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RESEARCH ARTICLE

SPECTROPHOTOMETRIC DETERMINATION OF LANSOPRAZOLE IN PURE AND PHARMACEUTICAL FORMS USING TRIPHENYL METHANE DYES

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ABSTRACT

Four simple and sensitive extractive spectrophotometric methods have been described for the assay of Lansoprazole either in pure form or in pharmaceutical formulations. The developed methods involve formation of coloured chloroform extractable ion-pair complexes of the drug with bromothymol blue (BTB), bromophenol blue (BPB), bromocresol purple (BCP) and bromocresol green (BCG) in acidic medium. The extracted complexes showed absorbance maxima at 419, 417, 416 and 414nm with use of the cited reagents, respectively. The stoichiometry of the complex is found to be 1:1 in each case. Beer's law is obeyed in the concentration ranges 2.5-25, 4.0-30, 4.0-40 and 4.5-45µg/ml with BTB, BPB, BCP and BCG respectively. The effect of concentration of dye, pH, and interference of excipients have been studied and optimized. The limits of detection and quantification have been determined for four methods. All the four 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

Lansoprazole, a benzimadazole derivative, chemically 2-[(Methyl-4-(2,2,2-trifluoroethoxy) pyridine-2-yl) methyl sulfinyl]-1H-benzimadazole (I) is a proton pump inhibitor (Sweetman SC, 2005) that suppresses gastric acid secretion by specific inhibition of the enzyme system of hydrogen/potassium adenosine triphosphatase (H^+/K^+ ATPase) at the secretory surface of the gastric parietal cell. Lansoprazole is used in the treatment of various gastric disorders such as gastric and duodenal ulcers and in pathological hyper secretory conditions (Oliveira CH *et al*., 2003). Lansoprazole is extensively metabolized in the liver and the latest reports include the indication of healing and risk reduction in non-steroidal anti-inflammatory drug-associated gastric ulcers (Ohashi *et al*., 1995). It is also used in the treatment of gastro esophageal reflux disease (Gunasekaran *et al*., 2002). Lansoprazole is one of the necessary components of dual – and triple therapy regimens for the eradication of Helicobacter pylori infection (Keith, 2000).

The methods such as HPLC (Unoa *et al*., 2005, Basavaiah *et al*., 2006), RP-HPLC (Bhavesh H Patel, 2007 and Bhavna Patel *et al*., 2009), RP-UPLC (Venkat Rao *et al*., 2013), HP-TLC (Pandya KK *et al*., 1997), LC-MS (Celso H *et al*., 2003),

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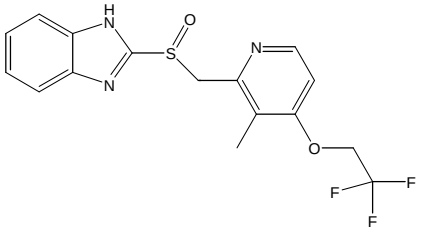


(I) Lansoprazole

Capillary electrophoresis (Dogrukol *et al*., 2001) and C13 NMR (DellaGreca *et al*., 2006) for the determination of Lansoprazole are available in the literature. The chemical features of the drug molecule offers a lot of scope for the development of new methods for its determination with better sensitivity, specificity, precision and accuracy. The reported chromatographic techniques require expensive experimental set-up and are not affordable in every laboratories for routine analysis. The literature survey revealed that, although, UV-vis spectrophotometric methods (Puratchikodi *et al*., 1996 and Alagar Raja *et al*., 2011) for the determination of Lansoprazole are available, a little attention was paid to the development of spectrophotometric methods for its determination using dyes. A report for the determination of Lansoprazole in pure and pharmaceutical formulations using Supracen Violet 3B and Topaeolin ooo is available in the literature (Uma Devi and Murali Krishna, 2013). Spectrophotometry is considered as the most convenient analytical technique because of its inherent

simplicity, low cost, and wide availability in most quality control laboratories. So the present study reports on newly developed and validated spectrophotometric estimation of Lansoprazole in bulk and pharmaceutical formulations using triphenyl methane dyes viz., bromothymol blue (BTB), bromophenol blue (BPB), bromocresol purple (BCP) and bromocresol green (BCG). The developed methods involve formation of coloured chloroform extractable ion-pair complexes of the drug with dyes in acidic medium.

