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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF PANTOPRAZOLE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

FARAAT ALI*

Translam Institute of Pharmaceutical Education and Research
Mavana Road Meerut U P India 250002

ABSTRACT

A simple, precise, sensitive and rapid reverse phase high performance liquid chromatographic method was developed for the estimation of Pantoprazole in bulk and pharmaceutical dosage forms. The method was developed by using Waters X-Bridge C-18(4.6×150 mm, 5µm) column; mobile phase consisting of ACN and bicarbonate buffer at pH 9.0; the flow rate of 1.0 ml/min and detection wavelength 214nm and eluted at 4.775 min. The linearity ranges were 25 to 125ppm for Pantoprazole. The method was validated as per ICH guidelines for various parameters like linearity, precision, accuracy, LOD, LOQ and robustness. The validated method was applied to the commercially available pharmaceutical dosage forms and obtained the desired result.

KEYWORDS: Pantoprazole, Acetonitrile, HPLC, Validation, Development.

FARAAT ALI
Translam Institute of Pharmaceutical Education and Research
Mavana Road Meerut U P India 250002

***Corresponding author**

INTRODUCTION

Pantoprazole chemically is 5-Sodium-(Difluromethoxy)-[(3,4DiMethoxy-2-Pyridinyl) methyl] sulphinyl]-1- H-benzimidazloe. Pantoprazole is in a class of drugs called proton pump inhibitors (PPI) which block the production of acid by the stomach. Proton pump inhibitors are used for the treatment of conditions such as ulcers, gastro esophageal reflux disease (GERD) and Zollinger-Ellison syndrome that are caused by stomach acid. Pantoprazole, like other proton-pump inhibitors, blocks the enzyme in the wall of the stomach that produces acid. By blocking the enzyme, the production of acid is decreased, and this allows the stomach and oesophagus to heal. It is a newer H^+ K^+ ATPase inhibitor, similar in potency and clinical efficacy to omeprazole, but is more acid stable and has higher oral bioavailability. It is also available for i.v. administration; particularly employed in bleeding peptic ulcer and for prophylaxis of acute stress ulcers. It has lower affinity for cytochrome P450 than omeprazole or lansoprazole: risk of drug interactions is minimal. It has an empirical formula of $C_{16}H_{15}F_2N_3O_4S$ and molecular weight of 383.37. The main purpose of the present study is to establish a relatively simple, single step, sensitive, validated and inexpensive HPLC method for the determination of Pantoprazole in pure form and in pharmaceutical dosage form, since most of the previous methods have been found to be relatively complicated and expensive.

MATERIALS AND METHODS

Equipment Used

The chromatographic separation was performed on Alliance Waters e2695 Separation Module integrated with Waters 2998 Photodiode Array Detector. A reverse phase X - Bridge C_{18}(4.6 X 150mm, 5µm, Make: Waters) was used. Empower Pro software was used for data acquisition. Schimadzu UV Visible spectrophotometer (Model-2450) and Mettler Toledo analytical balance were used.

Chemicals and Reagents

Pharmaceutical pure grade Pantoprazole sodium was obtained from Indian Pharmacopoeia Commission Ghaziabad. The Formulation was procured from local market. HPLC grade Acetonitrile, Ammonium hydroxide and Methanol were obtained from Sigma Aldrich Mumbai.

Optimized Chromatographic conditions

X-Bridge C_{18}(4.6 X 150mm, 5µm, Make: Waters) was used for the chromatographic separation at a detection wavelength 214nm. Flow Mode: Gradient, Mobile phase Acetonitrile and Ammonium bicarbonate buffer, pH 9.0 selected for elution. Flow rate was adjusted to 1ml/min and the injection volume was 5µL.

Preparation of mobile phase

ACN: Ammonium bicarbonate buffer – Ammonium bicarbonate 398 mg was weighed accurately and dissolved in 1000 ml water and pH was adjusted to 9.0 with ammonium hydroxide solution (1%v/v) and then filtered through 0.45µ filter. Water: Methanol (1:1) as diluent.

Preparation of standard solution

10 mg Pantoprazole was accurately weighed was taken to a 100 ml dried and cleaned volumetric flask. Then 50 ml of diluent was added, and sonicated. The volume was made up to 100 ml with the same solvent. This solution was yield 100 ppm or 100 µg/ml.

Optimization of HPLC method

HPLC method was optimized with an aim to develop an estimation procedure for the assay of Pantoprazole. For method optimization, different column and mobile phase were tried but acceptable retention time, good resolution and theoretical plates were observed with Acetonitrile and ammonium bicarbonate buffer pH 9.0 by using Waters X-Bridge C-18(4.6×150 mm, 5µm).

