Gadekar Sheetal *et al*, Int. Journal of Pharmaceutical Sciences and Medicine (IJPSM),
Vol.5 Issue. 9, September- 2020, pg. 17-33

ISSN: 2519-9889
Impact Factor: 3.426

QUANTITATIVE ESTIMATION OF CEFOTAXIME SODIUM BY RP – HP IN BULK AND PHARMACEUTICAL DOSAGE FORM

Gadekar Sheetal[1]; Tiwari Archana[2]

Gadekar Sheetal[1], Swami Vivekananda College Of Pharmacy, Khandwa Road, Indore, M.P. India
Email Address: sgadekar684@gmail.com
Tiwari Archana[2], Swami Vivekananda College Of Pharmacy, Khandwa Road, Indore, M.P. India
Email Address: archanatiwari@svcp.ac.in

ABSTRACT: ***The objectives required at each stage of drug development. High performance liquid chromatography is most accurate methods extensively used for the qualitative and quantitative analysis of drug product. Analytical method development and validation play vital role in the drug discovery, Drug development and manufacture of pharmaceuticals. Chromatography, although primarily a separation technique, is mostly employed in chemical analysis in which High-performance liquid chromatography (HPLC) is an extremely versatile technique where analytes are separated by passage through a column packed with micrometer-sized particles. Now a day reversed-phase chromatography is the most commonly used separation technique in HPLC. The reasons for this include the simplicity, versatility, and scope of the reversed-phase method commonly used separation technique in HPLC. This review covers the importance of RP-HPLC in analytical method development and their strategies along with brief knowledge of critical chromatographic parameters need to be optimized for an efficient method development.***

Keywords: ***reverse phase High-performance liquid chromatography, analytical method and development and validation***

INTRODUCTION:

Chromatography is probably the most powerful analytical technique available to the modern chemist. Its power arises from its capacity to determine quantitatively many individual components present in mixture by single analytical procedure [1, 2]. High-performance liquid chromatography (HPLC) is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture[3]. Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification.

ISSN: 2519-9889
Impact Factor: 3.426

Molecules that possess some degree of hydrophobic character, such as proteins, peptides and nucleic acids, can be separated by reversed phase chromatography with excellent recovery and resolution [4]. Method validation is the process used to confirm that the analytical procedure employed for a particular test is suitable for its proposed use. Results from method validation can be used to critic the quality, reliability and consistency of analytical results; it is an essential part of any good analytical practice [8]. High performance liquid chromatography is most accurate methods extensively used for the qualitative and quantitative analysis of drug product. Analytical method development and validation play vital role in the drug discovery, Drug development and manufacture of pharmaceuticals. It includes detection of the purity and toxicity of a drug substance. A number of chromatographic parameters have been evaluated in order to optimize the methods in the analysis of method development in HPLC. An appropriate mobile phase, column, column temperature, wavelength, and gradient are developed [9, 10 High Performance Liquid Chromatography (HPLC) is one of the most widely used analytical techniques in industry. It is used to separate and analyse compounds through the mass-transfer of analytes between stationary phase and mobile phase. The technique of HPLC uses a liquid mobile phase to separate the components of a mixture. The components themselves are first dissolved in a solvent and then forced to flow (via the mobile phase) through a column (stationary phase) under high pressure. The mixture is resolved into its components within the column and the amount of resolution is dependent upon the interaction between en the solute components and the column stationary phase and liquid phase. The interaction of the solute with the mobile and stationary phases can be manipulated through different choices of both solvent and stationary phases. HPLC can be divided into two broad categories, normal phase and reversed phase. For normal phase chromatography, a polar stationary phase (usually silica) is used to retain analytes, which are polar and mobile phase is non-polar (heptane, chloroform, hexane, cyclohexane), whilst reversed phase chromatographic separations are based upon forces between non-polar compounds and non- polar functional groups, which are bonded to the silica support and the mobile phase is polar (methanol, acetonitrile, water or buffer). The majority of applications today are based on reversed phase separations [9, 11, 12, 13].

NEED OF ANALYTICAL METHOD DEVELOPMENT AND VALIDATION:

- Available method may be too expensive, time consuming or energy intensive, or that may not be easily automated.
- Existing method may be too much error, contamination prone or they may be unreliable.
- There may be need for an alternative method to confirm, for legal or scientific reasons, analytical data originally obtained by existing methods.

