Int. J. Chem. Sci.: 9(3), 2011, 1353-1363
ISSN 0972-768X
www.sadgurupublications.com

ASSAY OF DICYCLOMINE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS BY EXTRACTIVE SPECTROPHOTOMETRY

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ABSTRACT

Four simple and sensitive extractive spectrophotometric methods have been described for the assay of dicyclomine hydrochloride either in pure form or in pharmaceutical formulations. The developed methods involve formation of coloured chloroform extractable ion-pair complexes of the drug with tropaeolin oo (TPoo) (Method-A), bromothymol blue (BTB), bromocresol green (BCG) and bromophenol blue (BPB) (Method-B) in acidic medium. The extracted complexes showed absorbance maxima in the range 410-415 nm for all four methods. Beer's law is obeyed in the concentration ranges 1.25-25, 2.0-25, 2.5-25 and 2.5-25 µg/mL with TPoo, BTB, BCG and BPB, 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 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.

Key words: Dicyclomine hydrochloride, Bromothymol blue, Bromocresol green Bromophenol blue, Tropaeolin oo, Spectrophotometry, International Conference on Hormonization (ICH).

INTRODUCTION

Spectrophotometric methods for determination of dicyclomine

Dicyclomine hydrochloride (CAS 77-19-0) is an important compound in pharmaceutical preparations. It is a tertiary amine with antimuscarinic effects[1]. It decreases spasms of the gastrointestinal tract, biliary tract, ureter, and uterus without producing

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characteristic atropinic effects on the salivary, sweat, or gastrointestinal glands, the eye, or the cardiovascular system except in the large doses[2]. Chemically, it is 2-diethylaminoethyl-bicyclohexyl-1-carboxylate hydrochloride[3]. Several methods have been reported for determination of the compound in bulk powder and pharmaceutical preparations, including microcrystallography[4], nuclear magnetic resonance[5] and gas-liquid chromatography[6,7]. Methods based on the spectrophotometric determination of dicyclomine hydrochloride using π -acceptors[8], p-chloranil[9], Van der Hoeve reagent[10], bromocresol green[11], and methylene blue[12] and with coloring reagent (KSCN/$CoCl_2$/sodium acetate/HCl) after thin-layer chromatographic separation have also been reported[13]. A nonaqueous titration method for determination of dicyclomine hydrochloride has also been reported by using 0.1N perchloric acid as a titrant and crystal violet as indicator. Literature survey on spectrophotometric determination of drugs revealed that certain acidic dyes act as complexing agents and form ion pair complexes with cation salts[14,15] 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 four simple and sensitive extractive spectrophotometric methods for the assay of dicyclomine hydrochloride. The methods are based on ion-pair complexation of drug with dyestuffs viz. bromothymol blue (BTB), bromophenol blue (BPB), bromocresol green (BCG) and Tropaeolin oo and subsequent extraction into chloroform and measuring the absorbance of coloured complex.

EXPERIMENTAL

Dicyclomine hydrochloride is procured from Symed Labs Limited, Hyderabad as a gift sample. The dyestuffs *viz.,* BPB, BTB, BCG and TPoo (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. 0.1 M HCl (for Method-A), sodium acetate-hydrochloric acid buffers[16] of pH 2.5, 2.8, 3.5 were prepared by mixing 50 mL 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 (for Method-B). 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 (25 mg/100 mL).

The spectra (Fig. 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.

Calibration curve

Different aliquots of drug solution were transferred into 125 mL separating funnel. To this, 6 mL of 0.1M HCl (for Method-A) and 5 mL of buffer (pH 2.5, 2.8, 3.5) (for Method-B), 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 in the range 410-415 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 (Fig. 2) are linear over the concentration ranges mentioned in the table and are within the permissible range of experimental errors. The optical characteristics and statistical data for the regression equation of the proposed methods are presented in Table 1.

Table 1: Optical characteristics and statistical parameters for the regression equation of the proposed methods

