Larix Publications

Journal of Pharma Research
http://www.jprinfo.com/
Vol. 13, Issue 02, 2024 ISSN: 2319-5622
USA CODEN: JPROK3

Review Article

METHOD DEVELOPMENT AND VALIDATION OF HPLC AS PER ICH GUIDELINES - A REVIEW

Pranjali S. Sonune [1], Vaishnavi S. Wagh [1], Pratik S. Kakode [1], Juned K. Bhawaniwale [1], Dr. A. Madhukar [2] *, Dr. K. Raja Rajeswari [3]

[1] B. Pharm IV Year, Department of Pharmacy, Vardhaman College of Pharmacy, Koli, Karanja (Lad), Washim, Maharashtra, INDIA.
*[*2] HOD & Professor, Department of Pharmaceutical Chemistry, Vardhaman College of Pharmacy, Koli, Karanja (Lad), Washim, Maharashtra, INDIA.*
[3] Principal & Professor, Department of Pharmaceutics, Vardhaman College of Pharmacy, Koli, Karanja (Lad), Washim, Maharashtra, INDIA.

Received on: 10-02-2024; Revised and Accepted on: 26-03-2024

ABSTRACT

Today, a wide range of industries, including the pharmaceutical, biotechnology, environmental, polymer, and food sectors, use high-performance liquid chromatography (HPLC) for separations and purifications. A little amount of liquid sample is injected into a moving stream of liquid (referred to as the mobile phase) that travels through a column filled with stationary phase particles to get the desired result. Different levels of retention of each component determine how a mixture separates into its constituent parts. An analytical method called high performance liquid chromatography (HPLC) is effective at separating, identifying, and quantifying a wide range of pharmaceuticals and their metabolites. Additionally, it is used to distinguish manufactured medications from contaminants associated with drugs. During the time of separation, to identify and measure the synthetic drug and to minimize additional contaminants. This review provides details on the several phases that go into developing and validating an HPLC technique. The ICH Guidelines for HPLC method validation address every aspect of performance characteristic related to validation, including robustness, range and limit of quantification, specificity, linearity, accuracy, precision, and linearity, as well as testing the suitability of the system.

Keywords: HPLC, ICH, Method Development and Validation.

INTRODUCTION:

Differential rates of elution are used in HPLC, an analytical technique, to resolve the solutes as they flow along a chromatographic column. The distribution of the mobile phase and stationary phase determines the manner of separation by this instrument. Eight fundamental parts make up the instrumentation: the column, detector, waste reservoir, mobile phase reservoir, solvent supply system, sample introduction device, computer, integrator, or recorder [1].

***Corresponding Author:**

Dr. A. Madhukar
HOD & Professor,
Department of Pharmaceutical Chemistry,
Vardhaman College of Pharmacy,
Koli, Karanja (Lad), Washim, Maharashtra, INDIA.
***E-Mail:** dr.amk2014@gmail.com*
***DOI:** https://doi.org/10.5281/zenodo.10890407*

This technique is widely used like spectroscopy and is a very powerful tool not only for analytical methods but also for preparative methods. Compounds of high-grade purity can be obtained by this method. Chromatography can be simply defined as follows: "It is the technique in which the components of a mixture are separated based upon the rates at which they are carried or moved through a stationary phase (column) by a gaseous or liquid mobile phase" [2].

The preferred technique for determining a new chemical entity's peak purity and tracking reaction changes in synthesis processes or scale up, assessing novel formulations and performing quality assurance/control on the finished pharmaceutical products [3]. The primary medication, any reaction impurities, all accessible synthesis intermediates, and any degradants are to be separated and quantified using the HPLC method [4].

High-Performance Liquid Chromatography (HPLC):

In the late 1960s and early 1970s, High-Performance Liquid Chromatography (HPLC) was created. It is now often used for

© 2012, JPR. All Rights Reserved

purifications and separations in many different contexts. Encompassing the biotechnology, food, polymer, pharmaceutical, and environmental sectors. In the last ten years, HPLC has emerged as the preferred technique for the analysis of a broad range of substances. The primary benefit of HPLC analysis over gas chromatography (GC) is that macromolecules can be analysed because the analytes do not need to be volatile. A tiny amount of liquid sample is injected into a moving stream of liquid (referred to as the mobile phase) and allowed to pass through a column that is filled with stationary phase particles to achieve HPLC. Various degrees of retention determine how a mixture separates into its constituent parts [2].

