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SPECTROPHOTOMETRIC DETERMINATION OF ALBENDAZOLE IN PURE FORM AND TABLET FORM

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ABSTRACT

Four simple sensitive and accurate spectrophotometric methods were developed for determination of Albendazole in pure and in tablet forms. The method (I) depends on reaction of drug and Vanillin reagent in acidic condition, the red colored product was measured λ_{max}. 562 nm. Beer's law was obeyed in the range of 10-110 µgml^{-1}. In method (II) 1,2-Naphthoquinone-4-sulphonate sodium reacts in alkaline mediam through nucleophilic substitution reaction producing an orange-brown colored product showing maximum absorption at 482 nm. Beer's law was obeyed in the range of 0.2-3 µgml^{-1}.Method(III) charge transfer complex was formed between Albendazole and Tetracyanoethylene measured at λ_{max}. 395 nm . Beer's law was obeyed in the range of 0.1-1 µgml^{-1}. Method (IV) based on bromination-oxidation reaction using bromate-bromide mixture with rhodamine B and thymol blue as reagents and measuring the absorbance of the unbleached dye at 555 nm and 545 nm. Beer's law was obeyed in the range 1.5-4.5 µgml^{-1} and 2-16 µgml^{-1} respectively.

KEYWORDS

Albendazole, Vanillin, 1,2-Naphthoquinone-4-sulphonate sodium, Tetracyanoethylene and Bromometric method

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INTRODUCTION

Albendazole (ALB) is chemically known as Methyl [5-(propylthio)-1H-benzoimidazol-2-yl] carbamate Albendazole is official in B.P. 2007[1], which describes a potentiometric titration with perchloric acid in formic acid – acetic acid medium. Albendazole is a benzimidazole drug used for the treatment of a variety of *parasitic worm's* infections[2]. It is widely used as *anthelmentic* having a wide spectrum of activity used for human and animal infections [3, 4]. When administered orally, it is quickly biotransformed into its active intermediate metabolite Albendazole-sulphoxide (ABZSO), which is then oxidized to the inactive form of

Albendazole- sulphone($ABZSO_2$). Because of their affinity for the parasite B-tubulin, both Albendazole and ABZSO show *anthelmentic* activity [5]

A literature survey revealed that Albendazole has been estimated in pharmaceuticals by Titrimetry,[6, 7] UV, spectrophotometry,[8-14]spectrofluorimetry,[15,16] voltammetry[17] and high performance liquid chromatography,[18-21]. The aim of the present work is to develop simple, sensitive and cost-effective spectrophotometric method for the determination of ALB in its pure form and tablet forms.

MATERIALS AND METHODS

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 v5.0.5. Spectronic Genesys® UV-VIS Spectrophotometer connected to an IBM PC computer load with FLWINLAB software

Material and Reagents

All chemicals used are of analytical reagent grade. Albendazole (Eipico Company, Egypt. 99%Purity). Alzental® tablets labeled to contain 200 mg of Albendazole. Batch No. 1302776 (Eipico, Egypt). Vanillin (EL Nasr Pharmaceutical Chemicals Company, Batch No.2011/1).

The solution was prepared by dissolving 4 gm.of Vanillin in 100 ml absolute methanol (99.8%) Sulphuric acid (97-99%, El Nasr pharmaceutical chemicals Company, Batch No.2012/3).

1,2-Naphthoquinon-4-sulphonate (NQS) 0.5%(w/v). 0.5 g of NQS was accurately weighed transferred into a 100 ml calibrated flask, dissolved in 20 ml distilled water, and make up the volume up to the mark with bidistilled water to obtain a solution of 0.5 % (w/v). The solution was freshly prepared and protected from light during the use.

Sodium hydroxide solution (Elgomhouria Chemicals company Batch No .1001/120).

7×10^{-2} M of sodium hydroxide is accurately weighed and transferred into 100 ml volumetric flask and made up to the mark with distilled water.

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 in bidistilled water was prepared.

Rhodamin B (Universal Fine Chemicals, India) 50µg/ml was dissolved in 20 ml bidistilled water then completed to 100 ml with bidistilled water (stable for 2 weeks at least).

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).

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 (Win lab, England) and 1.0 gm of potassium bromide (Win lab, England) in 100 ml bidistilled water (stable for 10 days at least).

