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International Journal of ChemTech Research
CODEN( USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.3, pp 1246-1254, July-Sept 2011

# Extractive spectrophotometric methods for determination of duloxetine hydrochloride in pharmaceutical formulations using acidic triphenyl methane dyes

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**Abstract:** Three simple and sensitive extractive spectrophotometric methods have been described for the assay of duloxetine hydrochloride either in pure form or in pharmaceutical formulations. The developed methods involve formation of colored chloroform extractable ion-pair complexes of the drug with bromothymol blue (BTB), bromophenol blue (BPB) and bromocresol green (BCG) in acidic medium. The extracted complexes showed absorbance maxima at 415 nm for all three methods. Beer's law is obeyed in the concentration ranges 2.5-25, 2.5-25 and 3.0-25. µg/ml with BTB, BPB 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 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.
**Keywords**: Duloxetine hydrochloride; Bromothymol blue; Bromophenol blue; Bromocresol green; Spectrophotometry, International Conference on Hormonization (ICH).

## Introduction

Duloxetine hydrochloride, chemically known as ( + ) – ( s ) – N – methyl – γ – (1- napthyloxy ) – 2 - thiophene propylamine hydrochloride, is an antidepressant agent [1] . It is indicated for the treatment of major depressive disorder. It is a selective serotonin and nor-epinephrine reuptake inhibitor for oral administration. Thorough survey of literature showed a few analytical methods has been reported for determination of duloxetine hydrochloride in human serum and biological fluids using tadem MS and LC-MS [2] methods and HPLC [3-5] methods and spectroflourimetric method [6,7] for the estimation of duloxetine hydrochloride in pharmaceutical preparations have been reported. Recently direct spectrophotometric determination of duloxetine hydrochloride has also been reported [8-9]. Literature survey on Spectrophotometric determination of drugs also revealed that certain acidic dyes *viz* BTB, BCG and BPB act as complexing agents and form ion pair complexes with cation salts [10] and form a basis for quantitative determination of drugs. The methods based on ion pair complexes extractable into a suitable organic solvent have been shown to be simple, sensitive, accurate and economical.

In this paper we report three simple and sensitive extractive spectrophotometric methods for the assay of duloxetine hydrochloride. The methods are based on ion-pair complexation of drug with dyestuffs such as

bromothymol blue (BTB), bromophenol blue (BPB) and bromocresol green (BCG) and subsequent extraction into chloroform and measure the absorbance of color complex.

## Experimental section

### Materials

Duloxetine hydrochloride is procured from Aurobindo Labs Limited, Hyderabad as a gift sample. The dyestuffs *viz.,* BTB, BPB and BCG (AR grade) supplied by SD Fine Chemicals Ltd. Mumbai, are used without any further purification. The dyestuffs were used as 0.025% solutions in doubly distilled water. Sodium acetate-hydrochloric acid buffers [11] of pH 2.5, 2.8 and 3.5 were prepared by mixing 50ml of 1.0M sodium acetate solution with 50.50, 49.50 or 46.25 ml, respectively, of 1.0 M HCl solution and diluted to 250 ml with doubly distilled water. The pH of each solution was adjusted to an appropriate value with the aid of a pH meter. Chloroform (HPLC grade) supplied by SD Fine Chemicals Ltd. Mumbai is used throughout the work. Stock solutions were prepared for all the dyes and drugs (25mg/100ml).

The spectra (Figure -1) of ion-pair complexes have been recorded on SHIMADZU 140 double beam spectrophotometer, Thermo Nicolet 1000 and also on ELICO 159 UV-Visible single beam spectrophotometer using quartz cells of 10 mm path length. An Elico model Li-120 pH meter was used for pH measurement.

### Methods

Different aliquots of drug solution were transferred into 125 ml separating funnel. To this 5 ml of buffer (pH 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 at least for 3 hrs is measured at 415 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 (**Figure – 2)** are linear over the concentration ranges are within the permissible range. The optical characteristics and statistical data for the regression equation of the proposed methods are presented in (**Table -1).**

