Review Article

Analytical Method Development and Validation

Rutik Nitin Patil[1], Bhupendra L Deore[2], Darshan Ganesh gurav[1]

From, [1]M.pharm, [2]PHD & M Pharm, Department of Quality Assurance, A.R.A College of Pharmacy, Dhule, India.

ABSTRACT

The main objective of the pharmaceutical business is to consistently and economically create goods with the requisite features and quality. The process of finding, creating, and assessing pharmaceutical formulations requires the creation of a methodology. The primary goal of this review article is to examine how the medication development and validation process was carried out, from the beginning of the formulation to the end of the commercial batch. Reliability of the results is essential whenever an analytical method is used to generate results for an array of samples related to medicine. In the pharmaceutical sector, good manufacturing practice (GMP) rules and good laboratory practices (GLP) are adhered to by means of defined validation policies, forms of validation, and validation criteria. Validation is crucial to the efficient operation of the pharmaceutical sector. Validation was done everywhere, from the raw material to the final, stable state. The procedure was appropriately designed, and the validation parameters—specificity, accuracy, quantitation, ruggedness, robustness, and system appropriateness testing—are specified in terms of limit of detection (LOD), limit of quantitation (LOQ), robustness, and ruggedness with reference to specific drug examples. Both the methodology and evaluation of stability make use of all the validation parameters.

Key words: Analytical validation, Method Development, Validation on HPLC, Separation technique, Various Parameters

These drugs may be entirely new or partially structurally modified versions of ones currently on the market. The aim of every analytical measurement is to generate data that is consistent, accurate, and reliable. Validated analytical methods are necessary to do this. Any legitimate analytical approach must include a means of assessing the precision, dependability, and uniformity of the statistical investigation utilizing its findings of method validation. Furthermore, the validation of analytical techniques is a must around numerous laws in addition to criteria pertaining to facilities. The date a medication is added to pharmaceutical protocols and the moment it was initially made available to consumers usually coincide [1].

In such circumstances, the pharmacopoeias could not have specified the requirements and testing methodologies for these drugs. Thus, it may be possible to develop fresher analytical methods for these drugs. In the process of finding, developing, and producing medications, analytical methods are crucial. Drug combination products, also known as combination products, combine the therapeutic benefits of two or more medications into a single item in order to fulfil the needs of individuals whose work is not formerly treated by them. This requires the invention and development of analytical methods. The analytical chemist in charge of creating and verifying analytical procedures may face significant challenges as a result of these combination products. To verify the identification, purity, potency, and effectiveness of pharmaceutical items, regulatory laboratories employ legitimate test techniques that are produced by these procedures. Identification and measurement of contaminants belong to one of the most crucial steps in creating pharmaceutical procedures for quality and safety [1].

Access this article online

Quick Response Code

Received – 20th July 2024
Initial Review – 28th September 2024
Accepted – 28th October 2024

- **Basic criteria for new method development for drug analysis**

a. The medication is unlikely to be listed as official in any pharmacopoeias.

b. Analytical methods for a medicine may not be permitted in publishing under any specific creation aspect due to interference through formulation excipients.

c. Due to patent laws, appropriate analytical techniques for the drug could not be accessible.

d. There could not be adequate analytical techniques obtainable for assessing the drug through your system.

- **Various reasoning for analytical technique**

Analytical techniques have progressed, and during analytical analyses, HPLC procedures produce vast amounts of data. Even though HPLC is a flexible separation method with many

Correspondence to: Rutik Nitin Patil. Department of Quality Assurance, A.R.A College of Pharmacy, Dhule, India.

Email: rutik.patil57@gmail.com

uses, its many variables, which must be precisely adjusted before each run, can occasionally make the procedure dangerous. Consequently, it becomes imperative to comprehend these methods on a deeper level. Furthermore, the process of optimizing Multiple variables that need to be controlled at the same time in order to achieve the required separations, such as the mobile phase's pH, buffer concentration, rate of flow, column temperature, detector raise length, etc [2]. The chemometric technique is highlighted in all of these studies as a new instrument in the arena of pharmaceutical analysis. The use of laboratory patterns to establish design spaces—a crucial element of the quality with the design approach of analytical methods—was recently covered by Rozet et al [3].

