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Simple Oxidimetric and High Performance Liquid Chromatographic Methods for the Determination of Pantoprazole

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Three simple and sensitive methods (A, B & C) have been described for the estimation of pantoprazole (PTP) in bulk samples and pharmaceutical formulations. Methods A and B are spectrophotometric methods based on the oxidation of PTP with oxidant (potassium permanganate, MnO_4^-, A; ferric chloride, Fe(III), B) followed by estimation of unreacted oxidant with Fast Green FCF (FGFCF, A) or reduced form of oxidant [Fe(II)] with potassium ferricyanide ($[Fe(CN)_6]^{3-}$, B). Method C is a HPLC method in which C_{18} column with a mobile phase consisting of acetonitrile and water (60 : 40) and beclomethasone dipropionate as an internal standard were utilised. The eluents were monitored at a detection wavelength of 289 nm. The results obtained are reproducible and are statistically validated.

Key Words: Oxidimetric, HPLC, Determination, Pantoprazole.

INTRODUCTION

Pantoprazole (PTP) is chemically known as 5-difluoro methoxy benzimidazol-2-yl-3,4-dimethoxy-2-pyridyl methyl sulphoxide. It is commercially available as pantoprazole sodium sesquihydrate. PTP is a proton pump inhibitor. It inhibits the secretion of gastric acid by irreversibly blocking the enzyme system of hydrogen/[potassiam 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 not official in IP, BP, USP, EP or JP. It is available in tablets form for oral administration. Literature survey reveals that only few methods based on HPLC[1–9] and visible spectrophotometry[10–12] were reported for this drug. It was observed that the structural features of PTP have not been fully exploited for designing such procedures. This paper presents three such analytical methods. A and B are visible spectrophotometric methods based on the reaction of PTP with MnO_4^--FGFCF, A; Fe(III)-($[Fe(CN)_6]^{3-}$), B. Method C is a HPLC method based on the usage of a column (C_{18}), mobile phase (acetonitrile and water, 60 : 40) and internal standard (beclomethasone dipropionate).

EXPERIMENTAL

A Systronics UV-Vis spectrophotometer-117 with 1 cm matched quartz cells was used for all the absorbance measurements. Systronics digital pH-meter was used for all pH measurements. Quantitative HPLC was performed on an isocratic High Pressure Liquid Chromatograph (Shimadzu) with LC-10AS pump, variable wavelength programmable UV/Vis detector SPD-10A, Chromatopac integrator C R6 A, 20 μL Rheodyne 7125 loop injector and RP C-18 column (250 mm × 4.6 mm I.D.; particle size 10 μm) was used. The HPLC equipment was operated at ambient

temperature. The attenuation was set at 6 and the range was set at 0.001 AUFS with a chart speed of 5 mm/min. The flow rate of mobile phase was maintained at 1.0 mL/min.

All chemicals used were of analytical grade. Aqueous solutions of $FeCl_3 \cdot 6H_2O$ (Qualigens: 0.9% w/v, 3.33×10^{-2} M), $K_3[Fe(CN)_6]$ (Qualigens: 0.1% w/v, 0.3×10^{-3} M), $KMnO_4$ (Qualigens: 0.0326% w/v, 2.063×10^{-3} M) in 2.0 M H_2SO_4, Fast Green FCF (FGFCF) (Chroma: 0.01% w/v, 1.236×10^{-4} M) in 1.0 M H_2SO_4 and Na_2SO_4 (Qualigens: 14.2% w/v, 1.0 M) were prepared in double distilled water. HPLC grade solvents such as acetonitrile and water were used. Two litres of mobile phase was prepared with mixture of acetonitrile and water in the ratio 3 : 2. This solution was filtered through 0.45 μm membrane filter and degassed before use.

Preparation of standard solutions

For visible spectrophotometry: About 100 mg of PTP was dissolved in pure distilled water to prepare stock standard solutions of 1.0 mg mL^{-1}. The solution was further diluted with distilled water stepwise to get different concentrations of working standard solutions in each method.

For HPLC: About 100 mg of PTP was dissolved in 100 mL of pure HPLC grade water and sonicated for about 30 min. It was further diluted with respective mobile phase in each procedure to prepare a standard solution of 100 μg mL^{-1}.

Internal standard solution: About 100 mg of beclomethasone dipropionate reference standard was dissolved in 100 mL pure HPLC grade methanol and sonicated for about 30 min. It was further diluted with respective mobile phase in each procedure to prepare an internal standard solution of 100 μg mL^{-1}.

Preparation of sample solutions

For visible spectrophotometry: About 20 tablets were pulverized and the powder equivalent to 100 mg of PTP was weighed, dispersed in 25 mL of IPA, sonicated for 30 min and filtered. The filtrate was evaporated and the residue was dissolved in 100 mL distilled water (1 mg mL^{-1}). It was used as stock sample solution and was further diluted as under standard solution preparation to get different concentrations of working standard solutions in each method.