ISSN: 2519-9889
Impact Factor: 3.426

- There may not be a suitable method for a particular analyte in the specific sample matrix.
- Existing method may not provide adequate sensitivity or regulatory requirements it is required [10.]

The need of validation of the analytical method development and validation emerged due to international competition, maintaining the standard of products in high commercial and market valve and ethical reasons. Various International Regulatory Agencies have set the standard and fixed the protocol to match the reference for granting approval, authentication and registration [3].

It is essential to employ well-characterized and fully validated analytical methods to yield reliable results in the laboratories while analyzing the registration batch and accelerated stability testing samples. It is also important to emphasize that each analytical technique has its own characteristics, which will vary from analyte to analyte. In these instances, specific validation criteria may need to be developed for each analyte.

CHROMATOGRAPHY

Chromatography is a technique employed for separation of the components of mixture by continuous distribution of the component between two phases. One phase moves (mobile phase) over the other phase (stationary phase) in a continuous manner. When the stationary phase is a solid support of adsorptive nature and mobile phase is liquid or gaseous phase it is called Adsorption Chromatography1Chromatography according to USP can be defined as a procedure by which solute are separated by a differential migration process in a system consisting of two or more phases, one of which move continuously in a given direction[10].

Theory of Chromatography[9]

Two theoretical approaches have been developed to describe the processes involved in the passage of solutes through a chromatographic system.

1. The Plate Theory[9]

According to Martin and Synge, a chromatographic system consists of discrete layers of theoretical plates. At each of these, equilibration of the solute between the mobile and stationary phases occurs. The movement of solute is considered as a series of stepwise transfers from plate to plate.

ISSN: 2519-9889
Impact Factor: 3.426

2. The Rate Theory[9]

This theory considers the dynamics of the solute particles as it passes through the void space between the stationary phase particles in the system as well its kinetic as it is transferred to and from the stationary phase. Phases of Chromatography[7]

1. Normal Phase Mode:[7]

In Normal Phase mode the stationary phase is polar and the mobile phase is non polar in nature. In this technique, non polar compounds travel faster and are eluted first. This is because of the lower affinity between the non polar compounds and the stationary phase. Polar compounds are 1retained for longer times because of their higher affinity with the stationary phase. These compounds, therefore take more times to elute. Normal phase mode of separation is therefore, not generally used for pharmaceutical applications because most of the drug molecules are polar in nature and hence take longer time to elute.

2. Reversed Phase Mode:[7]

It is the most popular mode for analytical and preparative separations of compound of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is non polar hydrophobic packing with octyl or octa decyl functional group bonded to silica gel and the mobile phase is polar solvent. The polar compound gets eluted first in this mode and non polar compounds are retained for longer time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for longer times and hence elute faster. The different columns used are Octa Decyl Silane (ODS) or C18, C8, C4, (in the order of increasing polarity of the stationary phase). An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complexation) to control retention and selectivity.

3. Ion Exchange Chromatography:[7]

The stationary phase contains ionic groups like NR_3^+ or SO_3^- , which interact with the ionic groups of the sample molecules. This is suitable for the separation of charged molecules only. Changing the pH and salt concentration can modulate the retention.

4. Ion Pair Chromatography:[7]

This technique is also referred to as Reversed Phase Ion Pair Chromatography or Soap Chromatography. It may be used for the separation of ionic compounds and this method can also substitute for Ion Exchange Chromatography. Strong acidic and basic compounds may be

ISSN: 2519-9889
Impact Factor: 3.426

separated by reversed phase mode by forming ion pairs (coulumbic association species formed between two ions of opposite electric charge) with suitable counter ions.

5. Affinity Chromatography:[7]

This technique uses highly specific biochemical interactions for separation. The stationary phase contains specific groups of molecules which can adsorb the sample if certain steric and charge related conditions are satisfied. This technique can be used to isolate proteins, enzymes as well as antibodies from complex mixtures.

