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A Research on Method Development and Assay for Rabeprazole Sodium by Chromatography Method

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Abstract Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis. Proteins can be purified based on characteristics such as size and shape, total charge, hydrophobic groups present on the surface, and binding capacity with the stationary phase. Four separation techniques based on molecular characteristics and interaction type use mechanisms of ion exchange, surface adsorption, partition, and size exclusion. Other chromatography techniques are based on the stationary bed, including column, thin layer, and paper chromatography. Column chromatography is one of the most common methods of protein purification.

Keywords Hydrophobic, Chromatography, Column chromatography, Protein

Introduction

High-Performance Liquid Chromatography (HPLC) has emerged as one of the most powerful and widely employed analytical techniques for the quantitative determination of pharmaceutical compounds. Its ability to provide high sensitivity, selectivity, and reproducibility makes it particularly suitable for the analysis of rabeprazole in bulk pharmaceutical dosage forms. However, the development and validation of an HPLC method specific to rabeprazole necessitate a comprehensive investigation to establish a robust and reliable analytical procedure [1,2].

The method development involves the systematic optimization of various parameters such as the mobile phase composition, column type, detection wavelength, and flow rate to achieve a well-resolved and efficient chromatographic separation of drug from potential impurities or excipients. This optimization is crucial not only for accurate quantification but also for minimizing analysis time and ensuring the cost-effectiveness of the analytical procedure [3].

Following method development, a thorough validation process is imperative to verify the reliability and suitability of the developed HPLC method. Method validation serves as a critical step in demonstrating that the analytical procedure is capable of providing accurate, precise, and reproducible results. The validation parameters typically include specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness. Each of these parameters contributes to the overall assessment of the method's performance and its applicability to routine pharmaceutical analysis [4].

Furthermore, the validation process must comply with regulatory guidelines, such as those outlined by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), to ensure that the developed method meets the required standards for pharmaceutical analysis [5].

This research aims to contribute to the field of pharmaceutical analysis by systematically developing and validating an HPLC method for the estimation of rabeprazole in bulk pharmaceutical dosage forms. The outcome of this research will provide a reliable analytical tool for quality control laboratories in the pharmaceutical industry, ensuring the accurate assessment of rabeprazole content in various formulations Pharmaceutical analysis plays a very prominent

role in quality assurance as well as quality control of bulk drugs and pharmaceutical formulations. Rapid increase in pharmaceutical industries and production of drug in various parts of the world has brought a rise in demand for new analytical techniques in the pharmaceutical industries [6].

As a consequence, analytical method development has become the basic activity of analysis. Recent development in analytical methods has been resulted from the advancement of analytical instruments. The improvement of the analytical method development and analytical instruments have reduced the time of analysis, increased precision and accuracy and reduced costs of analysis. As a consequence, most of pharmaceutical organizations are investing huge amount of money for the establishment of advanced analytical laboratories [7].

Analytical techniques are developed and validated for active pharmaceutical ingredients (API), excipients, drug products, degradation products and related substances, residual solvents, etc. As a result, it has become an integral part of the requirements of the regulatory organization. Analytical method development finally results in official test methods. These methods are used in quality control laboratories to ensure the identity, purity, safety, efficacy and performance of drug products. Regulatory authorities are placing greater emphasis on analytical methods in manufacturing. Drug approval by regulatory authorities requires the applicant to prove control of the entire process of drug development by using validated analytical methods and ultimately enhancing the safety and efficacy of the end products [8].

Analytical Method Development

Pharmaceutical analysis plays a very prominent role in quality assurance as well as quality control of bulk drugs and pharmaceutical formulations. Rapid increase in pharmaceutical industries and production of drug in various parts of the world has brought a rise in demand for new analytical techniques in the pharmaceutical industries. As a consequence, analytical method development has become the basic activity of analysis. Recent development in analytical methods has been resulted from the advancement of analytical instruments. The improvement of the analytical method development and analytical instruments have reduced the time of analysis, increased precision and accuracy and reduced costs of analysis. As a consequence, most of pharmaceutical organizations are investing huge amount of money for the establishment of advanced analytical laboratories. Analytical techniques are developed and validated for active pharmaceutical ingredients (API), excipients, drug products, degradation products and related substances, residual solvents, etc. As a result, it has become an integral part of the requirements of the regulatory organization. Analytical method development finally results in official test methods. These methods are used in quality control laboratories to ensure the identity, purity, safety, efficacy and performance of drug products. Regulatory authorities are placing greater emphasis on analytical methods in manufacturing. Drug approval by regulatory authorities requires the applicant to prove control of the entire process of drug development by using validated analytical methods [9].

