

Validated RP-HPLC method for rabeprazole and its stability studies

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ABSTRACT A reliable, fast isocratic reversed phase high performance liquid chromatography (RP-HPLC) method for the estimation of Rabeprazole in its tablet dosage forms and formulations in combination with different superdisintegrants warrants its quality assurance and also to apply to the stability studies of different rabeprazole formulations. The present study developed a validated RP-HPLC method as per ICH guidelines. The peaks were found to be symmetrical under the optimized chromatographic conditions using RP-C8 column and extensively used for the simultaneous estimation of Rabeprazole and its oxidative degradation products. The present method can be confidently used for single dosage enteric coated formulation of the drug rabeprazole. The stability studies of pure rabeprazole and its different forms with four different superdisintegrants showed that formulation with Polyplasdone XL gave the best stability under the test conditions. The present method solved all the difficulties developed due to the enteric coating of superdisintegrants and used for quality control and quality assurance of rabeprazole tablet dosage forms and its formulations.

Keywords: Rabeprazole, RP-HPLC, superdisintegrants, Polyplasdone XL, oxidative degradation products.

Introduction

Rabeprazole is an antiulcer and Zollinger-Ellison Syndrome drug in the class of proton pump inhibitors and is marketed by Janssen-Cilag as rabeprazole sodium (RS) under the brand names Aciphex and Pariet. Rabeprazole sodium (Figure 1), chemically 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl] methyl]sulfinyl]-1H-benzimidazole sodium salt¹, is specific inhibitor of the gastric hydrogen-potassium adenosine triphosphatase (H⁺, K⁺ ATPase) enzyme system at the secretory surface of gastric parietal cells. It inhibits the final transport of hydrogen ions (via exchange with potassium ions) into the gastric lumen.

Rabeprazole sodium is a yellowish-white solid and soluble in water, methanol, ethanol and ethyl acetate, but insoluble in ether and n-hexane. Its stability depends on

pH and degrades rapidly in acid media, but is more stable under alkaline conditions.

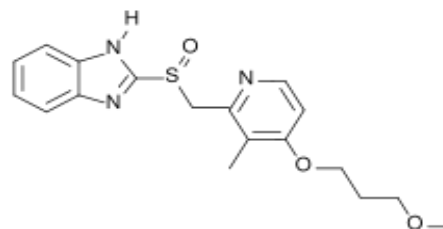


Figure 1: Structure of Rabeprazole sodium {2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl] methyl]sulfinyl]-1H-benzimidazole sodium salt}

The different analytical techniques reported so far for analysis of rabeprazole in biological samples and in pharmaceutical formulations included electrophoresis, UV-visible spectrophotometry, LC and LC-MS methods for enantioselective assay¹⁻¹¹.

Patel *et al.*³ did not get reproducible results with the enteric coated capsule formulation of rabeprazole with ISP excipient because measurement of its acid resistance was not considered for its stability and delivery. This study also did not perform stress analysis of the enteric coated tablet formulation³. A number of papers had been published for the HPLC assay of Rabeprazole but they were mostly in combination with antiemetic drug Domperidone or cholinergic drug Itopride.

Although Garcia *et al.*⁷ published an accurate and precise validated method for enteric formulation about rabeprazole, but the disadvantage of this method was lack of resolution with severe tailing.

So, the main objectives of the present study were to develop i) a simple, precise, sensitive, rapid and reproducible HPLC method for assay of rabeprazole, ii) to validate as per ICH guidelines and iii) to apply to the stability studies of the rabeprazole formulations.

Experimental

Reagents

Rabeprazole Standard (Aurobindo Pharma, Hyderabad), Rabeprazole coated tablet (ISP India Pvt. Ltd), acetonitrile (HPLC grade), sodium borate (Qualigens), Milli-Q water, Acdisol, SSG, PVPP XL, PVPP XL-10 (ISP India Pvt. Ltd), Karl Fischer reagent, and Whatmann filter paper were supplied from ISP (India) Pvt. Ltd, Hyderabad.

Instruments

A High Pressure Liquid Chromatographic (HPLC) system was used which was attached with a separation module 2695 system and equipped with 2996 photodiode array detector. The dissolution apparatus was from Venkel – Total solution 2 (Varian) and equipped with Agilent 8453UV spectrophotometer. Other small instruments such as Water content instrument - 795RFT titrator (metrohm), Compression machine – Cadmach, Coating machine - O'HARA Tech Labcoat system, Disintegration apparatus-ERWEKA, Friability apparatus-EWREKA, pH meter- Metrohm, Weighing Balance-Electronic balance (Mettler-Toledo) were available in the lab.

