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Selective RP-HPLC Determination of Pantoprazole in Pure and Pharmaceutical Dosage Forms

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A reverse phase high performance liquid chromatographic method has been developed for the estimation of pantoprazole in bulk and pharmaceutical formulations. The quantification was carried out using a techsphere silica column in isocratic mode, with mobile phase consisting of acetonitrile and water in the ratio of 60 : 40 (v/v). Beclomethasone dipropionate was used as an internal standard. The mobile phase was pumped at a rate of 1 mL/min and the detection was carried out at 289 nm. The linearity was found to be in the range of 3–15 µg/mL. The proposed method was found to be simple, precise, accurate, rapid and reproducible for the estimation of pantoprazole in pure and pharmaceutical dosage forms, *i.e.*, tablets.

Key Words: HPLC, Pantoprazole, Pharmaceutical dosage forms.

INTRODUCTION

Pantoprazole (PTP) is a proton pump inhibitor. Chemically, it is 5-difluoro methoxy benzimidazole-2-yl 3,4-dimethoxy-2-pyridyl methyl sulphoxide. It is commercially available as pantoprazole sodium sesquihydrate. It inhibits the secretion of gastric acid by irreversibly blocking the enzyme system of hydrogen/potassium adenosine triphosphatase (H^+/K^+ATPase), the 'proton pump' of the gastric parietal cell. It is used in conditions where inhibition of gastric secretion may be beneficial. It is available in tablet form for oral administration. Literature survey reveals that few methods based on HPLC[1–3] and visible spectrophotometry[4,5] were reported for this drug. The present investigation has been undertaken to develop a simple RP-HPLC method for the estimation of pantoprazole in tablet dosage forms.

EXPERIMENTAL

An isocratic high performance liquid chromatograph (Schimadzu) with two LC-10AS pumps, variable wavelength programmable UV/Visible detector SPD-10A, Chromatopac integrator C R6 A, 20 µL Rheodyne 7125 loop injector and RP Techsphere silica column (250 × 4.6 mm i.d.; particle size 10 µm) was used. Pantoprazole and beclomethasone dipropionate were the gift samples from

Emcure and Cipla Labs respectively. HPLC grade acetonitrile was purchased from Qualigens and triple distilled water was used.

Chromatographic conditions: The chromatographic column used was 250 × 4.6 mm Techsphere silica with 10 µm particles. Acetonitrile was filtered through 0.45-µm-membrane filter. The HPLC equipment was operated at ambient temperature. The flow rate of the mobile phase was maintained at 1 mL/min. Detection was carried out by UV detector at 289 nm and the injection volume was 20 µL.

Preparation of internal standard solution: About 100 mg of beclomethasone dipropionate was weighed accurately and dissolved in 100 mL of HPLC grade methanol and sonicated for 30 min. It was further diluted to prepare a standard solution of 100 µg/mL.

Procedure: About 100 mg of pure sample of PTP was weighed accurately and dissolved in 100 mL of HPLC grade water to get 1 mg/mL solution. The solution was sonicated for 30 min. It was further diluted to prepare a standard solution of 100 µg/mL. Subsequent dilutions of this solution were made after addition of beclomethasone dipropionate (100 µg/mL) as an internal standard (IS) to get concentrations of 3–15 µg/mL of PTP and 10 µg/mL of IS in each dilution. The solutions prepared as above were filtered through 0.45-µm-membrane filter and then 20 µL of filtrate was injected five times in to the column at a flow rate of 1 mL/min. The ratio of drug peak area to that of internal standard for each of the drug concentrations was calculated. The regression of the drug concentration over the ratio of drug peak area to that of internal standard was obtained. This regression equation was used to estimate the amount of PTP in tablet dosage forms.