TYPES OF HPLC

1. Reverse Phase HPLC:

Applications for reverse phase chromatography include analytical and preparative uses in the domain of biochemical purification and separation. Reversed phase chromatography may effectively separate molecules with varying degrees of hydrophobicity while maintaining good recovery and resolution [18]. Employs cyano as the stationary phase and C18 (ODS), C8, phenyl, Trimethyl Silane (TMS), and C8 as the mobile phase. For the majority of samples, particularly neutral or non-ionized chemicals that dissolve in organic mixes of water, it is the method of choice.

2. Normal Phase HPLC:

In this, the cyano, diol, and amino combinations of organic solvents for the mobile phase and columns One possible stationary phase is silica. It is the preferred method for preparative-scale HPLC and isomer mixtures, and the second option for lipophilic materials that don't dissolve well in water-organic combinations [12].

The HPLC principle:

One method of separation in HPLC is injecting a tiny amount of liquid sample into a tube that is filled with tiny particles, known as the stationary phase, with a diameter of 3 to 5 microns (μm). The sample's constituent components are pushed down the densely packed tube, or column, while the liquid, or mobile phase, is driven through the column by high pressure supplied by a pump. Column packing, which includes different chemical and/or physical interactions between the molecules of the constituents and the packing particles, keeps these components apart from one another. A low-through device (detector) that measures the amount of these separated components is found at the tube's (column) exit [5].

Instrumentation:

The contents of the mobile phase are kept inside a glass reservoir. In HPLC, the mobile phase, also known as the solvent, is often a combination of liquid components, both polar and non-polar, the concentrations of which vary based on the makeup of the sample. The components' ability to separate depends on the kind and makeup of the mobile phase. We utilize high-grade solvent for HPLC. For various HPLC types, different solvents are employed. In reverse-phase HPLC, the solvent is typically a combination of water and a polar organic solvent; in normal-phase HPLC, the solvent is typically non-polar. The mobile phase's solvent and inorganic salt purity must be met at all costs [6].

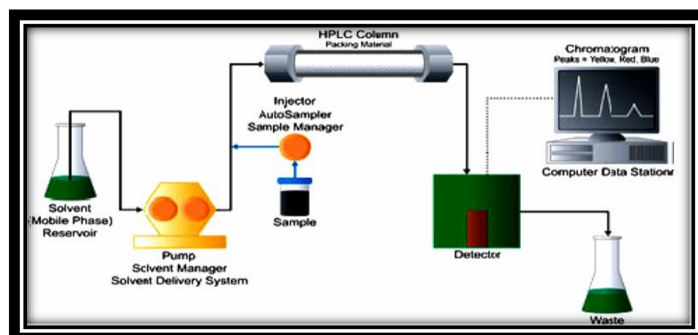

Fig 1: Isocratic HPLC system

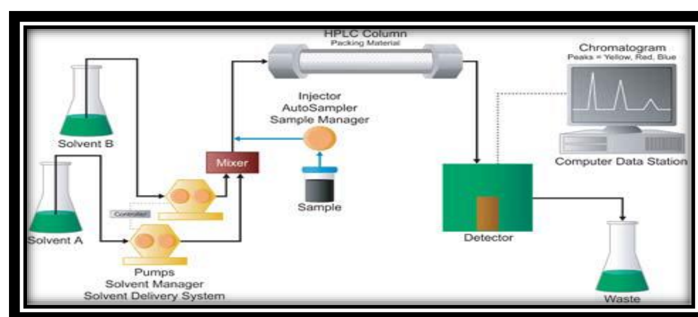

Fig 2: High Pressure Gradient HPLC System

1. Pump:

Primer Similar to the human heart, which continuously pumps blood throughout the body, an HPLC pump must deliver a low of mobile phase at a constant pressure and low rate even though the human heart can tolerate variations in blood pressure within a specified range under stress and strain. Errors in the findings may arise from modifications to either of these parameters. Put simply, the HPLC pump must be durable and capable of consistently producing repeatable\low properties from run to run [7].