Validation of the RP-HPLC method

The method validation was optimized as per the

ICH Q2 (B) guidelines.

System Suitability

System suitability test was carried out with an injection of a solution of 100ppm concentration. SST parameters like retention time obtained and calculated resolution, capacity factor, tailing factor, high efficiency theoretical plate (HETP) were reported in Table 1.

Linearity

For the establishment of linearity, five different concentrations (25ppm, 50ppm, 75ppm, 100ppm, 125ppm) of the drug were prepared for linearity studies. The calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients was under the acceptance limit that was not more than 1. The calibration curves for Pantoprazole was shown in figure 1 and their corresponding linearity parameter was given in Table 2.

Accuracy

To ensure the reliability and accuracy of the method, % recovery was observed using three different sample of Pantoprazole among these samples, one control sample was taken as having 150ppm concentration, other was a spiked sample of drug which is prepared by mixing of 2mg of drug and third one was the standard stock solution (100ppm). Five runs of each were made by 3µl injection volume. The results were given in Table 3.

Precision

For intermediate precision, sample was prepared six times on the same day and analyzed as per method. Two different concentration of Pantoprazole were prepared that is 150ppm and 200ppm. Six runs of each concentration were injected and % RSD was calculated which was under the limit that is not more than 2%. The results were given in Table 4.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. It was checked by subjecting the drug solution in different stress conditions like acid, base, peroxide but no interferences were found.

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

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. The Values were shown in Table 5.

Robustness

To verify the robustness of the method, slight changes to the chromatographic conditions were made to check for any changes in the chromatogram. The parameters included mobile phase composition, detection wavelength, flow rate and column temperatures were changed. The results are given in Table 6.

Assay of Quality Control Solutions

Quality control solutions for the Pantoprazole in the concentration range of low (50ppm), medium (125ppm), high (200ppm) was prepared and three injections of each concentration were made. The purpose of quality control solutions was to check the performance of the instrument before analysis of test solution and to confirm whether the instrument gave constant results or not, by comparing the data of standard solutions with that of quality control solutions.

RESULTS AND DISCUSSION

After a number of trials with Different, mobile phases were tested but adequate separation of Pantoprazole sodium was found in acetonitrile: 5mM Ammonium bicarbonate buffer (in gradient mode). Different composition of mobile phase, acetonitrile and ammonium bicarbonate buffer pH 9.0,the separation of peak was observed by altering pH of the mobile phase. The satisfactory separation of the drug with good resolution and short run time was achieved at pH 9.0. Low retention time of Pantoprazole and poor separation was achieved at pH 7.0, 8.0 and 10.0. So, Acetonitrile: 5mM Ammonium bicarbonate buffer (in gradient mode) at which

pH 9.0 was selected as mobile phase for method development. Maximum absorbance of Pantoprazole at 290.2nm was determined in mobile phase by utilizing Waters 2998 Photodiode Array Detector. Maximum peak height of the drug was obtained at 290.2 nm by injecting the 5µg/ml concentration of the drug sample and allowed to run at different wavelength. The best results were obtained with flow rate programming of selected mobile phase for the purpose of rapid analysis. Mobile phase was started at a flow rate of 1.0 ml/min which was continued for 1.0 min to 10.00 min. The validation of the developed and optimized HPLC method was carried out with respect to the parameters such as specificity, linearity, stability, accuracy, precision, limit of quantification (LOQ) and limit of detection (LOD) in the light of internationally accepted ICH guidelines.

Table 1
System Suitability Parameters of Pantoprazole

Parameters	Pantoprazole
HETP (mm)	38.624
Retention Time,R_t (min)	4.775
Resolution(Rs)	0.0
Capacity Factor (k)	0.0
Theoretical Plates	15692.11
Tailing factor	1.35
Separation	0.0

Table 2
Linearity data

Conc in ppm	Area count	R^2
25.00	557610	
50.00	1727915	
75.00	2753316	0.999
100.00	3855189	
125.00	5093405	

Table 3
Area of standard, control and spiked solution for % Recovery study

Area count			% Recovery	
Standard Sample	Control sample	Spiked Sample	Control sample	Spiked sample
2796704	2830101	2882141	98.81	97.03
2798878	2951509	2881163	94.80	97.14
2720244	2899977	2859189	93.80	95.14
2719572	2810585	2919898	96.76	93.13
2753316	2881569	2989650	95.54	92.04
Total = 13788714	15273741	14532041	479.71	474.48
Mean = 2757742.8	3054748.2	2906408.2	95.94	94.89
S.D.= 39028.34	56315.02	51398.06		
% RSD= 1.41	1.84	1.76		

