International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 977-988 www.ijprajournal.com ISSN: 2456-4494

A Review Article on Method Development and Validation of Verapamil by RP-HPLC Method

Raghuveersinh Parmar [1]*, Dr. Priyanka Patil [2], Dr.Chainesh Shah, [3], Dr.Umesh Upadhyay[4]

PG Scholar, M.PHARM (Q.A.), Sigma Institute of Pharmacy,Faculty of Pharmacy,Sigma University, Bakrol, Vadodara-390019

Director & Professor,Faculty of Pharmacy,Sigma University,Bakrol, Vadodara- 390019

P G Co-Ordinator & Professor, Sigma Institute of Pharmacy,Sigma University,BAKROL, Vadodara-390019

Dean of Sigma Institute of Pharmacy,Sigma University,Bakrol, Vadodara-390019

Date of Submission: 20-10-2024 Date of Acceptance: 30-10-2024

ABSTRACT:

High performance liquid chromatography, one of the most powerful analytical techniques utilized in the separation, identification, and quantification of complex mixtures. Reverse-phase or RP-HPLC is commonly practiced in high performance liquid chromatography because this technique has the advantages of versatility and appropriateness for more hydrophobic compounds. Fundamentals and Practices of Reversed-Phase HPLC: Part II -- RP-HPLC column configurations. It consists of the concept of separation, types of stationary and mobile phases with the variables concerned with the separation. This review discusses the necessity for development and validation of such methods along with its pharmaceutical and food industry purposes as well as environmental analysis benefits by RP-HPLC. Among the latest developments in RP-HPLC, new stationary and mobile phases for high-performance liquid chromatography based on hydrophobic interactions have also emerged. Changes like RP-HPL down-sizing techniques and coupled For have also gained prominence. In reversed-phase high-performance liquid chromatography, short for RP-HPLC, the separation of molecules takes place based on their hydrophobicity. Separation is based on the hydrophobic binding of the solute molecule from the mobile phase to immobilized hydrophobic ligands attached to the stationary phase, or sorbent.

Keywords: Verapamil hydrochloride; Revers Phase Chromatography (RP-HPLC); Validation, Calcium Channel Blocker, ICH Guidelines

I. INTRODUCTION:

A novel, economic, and time-effective stability-indicating reversed-phase high-performance liquid chromatographic method was developed for the determination of verapamil hydrochloride.

RP-HPLC: The mobile phase in Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is polar or slightly polar, whereas the stationary phase is non-polar. This technique of separation is based on hydrophobic interactions. The component that is most polar in a mixture of analytes will elute first, while the less polar are retained for a more extended duration by the non-polar stationary phase. This technique is mostly applicable in the separation of compounds based on hydrophobicity for better analytics in any field, such as pharmaceuticals, biochemistry, and environmental science.

What is verapamil?

Verapamil, α- [3-[[2-(3, 4-dimethoxyphenyl) ethyl]methylamino] propyl] -3, 4-dimethoxy-α- (1-methylethyl) benzene acetonitrile, a slow Calcium channel antagonist, which was originally introduced as an antianginal agent now widely used in cardiac dysrhythmias. Preclinical trials demonstrated that the concomitant therapy of verapamil had not only additive effects in lowering elevated blood pressure but also had a marked beneficial effect on concomitant cardiovascular diseases.

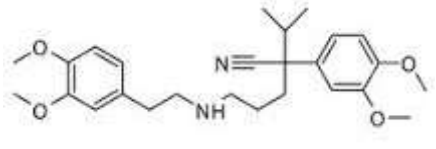
Verapamil is classified as one of the calcium-channel blockers. Verapamil is thus used to widen the muscles in the heart and blood vessels such that the heart does not have to pump forcefully. It also opens more channels so that the supplies of blood and oxygen to the heart may be raised as well as reduces the electrical activity in the heart to control the heartbeat. The extended tablets alone or with other drugs are also implemented to stop the onset of and cure irregular heartbeats.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 977-988 www.ijprajournal.com ISSN: 2456-4494

Marketed Products:

Drug Profile (Verapamil):

Summary: Verapamil is a non-dihydropyridine calcium channel blocker used in the treatment of angina, arrhythmia, and hypertension.

Brand Names	Calan, Isoptin, Tarka, Verelan
Generic Name	Verapamil
Classs	calcium-channel blockers
Appearance	A pale yellow, viscous oil
Solubility	Soluble in organic solvents like ethanol, DMSO, and dimethyl formamide (DMF)
pKa	8.6
Molecular Weight	454.6 g/mol
454.6 g/mol	C27H38N2O4
IUPA Name	2-(3,4-dimethoxyphenyl)-5-[2-(3,4-dimethoxyphenyl)ethyl-methylamino]-2-propan-2-ylpentanenitrile
Structure	
UV max	The UV max of verapamil is 232, 278 nm
Melting point	Verapamil crystals have a melting point of 131–133°

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 977-988 www.ijprajournal.com ISSN: 2456-4494

Classification of HPLC can be done as

• Chromatography: HPLC is classified into two types - analytical and preparatory depending on the size of the chromatographic column bed.
• Techniques: Size exclusion, affinity, and adsorption chromatography are some of the different chromatographic techniques.
• Type of separation: Based on the principle of separation, chiral phase, and ion exchange chromatography fall into this category.
• Elution technique: Isocratic and gradient separation methods separate chromatography.
• Phase mechanism: Two kinds of chromatography are there: normal and reverse phases, according to the ways of operation.

