the discrepancy between the real value and the outcome (or mean). The absolute method and the comparative method are the two workable approaches to assessing the correctness of analytical procedures.

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**Absolute method**

In order to verify the correctness of the technique under consideration, quantities of the elements are taken, and the procedure is followed according to the instructions given. % error is the discrepancy, commonly stated as parts per hundred (%), between the means of a sufficient number of outcomes and the actual quantity of ingredient present.

Since the ingredient in issue will be determined when other substances are present, it will be important to understand how the determination will turn out. Testing the impact of several likely substances in the selected samples in various concentrations will be necessary to achieve this. There are a few cases when the method's accuracy is determined by the separations (typically using chromatography or solvent extraction techniques).

**Comparative method**

One or more precise analytical techniques have identified the content of the target element in pharmaceutical formulations (or solid synthetic samples with the intended composition). Basically, if there are multiple distant (dissimilar) methods of analysis available for a particular constituent, such as chromatographic, spectrophotometric, gravimetric, or titrimetric, the agreement between at least two methods of essentially different character can be generally accepted as evidence that there isn't a significant systematic error in either method.

**Recovery experiments**

The sample is mixed with a known quantity of the component to be estimated, and the overall amount of the element present is determined by analysis. The recovery of the added component's quantity is determined by comparing the analytical findings for samples including and excluding the added ingredient. Our faith in the procedure's correctness is bolstered if the recovery is good.

Precision and accuracy are assessed by contrasting two processes.In [27]

To determine the correctness of the technique to be researched, the reference method—an existing method—must be compared with the test method.

Pupil t-test

F-testing

where sd is the standard deviation and di = xR (Reference method) – xT (Test method).

We may determine the significance of the variation between the reference and test methods' variances using the F-test. Assume for the moment that n1 duplicate measurements were made using test techniques, and n2 replicate measurements were made using reference methods. The estimates of ST2 (variance of the test method) and S 2 (variance of the reference method), if the null hypothesis is correct, should not vary significantly, and their ratio should not deviate significantly from unity. Actually, one makes advantage of the variance ratio.

F = ST2 / SR in 2

To get a result equal to or greater than unity, the F-ratio is often calculated by dividing the bigger variance by the smaller variance. At a particular confidence level, it may be concluded that there is no substantial difference in the accuracy of the processes if the computed F-value is less than the F-value from the F-table.

**Calibration**

The calibration and standardization process is a crucial component of any analytical method. The link between the analyte concentration and the analytical response (absorbance, peak area, peak height, etc.) is ascertained by calibration. Chemical standards are often used to achieve this. The only way to get high precision and accuracy is to adopt an effective calibration technique. The following calibration processes are often used in pharmaceutical analysis.

**External standard calibration**

A separate preparation of the external standard is made from the sample. When there are no matrix components interfering with the analyte solution, external standards are employed to calibrate devices and processes. An array of external standards is created, each carrying the analyte at a defined concentration. In the calibration procedure, three or more of these solutions are ideally employed. Getting the response signal as a function of the known analyte concentration allows for calibration. Plotting the data or fitting them to an appropriate mathematical equation, such the linear connection employed in the least squares approach, results in the preparation of a calibration curve. The next stage is the prediction phase, when the calibration curve or the best fit equation are utilized to forecast the unknown analyte concentration based on the response signal acquired for the sample.

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**Internal standard calibration**

An internal standard is a quantity of a chemical that is introduced to an unknown substance that differs from the analyte. To determine the amount of an analyte present, the analyte signal is contrasted and compared with the internal standard signal.

Typical augmentation

Standard addition involves adding known amounts of medication to unknown amounts. Determine the amount of analyte that was initially unknown by looking at the signal's growth. The response to the analyte in the standard addition technique is linear.

The least squares approach(28)

A linear response should ideally be attained throughout the calibration processes. One important statistical technique for fitting the data into a linear model is the least squares approach.

You may represent the connection between reaction (y) and concentration (x) using least-squares regression analysis. The general function may be used to illustrate the relationship.

f (x, a, b1, bm) = Y

where the function's arguments are a, b1, and bm.

We follow the rule that the dependent variable (the response measurements) is associated to the y values, and the controlled or independent variable (such as the concentration of a standard) is related to the x values. It follows that there is no inaccuracy in the X values. provided that the standard preparation errors are much less than the measurement error (which is often the case in analytical issues). It is necessary to estimate the values of the unknown parameters a, b1, and bm such that the model fits the experimental data points (xi, yi) as nearly as feasible. It is believed that a straight line represents the actual connection between x and y. Each observation pair's relationship (Xi, Yi) may be expressed as

The signal yi is made up of a random component (ei) and a deterministic component (predicted by the linear model). The estimations of a and b for the two values and must now be found. Finding the values of a and b for which ei2 is small can help with this. The discrepancies between the observed and anticipated yi values of the model are represented by the component ei. The intercept is represented by a, and the slope by b. The ei are referred to as the residuals.

## VI. CONCLUSION

This article offers guidance on how to carry out the validation process to demonstrate that the approach is appropriate for the goal for which it was designed and to guarantee the test method's capabilities. The parameters for method validation are defined and discussed in detail. The technique to validation is diverse and subject to interpretation, and the standards for validation change as medicines are developed, even if regulatory bodies have explicitly stated the needs. In the pharmaceutical sector, validation is a crucial process that is used to make sure that quality is ingrained in the operations that support the research and production of drugs.

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Volume 3, Issue 2, August 2023

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