Analytical methods

A stock solution 1000 mg/L RS reference standard was used throughout the study. The diluent was prepared by mixing sodium tetra borate (0.01M) buffer (pH 7) and acetonitrile in the ratio of 3:1 (v/v). A mobile phase consisted of water and acetonitrile in the ratio of 72:28 (v/v) was used at a flow rate of 1mL/min and the temp was maintained at 25°C.

Preparation of sample solution

The 1000 ppm sample was prepared by dissolving 100 mg rabeprazole taken after grinding 5 tablets of the each batch. Twenty mL of the stock solution was centrifuged at 3500 rpm for 10 minutes, filtered through 0.22 µm Millipore filter paper and used for analysis. The placebo (disintegrants) samples were prepared in the same way as mentioned above. The sample and placebo solutions were injected in triplicate separately for analysis.

The HPLC method was optimized against the effect of mobile phase, flow rate, column temperature, injection volume, run time and pH. The optimized HPLC instrumental conditions are given in Table 1.

Method validation

The accuracy of the method was determined by recovery experiment which was carried out by the standard addition method at three levels of 80, 100 and 120%. Each solution was injected in triplicate and the percentage recovery was obtained. The system suitability test was performed as per ICH guidelines. Six preparations were made individually using single batch of RS working standard as per test method and injected each solution in triplicate. The average RSD (%) values were calculated for precision.

Table 1: Optimized HPLC conditions for the elution of Rabeprazole

Parameters	Details
Mobile phase	Water: Acetonitrile
Column	YMC basic (C8, 150 x
Flow rate	1.0 mL/min
Column temperature	25°C
Injection volume	10 µL
Detector wavelength	302 nm
Run time	15 min
Retention time	10.1 min
pH	7
Diluent	0.01M Sodium borate: Acetonitrile (3:1 v/v)

The linear correlation between the peak area and the concentration of the compound was checked for each component. Data for five different concentrations of rabeprazole were prepared and analyzed. The correlation coefficient (*r*) was calculated. Limit of detection (LOD) for rabeprazole was found to be 1 ppm and limit of quantification (LOQ) was found to be 3 ppm for rabeprazole in this study. Robustness and ruggedness were determined between 2 different days, analysts and instruments¹¹⁻¹⁴.

Stability studies

The stability study of RS was undertaken to test its stability under three different conditions (40°C/75% RH, 25°C/60% RH and 30°C/65% RH). The samples were prepared by dissolving 100 mg from a stock of 5 grinded tablets of each batch in 100 mL of diluents solution. Then 20 mL of this solution was centrifuged at 3500 rpm for 10 minutes, filtered and analyzed. The placebo samples were prepared in the same way as mentioned above. The sample and placebo solutions were injected in triplicate separately for quantification of rabeprazole. The assays of the samples were determined by comparing the area counts of the standard as follows [%assay = (sample area * std. wt * Ave. wt. of 20 tablets)/std. area * sample wt. * label claim)]. The physical parameters such as moisture content, weight of each tablet, diameter and thickness of the tablets, and hardness were calculated as per ICH guidelines.

Oxidative degradation study

Degradation studies were carried out under acid hydrolysis, alkali hydrolysis and peroxide oxidation. For acid hydrolysis 5mL of 0.1 (N) HCl was added to standard solution, kept for one hour, and then neutralized with 0.1 (N) NaOH and volume was made up with the diluents. For alkali hydrolysis 5mL of 0.1 (N) NaOH was added to standard solution, kept for one hour, and then neutralized with 0.1 (N) HCl and volume was made up with the diluents. As superdisintegrants contained peroxide, the present study was also undertaken to ensure whether the peroxide had any effect on the stability of RS or not. For peroxide study 5mL of 3% H₂O₂ (volume %) was added to standard solution, kept for one hour, and volume was made up with the diluents.

Results

Rabeprazole sodium is unstable in neutral and acidic media. Therefore, when rabeprazole sodium is to be formulated into a preparation for oral administration, it should be enteric coated to prevent the decomposition with the gastric acid. However, an enteric coating being an acidic material is insoluble in acidic conditions, but soluble at neutral to alkaline conditions. For the stability studies this matter was considered in all stages and also mobile phase systems were chosen accordingly for validated HPLC method for identification and quantification of rabeprazole in its tablets and formulations. Validation of HPLC method was performed as per ICH guidelines as follows.