Table 4
Precision Data

S.NO.	Drug	Area count	
		150 ppm	200 ppm
1	Pantoprazole	3365721	3348860
2	Pantoprazole	3394474	3337011
3	Pantoprazole	3391168	3355903
4	Pantoprazole	3390694	3360310
5	Pantoprazole	3352994	3368085
6	Pantoprazole	3320306	3350830
7	Total	20215357	20120999
8	Average	3369226.16	3353499.83
9	SD	29150.36	10634.08
10	% RSD	0.865	0.317

Table 5

	LOD	LOQ
Pantoprazole	2.72 µg/ml	16.14 µg/ml

Table 6
Robustness Data

S.No.	Temperature	Flow	R.T. (min)	Area count
1	25^0C	0.9ml/min	5.099	2736253
2		1.0 ml/min	5.058	2579660
3		1.1 ml/min	4.813	2378943
4	30^0C	0.9ml/min	5.058	2958802
5		1.0 ml/min	4.968	2620244
6		1.1ml/min	4.803	2403367
7	35^0C	0.9ml/min	5.033	2938544
8		1.0 ml/min	4.954	2663118
9		1.1ml/min	4.781	2375035

Table 7
Data Analysis of Quality Control Solutions of Pantoprazole

Conc. (ppm)	Area count			Area mean	+S.D.	%CV
	Injection					
	1st	2nd	3rd			
50	1188752	1175231	1184630	1182871	6930.002	0.58
125	3196141	3171163	3219898	3195734	24370.05	0.76
200	5296077	5222124	5272699	5263633	37800.81	0.71

Table 8
Purity data

Name	R T (min)	Purity angle	Purity threshold	% Area	Height
Pantoprazole	**4.775**	**1.097**	**2.508**	**100.00**	**252257** µV