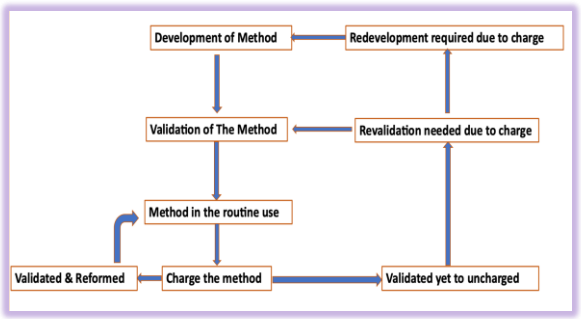


Figure 1: Life cycle of the analytical method [4]

Objectives:

1. Development and Validation of Analytical Methods: From the formulation phase all the way up to the manufacturing of commercial batches, analytical techniques are essential. To make sure that these techniques yield accurate and trustworthy findings, they must be researched and verified.
2. Importance of Validation: Validation guarantees that analytical procedures satisfy specified requirements for system appropriateness, ruggedness, robustness, limit of detection (LOD), limit of quantitation (LOQ), accuracy, specificity, and precision. These criteria are necessary to guarantee the quality of pharmaceuticals.
3. Regulatory Bodies: Validation procedures follow GMP guidelines and are recorded. In order to guarantee the security, effectiveness, and caliber of their goods, pharmaceutical companies must abide by these rules.
4. Stages of Validation: Testing is done on raw materials and final products in every stage of the production method to ensure their stability. This all-encompassing strategy guarantees that the procedure is validated and satisfies necessary requirements.
5. Method Development: In order to assure that analyses are sensitive, selective, and capable of reliably delivering accurate findings under a variety of the settings window, proper method development entails improving and refining these processes.
6. Routine and Stability Testing: Validation parameters are used in stability testing as well as routine analysis, as the latter evaluates the stability of the final product and the pharmaceutical ingredient that is active (API) over time [5,6,7].

METHOD DEVELOPMENT

The development and validation of analytical methods are crucial to the process of making discoveries and producing medications. These techniques are employed to guarantee drug goods' authenticity, potency, purity, and effectiveness. When creating procedures, there are several things to take into account. Initially, data on the analyte's physiochemical properties (*pKa*, log P, and solubility) are recorded, and if UV detection is required, the suitable detection mode is chosen, and if UV detection is needed, the appropriate mode of detection is determined. The validation of a stability-indicating HPLC method constitutes the principal focus of analytical development efforts. Trying to isolate and quantify the primary active ingredient, any reaction impurities, any synthetic intermediates that are accessible, and any degradants are the objectives of the HPLC-method [8, 9].

Criteria for the Development of New Analytical Method:

The identification of the product is predicated on the evaluation procedure of pharmaceuticals. The addition of a medicine to pharmacopoeias and its commercial release date frequently fall on the same day. This is brought on by new toxicity reports, patient resistance developing over time, potential concerns about the prolonged and increased use of these medicines, and the launch of more potent drugs by rival companies. The novel medication or combination of medications might not be classified in any pharmacopoeia. It's possible that analytical methods for quantifying the drug in body fluids are unavailable. The present tests may require the use of pricey materials and chemicals. It may also need time-consuming and sometimes unreliable extraction and separation processes [10, 11].

- **HPLC method development**

1. Recognizing the drug molecule's physicochemical characteristics.

When developing a technique, a medicinal molecule's physicochemical characteristics are crucial. The physical characteristics of the drug molecule, such as its pH, polarity, solubility, and *pKa*, must be studied in order to build a method. One of a compound's physical characteristics is polarity. It aids in the determination of the mobile phase's solvent and composition by the analyzer. The polarity of molecules that are provides an explanation for their solubility. Solvents that are polar, like water, and nonpolar, like benzene, do not combine. pH is characterized as the negative logarithm

of the h+ ion concentration to base 10. The pH scale is -log10[H3O+]. Sharp, symmetrical peaks are consistently observed in HPLC when ionizable analytes are selected at the proper *pH*. Quantitative analysis requires sharply symmetrical heights in order to ensure low relative standard deviations between injections, minimal detection limits, and predictable retention durations [12, 13].

