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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF PANTOPRAZOLE IN DEVELOPED TABLET DOSAGE FORM

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ABSTRACT

A simple reversed-phase HPLC method for determination of pantoprazole present in the formulated solid oral dosage form was developed. The proposed method utilizes a purospher® RP-18 end capped column (250x4.6 mm) in an isocratic separation mode using a mobile phase consisting of methanol : sodium acetate buffer (pH 5) (70:30 v/v) at a flow rate of 1 ml/min. Detection was carried out at 289 nm. Retention time of pantoprazole was 5.045 mins. The method was validated with respect to specificity, linearity, accuracy, precision, ruggednesss, and robustness as per the ICH guidelines. The proposed method is simple, precise, sensitive, and reproducible and is applicable for quantification of pantoprazole in a solid oral dosage form developed and formulated in our laboratory.

INTRODUCTION

Pantoprazole is a substituted benzimidazole derivative that belongs to the category of proton pump inhibitors (PPI's) and is used as an anti-ulcerant. It binds irreversibly to the proton pump H^+K^+ ATPase in the gastric parietal cell and inhibits gastric acid secretion[1]. Pantoprazole is official in IP[2], BP[3] and USP[4]. Literature survey reveals the availability of several analytical methods being developed and validated for pantoprazole alone or in combination with other drugs[5-10]. However, existing methods cannot be used for the developed formulation due to the possibility of interference caused by excipients. The present study describes a validated isocratic RP-HPLC method for pantoprazole in a solid oral dosage form developed and formulated in our laboratory.

MATERIALS AND METHODS

HPLC Instrumentation:

The HPLC analysis was carried out on Jasco Binary system with two PU2080 PLUS intelligent HPLC pumps, UV2075 PLUS intelligent UV detector, Solvent mixing module MX-2080-31, Rheodyne® manual injector system, LCNet II / ADC system interface and Borwin® Chromatography software, Jasco Corporation, Japan and Purospher® RP-18 end capped column(250x4.6mm, packed with 5µm).

Materials and Reagents:

Pantoprazole sodium sesquihydrate standard was obtained as a gift sample from Elder Pharmaceuticals pvt. Ltd (Navi Mumbai, India). HPLC grade methanol, sodium acetate used were purchased from S.D. Fine Chemicals (Mumbai, India). Water used was double distilled vacuum filtered.

Chromatographic conditions:

Chromatographic separation was carried out on a Purospher® RP-18, 250mmx4.6mm, 5µm column. Mobile phase consisting of methanol and sodium acetate pH 5 was pumped at a flow rate of 1 ml/min. The elution was monitored at 289 nm and the injection volume was 20µl.

Preparation of mobile phase:

Methanol and sodium acetate used for the mobile phase were filtered through a 0.45 µm membrane filter (Ultipore N-66R Nylon; Pall Corp.) and degassed by ultrasonication for 15 min.

Preparation of standard solution:

Accurately weigh about 10mg of pantoprazole and transfer into a 100ml volumetric flask. Add about 50ml of mobile phase and sonicate for 10 min to dissolve it completely and adjust the volume with mobile phase to get stock solution of 100µg/ml. The working standard solution of

pantoprazole was prepared by diluting 1ml stock solution to 10ml with mobile phase to obtain a solution containing 10µg/ml pantoprazole.

Preparation of sample solution:

Weigh 20 pantoprazole tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10mg of pantoprazole into a 100ml volumetric flask. Add about 50ml of mobile phase, sonicate to dissolve it completely and make volume upto the mark with mobile phase to get a stock solution of 100µg/ml. Mix well and filter through 0.45µm membrane filter. The working sample solution of pantoprazole was prepared by further diluting 1ml stock solution to 10ml with mobile phase to obtain a solution containing 10µg/ml pantoprazole.

Analysis of developed formulation:

Assay of developed formulation containing pantoprazole was performed by preparing the sample solutions as described earlier in the preparation of the sample. Five injections of above sample and standard solutions were injected. The assay of the developed formulation sample was calculated by comparing the areas of standard and sample peaks.

VALIDATION OF THE METHOD

The method was validated for system suitability, linearity, accuracy, precision, robustness, limit of detection and limit of quantification in accordance with ICH guidelines[11].

System suitability study:

For this study, 20µl blank solution [methanol: sodium acetate (70:30)] was injected and run for 10 mins. After this, 20µl standard solutions in five replicates were injected, and the RSD of the resultant peak areas was calculated.

