Asian Journal of Chemistry Vol. 17, No. 2 (2005), 1025–1030

Development of an HPLC Method for the Estimation of Rabeprazole in Bulk and its Pharmaceutical Formulations

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A simple and sensitive reversed-phase high-performance liquid chromatographic method (RP-HPLC) has been developed for the estimation of rabeprazole in bulk and its pharmaceutical formulations. Separation of rabeprazole and tinidazole (internal standard) was achieved on a Hypersil BDS C-18 (250 × 4.6 mm, packed with 5 micron) reversed-phase column, using UV detection at 284 nm. The mobile phase consisted of methanol : water (60 : 40 v/v). The analysis was performed in less than 6 min, with a flow rate of 1.0 mL/min. The calibration curve was linear in the range of 0.1 to 50 μg/mL. The intra and inter-day variation was found to be less than 1% showing high precision of the assay method. The mean recovery of the drug from the solutions containing 2, 6, 10 μg/mL was 99.97 ± 0.78% indicating high accuracy of the proposed HPLC method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining rabeprazole in bulk drug samples or in capsules.

Key Words: Rabeprazole, Tinidazole, Pharmaceutical formulations, Reversed-Phase HPLC.

INTRODUCTION

Rabeprazole[1] (RA) is a 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl] methyl] sulfinyl]-1H-benzimidazole sodium salt. Rabeprazole is tne fourth proton pump inhibitor and it is used in the management of acid-related disorders.

The literature survey reveals that few HPLC[2], LC-MS[3], LC-NMR[4] and capillary electrophoresis[5] were reported for its analytical monitoring in either biological fluids or formulations. The present paper reports a sensitive and precise HPLC method for the estimation of RA in bulk samples and pharmaceutical formulations by using a C18 column [(Hypersil BDS C-18 (250 × 4.6 mm, packed with 5 micron) (Thermo Hypersil)]; mobile phase combination is methanol : water 60 : 40 and internal standard is tinidazole.

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EXPERIMENTAL

All the reagents and solvents were of analytical and HPLC grade supplied by E. Merck. Quantitative HPLC was performed on a gradient HPLC Shimadzu LC-10 AVP system with Shimadzu 10AT VP series HPLC pump, SIL 10AD VP series auto sampler equipped with a 20 μL sample loop and SPD 10A VP dual absorbance detector. The output signal was monitored and integrated using Shimadzu Class-VP Version 6.12 SPI software.

Mobile phase and stationary phase and internal standard: A mixture of methanol : water (60 : 40) was used as a mobile phase. A Hypersil BDS C-18 column (250 × 4.6 mm, packed with 5 micron) was used as stationary phase. Tinidazole is used as an internal standard.

Preparation of standard drug solution: Stock solution of the drug and internal standard were prepared by dissolving 100 mg of rabeprazole and 100 mg of internal standard (tinidazole) separately in 100 mL volumetric flasks containing 70 mL of water (triple distilled water), sonicated for about 15 min and then made up to volume with water. Daily working standard solutions of RA and internal standard were prepared by suitable dilution of the stock solution with appropriate mobile phase.

Preparation of sample drug solution for pharmaceutical formulations: 20 tablets were weighed to get the average tablet weight and pulverized. The sample of the powdered tablet, claimed to contain 100 mg of active ingredient, was extracted with triple distilled water and made to volume to get a stock solution of 1 mg/mL. This solution was filtered through a 0.45 μm membrane filter. This solution was further diluted stepwise with mobile phase as under preparation of standard solutions to get different concentrations required.

Method: The contents of the mobile phase were filtered before use through 0.45 μm membrane filter, degassed with a vacuum pump and pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. 10 sets of the drug solutions were prepared in mobile phase containing RA at a concentration of 0.1 to 50 μg/mL along with a fixed concentration 4 μg/mL of internal standard. Then 20 μL of each of standard and sample solutions were injected for six times and the retention time, average peak areas and peak area ratios of component area to that of internal standard were recorded. The amount of drug present in each pharmaceutical formulation was calculated through peak area ratio of component to that of internal standard by making use of the standard calibration curve.

Optimized chromatographic conditions

The optimized chromatographic conditions were as follows:

Parameters	Method
Stationary phase (column)	((Hypersil BDS C-18 (250 × 4.6 mm, packed with 5 micron) (Thermo Hypersil)
Mobile phase	Methanol : water 60 : 40
Flow rate (mL/min)	1.0
Column back pressure (psi)	1200
Run time (min)	6
Column temperature (°C)	Ambient
Volume of injection loop (μL)	20
Detection wavelength (nm)	284
Internal standard	Tinidazole
Drug RT (min)	4.41
Internal standard RT (min)	2.16

RESULTS AND DISCUSSION

Chromatography: Initially a mobile phase consisting of methanol and water in the ratio of 40 : 60 were tried. Early elution with tailing of peaks was observed in the above condition. Then the composition of mobile phase was changed to 50 : 50 under these conditions broad peak shape and pronounced tailing was observed. For the same mobile phase, if the ratio was changed to 60 : 40, rabeprazole was eluted at around 19.47 min with symmetric peak shape.

The chromatograms indicating the separation of RA and internal standard (tinidazole) with RP C_{18} column and mobile phase have been given in Fig. 1. Blank samples tested by same procedure showed no interfering peaks.