Because of its physiological significance, the quantitative determination of Lansoprazole 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.*, bromothymol blue (BTB), bromophenol blue (BPB), Bromocresol purple (BCP) and bromocresol green (BCG) are not reported yet. This prompted the authors to develop extractive spectrophotometric methods for the determination of Lansoprazole using above mentioned dyes. In this paper we report four simple and sensitive extractive spectrophotometric methods for the assay of Lansoprazole. These methods are based on ion-pair complexation of drug with dyestuffs such as BTB, BPB, BCP 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.

MATERIAL AND METHODS

Lansoprazole was procured from Srini Pharmaceuticals Limited, Hyderabad as a gift sample. The dyestuffs *viz.*, BTB, BPB, BCP 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 2.8, 2.5, 2.5, and 3.5 were prepared by mixing 50ml of 1.0*M* sodium acetate solution with calculated volume 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. The spectra (Fig 1a, 1b, 1c and 1d) 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.

Calibration curve

Different aliquots of drug solution were transferred into 125 ml separating funnel. To this 5 ml of buffer (*p*H 2.8, 2.5, 2.5 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 419 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

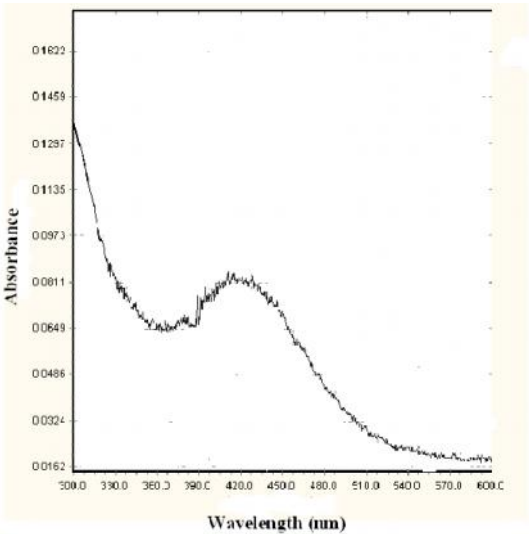


Fig 1a. Absorption spectrum of Lansoprazole-bromothymol blue(BTB) complex extracted into 10 ml chloroform
[drug] = 25 μg ml^{-1} + 5 ml of 0.025% BTB + 5 ml of pH 2.8 buffer

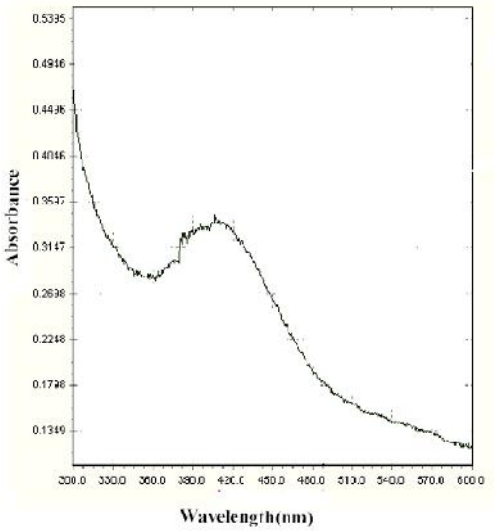


Fig 1b. Absorption spectrum of Lansoprazole-bromophenol blue(BPB) complex extracted into 10 ml chloroform
[drug] = 25 μg ml^{-1} + 5 ml of 0.025% BPB + 5 ml of pH 2.5 buffer

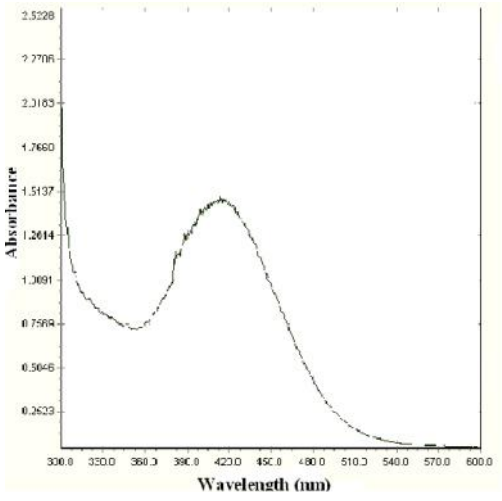


Fig 1c. Absorption spectrum of Lansoprazole-bromocresol purple (BCP) complex extracted into 10 ml chloroform
[drug] = 25 μg ml^{-1} + 5 ml of 0.025% BCP + 5 ml of pH 2.5 buffer