Calibration curve (linearity of the HPLC method):

The calibration curve was constructed by plotting concentrations of pantoprazole versus peak areas, and the regression equations were calculated. The linearity of the method was investigated by using concentrations of 8, 9, 10, 11, 12, 15, 20 and 25µg/ml. These concentrations were prepared by diluting appropriate volumes of working standard with mobile phase. The retention time of pantoprazole was 5.07 mins.

Accuracy:

Accuracy of the method was performed at three different levels, in which sample stock solutions of 10µg/ml were spiked with standard drug solution containing 80, 100 and 120% respectively. Three replicate samples of each concentration level were prepared and the percent recovery at each level (n=3), and mean percent recovery (n=9) were determined. The mean recovery was 99.99 percent.

Precision (repeatability):

Repeatability of the method was checked by injecting replicate injections of 10µg/ml of the sample solution for six times on the same day as intraday precision study of pantoprazole and the percent RSD was found to be 0.98.

Intermediate precision (ruggedness):

Interday variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analysed by different analyst on different day and different column. Ruggedness was also expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results obtained employing different analyst.

Robustness:

Robustness of the method was demonstrated by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 to 0.9 ml/min and from 1.0 to 1.1 ml/min. The composition of mobile phase was changed from 70:30 to 66.5:30 and from 70:30 to 73.5:30. The sample solutions described for the robustness study were applied onto the column in triplicate, and the responses were determined.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD and LOQ of pantoprazole was calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

LOD = 3.3 x σ/S, LOQ = 10 x σ/S.

Where, σ = standard deviation of response, S = slope of regression equation.

Specificity:

Specificity of the method was demonstrated by injecting the blank solution, standard solution, sample solution and the responses were determined.

RESULTS AND DISCUSSION

The main objective of this work was to develop and validate RP-HPLC method for pantoprazole in tablet dosage form. Several mobile phase compositions were tried. A satisfactory separation and good peak symmetry were obtained by using the described methanol : sodium acetate pH 5 (70 : 30 v/v). Quantification was achieved with UV detection at 289 nm. Representative chromatograms of standard pantoprazole and pantoprazole in developed tablet formulation are shown in Figure 3 and Figure 4 respectively. System suitability tests were carried out on freshly prepared standard solutions (n = 5) containing pantoprazole. System suitability parameters obtained with 20 µL injection volumes are summarized in Table 1.

Linearity regression data are summarized in Table 2, which shows a good linear relationship between concentration of pantoprazole and peak areas was obtained over a concentration range of 8-25 µg/mL Figure 2. The correlation coefficient (r^2) was found to be 0.999 for pantoprazole which ensures that a good correlation existed between the peak area and analyte concentration. The LOD was found to be 0.6 µg/ml for pantoprazole. LOQ was found to be 1.9 µg/ml. These values indicate that the method is sensitive.

Accuracy of the method was calculated by recovery studies at three levels by spiking method as shown in Table 3. The mean recovery of the added standard drug was 99.99 percent. This means recovery value is well within the range of 98-100%, indicating the method is accurate.

In the precision studies, RSD of mean assay values was found to be 0.98 for pantoprazole. These % RSD values which are well below 2% indicate that the repeatability of this method is satisfactory. Thus there exists a closeness of agreement in repeated measurements of peak response. The intermediate precision study revealed that the method is rugged with %RSD values of 0.23 for performing the analysis by changing the column, analyst and performing the analysis on another day respectively. As evident the RSD values of the data obtained are well below 2% indicating that method can be repeated successfully on different day, on different column and by different analyst. Robustness studies indicate that the developed method is robust and is not affected by deliberate changes in the method parameters. Hence it can be concluded that the developed RP-HPLC method is a rapid method for estimation of pantoprazole raw material as well as dosage form. This method can be used for both, qualitative and quantitive estimation.

CONCLUSION

The proposed RP-HPLC method is accurate, precise, sensitive, selective and rapid for the determination of pantoprazole in tablet dosage form developed and formulated in our laboratory.

