RESEARCH ARTICLE

Rapid and Sensitive Spectrophotometric Measurement of Non-Specific Beta Blocker Propranolol Hydrochloride Using Three Sulphonphthalein Dyes In Pure Form, Pharmaceuticals and Human Urine

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Rapid and Sensitive Spectrophotometric Measurement of Non-Specific Beta Blocker Propranolol Hydrochloride Using Three Sulphonphthalein Dyes In Pure Form, Pharmaceuticals and Human Urine

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Abstract
Three sensitive, selective, rapid and easily reproducible spectrophotometric methods (A-C) have been developed for the determination of propranolol hydrochloride (PPH) as a hydrochloride free base propranolol (PPL) in bulk sample and in its dosage forms. These methods are based on ion-pair formation between propranolol as a hydrochloride and free base and three acidic (sulphonphthalein) dyes; namely bromothymol blue (BTB), bromocresol green (BCG) and bromocresol purple (BCP) which induces an instantaneous bathochromic shift of the maximum in the drug spectrum. The colored products are measured at 420 nm (PPL-BTB complex and PPL-BCP complex) and 425 nm (PPL-BCG complex). The reactions were extremely rapid at room temperature and the absorbance values remained constant for 90 min (method B), and over 24 hrs (method A and C). Conformity to Beer's law in the range 0.4-7.0 µg $ml^{-1}$for methods A and B and 0.5-8.4 µg $ml^{-1}$for method C enabled the assay of dosage forms of the drug. The proposed methods were compared with a reference method; the results obtained were of equal accuracy and precision. In addition, these methods were also found to be specific for the analysis of PPH in the presence of excipients, which are co-formulated in the drug. Satisfactory results were obtained when applied to spiked human urine. A more detailed investigation of the propanolol hydrochloride ion pair complexes were made with respect to its composition indicated by stability constant values.

Keywords: Propranolol hydrochloride; spectrophotometric assay; ion-pair complexes; pharmaceuticals; human urine.

1. Introduction

Propranolol hydrochloride (PPH), (2RS)-1-[(1-Methylethyl)amino]-3-(naphthalen-1-yloxy)propan-2-ol hydrochloride (Figure 1), is a highly effective antihypertensive and antianginal drug. Being a nonselective prototype beta-adrenergic receptor-blocking agent, it possesses no other autonomic nervous system activity and specifically competes with beta-adrenergic receptor-stimulating agents for available receptor sites. The drug is widely used in clinical practice for the treatment of cardiac arrhythmia, hypertension and angina pectoris [1], dysfunctional labour, anxiety and migraine [2, 3]. It is also abused in sports involving little physical activity to reduce cardiac, contraction, heart rate and coronary blood flow [4]. Therefore, it has been included in the list of forbidden substances by the International Olympic Committee [5]. Monitoring of propranolol in biological fluids is important not only in clinical practice but also in the field of doping control. The drug is official in BP [6] and USP [8], which describe UV-spectrophotometric methods for the assay of PPH after extraction into methanol, and also in IP [7] which describes a potentiometric titration of drug in ethanol with 0.1 M NaOH.

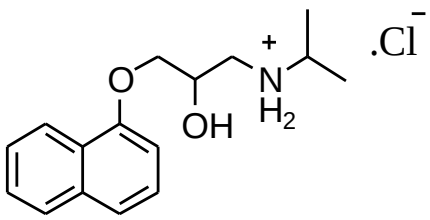


Figure 1: Structure of propranolol hydrochloride.

Due to its therapeutical and pharmacological relevance, several methods have been reported for PPH and include thin layer chromatography [9], UV-spectrophotometry [10-13], fluorimetry [14], voltammetry [15] and chemiluminometry [16,17]. These techniques involve an expensive experimental set up and are not always easily accessible. One titrimetric [18] and a few visible spectrophotometric [19-34] methods have also been reported.

Visible spectrophotometry, because of its simplicity and cost-effectiveness, sensitivity and selectivity and fair accuracy and precision is routinely used in many industrial quality control laboratories. Several visible spectrophotometric methods based on different reaction schemes are found in the literature for PPH.

A method for the assay of PPH using diazotized 4-amino-3,5-dinitrobenzoic acid (ADBA) as the chromogenic derivatizing reagent reported by Idowu *et al.* [19]. Bhandari *et al.* [20] reported a method based on the reaction of PPH with 1-chloro-2,4-dinitrobenzene, forming a complex, which absorb maximally at 314.6 nm. In a method reported by Golcu *et al.* [21], PPH was reacted with copper (II) or cobalt (II) and the coloured complexes were measured at 548 or 614 nm. El-Ries *et al.* [22] proposed two spectrophotometric methods based on the charge-transfer complex reaction of PPH with π-acceptors, tetracyanoethylene (TCNE), or chloranilic acid (CLA) to give highly coloured complex species which are quantitated spectrophotometrically, absorbing maximally at 415 or 510 nm. Salem [23] used similar reactions for the spectrophotometric determination of PPH which are based on the reaction of PPH as n-electron donor with the sigma-acceptor iodine and π-acceptors such as 7,7,8,8-tetracyaniquinodimethane, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, tetracyanoethylene, bromanil and chloranil. The resulting CT complexes were measured at 365, 840, 420, 470, 450 and 440 nm, respectively. Hussain *et al.* [24] reported a method based on the redox reaction of PPH with cerium (IV) in H_2SO_4 medium on heating and the developed color was measured at 478 nm. El-Emam *et al.* [25] reported a method based on oxidative-coupling reaction in which a mixture of an acidic solution of MBTH and PPH was treated with cerium (IV) and the resulting orange colour peaking at 496 nm was measured.