1. Size exclusion chromatography:

Size Exclusion Chromatography (SEC) or gel permeation or gel filtration chromatography is a technique which separates molecules on the ground of size. This requires gentle conditions and is mostly used to decide quaternary and tertiary structures of amino acids and proteins. It is also referred to for measuring molecular weight of polysaccharides to study the structural features in different scientific and analytical applications effectively.

2. Ion exchange chromatography:

It works on the basis of the retention of solute ions thirsty for charged sites on the stationary phase. Those ions instead which are of similar charges get retarded. This approach is majorly needed for the purification of water and protein ion-exchange chromatography, Ligand-exchange chromatography, high pH anion-exchange chromatography of carbohydrates and oligosaccharides. It is versatile and therefore, is highly demanded in various fields where selective separation and analysis is desired depending on the charged properties of the individual substances.

3. Bio-affinity chromatography:

Affinity chromatography is the type of chromatography which mainly depends upon reversible ligand protein associations. The proteins which show high affinity are attached to the column-bound ligands which are ordered to a solid support thereby containing a matrix conjugated to the biomolecule of interest to be purified and separated. Bio-affinity is a selective method with high specificity and the proteins are then separated and subsequently purified based on the specific interactions by this approach making it a method of high importance in the field of protein purification and Biochemistry.

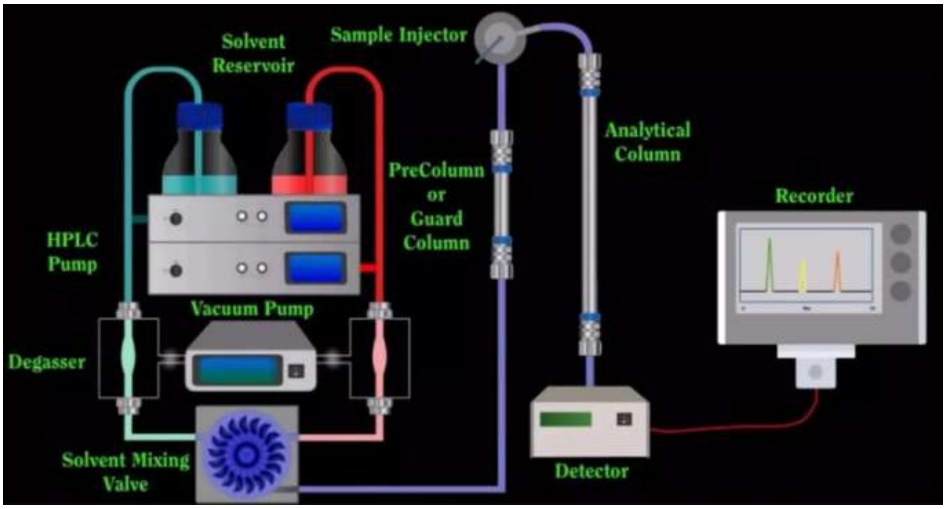
4. Normal phase chromatography:

In normal phase chromatography, a polar stationary phase and non-polar mobile phase are used. The polar analyte, therefore, gets retained strongly by the positively charged stationary phase. Polar solute molecules have longer elution times due to increased interaction with the adsorbent phase. The common modification of commercially available silica for use as the stationary phases in normal-phase chromatography includes cyanopropyl, aminopropyl, and diol. These are usually packed in columns from 150-250 mm long, with 4.6 mm internal diameter. During elution, polar compounds take longer, and thus non-polar components pass through the silica column quicker.

5. RP-HPLC. (Reverse-Phase HPLC)

In RP-HPLC, the mobile phase is polar or slightly polar, and the stationary phase is non-polar. The separating process is entirely due to hydrophobic interactions. In a mixture, less polar analytes interact longer with the non-polar stationary phase, thus the most polar component elutes first. RP-HPLC is one of the commonly used techniques for separating compounds through their hydrophobicity, making it widely used in application in pharmaceuticals, biochemistry, and environmental science.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 977-988 www.ijprajournal.com ISSN: 2456-4494