Parameters	Extraction methods with			
	BPB	**BCG**	**BTB**	**TPoo**
λ_{max} (nm)	415	415	415	410
Beer's law limit (μg mL^{-1})	3.0-25	2.5-25	2.0-25	1.25-22.5
Molar absorptivity (L mol^{-1} cm^{-1})	2.12 x 10^4	2.30 x 10^4	2.45 x 10^4	2.87 x 10^4
Formation constant, K, M^-	1.94 x 10^4	2.45 x 10^4	3.05 x 10^4	3.90 x 10^4
Sandell sensitivity (μg cm^{-2})	0.0162	0.0150	0.0140	0.0120
Slope (Specific absorptivity), b	0.06140	0.0666	0.07102	0.08313
Intercept (a)	-0.02	-0.05	-0.06	0.0054
Correlation coefficient (r)	0.9995	0.9991	0.9998	0.9999
Standard deviation of intercepts (% n = 6)	0.02	0.018	0.015	0.012
Limit of detection, μgmL^{-1}	1.0	0.8	0.69	0.4
Limit of quantification, μgmL^{-1}	3.0	2.4	1.8	1.2
Regression equation	Y = 0.0614 xC-0.02	Y = 0.0666 xC-0.05	Y = 0.0710 xC-0.06	Y = 0.08313 xC+0.0054

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

[b]Six replicate samples.

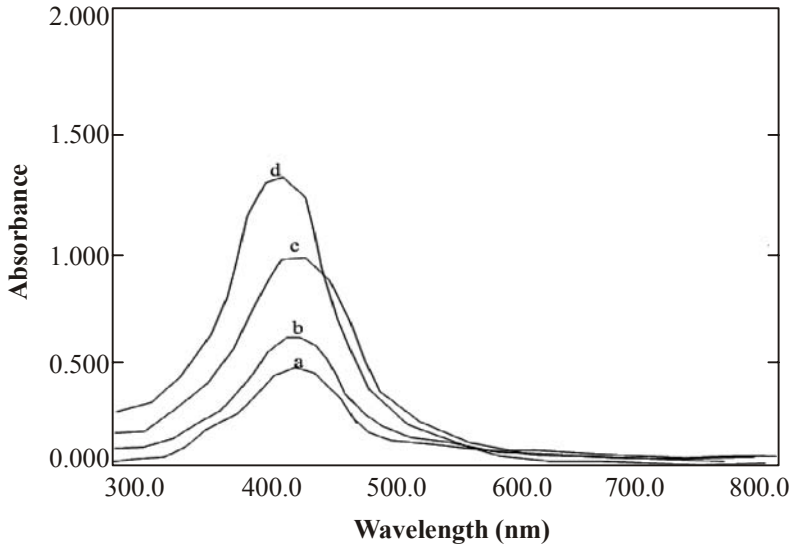


Fig. 1: Absorption spectra of dicyclomine hydrochloride-dye complexes (a) Drug = BPB (b) Drug = BCG (c) Drug = BTB (d) Drug = TPoo

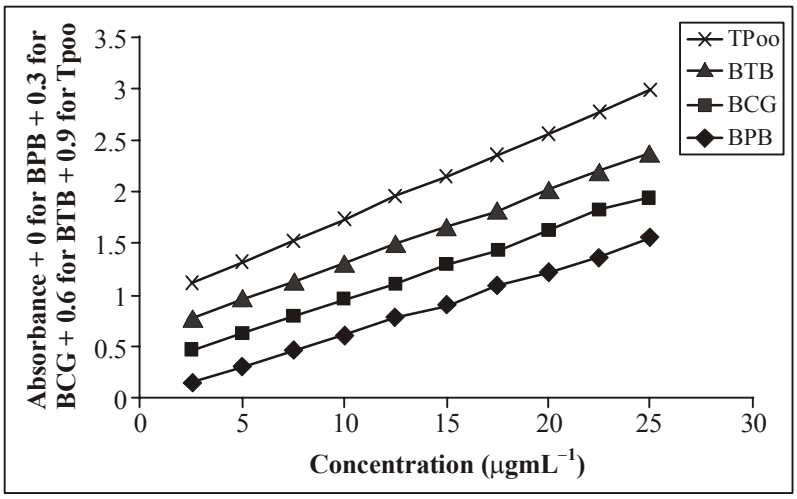


Fig. 2: Calibration graphs of drug-dye ion pair complexes

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).

Table 2: Application of proposed methods for the analysis of dicyclomine hydrochloride in pure form

Taken (µg mL^{-1})	Found (µg mL^{-1})				Proposed method recovery (%)				Reference method
	BPB	BCG	BTB	TPoo	BPB	BCG	BTB	TPoo	Recovery (%)
2.5	2.4	2.52	2.46	2.49	96	100.8	98.4	99.6	99.89
5.0	4.9	4.95	5.02	4.92	98	99	100.4	98.4	99.28
7.5	7.45	7.52	7.42	7.51	99.3	100.2	98.9	100.1	99.76
10.0	10.05	9.98	9.95	9.94	100.5	99.8	99.5	99.4	99.65
RSD (%)					1.956	0.75	0.866	0.717	1.96
Mean ± SD					98.45 ± 1.92	99.95 ± 0.75	99.3 ± 0.860	99.37 ± 0.713	99.64 ± 0.26
t-test					0.317	0.309	0.581	0.357	2.31
F-test					0.0083	0.116	0.082	0.134	6.39