The system's column and detector force the mobile phase through after a pump draws it out of the solvent reservoir. Depending on certain variables, such as column, dimensions, the stationary

© 2012, JPR. All Rights Reserved

phase's particle size, the mobile phase's low rate and composition, and operating pressures of up to 42000 kPa (about 6000 psi) can all be produced. The pump's job is to push a liquid through the liquid chromatography system at a precise low rate, measured in millilitres per minute (mL/min). This liquid is known as the mobile phase. In HPLC, 1-to 2-mL/min are considered normal low rates. Normal pumps are capable of pressures between 6000 and 9000 psi (400 and 600 bar). A pump can provide an isocratic mobile phase composition that is consistent throughout the chromatographic experiment [8].

The following qualities are appropriate for a pump:

• Resistance to corrosion and compatibility with solvents

• Easy replacement of worn-out parts;

• Constant low delivery independent of back pressure;

• Low dead volume for minimal issues during solvent changeover.

• Syringe-type pumps, constant pressure pumps, and reciprocating piston pumps are the three most widely used types of pumps.

2. Injector:

An automated injection system or a single injection can be used as the injector. For an HPLC system, an injector should inject the liquid sample at a high pressure (up to 4000 psi) and with good repeatability, ranging from 0.1 to 100 mL in volume. Additionally, the injector needs to be resilient to the high liquid system pressures. When a user has a large number of samples to evaluate or when manual injection is impractical, an autosampler is an automated version. Samples are injected into the mobile phase stream at a consistent volume using injectors. To sustain a high degree of accuracy, injection must be inert and repeatable. The precise volume of sample injected into HPLC [8].

3. Columns:

Typically composed of polished stainless steel, columns range in length from 50 to 300 mm and have an internal diameter ranging from 2 to 5 mm. Typically, they have a stationary phase with particles ranging in size from 3 to 10 µm. One term used to describe columns that have an interior diameter of less than 2 mm is "microbore column." Ideally, during an analysis, the temperature of the column and the mobile phase should remain constant. The stationary phase of the column, which is referred to as the "heart of the chromatograph," uses a variety of physical and chemical characteristics to separate the sample components of interest.

At normal low speeds, the high back pressure is caused by the minute particles inside the column. The mobile phase needs to be forced through by the pump.

Types of Columns:

Guard columns: Before the analytical column, a guard column is added to extend its lifespan by eliminating pollutants and particle matter. From the solvents as well as parts of the sample that bind to the stationary phase permanently. To reduce the amount of solvent lost from the analytical column, the guard column works to saturate the mobile phase with the stationary phase.

Although the guard-column packing's particle size is typically bigger, its composition is identical to that of the analytical column. The guard column is either disposed of or repacked and replaced with a fresh one after it has gotten contaminated.

Analytical Column: The core of high-performance liquid chromatography is analytical columns. The length of liquid chromatographic columns varies from 10 to 30 cm. Typically, the columns are straight, with extra length added as necessary by joining two or more columns. Liquid columns typically have an inner diameter of 4 to 10 mm, while packing particles typically have a size of 5 or 10 m. The most widely used column in use right now is 25 cm long, 4.6 mm in diameter, and filled with 5 mm particles. These columns hold between 40,000 and 60,000 plates per meter [9].

4. Detectors:

Analytes are detected as they elute from the chromatographic column by the HPLC detector, which is positioned at the end of the column. Detectors for evaporative light scattering, UV spectroscopy, fluorescence, mass spectrometry, and electrochemistry are frequently utilized. The individual molecules that elute (come out of the column) can be seen by the detector.

The purpose of a detector is to quantify the number of those molecules so that the chemist may examine the sample's constituent parts quantitatively. The liquid chromatogram, or graph of the detector response, is produced by a recorder or computer using an output from the detector. For the components that the column separates, a detector offers the necessary sensitivity in addition to a specific response. It must remain constant regardless of how the composition of the mobile phase varies [10].

METHOD OF DEVELOPMENT

The process is follows the following steps:

Step 1 - Selection of the HPLC method and initial system

© 2012, JPR. All Rights Reserved

Step 2 - Selection of initial conditions

Step 3 - Method optimization

Step 4 - Method validation

Step 1: Choosing the HPLC technique and starting system:

The first thing to do when creating an HPLC technique is to always look through the literature to find out if the separation has been done before and, if so, under what circumstances. By doing this, you may avoid wasting time on pointless experiments.