Working of RP-HPLC

FUNDAMENTALS OF RP-HPLC

In addition to column selection and mobile phase choice, a variety of chromatographic parameters are also applied to assess the efficiency of RP-HPLC separations. While the retention time is defined as the time between injection and elution of an analyte from the column, the selectivity factor defines the ratio of the distance between two peaks. Resolution measures the space between two successive peaks, while a compound's relative affinity for the stationary phase is measured by means of its capacity factor. The choice of stationary and mobile phases is the first step in preparing a successful separation using the RP-HPLC technology. Here, the choice of a stationary phase that favors interaction with the chosen mobile phase and will effectively retain the analyte to be targeted is important. It is very essential to choose a mobile phase that elutes the target analyte from the column and favors the stationary phase selected. A few changes in some parameters like column temperature and pH of the mobile phase, flow rate, or gradient elution may provide good separating conditions. Optimization is the process to obtain the maximum resolution and the lowest time required for analysis and the solvent consumption in optimum amounts. Validations are essentially required to ensure that the RP-HPLC methods are reliable, reproducible, and precise. Some of the parameters examined during validation are accuracy, precision, linearity, robustness, and limits of detection and quantification. There are a variety of applications for RP-HPLC, such as food analysis, pharmaceuticals, and environmental analysis. Impurities and degradation products in drug molecules and drug products are detected and separated with the use of RP-HPLC analysis. Among other applications, in the food industry, RP-HPLC is often applied for the analysis of chemical composition and general quality of a product. RP-HPLC is an instrument to be used for identification and determination of pollutants in environmental samples. Such latest developments of RP-HPLC include downsizing, advent of LC-MS as hyphenated methodologies, and newly designed stationary and mobile phases. New stationary and mobile phases can provide a more selective and efficient separation of complex compounds. Miniaturized RP-HPLC can handle much smaller sample volumes, which in turn reduce the run time and solvent consumption. Hybrid techniques offer even better sensitivity and accuracy as they can combine the advantages of both RP-HPLC and mass spectrometry for the detection of analytes. In essence, RP-HPLC is one of the most important techniques of analytical chemistry, particularly in the study of medicines. The development of accurate and reliable RP-HPLC techniques needs to be developed to determine the quality, safety, and efficiency of drug products. There is a need to give very careful consideration to the stationary and mobile phases, and separation parameters need fine-tuning, while RP-HPLC methods need to be validated in order to achieve perfect separation. In the analytical environment, RP-HPLC has been changing and becoming more adaptable and potent by incorporating new stationary and mobile phases, downsizing, and hyphenated techniques.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 977-988 www.ijprajournal.com ISSN: 2456-4494

METHOD DEVELOPMENT IN RP-HPLC

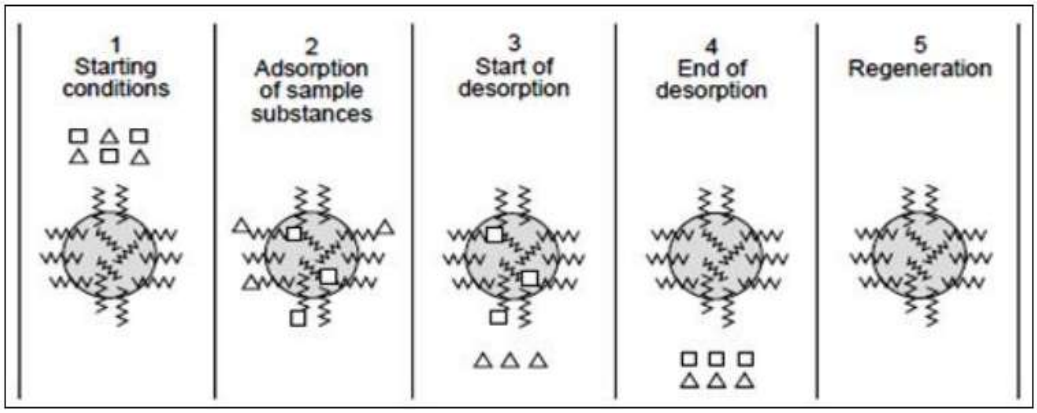
A method development process, therefore, includes the optimization of stationary and mobile phases, optimization of separation conditions, and determination of chromatographic parameters. It broadly depends on the choice of the sample's stationary and mobile phases, determined on polarity, solubility, and acid-base characteristics. Separation conditions must be optimized by pH, solvent strength, and temperature among others. The choice of stationary and mobile phases is actually the most important step in the development of a method for effective separation. The stationary phase is always a hydrophobic material, such as C18, C8, or phenyl, interacting with the analytes based on their hydrophobic nature. Usually, it is a mixture of an aqueous phase and an organic solvent, such as acetonitrile or methanol. It depends on the properties of the sample and the separation which needs to be achieved in the choice of the composition of the mobile phase. The optimization of separation conditions depends on pH, solvent strength, and temperature for attaining the desired separation. The variation of pH in the mobile phase may result in a change in its ionization state affecting the degree of retention on the column. The organic solvent to aqueous phase ratio controls solvent strength, and the effect of temperature on selectivity and resolution is typical of effects between column materials and analytes. Once separation conditions have been optimized, determination of chromatographic parameters will be essential for the validation of reliability and accuracy in any proposed method. Retention time is also the most common chromatographic parameter used for the identification of analytes in the sample. Selectivity It is the measure of separation obtained between two analytes. This can be calculated from the relative retention times of the two analytes. Resolution It is a measure of separation existing between two adjacent peaks. It is therefore calculated through measurement of the distances between the two peaks and their respective widths. The method development in RP-HPLC involves a proper choice of both the stationary and mobile phases; optimization of separation conditions: and determination of chromatographic parameters to obtain a reliable method of separation of the analytes. The optimization of separation conditions and determination of chromatographic parameters are seen to be crucial for the reliability and precision of the method in pharmaceutical analysis.

