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PERSPECTIVES ON ANALYTICAL METHOD VALIDATION-STRATEGIES AND IMPORTANCE IN PHARMACEUTICAL RESEARCH

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ABSTRACT

Providing proof that a certain technique, method, equipment, material, activity, or system operates as intended under a specific environment and also offers the necessary degree of accuracy, precision, sensitivity, ruggedness, and other properties is the process of validating. A process, element, substance, activity, or system can be validated if it can show that it performs as intended under a certain set of circumstances. When used in reference to an analytical technique, it means that a method consistently produces the desired results whether it is used by the same individuals or other individuals, in the same or various laboratories, with different chemicals or equipment, etc. One of the most crucial tasks that must be completed during the phases of drug discovery, development, and, eventually, approval for sale on the market, is the design of analytical procedures. From formulation research on up to commercial batch manufacturing, designing, optimising, and verifying the pharma product's process is crucial. As a result, the development and manufacturing phases are included in this time frame. The development of drug product methods seeks to offer practical methods for assessing specificity, selectivity, the limit of quantitation, the limit of detection, accuracy, linearity, range, and precision, as well as recovery and solution stability. This article examines the methodology for validating analytical approaches and their significance.

KEYWORDS: Validation, Analytical Methods, HPLC, ICH, USP, BP.

INTRODUCTION

Validation is the process by which a research facility checks to see if the execution qualities of the strategy meet the needs of the proposed application. Before studying any sample, it is necessary to get approval for all proposed investigation methods. The quality of pharmaceutical products needs to stay high so that they can be used safely and effectively. The way things are put together now suggests that quality should be built into the item. You can't count on testing alone to make sure an item is satisfactory.[1] Analytical methods that contain quality criteria must be used to test these items. These quality criteria are incorporated into the process through validation. A crucial yet time-consuming activity for most laboratories is the validation of analytical techniques. Despite this, it is cost-effective, reduces repetitive repeats, and ultimately improves time management.[2]

Analytical techniques must be validated or revalidated when transferred from one lab to another, especially if the strategy parameters for which the technique has been authorised have changed. It is defined as the separation of solutes by a strong differential relocation process in a structure made up of at least two flexible stages, one which moves continuously under a given control and where the different substances exhibit different mobilities due to differences in ingestion, segment, solvency, vapor weight and the atomic sizes and thicknesses of the ionic charge layer. The type of chromatography in which a liquid mobile phase is used is known as "liquid chromatography".[3] In modern HPLC, the mobile phase is elevated at high pressure and pushed through small particle cylinders. Degree of interaction between stationary phase and solute component determines separation. Eluting first is the component that binds to the stationary phase least strongly. HPLC is a popular pharmaceutical analysis technology. Rapid analysis, higher sensitivity, excellent resolution, simple sample recovery, and exact and consistent results are offered by HPLC.[4]

Review of validation definitions

The FDA regulates laboratories, and in order to comply by FDA regulations, these laboratories must verify their analytical procedures. According to the Code of Federal Regulations, there must be data demonstrating that the testing methods meet standards for accuracy and reliability.[5] The International Conference on Harmonization (ICH) recognised and addressed the requirement for standard nomenclature and grasp of essential elements in ICH Guideline Q2(R1), "Validation of Analytical Procedures: Text and Methodology.".[6] The ICH article identifies the components required for validation and makes suggestions.

Additionally, in accordance with ICH Guideline Q2(R1), rather than providing instructions on how to conduct the validations, the material is intended to assist in resolving any inconsistencies between the different compendia and regulators of the European Union, Japan, and the United States. The United States Pharmacopoeia (USP) has added the ICH guidelines from Q2(R1) to its general chapter <1225>, "Validation of Compendial Procedures," to the fullest extent practical.[7] The validation parameters required by the ICH include linearity, accuracy, range, specificity, precision, quantitation limit, detection limit, robustness, and system suitability. Any deviations from USP<1225> have been documented for each element, pharmaceutical product or one or more specifically chosen components.

