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Analytical Study of Rabeprazole Sodium in Formulation by Using HPLC

Asha Ranjani V[1]*, Chandrasekhar B[1], Bharath Kumar KS[2], Padmanaba Reddy Y[2] and Prabhakar T[1]

[1]Department of Pharmaceutical Analysis, MLR Institute of Pharmacy, Jawaharlal Nehru Technological University-Hyderabad, India

[2]Department of Pharmaceutical Analysis, Jawaharlal Nehru Technological University-Ananthapur, India

[3]Department of Pharmaceutical Analysis, University College of Pharmaceutical Sciences, Andhra University, India

***Corresponding Author:** Asha Ranjani V, Department of Pharmaceutical Analysis, MLR Institute of Pharmacy, Dundigal, Quthbullapur, Hyderabad, India.

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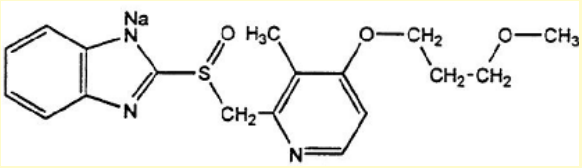
Abstract

The objective of this present work is to develop a simple, sensitive, precise method for the determination of Rabeprazole sodium in raw material and tablet dosage form were validated using RP-HPLC. The optimum wave length for detection was 284 nm at which better detector response for drug was obtained. The system with buffer, methanol (30:70% v/v) with 0.9 ml/min flow rate is quite robust. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The correlation coefficient was found to be 0.998. The mean recoveries were found in the range of 98.0-102.0% The LOD and LOQ were found to be 2.96 and 10.1 respectively. The proposed method was validated in accordance with ICH parameter and the results of all methods were very close to label value of commercial pharmaceutical formulation.

Keywords: *Method development; System suitability; Rabeprazole; RP-HPLC; Method validation*

Introduction

Rabeprazole is an antiulcer drug in the class of proton pump inhibitors. It is a prodrug in the acid of the parietal cells; it turns in to active sulphenamide form. Rabeprazole inhibits the H^+, K^+ and ATPase of the coating gastric cells. Rabeprazole sodium chemically known as 2-[{4-(3-methoxy propoxy) -3-methoxy pyridine-2-yl} methyl sulfinyl]-H-bezimidazole. It has an empirical formula of $C_{18}H_20N_3O_3S$ and a molecular weight of 359.4 Rabeprazole which is structurally related omeprazole, is a substituted benzmidazole, and acts as a proton pump inhibitor (PPI) that suppresses gastric acid secretion through an interaction with(H+/K+)-ATPase in gastric parietal cells. Like other PPIs (omeprazole, lansoprazole and pantoprazole), rabeprazole is effective in the treatment of various peptic diseases, including gastric and duodenal ulcer, gastroesophageal reflux disease and Zollinger-Ellison syndrome. Rabeprazole may also be used with antibiotics to get rid of bacteria that are associated with some ulcers. It inhibits the final transport of hydrogen ions in to the gastric lumen. Literature studies reported various methods for the determination of Rabeprazole in tablet dosage form. These studies revealed few methods and validated a dissolution test for Rabeprazole sodium in coated tablets using RP-LC method. Majority of these were stated I the determination of Rabeprazole and its metabolites using buffer solution and biological fluids for therapeutic monitoring of rabeprazole. An attempt was done to develop a new, accurate, precise method in accordance to ICH guidelines [1,2].



Chemical structure of Rabeprazole sodium

Experimental

Instrumentation

Analytical weighing balance

HPLC system: Waters 2695 High performance liquid chromatography equipped with auto sampler and dual absorbance detector with Empower soft ware.

Column: C18 (4.6* 150 MM, 3.5 µm, XTerra)

UV Spectrophotometer: Lab India 3000

Sonicator: (Sonica 2200MH)

Vacuum filter pump (model X15522050 of Millipore)

pH meter (Metler, Toledo)

Millipore filtration kit

Reagents used for the study

Methanol: HPLC grade

Water: Milli-Q-grade

Potassium di hydrogen phosphate: AR grade

Sodium hydroxide: AR grade

Assay method development: The objective of this work is to optimize the assay method for the estimation of Rabeprazole based on the literature survey. Few trials are done to make the optimization. Following conditions are selected for method optimization [3].