2. The chromatographic conditions chosen: **Selection of column**:

In method development, choosing the stationary phase or column is the first and most crucial step. Without a reliable, high-performing column, it is difficult to design a robust and repeatable procedure. Stable and repeatable columns are crucial to prevent issues with irreproducible sample retention through method development. All samples may normally be separated using a C8 or C18 column, which is highly recommended. These columns are built from carefully purified, lower-acid silica as well as being particularly developed for the separation from basic chemicals [14]. The primary ones are the column diameters, the silica substrate qualities, and the bonded stationary phase features. For a number of physical reasons, silica-based packing is preferred in the majority of current HPLC column [15].

3. Improving the mobile stage

a) Buffer Selection:

The intended pH determines which buffer to use. When reversed phase on silica-based packaging, the usual pH range is 2 to 8. Since buffers regulate pH best at their *pKa*, it is critical that the buffer's *pKa* be somewhat near the intended *pH*. Generally speaking, pick a buffer with *a pKa* value.

General consideration for buffer selection:

a. Phosphate seems to be more soluble in methanol/water than in acetonitrile or THF/water.

b. Due to their significant hygroscopicity, some salt buffers may result in chromatographic changes, including greater tailing of basic compounds and perhaps different selectivity.

c. Ammonium salts tend to dissolve more readily in organic/H20 mobile phases [16].

b) Effect of pH

The *pKa* of the ionizable analyte should be used as a guide to determine the proper mobile-phase *pH*. By doing this, it is guaranteed that the desired analyte is ionized or neutral. One of the most useful tools in the chromatographer's toolbox is the ability to modify the pH of the mobile phase. This feature enables retention and selectivity to be changed simultaneously, offering a tactical way to maximize separation conditions, especially for important component pairings in the sample. In order to customize chromatographic conditions and provide the required separation results, pH modification is essential [17].

c) Effect of organic modifier:

In reverse phase HPLC, choosing the kind of organic modifier is rather straightforward. Methanol and acetonitrile are the most common options (THF is rarely used). Since it would not be possible to elute every component between k (retention factor) 1 and ten using a single solvent strength during isocratic conditions, gradient elution is typically used with complicated multicomponent samples [18].

Choose the detector

The HPLC's detector is an essential component. The chemical composition of the analytes, potential interference, required detection limit, detector availability, and/or detector cost all affect the detector choice. The UV-visible detector is a UV-visible dual-wavelength absorbance detector that is flexible and stays HPLC. The high sensitivity of this detector is required for routine UV-based applications, such as low-level impurity detection and quantitative analysis. The photodiode array (PDA) detector offers advanced optical detection with aqueous analytical HPLC, preparative HPLC, or LC/MS system solutions. The developments in optics and integrated software allow for the achievement of high spectrum and chromatic sensitivity. The ideal choice for analysing components with little to no UV absorption is the Refractive Index (RI) detecting device because of its exceptional sensitivity, stability, and reproducibility. The Multi-Wavelength Fluorescence Detector can measure minute quantities of target chemicals and offers high sensitivity and selectivity fluorescence detection [19, 20].

Technique of separation

a) Isocratic separations

Analyte-eluent and analyte-stationary-phase interactions remain stable over the course of the run when there is an isocratic, constant eluent composition. This also guarantees equilibrium conditions in the column and the real velocity of compounds flowing through the column. Despite the low separation power—the amount of compounds that might be resolved—this increases the predictability of isocratic separations. The peak capacity is modest, and the resulting peak widens as the length of time the component is kept on the column [21].

b) Gradient separation

A system's separation power is greatly increased by gradient separation, mostly due to the abrupt rise in apparent efficiency (reduction in peak width). Peak width decreases in the situation when the chromatographic zone's tail is constantly affected by a more potent eluent composition. Peak width is a function of gradient slope, which measures how quickly the eluent composition changes. Shifting the Gradient Since it might not be possible to elute every component between k

(retention factor) from 1 to 10 using only one solvent's strength during isocratic conditions, gradient elution is used for complicated multicomponent samples. If the ratio is less than 0.25, isocratic would be sufficient; if it is greater than 0.25, gradient will be advantageous [22, 23].

