Research Article

Development and Validation of Stability Indicating Assay Method (Siam) For Rabeprazole in Rabeprazole Sodium Delayed Release Tablets Using HPLC

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ABSTRACT

A stability-indicating assay method (SIAM) method was developed for the assay of Rabeprazole (RBZ) in tablet dosage formulations using high performance liquid chromatographic (HPLC). Isocratic separation was achieved on Inertsil ODS 3V (250 x 4.6mm), 5µ, using a mobile phase of Ammonium Acetate buffer (pH 7.0): ACN (50:50), at a flow rate of 1 ml/min and UV detection at 282 nm. retention time of 4.72 min with sharp symmetrical peak. For specificity, linearity, precision, accuracy, precision, robustness, the method was validated. The drug has been subject to conditions of stress, including hydrolysis, oxidation, photolysis and thermal degradation. Full separation was achieved for the parent drug and the degradation products, with a total runtime of 10 min with eluting of the parent compound at approximately 4.72 min. For the study of RBZ in delayed release tablet dosage form, the method was successfully used.

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INTRODUCTION

Rabeprazole sodium (RBZ) shown in Figure1, is, 1H-Benzimidazole, 2-[[[4-(3-methoxypropoxy pyridinyl] methyl] sulfinyl], sodium salt. RBZ blocks the last step to secrete gastric acid. RBZ is protonated, picked up and converted into active sulfenamide in gastric parietal cells. When studied in vitro, RBZ is chemically activated at pH 1.2 with a half-life of 78 seconds.Literature review shows that some analytical procedures have been taken into account for estimation of RBZ as stability indicating and in biological fluids or in combination with different drugs in pharmaceutical dosage forms [1].

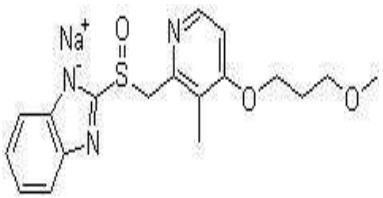

Figure 1: Rabeprazole sodium

EXPERIMENTAL

Materials

RBZ (99.9% purity) used as a standard for studies was provided from Biotech Solutions, New Delhi. Water and methanol (Merck ltd) used were HPLC grade, formic acid, acetonitrile (ACN), 30% Hydrogen peroxide with analytical reagent grade,provided by M/S SD Fine Chemicals, Mumbai, India.

Sample preparation was done in mobile phase solvent made by combining Ammonium Acetate buffer (pH 7.0): ACN (50:50). The tablet formulation of RBZ used for the validation of method accuracy, precision, and specificity were purchased from the local market, having brand name Acistaldelayed release tablet manufactured by Unichem Laboratories Ltd.with labeled claim 20mg of drug.

Instrumentation

RP-HPLC Shimadzu (LC 20 AT VP) model which consisted of an LC-20AD solvent delivery module, SPD-M20A prominence diode array detector, a rheodyne injector (model 7125, USA) valve fitted with a 20µl loop. The machine was operated using a SCL-10A system controller and a personal computer with chromatographically mounted shimadzu software (LC Solution, Published 1-11SP1).

Chromatographic conditions

The column used was anInertsil ODS 3V (250 x 4.6mm), 5µ. The mobile phase was 50:50 % (v/v) Ammonium Acetate buffer (pH 7.0): ACN. The flow rate was 1 ml/min, the wavelength was at 282 nm, and the injection volume was 20 µl. At room temperature, the column was kept at 10 minutes. The results were calculated using counts of peak areas.

Standard preparation

Standard solution of RBZ was prepared. An accurately weighed quantity of about 100 mg of RBZ was dissolved in 50:50 % (v/v) Ammonium Acetate buffer

(pH 7.0): ACN and diluted to 100ml. Working standard was prepared by dissolving 5ml of above Solution diluted to 50.0 ml with mobile phase to get concentration of 100 µg/ml [2].

Assay sample preparation

Weighed 20 tablets and measured the average weight. The tablets have been broken to fine powder. For 15 minutes, the precisely weighed amount of powder tablet was diluted by diluent for 100 mg of medication and the volume by diluting was made to 100.0 ml and filtered, and 0.1 ml of transparent filtrate was diluted to 10.0 ml by mobile process (conc.10 µg/ml). Similarly, five sample replication solutions have been prepared.