Method validation strategy[8]

When planning and carrying out validation, the following process should be used:

- Make a plan or set of instructions.
- Define the intended use and scope of the procedure.
- Establish quality measures and assessment criteria.
- Validation of research
- Check equipment performance.
- Specify quality standards and reagents
- Pre-validation trials should be conducted.
- If necessary, alter the parameters of the procedure and/or the approval criteria.
- Perform exhaustive internal and external validation.[9,10]

Significances in pharmaceutical research[11]

It has significantly influenced the growth of analytical science and all of its many applications in areas such as forensics, food, polymers, plastics, therapeutic domains, etc. Components of pharmaceutical medicines meet the criteria nicely.[12] Among the various products that may be consistently examined by HPLC method validation are biochemical, metabolic, and non-volatile substances.[13] It offers a useful method for analysing "labile natural compounds." Additionally, sample introduction and preparation are optimized in pharmaceutical research, along with excellent separation speeds and resolution. The method validation extracts and optimises all of those quite sensitive detections.[14]

Purpose of analytical method development[15]

Drug analysis reveals the identity, description, and quantification of the medicine in mixtures such as bodily fluids and undefined amounts. There are a number of purposes for which analysis tools are used in the production and development of pharmaceuticals, the most important of which are obtaining data on the efficacy, impurity, bioavailability, stability, and impact of production parameters. The efficacy of pharmaceuticals is linked to the amount of active ingredient required, while impurity data is used to determine how safe a drug is.

Internal control seeks to eradicate defects at various phases of production. Management determines whether a product can be unhitched or discarded. The main topic is straightforward analytical approaches to complex formulations. Fast growth in pharmaceutical industries and constant medicine production in various regions of the world has increased demand for new analytical techniques; analytical methodology development has become a quality control laboratory's key activity.[16]

Validation process

Validation requires coordination across regulatory affairs, analytical development, quality assurance, and control. Validation must be well thought out. A few assay validation steps are listed. Due to the numerous equipment, operational considerations, columns, and eluent. HPLC method development looks to be difficult. Although the analytes characteristics influence the procedure, the following stages are often followed[17]

- Step 1 - HPLC technique and system decision
- Step 2 - Choose the introductory circumstances
- Step 3 - Optimizing specificity
- Step 4 - Structure enhancement
- Step 5 - Protocol assurance.

Some of these procedures might not be carried out at all if the substance and analytes are not acceptable for HPLC analysis. Steps 3 and 4 might be avoided if step 2 reveals a good separation. The amount of technique validation (step 5) needed will vary depending on the intended purpose. Contrary to a quality control method, a one-time use validation technique has distinct validation standards. The following elements should be considered while developing an HPLC method. For instance, altering the mobile phase's makeup is the greatest method to increase selectivity. Selectivity would hardly be affected and only slightly changed by temperature. The pH has little to no impact on the retention of weak acids and bases.

Step 1 - HPLC technique and system decision

The initial stage in developing a novel HPLC technique is a literature review. This will show if the separation has been performed in the past and under what circumstances. By doing this, time that would have been wasted on pointless research is saved. It would be ideal to analyse the sample using reverse phase HPLC, however due to the sample's polar analytes, Normal phase(NP- HPLC) would be much more challenging.[18]

Ion pairing and reverse phase ion inhibition (for weak acids or bases) should be utilised for acidic or basic analytes, respectively. The stable phase is the C18-bond. Polar and nonpolar analyte isomers can be distinguished using normal phase HPLC. Working with pure silica is more challenging than working with cyano-bonded stages. An effective method for analysing anions and cations is ion exchange chromatography. Using size validity discrimination, compounds with high molecular weights are considered.

Progressive Chromatography: This stage is not required if the sample has more than 20–30 components since the resolution can handle more peaks. This is caused by gradient HPLC's fixed peak width (Peak width in isocratic HPLC increases according to retention time). This method can also be applied to samples containing active analytes that exceed the allowable range of 0.5–15 capacity factors. Particularly for long-retention analytes, its constant peak width increases sensitivity (Peak height and peak width have an inverse relationship for a given peak area.). In acetonitrile-water with 1% trifluoroethanol acid, reverse phase gradient HPLC studies peptides and small proteins. It examines samples well using gradient HPLC.

Dimensions of the columns: The great majority of samples can have their method development time decreased by employing short columns (10-15 cm). Using these columns results in shorter retention and equilibrium times. The initial flow rate should be in the range of 1-1.5 mL/min. The optimal particle size for packing is between 3 and 5 nm.

Detectors: Consider the following factors. Are chromophores in analytes UV-detectable, is the use of more preferred detection necessary? What are the detection thresholds, and will sample derivatization enhance chromatography/detectability.