Procedure for the assay of dosage forms

3 Tablets of 20 mg dicyclomine 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.

Table 3: Application of proposed methods for the analysis of dicyclomine hydrochloride in pharmaceutical form

Taken (µg mL^{-1})	Proposed methods								Reference method
	Found (µg mL^{-1})				Recovery (%)				Recovery (%)
	BPB	BCG	BTB	TPoo	BPB	BCG	BTB	TPoo	
Dicyclomine 20 mg/tablet									
5	5.01	5.08	4.96	4.98	100.2	101.6	99.2	99.6	99.89

Cont…

Taken (µg mL^{-1})	Proposed methods								Reference method
	Found (µg mL^{-1})				Recovery (%)				Recovery (%)
	BPB	BCG	BTB	TPoo	BPB	BCG	BTB	TPoo	
9	8.98	9.06	8.99	9.11	99.7	100.6	99.8	101	99.28
13	12.89	12.95	13.01	12.87	99.15	99.6	100.0	99	99.76
18	18.02	17.84	17.98	18.10	100.1	99.1	99.8	100.5	99.65
RSD (%)					0.47	1.09	0.31	0.85	1.96
Mean ± SD					99.78 ± 0.47	100.2 ± 1.10	99.7 ± 0.3	100.0 ± 0.89	99.64 ± 1.26
t-test					0.612	0.370	0.84	0.54	2.31
F-test					0.35	0.04	0.65	0.073	6.39

Table 4: Excipients

S. No.	Excipients	(µg/mL)
1	Microcrystalline cellulose	50
2	Magnesium stearate	30
3	Lactose monohydrate	25

RESULTS AND DISCUSSION

Dicyclomine hydrochloride forms ion-pair complexes in acidic buffer with dyestuffs such as bromothymol blue (BTB), bromocresol green (BCG), bromophenol blue (BPB) and Tropaeolinoo (TPoo). These complexes are quantitatively extracted into chloroform. Ion-pair complexes of drug with BTB, BPB, BCG and TPoo absorbed in the range 410-415 nm. The reagent blank under similar conditions showed no absorption.

In order to establish molar ratio between dicyclomine hydrochloride and dyestuffs used, the Job's method of continuous variation[17] 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[18,19] were also estimated and found to be 1.9493 x 10^4, 2.450 x 10^4, 3.056 x 10^4 and 3.9062 x 10^4 M^{-1} for complexes with BPB, BCG, BTB and TPoo, respectively.

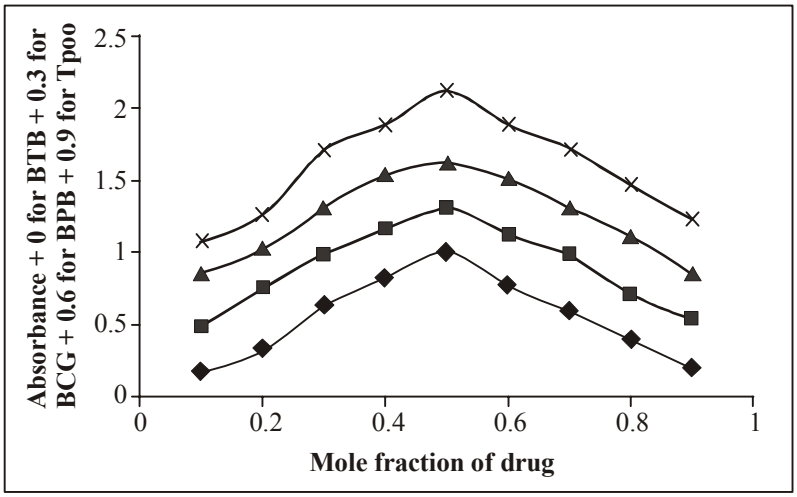
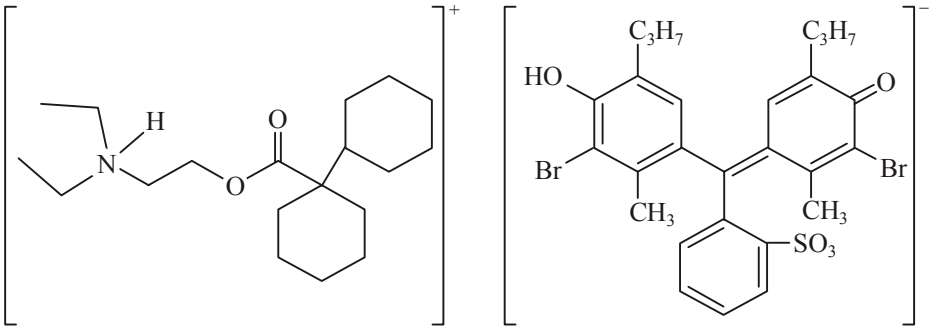
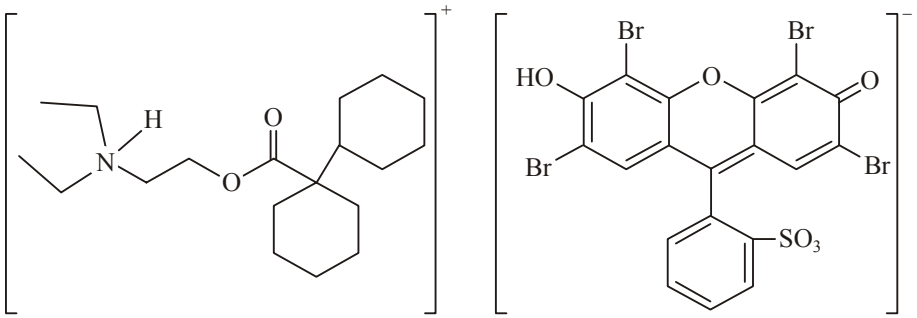