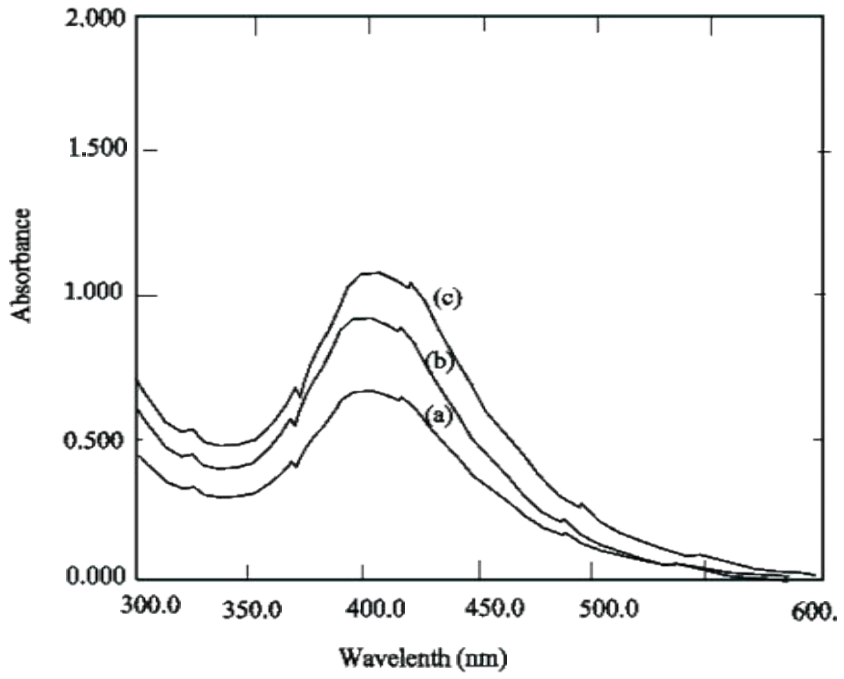



Fig. 1— Absorption spectra of duloxetine hydrochloride - dye complex extracted into 10 ml chloroform: (a) drug = 10 µg ml $^{-1}$ + 5ml of 0.025 % BTB + 5ml of pH 2.8 buffer. (b) drug = 15.0 µg ml $^{-1}$ + 5ml of 0.025 % BPB + 5ml of pH 2.5 buffer (c) drug = 20 µg ml$^{-1}$ + 5ml 0.025 % BCG + 5ml of pH 3.5 buffer.

**Table1.-Optical Characteristics and Statistical for the Regression Equation of the proposed methods**

| Parameters | Extraction Methods with | | |
|---|---|---|---|
| | BTB | BPB | BCG |
| Λmax(nm) | 415 | 415 | 415 |
| Beers law limit (µgml-1) | 2.5-25.0 | 2.5- 25.0 | 2.5-25.0 |
| Molar absorbvity (Lmol-1 cm-1) | 18514.7 | 17268.09 | 17078.36 |
| Formation constant , K, $M^{-1}$ | $2.4 \times 10^4$ | $1.709 \times 10^4$ | $1.281 \times 10^4$ |
| Sandells sensitivity(µg $cm^{-2}$) | 0.0160 | 0.0172 | 0.0174 |
| Slope (specific absorbtivity ), b | 0.0523 | 0.05807 | 0.0574 |
| Intercept (a) | 0.068 | -0.0887 | -0.0504 |
| Correlation coefficient ( r) | 0.9999 | 0.9990 | 0.9976 |
| Standard deviation (% n=6) | 0.0055 | 0.0108 | 0.0148 |
| Limit of detection , µg $ml^{-1}$ | 0.3470 | 0.614 | 0.969 |
| Limit of quantification, µg $ml^{-1}$ | 1.041 | 1.842 | 2.90 |
| Regression Equation<br>Y = Absorbance Y= bC + a<br>C = Concentration b = slope<br>a = intercept | Y = 0.0523C + 0.068 | Y= 0.058C - 0.088 | Y = 0.0574C - 0.0504 |