Working solution was freshly prepared daily by diluting 5 ml of stock solution (50 µg/ml in case Rhodamine B) and 3.5 ml of stock solution to 100 ml with bidistilled water (35 µg/ml in case of Thymol blue).

General procedures

Preparation of standard drug solutions for methods I, II and III

Stock solutions of Albendazole were prepared by dissolving 100 mg, 20 mg and 20 mg of pure drug in 10 ml concentrated HCL and diluting to 100 ml in calibrated flask with methanol in volumetric flasks for methods I. II. and III, respectively.

Working solutions of lower concentrations (0.2 $mg.ml^{-1}$, and 0.2 $mg.ml^{-1}$.) were prepared by further dilution of stock solutions with methanol for method method II, and method III, respectively.

Preparation of standard drug solutions for method IV

Stock solution of Albendazole was prepared by dissolving 100 mg and 50mg of the pure drug in 10 ml HCl and diluting to 100 ml in calibrated flask with methanol for method IV.1. and IV.2. Working solution of lower concentration (1 $mgml^{-1}$ and 0.5

$mgml^{-1}$) was prepared by further dilution of stock solution with methanol.

Procedures

Method I (vanillin)

To a series of 10 ml calibrated flasks, an increasing volume covering the concentration range (10-110) µg ml^{-1} of Albendazole solution were transferred, followed by addition of 1.5 ml of 4 % Vanillin and 2 ml of concentrated H_2SO_4 with occasional shaking and heated on a water bath 50°C for 15 min. and cooled to room temp, finally the volume was brought up to mark with absolute methanol. The absorbance was measured at 562 nm. Versus 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 Beer's law data.

Method II (1,2-Naphthoquinon-4-sulphonate)

To a series of 10 ml calibrated flasks ,an increasing volume covering the concentration range (0.2-3) µg ml^{-1} of Albendazole solution were transferred, followed by addition of 1ml of 0.5 % NQS and 1ml of 0.07 M NaOH with occasional shaking and left the solution 15 min at room temperature , finally the volume was brought up to mark with bidistilled water. The absorbance was measured at 482nm versus reagent blank. A calibration graph was prepared by plotting the measured absorbance versus concentration.

The concentration of the unknown samples was read from the calibration graph or computed from the regression equation derived using the Beers data.

Method III (Tetracyanoethylene)

To a series of 10 ml calibrated flasks, an increasing volume covering the concentration range (0.1-1) µg ml^{-1} of Albendazole solution were transferred, followed by addition of 1ml of 5×10^{-3} M Tetracyanoethylene and 1ml of NaHCO3 with occasional shaking and diluted to mark with bidistilled water. The solution was then left for 10 min in ice bath. The absorbance was measured at 395 nm versus 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 data.

Method IV.1 (Rhodamin B)

To 0.6 ml bromate - bromide working solution in 10 ml volumetric flasks, add (1.5- 4.5 µgml^{-1}) of Albendazole solution then acidify using 0.4 ml of 5 M HCl, close flasks and stand for 10 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 555 nm.

Method IV.2 (Thymol blue)

To 1.2 ml bromate - bromide working solution in 10 ml volumetric flasks, add (2-16) µg ml^{-1} of Albendazole solution then acidify using 0.2 ml 5 M HCl, 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 545nm

Pharmaceutical preparation

For Alzental tablets

Twenty tablets of Alzental tablets were weighed and finely powdered. An accurately weighed amount of the powder equivalent to the concentration of Albendazole in method I, II, III, IV.1 and IV.2 were dissolved in 10 ml of hydrochloric acid, 20 ml methanol in beaker and stirred for about 5-10 min, filtered through whatman filter paper to remove the in soluble matter. The residue was washed with 10 ml portions of methanol three times, the filtrate collected and completed with methanol to 100 ml in a volumetric flask. Aliquots from these solutions equivalent to those in authentic samples were used for the application of the proposed methods applying standard addition techniques.