Preparing samples for technique development

It is necessary for the drug material under analysis to be stable in solution (diluent). The solutions should be prepared in amber flasks during the early stages of method development until it is shown that the active ingredient is stable at room temperature and does not deteriorate under typical laboratory circumstances. It is advised to filter the sample solution using 0.22 / 0.45 µm pore-size filtration to remove any particles. For HPLC analysis, filtration is a preventative maintenance technique [24, 25].

Optimization of methods

Determine the shortcomings of approach and use experimental design to strengthen it. Evaluate the approach's effects on different samples, equipment setups, and environmental conditions. This iterative process aids in the methodology's improvement and ensures that high-performance liquid chromatography (HPLC) analysis is robust, reliable, and applicable under a variety of conditions [26].

Validation of methods

The procedure of laboratory testing is carried out to show that an analytical method's performance characteristics match the demands of the planned analytical application is known as validation. Any new or revised procedure has to be validated to make sure it consistently yields reproducible and trustworthy results, regardless of whether it is performed by several operators in various laboratories using the same Utilizing equipment that satisfies requirements, is operational, functional, and accurately calibrated is crucial to the validation process. Analytical methods are carefully examined during the validation process, after which they are either approved for use or rejected if they don't match the necessary standards. This ensures the correctness and dependability for statistical results in multiple uses [27, 28].

Validation

Analyst interpretation is used to validate methods because there is no industry standard for assay validation. Industry committees, regulatory bodies, and the International Conference on Harmonization (ICH) of technical standards for the registration of medicines in human use have all placed a great deal of emphasis on method validation. Methods that have been verified by multiple laboratories are offered by the US Environmental Protection Agency (US EPA), the Resource Conservation and Recovery Act (RCRA), the American Association of Official Analytical Chemists (AOAC), the US Environmental Protection Agency (USP), and other scientific associations [29]. The Food and Drug Administration (US FDA) has put forward criteria on the submission of analytical and sample data for the purpose of technique validation. Particular criteria have been established by the United States Pharmacopoeia (USP) for technique validation and substance assessment [30].

The four most prevalent types of analytical processes are the focus of the debate on their validity.

1. Tests for identification.
2. Measurements that quantify the amount of contaminants.
3. Limit assessments for impurity control.
4. Quantitative analyses of the active ingredient in drug substance or product samples, as well as other specific components within the drug product [31].

Various need to be validated.

1. Whenever the condition for which the technique has been validated changes, such as an instrument with new features, prior to its introduction into regular usage.
2. Whenever modifications are made to the procedure that go beyond its initial parameters [32].

Various parameters are given below:

a) Accuracy

Accuracy is the level to which the value being measured agrees with the true or recognized value. In practical terms, accuracy is the discrepancy between the actual value and the mean value that was produced. To ascertain accuracy, the process is applied to samples with known analyte concentrations. Accuracy is computed as a share of the analyser retrieved by the assay based on test results. It is commonly expressed as the assay-based recovery of the additional analyte levels that are known; this indicates the degree to which the real values are captured by the analytical method [33, 34].

b) Precision

In analytical procedures, precision is associated with the degree of agreement and dispersion between several measurements taken under particular circumstances from different samples of the same homogenous material. A crucial factor in determining the repeatability of the entire analytical process is precision. Precision consists of two elements: intermediate precision and repeatability. Repeatability is the difference between two measurements made by one analyst using the same tool These precision assessment components guarantee a thorough comprehension of the analytical method's repeatability and dependability across various operators and settings [35, 36].

%RSD=std dev.*100/mean

c) Linearity

A technique for analysis is said to be linear if it can yield test results that are precisely matched to the concentration of any analyte in the sample. One way to demonstrate linearity on the test material is to dilute a standard stock solution, or another method is to weigh each component separately in synthetic blends of the test product. The linearity is confirmed via injecting five to six times at least five standards at doses between 80 and 120 percent of the estimated concentration range. A precise mathematical computation must determine the proportionality of the reaction, or it must be proportional right away to the analyte concentrations.

d) Limit of Detection [LOC]

Analysing an analyte's threshold for detection using a single method is the easiest approach to quantifying it in a sample when it can be identified but not accurately measured. Is typically used to express the LOD. Determination: For non-instrumental approaches, the lowest level at which the analyte may be reliably detected is established, and samples with known concentrations of the analyte are analysed to determine the detection limit. ICH Standards: The ICH outlines a standard procedure that involves comparing the observed signal between samples that have known analyte concentrations and those that are blank. It is determined what the analyte's minimal concentration is at which it may be accurately identified. LOD computations pertaining to instrument sensitivity