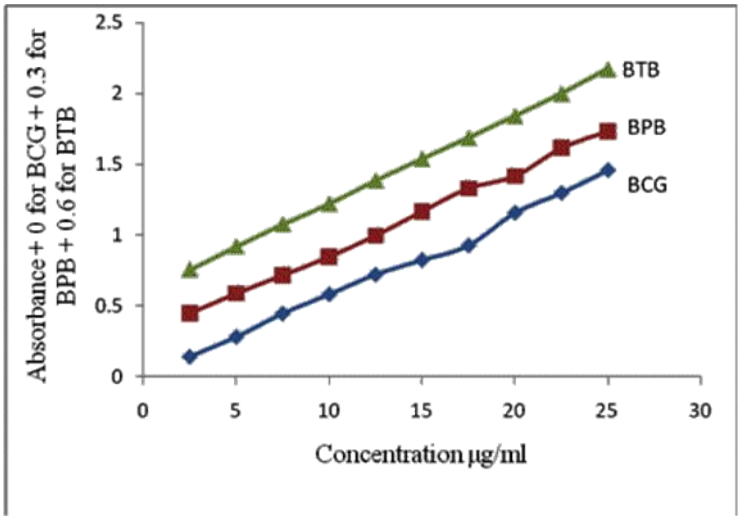



Fig .2 - Calibration graphs of Drug - Dyes ion pair complexes

**Table 2. Application of proposed methods for the analysis of duloxetine hydrochloride in pure form**

| Taken ($\mu gml^{-1}$) | Proposed methods | | | | | | Referance method |
|---|---|---|---|---|---|---|---|
| | Found ($\mu gml^{-1}$) | | | Recovery (%) | | | Recovery (%) |
| | BTB | BPB | BCG | BTB | BPB | BCG | |
| 4 | 4.00 | 4.01 | 3.98 | 00 | 100.25 | 99.5 | 100.9 |
| 8 | 8.01 | 7.98 | 7.96 | 100.12 | 99.75 | 99.5 | 100.3 |
| 12 | 12.00 | 12.02 | 12.10 | 100 | 100.16 | 100.8 | 98.3 |
| 16 | 15.96 | 16.11 | 16.20 | 99.75 | 100.6 | 101.25 | 100.29 |
| RSD (%) | | | | 0.1550 | 0.348 | 0.896 | 1.010 |
| Mean ± SD | | | | 99.96 ± 0.155 | 100.19 ± 0.349 | 100.26 ± 0.899 | |
| t- test | | | | 0.745 | 0.704 | 0.974 | |
| f – test | | | | 0.7118 | 0.084 | 0.0084 | |

**Procedure for the assay of pure drug:**
Four 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 (Table -2) for six replicates.

**Procedure for the assay of dosage forms:**
Five tablets of symbal 20 mg 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 in six replicates.

## Results and Discussion

Duloxetine hydrochloride forms ion-pair complexes in acidic buffer with dyestuffs viz; bromothymol blue (BTB), bromophenol blue (BPB) and bromocresol green (BCG) and these complexes are quantitatively extracted into chloroform. Ion-pair complexes of drug with BTB, BPB and BCG absorbed maximally at 415nm. The reagent blank under similar conditions showed no absorption.

In order to establish molar ratio between duloxetine hydrochloride and dyestuffs used, the Job's method of continuous variation [12] has been applied. In this method, solutions of drug and dyestuff with identical molar concentrations [$8 \times 10^{-5} M$] 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] (Figure -3). This measurement showed that 1:1 complex was formed with each dyestuff. The formation constants[13,14] were also estimated and found to be $2.4 \times 10^4$, $1.709 \times 10^4$ and $1.281 \times 10^4$ $M^{-1}$ for complexes with BTB, BPB and BCG respectively.

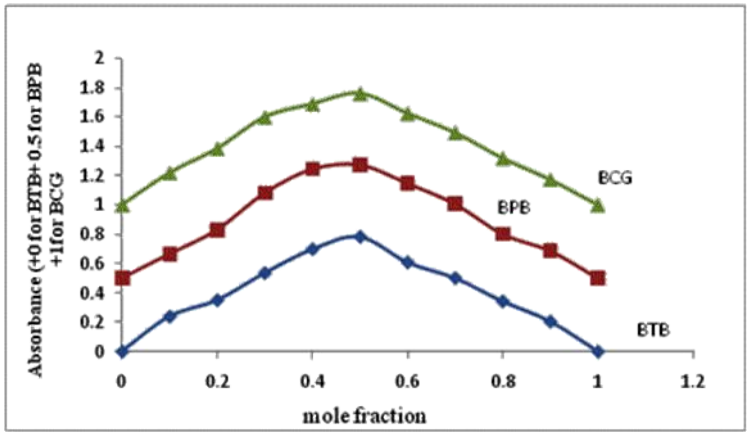
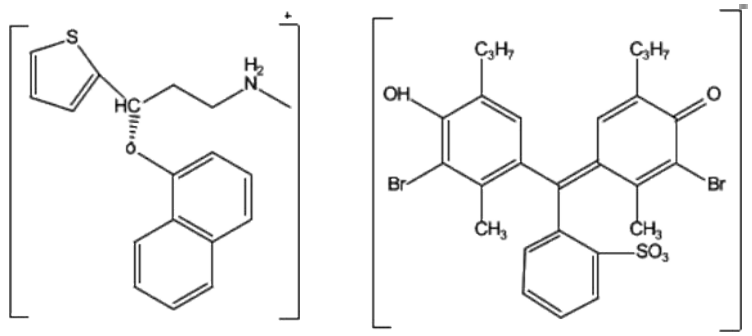
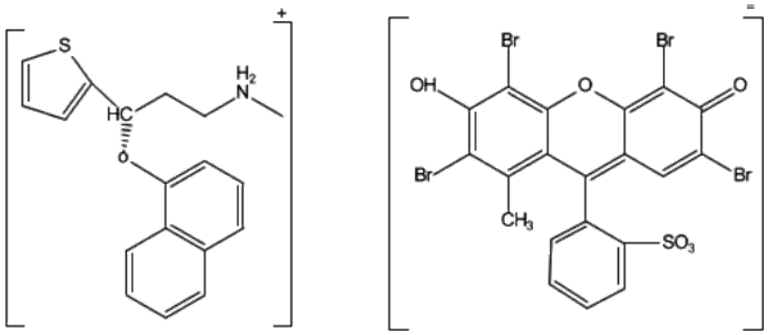


