Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2013, 5(4):99-104
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Analytical method development and method validation for the estimation of pantoprazole in tablet dosage form by RP-HPLC

B. Siddartha[1]* and I. Sudheer Babu[2]

[1]Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy, Maisammaguda, Secunderabad
[2]Department of Pharmaceutical Analysis, Sir C. R. Reddy College of Pharmaceutical Sciences, Eluru

---

ABSTRACT

A simple, sensitive and rapid reverse phase high performance liquid chromatographic method was developed for the estimation of Pantoprazole in pure and in pharmaceutical dosage forms. A BDS Thermohypersil Symmetry C8 column (250 x 4.6mm x 5 µ) was used with a mobile phase containing a mixture of Methanol and Dipotassium hydrogen phosphate buffer adjusted to pH-9 with ortho phosphoric acid in the ratio of 50:50. The flow rate was 1.2ml/min and effluent was monitored at 226nm and eluted at 4.189min. Calibration curve was plotted with a range from 50-150 µg/ml for Pantoprazole. The assay was validated for the parameters like specificity, system suitability, precision, accuracy, robustness and ruggedness parameters. The proposed method can be useful in the routine analysis for the determination on Pantoprazole in pharmaceutical dosage form.

Keywords: Pantoprazole, Reverse phase, HPLC, Calibration curve, RP-HPLC, Validation.

INTRODUCTION

Chemically, Pantoprazole is 6-(difluoromethoxy)-2-{[(3, 4-dimethoxypyridin-2-yl) methane] sulfinyl}-1H-1, 3-benzodiazole. Pantoprazole is a proton pump inhibitor drug used for short-term treatment of erosion and ulceration of the esophagus caused by gastro esophageal reflux disease [1]. Pantoprazole is a proton pump inhibitor (PPI) that suppresses the final step in gastric acid production by forming a covalent bond to two sites of the (H^+,K^+)- ATPase enzyme system at the secretory surface of the gastric parietal cell[2]. This effect is dose- related and leads to inhibition of both basal and stimulated gastric acid secretion irrespective of the stimulus. 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.

Drugs are available in tablet dosage from as Pantoprazole 40mg (Pantocid®) in the market. Literature survey revealed that Pantoprazole has been estimated with other drugs by spectrophotometry [3-8], TLC [9], HPTLC [10-12].

MATERIALS AND METHODS

Reagents

Pantoprazole was kindly supplied by Dr. Reddy Labs (Hyderabad, A.P., and India). Methanol (HPLC grade,Merck). Ortho phosphoric acid was purchased from Qualigens Fine Chemicals, Mumbai. All the other reagents were of AR grade.

Instrumentation
The LC system consisted of a Waters model 515, PDA detector 2998 with 20 µL sample loop. The output signals were monitored and integrated using Empower 2 software.

Chromatographic conditions
The elution was isocratic and the mobile phase consisted of a mixture of buffer and methanol (50: 50, v/v). The buffer was prepared by dissolving 17.418g of dipotassium hydrogen phosphate in 1000 ml water adjusted with ortho phosphoric acid to pH 9.0 ± 0.1. The buffer was filtered through a 0.45-µm (HVLP, Germany) membrane filter. The mobile phase was also filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. A Thermohypersil BDS C8 column (250 x 4.6mm x 5 µ) was used for determination. The flow rate was 1.2 ml min-1 and the column was operated at ambient temperature (~25 ^{o}C). The volume of sample injected was 20 µL. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The UV detector was set at wavelength of 226 nm. A typical chromatogram of Pantoprazole hydrochloride is shown in (Fig. 1).