Figure 1
Linearity graph between absorbance and concentration

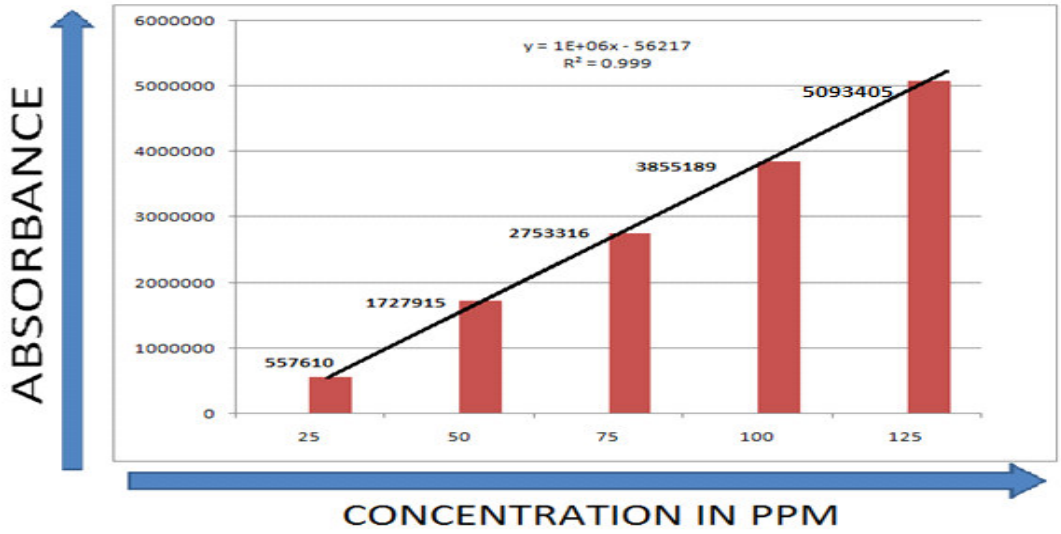


Figure 2
Purity plot of Pantoprazole after development

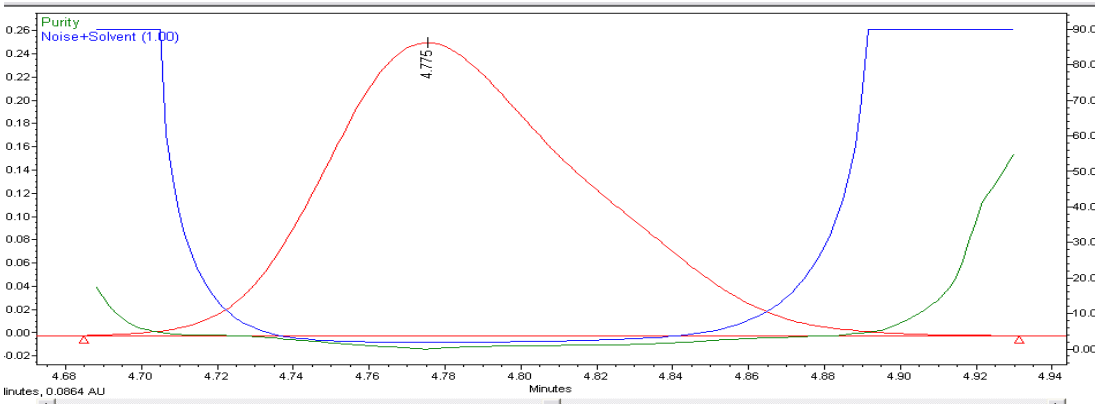


Figure 3
Chromatogram showing the response of Pantoprazole after analytical method development

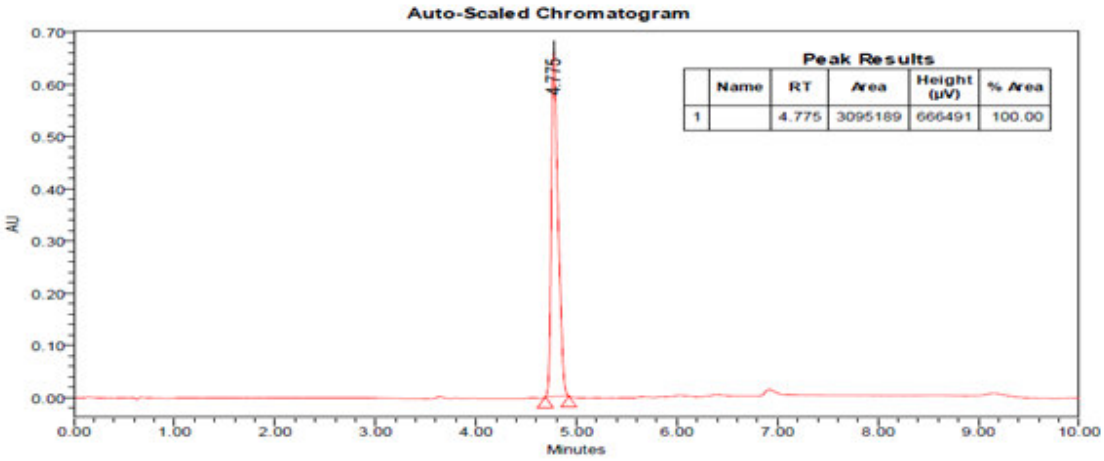
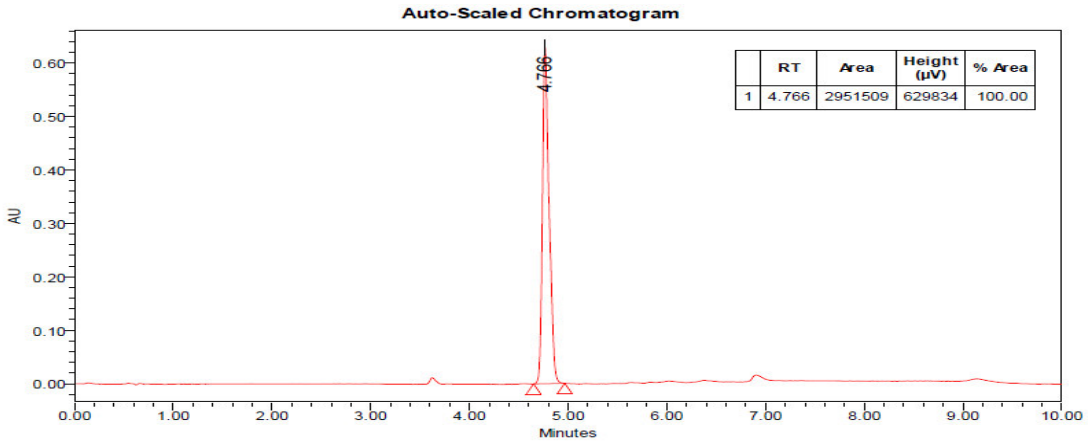


Figure 4
Chromatogram of control sample



CONCLUSION

The developed and validated HPLC method offered a simple, accurate and fast quantitative determination of Pantoprazole sodium from its formulation and API. According to the ICH guidelines, all validation parameters were found to be within the limits. The developed method offered several advantages such as rapidity, simple mobile phase and sample preparation step whereas improved sensitivity made it specific and reliable for its intended use. This method can be applied to the analysis of various pharmaceutical dosage forms.

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