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SPECTROPHOTOMETRIC DETERMINATION OF BISACODYL IN PURE FORM AND TABLET FORM

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ABSTRACT

Two simple, rapid and sensitive spectrophotometric methods were proposed for determination of bisacodyl in pure and in tablet forms. The method I depended on reaction of drug and tetracyanoethylene by charge transfer complex with maximum absorbance at 398 nm. Beer's law was obeyed in the range 0.05-0.3 μgml^{-1} . Method II based on bromination-oxidation reaction using bromate-bromide mixture with thymol blue and methyl orange as reagents and measuring the absorbance of the unbleached dye at 545 nm and 510 nm. Beer's law was obeyed in the range 4-24 μgml^{-1} and 4-10 μgml^{-1} . Under optimized conditions, the experimental conditions were optimized and Beer's law was obeyed over the applicable concentration ranges. The methods were applied successfully to the tablets containing bisacodyl. The results obtained are in good agreement with those obtained using official and reference methods.

KEYWORDS

Bisacodyl, Tetracyanoethylene, Thymol blue and Methyl orange methods.

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INTRODUCTION

Bisacodyl is a laxative usually administrated on a short-term basis for treatment of constipation, for evacuation of the bowel before surgery and radiological examination of the abdomen. Or endoscopy before or after surgical operation using barium enemas, when give as suppositories, it stimulates the rectal mucosa, which increases peristaltic movements and causing defecation in 15-30 minutes¹⁻³.

Bisacodyl is official in B.P. 2009⁴ and USP 2000⁵ where USP contains an assay for bisacodyl tablets which uses a C₁₈ chemically bonded silica gel

column and ultraviolet (UV) absorption detection at 265nm.

A literature survey revealed that bisacodyl has been estimated in pharmaceuticals by UV spectrophotometry⁶⁻⁸, Voltammetry⁹ and High performance liquid chromatography¹⁰⁻¹². Most of the reported method require expensive instrument setup, expertise personnel, and complicated procedure.

EXPERIMENTAL

APPARATUS

Labomed Spectro UV-VIS Double Beam (UVD-2950) Spectrophotometer with matched 1cm quartz cells connected to Windows compatible computer using UV Win. 5 Software v 5.0.5. **Spectronic Genesys** UV-VIS Spectrophotometer connected to an IBM PC computer load with FLWINLAB software.

MATERIALS AND REAGENTS

All chemicals used are of analytical reagent grade.

Bisacodyl (Alexandria, Egypt.99% Purity).

Bisadyl tablets labeled to contain 5 mg of bisacodyl per tablet. Batch No.506387 (Alexandria, Egypt).

Tetracyanoethylene (98%, B.No:138050100 ACROS Chemicals company). Solution of 5×10^{-3} M dissolved in 100 ml acetonitrile.

Sodium bicarbonate (98%, B.No:34090 El Nasr pharmaceutical chemicals company). Solution of 1×10^{-3} M dissolved in 100 ml water was used.

Thymol Blue (Universal Fine Chemicals, India) 100 µg/ml was dissolved in 20 ml ethanol then completed to 100 ml with same solvent (stable for 2 weeks at least).

Methyl Orange 60 µg/ml (Universal Fine Chemicals, India) was dissolved in 20 ml methanol then completed to 100 ml with bidistilled water (stable for 2 weeks at least).

5 M HCl (El-Nasr Chemicals, Egypt) was prepared by diluting 225 ml of concentrated HCl (34%) to 500 ml.

Bromate / Bromide stock solution was prepared by dissolving 0.1 gm of potassium bromate (Winlab, England) and 1.0 gm of potassium bromide (Winlab, England) in 100 ml bidistilled water (stable for 10 days at least).

Working solution was freshly prepared daily by diluting 3.5 ml of stock solution to 100 ml with bidistilled water to give final concentration (35 µg/ml in case of thymol blue) and diluting 2.5 ml of stock solution to 100 ml with bidistilled water to give final concentration (25 µg/ml in case of methyl orange).

Principle

Preparation of standard Stock solutions (10, 100 and µg/ml)

Preparation of standard drug solutions for method I, II.1. II.2.

Stock solutions of bisacodyl were prepared by dissolving 10 mg, 100 mg and 50 mg of the pure drug in 100 ml methanol in volumetric flasks for method I, method II.1. and method II.2., respectively. Working solution of lower concentration (0.1 µg/ml, 1 µg/ml and 0.5 µg/ml) was prepared by further dilution of stock solution with methanol for method I., method II.1. and method I.2. respectively.

Procedures

Method I: General procedures for the determination of bisacodyl through charge transfer complex reaction with TCNE:-

Method I. (TCNE)

To a series of 10 ml calibrated flasks, an increasing volume covering the concentration range (0.05-0.3) µg/ml of bisacodyl solution are transferred, followed by addition of 1 ml of 5×10^{-3} M tetracyanoethylene and 0.8 ml of NaHCO₃ with occasional shaking and diluted to mark with bidistilled water. The solution was left for 10 min. in ice bath. The absorbance was measured at 398 nm versus a reagent blank. A calibration graph was prepared by plotting the measured absorbance versus concentration. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using the Beers law data.