LOD(mg/L)=3×Noise/Signal

The linearity samples' lowest concentration A 2:1 or 3:1 signal-to-noise ratio is often considered adequate [37, 38, 39].

e) Limit of Quantification (LOQ)

Minimal level of analyte for the purpose of evaluating low analyte levels in test matrices; this limit functions as a quantitative test parameter. The quantitation limit serves as a threshold for accurate quantitative measurements on analytical procedures and is essential for detecting contaminants and/or impurities in samples [40].

A single analytical procedure's limit of quantitation, also known as its quantitation limit, is the smallest quantity of analyte in an experiment that is capable of being quantitatively measured with appropriate precision and accuracy. The LOQ is often determined from a S/N ratio (10:1) for analytical processes that display baseline noise, and it is typically validated by injecting standards that both meet an acceptable percent absolute standard deviation and this S/N ratio [41, 42].

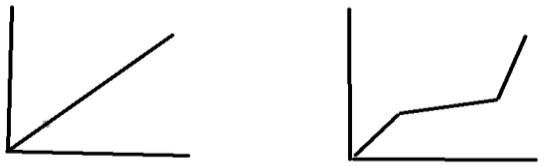
f) Specificity

Every development step should see a specificity in the analytical method. The method should be able to conclusively determine if the target analyte is present in the presence of every anticipated component, including sample blank peaks, excipients, degradants, and sample matrix [43]. To ascertain how long each medication would remain in the sample and in a combination, specificity testing was done. When the standard medications were tested separately, their respective retention times were found to be 3.750 min for nitazoxanide and 1.533 min for ofloxacin. When the drugs were tested together, their respective retention times were found to be 3.760 min with nitazoxanide and 1.542 min for ofloxacin.

g) Range

The analytical techniques range is the range of a compound's concentration inside the sample between its highest and minimum levels at which the linearity, precision, and accuracy of the analytical method can be shown to be sufficient. The range is often given in percentages or parts per million, much like the test results generated by the analytical technique.

1. 80–1200% of the test concentration is used in the experiment.
2. Consistency of content and test concentration between 70–130%
3. Impurities: 120% of the recommended level is the reporting threshold for impurities. test and impurities: up to 120% of the test-specific reporting criteria.
4. Q-20% solubility to 120% [44].



a) Linear b) Non-Linear

Figure 2. Plot for ranges of an analytical technique

h) Robustness

The robustness of an analytical method is defined as the degree of repeatability of test results via the same samples analysed under various standard test conditions, which could include different operators, different laboratories, and possibly different operational along with environmental conditions, while staying within the assay's defined parameters. Testing for ruggedness is typically suggested when the process will be used in several labs. Ruggedness is evaluated by calculating the degree of outcome repeatability as a function of the test variable. This reproducibility could be used to gauge the analytical method's ruggedness by comparing it to the assay's accuracy under typical conditions [45].

i) System suitability determination

Tests for system compatibility are an essential component of liquid chromatographic techniques. They are employed to confirm that the chromatographic systems detection sensitivity, resolution, and repeatability are sufficient for the intended analysis. The foundation of the testing is the idea that

the tools, electronics, processes involved in the analysis, and materials to be examined all work together as a cohesive system that is capable of being assessed as such. Measurements have been made of peak resolution, number of theoretical plates can, peak tailing, and capacity to assess the applicability of the used technique [45].

j) Forced Degradation Studies

In order to purposefully deteriorate the sample, stress experiments or forced degradation are conducted. By producing possible degradation products, these investigations assess an analytical method's capacity to quantify an active chemical and its breakdown products independently. Drug materials are subjected to acid, base, heat, light, and oxidizing agents during method validation, resulting in 10% to 30% degradation of the active ingredient. Forced degradation studies are conducted for various reasons, such as developing and validating stability-indicating methodology, identifying drug substances as well as drug product degradation pathways, and differentiating between degradation products in formulations related to drug substances and those related to non-drug substances [45].

k) Stability Studies

The durability of both samples and standards is determined during validation under typical circumstances, under typical storage settings, and occasionally within the instrument to ascertain whether specific storage conditions—such as refrigeration or light protection are required [45].