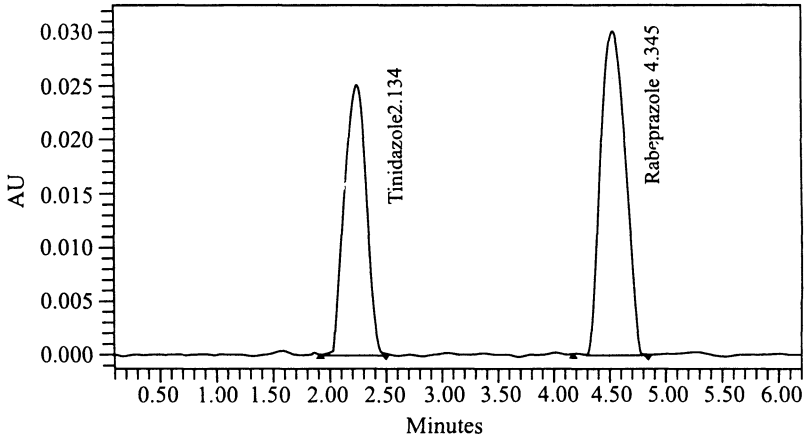

Fig 1. A typical chromatograph of Rabeprazole

System suitability: To ascertain the effectiveness of system suitability test, seven replicate injections of freshly prepared standard stock solution of RA (5 μg/mL) were injected into the chromatograph and the relative standard deviation (RSD) of peak areas was calculated. Observed RSD was 0.29% (USP limit is not more than 2%). The peak purity of the peak due to RA was tested using UV detector and was found to be pure.

Detection characteristics: To test whether the RA has been linearly eluted from the column, different amounts of RA were taken and a fixed quantity of internal standard solution was added separately and all the solutions were analyzed. The peak area ratios of component area to that of internal standard area were calculated and the values are graphically represented (Table-1). The linear fit of the system was illustrated graphically. Least square regression analysis was carried out for the slope, intercept and correlation coefficient. The results are presented in Table-2.

TABLE-1
CALIBRATION CURVE POINTS OF THE PROPOSED METHOD FOR ESTIMATION OF RABEPRAZOLE

Concentration of the solution (μg/mL)	Peak area ratio*	CV (%)
0.1	0.0715	0.32
0.25	0.1462	0.45
0.5	0.2851	0.42
1.0	0.5635	0.27
5.0	2.8351	0.98
10.0	5.6748	0.94
25.0	14.1991	1.21
50.0	28.4031	1.52

*Mean of six determinations
Regression equation : Y = 0.00607 + 0.56546X, (r = 0.9999).

TABLE-2
OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED HPLC METHODS FOR RA

Parameter	Method
Detection wavelength (nm)	284
Linearity range (μg/mL)	0.1–50
Detection limits (μg/mL)	0.02171
Regression equation (Y = a + bC)	
Slope (b)	0.56546
Standard deviation of slope (S_b)	0.00178
Interccpt (a)	0.00607
Standard deviation of intercept (S_a)	0.00409
Standard error of estimation (S_e)	0.00734
Correlation coefficient	0.9999

Precision: The precision of each method was ascertained separately from the peak area ratios obtained by actual determination of eight replicates of a fixed amount of drug and internal standard. The per cent relative standard deviation was calculated for RA (Table-2). The precision of the assay was also determined in terms of intra- and inter-day variation in the peak areas for a set of drug solutions on three different days. The intra- and inter-day variation in the peak areas ratio of the drug solution to that of internal standard was calculated in terms of coefficient of variation (CV) and the results are presented in Table-3.

TABLE-3
INTER- AND INTRA-DAY PRECISION FOR RABEPRAZOLE ASSAY IN PHARMACEUTICAL DOSAGE FORMS BY THE PROPOSED HPLC METHOD

Concentration of RA (μg/mL)	Observed concentration of RA (μg/mL)			
	Intra-day		Inter-day	
	Mean (n = 5)	% CV	Mean (n = 5)	% CV
2	1.987	0.59	2.036	0.78
6	6.255	0.35	6.374	0.39
10	10.281	0.34	10.395	0.74

Accuracy: To determine the accuracy of each proposed method, different amounts of bulk sample of the drug within the upper and lower limits were taken and analyzed by the proposed method. The results (per cent error) are recorded in Table-4.

TABLE-4
ESTIMATION OF RABEPRAZOLE TABLETS

Pharmaceutical formulation	Labelled amount (mg)	Amount obtained by proposed method	% Recovery of proposed method
Tablet	20.0	19.92	99.52
Tablet	20.0	19.82	99.11

Analysis of formulations: To find out suitability of each proposed method for the assay of pharmaceutical formulations (tablets), containing RA, were analyzed by the proposed method. The results are recorded in Table-4.

Recovery studies: Recovery studies were conducted by analyzing each pharmaceutical formulation in the first instance for the active ingredient by each proposed method. Known amount of pure drug was then added to each of the previously analyzed formulations and the total amount of the drug was once again determined by the proposed method after bringing the active ingredient concentration within the limits (Table-4).

Interference studies: The effect of wide range of excipients and other additives usually present in the formulation of RA in the determination under optimum conditions were investigated. The common excipients like starch, talc, magnesium state, methyl and propyl parabens, cellulose derivatives and propylene

glycol have been added to the sample and injected. They have not disturbed the elution or quantification of drug or internal standard. In fact many have no absorption at this UV maximum.

Conclusion

The proposed method is simple, precise, accurate and rapid for the determination of rabeprazole from bulk and its pharmaceutical formulations. Hence it can be easily and conveniently adopted for the routine quality control analysis.

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(*Received*: 1 July 2004; *Accepted*: 3 January 2005) AJC-4044