A high probability of successfully analysing the sample is necessary when choosing an HPLC system; for instance, if the sample contains polar analytes, reverse phase HPLC would provide sufficient retention and resolution, while normal phase HPLC would be far less practical [11].

Sample collection and preparation: Ideally, the sample should disintegrate during the first mobile phase. If this isn't feasible because Formic acid, acetic acid, or salt can be added to the sample to promote solubility if there are stability or solubility issues. As long as the volume of the loaded sample is less than the volume of the column, these additives typically have no influence on the separation. Applying huge sample volumes may only result in one or more additional peaks eluting in the void volume following sample injection. A crucial component of HPLC analysis is sample preparation, which aims to produce a homogenous, repeatable solution fit for column insertion [12].

Step 2: Choosing the starting conditions:

In this stage, the ideal circumstances to sufficiently retain every analyte are found; that is, guarantees that no analyte has a capacity factor exceeding 10–15 (high retention causes long analysis times and broad peaks with poor detectability) and no analyte has a capacity factor less than 0.5 (poor retention could result in peak overlaps) [11].

Step 3: Optimizing the Method:

After obtaining the proper separations, the experimental conditions should be adjusted to obtain the required separations and sensitivity. Planned/systemic inspection of parameters such as pH (if ionic), mobile phase components and ratio, gradient, flow rate, temperature, sample quantities, injection volume, and diluents solvent type will result in stability suggesting assay experimental circumstances [13].

Step 4: Method validation:

Laboratory investigations establish a process called validation of an analytical method.

Analyse whether the procedure's performance is unique and meets the needs for the purpose for which it is intended. The planned and methodical gathering of validation data by the applicant to support analytical procedures is the first step in the methods validation process for analytical procedures [14].

METHOD OF VALIDATION

Parameters of Method Validation:

The following are analytical performance characteristics which may be tested during methods validation:

1. Specificity
2. Accuracy
3. Precision
    a) Repeatability
    b) Intermediate precision
    c) Reproducibility
4. Limits of Detection and Limit of Quantitation (LOD & LOQ)
5. Linearity
6. Range
7. Robustness
8. Ruggedness

1. Specificity:

Specificity is the ability to assess unequivocally, the analyte in the presence of elements that could be anticipated to exist. These often include matrix, degradants, and contaminants, among other things. Lack of individual specificity further supplementary analytical procedure(s) may be used to make up for the analytical technique. The following are the implications of this definition: Identification: To confirm an analyte's identity [15].

2. Accuracy:

The degree to which a measured value resembles the true or recognized value is called accuracy. Reliability shows the difference between the actual value and the mean value that was discovered. Samples containing known concentrations of analyte are subjected to the procedure in order to ascertain it. To make sure there is no interference, these should be compared to blank and standard solutions. Next, using the test results as a percentage of the analyte recovered by the assay, the accuracy is

© 2012, JPR. All Rights Reserved

computed. The recovery by the test of known, added amounts of analyte is a common way to express it [16].

3. Precision:

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

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

b) Intermediate Precision: Intermediate precision expresses within laboratories variations: different days, different analysts and different equipment, etc.

c) Reproducibility: Reproducibility expresses, the precision between laboratories (collaborative studies, usually applied to standardization of methodology) [15].

4. Limits of detection and Limit of Quantitation:

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, not quantified. It is expressed as a concentration at a specified signal: noise ratio, 2 usually 3:1. The limit of quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. The ICH has recommended a signal: noise ratio 10:1. LOD and LOQ may also be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve(s) at levels approximating the LOD according to the formulae: LOD53.3(SD/S) and LOQ510(SD/S) [11].

5. Linearity:

Linearity is the ability of analytical procedure to obtain a response that is directly proportional to the concentration (amount) of analyte in the sample. If the method is linear, the test results are directly or by well-defined mathematical transformation proportional to concentration of analyte in samples within a given range. Linearity is usually expressed as the confidence limit around the slope of the regression line.

6. Range:

Range is defined as the interval between the upper and lower concentrations of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity [13].

7. Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [15].

