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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF PANTOPRAZOLE

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Pantaprazole in pharmaceutical dosage form. Isocratic elution at a flow rate of 1.0 mL min -1 was employed on a Symmetry C18 column at ambient temperature. The mobile phase consisted of acetonitrile: phosphate buffer 60:40 (v/v) and the detection wavelength were at 234 nm. Linearity was observed in concentration range of 50-175 µg/mL. The retention time for Pantaprazole was 2.28 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Pantaprazole in pharmaceutical dosage forms.

Keywords: Estimation, Method development, Pantaprazole, RP-HPLC, Validation.

INTRODUCTION

The proton-pump inhibitor pantoprazole inhibit gastric acid by blocking the H+/K+-adenosine triphosphatase enzyme system (the proton pump) of the gastric parietal cell [1]. It is used for short-term treatment of erosion and ulceration of the esophagus[2]. The pantoprazole oral dosage forms are supplied in enteric-coated tablets.Different analytical methods are reported in the literature for the assay of pantoprazole in dosage forms and in biological fluids including spectrophotometry[3-8], TLC9, HPTLC[10-12]. Pantoprazole sodium is chemically Sodium 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2- pyridinyl) methyl] sulfinyl]-1H-benzimidazole sesquihydrate. It has an empirical formula of $C_{16}H_{15}F_2N_3O_4S$ and molecular weight of 383.37. The aim of this work was to develop new and validated, simple and reproducible RP-HPLC method allowing the estimation of in dosage forms and human plasma samples.

Instrumental and analytical conditions

The HPLC analysis was carried out on Waters HPLC system (2695 module**)** equipped with 2487 dual lambda detector with auto Sampler and running on Waters Empower software. The column used is Symmetry C18 (150 × 4.6 mm, packed with 5 µm) and detection was performed at 234 nm. The injection volume of sample was 20 µL and the run time was 6 minutes. An isocratic mobile phase containing acetonitrile and 0.02 M phosphate buffer at 60: 40 (v/v) at the pH 3.5 was carried with the flow rate at 1.0mL min-1. The mobile phase was filtered through 0.45µm membrane filter and degassed before use.

Reagents and chemicals

Pantaprazole working standard was obtained from Merck (India). Tablets were purchased from local pharmacy manufactured by Novartis. Ultra pure water was obtained from a millipore system. HPLC grade acetonitrile was obtained from Merck (India) limited. All other chemicals used were AR grade.

Preparation of mobile phase

Dissolved 2.72 g of Potassium Di hydrogen orthophosphate in 1000 mL of water and mixed, pH adjusted to 3.5 using ortho phosphoric acid, sonicated to degas the buffer. Transferred 600 volumes of acetonitrile and 400 volumes of buffer into a 1000 volumes mobile phase bottle and mixed. Then sonicated up to 15 minutes for degas the mobile phase and filtered through 0.45 µm filter under vacuum. The same mobile phase was used as diluent.

Preparation of Standard Solution

Accurately weighed about 10 mg of Pantaprazole and transferred into a 10mL volumetric flask and 7 mL of diluent was added and sonicate to dissolve it completely and the volume was adjusted with the mobile phase to get stock solution of 1000 µg/mL. Then 1 mL of stock solution is transferred into 10 ml volumetric flask and make up to volume with mobile phase and filter through 0.45 µm filters, which gives a solution of strength 100 µg/mL.

Preparation of sample solution

Weigh 20 Pantaprazole tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 50 mg of Pantaprazole into a 50 ml volumetric flask. Add about 25ml of diluent, sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 1 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 µm filter.

Method Validation

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, limit of detection, limit of quantification, robustness and system suitability.

Linearity

From the standard stock solution, the various dilutions of Pantaprazole in the concentration of 50, 75, 100, 125, 150 and 175µg/mL were prepared. The solutions were injected using 20 µL injection volumes in to the chromatographic system at the flow rate of 1.0 mLmin-1 and the effluents were monitored at 234 nm, chromatograms were recorded. Calibration curve of Pantaprazole was obtained by plotting the peak area ratio versus the applied concentrations of Pantaprazole, given in table 1. The linear correlation coefficient was found to be 1, shown in figure2.

