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RESEARCH ARTICLE

EXTRACTIVE SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF PANTOPRAZOLE USING ACIDIC TRIPHENYLMETHANE DYES

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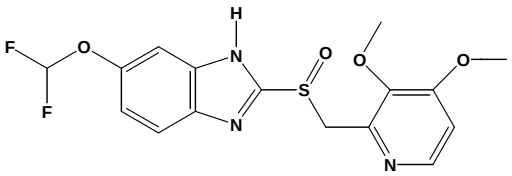
ABSTRACT

Three simple and sensitive extractive spectrophotometric methods have been described for the assay of pantoprazole either in pure form or in pharmaceutical formulations. The developed methods involve formation of coloured chloroform extractable ion-pair complexes of the drug with Bromo Thymol Blue (BTB), Bromo Phenol Blue (BPB) and Bromo Cresol Green (BCG) in acidic medium. The extracted complexes showed absorbance maxima at 412 nm for all three methods. Beer's law is obeyed in the concentration ranges 2.0-25, 2.0-20 and 2.5-25 µg/mL with BTB, BPB and BCG, respectively. The effects of concentration of dye, pH and interference of excipients have been studied and optimized. The limits of detection and quantification have been determined for three methods. All the three methods have been validated as per the guidelines of ICH. The methods have been applied to the determination of drug in commercial tablets and results of analysis were validated statistically through recovery studies.

INTRODUCTION

Pantoprazole (I) is chemically known as 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole (The Merck index, 1997).



(I)

Pantoprazole inhibits H^+ K^+ AT Phase pump function thereby healing the acid related conditions. Pantoprazole is chemically more stable than omeprazole and lansoprazole in neutral to slight acidic conditions, but under strongly acidic medium, active species is formed. Pantoprazole like omeprazole and lansoprazole also has a role in the eradication of Helicobacter Pylori (CIMS, 2005). The literature survey revealed that only few methods are available for the determination of Pantoprazole in dosage forms and include HPLC (Ding *et al.*, 2006, 2004, Hue-Hui *et al*., 2000), HPTLC (Agbaba *et al.*, 2004), UV spectrophotometry (Karljikovic *et al*., 2003) and chemometry (Wahbi Abdel *et al*., 2002). Visible spectrophotometry, because of its simplicity, cost-effectiveness, sensitivity, selectivity, fair accuracy and precision, has remained competitive in pharmaceutical analysis. In a method reported (Salama *et al.*, 2003). Pantoprazole was quantified by stability-indicating procedure through chelation with iron(III) in aqueous-ethanol medium to form an orange chelate with peak at 455 nm. The method is applicable over 30-300 µg mL^{-1}range of drug concentration. In another report (Moustafa, 2000), two methods based on charge transfer complexation reaction using 2, 3-dichloro-5, 6-dicyano-1, 4 - benzo quinone (DDQ) were described. The spectrophotometric determination of Pantoprazole using N-Bromosuccinimide, methyl orange and indigo carmine as reagents was carried out (Basavaiah, 2009). Because of its physiological significance, the quantitative determination of Pantoprazole attracted the attention of analytical chemists and almost all analytical methods have been applied to accomplish the purpose. However methods on spectrophotometric determination of this drug involving ion-pair complexes with common and versatile acidic dyes *viz.*, Bromo Thymol Blue (BTB), Bromo Phenol Blue (BPB) and Bromo Cresol Green (BCG) are not reported yet. This prompted the authors to develop extractive spectrophotometric methods for the determination of Pantoprazole using above mentioned dyes. In this paper we report three simple and sensitive extractive spectrophotometric methods for the assay of Pantoprazole. The methods are based on ion-pair complexation of drug with

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dyestuffs such as BTB, BPB and BCG and subsequent extraction into chloroform and to measure the absorbance of colour complex. The proposed methods have the advantages of speed and simplicity besides being accurate and precise, and can be adopted by the pharmaceutical laboratories for industrial quantitative analysis.