Method II: General procedures for the determination of the pure drug through bromometric method using Thymol blue and Methyl orange:-

Method II.1. (Thymol blue)

To bromate - bromide 1.4 ml working solution in 10 ml volumetric flasks, add (4-24) µg/ml of bisacodyl

solution then acidify using 5 M HCl, add 0.6 ml, close flasks and stand for 10 minutes, add 1 ml dye working solution then stand for another 4 minutes and complete to mark with bidistilled water then measure absorbance against reagent blank at 545 nm.

Method II.2. (Methyl orange)

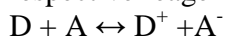
To 1 ml bromate - bromide working solution in 10 ml volumetric flasks, add (4-10) µg/ml of bisacodyl drug solution then acidify using 0.4 ml 5 M HCl, close flasks and stand for 15 minutes, add 1 ml dye working solution then stand for another 2 minutes and complete to mark with bidistilled water then measure absorbance against reagent blank at 510 nm.

Pharmaceutical preparation (tablets)

Twenty tablets of Bisadyl tablets were weighed and finely powdered. An accurately weighed amount of the powder equivalent to the concentration of the pure drug in the method was extracted with methanol three times, the filtrate was collected and transferred to 100 ml volumetric flask and completed to the mark with methanol. Aliquots from this solution equivalent to that in authentic sample were used for the application of the proposed method applying standard addition technique techniques for method I.1. and method II.1.2.

RESULTS AND DISCUSSION

Bisacodyl reacts with TCNE in alkaline medium through charge transference complex reaction (an electron donor (D) to an electron acceptor(A) producing yellow colored product showing maximum absorption at λ_{max} 398 nm against respective reagent blank are shown in Figure No.2.



The proposed spectrophotometric methods are indirect and are based on the determination of the residual bromine (insitu generated) after allowing the reaction between drug and a measured amount of bromine to be complete. The surplus bromine was determined by reacting it with a fixed amount of either thymol blue or methyl orange dye. The methods rely on the bleaching action of bromine on the dyes due to oxidative destruction of these dyes when added in increasing amounts to a fixed amount

of insitu generated bromine, consume the latter proportionately with a concomitant fall in the concentration of bromine. When a fixed amount of dye is added to the decreasing amounts of bromine, a concomitant increase in the concentration of dye results. Consequently, a proportional increase in the absorbance at the respective λ_{max} is observed with increasing concentration of each drug.

The insitu generation of bromine is carried out using a mixture of potassium bromate and potassium bromide in presence of 5 M HCl according to the following equation:



Absorption spectra

Absorption spectra for determination of bisacodyl was studied over range of 200 - 800 nm. After oxidation of drug and portions of dyes with bromine, residual unoxidized thymol blue and methyl orange are absorbed at 545 and 510 nm Figure No.3.

Study of the experimental parameters

The different experimental parameters affecting the development of the reaction products were carefully studied and optimized. Such factors were changed individually while others were kept constant. These factors include reagent concentration, effect of base, temperature and time.

Factors for method I

i- Effect of solvent

Different solvents were investigated in order to select the suitable for TCNE method. These solvents included acetonitrile, absolute ethanol and methanol. It is found that acetonitrile is considered to be an ideal solvent for this experiment because it has a suitable solvating power for TCNE as well as producing more stable and reproducible absorbance.

ii- Effect of reagent volumes

The effect of changing the TCNE concentration on the absorbance of solution containing a fixed amount of drugs were studied. It is evident that the absorbance increases with increasing TCNE concentration and reached maximum on using 1ml of 0.064 % (w/v), 5×10^{-3} M TCNE achieves a suitable volume for maximum color intensity Figure No.7.

iii- Effect of base

Therefore, different bases such as sodium hydroxide, potassium hydroxide, sodium carbonate and sodium bicarbonate of $1 \times 10^{-3} \text{M}$ concentration were examined in order to obtain high sensitivity. It was found that 0.8 ml of sodium bicarbonate gave maximum color intensity and beyond these amounts, the absorbance would be decreased. Therefore, 0.8 ml of $1 \times 10^{-3} \text{M}$ was chosen as the optimum concentration of sodium bicarbonate Figure No.8.

iv- Effect of Time and Temperature

The reaction time was determined by following the color development in different temperatures. It was observed that the absorbance reached maximum after left the solution 10 min in ice-bath and remained stable for at least 30 min. This temperature and reaction time were chosen for color development.

Study of the experimental parameters for method II

i- Effect of 5M HCl volume

5 M HCl was used throughout experiments and it was found that 0.6 ml or 0.4 ml with thymol blue and methyl orange is the appropriate acid volume and increasing HCl volume results in a decrease in absorption Figure No.9.

ii- Effect of volume of $35 \mu\text{g ml}^{-1}$ and $25 \mu\text{g ml}^{-1}$ bromate-bromide

Bromate - bromide volume was studied by varying the reagent volume while other factors were held constant. It was found that of $35 \mu\text{g ml}^{-1}$ of 1.4 ml and $25 \mu\text{g ml}^{-1}$ of 1 ml of bromine is sufficient for its bleaching action in case of thymol blue (method II.1.) and methyl orange (method II.2). using these stated concentrations Figure No.10.

iii- Effect of the reaction time

Time required to brominate and oxidize the drug before addition of dye and time required to irreversibly oxidize dye after its addition was studied. The bromination reaction was found to be complete in 10 minutes or 15 minutes with thymol blue and methyl orange while contact times up to 25 minutes had been examined and no further bromination was detected. A contact time of 4 minutes (in case of thymol blue) and 2 minutes (in case of methyl orange) was necessary for the

bleaching of the dye colour by the residual bromine and the colour of the dyes remains stable for at least two hours after mixing with the reaction mixture.