6. Size Exclusion Chromatography:[7]

It separates molecules according to their molecular mass. Largest molecules are eluted first and the smallest molecules last. This method is generally used when a mixture contains compounds with a molecular mass difference of at least 10%. This mode can be further subdivided into gel permeation chromatography (with organic solvents) and gel filtration chromatography (with aqueous solvents).

Separation Techniques[7]

1. Isocratic Elution: When the mobile–phase composition does not change throughout the course of the run, it is said to be isocratic. A mixed mobile phase can be delivered at a constant ratio by the pumps themselves or the solvent mixture can be prepared prior to analysis and pumped through a single reservoir. This is the simplest technique and should be the method of first choice when developing a separation.

2. Gradient Elution: HPLC can be performed with changes in composition of mobile phase over time (gradient elution). The elution strength of the eluent is increased during the gradient run by changing polarity, pH or ionic strength. Gradient elution can be a powerful tool to separate mixtures of compounds with widely different retention.

Quantitative Analysis in HPLC[7]

Three methods are generally used for quantitative analysis.

1. External Standard Method[7]

The External Standard Method is the simplest of the three methods. The accuracy of this method is dependent on the reproducibility of the injection of the sample volume. To perform this method, a standard solution of known concentration of the compound of interest is prepared. A fixed amount, which should be similar in concentration to the unknown, is injected. Peak height or area is plotted versus the concentration for each compound. The plot should be linear and pass

ISSN: 2519-9889
Impact Factor: 3.426

through the origin. The concentration of the unknown is then determined based on Regression Equation.

2. Internal Standard Method[7]

The Internal Standard Method tends to yield the most accurate and precise results. In this method, an equal amount sample, is added to both the sample and standard solutions. The internal standard selected should be chemically similar to the analyte, have a retention time close to that of the analyte and derivatise in a similar way to the analyte. For biological samples, the internal standard should extract similarly to the analyte without significant bias toward the internal standard or the analyte. Additionally, it is important to ensure that the internal standard is stable and that it does not interfere with any of the sample components. The internal standard should be added before any preparation of the sample so that extraction efficiency can be evaluated. Quantification is achieved by using ratios of peak height or area of the component to the internal standard.

3. Standard Addition Method[7]

In the standard addition method, a known amount of the standard compound is added to the sample solution to be estimated. This method is suitable if sufficient amount of the sample is available and is more realistic in the sense that it allows calibration in the presence of excipients or other components.

System Suitability Parameters[7]

System suitability parameters are used to determine the limits of the suitability of the chromatographic system. This approach facilitates the investigation of the worst case scenario, which reflects minimum performance standard used to ensure that the chromatography is not adversely affected. SST parameters studied are as follows:

1. Resolution (Rs):[7] Resolution is the parameter describing the separation power of the complete chromatographic system relative to the particular components of the mixture. The resolution, Rs, of two neighboring peaks is defined as the ratio of the distance between two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of Rs is 1.5.

2. Capacity Factor (k'): [7] Capacity factor is the ratio of the reduced retention volume to the dead volume. Capacity factor, k', is defined as the ratio of the number of molecules of solute in the stationary phase to the number of molecules of the same in the mobile phase. Capacity factor is a measure of how well the sample molecule is retained by a column during an isocratic separation. The ideal value of k' ranges from 2-10.

ISSN: 2519-9889
Impact Factor: 3.426

3. Selectivity (a):[7] The selectivity (or separation factor), a, is a measure of relative retention of two components in a mixture. Selectivity is the ratio of the capacity factors of both peaks, both peaks, and the ratio of its adjusted retention times. Selectivity represents the separation power of particular adsorbent to the mixture of these particular components. This parameter is independent of the column efficiency; it only depends on the nature of the components, eluent type, and eluent composition, and adsorbent surface chemistry. In general, if the selectivity of two components is equal to 1, then there is no way to separate them by improving the column efficiency. The ideal value of a is 2.

4. Column Efficiency:[7] Efficiency, N, of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Similar the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 5,000 to 100,000 plates/meter are ideal for a good system.

Peak asymmetry factor (Tf):[7] Peak asymmetry factor(Tf), can be used as a criterion of column performance. The peak half width, b, of a peak at 10% of the peak height, divided by the corresponding front half width, a, gives the asymmetry factor. For a well packed column, an asymmetry factor of 0.9 to 1.1 should be achievable.