There are two types of sensors used for trace analysis: fluorescence and electrochemical. Apart from this Refractive Index is the best option since it can withstand high concentrations without overwhelming the detector.

UV radiation: For the most sensitive results, choose max, which finds all sample parts that contain chromophores. Due to more detector noise, UV wavelengths below 200 nm should not be used. Higher wavelengths give you more choice.

Fluorescence frequency: The wavelength of excitation pinpoints the activation maximum or maximal emissions intensity. The emission intensity is determined by the maximum excitation. The starting system can be chosen based on sample and analyte evaluation, experience, literature data, empirical methods and expert system software.

Step 2 - Choose the introductory circumstances.

By ensuring that no analyte has a capacity factor of less than 0.5 (Poor retention might lead to peak overlapping), this step creates the ideal circumstances for successfully retaining all analytes (Long analytical times and broad peaks with low detectability are caused by high retention).

Solvency is the ability of a solvent to remove analytes from the column, whereas "mobile phase" refers to the liquid that contains the solvent in a column. Organic modifiers are used in reverse phase HPLC as the most polar solvent and as the strongest solvent in normal phase HPLC with water mobile phases. Decide on the solvent concentration that is best for your experiment. The solvent strengths of several samples are probably consistent with the capacity restrictions. pH and ion pairing reagents, or any or both, may have an impact on the retention of the samples. If a sample includes several analytes (between 20 and 30), or a wide range of analyte retention, gradient elution will be required to prevent excessive retention. Moreover, Determination of initial conditions like running two different gradients at different times is a good idea. Use a methanol/water or acetonitrile/water binary system (or aqueous buffer).[19]

Step 3 - Optimizing specificity

This stage promotes selectivity (peak spacing). It's crucial to look at the compositions of the mobile and stationary phases. Only those optimization factors that are thought to significantly affect selectivity should be put to the test. When selecting them, analyzer kind must be taken into account. This classification of analytes is useful.

Parameters for optimization can be defined after analyte types are known. Because it is simpler and more basic, optimising the parameters of the mobile phase is typically preferred

over optimising the parameters of the stationary phase. Acetonitrile/water (or aqueous buffer) or methanol/water systems should be utilised in HPLC to improve gradient selectivity (or aqueous buffer). If the selectivity is poor, an organic modifier may be used.

Step 4 - Structure enhancement

After achieving acceptable selectivity, this is used to optimize resolution and analysis time. Column diameter, particle size, and flow rate are significant. Modifying these parameters won't affect capacity or selectivity.[20]

Step 5 - Protocol assurance

Pharmaceutical analysis requires the validation of analytical procedures since the efficacy and safety of each batch depend on its quality. The capacity of analytical instruments to show any deviation from target criteria under specific circumstances and at a certain degree of sensitivity is necessary for regulating this quality. Regulatory bodies are increasingly requiring analytical technique validation for marketing authorizations; guidelines exist. Before it can be chosen or created, an analytical approach has to be verified. Selecting a method is the first step in creating an analytical technique. It is crucial to evaluate how thoroughly and accurately something will be observed.

Method development and validation are separate steps that follow method selection. Ingredients, excipients, intermediates, bulk items, and finished products should be examined using methods that ensure viable results. Prior to validating a test technique, the criteria must be specified. Analytical procedures must be developed in accordance with GMP and GLP standards (Q2A and Q2B). Both the FDA and the USP cite the International Conference on Harmonization (ICH) as their primary source of guidance. Qualities including accuracy, repeatability (intermediate precision), specificity (detection and quantitation limits are among the most often used) and linearity are also prevalent in analytical validation. Method validation must be specified before it can be used.

Components of method validation

Methods validation often tests the following analytical performance characteristics.[21] Specificity, linearity, range, precision, accuracy, detection and quantification limits, robustness, and toughness Appropriate for the system. What to pick and how to pick the extent to which an analytical method can measure the analyte even while other objects are in the way is referred to as its "selectivity." Impurities, degradants, matrix, and other substances

could also be present. People frequently use the terms "specificity" and "selectivity" interchangeably. Specific means that a method only produces results for one analyte, whereas selective means that it produces findings for multiple chemical entities that may or may not be distinguishable from one another. "Specificity is the pinnacle of Selectivity," asserts the International Union of Pure and Applied Chemistry (IUPAC). The IUPAC advises against using the phrase "specificity." They should instead use the term "selectivity." By separating the analyte from other possible components including degradants, contaminants, excipients, and so on, the specificity of the chromatographic technique is examined. There have also been investigations of forced degradation to put the method to the test. When impurities are scarce, forced degradation studies become even more crucial. The sample is stressed during forced degradation tests because it is subjected to humidity, heat, light, oxidation, and acid/base hydrolysis.