RESULTS AND DISCUSSION

Method I:

Enamine is formed by a condensation reaction of secondary amine and an aldehyde or ketone in the presence of an acid catalyst[22]. The formation of enamine forms the basis for the spectrophotometric determination of compound of pharmaceutical significance. Vanillin, an aromatic aldehyde, has been applied to quantification of drug with

secondary amino group in acidic medium. The proposed method is based on the formation of chromogenic enamine between the secondary amine group of Albendazole and aldehyde group of Vanillin. The most probable condensation step for the formation of enamine between drugs and Vanillin is presented in Scheme No.1. The absorption spectrum of the chromogen formed between Albendazole and Vanillin was recorded between 450 nm to 650 nm against respective reagent blank are shown in Figure No.2. The red-colored enamine exhibits λmax at 562 nm of Albendazole. The reagent blank showed negligible absorbance at 562 nm.

Method II

The NQS reagent reacts with Albendazole contain of secondary amino group, it involves in yielding colored produced by nucleophilic displacement of the sulfonic acid group of 1,2-Naphthoquinon-4-sulfonic acid in alkaline medium, Scheme No.2. The selected drugs were found to react instantaneously with NQS under the experimental conditions, to form an orange colored product exhibiting λ_{max} at 482 nm and shown in Figure No.3. Under the optimum reaction conditions the absorbance was found to obey Beers Law.

Method III (TCNE)

Albendazole is easy to be determining by spectrophotometry based on color charge transfer (CT) complexes with TCNE.

The absorption spectrum of the complex formed between albendazole as electron donors and TCNE as electron acceptors was recorded between 250 nm to 500 nm against respective reagent blank and shown in Figure No.4. The yellow-colored exhibits λ_{max} at 395 nm of Albendazole. The reagent blank showed absorbance at 330 nm.

Method IV

The proposed spectrophotometric methods are indirect and are based on the determination of the residual bromine (insitu generated) after allowing the reaction between Albendazole and a measured amount of bromine to be complete. The surplus bromine was determined by reacting it with a fixed amount of either Rhodamine B or Thymol blue dyes. 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 λmax is observed with increasing concentration of 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[25].

$$5Br^- + BrO_3^- + 6H^+ \longrightarrow 3Br_2 + 3H_2O$$

Absorption spectra

Absorption spectrum for determination of Albendazole was studied over range of 200 – 800 nm. After oxidation of drug and portions of dyes with bromine, residual unoxidized Rhodamine B and Thymol blue are absorbed at 555 and 545 nm Figure No.5.

Factors for method I (vanillin)

i- Effect of the reagent concentration

Vanillin is slightly soluble in water but soluble in absolute methanol. The effect of Vanillin on the sensitivity of the reaction was studied. It was observed that when 0.5-3 ml of 4% (w/v) was examined, 1.5ml Vanillin give maximum chromogen red colored product Figure No.6.

ii- Effect of acid volume

The reaction was very slow in dilute acid medium, thus concentrated sulphuric acid was used .The intensity of red colored product was found to be maximum on using 2 ml of sulphuric acid Figure No. 7.

iii- Effect of time

The color development slowly at room temperature, on heating the reaction mixture on thermostatically controlled water-bath an increase in the intensity of the produced color was observed, the absorbance reached maximum after heating in a water bath at (55±5°C) for 15 min and remained stable for at least 30 min .

Factors for method II (NQS)

i- Effect of the reagent concentration

NQS is soluble in water. The effect of changing the concentration on the absorbance of solution containing a fixed amount of Albendazole was studied. It is evident that the absorbance increases with increasing NQS concentration and reached maximum on using 1ml of 0.5 % (w/v) NQS achieves a suitable volume for maximum color intensity Figure No.8.

ii- Effect of alkalinity

Different bases of different molarity such as sodium hydroxide, potassium hydroxide of 7×10^{-2} M (0.28 g) concentration were examined in order to obtain high sensitivity. It was found that 1 ml of sodium hydroxide gave maximum color intensity of Albendazole and beyond these amounts, the absorbance would be decreased. Therefore, 1 ml of 7×10^{-2}M was chosen as the optimum concentration of sodium hydroxide Figure No.9.

iii- Effects of temperature and reaction time

The reaction time was determined by following the color development at room temperature and thermostatically controlled water-bath at different temperatures. It was observed that the absorbance reached maximum after leaving the solution 10 min at room temperature .This temperature and reaction time were chosen for color development. It was found that the absorbance of the chromogen remained stable for at least 1 hour.