Validation of the proposed method

The validity of the proposed methods was tested regarding linearity, range, limit of detection, limit of quantification, accuracy, precision, robustness and specificity according to ICH recommendations¹³.

Linearity and range

The calibration graph obtained by plotting the values of the absorbance versus the final concentrations ($\mu\text{g/ml}$) was found to be rectilinear over the concentration ranges cited in the table No.1, Figures No.4-6.

The calibration graph was described by the equation:

$$Y = a + bX$$

(Where Y=absorbance, a=intercept, b=slope, X=concentration in $\mu\text{g/ml}$).

Correlation coefficient, intercept and slope for the calibration data are summarized in Table No.1.

Limit of detection and limit of quantification

Limit of detection (LOD) were determined by evaluating the lowest concentrations of the analyte that can be detected according to the following equation:

$$\text{LOD} = 3.3 \text{ S/K}$$

Limit of quantification (LOQ) were determined also by establishing the lowest concentrations that can be quantitated according to the following equation:

$$\text{LOQ} = 10 \text{ S/K}$$

Where S is the standard deviation of the three replicate determination values under the same conditions as for the sample analysis in the absence of analyte and K is the sensitivity, namely, the slope of calibration graph. The results are summarized in Table No.2.

Accuracy and precision

Accuracy was evaluated as percentage relative error between the measured concentration for bisacodyl. The accuracy of the proposed methods was checked by performing recovery experiments through standard addition technique. The results are shown in Tables No.3 and 4 are compiled and show that the accuracy is good. The precision of the method was calculated in term of intermediate precision (intraday and interday). Two different concentration

five times of bisacodyl was analyzed during the same day (intra-day precision) and five consecutive days (inter-day precision). The standard analytical errors, relative standard deviations (RSD) and recoveries obtained by the proposed method were found to be acceptable. The results are summarized in Table No.6.

The standard analytical errors, relative standard deviations (RSD) and recoveries obtained by the proposed method were found to be acceptable.

Robustness

Robustness of the method was examined by small changes in the method variables such as change reagent concentration (± 0.05 ml), volume of base (± 0.05 ml), volume of acid (± 0.05 ml), volume of

bromated- bromine mixture (± 0.05 ml), volume of dye(± 0.05 ml) and reaction time (± 2 minutes). The results are shown in Table No.7.

The minor changes that may take place during the experiment didn't affect the absorbance of the reaction products.

Analysis of tablets

The proposed methods were applied to the analysis of the drug in tablets and the results were statistically compared with reference method⁷ by calculating Student's *t*- and F-values. The evaluated *t*- and F-values were less than the tabulated values at the 95% confidence level. The results are listed in Table No.5.

Table No.1: Analytical parameters of the reaction of bisacodyl with TCNE, Thymol blue and Methyl orange

Parameters	Method I. (TCNE)	Method II.1. (Thymol blue)	Method II.2. (Methyl orange)
λ_{max} , nm	398	545	510
Volume of the dye (ml)	-	1	1
Volume of 5M HCl (ml)	-	0.6	0.4
Volume of 35 μgml^{-1} and 5 $0\mu\text{gml}^{-1}$ bromate-bromide mixture (ml)	-	1.4	1
Time required to oxidize the drug before dye addition (min.)	-	10	15
Time required to irreversibly oxidize the dye (min.)	-	4	2
Volume of 1×10^{-3} M NaHCO ₃ (ml)	0.8	-	-
Reagent Conc. % w/v	0.064	-	-
Reagent volume (ml)	1	-	-
Temperature (°C)	Ice bath	-	-
Reaction time (minutes)	10	-	-
Diluting solvent	Bidistilled water	Bidistilled water	Bidistilled water
Beer's law limits ($\mu\text{g/ml}$)	0.05-0.3	4-24	4-10
Regression equation*	Slope (b)	2.8103	0.0252
	Intercept (a)	0.0012	0.0196
Correlation coefficient	0.9998	0.9999	0.9993

*A = a + bC where A is absorbance, C is the concentration of the drug in $\mu\text{g/ml}$.

Table No.2: Statistical data for the reaction of bisacodyl with TCNE, Thymol blue and Methyl orange

Parameters	Method I. (TCNE)			Method II.1. (Thymol blue)			Method II.2. (Methyl orange)		
	Conc. taken µg/ml	Conc. found µg/ml	Recovery %	Conc. taken µg/ml	Conc. found µg/ml	Recovery %	Conc. taken µg/ml	Conc. found µg/ml	Recovery %
	0.05	0.04975	98.5	4	3.9444	98.61	4	4.0369	100.92
	0.1	0.10134	101.34	8	8.0317	100.39	5	4.9666	99.33
	0.15	0.14867	99.11	12	11.9603	99.67	6	6.0035	100.06
	0.2	0.19919	99.60	16	16.1269	100.79	7	6.9570	99.39
	0.25	0.25150	100.6	20	20.0556	100.28	8	7.9821	99.78
	0.3	0.29954	100.85	24	23.9047	99.60	9	9.0309	100.34
	---	---	---	---	---	---	10	9.9964	99.96
Mean*	---	---	99.99	---	---	99.89	---	---	99.97
N	---	---	6	---	---	6	---	---	7
SD	---	---	0.8240	---	---	0.7734	---	---	0.5540
RSD	---	---	0.8240	---	---	0.7742	---	---	0.5541
SE	---	---	0.3363	---	---	0.3157	---	---	0.20905
Variance	---	---	0.6789	---	---	0.59807	---	---	0.3069
LOD, µgml ⁻¹	---	---	0.01214	---	---	1.1556	---	---	1.11137
LOQ, µgml ⁻¹	---	---	0.04047	---	---	3.8521	---	---	3.7045
Sandell's sensitivity(µgml ⁻¹ per 0.001A)	---	---	0.0001257	---	---	0.0475	---	---	0.0194
Apparent Molar absorptivity** LMol ⁻¹ cm ⁻¹	---	---	1018886	---	---	9817.826	---	---	16861.85