EXPERIMENTAL

MATERIAL AND METHODS

Pantoprazole was procured from Srini Pharmaceuticals Limited, Hyderabad as a gift sample. The dyestuffs *viz.*, BTB, BPB and BCG (AR grade) supplied by SD Fine Chemicals Ltd. Mumbai, were used without any further purification. The dyestuffs were used as 0.025% solutions in doubly distilled water. Sodium acetate-hydrochloric acid buffers (Britton, 1942) of *p*H 2.5, 2.8, and 3.5 were prepared by mixing 50ml of 1.0*M* sodium acetate solution with 50.50, 49.50 and 46.25 ml respectively, of 1.0 *M* HCl solution and diluted to 250 ml with doubly distilled water. The *p*H of each solution was adjusted to an appropriate value with the aid of a *p*H meter. Chloroform (HPLC grade) supplied by SD Fine Chemicals Ltd. Mumbai was used throughout the work. Stock solutions were prepared for all the dyes and drugs (25mg/100ml). The spectra (Fig. 1) of ion-pair complexes have been recorded on Elico double beam SL 210 spectrophotometer using quartz cells of 10 mm path length. An Elico model Li-120 *p*H meter was used for *p*H measurement.

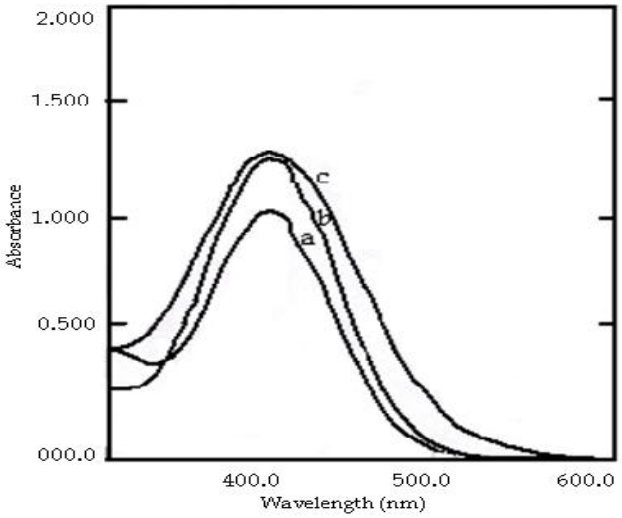


Fig. 1. Absorption spectra of Pantoprazole-dye complex extracted into 10 ml chloroform

a. drug = 20 µg ml^{-1} + 5 ml of 0.025% BTB + 5 ml of pH 2.8 buffer
b. drug = 17.5 µg ml^{-1} + 5 ml of 0.025% BPB + 5 ml of pH 2.5 buffer
c. drug = 17.5 µg ml^{-1} + 5 ml of 0.025% BCG + 5 ml of pH 3.5 buffer

Calibration curve

Different aliquots of drug solution were transferred into 125 ml separating funnel. To this 5 ml of buffer (*p*H 2.5, 2.8 and 3.5), 5 ml of dye were added and total volume was made up to 20 ml with water. 10 ml of chloroform was added and the contents were shaken for 5 min. The two layers were allowed to separate for 5 min. The organic layer was separated and absorbance of yellow colored solution which is stable atleast for 3 hrs is measured at 412 nm against blank similarly prepared. The same procedure of analysis is followed either for assay of pure drug or for dosage form. The calibration graphs (Fig. 2) are linear over the concentration ranges and are within the permissible range. The optical characteristics and statistical data for the regression equation of the proposed methods are presented in Table 1.

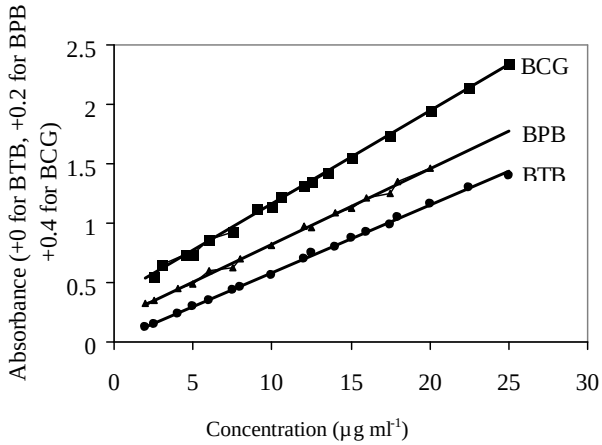


Fig. 2. Calibration graphs for Drug-BTB, BPB & BCG ion pair complexes

Procedure for the assay of pure drug

Five different solutions of pure drug in the range of calibration curve were selected and the recovery experiments were performed. The recoveries and their relative standard deviations are tabulated in Table 2.