VALIDATION OF RP-HPLC METHODS:

To ensure the validity and reliability of the established methods, validation of the RP-HPLC method is compulsory. During the validation process, parameters like linearity, accuracy, precision, and robustness are to be evaluated. Accuracy means that the obtained value is close to the true value, while precision is defined as the degree of reproducibility of the results. Linearity is the ability of the method to give a linear response over a range of concentrations, and robustness is the ability of the method to produce consistent results even for small variations in method parameters. Validation of RP-HPLC methods is an important step in developing reliable and accurate analytical methods. Validation enables the performance of the method to be checked and to be evaluated whether the selected method is suitable for its actual purpose. Parameters evaluated in the method validation of RP-HPLC are accuracy, precision, linearity, and robustness. Accuracy In RP-HPLC, accuracy and precision are two important parameters for the method validation since it reflects the reliability and reproducibility of the analytical technique. Often, accuracy is measured by spiking the sample with a known amount of the analyte of interest and comparing it to the expected value. Such an approach gives great utility in cases whereby the true value of the analyte is unknown and the sample matrix may be complex, thereby affecting the recovery of the analyte. Accuracy can also be defined as percent recovery or absolute deviation of the measured value to the expected value. Precision, on the other hand, may refer to the closeness with which repeated analyses of the same sample under controlled conditions yield closely reproducible results. This can also be assessed on the basis of a relative standard deviation (RSD) of the data obtained, expressed as a ratio of standard deviation to the mean of measurements. Low RSD indicates higher precision and is generally preferred in analytical techniques. Accuracy and precision are dependent upon various parameters like quality of analytical instruments, working of the chromatographic system, stability of the analyte, and the skill level of the operator. It is essential to validate the method for accurate and precise results before applying it to real sample analysis. Validation of a method involves assessing several parameters among them linearity, limit of detection, limit of quantification, specificity, robustness, ruggedness among others. Validating the method, therefore puts the analyst in a better

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 977-988 www.ijprajournal.com ISSN: 2456-4494

position to know if the method is fit for the intended purpose and if the results obtained are reliable and reproducible.



PRINCIPLE OF REVERSED PHASE CHROMATOGRAPHY WITH GRADIENT ELUTION

Analytical Method Development:

- Materials Required
- Verapamil hydrochloride: Pure form of the drug.
- Solvent: Methanol or acetonitrile, depending on the method.
- Buffer solution: Potassium dihydrogen phosphate or sodium acetate.
- Volumetric flasks: For solution preparation.
- Pipettes and syringes: For accurate measurement and transfer of liquids.
- Filtration apparatus: 0.45 µm membrane filter.

Procedure for Method Development

1. Preparation of Buffer Solution

For potassium dihydrogen phosphate buffer:

Dissolve 6.8 g of potassium dihydrogen phosphate in sufficient water to make a total volume of 1000mL.

Adjust the pH to 2.2 using glacial acetic acid.

2. Preparation of Stock Solution

Weigh accurately 240 mg of verapamil hydrochloride.

Transfer it to a 100 mL volumetric flask.

Add approximately 30 mL of methanol or acetonitrile and shake until completely dissolved.

Dilute to volume with the same solvent to achieve a concentration of 240 µg/mL.

3. Preparation of Working Standard Solution

Acetonitrile). This results in a working solution with a nominal concentration of 24 µg/mL.

4. Filtration

Filter the prepared solutions through a 0.45 µm membrane filter to remove any particulate matter that could interfere with the HPLC analysis.

5. Calibration Standards

Prepare calibration standards by diluting the stock solution to various concentrations (e.g., 0.5–18 µg/mL) in the mobile phase, which may consist of a mixture such as methanol and buffer (e.g., 70:30 v/v) depending on the specific method used

6. HPLC Setup

Equilibrate the HPLC column with the mobile phase for at least 30 minutes before injecting samples.

Inject a suitable volume (typically around 10 µL) of the standard or sample solution into the HPLC system.

7. Data Collection and Analysis

Monitor the elution using UV detection at an appropriate wavelength (for verapamil, typically around 278 nm) and record chromatograms for analysis

8. Validation

Validate the method according to ICH guidelines, assessing parameters such as linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) by analyzing multiple replicates

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 977-988 www.ijprajournal.com ISSN: 2456-4494

Analytical Method Validation:

To validate a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for verapamil, it is essential to follow the guidelines set forth by the International Council for Harmonisation (ICH), particularly ICH Q2(R1). The validation process ensures that the analytical method is suitable for its intended purpose and consistently produces reliable results. Below are the key steps and parameters involved in the method validation of verapamil using RP-HPLC.

Method validation Validation is laboratory testing required to be performed that confirms the analytical method performance characteristics to meet the requirements of a given analytical application. Any new or changed method regardless of the operators using it, and the same or different equipment, and laboratories shall be validated for consistent repeated reliable results produced. It is the specific methodology, with its intended uses, that drives the type of validation program needed to ensure that the analytical process is robust and fit for the purpose in different settings. Results from method validation are an important component of any robust analytical procedure-evaluation of quality, consistency, and reliability of the results obtained. Critical to the validation process is equipment that complies with specified requirements, is calibrated correctly, and operates and functions. The validation process ensures that analytical methods are fully studied to be validated for use or invalidated if they do not meet standards needed. This assures the accuracy and dependability of analytical results in various applications. The following are typical parameters recommended by the FDA, USP, and ICH.