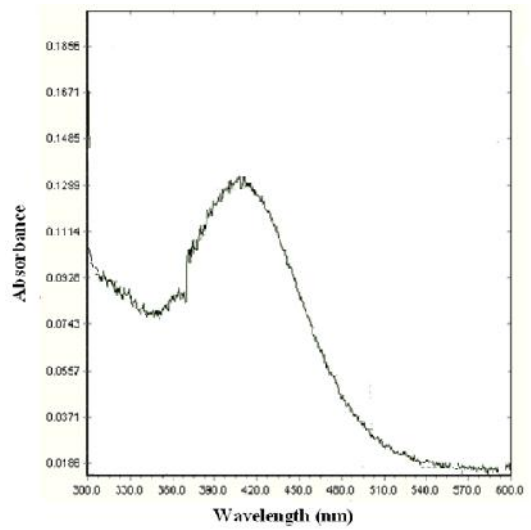
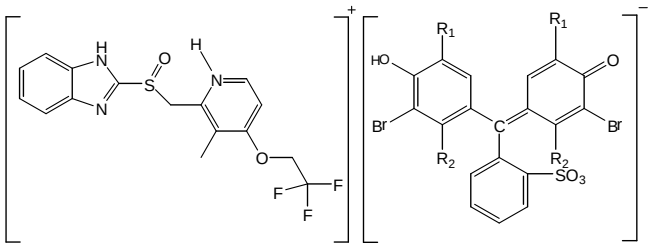


Fig 1d. Absorption spectrum of Lansoprazole-bromocresol green (BCG) complexextracted into 10 ml chloroform
[drug] = 25 µg ml^{-1} + 5 ml of 0.025% BCG + 5 ml of pH 3.5 buffer



Scheme 1 Lansoprazole-dye ion pair complex

Bromothymol blue : R_1 = isoporopyl, R_2 = -CH_3
Bromophenol blue : R_1 = -Br, R_2 = -H
Bromocresol purple: R_1= -CH_3, R_2= -H
Bromocresol green : R_1 = -Br, R_2 = -CH_3

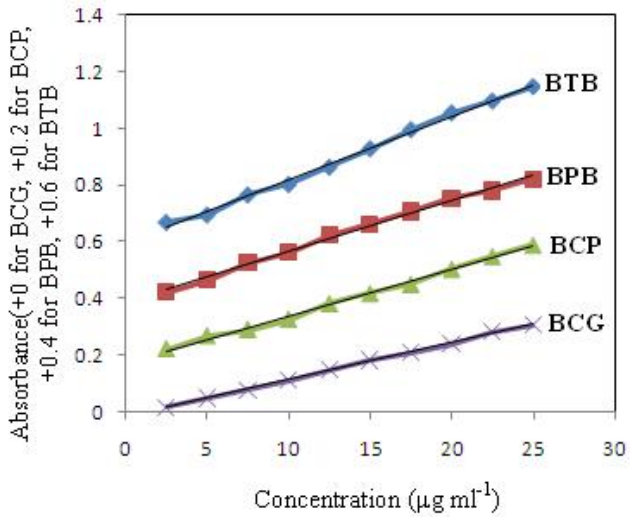


Fig 2. Calibration graphs for Drug-BTB, BPB, BCP & BCG Ion-pair complexes

(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.

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 Lanzol 15mg 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

Lansoprazole forms ion-pair complexes in acidic buffer with dyestuffs such as bromothymol blue (BTB), bromophenol blue (BPB), Bromocresol pruple (BCP) and bromocresol green (BCG) and these complexes are quantitatively extracted into chloroform. Ion-pair complexes of drug with BTB, BPB, BCP and BCG absorbed maximally at 419, 417, 416 and 414 nm respectively (Fig. 1a, 1b, 1c and 1d). The reagent blank under similar conditions showed no absorption. Lansoprazloe contains pyridine nitrogen which is protonated in acid medium, while sulphonic acid group is present in BTB, BPB, BCP and BCG, that is the only group undergoing dissociation in the *p*H range 1-6. 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 Lansoprazole forms ion-pairs with the dyestuffs which are quantitatively extracted into chloroform. The possible reaction mechanism is proposed and given in Scheme 1.