In addition to direct methods described above, several indirect methods based on a variety of reaction chemistries are also found in the literature. A spectrophotometric method proposed by Basavaiah *et al.* [26] makes use of the reaction between chloride of PPH and mercury(II) thiocyanate in which thiocyanate ions displaced complexed with iron(III) for subsequent measurement at 460 nm. In a spectrophotometric method reported by Basavaiah *et al.* [27], the unreacted cerium(IV) sulphate was treated with iron(II) and the iron(III) was complexed with thiocyanate and measured at 480 nm. Similar method reported by Basavaiah *et al.* [28] is based on the oxidation of PPH by a known excess of CAT in acid medium followed by determination of the unreacted oxidant by reacting with metal and sulphanilic acid. The same authors reported another spectrophotometric method in which the unreacted oxidant metavanadate was determined by reacting with diphenylamine, and the absorbance measured at 560 nm [29]. A method reported by Basavaiah *et al.* [30] involves the addition of a known excess of bromate-bromide mixture to an acidified solution of the drug and determination of the unreacted bromine by its bleaching action on methyl orange acid color and the absorbance measured at 510 nm. El-Didamony [31] reported three methods based on oxidation-bromination reaction of PPH by bromine, generated *in situ* by the action of acid on a bromate-bromide mixture, followed by determination of unreacted bromine by three different reaction schemes. In one method the residual bromine was determined by indigo carmine dye. In the other two methods, the residual bromine was determined by treating with a known excess of iron(II) and the resulting iron(III) was complexed with thiocyanate or the residual iron(II) with 1,10-phenanthroline. Gowda *et al.* [32] reported two procedures, similar to the above, in which PPH was oxidized by a known excess of NBS in H_2SO_4 medium followed by the reaction of unreacted oxidant with promethazine hydrochloride (PH) or methdilazine hydrochloride (MDH) to yield red coloured products with absorption maximum at 515 or 513 nm. Two methods described by Al-Attas *et al.* [33] based on the oxidation of PPH by a known excess of N-bromosuccinimide (NBS), in an acidic medium followed by the reaction of excess oxidant with amaranth dye. Sastry *et al.* [34] devised one more method by treating PPH with a known excess of NBS in HCl medium, and after 10 min, the unreacted oxidant was determined by reacting with celestine blue and measuring the absorbance at 540 nm.

The above reported methods suffer from disadvantages like heating step, slow reaction, extraction step, multi step reactions, tedious control of experimental variables and less sensitivity. The present investigation involves the development of accurate, reproducible, and adequately sensitive three spectrophotometric methods based on the formation of ion- pair complexes between hydrochloride free propranolol (PPL) with sulphonphthalein dyes namely bromothymol blue (BTB), bromocresol green (BCG) and bromocresol purple (BCP). The proposed methods were applied to the determination of PPH in pharmaceutical formulation and in human urine. No interference was observed in the assay of PPH from common excipients found in pharmaceutical

formulation and other biological materials present in urine. These methods are validated by the statistical data. These methods provide economic procedures, less time consuming, and more sensitive compared with other reported spectrophotometric methods (Table 1). The proposed methods are simple and suitable for routine determination of PPH in quality control laboratories.

Table 1: Comparison of the proposed and the existing visible spectrophotometric methods.

Reagent/s	λ_{max}, nm	Beer's law range, µg ml^{-1} (ε in l mol^{-1} cm^{-1})	Remarks	Ref.
4-amino-3,5-dinitrobenzoic acid	470	1.0-8.0	Heating required	19
1-chloro-2,4-dinitrobenzene	314.6	-	-	20
Copper(II) or Cobalt(II)	548/614	$2x10^{-5}$-$1×10^{-2}$ M	-	21
Tetracyanoethylene, chloranilic acid	415 510	-	Use of large quantity of organic solvents	22
σ and π-acceptors	-	4-120	Use large quantity of organic solvents; less sensitive	23
Cerium(IV)	478	15-350	Involves boiling for 25 min; less sensitive	24
Cerium(IV)-MBTH	496	1-10	Uses an expensive chemical	25
Mercury(II) thiocyanate-iron(III)	460	10-50 ($2.63×10^3$)	-	26
Cerium (IV)-iron (II) sulphate-thiacyanate	480	0.0-5.0 ($3.6×10^4$)	Multi-step reaction	27
Chloramine-T-metol and sulphanilic acid	520	0.0-3.0 ($7.1×10^3$)	Less sensitive, multi step reaction, critical pH.	28
Sodium metavanadate-diphenylamine	560	0.0-4.0 ($5.33×10^4$)	Multi-step reaction	29
Bromate-bromide-methyl orange	510	0.5-3.5 ($6.66×10^4$)	Multi-step reaction	30
Bromate-bromide a)Indigo carmine b)Fe(III)+thiocyanate c)Fe(III)+1,10-phenanthroline	 610 480 510	 1.0-13.0 4.0-12.0 2.0-9.0	Multi-step reaction	31
a)NBS-PH b)NBS-MDH	515 513	0.5-12.5 ($1.36x10^4$) 0.3-16.0 ($2.55x10^4$)	Multi-step reaction	32
a)N-bromosuccinimide-amaranth		0.2-6.4	Multi-step reaction	33
NBS-Celestine blue	540	0.4-3.0	Multi-step reaction	34
a) Bromo thymol blue (BTB) b) Bromo cresol green (BCG) c) Bromo cresol purple (BCP)	420 425 420	0.4-7.0 ($ε = 3.55×10^4$) 0.4-7.0 ($ε = 3.12×10^4$) 0.48-8.4 ($ε = 2.94×10^4$)	Simple, rapid, sensitive, selective and no heating step. Use of single reagent and no extraction step involved.	Proposed methods

2. Methods

2.1 Instrument

All the absorbance measurements were made using a Systronics model 106 digital spectrophotometer provided with 1cm matched quartz cells.

2.2 Materials

Pharmaceutical grade propranolol hydrochloride (PPH) was received from Cipla India Ltd., Mumbai, India, as a gift and used as received. The following formulations were obtained from commercial sources and subjected to analysis: Monoprolol-20 (Cosmo Life Sciences Ltd., India), Ciplar – 40 (Cipla Ltd., India) and Betacap-40 from (Sun Pharma Ltd., India).

2.3 Reagents and chemicals

All the reagents and solvents used were of analytical-reagent grade. Bromothymol blue (BTB), bromocresol green (BCG) and bromocresol purple (BCP) (all from Loba Chemie Ltd., Mumbai, India) solutions were prepared daily as 0.1% BTB and BCP and 0.05% BCG in dichloromethane.

2.4 Standard stock solution of PPL

Pure propranolol hydrochloride (11.4 mg) was dissolved in 20 ml water in a 125 ml separating funnel, 5 ml of liquid ammonia was added followed by 20 ml of dichloromethane. The content was shaken for 15 minutes. The lower organic layer was collected in a beaker containing anhydrous sodium sulphate. The water-free organic layer was transferred into a 100 ml calibrated flask and diluted to the volume with the same solvent to get 100 µg ml^{-1} with respect to PPL (hydrochloride free propranolol). This solution was diluted appropriately with dichloromethane to get working concentrations of 10 µg ml^{-1} in both the methods A and B, and 12 µg ml^{-1} for use in method C, respectively.