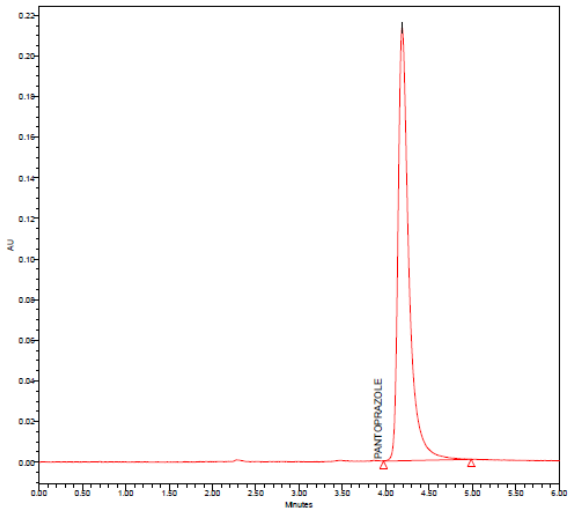

Fig 1: Chromatogram of Pantoprazole

Diluent: Water

Standard Preparation
Stock solution of Pantoprazole was prepared by dissolving 16mg of Pantoprazole in 50 ml volumetric flask add few ml of water. Sonicate it for 30minutes and make up with water.

Sample Preparation
About 10 Tablets were taken and their average weight was calculated. The Tablets were crushed to a fine powder and dose equivalent to 16mg was transferred to a 50 ml volumetric flask, dissolved in water and filtered through 0.45 µ membrane filter to get concentration of 320µg/ml.

Validation of method
The method developed here was validated as per ICH guidelines [13-14] for its accuracy, linearity, precision, specificity, robustness, and ruggedness, limit of detection and limit of quantification by using the following procedures.

Linearity
Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Pantoprazole at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance and concentration of the drug. The response was found to be linear in the range 160-480 µg/ml for Pantoprazole (Fig 2).

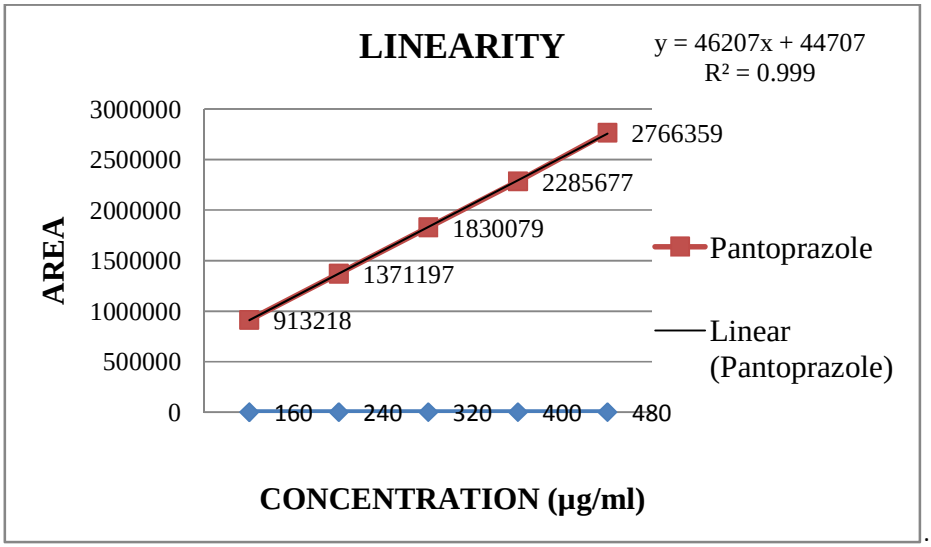

Fig 2: Linearity of Pantoprazole

Accuracy

Accuracy was performed in triplicate for various concentrations of Pantoprazole equivalent to 50%, 100% and 150% of the standard amount was injected into the HPLC system per the test procedure. The average % recovery of Pantoprazole was calculated. The data is given in Table 1.

Table 1: Accuracy data

S. No.	Spiked level	Amount Present (µg/ml)	Amount Added (µg/ml)	%Recovery	Std. Dev	%RSD
1(n=6)	50%	177.655	180.4227	98	5048	0.5
2(n=3)	100%	323.8333	320.26	101	12353	0.7
3(n=6)	150%	480.54	485.5933	99	9790	0.4

Precision

Six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure (Table 2).

