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A NOVEL DIFFERENCE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF PANTOPRAZOLE IN TABLET DOSAGE FORM

B. Shrestha[1], A. Koirala[1], S. Basnett[1], H. Basnett[2*]

1. Department of Pharmaceutical Analysis and Quality Assurance, Himalayan Pharmacy Institute, Majhitar, East Sikkim, India.

2. Department of Pharmacognosy, Himalayan Pharmacy Institute, Majhitar, East Sikkim, India.

ABSTRACT: A new, simple, accurate and highly sensitive difference spectrophotometric method was proposed for the determination of pantoprazole in tablet dosage form. The method is based on the measurement of difference absorbance of pantoprazole in 0.1M HCl and 0.1M NaOH. The measured value is the amplitude of the maxima and minima between two equimolar solutions of pantoprazole in different chemical forms, which exhibit different spectral characteristic i.e. λ_{max} of 284 nm in 0.1 M HCl and λ_{max} of 295 nm in 0.1M NaOH. The method was linear over the concentration range of 5-50 µg/mL of the analyte with the correlation coefficient value of 0.995 and regression equation of y=0.022x+0.036. The method was also accurate and the percentage recovery of the spiked drug was found to be between the ranges of 98.3-102.4%. The results obtained of the proposed method was statistically validated as per ICH guideline and successfully applied for the analysis of pantoprazole in tablet dosage form.

KEYWORDS: Difference Spectrophotometry, Pantoprazole, Validation, ICH guideline.

Corresponding Author: Dr. Hema Basnett* Ph.D.

Department of Pharmacognosy, Himalayan Pharmacy Institute, Majhitar, East Sikkim, India.

Email Address: hemabasnett_gtk@yahoo.co.in

1.INTRODUCTION

Pantoprazole is a proton pump inhibitor (PPI) that decreases secretion of gastric acid. Like other PPIs, pantoprazole exerts its pharmacodynamic actions by binding to the proton pump (H^+,K^+-adenosine triphosphatase) in the parietal cells, but, compared with other PPIs, its binding may be more specific for the proton pump. Pantoprazole has similar efficacy to other PPIs in the healing

of gastric and duodenal ulcers, as well as erosive esophagitis, and as part of triple-drug regimens for the eradication of *Helicobacter pylori* from the gastric mucosa [1]. Pantoprazole is chemically, 6-(Difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methylsulfinyl]-1H- benzimidazole [2].

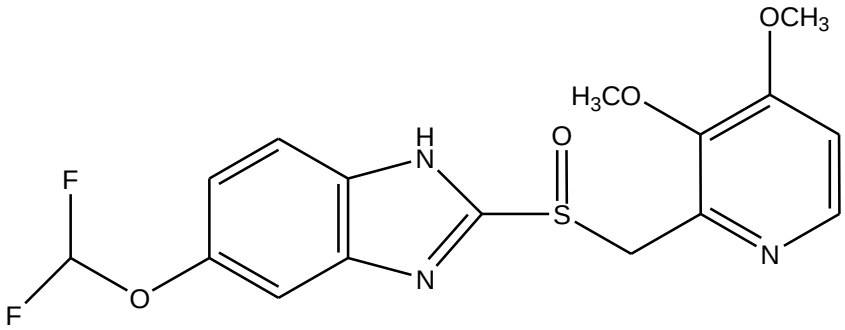

Fig. 1: Chemical structure of Pantoprazole

Various analytical methods such as spectrophotometery [3, 4] HPLC [5,6,7,8,9,10,11,12,13] voltammetry [14]**,** TLC [15,16] HPTLC [17,18,19] polarography [20,21] capillary electrophoresis [22,23] gas chromatography [24], thermogravimetry [25], amperometry [26] exists for the determination of pantoprazole in pharmaceutical formulations either alone or in combination with other drugs. HPLC methods are more preferable technique for drug analysis due to their selectivity and specificity. However, the high cost of the instrument makes it difficult to be maintained by all laboratories. It is easier for the laboratories to maintain spectrophotometers; however the ordinary spectrophotometric methods are not sufficiently selective and sensitive. The sensitivity and selectivity of a HPLC method can be achieved by employing difference spectrophotometric technique, as in this technique the absorbance of the same concentrations of solutions at different maxima are added to each other. Thus the amplitude obtained in the difference method is higher as compared to simple UV method for same concentration of chromogen, [27] which makes the method more sensitive**.** Moreover, the technique is simple and can be completed quickly thus making it cost effective. From the literature survey, it has been found that no difference spectroscopic method is reported, thus the authors have developed a simple, accurate and sensitive difference spectroscopic method for the determination of pantoprazole in tablet dosage form in order to achieve the sensitivity and selectivity of HPLC method by using an ordinary spectrophotometer.