2.5 Recommended Procedures

2.5.1 BTP method (method A)

Different aliquots (0.2, 0.5, 1.0, 2.0, 3.0 and 3.5 ml) of a standard PPL (10 µg ml^{-1}) solution were transferred into a series of 5 ml calibrated flasks using a micro burette and to each flask was added 1 ml of 0.1% BTB solution. The mixture was diluted to the volume with dichloromethane and mixed well. The absorbance of resulting yellow colored solution was measured at 420 nm against a reagent blank.

2.5.2 BCG method (method B)

Varying aliquots (0.2, 0.5, 1.0, 2.0, 3.0 and 3.5 ml) of a standard PPL (10 µg ml^{-1}) solution were transferred into a series of 5 ml calibrated flasks using a micro burette and to each flask was added 1 ml of 0.05% BCG solution. The mixture was diluted to the volume with dichloromethane and mixed well. The absorbance of resulting yellow colored solution was measured at 425 nm against a reagent blank.

2.5.3 BCP method (method C)

Into a series of 5 ml calibration flasks, aliquots (0.25, 0.5, 1.0, 2.0, 3.0 and 3.5 ml) of standard PPL solution (12 µg ml^{-1}) equivalent to 0.6 – 8.4 µg ml^{-1} PPL were accurately transferred and the total volume in each flask was brought to 3.5 ml by adding dichloromethane. To each flask 1 ml of 0.1 % BCP solution in dichloromethane was added and mixed well. The absorbance of the yellow colored ion-pair complex was measured at 420 nm against the reagent blank.

2.5.4 Procedure for dosage forms

Ten tablets or content of ten capsules were weighed accurately and ground into fine powder. An amount of the powder equivalent to 11.4 mg of PPH was weighed into a 125 ml separating funnel. The extraction procedure used for pure drug was followed for tablet/capsule powder. The resulting solution was diluted to get working concentration (10 and 12 µg ml^{-1} PPL) and suitable aliquots were analyzed following the procedures described above.

2.5.5 Procedures for selectivity study

A placebo blank of the composition: talc (100 mg), starch (50 mg), acacia (50 mg), methyl cellulose (100 mg), sodium citrate (50 mg), magnesium stearate (100 mg), and sodium alginate (50 mg) was prepared and 20 mg was extracted with dichloromethane and solutions were made as described under preparation of dosage forms. A convenient aliquot of solution was subjected to analysis following the recommended procedures.

To the 20 mg of the placebo blank, 11.4 mg of PPH was added and homogenized. The solution of the synthetic mixture equivalent to 100 µg ml^{-1} of PPL was prepared as described earlier. The resulting solution was assayed (n = 5) by all the three methods after appropriate dilution.

2.5.6 Procedure for spiked human urine

Five ml of PPH free urine taken in a 125 ml separating funnel was spiked with 20 ml of aqueous solution containing 11.4 mg of pure PPH and to the same solution, 5ml of liquid ammonia was added followed by 20 ml of ethyl acetate. The content was shaken for 15 min. The lower aqueous layer was discarded and the upper organic layer was collected in a beaker containing anhydrous sodium sulphate. The water-free organic layer was transferred into a dried beaker and evaporated on a hot water bath. The dry residue was dissolved in dichloromethane in a 100 ml calibrated flask, and diluted to the mark with solvent. This solution was diluted appropriately with dichloromethane to get working concentrations. An aliquot of resulting solution was analyzed following the procedures described above.

2.5.7 Procedure for stoichiometric relationship

Job's method of continuous variations of equimolar solutions was employed: 5.78×10^{-5} M each of PPL and BTB in dichloromethane (Method A) solutions, 5.78×10^{-5}M each of PPL and BCG in dichloromethane (Method B) solutions and 1.93×10^{-4}M each of PPL and BCP in dichloromethane (Method C) solutions were prepared separately. A series of solutions was prepared in which the total volume of PPL and dye was kept at 5 ml. The drug and reagent were mixed in various complementary proportions (0:5, 1:4, 2:3, …..5:0, inclusive) and completed as directed under the recommended procedures. The absorbance of the resultant ion-pair complex was measured at 420 nm in method A and C, and 425 nm in method B.

3. Results and Discussion

The mechanism for the extraction-free ion pair complex formation between nitrogenous compound and sulphonphthalein acid dyes were recently reported [35-37]. Similar reaction mechanism (Scheme 1) for ion pair complex formation between PPL and BTB, BCG or BCP is proposed. The PPH contains a secondary aliphatic amino group which forms ion-pair complex with sulphonphthalein acid dyes, BTB, BCG and BCP. Since ion-pair complex forms in non-polar solvents, the insolubility of PPH in any of the non-polar solvents was overcome by using PPH in its base form, PPL.

The dyes employed have insignificant absorbance (Figure 2). The formation of intense yellow colored product with an absorption maximum at 425 or 420 nm is due to an opening of lactoid ring and subsequent formation of quinoid group [38]. It is supposed that the two tautomers are present in equilibrium but due to strong acidic nature of the sulphonic acid group, the quinoid moiety must predominate.

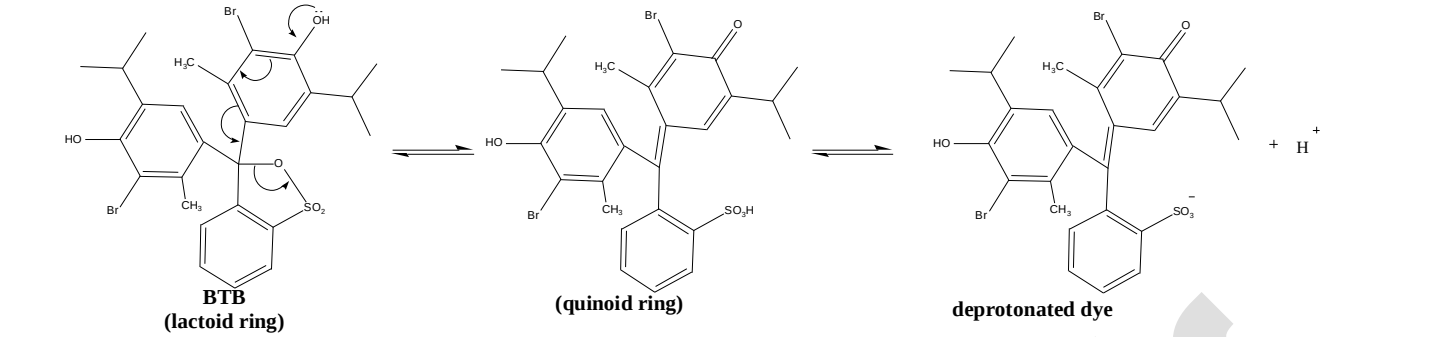
3.1 Optimization of experimental variables