Table 2: Precision data of 320µg/ml

S. No.	Concentration(µg/ml)	Injection	Retention time (mins)	Area
1	320	1	4.145	1832313
2	320	1	4.130	1830849
3	320	1	4.134	1821500
4	320	1	4.138	1822036
5	320	1	4.127	1825175
6	320	1	4.119	1827385
Mean				1826543
Std.Dev				4476
%RSD				0.2

Limit of Detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. LOD and LOQ were found to be 0.1958µg/ml and 0.5934µg/ml respectively.

Robustness and Ruggedness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of Pantoprazole was noted. The factors selected were flow rate and variation in the mobile phase composition. The results remained unaffected by small variations in these parameters (Table 3 and Table 4). Ruggedness of the

method was checked by using different analysts and instruments. The relative standard deviation of the results obtained from different analysts and instruments was <2.0% (Table 5 and Table 6).

Table 3: Robustness data relating to change in flow rate: (1.2ml/min)

S. No.	Flow rate (ml/min)	Injection	Retention time (min)	Area
1	flow rate-1-(1.1ml)	1	4.22	1832367
2	flow rate-2-(1.3ml)	1	4.02	1830235
Mean				1831301
Std dev				1508
%RSD				0.1

Table 4: Robustness data relating to change in mobile phase composition (Buffer: MeOH :: 50:50)

S. No.	Mobile phase	Injection	Retention time (min)	Area
1	m.p-1(49:51)	1	4.21	1835323
2	m.p-2(51:49)	1	4.08	1831152
Mean				1833238
Std.Dev				2949
%RSD				0.2

Table 5: Ruggedness data (Intraday)

S. No.	Sample name	Injection	Retention time (min)	Area
1	Intraday-1	1	4.181	1834304
2	Intraday-2	1	4.161	1837299
3	Intraday-3	1	4.173	1835731
4	Intraday-4	1	4.159	1832070
5	Intraday-5	1	4.190	1833121
6	Intraday-6	1	4.165	1836883
Mean				1834901
Std.Dev				2093
% RSD				0.1

Table 6: Ruggedness data (Instrument to Instrument)

S. No.	Sample name	Injection	Retention time (min)	Area
1	Inst-Inst-1	1	4.172	1835420
2	Inst-Inst-2	1	4.184	1834210
3	Inst-Inst-3	1	4.191	1837263
4	Inst-Inst-4	1	4.165	1832120
5	Inst-Inst-5	1	4.179	1831314
6	Inst-Inst-6	1	4.184	1833004
Mean				1833889
Std.Dev				2208
% RSD				0.1

Specificity and Selectivity

Specificity and selectivity were studied for the examination of the presence of interfering components. It was checked by subjecting the drug solution in different stress conditions like Acid, Base, Peroxide and the degradation was noted (Table 7, Table 8 and Table 9).

Table 7: Specificity testing (Acid stress)

	Pantoprazole					
S. No	Concentration (µg/ml)	Time (hrs)	Retention time (min)	Area	% Assay	% Degradation
1	100	0	4.127	1825170	100	
2	100	0	4.058	1749762	94	-4

Table 8: Specificity testing (Base stress)

	Pantoprazole					
S. No	**Concentration (µg/ml)**	**Time (hrs)**	**Retention time (min)**	**Area**	**% Assay**	**% Degradation**
1	100	0	4.120	1826110	99	
2	100	24	4.071	1516662	82	-16

Table 9: Specificity testing (Peroxide stress)

	Pantoprazole					
S. No	**Concentration (µg/ml)**	**Time (hrs)**	**Retention time (min)**	**Area**	**% Assay**	**% Degradation**
1	100	0	4.132	1826110	99	
2	100	24	4.068	1425037	78	-20

System suitability

System suitability and chromatographic parameters were validated such as number of theoretical plates, asymmetry factor and tailing factor were calculated (Table 10).