2. MATERIALS AND METHODS

2.1. Reagents and Chemicals: Standard pantoprazole was obtained as a gift sample from Alkem Laboratories, Sikkim, India. Sodium hydroxide and hydrochloric acid was purchased from S.d Fine Chem. Ltd, Mumbai, India and prepared as per Indian Pharmacopeia (IP) 1996. Commercially available tablet Pan40 (Alkem Laboratories Ltd. Sikkim, India) containing 40mg of pantoprazole was procured from the local market.

2.2. Instruments: A double beam UV-Visible spectrophotometer (Shimadzu, Japan) model no: 1800, with a pair of matched quartz cell of 1cm were utilized for spectrophotometric measurements.

2.3. Preparation of standard acids and base: It was prepared and standardize as per IP 1996. [28]

2.4. Preparation of standard solution: Weighed accurately 10 mg of pantoprazole and transferred in a 50 mL volumetric flask, dissolved with 30 mL distilled water and made up the volume with distilled water. This is the stock solution. From the stock solution accurately pipetted out 5 mL and transferred in a 50 mL volumetric flask and made up the volume with 0.1M HCl. Similarly, again pipetted out 5 mL from the stock solution and transferred to another 50 mL volumetric flask and made up the volume with 0.1M NaOH.

2.5. Preparation of sample solution: Weighed and powdered 20 tablets. Weighed accurately 10 mg equivalent of pantoprazole and transferred in a 50 mL of volumetric flask, dissolved with 30 mL distilled water and made up the volume with distilled water. Filter the solution through a whatman filter paper. Then, pipetted out 5 mL of filtrate and transferred to a 50 mL volumetric flask and made up the volume with 0.1M HCl. Once again pipetted out 5 mL of the filtrate and transferred to another 50 mL volumetric flask and made up the volume with 0.1M NaOH.

A difference spectrum was obtained by keeping pantoprazole in 0.1M HCl in reference cell and pantoprazole in 0.1M NaOH in sample cell.

2.6. Analytical method validation: It was performed as per ICH guidelines [29].

2.6.1. Linearity: The linearity of the method was established by constructing a calibration curve over a concentration range of 5-50 µg/mL of pantoprazole. Difference absorbance against the corresponding analyte concentration was plotted and slope, intercept and correlation co-efficient were determined using linear regression analysis.

2.6.2. Precision: Intra-day precision was reported as %RSD for three replicate samples at three different concentration levels against a qualified standard drug. Inter day precision was also carried out similarly but in two different days and the %RSD was calculated.

2.6.3. Accuracy: The accuracy was evaluated in triplicate by adding pure drug of pantoprazole in already analyzed sample solution. Known amount of pantoprazole standard solutions (10, 20, 30 µg/mL) was added to the already analyzed sample solution and the analysis was carried out. The total amount of drug present was determined by the proposed method and the % recovery of pure drug was calculated.

2.6.4. Assay of the tablet dosage form: Solutions of the tablet dosage form was prepared as mentioned above for the preparation of the sample solution and %purity was calculated based upon the standard purity.

3. RESULTS AND DISCUSSION

3.1. Method development: Simple and sensitive method development has become necessary in recent years due to the importance of quality control of drugs and drug products. The selectivity and accuracy of spectrophotometric analysis of samples containing absorbing interferents may be improved by the technique of difference spectrophotometry. The essential features of difference spectrophometry is that the measured value is the difference absorbance between two equimolar solutions of the analyte in different chemical forms which exhibits different spectral characteristics and spectral properties can be altered by adjustment of pH[30]. Many substances containing the ionisable functional groups like phenols, amines and aromatic carboxylic acids, shows different characteristics in two different states i.e. acidic or basic medium. As shown in the Fig. 1, structure of pantoprazole consists of secondary amine (N-H) as one of the functional group, thus in 0.1M HCl having pH 1, pantoprazole shows λ_{max} at 284nm due to the loss of lone pair of electrons in nitrogen, whereas in 0.1M NaOH having pH 13, it shows λ_{max} at 295nm as such phenomenon does not exist here. This indicates that pantoprazole shows two different characteristics in two different solvents, as shown in Fig. 2.