A peak homogeneity or peak purity test can be used to figure out how selective a chromatographic method is. The peak purity test shows that none of the sample components are coming out at the same time. For this, PDA or MS detectors are used to measure the purity of the peak. In the validation report, there should be representative chromatograms with peaks marked, as well as information about the resolution, plate count, and tailing factor.

Linearity

Testing for linearity means that the technique may provide findings that are proportionate to the dilute solution throughout a particular range. As part of HPLC processes, a linear relationship is established between detector response and sample concentration. There are two ways to show the connection: directly on the substance, by adding standard stock to dilute it, or by weighing each sample individually. Plotting signals against concentration of the analyte or content is a good way to check for linearity. If there is a linear connection, relevant statistical techniques, such as regression analysis, should be used to evaluate test data. Mathematical estimations of linearity may be made using data from the linear regression. The variation around the regression line's slope is a frequent way to represent it. Analytical reactions may need to be defined by a concentration-dependent function in some instances.

Precision

In order to determine a method's accuracy, it is necessary to take several measurements of the same homogeneous sample under the same circumstances. It's possible to have precision that

is repeatable, intermediate, or even replicable. Over a short period of time, repeatability is a measure of accuracy. This is what we mean by precision inside the test itself. In order to assess the procedure's accuracy, six samples are analysed in triplicate at 100% concentration, or three samples are analysed in triplicate at three different concentrations. It requires repeated sampling. Intermediate precision expresses variety in labs, such as different days, analysts, and equipment. The USP defines it as "ruggedness." How much intermediate precision is needed depends on how the procedure will be used. For intermediate precision, utilize an experimental design. Multiple sample and reference solutions are utilized to assess intermediate precision. Comparing lab results precisely is called reproducibility. It's usually evaluated when technology or procedures are transferred. Interlaboratory tests evaluate it. Precision is expressed as confidence intervals, standard deviation and relative standard deviation. For important analytes, RSD should be less than 2%. RSD 5-10% is acceptable for low-level pollutants.

Range

Within a certain range, or the analytes maximum and lowest concentrations in the sample, an analytical technique can precisely quantify an analytes levels. Linearity studies are used to figure out the procedure's range, which is then used to figure out how the procedure is actually used. Consider the following minimum ranges:

- For the assay method, 80–120 percent of the test concentration is usually covered.
- Depending on the dosage form, cover at least 70% to 130% of the test concentration to make sure the content is the same.
- ±20% the range given when testing dissolution.
- For figuring out how much impurity there is, from the reported amount of impurity to 120% of the specification. When accuracy, precision, and linearity criteria are met, the range of a method is known for sure.

Accuracy

An analytical method's accuracy is how closely the obtained value matches a true or reference value. No measuring method is perfect, hence the true value can't be determined. By analyzing a known sample's concentration, you can determine the true value for measuring accuracy. Most accuracy investigations involve determining how much analyte was in a manipulated sample or comparing results to a pure reference material. If no placebo is available, conventional addition is utilized. With impurity counting methods, a sample with

a known impurity count is employed. To assess accuracy, at least nine measurements at three concentration levels (three concentrations/three replicates for each analytical technique) should be employed.

The proportion of a known analyte added to the mixture that the assay recovered, or the variation between the mean and the recognized true value, when combined with confidence intervals, are used to assess the test's accuracy. Fill in all the gaps. Sample matrix, processing technique, and analyte concentration all influence recovery. Accuracy levels vary from 97% to 100% for substances and commodities that include drugs. To calculate impurities, 50–150 percent of the average recovery might be utilised.[22]

Limit of detection

A sample's limit of detection is the smallest quantity of analyte that can be detected but not accurately quantified. Setting a detection limit is possible in various approaches. SNR is the simplest method. The signal-to-noise ratio is calculated by comparing the recorded signals from samples having a known low concentration of analyte to blank samples. There should be a 3:1 or 2:1 signal-to-noise ratio in the detection limits. The alternative method uses standard deviation and slope. Detection thresholds are set at

$$LOD = 3.3\frac{\sigma}{s}$$

Where,

σ = the response's standard deviation,

S = the calibration curves' slopes (The slope may be estimated from the calibration curve of the analyte).