Fig. 3: Job's Continuous study of drug-dye system [Drug] = [Dye] = 8 x 10^{-5} M

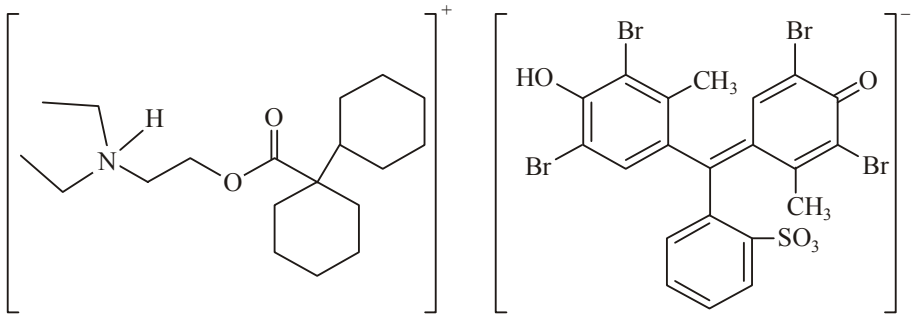
Dicyclomine hydrochloride contains tertiary amino groups. Hence, We propose the protonation of tertiary nitrogen in acidic medium. The sulphonic acid group present in BTB, BPB, BCG and TP oo that is the only group undergoing dissociation in the pH range 1-5. Finally, the protonated dicyclomine hydrochloride forms ion-pairs with the dyestuffs, which are quantitatively extracted into chloroform. The possible reaction mechanisms are proposed and given in **Scheme 1**.



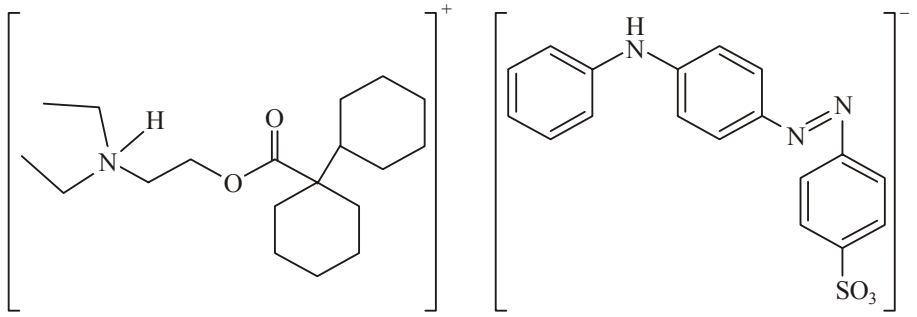
Dicyclomine HCl bromothymol blue complex



Dicyclomine HCl bromophenol blue complex



Dicyclomine HCl bromocresol green complex



Dicyclomine HCl tropaeolinoo complex

Scheme 1

The influence of pH on the ion-pair formation of dicyclomine hydrochloride with various dyestuffs has been studied using 0.1M HCl (for Method-A) and sodium acetate-hydrochloric acid buffer (for Method-B). The results are shown in Fig. 4. It is evident that absorbance of complexes with TPoo, BTB, BPB and BCG was found to be constant within the pH ranges 1.0-1.5, 2.2-3.3, 2.0-3.0, 3.0-4.0, respectively. Thus, all the absorbance measurements were made at pH 1.28, 2.8, 2.5 and 3.5 with TPoo, BTB, BPB and BCG, respectively.