3.1.1 Absorption spectra

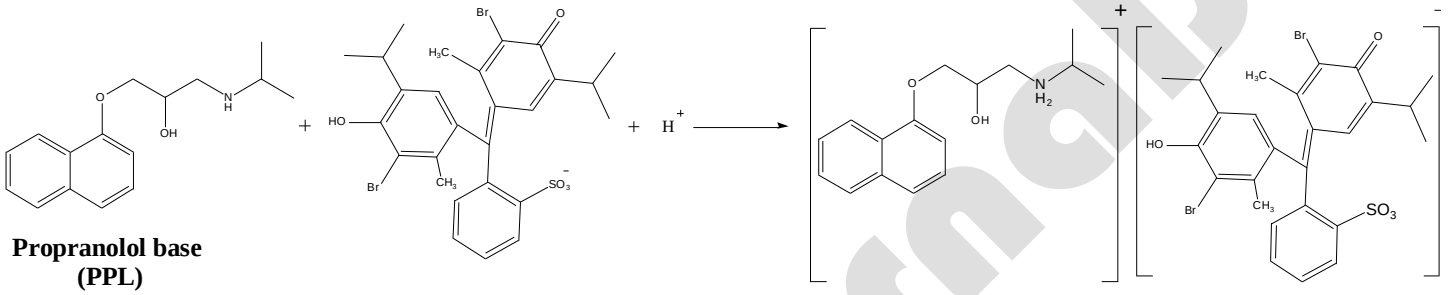
The absorption spectra of the ion-pair complexes, formed between PPL and each of BTB BCG and BCP, were recorded at 370-540 nm against respective reagent blank and the same are shown in Figure 2. The yellow ion-pair complexes showed maximum absorbance at 420 nm for PPL-BTB and PPL-BCG, and 425 nm for PPL-BCP (Figure 2). The measurements were thus made at these wavelengths.

3.1.2 Effect of solvents

The organic solvent exhibiting minimum blank absorbance in the presence of sulphonphthalein dyes alone is the ideal solvent for extraction-free ion pair technique. In order to select a suitable solvent for preparation of the reagent solutions used in the study, the reagents were prepared separately in different solvents such as 1,4-dioxane, chloroform, acetonitrile, acetone and dichloromethane, and the reaction of PPL with BTB, BCG or BCP was followed. Among the organic solvents studied (Figure 3), the order of blank absorbance for all the dyes was: dichloromethane <chloroform <acetonitrile <1,4-dioxan <acetone. Therefore, dichloromethane was chosen for further investigation. Similarly, the effect of the diluting solvent was studied for all the methods and the results showed that none of the solvents except dichloromethane formed sensitive and stable colored species. Therefore, dichloromethane was used for dilution throughout the investigation.



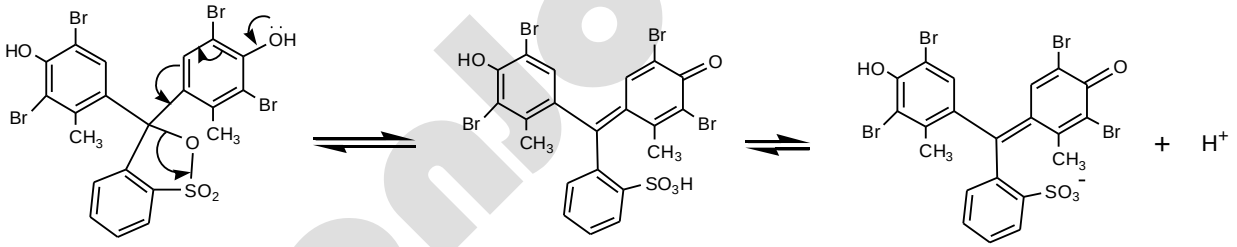
BTB (lactoid ring) **(quinoid ring)** **deprotonated dye**



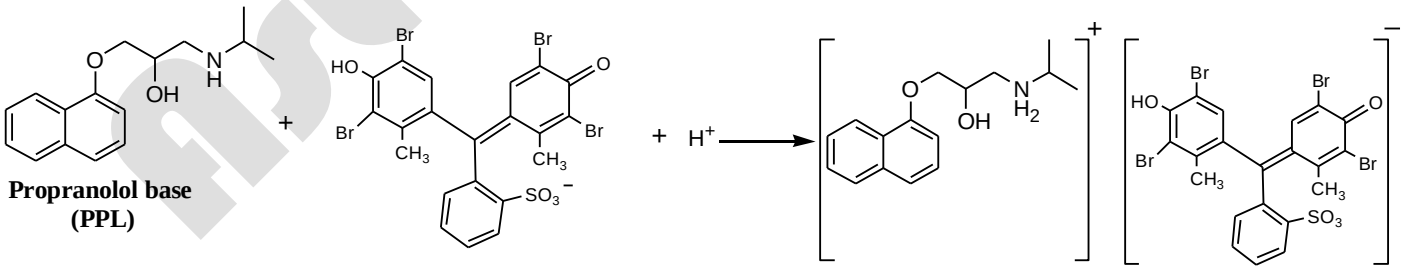
Propranolol base (PPL)

1:1 ion-pair complexes of PPL-BTB (measured at 420 nm)

(a)



