ISSN 2349-7203

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

HUMAN

Human Journals
Research Article
July 2016 Vol.:6, Issue:4

A New Method Development and Validation of Rabeprazole Sodium in Formulation by Using HPLC

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

V.ASHA RANJANI*[1], J.PRAVEEN KUMAR[1], K.S.BHARATH KUMAR[2]

[1] *MLR Institute of Pharmacy, Dundigal (V), Quthubullapur (M), Hyderabad-43, Telangana.*

[2] *Jawarhalal Nehru Technological University, Ananthapur., India.*

Submission: 30 June 2016
Accepted: 5 July 2016
Published: 25 July 2016

Keywords: Method development, System suitability, Rabeprazole, RP-HPLC, Method validation

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 was validated using RP-HPLC. The optimum wavelength for detection was 284nm 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.

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 into active sulphenamide form. Rabeprazole inhibits the H+, K+, ATPase of the coating gastric cells. Rabeprazole sodium is chemically known as 2-[{4-(3-methoxy propoxy) -3-methoxy pyridine-2-yl methyl sulfinyl] –H-benzimidazole. It has an empirical formula of C18H20N3O3S and a molecular weight of 359.4 Rabeprazole which is structurally related to omeprazole, is a substituted benzimidazole, 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 into 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 in 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.

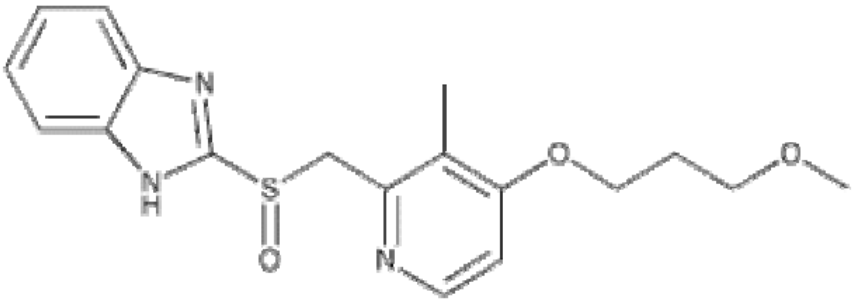

Fig: 1 Chemical structure of Rabeprazole sodium

MATERIALS AND METHODS

Experimental

Instrumentation

Analytical weighing balance

HPLC system: Waters 2695 High performance liquid chromatography equipped with autosampler and dual absorbance detector with Empower software.

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 dihydrogen 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.

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

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

Chromatographic conditions:

Flow rate: 0.9ml/min

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

Detector wavelength: 284nm

Column temperature: Ambient

Injection volume: 20µl

Run time: 5 min.

Diluents: mobile phase

Preparation of Rabeprazole standard and sample solution:

Standard Solution Preparation:

Accurately weigh and transfer 10mg 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 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm 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 0.45µm filter. 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. Inject 20µl 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 fig 2.

Calculation:

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

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 quantification (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 (20µl) 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.

Linearity:

Preparation of stock solution: Accurately weigh and transfer 10 mg of rabeprazole API sample into 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, 60µg/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 into 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 40ug/ml solution:

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

Preparation of sample solutions:

For preparation of 50% solution: Accurately weigh and transfer 5.0 mg of Rabeprazole API sample into 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 into 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 into 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 into 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 150% solution: Accurately weigh and transfer 15 mg of Rabeprazole API sample into 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 into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45um filter.

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 40ug/ml solution:

Further pipette 0.4 ml of the above stock solution into 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 into 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

Limit of Detection:

Preparation of 40ug/ml solutions:

Accurately weigh and transfer 10 mg Rabeprazole working standard into 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 into a 10 ml volumetric flask and dilute up to the mark with the diluent. Mix well and filter through 0.45 um filter.

Preparation of 0.7% solution at specification level (0.28ug/ml solution):

Pipette 1 ml of 10ug/ml solution into a 10 ml of volumetric flask and dilute up to the mark with the diluent.

Limit of Quantification:

Preparation of 40ug/ml solutions: Accurately weigh and transfer 10 mg Rabeprazole working standard into 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 into a 10 ml volumetric flask and dilute up to the mark with the diluent. Mix well and filter through 0.45 um filter.