For HPLC: 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 and the filtrate was further diluted with mobile phase to prepare a solution of 100 μg mL^{-1}.

Assay Procedures

Method A: To each of 25 mL volumetric flasks, aliquots (1.0–5.0 mL, 100 μg/mL) of standard solution and 0.5 mL of $KMnO_4$ solution were added successively and kept aside for 5 min at room temperature. Then 4.0 mL of FGFCF solution and 4.0 mL of Na_2SO_4 solutions were added, made up to 25 mL with distilled water, mixed thoroughly and the absorbances were measured after 5 min at. 625 nm against distilled water blank. A blank experiment was also carried out in the similar manner omitting the drug. The decrease in absorbance corresponding to consumed $KMnO_4$ and in turn the PTP concentration was obtained by subtracting the decrease in absorbance of the test solution (dye minus test) from that of the blank solution (dye minus blank). The amount of PTP in a sample was obtained from the Beer Lambert plot.

Method B: Aliquots of standard solution (1.0–5.0 mL, 250 μg/mL) were

placed separately in a series of 10 mL volumetric flasks. Then solutions 1.0 mL each of 0.5 M HCl, Fe(III) and $K_3[Fe(CN)_6]$ were added successively and total volume in each flask was made to 10.0 mL with distilled water. The absorbance was measured after 45 min at 770 nm against reagent blank. The amount of PTP in a sample solution was obtained from the Beer-Lambert plot.

Method C: In a series of 10 mL volumetric flasks, 0.3 to 1.5 mL of above standard solution was transferred and 1.0 mL of internal standard solution was added to each flask. The total volume in each flask was made up to 10 mL with mobile phase and filtered through 0.45 μ membrane filter. Initially the mobile phase was pumped for about 30 min to saturate the column thereby to get the baseline corrected. Then twenty micro litres of each of the standard and sample solutions were injected for five times and the peak area ratios (ratio of component area to that of internal standard) were calculated. The amount of PTP present in a sample was calculated through the standard graph constructed by using internal standard ratio method.

RESULTS AND DISCUSSION

The optimum conditions for the development of all the methods were established by varying the parameters one at a time and keeping others fixed and observing the effect produced. The linearity range (μg/mL) was found to be 4–20, 25–150 and 0.06–0.3 for methods A, B and C respectively. Linear regression equations Y = –0.0036 + 0.0503C; 0.001 + 0.0072C and –0.0028 + 0.0041C were obtained with correlation coefficients of 0.99999, 0.99996 and 0.99999 for methods A, B and C respectively. The per cent relative standard deviation was found to be 0.4119, 0.3649 and 0.0718 for methods A, B and C respectively. The per cent range of errors (95% confidence limit) was found to be 0.344, 0.305 and 0.060 for methods A, B and C respectively.

For method C, as shown in Fig. 1, drug and internal standard were eluted in 5

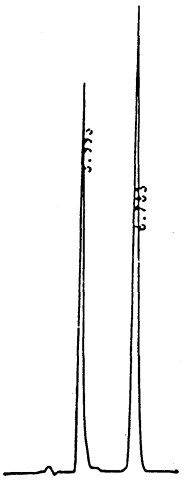

Fig. 1. Model chromatogram for method C

min. and 7 min respectively. Blank samples tested by the same procedure showed no interference peaks.

To confirm the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analysed pharmaceutical preparations and the mixtures were analysed by proposed methods. The results obtained by the proposed and reported methods are given in Table-1. The excipients and diluents present usually in pharmaceutical preparations did not interfere. The results indicate that the proposed methods are sensitive (C > A > B), accurate and reproducible and can be used for the routine determination of PTP in bulk as well as in its pharmaceutical preparations.

TABLE-1
ASSAY AND RECOVERY OF PTP IN PHARMACEUTICAL FORMULATIONS

Tablets	Labelled amount (mg)	Amount found by proposed methods (mg) ± S.D.			Amount found by reference method[10] (mg)	% Recovery by proposed methods ± S.D.*		
		A	B	C		A	B	C
1	40	40.05 ± 0.29	39.91 ± 0.22	40.03 ± 0.12	40.00 ± 0.20	99.88 ± 0.19	99.80 ± 0.98	100.20 ± 0.48
2	40	39.96 ± 0.23	40.04 ± 0.34	39.95 ± 0.46	39.95 ± 0.55	100.16 ± 0.98	99.47 ± 0.66	100.23 ± 0.44
3	40	39.97 ± 0.21	39.94 ± 0.34	40.21 ± 0.14	39.24 ± 0.46	99.78 ± 0.97	99.72 ± 0.71	99.46 ± 0.22
4	40	40.04 ± 0.34	40.11 ± 0.33	39.44 ± 0.12	40.65 ± 0.36	99.58 ± 0.24	99.58 ± 0.64	100.26 ± 0.18

*Average of five determinations.

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