Validation:

Method validation is the process by which it is established that performance characteristics of the method meet the requirements for the intended analytical applications. Methods need to be validated or revalidated before their introduction into routine use. The International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use2 has developed a text on the validation of analytical procedures. The United States Food and Drug Administration (USFDA) have proposed guidelines on submitting samples and analytical data for methods validation5-7. The United States Pharmacopoeia (USP) has published specific guidelines for method validation for compound evaluation8. The document includes definitions for eight validation[14]

Definition:

- Documented evidence that the manufacturing process consistently produces product that meets predetermined specifications.
- Manufacturing process validation consists of successfully manufacturing at least three full-scale batches in succession, which pass all in-process and product quality attributes.

ISSN: 2519-9889
Impact Factor: 3.426

Benefits of process validation

- Increased throughput
- Reduction in rejections and reworks
- Reduction in utility costs
- Avoidance of capital expenditures
- Fewer complaints about process related failures
- Reduced testing in process and finished goods
- More rapid and accurate investigations into process deviations
- More rapid and reliable start-up of new equipment
- Easier scale-up from development work
- Easier maintenance of the equipment
- Improved employee awareness of processes
- More rapid automation

Validation Process:

Successful validation requires cooperative efforts of several departments of organization including regulatory affairs, quality control, quality assurance and analytical development. Therefore a well-planned process should be followed during validation. Possible steps for a complete method validation are listed below:

Steps in method validation [16-17]:

1. Develop a validation protocol or operating procedure for the validation
2. Define the application, purpose and scope of the method
3. Define performance parameters and acceptance criteria
4. Define validation experiments
5. Verify relevant performance characteristics of equipment
6. Qualify materials, e.g. standards and reagents
7. Perform pre-validation experiments
8. Adjust method parameters and acceptance criteria if necessary
9. Perform full validation experiments
10. Develop SOPs for executing method in routine
11. Define criteria for revalidation
12. Define type and frequency of system suitability tests for routine
13. Document validation experiments and results of validation.

ISSN: 2519-9889
Impact Factor: 3.426

Validation protocol

Validation protocol prepared is a document that indicates the company's approach for validation[16]. It ensures consistent and efficient execution of validation projects and also answers auditor during audits. The validation protocol is an ideal tool for training all the employees working for validation. The validation protocol should include:

1. Introduction: Firms validation policy, general description
2. Organizational structure: Description of all personal responsibilities for all validation activities
3. Process and product description: Makes a brief description of the process and product or reference to adequate documents.
4. Specific process considerations: describes critical characteristics of the process.
5. Key acceptance criteria: General statement on acceptance criteria for the process.
6. Documentation format: The format used for protocol and report is described.
7. Required SOPs: a list of relevant SOPs should be mentioned.
8. Planning and Scheduling: describes the resources, equipments and chemicals to be used, including time plan of the project.
9. Change control: includes description or reference to the critical parameters variations in the process or product.

Validation Parameters

The analytical methods which need to be validated are classified as per ICH are classified as Following : [18]

Identification tests: To ensure identity of an analyte. Quantitative test for impurities: to accurately and quantitatively reflect the purity of a sample

Limit test for impurities: to reflect purity characteristics of the sample

Assay of drug substance and drug products: to measure accurately and quantitatively the analyte present in the sample. These methods also include analysis for content uniformity an measurement of analyte from dissolution samples. The characteristics which need to be validated for the different types of method are also mentioned in ICH guidelines[18].

ISSN: 2519-9889
Impact Factor: 3.426

These are tabulated below in table 1.

characteristics	Identification	Test for impurities		Assay
		Quantitative	Limit test	
Accuracy	X	√	X	√
Precision		√	X	√
Repeatability	X	√		√
Intermediate precision	X	√	X	√
Specificity	√	√	√	√
Detection limit	X	X	√	X
Quantitation limit	X	√	X	X
Linearity	X	√	X	√
Range	X	√	X	√

√ indicate this need to be evaluated

x indicate this need not to be evaluated

The united state pharmacopoeia (USP) has classified these methods into four categories and also specifies which parameters to be considered for validation of different types of methods [7].

Category I: Analytical methods for quantitation of measurement of bulk drug substances or active ingredients including preservatives in finished pharmaceutical products.