Precision

Repeatability of the method was checked by injecting replicate injections of 100 µg/mL of the solution for six times on the same day as intraday precision study of Pantaprazole and the % RSD was found to be 0.15, given in table 2.

Accuracy

Pantaprazole reference standards were accurately weighed and added to a mixture of the tablets excipients, at three different concentration levels (50%, 100% and 150%). At each level, samples were prepared in triplicate and the recovery percentage was determined and presented in table 3.

Specificity

Spectral purities of Pantaprazole chromatographic peaks were evaluated for the interference of the tablet excipients as per the methodology. In the work, a solution containing a mixture of the tablet excipients was prepared using the sample preparation procedure to evaluate possible interfering peaks and no interference peaks were observed.

Robustness

To determine the robustness of the method, two parameters (flow rate, composition of mobile phase) from the optimized chromatographic conditions were varied. Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of parameters were introduced. Thus the method showed to be robust which is shown in table 4.

Ruggedness

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results obtained employing different analyst.

Detection and quantitation limits

According to the determined signal-to-noise ratio, Pantaprazole presented limits of detection of 0.06µg/mL and limits of quantitation of 0.8µg/mL, where the compounds proportion found in the sample solutions injected on to the chromatograph. However, the objective of the method is the quantitation of Pantaprazole so that the values obtained should be considered as the limit of method sensitivity.

System Suitability

System suitability tests were carried out on freshly prepared standard stock solutions of Pantaprazole and it was calculated by determining the standard deviation by injecting standards in six replicates at 6 minutes interval and the values were recorded and the system suitability parameters are shown in table 5.

RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Pantaprazole was preferably analyzed by reverse phase chromatography and accordingly C18 column was selected. The elution of the compound from the column was influenced by polar mobile phase. The ratio of the acetonitrile to phosphate buffer was optimized to give symmetric peak with short run time. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase of acetonitrile: phosphate buffer at the ratio of 60:40 (v/v). The retention time of Pantaprazole was found to be 2.28 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters are given in Table 5. A typical chromatogram showing the separation of Pantaprazole is shown in figure 3.

Table 1: Linearity of Pantaprazole

Concentration (µg/mL)	Average area
50	641426
75	969169
100	1286672
125	1607741
150	1932957
175	2257921

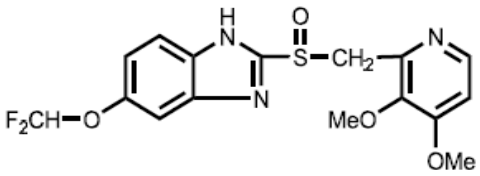

Fig. 1: Structure of Pantaprazole

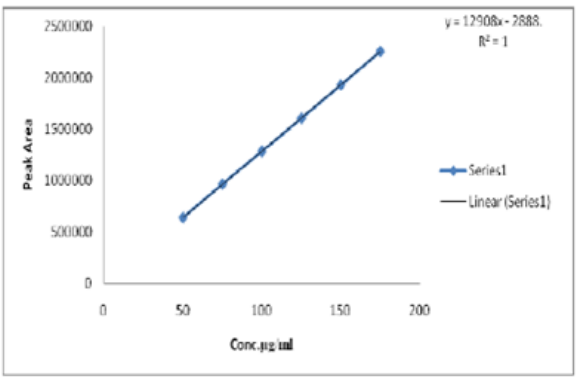

Fig. 2: Linearity curve of Pantaprazole

Table 2: Precision of Pantaprazole

Injections	Area
1	1278827
2	1280763
3	1283837
4	1280931
5	1283027
6	1279532
Mean	1281153
SD	1947.051
%RSD	0.1517