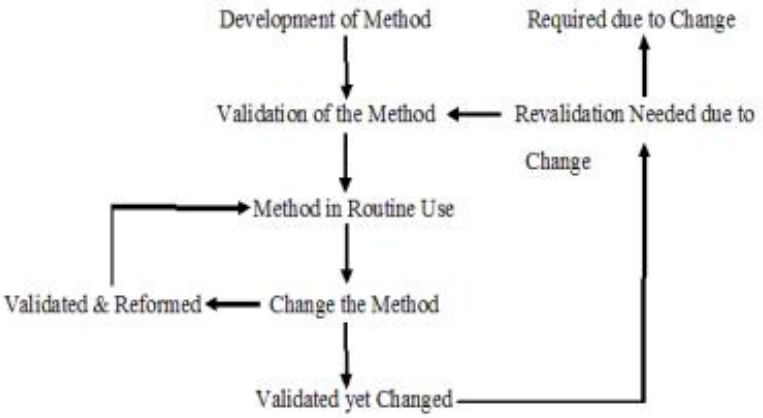


Figure 1: Life cycle of analytical method

Purpose of Analytical Method Development

Drug analysis reveals the identification characterization & determination of the drugs in mixtures like dosage forms & biological fluids. During manufacturing process and drug development the main purpose of analytical methods is to provide information about potency, bioavailability, stability and effect of manufacturing parameters to ensure that the production of drug products is consistent. The concept of quality control is intended to examine and identify a genuine and right product by series of measures designed to avoid and get rid of errors at varied stages in production. To take a decision to release or discard a product is based on one or more sorts of control actions. Providing simple and analytical process for various complex formulations is a subject matter of utmost importance. Rapid increase in pharmaceutical industries and constant production of drug in various parts of the world has brought a quick rise in demand for new analytical techniques in the pharmaceutical industries as a consequence; analytical method development has become the basic activity of analysis in a quality control laboratory. The reasons for the development of novel methods of drug analysis are:

a) When there is no official drug or drug combination available in the pharmacopoeias.

b) When there is no decorous analytical process for the existing drug in the literature due to patent regulations.

c) When there are no analytical methods for the formulation of the drug due to the interference caused by the formulation excipients.

d) Analytical methods for the quantization of the analyte in biological fluids are found to be unavailable.

e) The existing analytical procedures may need costly reagents and solvents. It may also involve burdensome extraction and separation procedures.[10]

Analytical Method Validation Procedure

The steps involved in development, validation and determination of validation parameters, also termed analytical performance characteristics, depend upon the type and nature of the analytical method. Pharmaceutical analytical methods are categorized into five general types, namely, identification tests, potency assays, impurity tests, impurity tests and specific tests. The first four tests are universal tests, but the specific tests such as particle-size analysis and X-ray diffraction studies are used to determine specific properties of the active pharmaceutical ingredient (API) or the drug product. A method has to be validated when it is necessary to verify whether its performance parameters are adequate for use for a particular analytical problem. For example,

(a) When a new method is developed for a specific problem

(b) When indications exist that an established method is changing with time

(c) When an established method is revised to incorporate changes/improvements or to extend it for another purpose

(d) When an established method is used in a different laboratory, or with different analysts or different instrumentation

(e) To demonstrate the equivalence between two methods.