Preparation of Buffer: Weigh accurately 1.56 g of potassium di hydrogen phosphate and dissolve it in 500 ml of Milli-Q-water. Adjust the pH to 5.5 with sodium hydroxide, filter through 0.45 µ membrane filter and de gas.

Mobile phase: pH 5 buffer: Methanol (30:70)

Chromatographic conditions:

Flow rate: 0.9 ml/min

Column: C18 (X Terra 4.6* 150 mm, 3.5µm)

Detector wave length: 284 nm

Column temperature: Ambient

Injection volume: 20 µl

Run time: 5 minutes

Diluents: mobile phase

Preparation of Rabeprazole standard and sample solution

Standard Solution Preparation: Accurately weigh and transfer 10 mg of Rabeprazole Working standard into a 10 ml volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). 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 0.45 µm filter.

Sample Solution Preparation

Weigh 5 Rabeprazole Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Rabeprazole into a 10 ml volumetric flask. Add about 7 ml 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 0.45 µm filter. Inject 20 µl of the standard, sample into chromatographic system and measure the area for the Rabeprazole peak and calculate the % assay by using the formula. Values are mentioned in table1 and chromatograms represented in figure 2.

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

Method Validation

The method was validated for the parameters like linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy ,precision, ruggedness, robustness, system suitability parameters were also calculated. To evaluate the linearity different concentrations of sample solutions were prepared from stock solution and correlation coefficient was calculated. The samples were injected (20 µl) and signals from the samples were recorded at 2.65 min which were compared with those of blank. LOD and LOQ values were calculated as signal-to-noise ratio of 3:1 and 10:1 respectively. To determine accuracy of the method, sample solution of rabeprazole sodium at three different concentration levels were prepared and analyzed [4-7].

Linearity

Preparation of stock solution: Accurately weigh and transfer 10 mg of rabeprazole API sample in to a 10 ml volumetric flask add about 7 ml of diluent and sonicate to dissolve it completely to make up the volume up to mark with solvent. From this 20, 30, 40, 50, 60 µg/ml concentrated solutions are prepared. Linearity plot was represented in figure 2.

Accuracy

Preparation of stock solution: Accurately weigh and transfer 10 mg of Rabeprazole working standard in to a 10 ml volumetric flask add about 7 ml of diluent and sonicate to dissolve it completely and make up to volume with the same solvent. Preparation of 40 ug/ml solution: Further pipette 0.4 ml of the above stock solution in to a 10 ml volumetric flask and dilute up to the mark with the diluent. Mix well and filter through 0.45um filter.