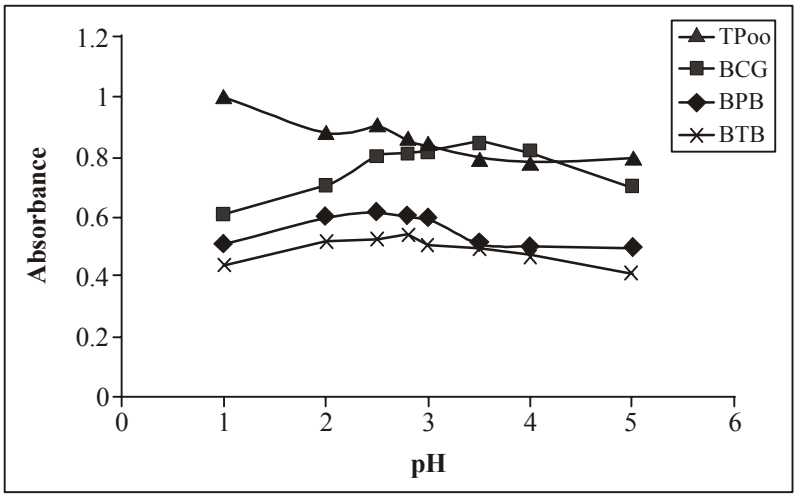


Fig. 4: Effect of pH; [Drug] = 12.5 µg/mL, [Dye] = 5 mL of 0.025%

The effect of dyestuff concentrations was also studied by adding different volumes of dyestuff to a constant amount of dicyclomine hydrochloride (25 µg mL^{-1}). It is apparent from Fig. 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.

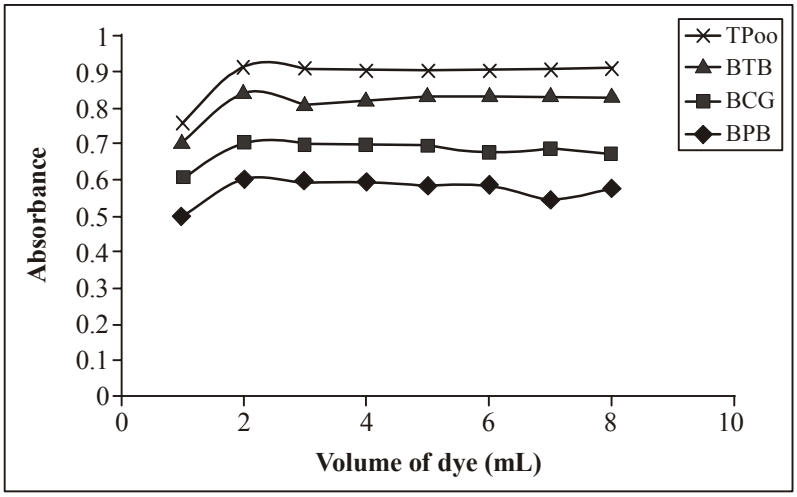


Fig. 5: Influence of volume of 0.025% dye; [Drug] = 12.5 µg/mL

A systematic study of the effect of foreign species present along with dicyclomine hydrochloride on the determination of dicyclomine hydrochloride at 12.5 µg mL^{-1} level 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 dicyclomine 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.

Validation of the proposed method

All the three proposed methods have been validated in terms of guideline proposed by ICH[20] 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 12.5 μg mL^{-1} of dicyclomine hydrochloride were made. The coefficient of variation was found to be less than 1.9% for all the procedures.

The proposed methods have been successfully applied to the determination of dicyclomine hydrochloride in pharmaceutical preparations. The performance order of the proposed methods is TPoo > BTB > BCG > BPB. The results obtained and shown in Table 3 were compared to those obtained by a reference method[8] 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 dicyclomine hydrochloride in pure form and in formulation.

ACKNOWLEDGEMENTS

The authors are grateful to Head, Department of Chemistry and Principal, Nizam College and St. Anns College for providing facilities.

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Revised : 06.05.2011 *Accepted : 09.05.2011*