1. Specificity
2. Linearity & Range
3. Precision I. Method precision (Repeatability) II. Intermediate precision (Reproducibility)
4. Accuracy (Recovery)
5. Solution stability
6. Limit of Detection (LOD)
7. Limit of Quantification (LOQ)
8. Robustness
9. Range
10. System suitability

1. Selectivity and specificity Selectivity and specificity are sometimes used as synonymous terms while validating the method. Specificity is the ability to uniquely identify the analyte in the presence of other species that could potentially be present. It is the ability to distinguish the analyte with 100% assurance in the presence of a possible interfering species. It compares the results obtained using test samples containing contaminants, degradation products, or placebo ingredients with the results obtained using a sample prepared in the absence of such elements. It controls and evaluates the selective ability of a method to identify and quantify the analyte of interest against possible interferences in order to validate the reliability and the accuracy of an analytical method.

2. Linearity and range Linearity in an analytical process describes its ability to generate test results proportional to the concentration of analytes in the sample over a set range for that specific analytical technique. Therefore, it is critical to establish the relationship at multiple points across the range of the analytical technique. Dilution of a normal stock solution that contains the constituent parts of the medicinal product directly to show linearity on the drug substance. Usually, for establishing linearity, the confidence interval around the slope of the regression line is used. ICH guideline has proposed at least five concentrations for establishing linearity. The range of an analytical method can be considered as the interval that lies between the higher and lower values that have been proved to be determined with precision, accuracy, and linearity by the method. This wide evaluation will ensure the reliability and validity of the analytical method over a defined concentration range.

3. Precision in analytical procedures is the closeness or scatter of a group of measurements that was conducted under controlled conditions between different samplings of the same homogenous matrix. It is an important parameter in ascertaining the repeatability of a whole analytical procedure. Further, the term precision encompasses two types: repeatability and intermediate precision. Repeatability is defined as the variation of a single analyst on the same instrument. It does not differentiate between variance introduced by the sample preparation procedure and that caused by the instrument or system. In validation, several replicates of an assay composite sample are analyzed with the analytical procedure to determine repeatability, and a recovery value is calculated. Intermediate precision refers to the fluctuation that occurs within a laboratory on different days, with different instruments, and involving different analysts. These requirements of accuracy measurement ensure a comprehensive understanding of the reliability and reproducibility

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 977-988 www.ijprajournal.com ISSN: 2456-4494

of the analytical method under different conditions and by other operators.

4. Accuracy is that measure of the closeness of agreement among a series of measurements or values. It is the degree to which a measured value agrees with the true or accepted value. In practice, accuracy is defined as the difference between the true value and the mean value obtained. This method is applied to samples with known concentrations of analyte and compared with blank and standard solutions in order to ascertain no interference. Accuracy is calculated as a percentage of the analyte recovered by the assay based on test results. It is often stated as the recovery, at assay, of known, additional analyte levels, providing a measure of how well the analytical method reflects the true values.

5. Solution stability During validation, the stability of standards and samples is assessed under different conditions: normal settings, standard storage conditions, and sometimes within the instrument. This evaluation will determine if certain storage requirements such as refrigeration or protection from light are necessary to ensure that standards and samples remain stable. The realization of the impact that storage conditions have on stability is key for reliability and integrity in analytical results over time and will advise on how substances involved in the analysis should be handled and stored.

6. Limit of Detection (LOD) The smallest detectable but not necessarily quantitatively determinable amount of analyte in a sample characterized the sensitivity of an analytical method. The term LOD refers to the least concentration of analyte that can be recorded as detected, and hence it serves as a basic indicator of the analytical method's sensitivity.

7. Limit of Quantification (LOQ) The quantitation limit is the lowest quantity of an analyte in a sample that can be accurately measured quantitatively by a particular analytical system. This becomes a quantitative test parameter for determining low analyte levels in test matrices. It is vital for the identification of impurities and/or contaminants found in samples and sets a threshold beyond which reliable quantitation of measurements taken in analytical

8. Robustness: The robustness is the ability of an analytical procedure, in terms of reliability under normal conditions and its ability to resist small, deliberate departures from the method. It, therefore, assures that a developed analytical method will remain reliable and give consistent results even when minor variations or deliberate changes to its parameters are introduced. This makes the method less susceptible to change and variability, adding up to the reliability of the method in real world analytical procedures.

9. Range The range of an analytical method is the interval, in which the higher and lower values of an analyte have been demonstrated with enough linearity, precision, and accuracy. This is usually determined based on a linear or nonlinear response curve and is expressed in the same units as those of test findings. There must be a defined range for getting the proper assessment and reporting of results within the established concentration limits, valid and reliable within the scope of the established method.

10. System Suitability the system suitability tests are a part and parcel of liquid chromatographic methods. In this respect, they ensure that the repeatability as well as the resolution and detection sensitivity of the chromatographic system is adequate for the intended analysis. These tests take their theory from the fact that all devices, electronics, together with procedures to be adopted in the analysis, and the samples, are constituents of a system, which consequently can be evaluated as one unit. Significant parameters of studies to be involved in system suitability tests include peak resolution, the number of theoretical plates, peak tailing, and capacity, to determine whether the method for the analytical process used is appropriate and competent enough. This wide evaluation confirms the fact that the system offered is reliable and appropriate for the intended chromatographic study to ensure that all analyses performed will be accurate and reproducible.