Table 3: Accuracy of Pantaprazole

% Conc	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	5.0	4.96	99.2%	99.17%
100%	10.0	9.96	99.6%	
150%	15.5	14.8	98.7%	

Table 4: Robustness of Pantaprazole

Parameters	Adjusted to	Average Area	R_t	SD	% RSD
Flow rate as per method 1.0mL/min	0.8 mL/min	1295159	2.291	5918.5	0.45
	1.0mL/min	1289713	2.289	4993.7	0.39
	1.2ml/min	1297130	2.284	3887.0	0.30
Mobile phase composition Acetonitrile: Buffer (60:40)	Acetonitrile: Buffer (55:45)	1297717	2.279	3475.6	0.28
	Acetonitrile: Buffer (60:40)	1294644	2.282	4987.4	0.38
	Acetonitrile: Buffer (65:35)	1303208	2.284	5215.3	0.40

Table 5: System Suitability for Pantaprazole

Concentration	Injection	Area	R_t
100 µg/mL	Injection-1	1287115	2.285
	Injection-2	1283496	2.287
	Injection-3	1290171	2.288
	Injection-4	1289663	2.286
	Injection-5	1289921	2.289
	Injection-6	1290228	2.288
Statistical	Mean	1288432	2.287167
Analysis	SD	2686.291	0.001472
	%RSD	0.21	0.06
	Tailing Factor	1.6	
	Plate Count	2496.6	

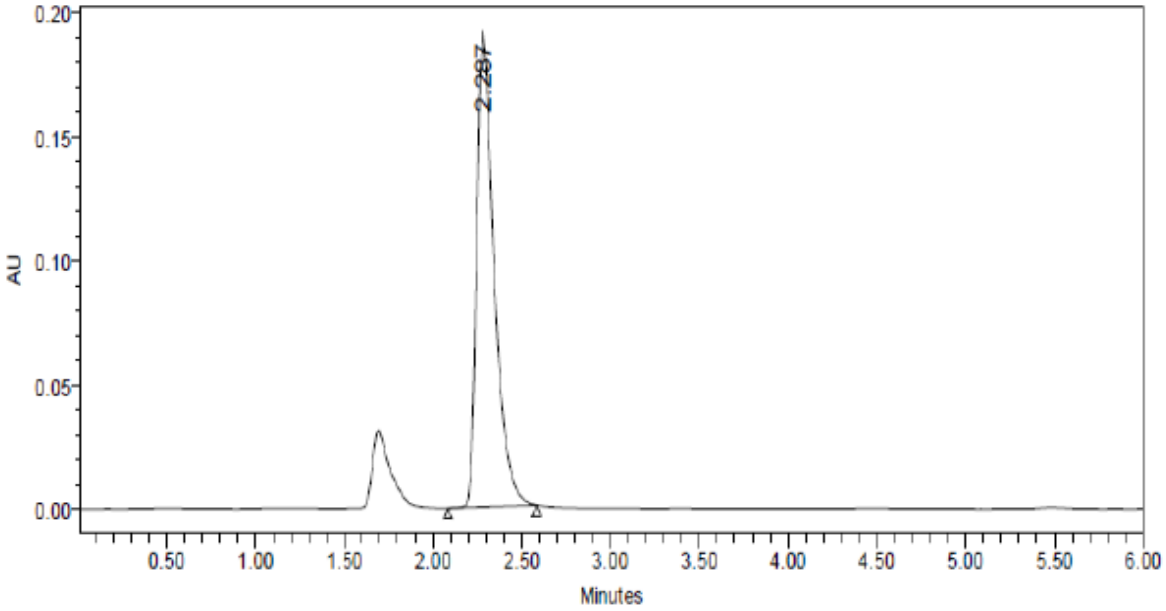

Fig. 3: Standard Chromatogram of Pantaprazole

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Pantaprazole in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Therefore, it is suitable for the routine analysis of Pantaprazole in pharmaceutical dosage form.

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