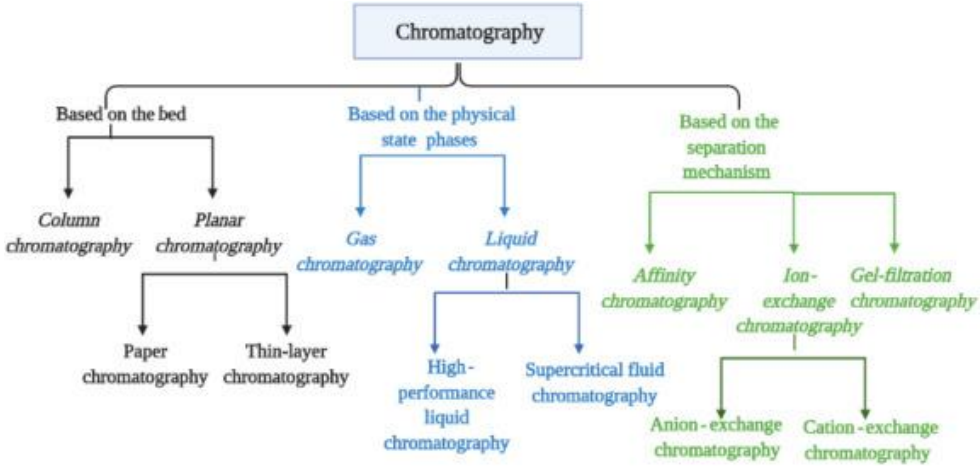
The extent of validation or revalidation required would depend on the nature of the changes made in reapplying a method to different laboratories, instrumentation or operators, and the circumstances in which the method is going to be used. Some degree of validation is always appropriate even when using apparently well-characterized standard or published methods. A well-developed method should be easy to validate. As the development of the method and the validation process advance, the information gathered is captured in the design and subsequent improvement of the method. Ideally, the validation protocol should be written only following a thorough understanding of the method's capabilities and intended use. The validation protocol will list the acceptance criteria that the method can meet. Any failure to meet the criteria will require that a formal investigation is conducted.[11]

Validation Parameters

(a) *Specificity*: It is the ability of the method to measure the analyte response accurately in the presence of all potential sample components, referred to as the sample matrix.

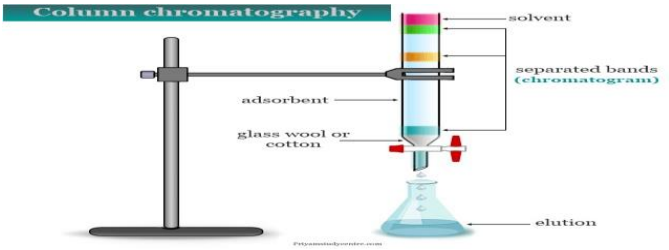
(b) *Linearity and Range*: The linearity of an analytical method refers to the ability to elicit test results that are, either directly or by well-defined mathematical transformations, proportional to the concentration of analyte in the sample over the entire range of interest.
(c) *Accuracy*: The accuracy of an analytical method is the closeness of the result obtained to the true value.
(d) *Precision*: The precision of the method is defined as the degree of scatter of individual test results of multiple measurements of a homogenous sample.
(e) *Limit of detection*: Limit of detection (LOD) is the lowest concentration of analyte that can be reliably detected using the method but not necessarily quantified. The LOD of a method should be established quite early in the method development-validation process and its determination should be repeated using the specific wording of the final procedure.
(f) *Limit of quantitation*: The limit of quantitation (LOQ) is the concentration at and above which the analyte can be reliably quantitated with a previously defined level of certainty.
(g) *Robustness*: Robustness is the ability of the method to remain unaffected by small changes in method parameters carried out deliberately or otherwise during the validation/usage of analytical methodology.
(h) *Ruggedness*: Ruggedness is not defined by ICH guidelines. It is defined by the USP as the degree of reproducibility of test results obtained by analysis of the same samples under a variety of conditions.
(i) *System suitability*: The system has to be tested for its suitability for the intended purpose.
(j) *Stability*: During the earlier validation studies, the method developer gained some information on the stability of reagents, mobile phases, standards, and sample solution.[12]