Category II: Analytical methods for determination of impurities in bulk drugs or for the determination of degradation compounds in finished pharmaceutical products.

Category III: Analytical methods for the determination of performance characteristics (e.g. dissoldissolution, drug release).

Category IV: identification tests.

ISSN: 2519-9889
Impact Factor: 3.426

Table 2: characteristics required for the validation as per USP

analytical performance characteristics	Category I	Category II		Category III	Category IV
		Quantitative	Limit test		
Accuracy	√	√	*	*	
Precision	√	√		√	
Specificity	√	√	√	*	√
Limit of detection			√	*	
Limit of quantitation		√		*	
Linearity	√	√		*	
Range	√	√	*	*	
Ruggedness	√	√		√	

√ indicates the parameter need to be considered

* indicates parameter may be considered depending on the nature of the test.

The parameters for validation need to be selected as per the regulatory requirements. The parameters considered in chromatographic method validation are discussed below.

Selectivity and Specificity

Selectivity of the analytical method is defined as the degree to which a method can quantify the analyte in the presence of inerferents[20]. The other components which may be present include impurities, degradants, matrix, etc. The term specificity and selectivity is often used interchangeably. The term specific generally refers to a method that produces a response for a single analyte only, while the term selective refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other.

The International Union of Pure and Applied Chemistry (IUPAC) have expressed the view that "Specificity is the ultimate of Selectivity'. The IUPAC discourages use of the term specificity and instead encourages the use of the term selectivity[21].

ISSN: 2519-9889
Impact Factor: 3.426

Linearity

Linearity of a method is its ability to obtain test results that are directly proportional to the sample concentration over a given range. For HPLC methods, the linear relationship between detector.response (peak area and height) and sample concentration is determined. The relationship can be demonstrated directly on drug substance by dilution of standard stock or by separate weighing of the sample components, using the proposed procedures.

Table 3: Linearity ranges and Acceptance criteria for various pharmaceutical methods

Test	Linearity levels and ranges	Acceptance criteria
Assay	Five levels, 50-150% of label claim	Correlation coefficient, R ≥0.999
Dissolution	Five to eight levels, 10-150% of label claim	% y intercept NMT 2.0% R ≥0.99
Related substances	Five levels, LOQ to acceptance criteria1	% y intercept NMT 5.0% R ≥0.99

Precision

Precision of an analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Repeatability is the precision under the same operating conditions over a short interval of time. It is also termed as intra-assay precision. The precision data is generally expressed in the form of standard deviation, relative standard deviation and confidence intervals. To ensure precision of method for major analytes, RSD should be ≤ 2 %. For low level impurities, RSD of 5-10 % is usually acceptable [23].

The precision data is generally expressed in the form of standard deviation, relative standard deviation and confidence intervals. To ensure precision of method for major analytes, RSD should be ≤ 2 %. For low level impurities, RSD of 5-10 % is usually acceptable [23].

ISSN: 2519-9889
Impact Factor: 3.426

Range

Range of an analytical method is the interval between the upper and lower concentration of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The range is normally derived from the linearity studies and depends on the intended application of the procedure. The following minimum specified ranges should be considered[24]:

• For the assay method, normally covering from 80 to 120 percent of the test concentration.

• For content uniformity, covering minimum of 70 to 130 percent of the test concentration, based on the nature of the dosage form.

• For dissolution testing, ±20 % over the specified range.

• For impurity determination, from reporting level of impurity to 120 % of the specification. The range of a method is confirmed when linearity, accuracy and precision criteria are fulfilled[25].

Accuracy

The accuracy of an analytical method expresses the closeness of agreement between the value accepted either as a conventional true value or an accepted reference value and the value found. Practically no measurement process is ideal, therefore, the true or actual value cannot be exactly known in any particular measurement. The accepted true value for accuracy assessment can be assessed by analyzing a sample with known concentration. The accuracy studies are usually carried out by determining the recovery of the spiked sample of analyte into the matrix of the sample (a placebo) or by comparing the result to the results of a certified reference material of known purity.