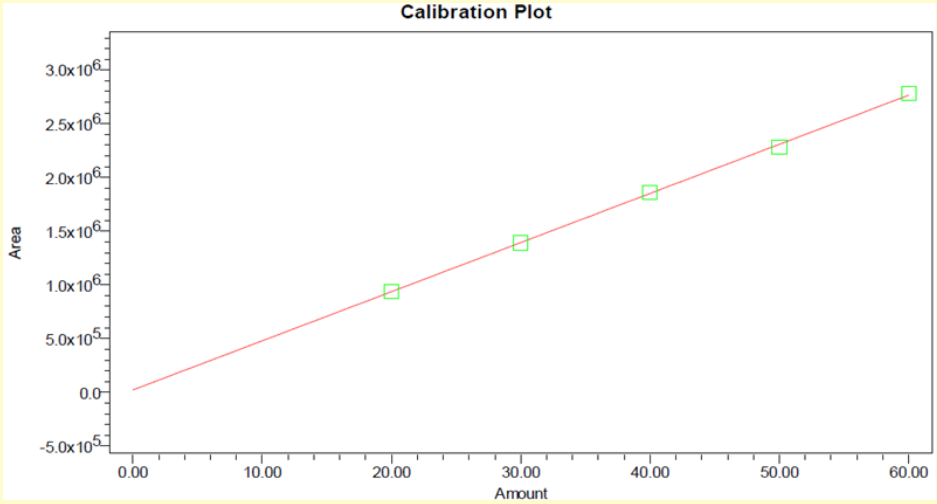


Figure 1: *Linearity plot for Rabeprazole.*

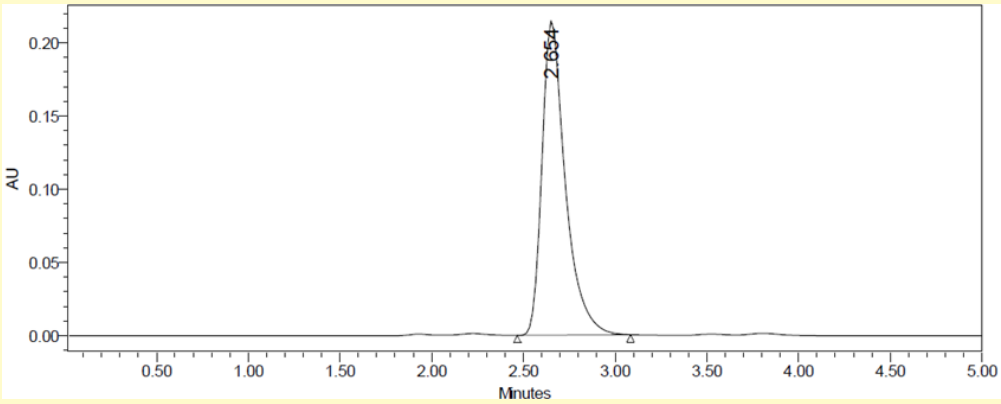


Figure 2: *Chromatogram of standard.*

Preparation of sample solutions: For preparation of 50% solution: Accurately weigh and transfer 5.0 mg of Rabeprazole API sample in to a 10 ml volumetric flask add about 7 ml of diluent and sonicate to dissolve it completely and make up the volume with the same solvent. Further pipette 0.4 ml of the above stock solution in to a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 um filter.

For preparation of 100% solution: Accurately weigh and transfer 10 mg of Rabeprazole API sample in to a 10 ml volumetric flask add about 7 ml of diluent and sonicate to dissolve it completely and make up to volume with the solvent. Further pipette 0.4 ml of the above stock solution in to a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

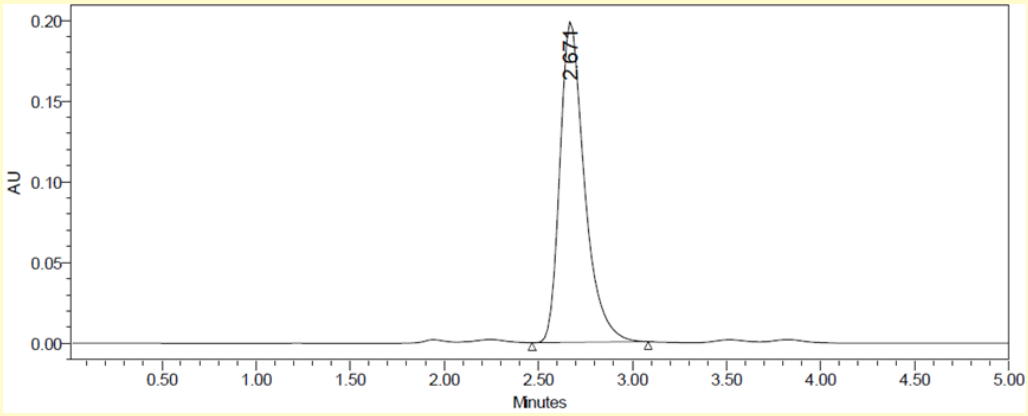


Figure 3: *Chromatogram of sample.*

For preparation of 150% solution: Accurately weigh and transfer 15 mg of Rabeprazole API sample in to a 10 ml volumetric flask add about 7 ml of diluent and sonicate to dissolve it completely and make up to volume with the solvent. Further pipette 0.4 ml of the above stock solution in to a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter [8-11].