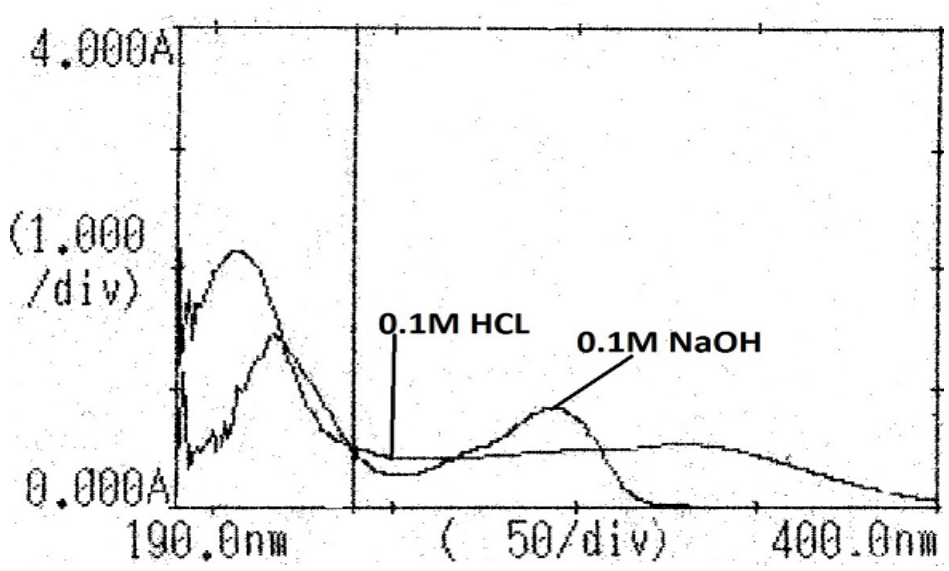

Fig. 2: Overlaid spectra of standard pantoprazole in 0.1M HCl and 0.1M NaOH

3.2. Optimization of strength of solvent: We have performed the analysis using various strengths of HCl and NaOH (0.01M, 0.05M, 0.1M, 0.15M, and 0.2M). The difference absorbance data as shown in table 1 were plotted against the various strengths of the solvent to determine the most suitable strength of the solvents. As can be seen from the Fig. 3, the highest difference absorbance is obtained in strength of 0.1M, thus it was selected for the method. The pH of the 0.1M HCl was found to be 1.0 and the pH of 0.1M NaOH was 13.0.

Table 1: Difference absorbance obtained at various strengths of HCl and NaOH

Strength of HCl and NaOH	Difference Absorbance
0.01M	0.416
0.05M	0.427
0.1M	0.501
0.15M	0.454
0.2M	0.426

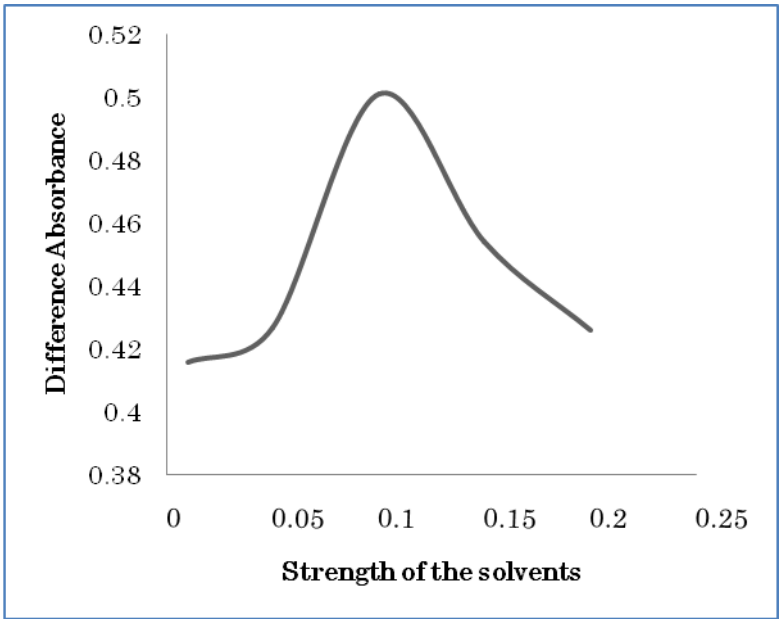

Fig. 3: Difference absorbance of pantoprazole in various strengths of HCl and NaOH

3.3. Method Validation

3.3.1. Precision: The precision of the method was determined by intra-day and inter-day precision studies by taking three different concentrations of sample. Values of %RSD for intra-day were 1.5, 0.9, and 1.9 and for inter-day was 1.5, 1.9, and 0.9 for 10, 20, and 30 µg/mL concentrations respectively, as shown in table 2.