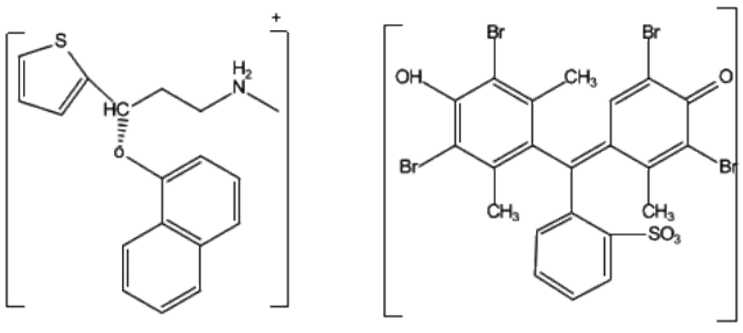

Fig . 3 - Continuous - variation study of drug - dye system.
[Drug ] = [Dye] = 8 x 10-5




Duloxetine - Bromothymol blue complex




Duloxetine - Bromophenol blue complex




Duloxetine - Bromocresol green complex

**(Scheme – 1)**

**Table 3.-Application of proposed methods for the analysis of duloxetine hydrochloride in pharmaceutical form**

| Taken (µgml-1) | Proposed methods | | | | | | Reference method |
|---|---|---|---|---|---|---|---|
| | Found (µgml-1) | | | Recovery (%) | | | |
| | BTB | BPB | BCG | BTB | BPB | BCG | Recovery (%) |
| Symbal 20mg/tablet | | | | | | | |
| 5 | 5.02 | 5.06 | 4.9 | 100.4 | 100.2 | 98 | 100.97 |
| 10 | 10.07 | 10.06 | 10.01 | 100.7 | 100.6 | 100.1 | 100.3 |
| 15 | 14.9 | 14.4 | 15 | 99.3 | 98.6 | 100 | 98.3 |
| 20 | 19.8 | 20.1 | 20.04 | 99.0 | 100.2 | 100.2 | 100.29 |
| RSD (%) | | | | 0.8276 | 1.1237 | 1.0582 | 1.0108 |
| Mean ± SD | | | | 99.8 ± 0.826 | 100.2 ± 1.126 | 99.5 ± 1.053 | 100.04± 1.0113 |
| t-test | | | | 0.8354 | 0.9718 | 0.7129 | |
| f-test | | | | 0.596 | 0.7336 | 0.8832 | |