Factors for method III (TCNE)

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 was 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 drug was 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. 10.

iii- Effect of base

Different bases such as sodium hydroxide, potassium hydroxide, sodium carbonate and sodium bicarbonate of 1×10^{-3}M concentration were examined in order to obtain high sensitivity. It was found that 1.0 ml of sodium bicarbonate gave maximum color intensity and beyond these amounts, the absorbance would be decreased. Therefore, 1 ml of 1×10^{-3}M was chosen as the optimum concentration of sodium bicarbonate Figure No.11.

iv- Effect of time and temperature

The reaction time was determined by following the color development in ice-bath, at room temperature and thermostatically controlled water-bath at different temperatures. It was observed that the absorbance reached maximum after 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 IV

i- Effect of 5 M HCl volume

5 M HCl was used throughout experiments and it was found that 0.2 ml with Thymol blue and 0.4 ml with Rhodamine B is the appropriate acid volume and increasing HCl volume results in a decrease in absorption Figure No.12.

ii- Effect of concentration of bromate-bromide mixture:

Bromate - bromide concentration was studied by varying the reagent volume while other factors were held constant. It was found that 35 µg ml^{-1}(1.2 ml) and 50 µg ml-1 (0.6 ml of bromine was sufficient for its bleaching action in case of Thymol Blue and Rhodamine B using these stated concentrations. Figure No.13.

iii- Effect of the reaction time:

Time required to brominates 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 with Rhodamine B and Thymol blue while contact times up to 25 minutes had been examined and no further bromination was

detected. A contact time of 2 minutes *(*in case of Rhodamine B) and 4 minutes(in case of Thymol blue) 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 methods

The validity of the proposed methods was tested regarding linearity, range, limits of detection, limits of quantification, accuracy, precision, robustness and specificity according to ICH recommendations [26].

Linearity and range

The calibration graphs obtained by plotting the values of the absorbance versus the final concentrations (µg/ml) were found to be rectilinear over the concentration ranges cited in the Table No.1.

The calibration graph was described by the equation:

$Y = a + bX$

(Where Y=absorbance, a=intercept, b=slope, X=concentration in µg/ml).

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

Limits of detection and limits of quantification

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

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

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

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

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 Tables No.2 and 3.

Accuracy and precision

Accuracy was evaluated as percentage relative error between the measured concentration for albendazole. The accuracy of the proposed methods was checked by performing recovery experiments of the dosage forms through standard addition technique. The results are shown in Tables No.4, 5 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 were repeated five times of albendazole and 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. 7.

Robustness and Ruggedness

Robustness of the method was examined by small changes in the method variables such as change in the volume of the reagent (±0.05ml), change in bromated- bromine mixture and dye (±0.05ml), change in volume of base (±0.05ml), change in reaction time (±2min) and change in the volume of the acid (±0.05 ml). The results are listed in Table No. 8.

The ruggedness was analysis by two different analyst and on two different instruments by same analyst. The intermediate precision, expressed as % RSD, which is a measure of ruggedness was within the acceptable limits as shown in the Table No .9.

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

Analysis of pharmaceutical preparations:

The proposed methods were applied to the analysis of the drug in dosage forms and the results were statistically compared with reference method, [8]by calculating Student's *t*- test 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.6.

Table No.1: Analytical parameters for spectrophotometric determination of Albendazole through the proposed methods

Parameters		Method I (Vanillin)	Method II (NQS)	Method III (TCN)	Method IV.1. (Rhodamine B)	Method IV.2. (Thymol blue)
λmax, nm		562nm	482nm	395nm	555 nm	545 nm
Volume of H_2SO4, ml		2ml	.-	-	-	-
Volume of 1×10^{-3} $MNaHCO_3$(ml)		-	-	1ml	-	-
Volume of 5M HCl (ml)		-	-	-	0.4ml	0.2ml
Volume of bromate-bromide mixture (ml)		-	-	-	0.6ml	1.2 ml
Volume of 0.07M NaOH(ml)		-	1ml	-	-	-
Reagent Conc.		4%*w/v*	0.5%*w/v*	0.064%*w/v*	50µg/ml	100 µg/ml
Time required to oxidize the drug before dye addition (min.)		-	-	-	10 min.	10 min.
Time required to irreversibly oxidize the dye (min.)		-	-	-	2 min.	4min.
Reagent volume (ml)		1.5 ml	1ml	1 ml	1 ml	1 ml
Temperature (^oC)		55±5**°C**	25±5**°C**	Ice bath	-	-
Reaction time (minutes)		15 min.	15 min.	10 min.	-	-
Diluting solvent		Methanol	bidistilled water	bidistilled water	bidistilled water	bidistilled water
Beer's law limits (µg.ml^{-1})		10-110 µg/ml	0.2-3 µg/ml	0.1-1 µg/ml	1.5-4.5 µg/ml	2-16 µg/ml
Regression equation*	**Slope (b)**	0.0065	0.299	0.878	0.251	0.0433
	Intercept (a)	0.0564	0.057	0.044	-0.235	0.0498
Correlation coefficient		0.9999	0.999	0.999	0.999	0.999