Table No.3: Application of standard addition technique for the determination of Bisacodyl (Bisadyl) through reaction with TCNE

S.No	Statistics	Bisadyl [®] tablets			
		Conc. added form pure drug (µg/ml)	Conc. taken from Bisadyl [®] (µg/ml)	Conc. found (µg/ml)	Recovery**%
		0.05	0	0.05119	102.38
		0.05	0.05	0.09949	99.49
		0.05	0.1	0.14816	98.77
		0.05	0.15	0.19863	99.32
		0.05	0.2	0.25162	100.65
1	Mean recovery*				100.12
2	N				5
3	S.D.				1.4346
4	R.S.D.				1.4328
5	V				2.0582
6	S.E.				0.6416

Table No.4: Application of standard addition technique for the determination of Bisacodyl (Bisadyl) using Thymol blue and Methyl orange

Items	Bisadyl® tablets							
	Thymol blue				Methyl orange			
	Conc. added form pure drug (µg/ml)	Conc. taken from Bisadyl (µg/ml)	Conc. found (µg/ml)	Recovery* %	Conc. added form pure drug (µg/ml)	Conc. taken from Biadyl (µg/ml)	Conc. found (µg/ml)	Recovery* %
	4	0	3.96	99	4	0	4.03659	100.91
	4	4	8.04	100.5	4	1	4.9878	99.76
	4	8	12.16	101.33	4	2	6.0488	100.81
	4	12	15.92	99.5	4	3	7.0244	100.35
	4	16	20	100	4	4	8.0731	100.91
Mean*	---	---	---	100.07	---	---	---	100.55
N	---	---	---	5	---	---	---	5
S.D.	---	---	---	0.90216	---	---	---	0.502
R.S.D.	---	---	---	0.90156	---	---	---	0.499
V	---	---	---	0.81389	---	---	---	0.252
S.E.	---	---	---	0.40347	---	---	---	0.225

Table No.5: Statistical data for the determination of Bisadyl by the proposed method compared with the reference methods

S.No	Statistics	Reference method ⁷	proposed method of TCNE	proposed method of Thymol blue	proposed method of Methyl orange
1	Mean recovery*± SD	100.87±1.071	100 .12± 1.44	100.07± 0.9022	100.55 ± 0.502
2	N	5	5	5	5
3	Variance	1.147	2.058	0.814	0.252
4	S.E.	0.480	0.642	0.404	0.225
5	t-test**	---	0.934(2.306) ^a	1.294(2.303)a	0.606(2.306) ^a
6	F-ratio**	---	1.808(5.05) ^b	1.407(5.05)b	4.54(5.05) ^b

a and b are Theoretical Student *t-values* and F- ratio at p=0.05, *Mean of three different experiments.

Table No.6: Results of the intraday and interday precision for the determination of Bisacodyl with TCNE, Thymol blue and Methyl orange

S.No	Item	conc.µg/ml	Intraday		Interday	
			mean ± SD	RSD	mean± SD	RSD
1	TCNE,	0.05 µg/ml	100±0.940	0.940	100.29±1.169	1.166
		0.15 µg/ml	100.17±0.620	0.619	100.45±0.739	0.736
2	Thymol blue	8µg/ml	99.92± 0.844	0.845	100.07± 1.173	1.169
		20µg/ml	99.82± 0.868	0.870	100.06± 1.049	1.048
3	Methyl orange	7µg/ml	100.04± 0.766	0.762	99.88± 1.25	1.26
		10µg/ml	100.048± 0.606	0.606	100.35± 0.606	0.604

Table No.7: Results of the robustness for the determination of Bisacodyl with TCNE, Thymol blue and Methyl orange

S.No	<i>Robustness</i>			
	% of recovery ± SD			
	Item	TCNE	Thymol blue	Methyl orange
1	Reagent +0.05 ml	100.43±0.537	100.87±1.003	100.45±0.578
2	Reagent -0.05 ml	98.29±0.969	98.95±0.803	99.95±0.812
3	HCl+0.05 ml	-	100.04±0.649	99.95±0.593
4	HCl-0.05 ml	-	101.53±0.979	100.06±0.735
5	Br2+0.05 ml	-	98.87±0.734	100.49±1.79
6	Br2-0.05 ml	-	101.78±1.058	100.31±1.29
7	NaHCO3+0.05 ml	99.47±0.624	-	-
8	NaHCO3-0.05 ml	101.084±0.838	-	-
9	Time+2 min.	99.47±0.624	99.80±0.635	99.78±0.680
10	Time-2 min.	100.66±0.557	100.79±0.773	99.87±0.585