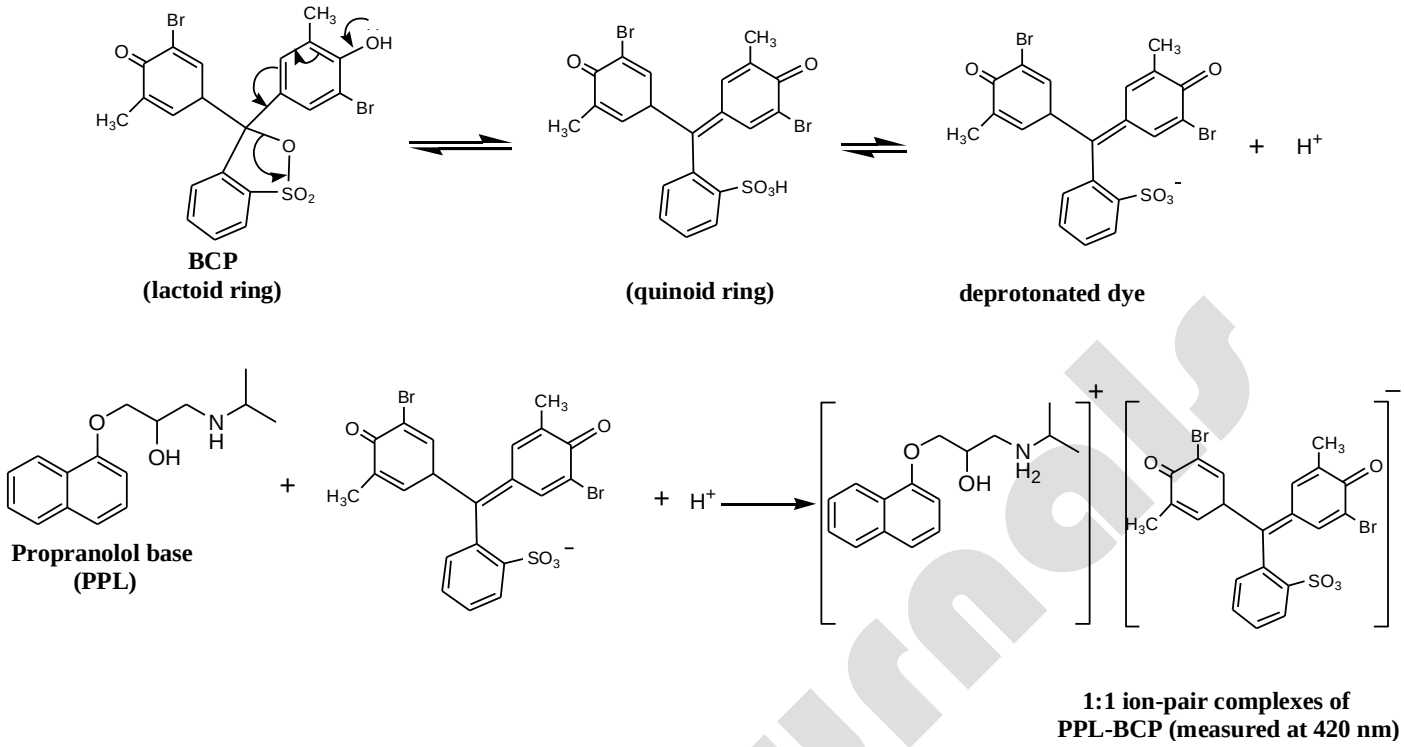
BCG (lactoid ring) **(quinoid ring)** **deprotonated dye**



Propranolol base (PPL)

1:1 ion-pair complexes of PPL-BCG (measured at 425 nm)

(b)



Scheme 1: (a) Tentative reaction mechanism for PPL-BTB ion-pair complex formation,
(b) Tentative reaction mechanism for PPL-BCG ion-pair complex formation,
(c) Tentative reaction mechanism for PPL-BCP ion-pair complex formation.

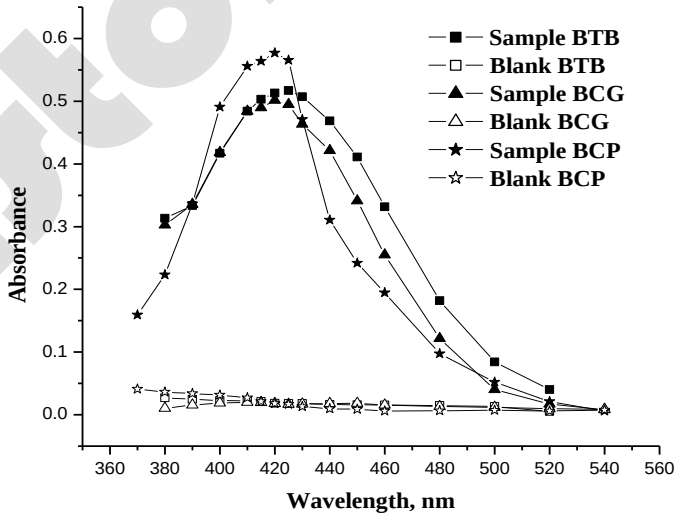


Figure 2: Absorption spectra (4 µg ml^{-1} of PPL in both methods A and B, and 4.8 µg ml^{-1} of PPL in method C)

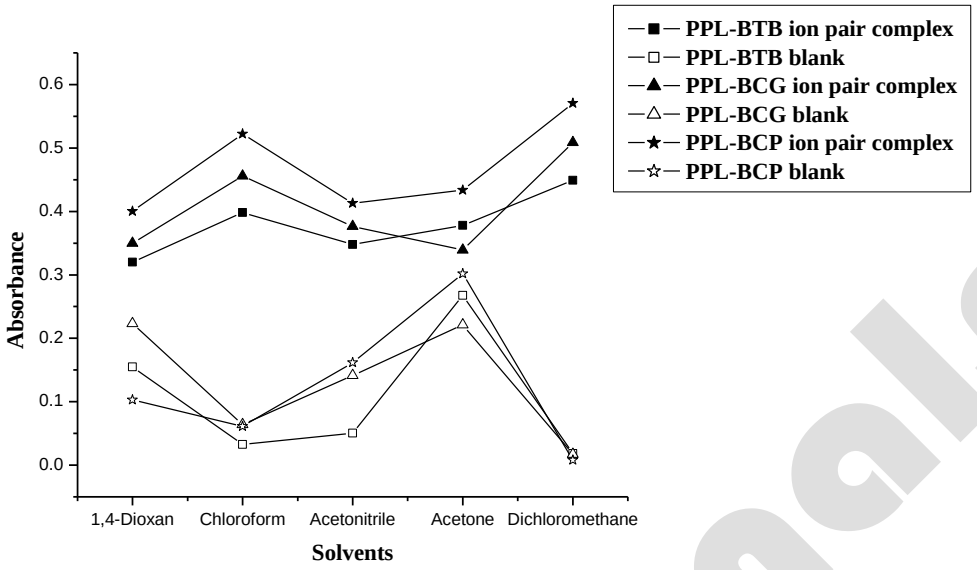


Figure 3: Effect of solvents on the formation of PPL-BTB complex (3.2 µg ml^{-1} PPL), PPL- BCG complex (4.0 µg ml^{-1} PPL) and PPL-BCP complex (4.8 µg ml^{-1} PPL).