Table 10: System suitability parameters

Validation parameters	**Pantoprazole**
Linearity range	160-480
Regression equation	Y = 46207x+44707
Correlation Coefficient(r^2)	0.999
Accuracy	99-101
Precision (%RSD)	0.2
Robustness (%RSD)	
Flow rate	0.1
Mobile phase	0.2
Ruggedness (%RSD)	
Intraday	0.1
Instrument to Instrument	0.1

RESULTS

A reverse-phase column procedure was proposed as a suitable method for the determination of Pantoprazole dosage form. The chromatographic conditions were optimized by changing the mobile phase composition, pH, and buffers used in the mobile phase. Different ratios were experimented to optimize the mobile phase. Finally transfer 17.418g of K_2HPO_4 into beaker. Dissolve and dilute volume with water and adjusted pH to 9 with Ortho Phosphoric Acid (OPA) and methanol in the ratio of 50:50 was used this mobile phase showed good resolution of Pioglitazone peak. The wavelength of detection selected was 226 nm, as the drug showed optimized absorbance at this wavelength. By our proposed method the retention time of Pioglitazone was about 4.189 minute and none of the impurities were interfering in its assay.

DISCUSSION

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate and can thereby easily adopted for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in pharmaceutical formulation with virtually no interference of additives. Hence the proposed method can be successfully applied in estimation of Pioglitazone in marketed formulation.

CONCLUSION

The proposed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. Many samples can be suitably analyzed for the routine analysis of Pantoprazole in bulk and its Tablet dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

Acknowledgements
The authors are thankful to Malla Reddy College of Pharmacy Maisammaguda for providing all facilities to complete the work. Dr. Reddy Labs for providing the Pantoprazole, S.D.Fine Chem. Ltd, Mumbai for providing triethyl amine, Qualigens Fine Chemicals, Mumbai for providing ortho phosphoric acid.

REFERENCES

[1] Ewin K.J., "Goodman & Gilman's. The Pharmacological Basis of Therapeutics", 10th ed., McGraw-Hill Inc., London, **2001**, p. 1007.
[2] Ritter j. m., Lewis L.D., Mant T.G.K., "A Textbook of Clinical Pharmacology", 4th ed., Arnold LTD London, **1999**, p. 365.
[3] Ozaltin N., Kocer A., *J. Pharm. Biomed. Anal*.,**1997**, 16, 337-342
[4] Sastry C.S.P.., Naidu P.Y., Murty S.S.N.,*Talanta*, **1997**, 44, 1211-1217.
[5] Meyyanathan S.N., Raj J. R. A., Suresh B.,*Indian Drugs*, **1997**, 34, 403-406.
[6] Moustafa A.A. M., *J.Pharm. Biomed Anal*., **2000**, 22, 45-58.
[7] Wahbi A. A. M., Abdel-Razak O., Mahgoub Gazy A. A. H., Moneeb M.S., *J.Pharm. Biomed Anal*., **2002**, 30, 1133-1142.
[8] Salama F., Abasawy N. E.I., Abdel Razeq S.A.,Ismail M.F., Fouad M.M., *J.Pharm. Biomed Anal*., **2003**, 32, 1019-1027.
[9] EI Sherif Z.A., Mohamed A. O., EI-Bardeicy M.G., EI-Tarras M. F., *Spectroscopy Lett.*, **2005**, 38, 77-93.
[10]Renger B., J. *AOAC. Int.*, **1993**, 76, 7-13.
[11]Argekar A.P., Kunjir S.S., J *Planar-Chromator.Mod.*, **1996**, 9, 296-299.
[12]Pandya K.K., Mody V.D., Satia M.C., Modi I.A., Modi R.I., Chakravarthy B. K., Gandhi T.P.,J Chromatog. B. *Biomed. App* **1997**, 693, 199-204.
[13]ICH Q2B: Validation of Analytical Procedures: Methodology, May (**1997**).8.
[14]International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceutical for Human Use (ICH) Q2B (**1996**).Validation of Analytical Procedures, Methodology.