System suitability test was carried out and the %RSD values for both retention time and area were found to be good and within the limits as per ICH guidelines (Table 2). Accuracy test showed that the %RSD and % Recovery values were 100.49 ± 0.86 which was within the limit (95-105%) as per ICH guidelines. Method Precision showed that the average %RSD value was 1.1 which implied that the reproducibility of the proposed method was good. The linearity was investigated in the range of 100 to 1250 ppm using five different concentrations. The areas obtained at 302 nm for Rabeprazole were fitted to a straight line by the method of least squares. Linear regression analysis for rabeprazole was found to be 0.9997. The LOD and LOQ value for rabeprazole was found to be 1 ppm and 3ppm.

The results on robustness indicated that changing the temperature (± 2), wavelength (± 2) had no large effect on the chromatographic behavior of rabeprazole respectively. Alteration of the flow rate and mobile phase composition caused

significant variation of retention time and affect the chromatographic behavior of rabeprazole of the method. Therefore this method was very much specific towards flow rate and mobile phase composition.

Rabeprazole was found to be unstable in acidic media but stable in alkaline media and it was prone to be oxidized when it was treated with oxidizing agent.

The value of RSD (%) was found to be below 2.0% which showed ruggedness of developed HPLC method. Four different excipients (Ac-di-sol, SSG, PVPP-XL and PVPP-XL-10) were used for the preparation of tablets and assay of all types showed that up to 3 month's stability study PVPP-XL and Ac-di-sol were showing good results then SSG and PVPP-XL-10. Excipients Ac-di-sol and PVPP-XL containing drugs were within the limit 96.6-105.6%.

The measurement of moisture content showed that the results were within the limit i.e. it was between 3-4.5%. The study on uniformity of tablet weight showed that the wt. were within the limits ($120\text{mg} \pm 7.5\%$). Similarly study results on hardness were within the specified limit (140-160). The results on diameter of rabeprazole tablets were all within the limit (i.e. $\text{wt} \pm 0.1$). The results on thickness were within the limits (3.1 ± 0.2 mm).

Discussion

The present study aimed to develop a validated HPLC method for identification and quantification of rabeprazole in its tablets and formulations with or without superdisintegrants as enteric coatings. Different proportions of water and acetonitrile were used to optimize retention time and resolution. A proportion of water:methanol (as 72:28 v/v) showed a good resolution at a retention time of 10.1 min. The method was validated against accuracy/trueness, precision, repeatability, intermediate precision, specificity/selectivity,

ruggedness, robustness, linearity and recovery as per ICH guidelines 12-13. All the results (Table 2) were within the specifications suggested by ICH guidelines 12-13. In addition LOD and LOQ were calculated using this validated method

and were 1 ppm and 3 ppm respectively. Also, the method was applied to identify and quantify rabeprazole and its oxidative products if any in the presence of superdisintegrants.

Table 2: Comparison between results obtained by validated method and ICH guidelines

Characteristics	Acceptance Criteria	Observed Results
Accuracy/trueness	Recovery 95-105%	99.77-101.69
Precision	RSD < 2%	1.1-1.4%
Repeatability	RSD < 2%	Complies
Intermediate precision	RSD < 2%	<1%
Specificity/Selectivity	No interference	Complies
Ruggedness	% RSD not more than 2.0%	0.15-1.01
Robustness	% RSD not more than 2.0%	Complies
Detection limit	Signal to noise ratio (S/N = 3:1)	1.48
Quantization limit	Signal to noise ratio (S/N = 10:1)	4.6
Linearity	Correlation coefficient (r > 0.999)	r = 0.9997
Recovery	Recovery 95-105%	96.6-105.6

A disintegrant is a substance which increases the surface area of the tablet particles after rupturing tablet walls rapidly in contact with gastrointestinal fluids, thereby increasing the rate of absorption of the active ingredient and producing the desired therapeutic action. Four different super disintegrants Ac-di-sol, Polyplasdone XL, Polyplasdone XL-10 and Sodium starch Glycolate were used as excipients for enteric coatings of rabeprazole tablets. A thorough stability studies were carried out to check the effects of these excipients under various stress conditions on stability of rabeprazole tablets and its different formulations.