Stoichiometry

In order to establish molar ratio between Lansoprazole 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 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 1.42x 10^6, 1.69x 10^6, 1.75x 10^6 and 1.87x 10^6 K M^{-1} for complexes with BTB, BPB, BCP and BCG respectively.

Optimization of the factors affecting the absorbance

The influence of *p*H on the ion-pair formation of Lansoprazole with various dyestuffs has been studied using sodium acetate-hydrochloric acid buffer. The results are shown in Fig.4. It is

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

Parameters	Extraction methods with[b]			
	BTB	BPB	BCP	BCG
λ_{max} (nm)	419	417	416	414
Beer's law limit (µg ml^{-1})	2.0-25	2.0-20	2.0-2.5	2.5-25
Molar absorptivity (L mol^{-1} cm^{-1})	18596	20333	22630	25622
Formation constant, K, M^{-1}	$1.42x10^6$	$1.69x10^6$	$1.75x10^6$	$1.87x10^6$
Sandell sensitivity (µg cm^{-2})	0.0166	0.0143	0.0133	0.0122
Slope (specific absorptivity), b	0.0602	0.0699	0.0752	0.0819
Intercept (a)	0.00784	-0.0227	0.0213	-0.0139
Correlation coefficient (r)	0.999	0.998	0.999	0.998
Standard deviation of intercepts (% n=6)	0.0045	0.0078	0.0102	0.0116
Limit of detection, µgml^{-1}	0.2466	0.3682	0.4476	0.4673
Limit of quantification, µgml^{-1}	0.7475	1.1158	1.3563	1.4163
Regression equation[a]	Y=0.0602C± 0.00784	Y=0.0699C± 0.0227	Y=0.0752C± 0.0213	Y=0.0819C± 0.0139

[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 Lansoprazole in pure form

Taken (µg ml^{-1})	Proposed methods								Reference method
	Found (µg ml^{-1})				Recovery (%)				Recovery (%)
	BTB	BPB	BCP	BCG	BTB	BPB	BCP	BCG	
6	6.07	5.97	6.17	5.95	101.16	99.50	102.83	99.16	98.75
10	9.97	10.12	10.32	9.96	99.7	101.2	103.2	99.6	101.02
14	14.34	13.95	13.85	14.12	100.32	99.64	98.92	100.85	101.88
18	18.05	17.75	18.12	17.65	100.27	98.61	100.66	98.05	101.06
22	21.98	22.04	21.88	22.02	99.9	100.18	99.45	100.09	100.12
									99.84
									101.58
									101.85
RSD (%)					0.5589	0.9546	1.9191	1.0518	1.10638
Mean±SD					100.27 ±0.56	99.82 ±0.95	100.02 ±1.9	99.55 ±1.04	100.76 ±1.106
t-test					1.0542	0.2368	0.2348	0.2218	
F-test					0.3256	0.4265	0.5489	0.4866	

Table 3. Application of proposed methods for the analysis of Lansoprazole in pharmaceutical form

Taken(µg ml^{-1})	Proposed methods								Reference method
Lanzol15mg	Found (µg ml^{-1})						Recovery (%)		Recovery (%)
	BTB	BPB	BCP	BCG	BTB	BPB	BCP	BCG	
4	3.92	4.02	3.98	4.09	98.0	100.5	99.5	102.25	98.98
8	8.12	7.88	8.02	8.07	101.5	98.5	100.25	100.87	101.06
12	11.88	12.12	12.16	12.28	99.0	101.0	101.33	102.33	101.12
16	16.13	16.06	15.97	15.96	100.8	100.3	99.81	99.75	101.25
20	20.09	19.88	19.73	20.03	100.45	99.4	98.65	100.15	101.16
									100.48
									99.96
									101.58
									101.45
RSD (%)					1.4819	0.9919	0.9900	1.1715	0.8509
Mean±SD					99.95 ±1.48	99.94 ±0.99	99.91 ±0.98	101.07 ±1.18	101.78 ±0.857
t-test					1.2527	0.2245	0.4564	0.2564	
F-test					0.4224	0.6258	0.4135	0.4356	