Procedure for the assay of dosage forms

Ten tablets of Pantop 40 mg each are powdered and dissolved in doubly distilled water and stirred thoroughly, filtered through a Whatman No. 42 filter paper. This solution was transferred into 100 ml standard volumetric flask and diluted with doubly distilled water as required. Different solutions of drug in the range of calibration curve were chosen and the assay was estimated using the calibration curve. The results of the recovery experiments are tabulated in Table 3.

RESULTS AND DISCUSSION

Pantoprazole forms ion-pair complexes in acidic buffer with dyestuffs such as Bromo Thymol Blue (BTB), Bromo Phenol Blue (BPB) and Bromo Cresol Green (BCG) and these complexes are quantitatively extracted into chloroform. Ion-pair complexes of drug with BTB, BPB and BCG absorbed maximally at 412 nm. The reagent blank under similar conditions showed no absorption. In order to establish molar ratio between Pantoprazole and dyestuffs used, the Job's method of continuous variation (Vosburgh and Cooper, 1941) has been applied. In this method, solutions of drug and dyestuff with identical molar concentrations (8 x 10^{-5}*M*) were mixed in varying volume ratios in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured and plotted against the mole fraction of

Table 1. Optical characteristics and statistical analysis for the regression equation of the proposed methods

Parameters	Extraction methods with[b]		
	BTB	BPB	BCG
λ_{max} (nm)	412	412	412
Beer's law limit (µg ml^{-1})	2.0-25	2.0-20	2.5-25
Molar absorptivity (L mol^{-1} cm^{-1})	18228	20527	24329
Formation constant, K, M^{-1}	1.53x10^6	1.76x10^6	1.83x10^6
Sandell sensitivity (µg cm^{-2})	0.0176	0.0159	0.0127
Slope (specific absorptivity), b	0.058	0.0632	0.0783
Intercept (a)	0.00876	-0.0127	-0.02397
Correlation coefficient (r)	0.9994	0.999	0.998
Standard deviation of intercepts (% n=6)	0.0049	0.0083	0.01276
Limit of detection, µgml^{-1}	0.2778	0.4289	0.537
Limit of quantification, µgml^{-1}	0.8336	1.2865	1.618
Regression equation[a]	Y=0.058C+0.00876	Y=0.0632C+0.0127	Y=0.0783C+0.02397

[a]With respect to $Y=bc+a$, where C is the concentration (µg ml^{-1}) and Y is absorbance
[b]Six replicate samples

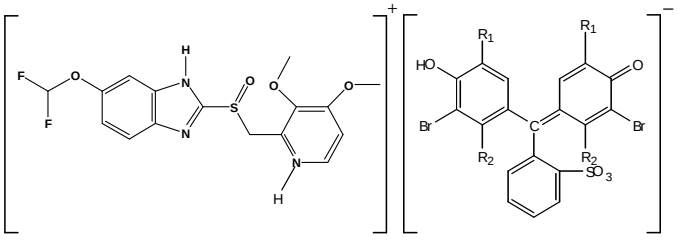
Table 2. Application of proposed methods for the analysis of Pantoprazole in pure form

Taken (µg ml^{-1})	Proposed methods						Reference method
	Found (µg ml^{-1})			Recovery (%)			Recovery (%)
	BTB	BPB	BCG	BTB	BPB	BCG	
3.5	3.56	3.54	3.54	101.82	101.34	101.32	98.25
6.5	6.63	6.63	6.48	102.05	102.13	99.73	101.25
9.5	9.53	9.72	9.62	100.32	102.41	101.26	101.65
12.5	12.58	12.46	12.63	100.66	99.72	101.1	101.16
15.5	15.8	15.54	15.92	101.99	100.26	102.76	100.6
							99.0
							103.58
							101.95
RSD (%)				0.8034	1.15	1.0638	1.6143
Mean±SD				101.4±0.81	101.18±16	101.23±1.07	101.07±1.63
t-test				1.454	0.1377	0.2218	
F-test				0.249	0.508	0.4356	