The significance of stability studies includes i) to assess therapeutic efficacy in the dosage form; ii) to find out any toxic rabeprazole was observed for different superdisintegrants coated rabeprazole the resolution was good for polyplasdone XL

product formed during decomposition process or not; iii) to predict whether instability lowers bioavailability of the dosage form or not, and iv) to monitor whether the changes in the physical appearance of the dosage form cause to lose confidence of the patients on the drug products. In the present study the effect of pH on stability of rabeprazole and its formulations was checked and rectified the reproducibility problem that was appeared in the study of Patel *et al.*³ (2007). Rao *et al.*¹⁴ also developed an isocratic HPLC method for the estimation of rabeprazole in tablet dosage form, but it was not suitable for the present study since enteric coating interfered in the determination and resolution was not good. Although very minor variation in retention time of coated rabeprazole tablets (Figure 6). Also, it was applied for identification and quantification of rabeprazole in its tablet

forms and formulations and it was selective, accurate and reproducible in the presence of placebos/disintegrants and other

supporting ingredients as well as oxidative degradation products (Figure 2-7).

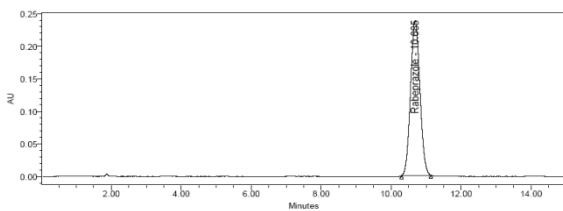


Figure 2: A representative chromatogram of the working standard.

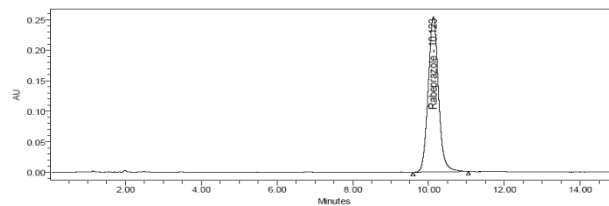


Figure 3: A representative chromatogram of the working sample of rabeprazole.

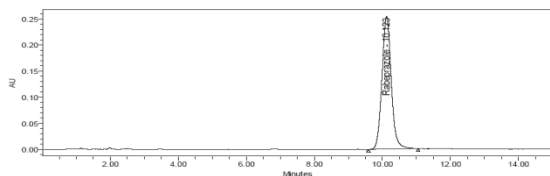


Figure 4: A representative chromatogram of the working sample of rabeprazole made with Ac-di-sol.

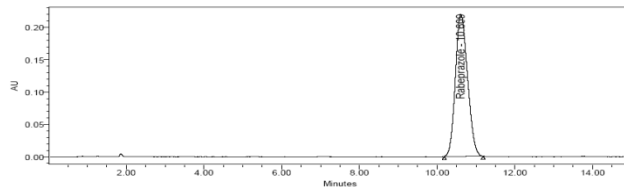


Figure 5: A representative chromatogram of the working sample of rabeprazole made with SSG.

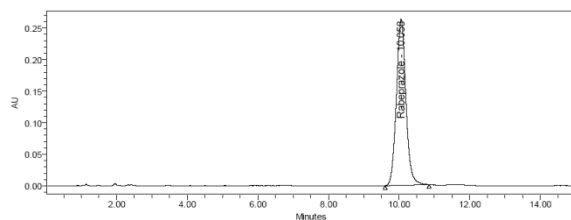


Figure 6: A representative chromatogram of the working sample of rabeprazole made with PVPP-XL.

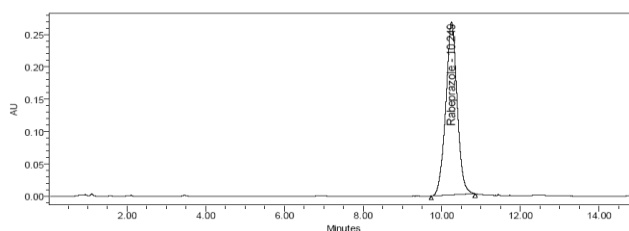


Figure 7: A representative chromatogram of the working sample of rabeprazole made with PVPP-XL10.

Conclusion

A reliable, fast isocratic RP-HPLC method for the estimation of Rabeprazole in tablets and its formulations has been developed and validated for various parameters proposed by ICH guidelines. The peaks were found to be symmetrical under the optimized chromatographic conditions. Validation parameters like specificity, system suitability, linearity,

range, accuracy, and precision and robustness results have showed that the proposed method is validated and can be used for the simultaneous estimation of Rabeprazole and its oxidative degradation products. A comparative three-month stability studies for rabeprazole formulations using four types of disintegrants are evaluated and as compared to others three, a formulation with Polyplasdone XL showed better result in terms of stability concerned.

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