ICH Guideline for method development and Validation:

The ICH (International Council for Harmonisation) guidelines for analytical method development and validation, particularly ICH Q14 and Q2(R2), provide a comprehensive framework for ensuring the reliability and regulatory compliance of analytical procedures used in the pharmaceutical industry. Here's an overview of the key aspects of these guidelines:

ICH Q14: Analytical Procedure Development

Overview

Objective: ICH Q14 outlines a science- and risk-based approach to developing analytical procedures

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 977-988 www.ijprajournal.com ISSN: 2456-4494

that are fit for their intended purpose, ensuring they can accurately measure attributes of drug substances and products.

Lifecycle Approach: The guideline emphasizes a lifecycle management approach that includes three main stages:

Procedure Design
Procedure Performance Qualification (PPQ)
Continued Procedure Performance Verification (CPV)

Key Components

Analytical Target Profile (ATP): This is a foundational element that specifies the intended purpose and performance characteristics of the analytical method, guiding its development and validation.

Minimal vs. Enhanced Approaches:

The minimal approach is traditional and acceptable for method development.

The enhanced approach incorporates more sophisticated techniques, such as Design of Experiments (DoE), to optimize method performance.

Risk Management

A significant focus is placed on identifying and mitigating risks throughout the method development process, which helps ensure robust methods that can withstand regulatory scrutiny.

Robustness and Parameter Evaluation

The guideline encourages evaluating the robustness of analytical procedures by assessing how variations in method parameters affect results.

ICH Q2(R2): Validation of Analytical Procedures

Overview

ICH Q2(R2) provides detailed guidance on validating analytical methods to ensure they are fit for their intended use, focusing on aspects such as specificity, accuracy, precision, linearity, range, and robustness.

Validation Parameters
Specificity: Ability to measure the analyte in the presence of other components.
Linearity: The relationship between concentration and response should be linear over a specified range.
Accuracy: The closeness of the measured value to the true value.
Precision: The degree of reproducibility or repeatability under normal operating conditions.
Limit of Detection (LOD) and Limit of Quantification (LOQ): Establishing the smallest amount that can be reliably detected or quantified.

Method Lifecycle Management

Both Q14 and Q2(R2) emphasize continuous improvement and adaptation of analytical methods based on ongoing performance data and changing regulatory environments.

II. RESULT:

Specificity: The method effectively separates verapamil from probable impurities and degradation products, making it suitable for the analysis of pharmaceutical formulations without excipients or any other interferences15.
Linearity: Good linearity was shown in a given concentration range in which correlation coefficients r^2 are always greater than 0.999, meaning very good linearity response for verapamil4.
Accuracy: The recovery studies were within the acceptable range of 98 to 102%, so the method could accurately quantify verapamil in many formulations24.
Precision: The method was highly precise with % RSD values that were well below the acceptable threshold of 2% thus showing repeatability with the measurements from multiple runs34.
Robustness: Small changes in the parameters of the method have caused no effect in the results, and the method seems to be robust and insensitive to small changes in the conditions of its use3.

Implications for Use

The validated RP-HPLC method is inexpensive and time-saving and, therefore, quite suitable for routine analysis in quality control laboratories. The faster production of results enhances productivity, thus allowing the examination of several samples within a short period. This method can also be used for bulk drug substances and finished pharmaceutical products to comply with regulatory requirements.

In conclusion, the RP-HPLC method developed for verapamil is a reliable analytical tool which supports quality assurance in pharmaceutical manufacturing and overall safety and efficacy of drug products. Further studies may include further

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 5 Sep - Oct 2024, pp: 977-988 www.ijprajournal.com ISSN: 2456-4494

optimization of the method or its application to other related compound

III. CONCLUSION:

The conclusion of the analytical method development and validation using RP-HPLC for verapamil usually focuses on a number of major findings and implications arising from the validation results. A structured conclusion according to the search results is as follows:

The developed RP-HPLC method of verapamil for estimation is validated reliable and effective routine quality control in pharmaceutical applications. The developed method validates according to the guidelines of ICH Q2(R1) and fulfills criteria for specificity, linearity, accuracy, precision, and robustness.

REFERENCES:

[1]. British Pharmacopoeia, 2009. Stationary office London, 2128-2129
[2]. Gerard, C., Siobhain, M., John, F., Timothy, G., 2009. Human Psychopharmacology 24 (3), 217-223.
[3]. International Conferences on Harmonization.Q2(R) Validation of Analytical Procedures.1994.
[4]. Stefan, R.J.; Van Staden, J.F.; Aboul-Enein, H.Y.; Electroanalysis 1999, 11, 192–194.
[5]. Rajan HV. Development and validation of HPLC method - A Review. International Journal of current research in pharmacy, 2015; 1(2): 55-68
[6]. VSK Yelamanchi. "Modified and residual determination method development and validations for active pharmaceuticals ingredient by RP HPLC." KL University, 2017.
[7]. KS Nataraj, GN Raju, AS Rao, SVS Kumar, S Divya. "European Journal of Pharmaceutical and Medical Research." European Journal of Pharmaceutical and Medical Research, vol. 4, no. 5, 2017, pp. 28-36.
[8]. Sultana, A., Singh, M., Kumar, A., Kumar, R., Saha, P., Kumar, R. S., & Kumar, D. (2022). To Identify Drug-Drug Interaction in Cardiac Patients in Tertiary Care Hospitals. Journal for Research in Applied Sciences and Biotechnology, 1(3), 146-152.
[9]. RC Prabhu, AMaruthapillai, GC Senadi. "Wise AQbD and an Orthogonality Approach to Develop a Short-Runtime Method for the Simultaneous Quantification of Bosentan and Impurities using UPLC Equipped." Analytical Chemistry, vol. 93, no. 6, 2021, pp. 3041-3049. doi: 10.1021/acs.analchem.0c05159.
[10]. S Sharmin, MH Sohrab, F Moni, F Afroz, et al. "Simple RP-HPLC method for Aceclofenac quantitative analysis in pharmaceutical tablets." Pharmacia, vol. 67, no. 2, 2020, pp. 79-85. doi: 10.3897/pharmacia.67.e51691.
[11]. SA Jadhav, SB Landge, SL Jadhav, et al. "Stability-indicating gradient RP-LC method for the determination of process and degradation impurities in bosentan monohydrate: An endothelin receptor antagonist." Chromatography Research International, vol. 2011.
[12]. Kirthi, A., Shanmugam, R., Prathyusha, M. S., & Kumar, S. S. (2014). A review on bioanalytical method development and validation by RP-HPLC. Journal of global trends in pharmaceutical sciences, 5(4), 2028- 2037
[13]. Subramanian Natesan et.al. Improved Rp-Hplc Method for the Simultaneous Estimation of Tranexamic Acid and Mefenamic Acid in Tablet Dosage Form. Pharm Anal Acta, 2011; 2: 115.
[14]. Vishnu P et.al. Simultaneous Estimation of Atorvastatin, Ezetimibe and Fenofibrate in Pharmaceutical Formulation by RP-LC-PDA. Pharm Anal Acta, 2010; 1: 111.
[15]. Lalit V. Sonawane and Sanjaykumar B. Bari Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Amoxicillin Trihydrate and Bromhexine Hydrochloride from Oily Suspension. Pharm Anal Acta, 2010; 1: 107.
[16]. LaxmanSawant et.al. Quantitative HPLC Analysis of Ascorbic Acid and Gallic Acid in PhyllanthusEmblica. J Anal Bioanal Techniques, 2010; 1: 111.
[17]. ICH Q2A. Text on Validation of Analytical Procedures, International Conference on Harmonization. Geneva; 1995.
[18]. Validation of Compendial Procedures, United State Pharmacopeia, USP 36 NF, 2010; 27
[19]. G. David Watson, Pharmaceutical Analysis (3rd Ed., Churchill Livingstone,

London: Harcourt Publishers Limited, Essex CM 20 2JE, 2012.
[20]. A.H. Beckett, and J.B. Stenlake, Practical Pharmaceutical Chemistry (4th Ed., Vol. I & II. CBS Publishers and Distributors, New Delhi, 2007.
[21]. T. Higuchi, and Brochman-Hansen, Pharmaceutical Analysis, (3rd edition, CBS Publishers and Distributors pvt. Ltd., New Delhi, 1997.
[22]. G. Oliver, R. Gerrit, and VZ. Maxmilian, Leading Pharmaceutical Innovation, „Trends and drivers for Growth in the pharmaceutical industry, (2nd Ed., Springer, 2008; 12-15.
[23]. Br. Jay, J. Kelvin, and B. Pierre, Understanding and Implementing Efficient Analytical Methods Development and Validation, 2003.
[24]. R.M. Christopher, and W.R. Thomas, Quality Systems approach to Pharmaceutical cGMP Development and validation of Analytical Methods, (1st Ed., 2005; 147-152.
[25]. R. Lloyd Snyder, J. Joseph Kirkland and L. Joseph Glajah, Practical HPLC method development (2nd Ed., 1997; 179-184.
[26]. B.K. Sharma, Instrumental method of chemical analysis (29th Ed., Meerut, Chromatography, HPLC, Goel Publishing House, 2013; 286- 385.
[27]. H.H. Willard, L.L. Merrit, J.A. Jr. Dean, and F.A. Jr. Settle, Instrumental Methods of Analysis (CBS Publishers, New Delhi, 1986.
[28]. R.A. Day, and A.L, Underwood, Quantitative Analyses, (5th Ed., Prentice Hall, New Delhi, 1986.
[29]. Macek and Karel, Pharmaceutical Applications of Thin Layer and Paper Chromatography, 1972; 62(6): 1032.
[30]. G. Ramana Rao, S.S.N. Murthy, and P Khadgapathi, Gas Chromatography to Pharmaceutical Analysis, Eastern Pharmacist, 1987; 30(353): 35
[31]. C.S.P. Sastry, T.N.V. Prasad and E.V. Rao, Recent applications of High-Performance Liquid chromatography in pharmaceutical analysis, Indian J. Pharm. Education, 1987; 21(37)
[32]. Ravisankar P, Gowthami S, and Devala Rao G, A review on analytical method development, Indian journal of research in pharmacy and biotechnology, 2014; 2(3): 1183-1195.
[33]. Ravisankar P, Rajyalakshmi G, DevadasuCh, and Devala Rao G, Instant tips for right and effective approach to solve HPLC trouble shooting, Journal of chemical and pharmaceutical sciences, 2014; 7(3): 259-274.
[34]. Jay Breaux, Kevin Jones, and Pierre Boulas, Development services development analytical and validation method Pharmaceutical technology, 2003; 27(1): 6-13.
[35]. E. Michael Swartz, and IrasKrull, Analytical method development and validation, CRC press, Marcel dekker, Inc., Madison Avenue, New York, 1997.
[36]. K. Yuri, and LB. Rosario, HPLC for pharmaceutical scientists, (John Wiley and Sons, Inc., Hoboken, New Jersey, 2007; 92-98.
[37]. G. P. Carr, and J. C. Wahlichs. „A practical approach to method validation in pharmaceutical analysis", J. Pharm, Biomed. Anal, 1990; 8: 613-618.
[38]. United States Pharmacopoeia,24, National Formulary 19, section„ Validation of compendial methods". US Pharmacopoeial convention, Rockville, Validation of analytical procedures text and methodology Q2 (R1), November, 2000; 2005.
[39]. International conference on harmonization (ICH) of technical requirements for registration of pharmaceuticals for human use, Validation of analytical procedures: Text methodology, (Q2 (R1) Geneva, 2005; 6-13.
[40]. Draft guidance analytical procedures and method validation, US food and drug administration, Centre for drugs and biologics, Department of Health and Human Services.
[41]. N.Toomula, A. Kumar, S.D. Kumar, V.S. Bheemidi, Development and Validation of Analytical Methods for Pharmaceuticals, J Anal Bioanal Techniques, 2011; 2(5): 1-4.
[42]. Sethi PD. Introduction – High Performance Liquid Chromatography, 1st edn, CBS Publishers, New Delhi, 2001; 1-28.
[43]. Julia T, Mena AJ, Aucoin MG, Kamen AA. Development and validation of a HPLC method for the quantification of