Table 3. Application of proposed methods for the analysis of Pantoprazole in pharmaceuticals form

Taken (µg ml^{-1})	Proposed methods						Reference method
Pantop 40mg	Found (µg ml^{-1})			Recovery (%)			Recovery (%)
	BTB	BPB	BCG	BTB	BPB	BCG	
3	3.02	3.02	3.09	100.72	100.73	103.3	98.28
6	6.12	6.08	6.07	102.08	101.36	101.3	101.26
9	9.02	9.12	9.28	100.24	101.38	103.11	101.66
12	12.13	12.26	11.96	102.78	102.2	99.73	102.25
15	15.09	15.17	15.03	100.61	101.18	100.26	101.16
							100.6
							99.0
							103.58
							101.95
RSD (%)				1.07	0.5239	1.5318	1.6143
Mean±SD				101.29±1.08	101.37±0.53	101.49±1.55	101.07±1.64
t-test				1.454	0.1377	0.2218	
F-test				0.249	0.508	0.4356	

the drug, [drug]/ [drug] + [dyestuff] (Fig.3). This measurement showed that 1:1 complex was formed with each dyestuff. The formation constants (Likussar and Boltz, 1971, Momoki *et al*., 1969) were also estimated and found to be 1.54x 10^6, 1.75 x 10^6 and 1.84x 10^6 K M^{-1} for complexes with BTB, BPB and BCG respectively. Pantoprazloe contains pyridine nitrogen which is protonated in acid medium, while sulphonic acid group is present in BTB, BPB and BCG, that is the only group undergoing dissociation in the *p*H range 1-5. The colour of such dyes is due to the opening of lactoid ring and subsequent formation of quinoid group. It is supposed that the two tautomers are present in equilibrium but due to strong acidic nature of the sulphonic acid group, the quinoid body must predominate. Finally the protonated Pantoprazole forms ion-pairs with the dyestuffs which are quantitatively extracted into chloroform. The possible reaction mechanism is proposed and given in Scheme 1.



Scheme 1:Pantoprazole-Dye complex
Bromothymol blue : R_1 = isoporopyl, R_2 = -CH_3
Bromophenol blue : R_1 = -Br, R_2 = -H
Bromocresol green : R_1 = -Br, R_2 = -CH_3

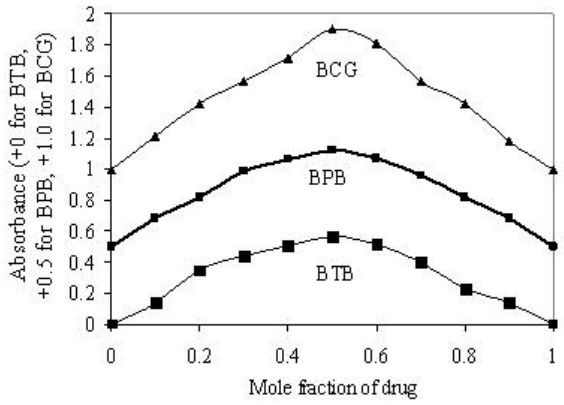


Fig. 3. Continuous-variations study of drug-dye systems [Drug] = [Dye] = 8x10^{-5}M

The influence of *p*H on the ion-pair formation of Pantoprazole with various dyestuffs has been studied using sodium acetate-hydrochloric acid buffer. The results are shown in Fig.4. It is evident that absorbance of complexes with BTB, BPB and BCG was found to be constant within the *p*H ranges 2.2-3.3, 2.0-3.0 and 2.8-3.8 respectively. Thus, all the absorbance measurements were made at *p*H 2.8, 2.5 and 3.5 with BTB, BPB and BCG respectively.