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Table 1 : System suitability test parameters for pantoprazole

Parameter	Pantoprazole
Retention time (mins)	5.045 ± 0.38
Theoretical plates	2801 ± 1.60
Asymmetry	1.59 ± 1.91
Area of response (µV. sec)	704080.6 ± 0.33

Table 2 : Regression analysis of calibration curve for pantoprazole

Parameter	Pantoprazole
Linearity range (µg/ml)	8 – 25
Regression equation	72255x + 22563
Correlation coefficient (R^2)	0.999
Slope	72255
X-intercept	72255
Y-intercept	22563

Table 3 : Summary of validation parameters for the proposed HPLC method of pantoprazole

Sr. No	Parameter	Pantoprazole
1.	LOD (µg/ml)	0.6
2.	LOQ (µg/ml)	1.9
3.	Accuracy (% recovery)	99.99 ± 0.91
4.	Precision	100.44 ± 0.98
5.	Ruggedness (Column change, Analyst change, Day change)	100.28 ± 0.23
6.	Robustness (Mobile phase comp. 66.5 : 30 v/v)	99.57 ± 0.95
7.	Robustness (Mobile phase comp. 73.5 : 30 v/v)	101.26 ± 1.42
8.	Robustness (0.9 ml/min Flow rate)	99.66 ± 0.83
9.	Robustness (1.1 ml/min Flow rate)	100.26 ± 0.02

FIGURES

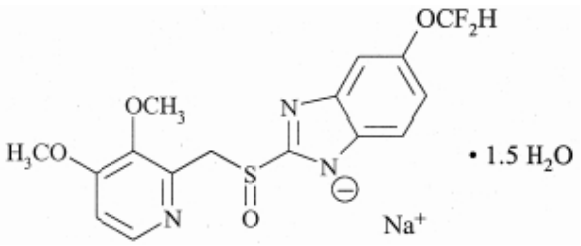

Figure 1 : Chemical structure of pantoprazole sodium sesquihydrate

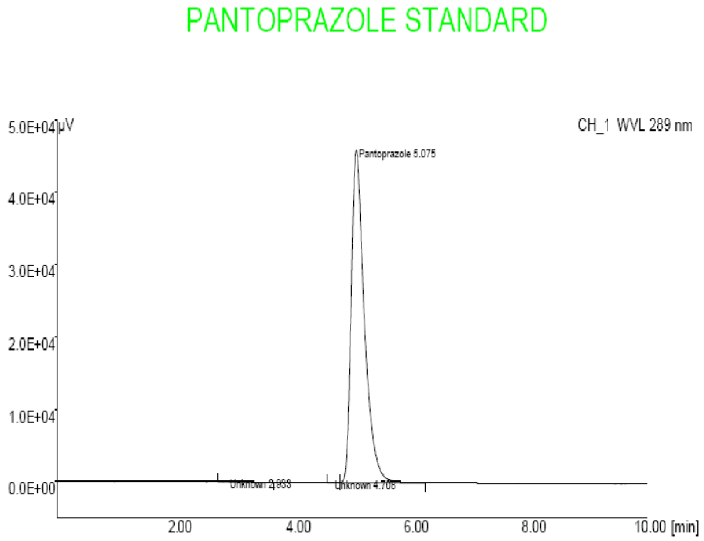

Figure 2 : Representative chromatogram of standard pantoprazole (R.T. 5.07 mins)

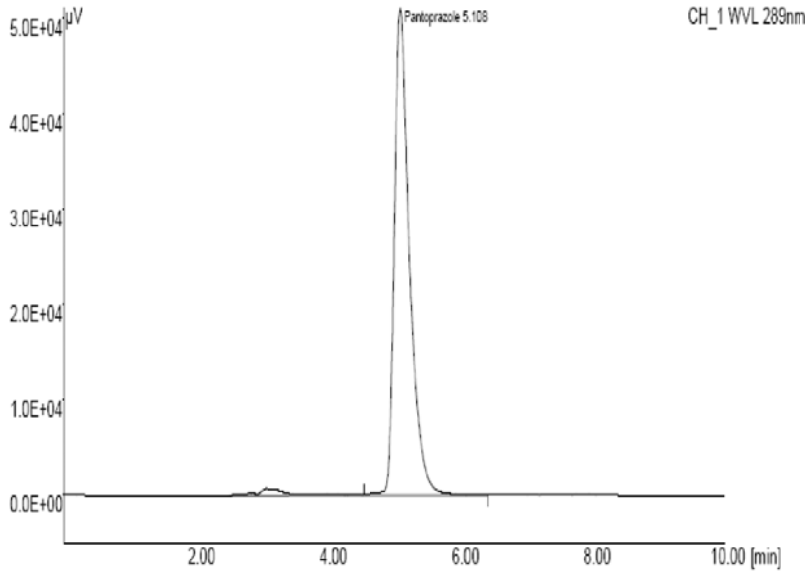

Figure 3 : Representative chromatogram of formulation containing pantoprazole (R.T. 5.10 mins)