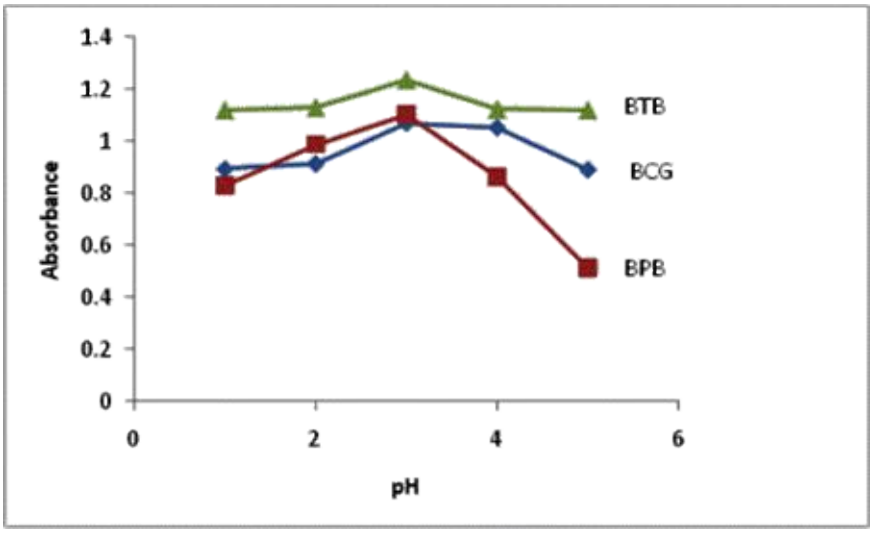



Fig 4 - Effect of pH

[Drug] = 20.0µg/ml, [Dye] = 5ml of 0.025%

Duloxetine hydrochloride contains secondary amino group. Hence we propose the protonation of secondary nitrogen in acidic medium, while sulphonic acid group is present in BTB, BPB and BCG, that is the only group undergoing dissociation in the pH range 1-5. The color 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 duloxetine hydrochloride forms ion-pairs with the dyestuffs which are quantitatively extracted into chloroform. The possible reaction mechanisms are proposed and given in (**Scheme 1).**

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

The effect of dyestuff concentrations was also studied by adding different volumes of dyestuff to a constant amount of duloxetine hydrochloride (20 µg $ml^{-1}$). It is apparent from (**Figure – 5**) that the maximum absorbance, in each case, was found with 2.0 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 duloxetine hydrochloride on the determination of duloxetine hydrochloride at 12.5 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 duloxetine hydrochloride solution of 12.5 µg $ml^{-1}$. (**Table- 4**) summarizes the results obtained. However, the drug content from the powdered tablets was extracted into chloroform, which completely removes any interference by the common excipients found in formulations.

**Table 4. Interferance study**

| Excipients | Tolerance limit ( µg $ml^{-1}$ ) |
|---|---|
| Gelatin | 50 |
| Hydroxy propyl methyl Cellulose acephate | 95 |
| Sodium lauryl sulphate | 95 |
| Sucrose | 140 |
| Sugar spheres | 45 |
| Talc | 70 |
| Titanium dioxide | 20 |
| Triethyl citrate | 40 |

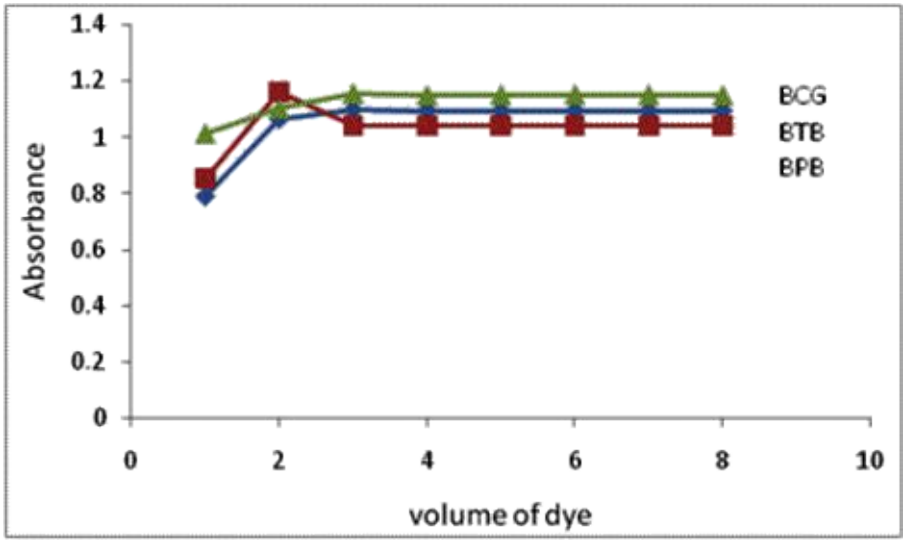



Fig 5 - Influence of volume of 0.025%Dye [ Drug] = 20.0µg /ml

## Validation of the proposed method:

All the three proposed methods have been validated in terms of guideline proposed by ICH[15] 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 12.5µg $ml^{-1}$ of duloxetine hydrochloride were made. The coefficient of variation was found to be less than 1.0% for all the procedures.

The proposed methods have been successfully applied to the determination of duloxetine hydrochloride in pharmaceutical preparations. The performance order of the proposed methods is BTB>BPB>BCG. The result obtained and shown in (**Table 3)** was compared to those obtained by a reference method [7] 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 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 duloxetine hydrochloride in pure form and in formulation.

## Conclusions

Duloxetine hydrochloride forms extractable ion pair complexes with triphenyl methane acidic dyes. Complexes are of 1:1 composition and have stability of $10^{4.}$ Calibration curves for quantification of the drug have been constructed and method is validated.

## Acknowledgements

The authors are grateful to Head, Department of Chemistry, Principal prof Naidu Ashok Nizam College and St Ann's College for providing facilities.

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