evident that absorbance of complexes with BTB, BPB, BCP and BCG was found to be constant within the *p*H ranges 2.2-3.3, 2.0-3.0, 2.0-3.0 and 2.8-3.8 respectively. Thus, all the absorbance measurements were made at *p*H 2.8, 2.5, 2.5 and 3.5 with BTB, BPB, BCP and BCG respectively. The effect of dyestuff concentrations was also studied by adding different volumes of dyestuff to a constant amount of Lansoprazole (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. A systematic study of the effect of foreign species present along with Lansoprazole on the determination of Lansoprazole 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 Lansoprazole 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	96
2	Starch	156
3	Lactose	126
4	Povidone	58
5	Silicon dioxide	84
6	Titanium dioxide	55

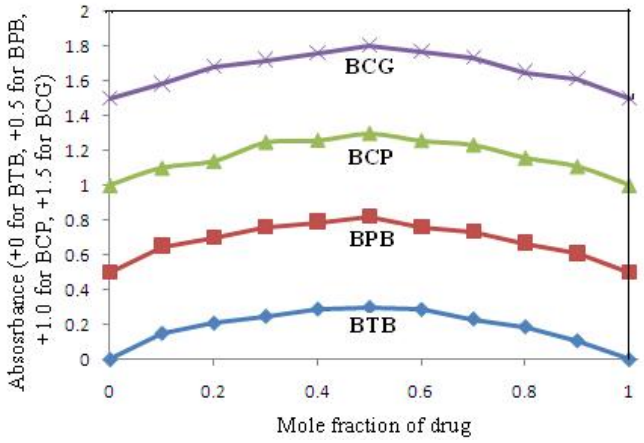


Fig 3. Continuous-variations study of drug-dye systems [Drug] = [Dye] = 8x10^{-5}M

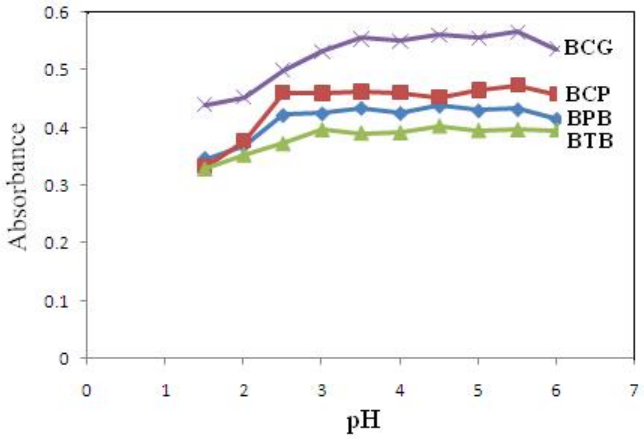


Fig. 4 Effect of P^{H} [Drug] = [8µg ml^{-1}, [Dye] = 5ml of 0.025%

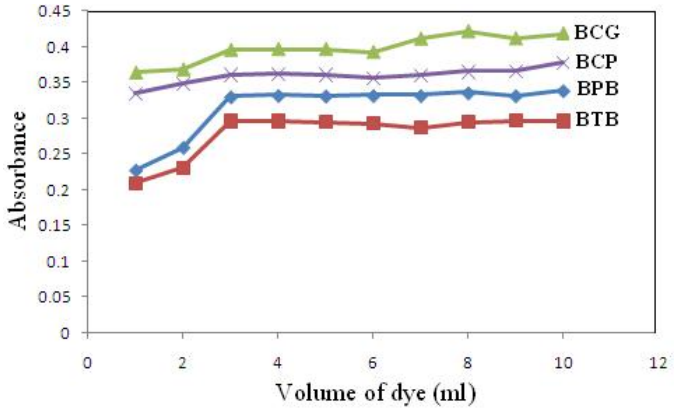


Fig 5. Influence of the volume of 0.025% Dye [Drug] = [8µg ml^{-1}]

Validation of the proposed method

All the four 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 Lansoprazole 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 Lansoprazole in pharmaceutical preparations. The performance order of the proposed methods is BCG>BCP>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.

Conclusion

In conclusion, Lansoprazole forms ion-pair complexes with acidic triphenylmethane dyes *viz.*, bromothymol blue, bromophenol blue, bromocresol purple 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 Lansoprazole in pure and formulation forms.

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