Table No.8: Results of Ruggedness for the determination of Bisacodyl using Tetracyanoethylene method

S.No	Item	% of recovery ± SD	
1	Bisacodyl	0.05 µg/ml	100.192±0.743
		0.2 µg/ml	101.71±0.946

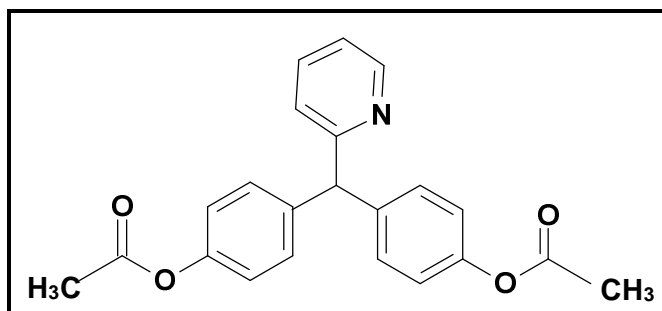


Figure No.1: Structure of bisacodyl 4, 4' (pyrid-2-yl methylene) bis (phenyl acetate)

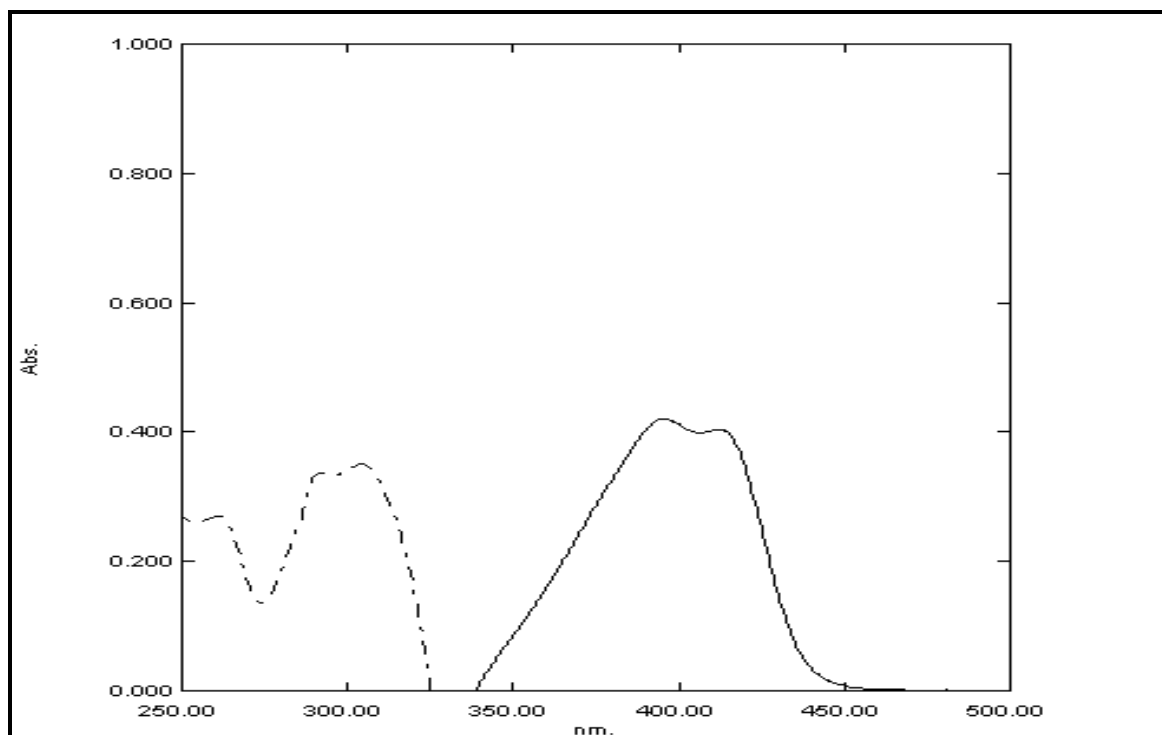


Figure No.2: Absorption spectra of (method I) for the reaction between TCNE and bisacodyl ($10 \mu\text{gml}^{-1}$) showing λ_{max} at 398nm against reagent blank