Precision

Preparation of stock solution: Accurately weigh and transfer 10 mg of Rabeprazole working standard into a 10 ml volumetric flask add about 7 ml of diluent and sonicate to dissolve it completely and make up to volume with same solvent.

Preparation of 40 µg/ml solution: Further pipette 0.4 ml of the above stock solution in to a 10 ml volumetric flask and dilute up to the mark with the diluent. Mix well and filter through 0.45 um filter.

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision/Ruggedness: To evaluate the intermediate precision of the method, precision was performed on different day by using different make column of same dimensions. Preparation of stock solution: Accurately weigh and transfer 10 mg of Rabeprazole working standard in to a 10 ml volumetric flask and dilute up to the mark with the diluent. Mix well and filter through 0.45 µm filter.

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits [12-15].

Limit of Detection

Preparation of 40 µg/ml solutions: Accurately weigh and transfer 10 mg Rabeprazole working standard in to a 10 ml volumetric flasks add about 7 ml of diluent and sonicate to dissolve it completely and make up to the mark with solvent. Further pipette 0.4 ml of the above stock solution in to a 10 ml volumetric flask and dilute up to the mark with the diluent. Mix well and filter through 0.45 µm filter.

Preparation of 0.7% solution at specification level (0.28 µg/ml solution): Pipette 1 ml of 10 µg/ml solutions in to a 10 ml of volumetric flask and dilute up to the mark with the diluent.

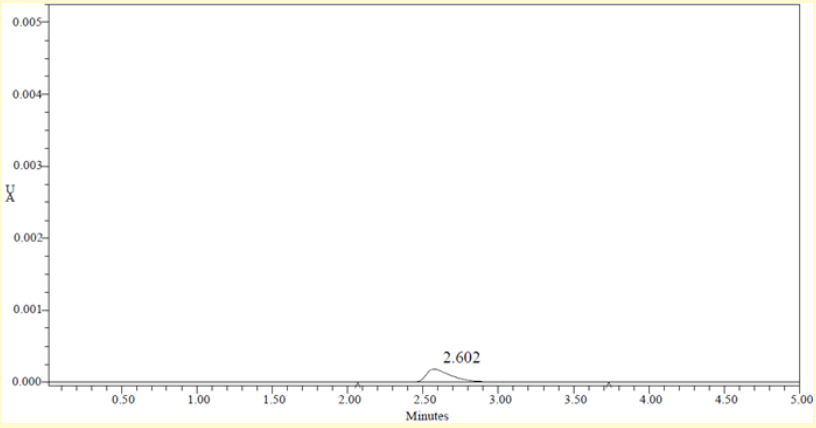


Figure 4: *Chromatogram of LOD.*

Limit of Quantification

Preparation of 40 µg/ml solutions: Accurately weigh and transfer 10 mg Rabeprazole working standard in to a 10 ml volumetric flasks add about 7 ml of diluent and sonicate to dissolve it completely and make up to the mark with solvent. Further pipette 0.4 ml of the above stock solution in to a 10 ml volumetric flask and dilute up to the mark with the diluent. Mix well and filter through 0.45 µm filter.

Preparation of 0.23% solution at specification level (0.092 µg/ml solution): Pipette 1 ml of 10 µg/ml solution in to a 10 ml of volumetric flask and dilute up to the mark with diluent.