CONCLUSION

The aim of every analytical measurement is to generate data that is consistent, accurate, and reliable. These techniques are employed to guarantee drug authenticity, potency, purity, and effectiveness. These state about various parameters and techniques, which shows that are suitable for the procedure and can be developed for the tests. In this article, we have discussed how validation was carried out with the use of specific validation parameters—including linearity, LOQ, LOD, range, robustness, and system suitability. In the pharmaceutical industry, validation is an essential technique that ensures quality is included in the processes that support drug development and production. It assures all parameters and conducts the phases of trails.

REFERENCES

1. FDA Guidance for Industry. Analytical Procedures and Method Validation, Chemistry, Manufacturing, and Controls Documentation, Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER). 2000:234
2. Vander Heyden Y, Nijhuis A, Smeyers-Verbeke J, *et al*. Guidance for robustness/ruggedness tests in method validation. Journal of pharmaceutical and biomedical analysis. 2001; 24(5-6):723-53.
3. Rozet E, Lebrun P, Hubert P, *et al*. Design spaces for analytical methods. TrAC Trends in Analytical Chemistry. 2013; 42:157-67.
4. Chauhan A. harti Mittu B, Chauhan P (2015) Analytical Method Development and Validation: A Concise Review. J Anal Bioanal Tech. 2015; 6(233):2.
5. Shabir GA, John Lough W, Arain SA, *et al*. Evaluation and application of best practice in analytical method validation. Journal of liquid chromatography & related technologies. 2007; 30(3):311-33.
6. Sharma S, Goyal S, Chauhan K. A review on analytical method development and validation. International Journal of Applied Pharmaceutics. 2018; 10(6):8-15.
7. Castelino LJ, Narayanan VA, Fernandes SD, *et al*. Good clinical practices: an Indian perspective. Research journal of Pharmacy and Technology. 2018; 11(7):3209-15.
8. Kavittha KY, Geetha G, Venkatnarayanan R. Development and validation of liquid chromatographic methods for the estimation of drugs in multi-component dosage forms. Pharmacie Globale. 2012; 3(11):1.
9. Sankar Rs. Text Book of Pharmaceutical Analysis. Rx publications; 2010.
10. Shu YZ. Recent natural products based drug development: a pharmaceutical industry perspective. Journal of natural products. 1998; 61(8):1053-71.
11. Chin WW, Parmentier J, Widzinski M, *et al*. A brief literature and patent review of nanosuspensions to a final drug product. Journal of Pharmaceutical Sciences. 2014; 103(10):2980-99.
12. S. Sood, R. Bala, N.S. Gill, Method development and validation using HPLC technique – A review, Journal of Drug Discovery and Therapeutics 2 (22) 2014, 18- 24.
13. Charde MS, Welankiwar AS, Kumar J. Method development by liquid chromatography with validation. International Journal of Pharmaceutical Chemistry. 2014; 4(02):57-61.
14. Isane SP, Waghmare SA, Kamble HV. A Review on Method Development, Validation, Optimization and Applications of HPLC.
15. Md Sabir A, Moloy M, Parminder BS. HPLC Method Development and Validatıon: A Review. Int Res J Pharm. 2013; 4:39-46.
16. Chandrul Kaushal K, Srivastava B. A process of method development: A chromatographic approach. J. Chem. Pharm. Res. 2010; 2(2):519-45.
17. Lindholm J. Development and validation of HPLC methods for analytical and preparative purposes (Doctoral dissertation, Acta Universitatis Upsaliensis).
18. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. John Wiley & Sons; 2012 Dec 3.
19. Dean JA. Analytical chemistry handbook. (No Title). 1995 Apr.
20. TROUBLESHOOTING L. Stability-Indicating Assays. LC• GC Europe. 2002 May: 274.
21. Pirok BW, Gargano AF, Schoenmakers PJ. Optimizing separations in online comprehensive two-dimensional liquid chromatography. Journal of separation science. 2018; 41(1):68-98.
22. Hong DD, Shah M. Development and validation of HPLC stability-indicating assays. Drugs and the Pharmaceutical Sciences. 2000; 107:329-84.
23. Gassmann O, Reepmeyer G, Von Zedtwitz M. Leading pharmaceutical innovation. Berlin: Springer. https://doi. org/10. 2008; 1007:978-3.