8. Ruggedness:

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, analyst, instruments and lots of regents, elapsed assay times, assay temperature or days. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst [17]

Table No. 1: Validation parameters acceptance criteria as per ICH guidelines

Characteristics	Proposed used acceptance criteria
Linearity	R2 > 0.99, similar response ratios
Precision system	RSD < 2%
Precision Method	RSD < 2%
Precision (Repeatability/Reproducibility)	RSD < 2%
Accuracy	FDA 98-102%, EPA 50-150%
Specificity	No interference
Limit of Detection	>2 times base line
Limit of Quantitative	Signal to Noise ratio = 10:1
Range	Concentration where data can be reliably detected

© 2012, JPR. All Rights Reserved

LITERATURE COLLECTION

METHOD DEVELOPMENT

Sample separation

Sample preparation

Chromatographic separation

Quantification

METHOD VALIDATION

1. System Suitability
2. Accuracy
3. Precision
4. Linearity
5. Limit of Detection (LOD)
6. Limit of Quantification (LOQ)
7. Robustness
8. Ruggedness
9. Specificity

Parameter used in HPLC:

1. Retention time:

This is the amount of time that passes between the injection point and the peak maxima's appearance. Additionally, it is defined as the time needed for 50% of an element that has to be removed from a column. It is expressed in seconds and minutes.

2. Retention volume:

The amount of carrier gas needed to extract 50% of the component from the column is known as the retention volume. Retention time multiplied by the low rate yields this result.

3. Separation factor:

Separation factor is the ratio of partition coefficient of the 2 components to be separated. S= Ka /Kb = (tb -to) / (ta -to) Where to = Retention time of unretained substance.

4. Resolution:

The degree of separation between two components and the achieved baseline separation are measured by resolution.

Rs is equal to 2 (Rt1-Rt2) / w1 + w2.

5. Theoretical Plate Equivalent to Height (HETP):

An imaginary or hypothetical unit of a column where the solute distribution between the stationary phase and mobile phase has reached equilibrium is called a theoretical plate.

It is often referred to as the column's functional unit. Any height can be used to represent the efficiency of separation in a theoretical plate. A lower HETP indicates greater column efficiency.

6. Efficiency:

The theoretical plates represent a column's efficiency.

16 Rt2 / w2 = n

as n is the number of theoretical plates.

W = peak width at and Rt = retention time

7. Asymmetry factor:

The center of a chromatographic peak should be symmetrical. However, in reality, the peak is not symmetrical and exhibits tailing or putting forward. Fronting is caused by stationary phase saturation and can be prevented by utilizing a smaller sample size. Because tailing results from more active adsorption sites, support pretreatment can get rid of it.

Applications of High-Performance Liquid Chromatography (HPLC)

The identification, quantification, and resolution of a can all be accomplished by HPLC Combine. The term "preparative HPLC" describes the procedure used to isolate and purify chemicals. This is not the case with analytical HPLC, where the goal is to learn more about the sample substance.

1. Detection of phenolic compounds in drinking water

2. Bio-monitoring of pollutants.

3. Measurement of Quality of soft drinks and water.

4. Sugar analysis in fruit juices.

5. Analysis of polycyclic compounds in vegetables.

6. Urine analysis, antibiotics analysis in blood.

7. Analysis of bilirubin, biliverdin in hepatic disorders.

Advantages and Disadvantages of HPLC:

Advantages:

• Fast and efficient separations (high-resolution power).

• Constant observation of the column effluent.

• It can be utilized for the analysis and separation of extremely intricate mixes precision in numerical measurements.

• Analysis that is repeated and repeatable using the same column.

© 2012, JPR. All Rights Reserved

• Excellently made are the separations by exclusion, partition, ion exchange, and adsorption.

Disadvantages:

• Column performance is very sensitive, which depends on the method of Packing.

• Further, no universal and sensitive detection system is available.

• Very costive, have low sensitivity for certain compounds, and some cannot be detected as they are irreversibly adsorbed.

CONCLUSION

The primary medication and any reaction contaminants must be separated and quantified using the HPLC technique. A liquid serves as the mobile phase in HPLC. The most popular type of HPLC is reversed-phase HPLC type. Reversed phase is characterized by a generally non-polar stationary phase and a moderately polar mobile phase. A number of sample phases are involved in method development; depending on the sample's characteristics, a column and detector are selected; the Sample is dissolved, extracted, purified, and filtered as needed.

An eluent survey (isocratic or gradient) is conducted. The survey is used to determine the type of final separation to be performed, as well as the preliminary conditions for it. Retention efficiency and selectivity are optimized as needed for the separation, and the method is validated in accordance with ICH guidelines.