The standard deviation of the blank gives σ. The calibration curve can estimate σ. To analyze the calibration curve, use a sample with an analyte around the detection limit. As a standard deviation, utilize a regression line's residual standard deviation or y-intercept standard deviation. The visual examination helps determine the detection limit. This method works with and without instruments. Analyzing samples with known analyte levels helps determine the LOD. Relevant chromatograms prove the detection limit.

Limit of quantitation

Quantitation limit is the least amount of analyte in a sample that can be determined quantitatively. Depends on detector sensitivity and sample preparation. The detection limit

and quantitation limit are found the same way. Its signal-to-noise ratio is 10:1. The formula uses response standard deviation and slope:

$$LOD = 10\frac{\sigma}{s}$$

Where,

σ = the response's standard deviation,

S = the calibration curves' slopes

The value of S and σ are estimated as for the detection limit.

As with LOD, LOQ is based on appearance. At LOQ, the analyte concentration should be accurately quantified. LOQ average recovery should be between 50 - 150%, and RSD should be less than 25%.[23]

Robustness

Robustness measures an analytical procedure's ability to withstand tiny, deliberate changes in method parameters and indicates its reliability during routine use. During technique development, it's partially evaluated. Robustness study identifies technique factors. Warnings should be included in method documentation. HPLC robustness studies change column temperature, flow rate, pH, and mobile phase composition. The most relevant parameter for each condition's system appropriateness is determined. Other robustness research characteristics are analytical solution stability and extraction time. Analytical solution stability is determined by exposing it to method conditions for 4 hrs, 12 hrs, 24 hrs, 48 hrs, etc. Initial value and solution stability time determine acceptance. Compounds, products, and impurities should differ by 2%. After sample filtration, study filter paper. Filtering sample solutions through papers.

System suitability

System suitability testing (SST) is used in many analytical processes. The tests judge equipment, analytical techniques, and samples as a whole. System suitability tests ensure a method's accuracy and precision at a given time. Before or during analysis, a method's appropriateness is tested. If each system suitability test passes acceptance criteria, the method is considered satisfactory. System suitability tests ensure that HPLC methods work every day. SST parameters include resolution (Rs), repeatability (percent RSD of peak response and retention time), column efficiency (N), and tailing factor (Tf), Other SST parameters are and

k.[24] The acceptance criteria respectively Rs >2.0, RSD<1.0% for five replications, N>2000, Tf < 2.0.