24. Br. Jay, J. Kelvin, and B. Pierre, Understanding and Implementing Efficient Analytical Methods Development and Validation, 2003.
25. Huber L. Validation of analytical methods and processes. InPharmaceutical process validation 2003 Mar 27 (pp. 550-567). CRC Press.
26. Mokhtar HI, Abdel-Salam RA, Hadad GM. Development of a fast high performance liquid chromatographic screening system for eight antidiabetic drugs by an improved methodology of in-silico robustness simulation. Journal of Chromatography A. 2015; 1399:32-44.
27. Guidance R. Validation of chromatographic methods. Center for Drug Evaluation and Research (CDER), Washington. 1994; 2.
28. ICH-Q2B. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, validation of analytical procedures: Methodology.
29. G. Ramana Rao, S.S.N. Murthy, and P Khadgapathi, Gas Chromatography to Pharmaceutical Analysis, Eastern Pharmacist, 1987; 30(353): 35. 49.
30. G. Ramana Rao, S.S.N. Murthy, and P. Khadgapathi, High Performance Liquid Chromatography and its Role in Pharmaceutical Analysis, Eastern Pharmacist, 1986; 29(346): 53.
31. R.A. Day, and A.L, Underwood, Quantitative Analyses, (5th Ed., Prentice Hall, New Delhi, 1986.
32. Macek K. Pharmaceutical applications of thin-layer and paper chromatography. (No Title). 1972.
33. Raunig DL, McShane LM, Pennello G, *et al*. Quantitative imaging biomarkers: a review of statistical methods for technical performance assessment. Statistical methods in medical research. 2015; 24(1):27-67.
34. González AG, Herrador MÁ. A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles. TrAC Trends in Analytical Chemistry. 2007; 26(3):227-38.
35. Ravisankar P, Rajyalakshmi G, Devadasu C, *et al*. Instant tips for right and effective approach to solve HPLC trouble shooting. Journal of chemical and pharmaceutical sciences. 2014; 7(3):259-74
36. Breaux J, Jones K, Boulas P. Analytical methods development and validation. Pharm. Technol. 2003; 1:6-13.
37. Guideline IH. Validation of analytical procedures: text and methodology. Q2 (R1). 2005; 1(20):05.
38. United States Pharmacopeial Convention. USP 33 NF 28: United States Pharmacopeia [and] National Formulary. Reissue. Supplement 2. a. United States Pharmacopeial Convention.
39. Tiwari G, Tiwari R. Bioanalytical method validation: An updated review. Pharmaceutical methods. 2010; 1(1):25-38.
40. Nirupa G, Tripathi UM. RP-HPLC analytical method development and validation for simultaneous estimation of two drugs nitazoxanide, ofloxacin and its pharmaceutical dosage forms. Int J ChemTech Res. 2012; 5(1):775-83.
41. Pagare R, Aher S, Bachhav R. A Simple Analytical Method Development and Validation of Azilsartan Medoxomil in Bulk and Pharmaceutical Dosage Form. 1-9.
42. Pharmacopeial Convention, Inc, 2440; 2003.Michel E. Swartz and Ira S Krull; Analytical method development and validation; Special Indian edition; Marcel Dekker, Inc.73-74.
43. Dadhich B, Goyal R, Agarwal D. A Review On: Development and Validation of HPLC in Pharmaceutical Dosage Form. Asian Journal of Pharmaceutical Research and Development. 2020; 8(4):110-21.
44. United States Pharmacopeial Convention. USP 33 NF 28: United States Pharmacopeia [and] National Formulary. Reissue. Supplement 2. a. United States Pharmacopeial Convention.
45. Alsante KM, Ando A, Brown R, *et al*. The role of degradant profiling in active pharmaceutical ingredients and drug products. Advanced drug delivery reviews. 2007; 59(1):29-37.

How to cite this article: Patil RN, Deore BL, Gurav DG. Analytical Method Development and Validation. Indian J Pharm Drug Studies. 2024; Online First.

Funding: None; *Conflicts of Interest: None Stated*