Estimation of PTP in tablet dosage forms: Two commercial brands of tablets were chosen for testing suitability of the proposed method to estimate PTP in tablet dosage forms. About 20 tablets were pulverized and the powder equivalent to 100 mg of PTP was weighed, dissolved in 100 mL of HPLC grade water and sonicated for about 30 min. The insoluble portion was filtered through a 0.45 µm membrane filter. The filtrate was further diluted to prepare a solution of 100 µg/mL. From the filtrate, different aliquots (3–15 µg/mL) were taken in separate 10 mL volumetric flasks. These solutions were spiked with suitable volume of the internal standard solution, such that the concentration of the internal standard in each was 10 µg/mL. The contents of the flask were made up to the volume with the mobile phase and mixed well. Each of these solutions (20 µL) were then injected five times into the column. The mean peak area ratios of the drug to the internal standard of five such determinations were calculated and the drug content in the tablets was quantified using the regression equation obtained from the pure sample.

RESULTS AND DISCUSSION

The present study was carried out to develop a simple, rapid, accurate and precise HPLC method for the analysis of PTP in pure and pharmaceutical dosage forms. The retention times for PTP and internal standard (beclomethasone

dipropionate) were 4.96 and 5.49 min respectively. Each of the samples was injected five times and the same retention times were observed in all cases. The ratio of the peak area of the PTP to peak area of internal standard for different concentrations set up as above were calculated and the average values for five such determinations are shown in Table-1. The peak areas of both the drug and internal standard were reproducible as indicated by low coefficient of variation (0.5700).

TABLE-1
CALIBRATION OF THE PROPOSED METHOD

Drug concentration (μg/mL)	Mean peak area ratio (n = 5)	Coefficient of variance (%) (CV)
3.0	0.490	0.19
6.0	0.997	0.21
9.0	1.480	0.18
12.0	2.012	0.17
15.0	2.495	0.22

Regression equation (from 3.0 to 15.0 μg/mL)
Y = 0.1674X + 0.0126 (r = 0.9999)

A good linear relationship (r = 0.9999) was observed between the concentration of the PTP and the respective ratio of peak areas. The calibration equation was found to be Y = 0.1674X + 0.0126 (where Y is the ratio of peak area of drug to that of internal standard, X = concentration of PTP). The intra-day and inter-day variations of the method were determined using five replicate injections of three different concentrations, which were prepared and analyzed on the same day and three different days over a period of two weeks, a low coefficient of variation was observed (Table-2). This shows that the present HPLC method is highly precise.

TABLE-2
PRECISION OF THE PROPOSED METHOD

Concentration of PTP (μg/mL)	Observed concentration of PTP (μg/mL)			
	Intra-day		Inter-day	
	Mean (n = 5)	CV (%)	Mean (n = 5)	CV (%)
6.0	6.02	0.19	6.04	0.22
9.0	8.99	0.24	9.01	0.23
12.0	12.04	0.20	12.05	0.18

To ensure the reliability and accuracy of the method, recovery studies were carried out by mixing a known quantity of drug with preanalyzed sample and contents were reanalyzed by the proposed method. The values are shown in Table-3. About 99.9% of PTP could be recovered from the preanalyzed samples indicating the high accuracy of the proposed HPLC method.

TABLE-3
RESULTS OF RECOVERY STUDY

Amount of drug added (μg)	Recovery from drug solution		Recovery from tablet formulation	
	Mean amount found (n = 5)	Mean % recovery	Mean amount found (n = 5)	Mean % recovery
3.0	3.02	100.60	2.99	99.60
9.0	9.01	100.10	9.02	100.22
15.0	14.98	99.86	15.01	100.06

The drug content in the tablets was quantified using the proposed analytical method. The mean amount of PTP in two different brands of tablet dosage forms is shown in Table-4. The absence of additional peaks in the chromatogram indicates the non-interference of the common excipients used in the tablets .The tablets were found to contain 99.57–99.67% of the labelled amount of drug. It can be concluded that the proposed HPLC method is sufficiently sensitive and reproducible for the analysis of PTP in pharmaceutical dosage forms within a short analysis time.

TABLE-4
ASSAY OF PTP IN TABLET DOSAGE FORMS

S. No	Labelled amount of drug (mg)	Mean (± s.d.) amount (mg) found by the proposed method (n = 5)	Mean (± s.d)% labelled amount (n = 5)
Tablet I	40	39.87 ± 0.24	99.675 ± 0.20
Tablet II	40	39.83 ± 0.35	99.575 ± 0.40

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