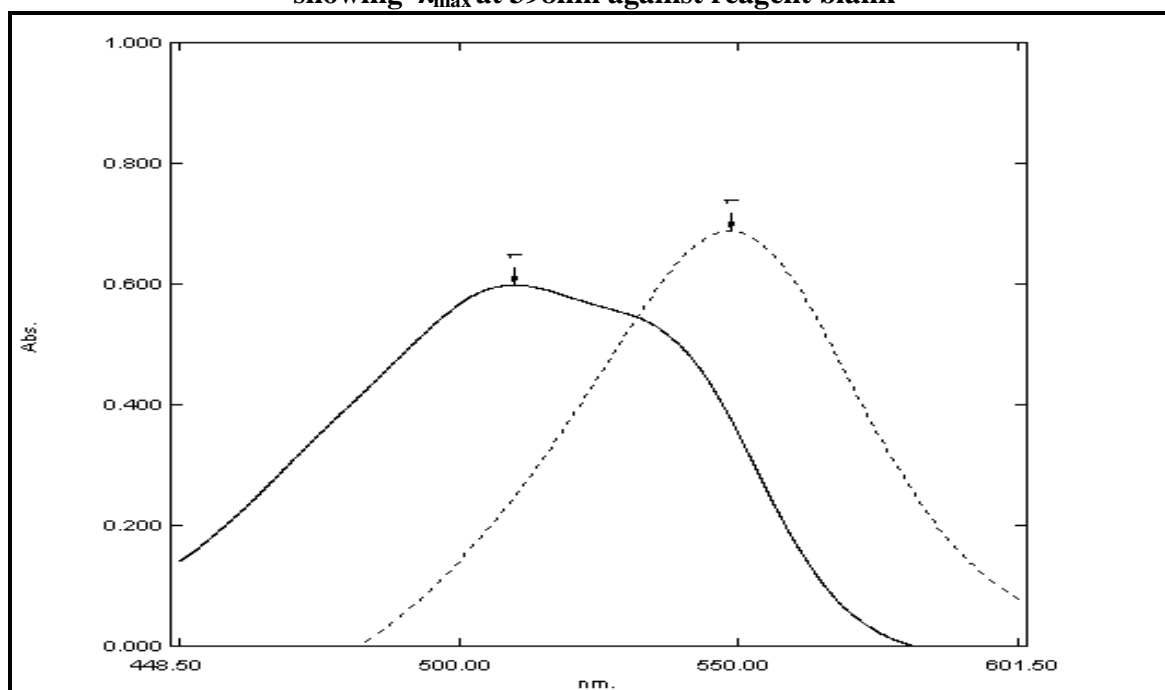


Figure No.3: Absorption spectra for reaction between Thymol blue and Methyl orange with Bisacodyl at 545 nm and 510 nm

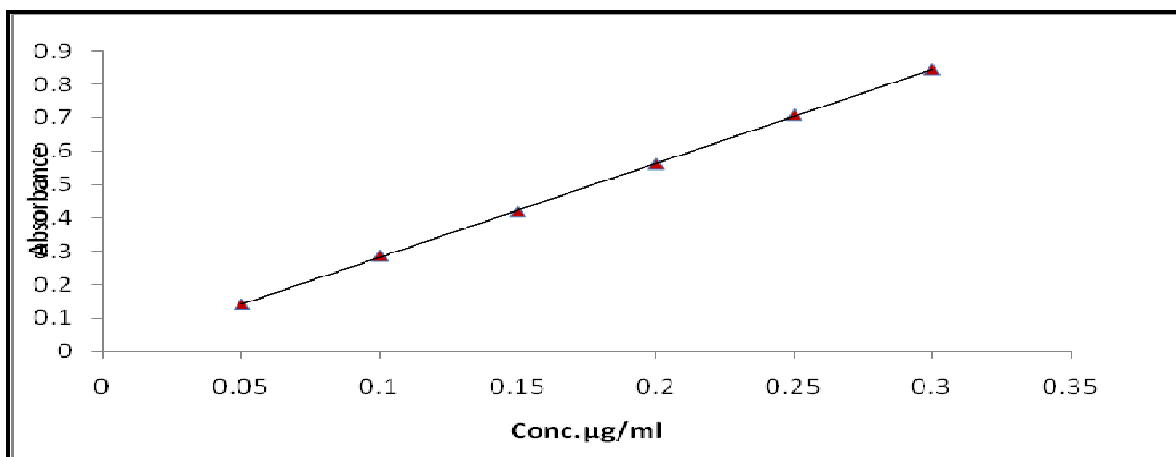


Figure No.4: Calibration curve for the reaction between TCNE and Bisacodyl at λ_{\max} 398 nm

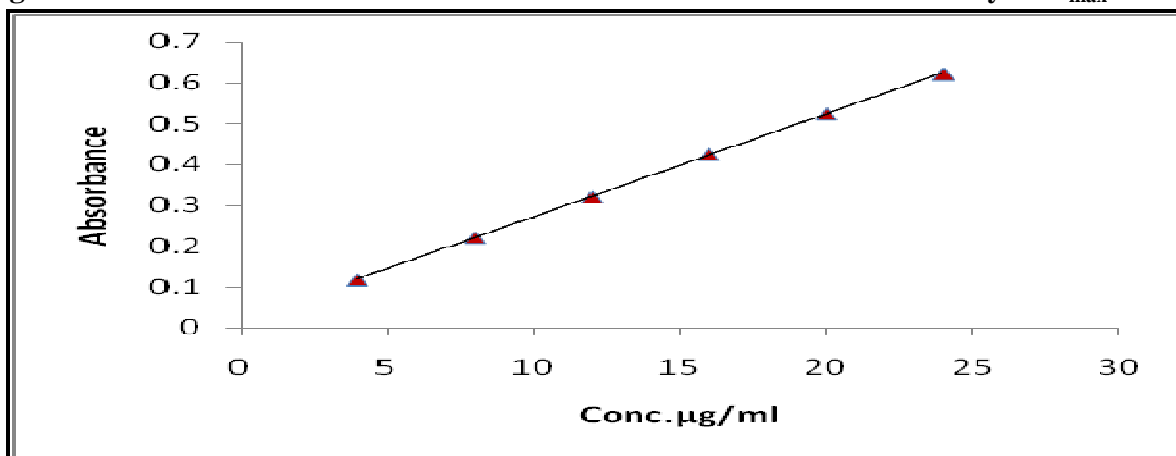


Figure No.5: Calibration curve for bromatometric determination of Bisacodyl in presence of Thymol blue at λ_{\max} 545nm