Forced degradation studies of tablet

RBZ delayed release tablet under different conditions was stressed to perform forced degradation studies in order to decide if the analytical method and assay is stability-indicating. RBZ is methanol-soluble and solvent in any sample. Both RBZ solutions prepared for use during studies on forced degradation were diluents in 1mg/ml RBZ starting diluents. [3].

Oxidation

50 mg of RBZ was dissolved in 50ml 30% H_2O (1mg/ml) and 10 ml of the above solution refluxed in round bottom flask on boiling water bath for 5 hr. Chromatogram is shown in Figure 2.

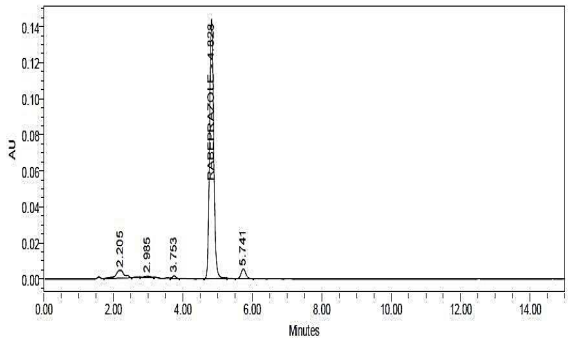

Figure 2: Oxidation degradation

Acid degradation studies

50 mg of RBZ was dissolved in 50ml of 0.1N hydrochloric acid (1mg/ml) and 10 ml of it was refluxed in round bottom flask on boiling water bath for 5 hr. Chromatogram shown in Figure 3.

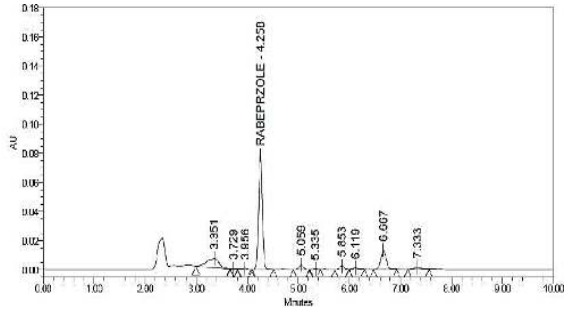

Figure 3: Acid degradation

Alkali degradation studies

50 mg of RBZ was dissolved in 50ml of 0.1N sodium hydroxide (1mg/ml) and 10 ml of it was refluxed in round bottom flask on boiling water bath for 5 hr. Chromatogram shown in Figure 4.

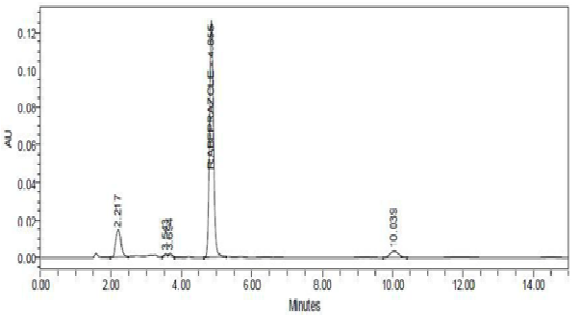

Figure 4: Alkali degradation

Neutral degradation studies

50 mg of RBZ was dissolved in 50ml of Distilled Water (1 mg/ml) and 10ml of the above solution was refluxed in round bottom flask on boiling water bath for 5 hr. Chromatogram shown in Figure 5.

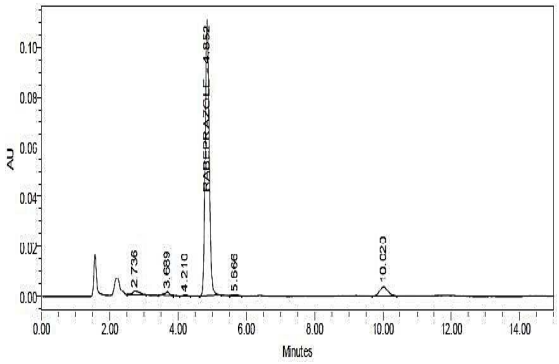

Figure 5: Neutral degradation

Temperature stress studies

Tablet and API powder were exposed to dry heat in an oven for 3 days at various temperatures (50^0C, 60^0C & 70^0C). The tablet and API powder were removed from the oven and the contents of 20 tablets were removed and mixed. An aliquot of powder equivalent to the weight of one tablet and API powder were then prepared for analysis as previously described. Chromatogram shown in Figure 6.

