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Spectrophotometric Determination of Pantoprazole by Precipitation Reactions and by HPLC

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Three simple and sensitive spectrophotometric methods (methods A, B and C) based on precipitation reactions and one HPLC method (method D) have been described for the estimation of pantoprazole (PTP) in pure form and its formulations. PTP forms insoluble molecular complexes with alkaloid precipitants like phosphomolybdic acid (PMA, method A), ammonium molybdate (AM, method B) or with iodine (I_2, method C) under acidic conditions. In addition, colour reactions have been combined to estimate each precipitant (and in turn PTP) either released from the complex (with PMA or AM) or the unreacted precipitant in the filtrate (I_2). They are based on the colour formation with cobalt nitrate - ethylene diamine tetraacetic acid disodium salt complex [Co(II)-EDTA, λ_{max}, 750 nm for PMA, method A], potassium thiocyanate (SCN^-, λ_{max} 450 nm for AM, method B) or *p*-N-methyl amino phenol sulphate-sulfanific acid (PMAP-SA, λ_{max} 525 nm for I_2, method C). Method D is an HPLC method in which silica column with a mobile phase consisting of acetonitrile, methanol and water (30 : 30 : 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: Spectophotometric determination, Pantoprazole, HPLC.

INTRODUCTION

Pantoprazole (PTP), chemically 5-(difluoromethoxy)-2-[[3,4-di-methoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole, is used in the treatment of ulcerative colitis. PTP acts by inhibiting gastric proton pump. Very few analytical methods have been reported in the literature for the determination of this drug based on HPLC[1–9] and visible spectrophotometric[10–12] methods. It was observed that the structural features of PTP have not been fully exploited for designing such procedures. This paper presents four such analytical methods. PTP undergoes quantitative precipitation in the form of molecular complexes with phosphomolybdic acid (PMA, method A), ammonium molybdate (AM, method B) or iodine (I_2) when used in excess. Colour reactions have been combined in addition to precipitation to estimate the precipitants and in turn PTP. They are based on

the colour formation with either the released precipitant from the molecular complex (PMA or AM) or the unreacted precipitant in the filtrate (I_2) with chromogenic reagents such as Co(II)-EDTA complex (for PMA)[13, 14], SCN^- (for AM)[15] or PMAP-SA (for I_2)[16]. Method D is a HPLC method using silica column, acetonitrile, methanol and water (30 : 30 : 40) as mobile phase and beclomethasone dipropionate as internal standard.

EXPERIMENTAL

A Systronics UV-Vis spectrophoto meter-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 CR6A, 20 μL Rheodyne 7125 loop injector and silica column (250 mm × 4.6 mm I.D.; particle size 10 μm) was used.

All chemicals used were of analytical grade. Aqueous solutions of PMA (Lobo: 1.0% w/v, 4.43×10^{-3} M), cobalt nitrate (Qualigens: 3.0% w/v, 1.03×10^{-1} M), EDTA (Qualigens: 4.0% w/v, 1.07×10^{-1} M), HCl (Qualigens: 0.01 M), AM (Qualigens: 2.0% w/v, 1.612×10^{-2} M), potassium thiocyanate (Qualigens: 5.0% w/v, 5.15×10^{-1} M), HCl (Qualigens : 5 M), I_2 (Qualigens: 0.1 % w/v, 3.94 x 10^{-3} M), PMAP (metal) (Loba: 0.3% w/v, 8.70×10^{-3} M), SA (IDPL: 0.2% w/v, 1.16×10^{-2} M) and phthalate buffer pH 3.0 (potassium acid phthalate-HCl) were prepared in double distilled water.

HPLC grade solvents such as acetonitrile, methanol and water were used. Two liters of mobile phase was prepared by mixing acetonitrile, methanol and water in the ratio 3 : 3 : 4. This solution was filtered through 0.45 μ 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 mobile phase 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 mobile phase 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 the 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: Aliquots of standard solution (1.0–5.0 mL, 1.0 mg/mL) were placed separately in a series of 10 mL centrifuge tubes. Then 3.0 mL of 0.01 M HCl and 0.5 mL of PMA solution were added and the total volume in each tube was made to 10.0 mL with distilled water, kept aside for 15 min at room temperature. All the tubes were centrifuged, the supernatant liquid was discarded and the precipitate was washed thoroughly thrice each with 1.0 mL of water and the precipitate was dissolved in 5 mL of acetone and collected in a series of 10 mL calibrated tubes respectively. Then 1.0 mL of EDTA solution and 1.0 mL of cobalt nitrate solution were added successively and heated for 10 min at 60°C in a water bath. All the tubes were cooled rapidly to room temperature and made up to 10 mL with distilled water, mixed thoroughly and the absorbances were measured at 750 nm against reagent blank. The amount of PTP in a sample solution was obtained from the Beer-Lambert plot.

Method B: Aliquots of standard solution (1.0–5.0 mL, 1.0 mg/mL) were placed separately in a series of 10 mL centrifuge tubes. Then 3.0 mL of 0.01 M HCl and 1.0 mL of AM solution were added and the total volume in each tube was made to 10.0 mL with distilled water, kept aside for 15 min at room temperature. All the tubes were centrifuged, the supernatant liquid was discarded and the precipitate was washed thoroughly thrice each with 1.0 mL of 50% aqueous alcohol and the precipitate was dissolved in 5 mL of acetone and collected in a series of 10 mL calibrated tubes respectively. Then 2.0 mL of 5.0 M HCl and 1.0 mL of potassium thiocyanate solution were added successively and heated for 10 min at 60°C in a water bath. All the tubes were cooled rapidly to room temperature and made up to 10 mL with distilled water, mixed thoroughly and the absorbances were measured at 750 nm against reagent blank. The amount of PTP in a sample solution was obtained from the Beer-Lambert plot.