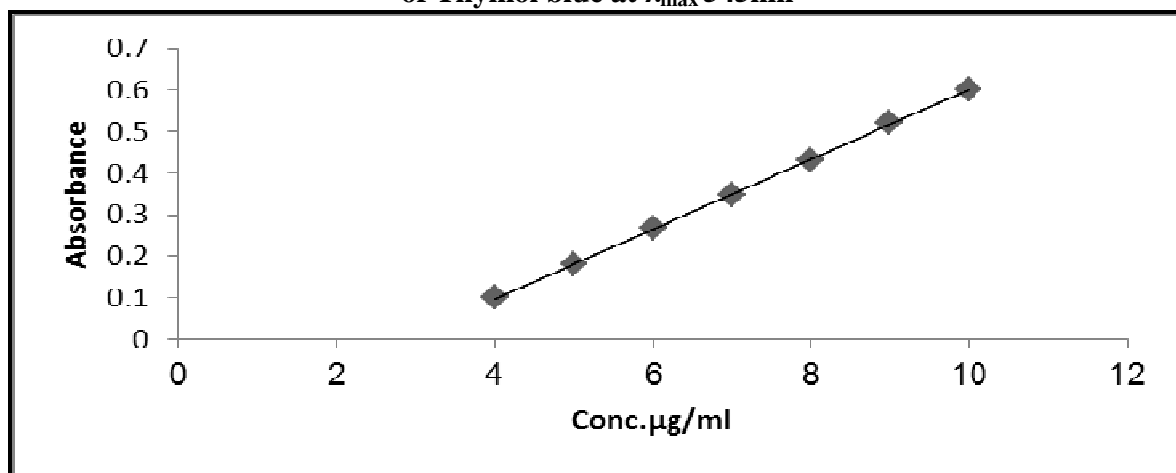


Figure No.6: Calibration curve for bromatometric determination of Bisacodyl in presence of Methyl orange at λ_{\max} 510 nm

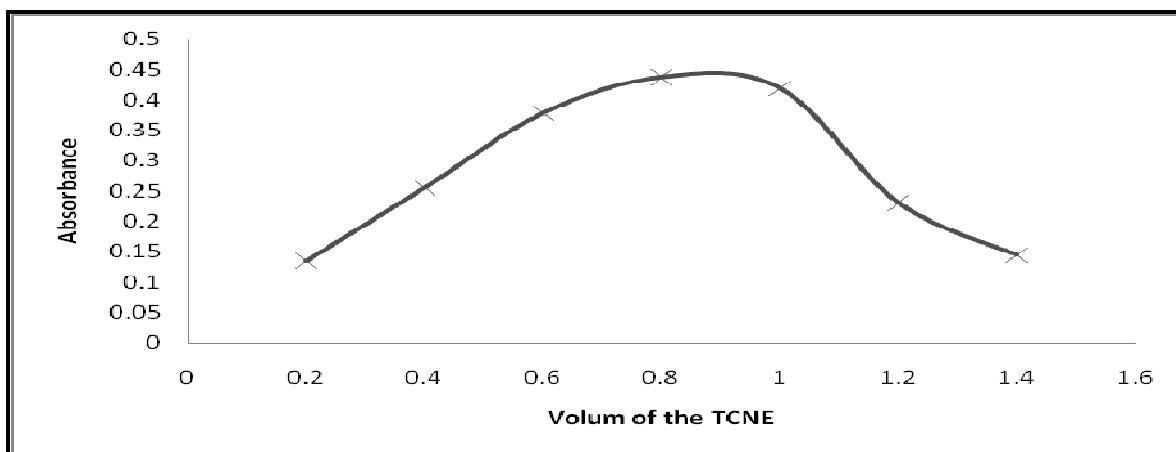


Figure No.7: Effect of volume of TCNE on the absorbance of (0.15µg ml⁻¹) Bisacodyl

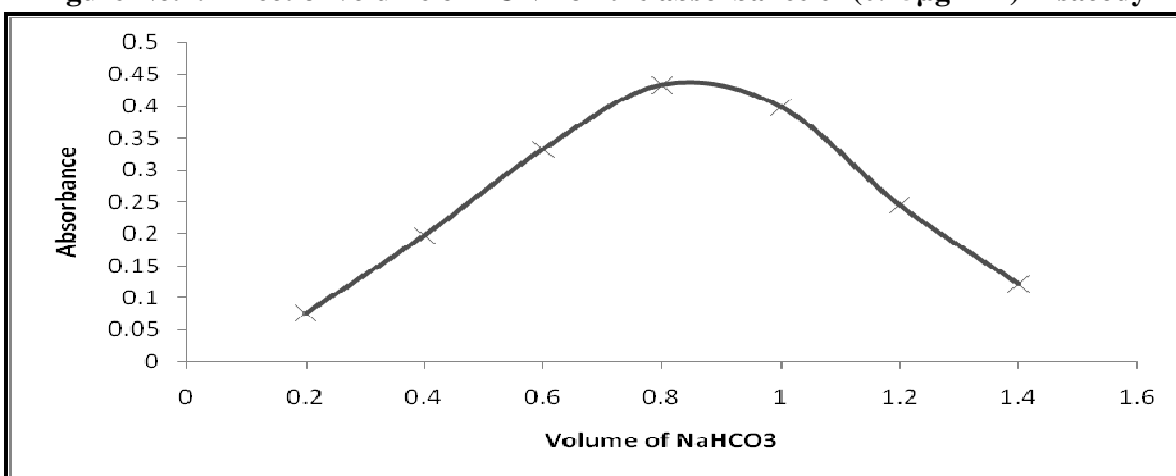


Figure No.8: Effect of base volume on the reaction of TCNE with (0.15µg ml⁻¹) Bisacodyl