baculovirus particles. J Chromatogr B. 2011; 879: 61-68.

[44]. Santhosh G, Nagasowjanya G, Ajitha A, Uma Maheswara Rao Y. HPLC method development and validation: an overview. International Journal of Pharmaceutical Research & Analysis, 2014; 4(2): 274-280.

[45]. Kayode J, Adebayo. Effective HPLC method development. Journal of Health, Medicine and Nursing, 2015; 12: 123-133.

[46]. Gad S. Pharmaceutical manufacturing handbook of regulations and quality. John wiley and sons, 2006.

[47]. T. Bhagyasree, N. Injeti, A. Azhakesan, U.M.V. Rao, A review on analytical method development and validation, International Journal of Pharmaceutical Research & Analysis, 2014; 4(8): 444-448.

[48]. V. Kumar, R. Bharadwaj, G.G., S. Kumar, An Overview on HPLC Method Development, Optimization and Validation process for drug analysis, The Pharmaceutical and Chemical Journal, 2015; 2(2): 30-40.

[49]. Nevado JJB et.al. Reliable and Sensitive SPE HPLC-DAD Screening of Endocrine Disruptors Atrazine, Simazine and their Major Multiresidues in Natural Surface Waters: Analytical Validation and Robustness Study Perfomance. J Chromatograph SeparatTechniq, 2014; 5: 215.

[50]. Nia Y et.al. Determination of Ti from TiO2 Nanoparticles in Biological Materials by Different ICP-MS Instruments: Method Validation and Applications. J NanomedNanotechnol, 2015; 6: 269.

[51]. Shrivastava, V.B. Gupta, HPLC: Isocratic or Gradient Elution and Assessment of Linearity in Analytical Methods, J AdvScient Res, 2012; 3(2): 12-20.

[52]. Weston A, Brown PR. HPLC and CE Principles and Practice. Academic press California, 1997.

[53]. Ngwa G. Forced Degradation Studies. Forced Degradation as an Integral part of HPLC Stability Indicating Method Development. Drug Delivery Technology, 2010; 10(5).

[54]. Mohamad T, Mohamad MA, Chattopadhyay M. Particle size role, Importance and Strategy of HPLC Analysis An update. International Archives of BioMedical and Clinical Research, 2016; 2(2): 5-11.

[55]. Raju, V. B., & Rao, A. L. (2011). A Simple and Sensitive RPHPLC Method for Estimation of Trandolapril in Bulk and Tablet Dosage Forms. Asian Journal of Research in Chemistry, 4(9), 1425-1427.

[56]. Sreekanth, N., Awen, B. Z., & Rao, C. (2010). HPTLC method development and validation of trandolapril in bulk and pharmaceutical dosage forms. Journal of Advanced Pharmaceutical Technology & Research, 1(2), 172.

[57]. Kowalczuk, D. (2005). Simultaneous high performance thin-layer chromatography densitometric assay of trandolapril and verapamil in the combination preparation. Journal of AOAC International, 88(5), 1525 1529.

[58]. Chytil, L., Štrauch, B., Cvačka, J., Marešová, V., Widimský, J., Holaj, R., &Slanař, O. (2010). Determination of doxazosin and verapamil in human serum by fast LC–MS/MS: Application to document non-compliance of patients. Journal of Chromatography B, 878(30), 3167-3173.

[59]. V. Kumar, R. Bharadwaj, G.G., S. Kumar, An Overview on HPLC Method Development, Optimization and Validation process for drug analysis, The Pharmaceutical and Chemical Journal, 2015; 2(2): 30-40.