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

RESULTS AND DISCUSSION

Linearity:

Acceptance criteria: Correlation coefficient should not be less than 0.99 Results were represented in following table 1

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

Assay:

Results are within the acceptance limits of 95-105%

Results were represented in following table 2

S.no	Rabeprazole	
01	Spl. Area	1884270
02	Std.Area	1807375
03	Std. Wt	10mg
04	Spl.Wt	10.1mg
05	LC	20mg
06	Avg.Wt	0.0204mg
07	Std.Purity	99.7
08	Assay %	99.5

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%

SI.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
	AVRG		1749729
	STDEV		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	0.6ml/min	2168.0	1.4
	0.8ml/min	2253.6	1.5
	1.0ml/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(80mcg)	2.669	1890004
Analyst(1)(80mcg)	2.672	1885751
Analyst(2)(80mcg)	2.672	1892673
Analyst(3)(80mcg)	2.667	1861622
Analyst(4)(80mcg)	2.668	1871563
AVRG		1880323
STDEV		13249.5
%RSD		0..70

Limit of Detection:

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 51µV

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

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.

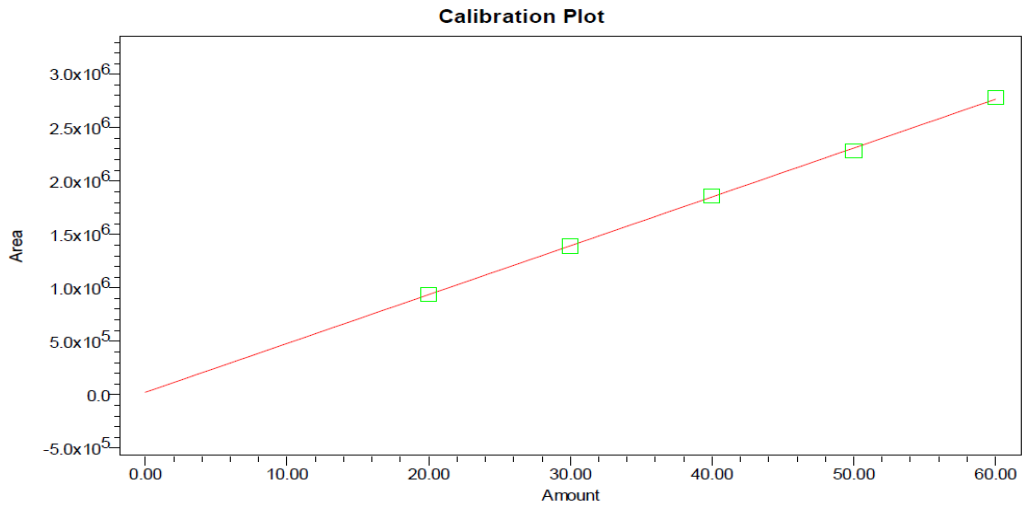

Figure 2: Linearity plot for Rabeprazole

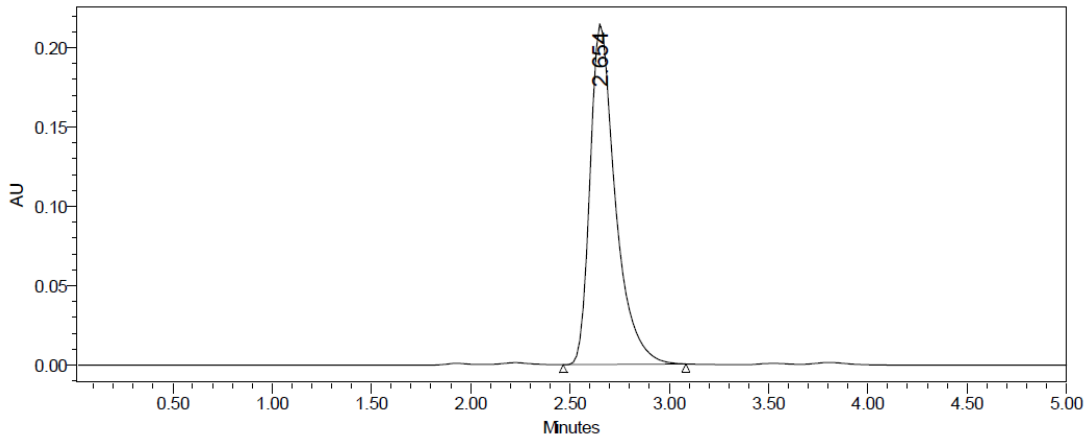

Figure 3: Chromatogram of standard

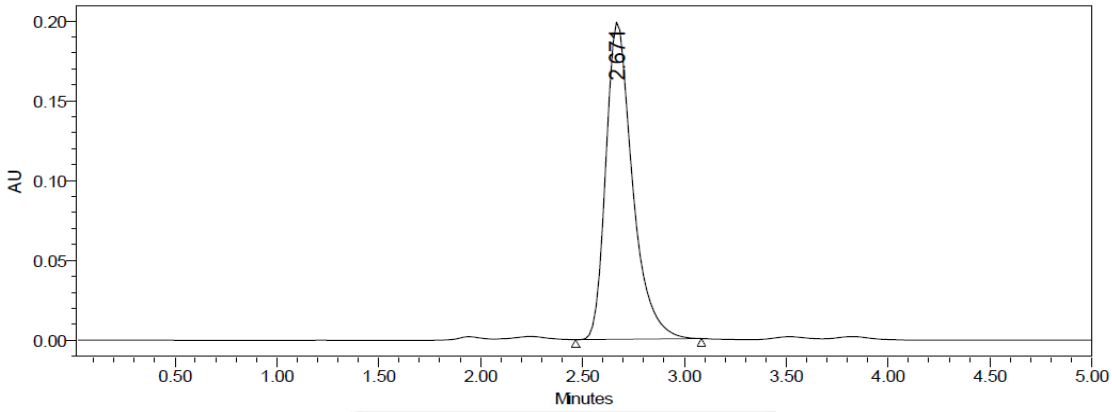

Figure 4: Chromatogram of sample

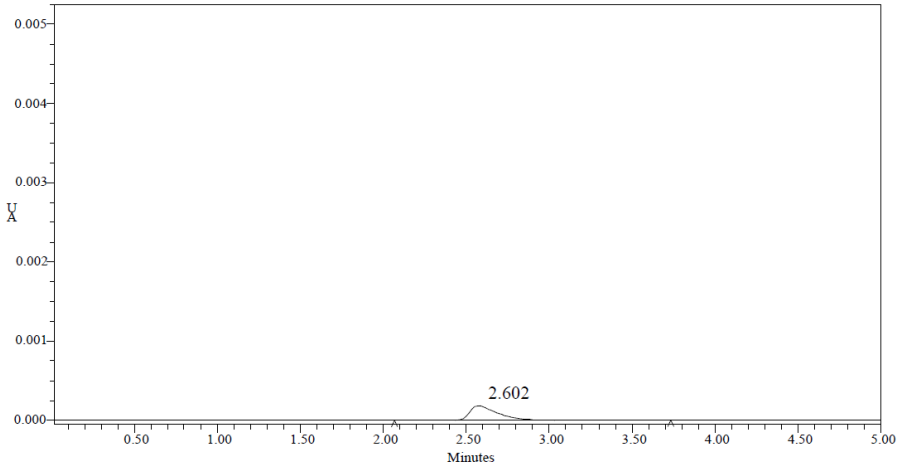

Figure 5: Chromatogram of LOD

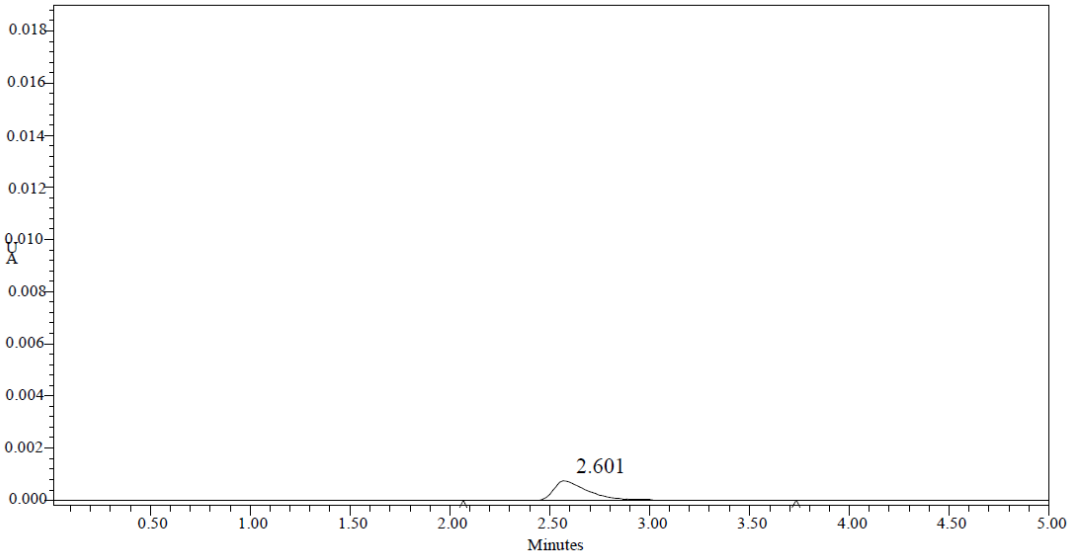

Figure 6: Chromatogram of LOQ

REFERENCES

1. Beckett, A.H. and Stenlake, J.B. Practical Pharmaceutical Chemistry, part B. 4th edn., CBS publishers and Distributors, New Delhi, 2002, 272-280.
2. Chatwal, R. Gurdeep and Sham, K. Anand. Instrumental Methods of Chemical Analysis. 5th Revised edn., Himalaya publishing House, Mumbai, 2000, 2, 160.
3. Skoog, DA and West, DM. Fundamentals of Analytical chemistry. 8th edn, Thompson Asia Pvt Ltd., Singapore, 2005, 314.
4. Sharma, B.K. Instrumental method of chemical Analysis. 17th edn. Krishna Prakashan Media Pvt. Ltd., Meerut, 1997, 8.
5. Sethi, P.D. HPLC Quantitative Analysis of Pharmaceutical formulations. 1st edn. CBS Publishers and Distributors, Delhi, 2001, 3-94.
6. Martindale, the Extra Pharmacopoeia, 3rd Ed., The Royal Pharmaceutical Society, 1996, 1237.