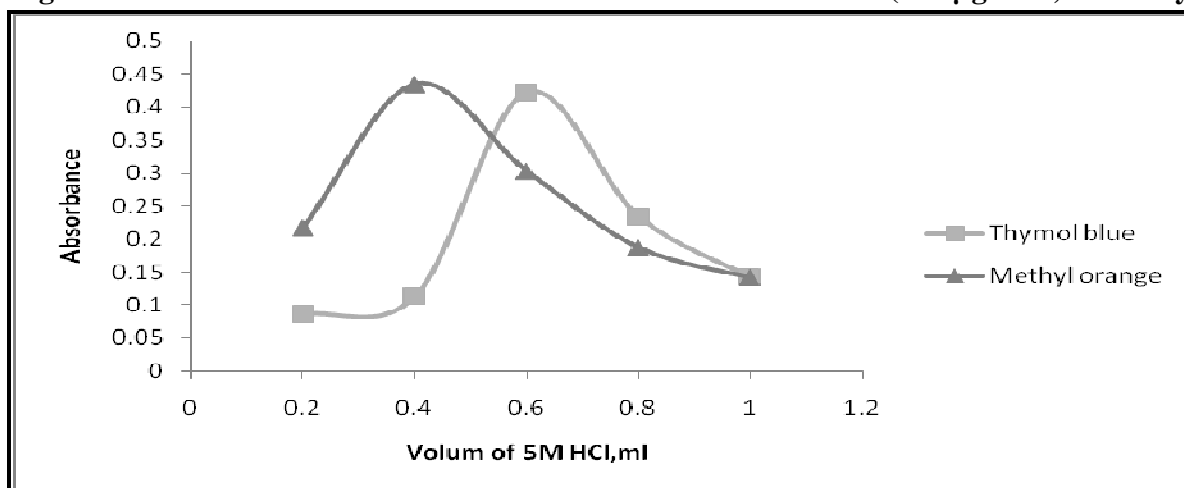


Figure No.9: Effect of volume of 5 M HCl on absorbance in case of Thymol blue and Methyl orange with (16 and 8 µgml⁻¹) Bisacodyl at λ_{max} 545 and 510 nm

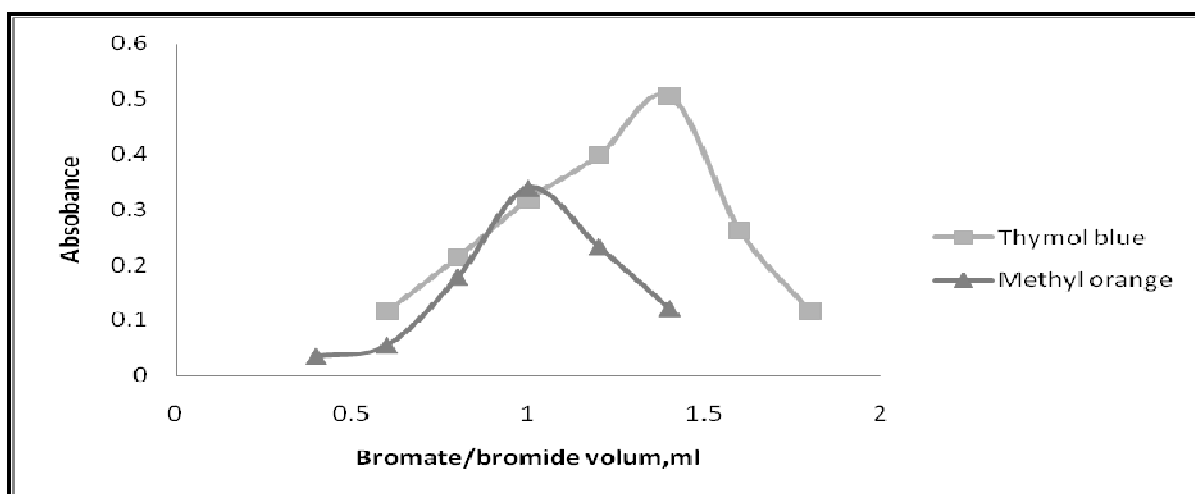


Figure No.10: Effect of volume Bromate-Bromide mixture (3.5 and $2.5 \mu\text{gml}^{-1}$) on absorbance in case of Thymol blue and Methyl orange with (20 and $7 \mu\text{gml}^{-1}$) of Bisacodyl at λ_{max} 545 and 510 nm

CONCLUSION

The proposed spectrophotometric method provided simple, sensitive, specific and analytical procedures for determination of Bisacodyl either in pure form or in its tablet form without interference from common excipients. The satisfactory sensitivity and reproducibility as well as the convenience and simplicity, make the proposed method suitable for routine analysis in quality control laboratories.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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