Chromatography

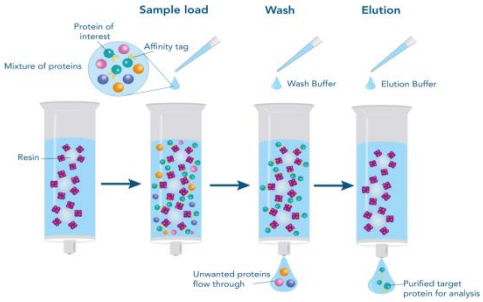


Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase.
Stationary phase: This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface a solid support".
Mobile phase: This phase is always composed of "liquid" or a "gaseous component."
Stationary phase in chromatography is a solid phase or a liquid phase coated on the surface of a solid phase. Mobile phase flowing over the stationary phase is a gaseous or liquid phase. The purpose of applying chromatography which is used as a method of quantitative analysis apart from its separation is to achieve a satisfactory separation within a suitable time interval.
The type of interaction between stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other.
Chromatography methods based on partition are very effective on separation, and identification of small molecules as amino acids, carbohydrates, and fatty acids.

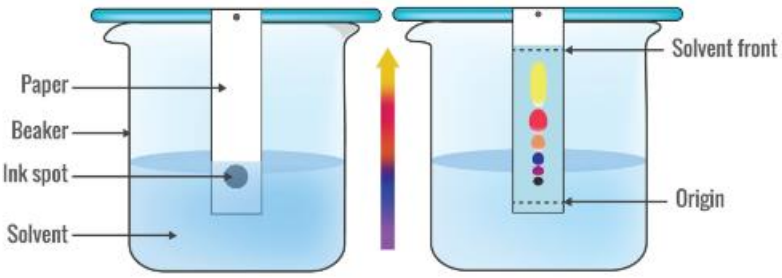
Column chromatography is used for the purification of biomolecules. On a column (stationary phase) firstly the sample to be separated, then wash buffer (mobile phase) are applied. Their flow through inside column material placed on a fiberglass support is ensured.[13]



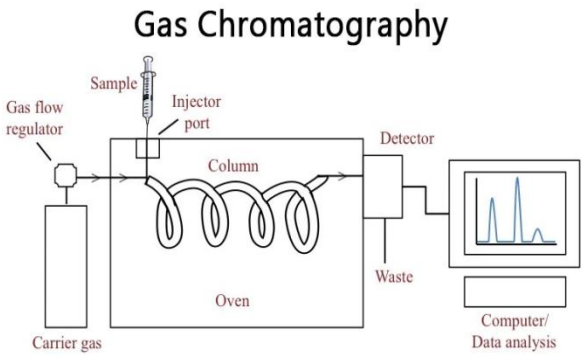
Affinity chromatography is a method for selective purification of a molecule or group of molecules from complex mixtures based on highly specific biological interaction between the two molecules. The interaction is typically reversible and purification is achieved through a biphasic interaction with one of the molecules (the ligand) immobilized to a surface while its partner (the target) is in a mobile phase as part of a complex mixture. Affinity chromatography is more effective in the separation of macromolecules as nucleic acids, and proteins.[14]



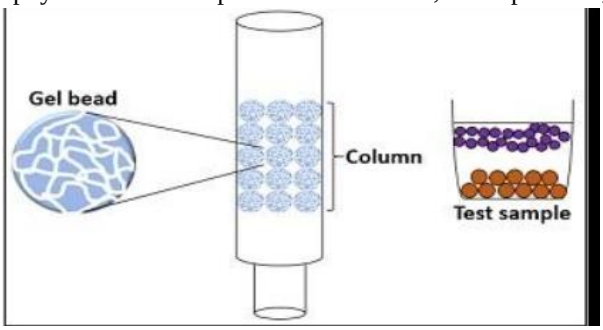
In *Paper chromatography* support material consists of a layer of cellulose highly saturated with water. In this method a thick filter paper comprised the support, and water drops settled in its pores made up the stationary "liquid phase." Mobile phase consists of an appropriate fluid placed in a developing tank. Paper chromatography is a "liquid-liquid" chromatography. Paper chromatography is used in the separation of proteins, and in studies related to protein synthesis.[15]