The development of analytical techniques for drug identification, purity assessment, and quantification has drawn a lot of interest recently in the field of analysis of pharmaceuticals. The development and validation of HPLC methods are generally described in this article. There was discussion of a broad and straightforward strategy for developing an HPLC method for compound separation. It is crucial to understand the physiochemical characteristics of the main component before developing any HPLC methods.

REFERENCES

1. Settle FA, In: Handbook of Instrumental Techniques for Analytical Chemistry. 1st ed, Singapore, Pearson Education Inc.2004.

2. Gerber F, Krummen M, Potgeter H, Roth A, Siff rin C, Spoendlin C. Practical aspects of fast reversed-phase high-performance liquid chromatography using 3 microm particle packed columns and monolithic columns in pharmaceutical development and production working under current good manufacturing practice. J Chromatogr A. 2004 May 21; 1036(2):127-33. doi: 10.1016/j.chroma.2004.02.056. PMID: 15146913.

3. S. Ahuja, H. Rasmussen, Development for Pharmaceuticals, Vol.8 Separation Science and Technology, Elsevier, New York 2007.

4. M.S. Azim, M. Mitra, P.S. Bhasin, HPLC method development and validation: A review, Int. Res. J. Pharm. 4(4) (2013) 39-46.

5. Gerber F, Krummen M, Potgeter H, Roth A, Siff rin C, Spoendlin C. Practical aspects of fast reversed-phase high-performance liquid chromatography using 3 microm particle packed columns and monolithic columns in pharmaceutical development and production working under current good manufacturing practice. J Chromatogr A. 2004 May 21; 1036(2):127-33. doi: 10.1016/j.chroma.2004.02.056. PMID: 15146913.

6. Bergh JJ, Breytenbach JC. Stability-indicating high-performance liquid chromatographic analysis of trimethoprim in pharmaceuticals. J Chromatogr. 1987 Jan 30; 387: 528-31. doi: 10.1016/s0021- 9673(01)94565-0. PMID: 3558640.

7. Haginaka J, Yasuda H, Uno T, Nkagawa T. Alkaline degradation and determination by high-performance-by-high-performance liquid chromatography. Chemical Pharmacy. Bullet. 1984; 32: 2752-2758

8. Fredj G, Paillet M, Aussel F, Brouard A, Barreteau H, Divine C, Micaud M. Determination of sulbactam in biological fluids by high-performance liquid chromatography. J Chromatogr. 1986 Nov 28; 383(1):218-22. doi: 10.1016/s0378-4347(00)83464-7. PMID: 3029153.

9. Polite L. Liquid chromatography: basic overview. In: Miller J, Crowther JB [eds], Analytical chemistry in a GMP environment: a practical guide. John wiley & sons, New York. 2000.

10. Rodenas V, Garcia MS, Sanchez-Pedreno C, Albero MI. Flow-injection spectrophotometric determination of frusemide or sulphathiazole in pharmaceuticals. Journal of Pharmacy and Biomedical Analyst. 1997; 15: 1687-1693.

11. Mohammad T et al., HPLC Method Development and Validation for Pharmaceutical Analysis- A Review. Internationale Pharmaceutica Sciencia. 2012, 2(3), 14.

12. Snyder LR, Kirkland JJ and Glajch JL. In: Practical HPLC Method Development. 2nd ed, 2001.

13. Vibha G et al., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012, 2(4), 22-23.

14. Bliesner DM. In: Validating Chromatographic Methods. John Wliey & sons Inc. 2006, 88-92.

© 2012, JPR. All Rights Reserved

15. Pawar PV et al., Development and validation of UV-HPLC method on tablet dosage form: a review. International Journal of Pharma Research and Development. 2011, 3(1), 187.

16. Hearn Perkin Elmer RA. Www.standardbase.com. In: A Guide to Validation in HPLC Based on the Work of G.M. Holland.

17. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).

18. Principles and Methods. In: Amesham Biosciences of Reversed Phase Chromatography. 6-8.

How to cite this article:

Authors Name. Dr. A. Madhukar. METHOD DEVELOPMENT AND VALIDATION OF HPLC AS PER ICH GUIDELINES - A REVIEW. J Pharm Res 2024; 13(02): 09-16 DOI: https://doi.org/10.5281/zenodo.10890407

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nils

© 2012, JPR. All Rights Reserved