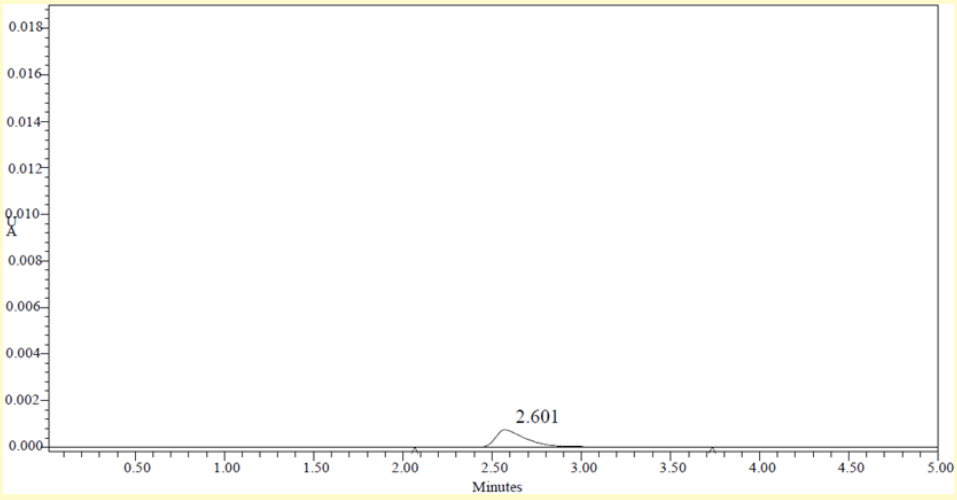


Figure 5: *Chromatogram of LOQ.*

Results and Discussions

Assay

Results are within the acceptance limits of 95-105%

Results were represented in following table 1

S No	Rabeprazole	
1	Sample Area	1884270
2	Standard Area	1807375
3	Standard Weight	10 mg
4	Sample Weight	10.1 mg
5	LC	20 mg
6	Average Weight	0.0204 mg
7	Standard Purity	99.7
8	Assay %	99.5

Linearity

Acceptance criteria: Correlation coefficient should not be less than 0.99

Results were represented in following table 2

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

Accuracy

Results are within the acceptance limits 0f 98-102.0%

% 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%	

Precision

Results are within the acceptance limits and area of five standard injections results should not be more than 2%

S No	Injection number (80 mcg/ml)	Retention Time of Rabeprazole	Area of Rabeprazole
1	Injection-1	2.669	1724358
2	Injection-2	2.668	1777933
3	Injection-3	2.655	1767353
4	Injection-4	2.666	1729271
5	Injection-5	2.662	1782024
	Average	-	1749729
	Standard Deviation	-	27398.33
	%RSD	-	1.5

Robustness

Results are within the acceptance limits and area of five standard injections results should not be more than 2%

Proposed Variations		USP Plate Count	USP Tailing
Variation in mobile phase composition	10% less	2854.3	1.4
	*Actual	2253.6	1.5
	10% more	2111.0	1.4
Variation in flow rate	0.6 ml/min	2168.0	1.4
	0.8 ml/min	2253.6	1.5
	1.0 ml/min	2074.3	1.4

Ruggedness

The results are within the acceptance limit, the proposed method is found to be rugged.

	Retention Time of Rabeprazole	Area of Rabeprazole
Standard (80 mcg)	2.669	1890004
Analyst (1) (80 mcg)	2.672	1885751
Analyst (2) (80 mcg)	2.672	1892673
Analyst (3) (80 mcg)	2.667	1861622
Analyst (4) (80 mcg)	2.668	1871563
Average	-	1880323
Standard Deviation	-	13249.5
%RSD	-	0.70

Limit of Detection

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 51 µV

Signal Obtained from LOD solution (0.7% of target assay concentration): 151 µV

S/N = 151/51= 2.96

Acceptance Criteria: S/N Ratio value shall be 3 for LOD solution.

Conclusion: The LOD for Rabeprazole was found to be 2.96.

Limit of Quantification:

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 51µV

Signal Obtained from LOD solution (0.23% of target assay concentration): 516 µV

S/N = 516/51 = 10.1

Acceptance Criteria: S/N Ratio value shall be 10 for LOQ solution.

Conclusion: The LOQ for Rabeprazole was found to be 10.1

Conclusion

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low cost RP-HPLC method. It is successfully applied for the determination of Rabeprazole in pharmaceutical preparation without the interferences of other constituents in the formulations.

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