3.1.3 Effect of dye concentration and reaction time

The effect of the dye-concentration on the intensity of the color developed at selected wavelengths was studied by measuring the absorbance of solutions containing a fixed concentration of PPL (2.4, 3.5 and 5.0 µg ml^{-1} in methods A, B and C, respectively) and different amounts (0.5 - 3.0 ml) of the respective dye of 0.1% BTB solution, 0.05% BCG solution and 0.1 % BCP solution. Maximum color intensity of the complex was achieved with 1 ml of dye solutions in all the methods and an excess dye slightly decreases the absorbance of the complex (Figure 4). The addition of the dye solution resulted in an immediate color development at room temperature for method A and B, and takes 5 min to develop color in method C. The formed ion-pairs were stable for at least 24 h in methods A and C and 90 min in method B.

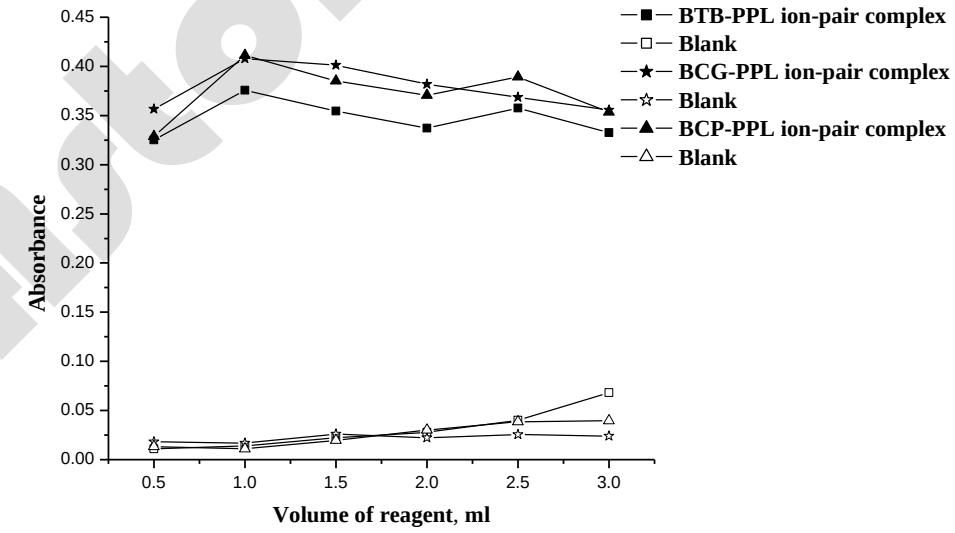


Figure 4: Effect of reagent concentration on the formation PPL-BTB complex (2.4 µg ml^{-1} PPL), PPL-BCG complex (3.5 µg ml^{-1} PPL) and PPL-BCP complex (5.0 µg ml^{-1} PPL).

3.1.4 Study of composition of ion-pair complex and its conditional stability constant

The composition and conditional stability constant of the PPL-BTB or PPL-BCG or PPL-BCP complex formed were evaluated by applying Job's method of continuous variations [39] using equimolar concentrations of PPL (prepared by following the general procedure) and the dye. The concentration of dye used in method A and B was 5.78×10^{-5} M, whereas concentration of PPL and dye is 1.93×10^{-4} M in method C. The experiments were performed by mixing equimolar solutions of drug and reagent by maintaining the total volume at 5.0 ml. The plots of the mole ratio between drug and reagent *versus* the absorbance values were prepared (Figure 5), and the results revealed that the formation of ion -pair complex between drug and reagent followed a 1:1 reaction stoichiometry. The conditional stability constant (K_f) of the ion-association complex was calculated from the continuous variation data using the following equation [40]:

$$K_f = \frac{A/A_m}{\left[1 - A/A_m\right]^{n+2} C_M (n)^n}$$

where A and A_m are the observed maximum absorbance and the absorbance value when all the drug present is associated, respectively. C_M is the mole concentration of drug at the maximum absorbance and n is the stoichiometry which BTB/ BCG/BCP ion associates with PPL. The log K_f values were found to be 7.21, 6.96 and 7.32 for BTB method, BCG method, and BCP method, respectively.