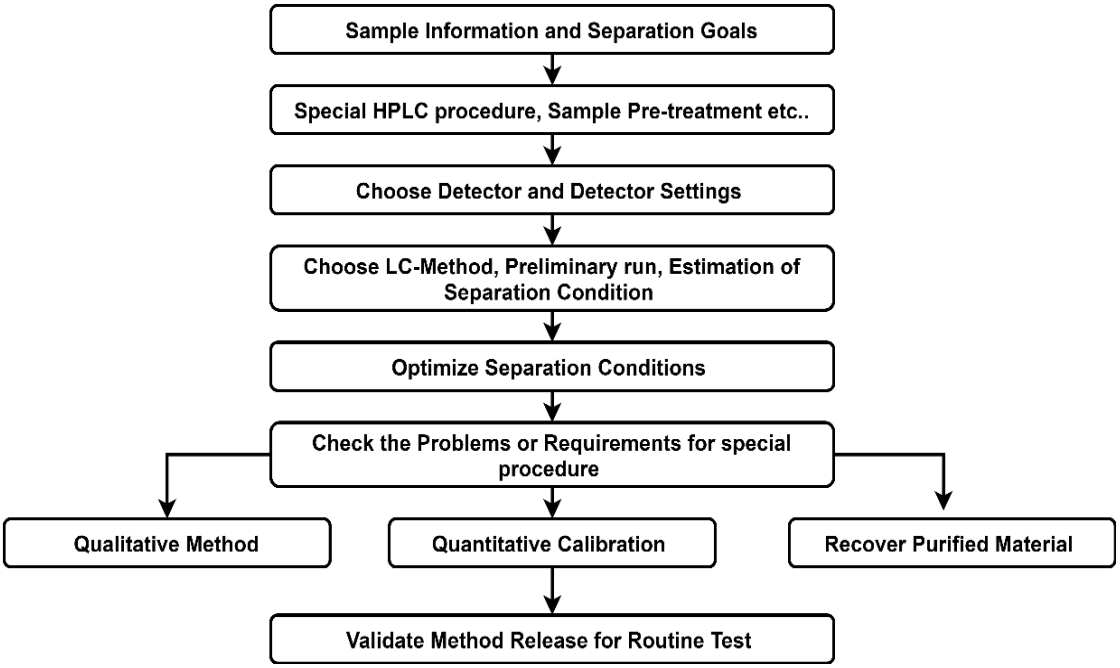

Fig. 1: The scheme for developing and validating HPLC methods.

Table 1: Parameters for method validation Comparison between BP/USP/ICH.[25]

Parameters	BP	USP[26]	ICH[27]
Precision	Yes	Yes	Yes
Accuracy	Yes	Yes	Yes
LOD	Yes	Yes	Yes
LOQ	Yes	Yes	Yes
Specificity	Yes	Yes	Yes
Linearity	Yes	Yes	Yes
Range	Yes	Yes	Yes
Robustness	Yes	Yes	Yes
Ruggedness	-	-	Yes
System suitability	Yes	Yes	Yes

Table 2: Parameters to take into account for various analytical techniques.[28]

Parameters	Quantification of API include Preservative	Impurities in the drug		Performance test (Dissolution, drug release)	Identification Test
		Qualitative test	Limit Test		
Precision	Yes	Yes	-	Yes	Yes
Accuracy	-	Yes	-	Yes	Yes
LOD	-	Yes	Yes	-	-
LOQ	-	Yes	-	-	-
Specificity	Yes	Yes	Yes	Yes	Yes

Linearity	Yes	Yes	-	Yes	Yes
Range	-	-	-		
Robustness	Yes	Yes	Yes	Yes	Yes
System suitability	Yes	Yes	Yes	Yes	Yes

Table 3: Pharmaceutical Linearity and Acceptance criteria.

Test	Linearity Levels and Ranges	Acceptance criteria
Assay	Five levels	Correlation coefficient
	50-150% of label claim	R ≥0.999
Dissolution	Five to eight levels	%y intercept NMT 2.0%
	10-150% of label claim	R ≥0.99
Related substance	Five levels	%y intercept NMT 5.0%
	LOQ to acceptance criteria	R ≥0.99

CONCLUSION

Finally we conclude that the process of validation is dynamic and ongoing. It begins prior to the activation of an instrument and continues after the technique has been created and conveyed. In this review paper, we discussed the significance of validating analytical methods as well as the many types of validation. We can see from what we've discussed so far that a crucial step in producing drugs is the validation of developed analytical procedures. Several crucial elements that are connected to achievement in various fields will support regulatory compliance. As the pharmaceutical business expands, various medications require various methods of analysis. To make sure the product is of good quality, the way it is tested must produce results that are accurate and easy to predict. For this, it must be shown that the method works. HPLC methods are the best way to test something because they work so quickly.

REFERENCE

1. Ostrove, S.A., *Chapter 4 - Getting Started*, in *How to Validate a Pharmaceutical Process*, S.A. Ostrove, Editor. Academic Press: Boston, 2016; 45-58.
2. Chan, C.C., *Principles and Practices of Analytical Method Validation: Validation of Analytical Methods is Time-consuming but Essential.* The Quality Assurance Journal, 2011; 14(3-4): 61-64.
3. García, P., et al., *Analytical Method Validation*, 2011.
4. Nikolin, B., et al., *High perfomance liquid chromatography in pharmaceutical analyses.* Bosn J Basic Med Sci, 2004; 4(2): 5-9.