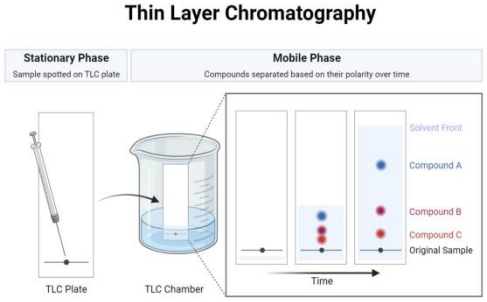
In *Gas Chromatography* separation is achieved by a series of partitions between a moving gas phase and a stationary liquid phase held in a small diameter tube (the column) after a mixture is injected as a narrow band. A detector then monitors the composition of the gas stream as it emerges from the column carrying separated components, and the resulting signals provide the input for data acquisition. GC can be applied to the analysis of mixtures, which contain compounds with boiling points from near zero to over 700 K, or which can be heated sufficiently without decomposition to give a vapor pressure of a few mmHg. Gas chromatography is applied for gases, and mixtures of volatile liquids, and solid material. [16]



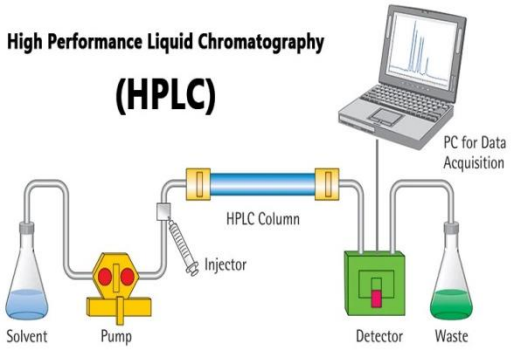
Gel Chromatography use dextran containing materials to separate macromolecules based on their differences in molecular sizes. This procedure is basically used to determine molecular weights of proteins, and to decrease salt concentration. Gel chromatography is used for the purification of RNA, DNA particles, and viruses.



Thin-layer chromatography (TLC) is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminum oxide, or cellulose (blotter paper). This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analyte ascend the TLC plate at different rates, separation is achieved.[17]



High performance Liquid Chromatography (HPLC) is one of the most popular and mature analytical techniques and by far the most widely used separation technique. It has been used in laboratories worldwide over the past 40-plus years for pharmaceutical sciences, clinical chemistry, food and environmental analyses, synthetic chemistry, etc. HPLC has gained its popularity mainly due to its reliability (use of pressure driven liquid support) and versatility (possibility of adjusting the composition of both mobile and stationary phases). The chromatographic mode or separation mechanism depends on the overall interactive relationships between the stationary phase, the mobile phase and the analyte.[18]



Application area of Chromatography in Medicine

Chromatography technique is a valuable tool for biochemists, besides it can be applied easily during studies performed in clinical laboratories For instance; paper chromatography is used to determine some types of sugar, and amino acids in bodily fluids which are associated with hereditary metabolic disorders. Gas chromatography is used in laboratories to measure steroids, barbiturates, and lipids. Chromatographic technique is also used in the separation of vitamins, and proteins.[19]

Materials and Methods

Instruments used for method development

Table 1: Instruments used in method development

Sr. No.	Instrument	Specification and Manufacture
1	HPLC	Make cyber lab, Dector:UV100 (08080463), Software-cyber lab
2	Analytical balance	Reptech Max
3	Ultra sonic cleaner	EIE instrument pvt. ltd. Ahmedabad
4	UV visible spectrophotometer	Model-UV 3000 Make lab India
5	pH Meter	Lab India

Table 2: System specifications of HPLC

Sr. No.	Name	Specification and Manufacture
1	Pump	LC-20AT
2	Detector	SPD-20A
3	Column	C18 ODS
4	Injector	Rheodyne injector (20ul Capacity)
5	Syringe	Hamilton (25ul)
6	Chromatographic software	Cyber lab

Table 3: Drug in use

Sr. No.	Name	Specification and Manufactures
1	Rabeprazole	Sun Pharma

Table 4: Reagents and chemicals

Chemical	Grade	Manufacture
Acetonitrile	HPLC	Finar reagent
Methanol	AR	Chemdise corporation
Water	HPLC	Finar reagent
Methanol	HPLC	Finar reagent
Potassium dihydrogen phosphate	AR	Merck, Rankem
Orthophosphoric acid	AR	Merck, Rankem
Tri ethyl amine	AR	Merck, Rankem

Development of assay method for Rabeprazole by HPLC

Identification of bulk drug

Identification of sample is the first step for method development. Information about the sample is necessary criteria for any method development.