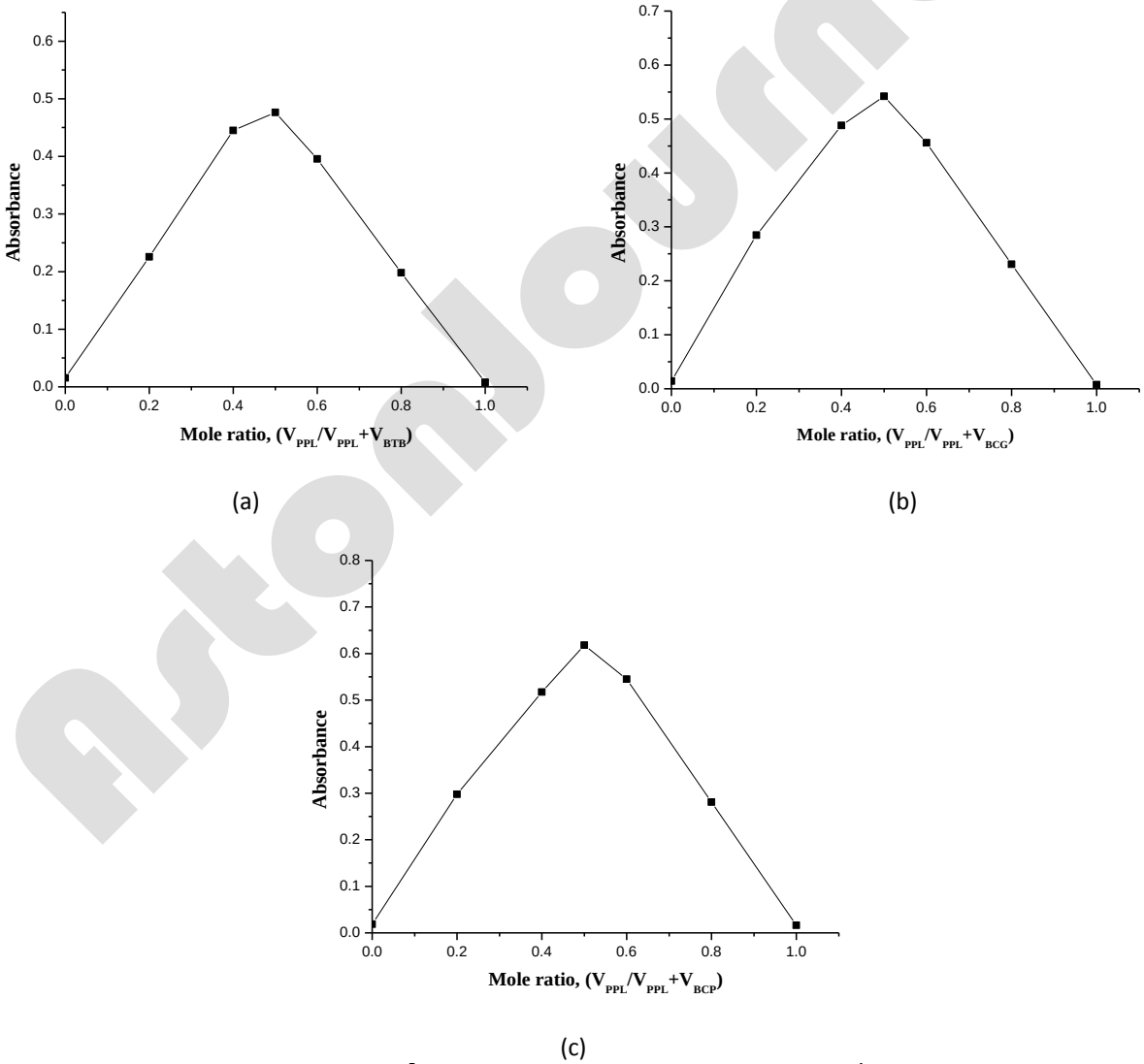


Figure 5: Job's plots obtained for (a) 5.78×10^{-5} M PPL and BTB ion-pair complex (b) 1.93×10^{-4} M PPL and BCG ion-pair complex and (c) 5.78×10^{-5}M PPL and BCP ion-pair complex.

3.2 Method validation

3.2.1 Analytical parameters

A linear relation was found to exist between absorbance and the concentration of PPL in the ranges given in Table 2. The calibration graph in each case is described by the equation:

$$Y = a + b X$$

where Y = absorbance, a = intercept, b = slope and X = concentration in µg ml^{-1}, obtained by the method of least squares. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 2. Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, the limits of detection and quantification calculated as per the current ICH guidelines [41] are compiled in Table 2 and are indicative of the sensitivity of the methods. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulae:

$$LOD=3.3\sigma/s \text{ and } LOQ=10\sigma/s$$

where σ is the standard deviation of five reagent blank determinations and s is the slope of the calibration curve.

Table 2: Regression and analytical parameters.

Parameter	BTB Method	BCG Method	BCP Method
λ_{max}, nm	420	425	420
Beer's law limits (µg ml^{-1})	0.4-7.0	0.4-7.0	0.6-8.4
Molar absorptivity (l mol^{-1} cm^{-1})	3.55×10^4	3.12×10^4	2.94×10^4
Sandell sensitivity* (µg cm^{-2})	0.0073	0.0083	0.0088
Limit of detection (µg ml^{-1})	0.03	0.04	0.06
Limit of quantification (µg ml^{-1})	0.10	0.12	0.18
Regression equation, Y**; Intercept (a)	-0.0160	-0.0089	0.0017
Slope (B)	0.1420	0.1276	0.1123
Correlation coefficient (r)	0.9982	0.9999	0.9991
Standard deviation of intercept (S_a)	0.01819	0.00346	0.01239
Standard deviation of slope (S_b)	0.00432	0.00082	0.00246

*Limit of determination as the weight in µg per ml of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm. **$Y = a + bX$, where Y is the absorbance, a is the intercept, b is the slope and X is the concentration in µg ml^{-1}.

3.2.2 Precision and accuracy

The precision of the methods was calculated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of PPL were analyzed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The RSD (%) values of intra-day and inter-day studies showed that the precision was good (Table 3). The accuracy of an analytical method expresses the closeness between the reference value and the found value. Accuracy was evaluated as percentage relative error between the measured concentrations and taken concentrations for PPL (Bias %). The results obtained are compiled in Table and show that the accuracy was good.

3.2.3 Robustness and ruggedness

The robustness of the all the methods was evaluated by making small incremental changes in the volume of dye (1 ± 0.2 ml) and contact time (5 ± 1 min) in method C, and the effect of the changes were studied on the absorbance of the ion-pair complex. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as % RSD (≤ 1.43 %). Method ruggedness was demonstrated having the analysis done by four analysts, and also by a single analyst performing analysis using four different cuvettes. Intermediate precision values (%RSD) in both instances were in the range 0.99 - 1.74 indicating acceptable ruggedness. These results are presented in Table 4.

Table 3: Evaluation of intra-day and inter-day precision and accuracy.

Method	PPL taken (μg ml^{-1})	Intra-day (n = 7)			Inter-day (n = 5)		
		PPL found[a] (μg ml^{-1})	%RSD[b]	%RE[c]	PPL found[a] (μg ml^{-1})	%RSD[b]	%RE[c]
BTB Method	2.00	1.98	0.77	1.10	2.02	1.41	1.00
	4.00	4.07	1.22	1.64	4.07	0.91	1.64
	6.00	6.11	1.08	1.83	6.12	0.78	2.03
BCG Method	2.00	2.02	1.66	1.26	2.03	1.54	1.32
	4.00	3.98	1.67	0.38	4.02	0.73	0.53
	6.00	6.02	1.86	0.38	6.04	1.57	0.70
BCP Method	2.40	2.41	0.54	0.22	2.43	0.86	1.31
	4.80	4.80	0.89	0.02	4.82	0.67	0.60
	7.20	7.18	0.81	0.28	7.23	1.74	0.37

[a.] Mean value of five determinations; [b.] Relative standard deviation (%); [c.] Relative error (%).

Table 4: Robustness and ruggedness.