3.3.2. Linearity: The calibration curve for pantoprazole was linear over the concentration range of 5-50 µg/mL. The correlation coefficient value obtained was 0.995 with the regression equation y = 0.022x + 0.036. The high value of correlation coefficient indicates the method is linear over the concentration range.

Table 2: Intra-day and Inter-day precision data of pantoprazole by difference spectrophotometric method

Parameters	Intra-day			Inter-day		
Drug concentration (µg/mL)	**10**	**20**	**30**	**10**	**20**	**30**
%Assay	99.8	101.0	99.4	99.4	99.4	99.0
	99.4	99.4	102.0	101.0	102.0	101.2
	102.1	99.6	98.3	98.1	98.3	99.5
% Mean	100.4	100.5	99.9	99.5	99.9	100.2
% RSD	1.5	0.9	1.9	1.5	1.9	0.9

3.3.3. Accuracy: The accuracy of the method was proven by recovery test. The method has shown good and consistent recoveries ranging from (98.3-102.4%) for pantoprazole confirming the accuracy of the method, as shown in table 3.

Table 3: Accuracy data for the determination of pantoprazole by difference spectrophotometric method

Concentration of sample (µg/mL)	Concentration of standard added (µg/mL)	Total concentration found (µg/mL)	Recovery% (n=3)	Mean Recovery%(n=9)	%RSD
20	10	30.1	100.3	99.9	1.2
		30.4	101.1		
		29.5	98.3		
20	20	40.4	101.0	101.0	1.6
		41.1	102.7		
		39.8	99.5		
20	30	49.4	98.8	100.5	1.4
		50.3	100.6		
		51.2	102.4		

3.3.4. Assay: Student t-test was performed for the results obtained and it was found to be insignificant, suggesting the results were not significantly different from the actual value. The results obtained were also evaluated at 95% confidence limit and it was found that almost all the results were within the limit indicating the accuracy of the method (Table 4). An overlaid difference spectra of the standard and the test pantoprazole is given in Fig. 4.

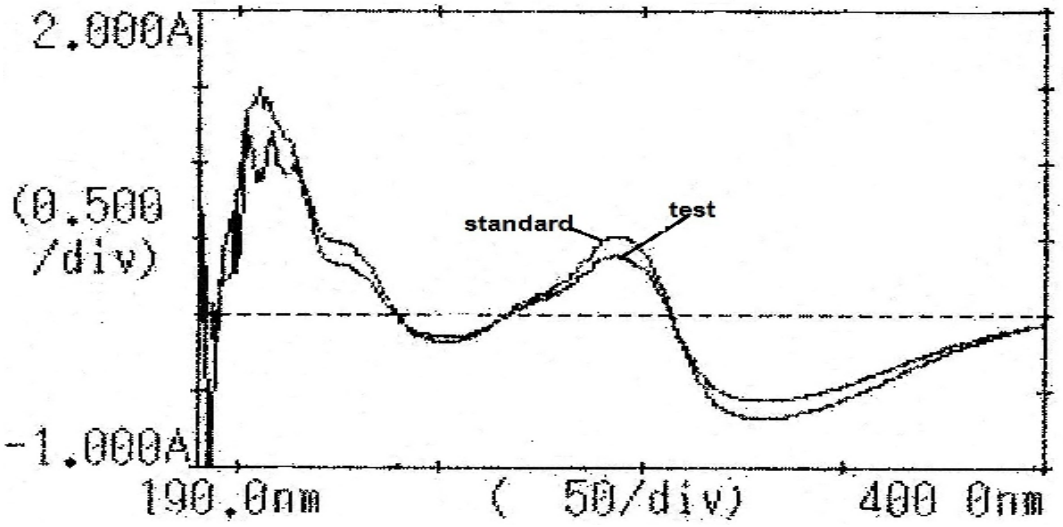

Fig. 4: Overlaid difference spectra of standard and test pantoprazole solutions

Table 4: Assay result of pantoprazole

Drug Name	Label claim	Mean Recovery ±S.D	95% Confidence Limit
Pantoprazole	40mg	100.2 ± 0.9	98.0-102.0%

4. CONCLUSION

The newly developed method was found to be accurate, precise, highly sensitive, specific and inexpensive at the same time. The method was validated as per the ICH guidelines for linearity, precision and accuracy and the results passed the criteria set forth by ICH guidelines. Hence the method stands validated and can be used for the routine quality control analysis of pantoprazole in the tablet dosage form.

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CONFLICT OF INTEREST

Authors have no any conflict of interest.

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