The identification of sample is carried out by different method. Based on the physical properties like appearance and melting point. While based on chemical properties like chemical structure, its formula identification was carried out by analytical method like chromatography.

Instrumentation

The HPLC system consisted of Waters Alliance (Waters Corporation, MA, USA) equipped with a Waters 2695solvent delivery module in a quaternary gradient mode and aWaters2669PDAdetector. Data acquisition was performed by the Empower 200 software operated on a Pentium IV microprocessor. Analysis was carried out at 284 nm with reversed phase Symmetry C18 (4.6x150mm,5µm, Make: XTerra) or equivalent column, using a mixture of phosphate buffer (pH5.5), methanol (30:70) as the mobile phase using a low pressure gradient mode with flow rate at 0.9ml/min. The mobile phase was degassed and filtered through 0.45 µm membrane filter before pumping into HPLC system.

Preparation of solutions:

Preparation of Phosphate buffer:

Weigh 7.0 grams of Potassium di-hydrogen phosphate into a 1000ml beaker, dissolve and diluted to 1000ml with HPLC water. Adjusted the pH to 5.5 with Sodium Hydroxide. Preparation of the mobile phase. Mix a mixture of above buffer300ml.(30%) and700ml of Methanol HPLC(70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 u filter under vacuum filtration.

Diluent Preparation:

Mobile phase used as diluent.

Preparation of Standard Solution:

Accurately weigh and transfer 10mg of Rabeprazole. Working standard into a 10ml volumetric flask add about7ml of Diluent and sonicate to dissolve it completely and make volume upto the mark with the same solvent. (Stock solution) Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Preparation of Sample Solution:

Weigh 5 Rabeprazole Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to10 mg of Rabeprazole into a 10 ml volumetric flask. Add about 7ml of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent.

Mix well and filter through 0.45 µm filter. Further pipette 0.4 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

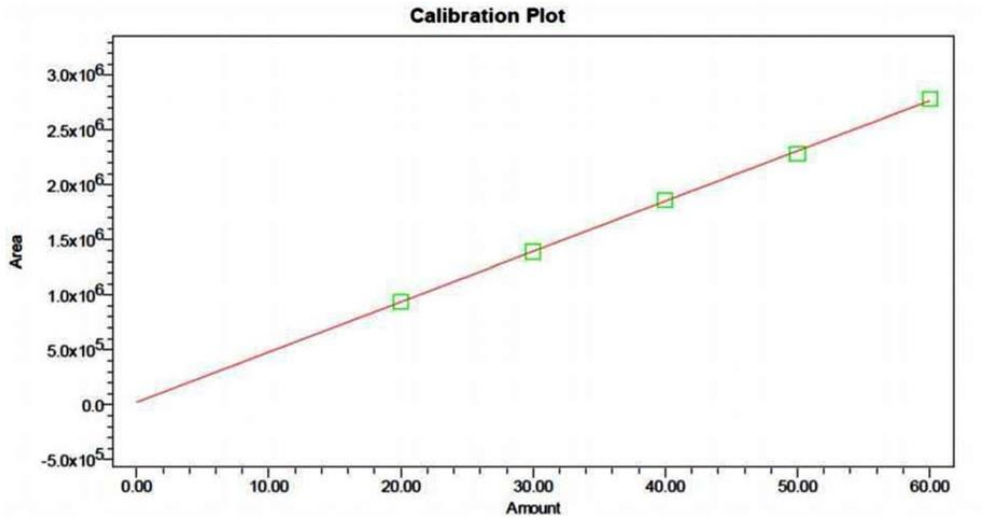
Chromatographic parameters:

Equipment: High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector.
Column : Symmetry C18(4.6x150mm,5mm, Make: XTerra) or equivalent
Flow rate: 0.9mL per min
Wavelength: 284 nm
Injection volume: 20 µl
Column oven: Ambient
Runtime: 5 min

Preparation of stock solution:

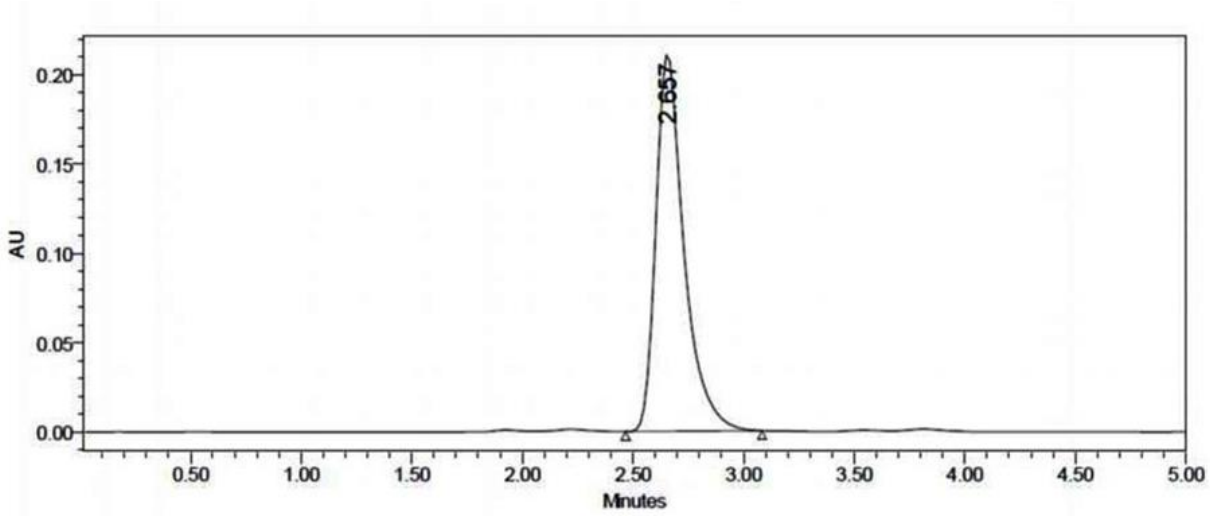
Accurately weigh and transfer 10mg of Rabeprazole API sample into a 10 mL volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume upto the mark with the same solvent. (Stock solution) Linearity was performed by taking from stock solution aliquots of 2, 3, 4, 5 and 6 ml were taken in10ml volumetric flasks and diluted upto the mark with mobile phase such that the final concentration of Rabeprazole in the range of 20-60˜g/ml. Volume of 20 sample was injected in five times for each concentration level and calibration curve was constructed by plotting the peak are a versus the drug concentration. As depicted below

S.No	Linearity Level	Concentration	Area
1	I	20µg/ml	939926
2	II	30µg/ml	1390971
3	III	40µg/ml	1860230
4	IV	50µg/ml	2285771
5	V	60µg/ml	2779976
Correlation Coefficient			1.000



Acceptance Criteria:

Correlation coefficient should be not less than 0.999.



Assay:

Assay was performed by accurately weighed amount of powder equivalent to 10.00 mg of rabeprazole was quantitatively transferred to 10 ml of calibrated flask with the aid of diluent. The volume was made up to mark, sonicate for 10min. From the stock solution aliquot of 0.1ml was taken in 10ml volumetric flasks and diluted upto the mark with mobile phase and filtered, such that the final concentrationofRabeprazolewas10 mg/ml.

Calculation:

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.\ Wt}{Label\ Claim} \times 100$$

Where:

AT= Peak Area of Rabeprazole obtained with test preparation.

AS=Peak Area of Rabeprazole obtained with standard preparation.

WS = Weight of working standard taken in mg.

WT= Weight of sample taken in mg.