Method	PPL taken, μg ml^{-1}	Method robustness		Method ruggedness	
		Parameters altered			
		Reagent volume, ml[a] RSD, % (n = 3)	Reaction time[b] RSD, % (n = 3)	Inter-analysts RSD, % (n = 4)	Inter-cuvettes RSD, % (n = 4)
BTB Method	2.00	1.06		1.74	1.38
	4.00	1.24		1.39	1.32
	6.00	0.93		1.18	1.09
BCG Method	2.00	1.31		1.56	1.41
	4.00	0.78		1.43	1.30
	6.00	1.09		1.55	1.39
BCP Method	2.40	0.91	1.01	1.16	0.99
	4.80	1.42	1.29	1.47	1.35
	7.20	1.37	1.43	1.29	1.12

[a]In all methods, the volume of reagent was 0.8, 1.0 and 1.2 mL. [b]The reaction time was 4, 5 and 6 min for method C.

3.2.4 Effect of co-formulated substances

The effect of co-formulated substances was tested by placebo blank and synthetic mixture analyses. A convenient aliquot of the placebo blank solution was subjected to analysis according to the recommended procedures. There was no interference by the inactive ingredients as indicated by the near blank absorbance in all the methods.

The analyses of synthetic mixture solution yielded percent recoveries which ranged between 98.99 – 102.1 and with standard deviation of 0.78 – 1.56. The results of this study indicated that the inactive ingredients did not interfere in the assay.

3.2.5 Application to analysis of spiked urine sample and pharmaceutical formulations

The proposed methods were successfully applied to the determination of PPL in spiked urine sample with mean percent recovery of 106.4 ± 1.85 (n=5), 108.1±2.12 (n=5) and 105.9 ± 0.93 (n=5), for BTB, BCG and BCP methods, respectively and the results of two representative tablet and capsule were statistically compared with those of the official method [7] by applying the Students t-test for accuracy and F-test for precision (Table 5). The reference method describes a potentiometric titration of ethanolic solution of PPH with sodium hydroxide. As can be seen from the Table 5, the calculated t-value and F-value at 95% confidence level did not exceed the tabulated values of

2.78 and 6.39, respectively, for four degrees of freedom. The results indicated that there is no difference between the proposed methods and the official method with respect to accuracy and precision.

Table 5: Results of analysis of tablets by the proposed methods.

Tablet brand name	Label claim mg/tablet	Found (Percent of label claim ±SD)[a]			
		Reference method	Proposed methods		
			BTB Method	BCG Method	BCP Method
Monoprolol-20	20	101.56 ± 0.64	101.89 ± 1.07 **t** = 0.59 **F**= 2.80	102.02± 0.93 **t** = 0.91 **F** = 2.11	100.77± 1.12 **t** = 1.37 **F** = 3.06
Ciplar-40	40	100.56 ± 0.75	101.35 ± 1.10 **t** = 1.33 **F**= 2.15	101.92 ± 0.98 **t** = 2.46 **F**= 1.71	100.96 ± 1.21 **t** = 0.63 **F**= 2.60
Betacap-40	40	100.19 ± 1.06	99.46 ± 1.42 **t** = 0.92 **F**= 1.79	100.28 ± 0.99 **t** = 0.14 **F**= 0.87	99.51 ±0.83 **t** = 1.13 **F**= 0.61

[a]Mean value of five determinations.
Tabulated t-value at the 95% confidence level is 2.78.
Tabulated F-value at the 95% confidence level is 6.39.

3.2.6 Recovery studies

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure PPH at three levels (50, 100 and 150% of that found in tablet powder) and the total was determined by the proposed methods. The percent recovery of pure PPH added was in the range 98.67– 102.3 % with standard deviation of 0.48 – 1.67 (Table 6) indicating that the recovery was good, and that the co formulated substance did not interfere in the determination.

Table 6: Results of recovery study by standard addition method.

Tablets studied	BTB Method				BCG Method				BCP Method			
	PPL in tablets, µg ml^{-1}	Pure PPL added, µg ml^{-1}	Total found, µg ml^{-1}	Pure PPL recovered*, Percent± SD	PPL in tablets, µg ml^{-1}	Pure PPL added, µg ml^{-1}	Total found, µg ml^{-1}	Pure PPL recovered*, Percent± SD	PPL in tablets µg ml^{-1}	Pure PPL added, µg ml^{-1}	Total found, µg ml^{-1}	Pure PPL recovered*, Percent± SD
Monoprolol-20	2.04 2.04 2.04	1.0 2.0 3.0	3.03 4.07 5.11	99.0 ±1.64 101.5±0.55 102.3±0.51	2.04 2.04 2.04	1.0 2.0 3.0	3.06 4.08 5.02	102.0±1.24 102.0±0.83 99.33±0.48	2.42 2.42 2.42	1.2 2.4 3.6	3.61 4.83 6.06	99.17±1.25 100.4±1.41 101.1±0.51
Ciplar-40	2.03 2.03 2.03	1.0 2.0 3.0	3.04 4.02 5.08	101.0±0.79 99.5±0.83 101.7±0.51	2.04 2.04 2.04	1.0 2.0 3.0	3.05 4.08 5.11	101.0±0.81 102.0±1.67 102.3±0.49	2.42 2.42 2.42	1.2 2.4 3.6	3.64 4.87 6.10	101.7±0.48 102.1±0.86 102.2±1.04
Betacap-40 (Capsules)	1.99 1.99 1.99	1.0 2.0 3.0	2.98 4.03 4.98	99.0±0.78 102.0±0.55 99.67±1.27	2.00 2.00 2.00	1.0 2.0 3.0	3.02 4.03 4.96	102.0±0.81 101.5±1.38 98.67±0.73	2.39 2.39 2.39	1.2 2.4 3.6	3.58 4.76 6.01	99.17±0.82 98.75±0.64 100.6±0.18

*Mean value of three determinations.

4. Conclusion

Unlike the chromatographic methods, the spectrophotometric methods are simple and are not of high cost. The importance lies in the chemical reactions upon which the procedures are based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility in the assay of a particular component in complex dosage formulations. The reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The described procedures are simple, rapid, accurate and precise in determining propranolol hydrochloride in their pharmaceutical preparations and in urine without interference from common excipients or biological matrix. The proposed methods involve single step reaction and do not involve heating or extraction step unlike many of the previously reported methods. Moreover, they do not require various elaboration treatment and tedious extraction procedures required in other traditional extractive spectrophotometric methods. These, in addition to satisfactory sensitivity and reproducibility compared to the official non-aqueous titrimetric and many other methods as well as the convenience and simplicity, make the methods applicable for routine analysis of the drug in pure form, tablets/capsules and urine.

Competing Interests

Authors have no competing interests.

Authors' Contributions

All authors contributed equally to this work.

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