5. *U.S. Food and Drug Administration. Title 21 Code of Federal Regulations (21 CFR Part 211.194(a)(2)).Laboratory Records*. 2022 [cited 2022 August-2022]; Available from: www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=211.194
6. *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Guideline Q2(R1): Validation of analytical procedures*. 2022; Available from: www.ich.org/LOB/media/MEDIA417.pdf.
7. *United States Pharmacopeial Convention Generalchapter<1225>. Validation of compendial procedures*. 2022 [cited 2022 August-2022]; Available from: http://pharmacopeia.cn/v29240/usp29nf24s0_c1225.html
8. Veerasamy, R., et al., *Validation of analytical methods - Strategies & importance.* International Journal of Pharmacy and Pharmaceutical Sciences, 2010; 2: 18-22.
9. Steyerberg, E.W., et al., *Internal and external validation of predictive models: A simulation study of bias and precision in small samples.* Journal of Clinical Epidemiology, 2003; 56(5): 441-447.
10. Olsen, M.K., K.M. Stechuchak, and K.E. Steinhauser, *Comparing internal and external validation in the discovery of qualitative treatment-subgroup effects using two small clinical trials.* Contemporary Clinical Trials Communications, 2019; 15: 100372.
11. Dong, M. and A. Llanas, *The Essence of Modern HPLC: Advantages, Limitations, Fundamentals, and Opportunities.* Lc Gc North America, 2013; 31: 472.
12. Emara, S., *Development of highly sensitive and specific HPLC assay for plasma morphine using direct injection technique and post-column derivatization.* Biomed Chromatogr, 1998; 12(1): 15-20.
13. Lazzarino, G., et al., *HPLC analysis for the clinical-biochemical diagnosis of inborn errors of metabolism of purines and pyrimidines.* Methods Mol Biol, 2011; 708: 99-117.
14. Sankar, R., et al., *applications in HPLC in pharmaceutical analysis.* International Journal of Pharmaceutical Sciences Review and Research, 2019; 59: 117-124.
15. Ramnarayanan, C., P. Kumar, and K.V. Bibhishan, *Analytical Method Development and Validation for Pre-Clinical Analysis.* Journal of Pharmaceutical Sciences and Research, 2010; 2: 795-803.
16. Hagel, L., G. Jagschies, and G. Sofer, *5 - Analysis*, in *Handbook of Process Chromatography (Second Edition)*, L. Hagel, G. Jagschies, and G. Sofer, Academic Press: Amsterdam, 2008; 127-145.
17. Shabir, G., *HPLC Method Development and Validation in Pharmaceutical Analysis, Handbook for Analytical Scientists*, 2013.

18. Azim, M., M. Moloy, and P. Bhasin, *HPLC METHOD DEVELOPMENT AND VALIDATION: A REVIEW.* International Research Journal of Pharmacy, 2015; 4: 39-46.
19. Raut, R. and J. Shaji, *HPLC method validation for quantification of tetrahydrocurcumin in bulk drug and formulation.* Future Journal of Pharmaceutical Sciences, 2021; 7(1): 42.
20. Yang, S.H., J. Wang, and K. Zhang, *Validation of a two-dimensional liquid chromatography method for quality control testing of pharmaceutical materials.* J Chromatogr A, 2017; 1492: 89-97.
21. Tentu, N.R., *Validation of Analytical Methods*, 2018.
22. Desai, N. and R.D. Senta, *Simultaneous Rp-HPLC determination of salicylamide, salicylic acid and deferasirox in the bulk API dosages forms.* Journal of Taibah University for Science, 2014; 4.
23. Green, J., *Peer Reviewed: A Practical Guide to Analytical Method Validation.* Analytical Chemistry, 2011; 68.
24. Ranher, S., et al., *A validated HPLC method for determination of Artesunate in bulk and tablet formulation.* Journal of Analytical Chemistry, 2010; 65: 507-510.
25. Pieńkowska, K., *Size exclusion chromatography with evaporative light scattering detection as a method for speciation analysis of polydimethylsiloxanes. II. Validation of the method for analysis of pharmaceutical formulations.* Journal of pharmaceutical and biomedical analysis, 2011; 56: 851-8.
26. Porter, D.A., *Review of USP chapter 〈1223〉 validation of alternative microbiological methods presented at the 2007 global conference on pharmaceutical microbiology.* American Pharmaceutical Review, 2007; 10: 76-81.
27. EMA. *ICH Q2(R2) Validation of analytical procedures,* 2022 [cited 2022 July-2022]; Available from: https://www.ema.europa.eu/en/ich-q2r2-validation-analytical-procedures.
28. Bridwell, H., et al., *Perspectives on Method Validation: Importance of Adequate Method Validation.* The Quality Assurance Journal, 2010; 13(3-4): 72-77.