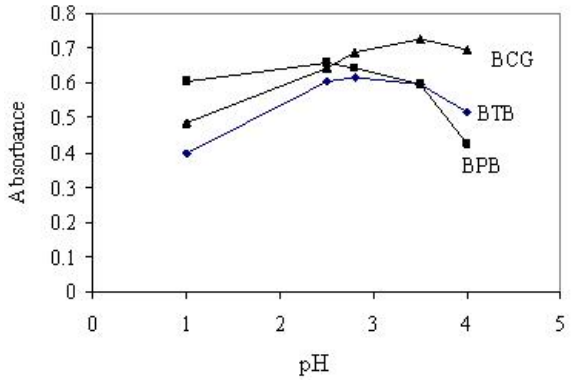


Fig. 4. Effect of pH [Drug] = [8µg ml^{-1}, [Dye] = 5ml of 0.025%

The effect of dyestuff concentrations was also studied by adding different volumes of dyestuff to a constant amount of Pantoprazole (8 µg ml^{-1}). It is apparent from Fig. 5 that the maximum absorbance, in each case, was found with 3.0 ml of dyestuff, beyond which absorbance was constant. Thus, 5 ml of each dyestuff was used for ion-pair formation throughout the experiment.

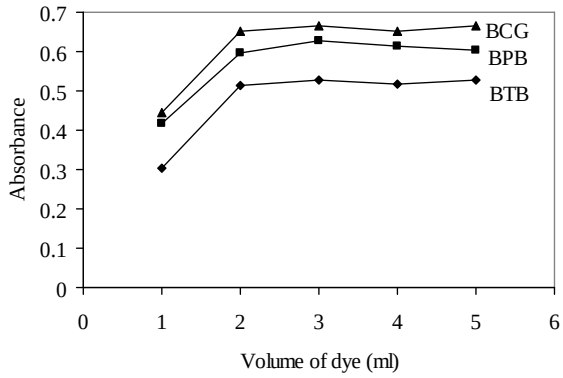


Fig. 5. Influence of the volume of 0.025% Dye [Drug] = [8µg ml^{-1}]

A systematic study of the effect of foreign species present along with Pantoprazole on the determination of Pantoprazole at 8 µg ml^{-1} levels was undertaken. This study was carried out by following the proposed procedures for a 10 ml sample system, by adding a known amount of foreign species to a Pantoprazole solution of 8 µg ml^{-1}. Table 4 summarizes the results obtained. However, the drug content from the powdered capsules was extracted into chloroform, which completely removes any interference by the common excipients found in formulations.

Table 4. Interference study

Sl. No	Excipients	Tolerance limit (µg ml^{-1})
1	Microcrystalline cellulose	87
2	Starch	171
3	Lactose	132
4	Povidone	54
5	Silicon dioxide	93
6	Titanium dioxide	45

Validation of the proposed method

All three proposed methods have been validated in terms of guideline proposed by International Conference on Harmonization (ICH, 1996) viz., selectivity, specificity, accuracy, precision, limits of calibration curve, LOD, LOQ, robustness, ruggedness and regression equation. The student t-test and variance F-test have been performed in comparison with a reference method. Table 1 summarizes the values for Beer's law limits, molar absorptivity, regression equation, correlation coefficients, relative standard deviation and recoveries. To test the reproducibility of the proposed methods, six replicate determinations of 10µg ml^{-1} of Pantoprazole were made. The coefficient of variation was found to be less than 1.2% for all the procedures. The proposed methods have been successfully applied to the determination of Pantoprazole in pharmaceutical preparations. The performance order of the proposed methods is BCG>BPB>BTB. The results obtained and shown in Table 2 and Table 3 were compared to those obtained by a reference method (ICH, 1996) by means of t-test at 95% confidence level. In all cases, the average results obtained by proposed methods and reference method were statistically identical, as the difference between the average values had no significance at 95% confidence level. The proposed methods are simple, sensitive and reproducible and can be used for routine analysis of Pantoprazole in pure form and in formulations.

Conclusion

In conclusion, pantoprazole forms ion-pair complexes with acidic triphenylmethane dyes *viz.*, bromothymol blue, bromophenol blue and bromocresol green in 1:1 proportion. These complexes are extractable into chloroform and offer a basis for assay of the drug. The developed methods are simple, sensitive, reproducible and can be used for routine analysis of pantoprazole in pure and formulation forms.

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