Method C: Aliquots of standard solution (1.0–5.0 mL, 200 μg/mL) were placed separately in a series of 10 mL centrifuge tubes. Then 3.0 mL of 0.01 M HCl and 3.0 mL of iodine solution were added and kept aside for 15 min at room temperature in a dark place. All the tubes were centrifuged, the supernatant liquid was collected in a series of 25 mL volumetric flasks. The precipitate was washed thoroughly thrice each with 3.0 mL of water, the washings also collected in the respective 25 mL volumetric flasks and the precipitate was discarded. Then 10 mL of buffer (pH 3.0), 1.5 mL of metal solution were added successively. After 2 min 2.0 mL of SA solution was added and kept aside for 5 min at room temperature and made up to 25 mL with distilled water, mixed thoroughly and the absorbances

were measured at 525 nm against distilled water blank. A blank experiment was also carried out in a similar manner omitting the drug. The decrease in absorbance and in turn the PTP concentration were obtained by subtracting the absorbance of the test solution from that of the blank solution. The amount of PTP in a sample was obtained from the Beer-Lambert plot.

Method D: In a series of 10 mL volumetric flasks, 0.3 to 1.5 mL of above stock standard solution was transferred and 1.0 mL of stock 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. 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 0.5 mL/min and the detection was carried out at 289 nm. Initially the mobile phase was pumped for about 30 min to saturate the column, thereby to get the baseline corrected. Then 20 μL of each of the standard solutions were injected 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 Beer's law limits, regression equation and correlation coefficient were obtained by the least squares method and these results are given in Table-1. The precision of each method was tested by analyzing eight replicate samples. The per cent standard deviation and the per cent range of error at 95% confidence level of each method are given in Table-1.

TABLE-1
OPTICAL CHARACTERISTICS AND PRECISION

Parameters	Method A	Method B	Method C	Method D
Beer's law limit /Linearity range	100–500 μg/mL	100–500 μg/mL	8–40 μg/mL	60–300 ng/20 μL
% Relative standard deviation	1.0290	0.3130	0.3210	0.3380
% Range of error 0.05 confidence limit	0.8600	0.2610	0.2690	0.2830
Correlation coefficient	0.9999	0.9999	0.9999	0.9999
Regression equation (Y*)				
Slope (a)	8.10×10^{-4}	1.38×10^{-3}	1.824×10^{-2}	8.00×10^{-5}
Intercept (b)	-0.14×10^{-3}	1.00×10^{-3}	-3.000×10^{-4}	-4.81×10^{-3}

*Y = b + aC, where "C" is concentration in 1 μg/mL and Y is absorbance unit.

Commercial formulations (tablets) containing PTP were successfully analyzed by the proposed methods. For establishing accuracy, recovery experiments were performed by adding different amounts of the pure drug to the pre-analyzed

formulation. These results are summarized in Table-2. The ingredients usually present in formulations of PTP did not interfere with the proposed analytical methods.

TABLE-2
ESTIMATION OF PTP IN PHARMACEUTICAL FORMULATIONS

Sample	Labelled amount (mg)	Amount obtained (mg)					Per cent recovery by proposed method*			
		Reference method[10]	Proposed method							
			A	B	C	D	A	B	C	D
1	40	40.00 ±0.20	40.00 ±0.26	39.95 ±0.20	40.14 ±0.19	40.00 ±0.17	100.25 ±0.08	100.32 ±0.52	99.93 ±0.96	99.82 ±0.49
2	40	39.95 ±0.55	40.07 ±0.32	40.05 ±0.22	40.09 ±0.28	39.78 ±0.27	100.51 ±0.13	99.88 ±0.77	99.51 ±0.94	100.28 ±0.44
3	40	39.24 ±0.46	40.06 ±0.28	40.10 ±0.22	40.08 ±0.21	40.12 ±0.18	99.85 ±0.74	99.96 ±0.75	99.64 ±0.96	99.78 ±0.36

* Average of five determinations.

Chemistry of coloured species

The methods A, B and C involve two steps. In method A, first step is the quantitative precipitation of PTP with PMA. Second step is the reduction of PMA (released from the adduct by acetone) by Co(II)-EDTA complex to generate molybdenum blue. In method B, first step is the quantitative precipitation of PTP with AM. Second step involves the formation of orange-yellow coloured complex due to the formation of $[Mo(NCS)_6]^{3-}$ by the reaction of potassium thiocyanate with the molybdate released from the precipitate on treatment with acetone. In method C, first step is the quantitative precipitation of PTP with I_2. Second step is the determination of excess I_2 (unreacted I_2) with PMAP-SA, which forms a charge transfer complex involving oxidized form of PMAP (*p*-N-methyl benzoquinine imine) and SA.

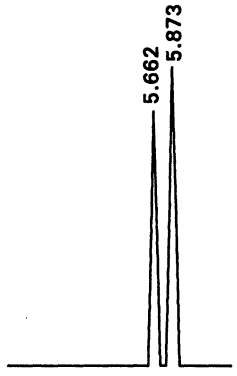

Fig. 1 Model chromatogram for method D

For method D, as shown in Fig. 1, drug and internal standard were eluted in 5.37 min and 5.87 min respectively. Blank samples tested by the same procedure showed no interference peaks. In conclusion the proposed methods are simple, sensitive, precise, reproducible and accurate and can be used for the routine determination of PTP in bulk as well as in pharmaceutical preparations.

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