Limit of Detection

The limit of detection of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantified as an exact value. The detection limit can be determined in different ways. The simplest approach is based on the signal to noise ratio. The signal to noise ratio is determined by comparing measured signals from samples with known low concentration of analyte with those of blank samples. The concentration showing signal to noise ratio between 3:1 or 2:1 is generally considered as acceptable detection limit. The other approach is based on the standard deviation of the response and the slope. The detection limit may be expressed as:

LOD = $3.3(Sy/S)$

Where, σ = the standard deviation of the response

ISSN: 2519-9889
Impact Factor: 3.426

S = the slope of the calibration curve The slope may be estimated from the calibration curve of the analyte. The σ can be estimated as the standard deviation of the2.6.

Limit of Detection The limit of detection of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantified as an exact value. The detection limit can be determined in different ways.

The simplest approach is based on the signal to noise ratio. The signal to noise ratio is determined by comparing measured signals from samples with known low concentration of analyte with those of blank samples. The concentration showing signal to noise ratio between 3:1 or 2:1 is generally considered as acceptable detection limit.

Limit of Quantitation

The Quantitation limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. It is mainly affected by the detector sensitivity and accuracy of sample preparation. The Quantitation limit can be determined in the similar way as that of the detection limit. It is the concentration showing signal to noise ratio of 10:1. Based on the standard deviation of the response and the slope it is calculated by the formula:

LOQ = $10(Sy/S)$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve The value of S and σ are estimated as for the detection limit.

The LOQ can also be established from the visual evaluation as the LOD. The analyte concentration should be quantifiable with acceptable accuracy and precision at LOQ level. Typical acceptance criteria for LOQ are mean recovery at this level between 50 – 150 % with % RSD of ≤ 25 %.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is partially evaluated during method development stages. The aim of the robustness study is to identify the criti operating parameters for the successful implementation of the method. These parameters should be adequately controlled and a precautionary statement included in the method documentation.

ISSN: 2519-9889
Impact Factor: 3.426

System suitability

System suitability testing (SST) is an integral part of many analytical procedures. The tests are based on the concept that the equipment, analytical operations and samples are the integral part of the system that can be evaluated as such. System suitability test provide the added assurance that on a specific occasion the method is giving, accurate and precise results. System suitability test are run every time a method is used either before or during analysis. The results of each system suitability test are compared with defined acceptance criteria and if they pass, the method is deemed satisfactory on that occasion. In case of HPLC methods, system suitability tests ensure the adequacy for performing the intended application on daily basis. The primary SST parameters considered are resolution (R s), repeatability (% RSD of peak response and retention time), column efficiency (N), and tailing factor (T f). The other SST parameters include retention factor (k) and separation factor (α). The limits which are considered for the SST parameters are listed table 5[23.]

Table 4: Limits for system suitability tests

SST	**Limits**
Resolution (R_s)	>2.0
Repeatability (RSD)	<1.0% for five replicates
Plate count (N)	>2000
Tailing factor (T_f)	≤2.0
Separation factor (α)	>1.0

Conclusion

This review describes about RP- HPLC technique. The growing pharmaceutical industry demands various analytical methods for various pharmaceutical products. To ensure quality of the product, The HPLC methods are the preferred methods of analysis due to their responsiveness. high-Performance Liquid Chromatography has a wide variety of uses in many fields such as analysis, and identification of pharmaceutical drug, impurities. Optimized method in validated with various parameter.

ISSN: 2519-9889
Impact Factor: 3.426

REFERENCES:

1. Scott RPW: Principles and Practice of Chromatography. Chrom-Ed Book Series, 2003; 1-2.
2. Chatwal GR, Anand SK : Instrumental Methods of Chemical Analysis. 5th edition 2004. 1.1-1.3, 2.566-2.2.575.
3. High performance liquid chromatography [Internet]. 2009 [Accessed 2009 Jan 20]. Available from: en. wikipedia.org/wiki/file:Agilent1200HPLC.jpg.html.
4. Renlund S, Erlandsson I, Hellman U, Silberring J, Wernstedt C, Lindström L, Nyberg F: Micropurification and amino acid sequence of -casomorphin-8 in milk from a woman with postpartum psychosis Peptides.β 1993;14 :1125-1132
5. Sanjay Kumar D and D.R. Harish Kumar: Department of Pharmaceutical Analysis, Published by 01 December, 2012
6. R. G. Sapkal College of Pharmacy, Anjaneri, Nashik – 422213, Maharashtra, India. Department of quality assurance techniques
7. Shethi P.D. Quantitative Analysis of Pharmaceutical Formulations, 1st Edition 2001, 11.
8. Potdar MA, Pharmaceutical Quality Assurance, 2nd Edition, Pune, Nirali Prakashan, 2007.
9. Martin A.J.P., Synge R.L.M. Biochem J 1941; 35:1358.
10. United States Pharmacopoeia and National Formulary, (24th) Asian Edition, The United States Pharmacopoeia Convention Inc., U.S.A. 2003, 2126.
11. International Conference on Harmonization (ICH), Q2B: Validation of Analytical Martindale, The Complete Drug Reference; Pharmaceutical Press: London 2002, 33rd edition, 321-323.
12. International Conference on Harmonization (ICH), Q2A: Text on Validation of Analytical Procedures: Definitions and Terminology, Vol. 60, US FDA Federal Register, 1995.
13. Quality Assurance of Pharmaceuticals, (A compendium of guidelines and related materials), (1997), Geneva, 119-124.Volume I, WHO,
14. Agalloco J. Validation: an unconventional review and reinvention, PDA J Pharm Sci Tech, 1995, 49, 175–179.
15. Lingnau J. Optimization and Validation of Manufacturing Processes, Drug Dev Ind Pharm, 1989, 15, 1029-1046.
16. Songara R. and Prakashkumar A. (2011), Overview of Analytical Method Validation in Pharmaceutical Industries. IJPI's Journal of Analytical Chemistry. 1(5).10-20.
17. Ravichandran V, Shalini S, Sundram K. M. and Harish R. (2010), Validation of Analytical Methods- Strategies and Importance. Int J Pharmacy and Pharm Sci. 2(3), 18-22.
18. ICH Q2A (1994), Text on Validation of Analytical Procedures. Geneva Q2A, in 2005 incorporated in Q2(R1)
19. USP32- NF27, General Chapter 1225, Validation of Compendial Methods, 2009.
20. IUPAC. (2002), Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis, IUPAC Technical Report. Pure Appl.Chem., 74(5). 15.
21. Shah B. P., Jain S., Prajapati K. K. and Mansuri N. Y. (2012), Stability Indicating HPLC Method Development: A Review. Int J Pharm Res Sci. 3(9), 2978-2988.
22. Dong M. W. (2006), Regulatory Aspects of HPLC Analysis: HPLC System and Method Validation, Modern HPLC for Practicing Scientists. 1 st ed. John Wiley and Sons. New Jersey.

ISSN: 2519-9889
Impact Factor: 3.426

23. Snyder L. R., Kirkland J. J. and Glajch J. L. (1997), Completing the Method: Validation and Transfer, in Practical HPLC Method Validation, 1 st ed. John Wiley and Sons. New York.240
24. Eurachem (1998), The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics. Available at www.eurachem.ul.pt.
25. imidar M. I., Heylen P. and Smet M. D. (2007), Method Validation in HPLC Method Development for pharmaceuticals. Edited by Satinder Ahuja and Henrik Rasmussen. Academic Press. London.

A Brief Author Biography

Sheetal Gadekar – Ms. Sheetal Gadekar, has obtained her B.pharma from N.M.T. Gujarati college of pharmacy indore, M.P. india, and presently pursuing M.pharma from Swami Vivekananda College Of Pharmacy, Khandwa Road, Indore, M.P. India her keen interest is in analytical pharmacology, and medicinal chemistry.

Archana Dubey Tiwari – Mrs. Archana Dubey Tiwari, has obtained her B.pharma Swami Vivekananda College Of Pharmacy, Khandwa Road, Indore, M.P. India M.pharma (pharmaceutical chemistry), with Hons from Swami Vivekananda College Of Pharmacy, Khandwa Road , Indore, M.P. India, Phd pursuing from sage university, indore M.P. She is presently working us associated Professor, department of pharmaceutical chemistry Swami Vivekananda College Of Pharmacy, Khandwa Road, Indore, her keen interest organic chemistry, biochemistry, pharmaceutical analysis and medicinal chemistry.