DS = Dilution of Standard solution.

DT = Dilution of sample solution.

P= Percentage purity of working standard.

System Suitability:

Tailing factor for the peak due to Rabeprazole in Standard solution should not be more than 2.0. Theoretical plates for the Rabeprazole peak in Standard solution should not less than 2000.

Drug name	USP tailing	USP theoretical plates
Rabeprazole	1.6	2183.4
Acceptance criteria	In between 0.5 to 2.0	Above 2000

Accuracy

Preparation of 40 g/ml solution:

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 m filter. It is taken as 100% target solution.

Accuracy and Recovery:

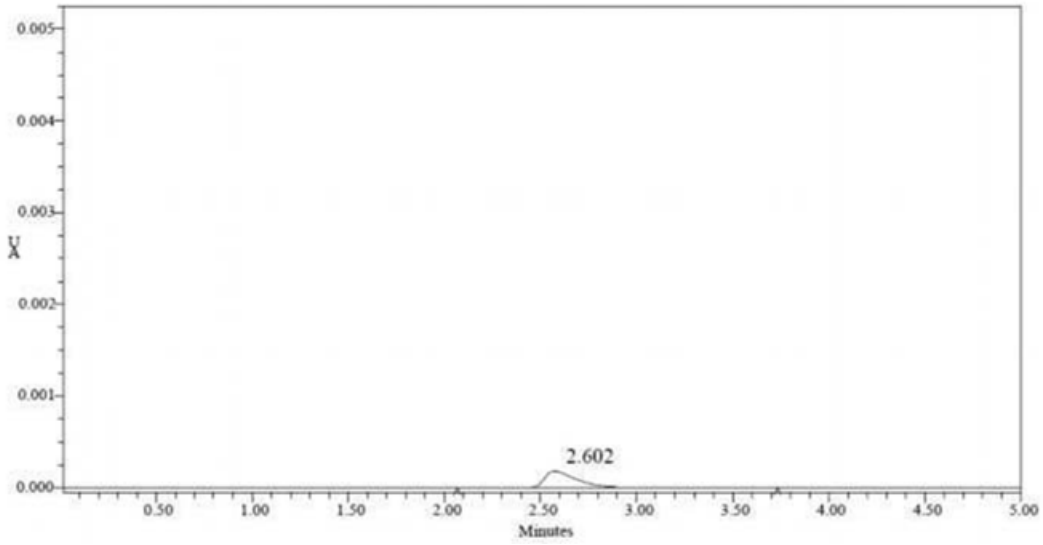
It was done by recovery study. Sample solutions were prepared by spiking at about 50%, 100% and 150 % of specification limit to Placebo and analyzed by the proposed HPLC method. Results are shown below

%Concentration(at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	951730	5.0	5.03	100.7%	99.8%
100%	1881869	10.0	9.95	99.5%	
150%	2815614	15.0	14.8	99.3%	

Limit of Detection:

Preparation of 0.7% solution At Specification level (0.28 g/ml solution):

Pipette 1mL of 10 g/ml from stock solution into a 10 ml of volumetric flask and dilute upto the mark with diluent. Further pipette 0.7mL of above diluted solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.



Acceptance Criteria:

- S/N Ratio value shall be 3for LOD solution.

Limit of Quantification:

Preparation of 0.23 % solution At Specification level (0.092 g/ml solution):

Pipette 1mL of 10g/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Further pipette 0.23 Ml of above diluted solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Acceptance Criteria

- S/N Ratio value shall be10 for LOQ solution.

Result and Discussion

A reverse-phase column procedure was proposed as a suitable method for the estimation of rabeprazole.

The chromatographic conditions were optimized by changing the mobile phase composition. Different ratios were experimented to optimize the mobile phase. Finally, buffer and acetonitrile in the ratio 55:45v/v was used as mobile phase, which showed good resolution of rabeprazole peak. The wavelength of detection selected was260nm, as the drugs optimized absorbance at this wavelength. By our proposed method the retention time of rabeprazole was about 2.399 min and 3.191mins and none of the impurities were interfering in its assay.

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