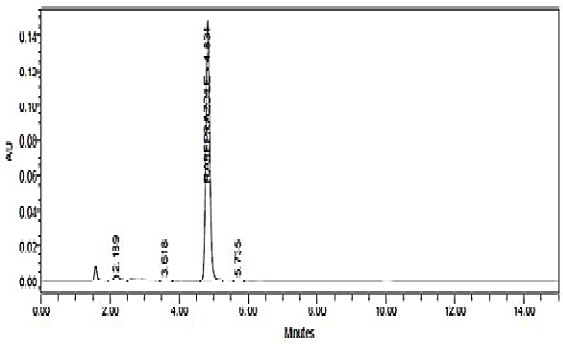

Figure 6: Temperature stress studies

Photostability

RBZ API and tablet contents RBZ were prepared and exposed to sun light to determine the effects of sunlight irradiation on the stability of RBZ in the solid state. 10 mg samples of RBZ, evenly spread in thin layer in a Petri-dish with thickness less than 2mm were kept in sunlight for different time intervals; for 3 days. Tablet samples were prepared in same manner. The samples for analysis were prepared as described previously. Chromatogram shown in Figure 7.

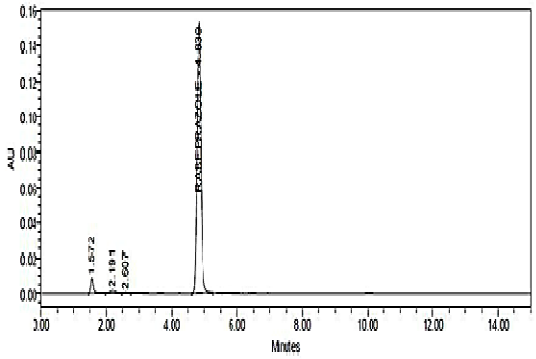

Figure 7: Photostability

RESULTS AND DISCUSSION

Development and optimization of the HPLC method

For optimum absorbance in the UV spectrophotometer the prepared standard solution was screened with a maximum absorption of 282 nm.

The chromatographic conditions were calculated in accordance with the given parameters and a blank chromatograph was taken before analysis to balance with the standing phase as indicated in the stable baseline. The injector of Rheodyne injector (20µl) was prepared for the standard solution and chromatograms for the drug were registered. By permutation and combination, various mobile phases were also checked by different flow rates.Mobile phase containing 50:50% (v/v) Ammonium Acetate buffer (pH 7.0): ACN at a flow rate of 1 ml/min found to have a reasonable retention time of approximately 4.72 min with a sharp symmetrical peak with a tailing factor below 2 and performance above 2000. The chromatogram reported five replicate injections separately. Periodically collected chromatograms of the sample solution over 30 hours, the findings indicate that the drug is sufficiently stable at the mobile phase. Table.1 displays the results. The same mobile phase, the stressed samples of the ammonium acetate buffer (pH 7.0) of 50:50 % (v/v), were analyzed.

Validation

In terms of parameters, including linearity, accuracy and precision, the method was validated [4].

Linearity

Stock standard solution portions of aliquot were diluted to 10.0 ml with 100 µg/ ml concentrations of the mobile phase. The chromatographic conditions with a steady baseline were set as stated earlier. Separately, standard solutions of various concentrations were injected and the chromatograms were reported and shown in Figure 8. By plotting the peak area against the concentration with excellent linearity, the calibration curve was built. [5].

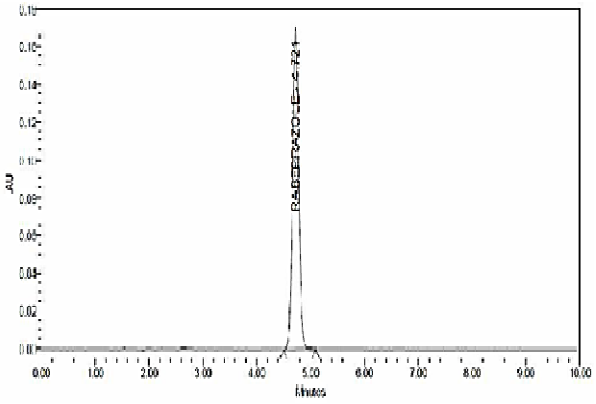

Figure 8: Standard chromatogram for RBZ

Precision

Analytical method precision was demonstrated as SD and RSD percentage of replicate series calculation. The precision of RBZ estimates was calculated by the proposed method using a repeated