7. Goodman and Gilman's, The Pharmacological Basis and Therapeutics, 10th Ed., 2001, 1007.
8. Langtry H D and Markham A, Drugs, 1999, 58, 725-742.
9. The Merck Index, 12th Ed., 1996, 1392.
10. Lakshmana Rao A, B.N.V.Ravi Kumar and G.G.Sankar .,Development of RP-HPLC Method for the Estimation of Rabeprazole in Pure and Tablet Dosage Form 2008, 5, 1149-1153
11. Palled MS, M Chatter, PNM Rajesh, AR Bhat Reverse phase high performance liquid chromatographic determination of rabeprazole in tablet dosage form 2006, 68, 3, 406-408
12. Uma Mahesh Karra* and Sanjeeva Yarkala ., A Simple and Validated Reverse Phase HPLC Method for the Determination of Rabeprazole in Pharmaceutical Dosage Forms 2010, 7(2), 569-577
13. Prasanna Reddy Battu and MS Reddy ., Development and Validation of RP-HPLC for the Rabeprazole sodium in Pharmaceutical formulations and Human Plasma Jan.-March, 2009;Page 49-51
14. Cassia V. Garcia, Clesio S. Paim, Martin Steppe and Elfrides E.S. Schapoval Development and validation of a dissolution test for rabeprazole sodium in coated tablets
15. Shirish R. Patel, L. J. Patel, Yogeshvar P. Thakkar, Nimesh D. Patel., Rabeprazole and Ondansetron in pharmaceutical dosage form by Reversed-phase HPLC 2010, 2, 3, 1531-1536.