analysis of homogenous tablet powder samples [6]. The performance of the test was analyzed in terms of both repetitiveness and reproductiveness. The injection of three samples with a 20µg/ml standard drug concentration was investigated for repetitiveness. The average amounts were 19.84 µg/ml SD at 0.351 and 0.353 RSD at the percentage. Intraday precision was tested by injection of the same 3 specimens of 20µg/ml standard pharmaceutical drugs at 3 different concentrations, leading to mean RBZ concentrations of 19.93µg/ml and related RSD concentrations of 0.531%.

The findings showed the mean concentration of 19.94 µg/ml and associated percent of RSD 0,511, shown in Table1 were based on the injection of three same samples on 3 different consecutive days.

Table 1: Intraday and Inter day precision studies

Intra Day	**Amount Estimated (µg/ml)**
T1 (20µg/ml)	19.90
T2 (20µg/ml)	19.84
T3 (20µg/ml)	20.06
Mean	19.93
±SD	0.106
%RSD	0.531
Inter Day	**Amount Estimated (µg/ml)**
D1 (20µg/ml)	20.06
D2 (20µg/ml)	19.86
D3 (20µg/ml)	19.92
Mean	19.94
±SD	0.102
%RSD	0.511

A comparison of the intra- and interday-test results for RBZ, carried out by two analysts, evaluated the robustness of the process.In the tablet formulation conducted by the same laboratory by the two analysts, the percentages of RSD values for RBZ intra and interday assays were 0.113 percent no greater, suggesting the robustness of the process. RBZ was 4.72 min by average retention time.

Accuracy

On the basis of recovery studies conducted by the traditional addition process, the accuracy of the proposed method was calculated. RBZ API (conc. 20 mg/ml) standard solution has been established. A pre-tested tablet powder equivalent of approximately 20 mg of RBZ was accurately measuring and the volume was balanced with methanol by weighing up to six different 10.0 ml

volumetric flasks. Six separate 10-ml volumetric flasks took 0.1 mL of the above solution, and the precisely known normal RBZ was added and 5.0 ml methanol were added. The flasks have been sonicated for 15 minutes, and the solutions have been filtered with methanol. The results showed a percent RSD of 0.348 with good precision, and the results are shown in Table 2.

Table 2: Results of recovery studies

Weight of tablet power (mg)	Amount added (mg)	Peak area	Average % recovered	% label claim
25	20.83	1812822		99.5
50	41.66	361933	100.20	99.89
100	62.49	939631		100.3
			Mean	99.77
			S.D.	0.347
			% RSD	0.348

Stability studies

Hydrolysis has been suspected of RBZ. The colour from light yellow to dark yellow was turned to stressed samples in both solid and solution condition. Under dry heat condition, RBZ was found to be stable. The treatment has been moderately stable to neutral hydrolysis. Drop of the drug at sunlight exposure was minimal, suggesting that the medication was stable in photos. Under acidic and fundamental stress conditions the drug was more unstable [7-8]. After refluxation at the first 2 hours, the concentration of RBZ decreased by more than 80% and 60% respectively. Displaying acid and base hydrolysis sensitivity of medications. RBZ has been found unstable for stress oxidation. More than 30% of the medication was degraded as it was refluxed with 30 percent H_2O for 5 hours. By determining RBZ and comparing it to newly prepared requirements, we determined the consistency of the stock solution. In the answer to the stock solution, no major change (<1 percent) compared to the newly prepared standard were noted [9].

Conclusions

To determine RBZ in the form of delayed release tablets, a validated stability-indicating HPLC analytical method has been developed. The results of stress tests carried out in compliance with the guidelines of the International Conference for Harmonization (ICH) indicate that the technique is selective and secure. The method proposed is simple, without buffer as a mobile step, which increases column age. Often economic as methanol and water are used as mobile stages. The process is cost efficient. For precise and reliable results, the process has been validated. The method will isolate the medicine from the products of degradation. The treatment is appropriate in delayed release tablet dose for the routine study of RBZ. The simplicity of the approach enables use in any laboratory for quality control. For analyzing the samples obtained during accelerated stability studies, the evolved HPLC method can be used to forecast expiry date of drugs.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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