*A = a + bC where A is absorbance, C is the concentration of the drug in µgml^{-1}

Table No.2: Statistical data for the determination of Albendazole with Vanillin, NQS and TCNE

Parameters	Method I (vanillin)			Method II (NQS)			Method III (TCNE)		
	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 %**
	10	9.9384	99.38	0.2	0.20067	100.34	*0.1*	0.10157	101.57
	30	29.9384	99.79	0.6	0.60134	100.22	0.2	0.20177	100.89
	50	50.2462	100.49	1	0.9987	99.87	0.3	0.30426	101.42
	70	70.4	100.57	1.4	1.40267	100.19	0.4	0.39877	99.69
	90	89.6308	99.59	1.8	1.80334	100.19	0.5	0.49898	99.79
	110	110.2461	100.22	2.2	2.18064	99.12	0.6	0.59349	98.91
				2.6	2.604675	100.18	0.7	0.69711	99.59
				3	3.00534	100.18	0.8	0.79617	99.52
							0.9	0.89638	99.59
							1	1.0114	101.14
Mean*			100.01			100.03			100.2
N			6			8			10
SD			0.4914			0.393			0.9424
RSD			0.4913			0.392			0.9404
SE			0.2005			0.139			0.00298
Variance			0.2415			0.154			0.8882
LOD, µgml^{-1}			2.820			0.0564			0.0238
LOQ, µgml^{-1}			9.401			0.1878			0.0795
Sandell's sensitivity(µgml^{-1}per 0.001A)			0.0694			0.0026			0.00048
Apparent Molar absorbitivity** $LMol^{-1}cm^{-1}$			2193.25			98906.41			268324.2

*Mean of three different experiments. **Calculated in the basis of molecular weight of the drug.

Table No.3: Statistical data for the determination of Albendazole using Rhodamine B and Thymol blue

Parameters	Method IV.1. (Rhodamine B)			Method IV.2. (Thymol blue)		
	Conc. taken µg/ml	Conc. found µg/ml	Recovery %	Conc. taken µg/ml	Conc. found µg/ml	Recovery %
	1.5	1.5019	100.13	2	2.0139	100.70
	2	1.9920	99.6	4	3.9769	99.42
	2.5	2.4780	99.12	6	6.0554	100.92
	3	3.0159	100.53	8	7.9723	99.65
	3.5	3.5179	100.51	10	10.0046	100.05
	4	4.0119	100.30	12	11.9215	99.35
	4.5	4.4781	99.51	14	14.0231	100.17
				16	16.0323	100.20
Mean recovery*			99.95			100.05
N			7			8
SD			0.5483			0.5682
RSD			0.545			0.5678
SE			0.2069			0.2007
Variance			0.3006			0.3228
LOD, µgml-1			0.3998			0.4840
LOQ, µgml-1			1.332			1.6132
Sandell's sensitivity (µgcm-2)			0.0112			0.03395
Apparent Molar absorptivity** LMol-1cm-1			42894.96			13739.79

*Mean of three different experiments. **Calculated in the basis of molecular weight of the drug.

Table No.4: Application of standard addition technique the determination of Albendazole in tablet form using Vanillin, NQS and TCNE

Items	**Alzental**											
	Vanillin				NQS				TCNE			
	Conc. added form pure drug (µg/ml)	Conc. taken from Alzental (µg/ml)	Conc. found (µg/ml)	Recovery* %	Conc. added form pure drug (µg/ml)	Conc. taken from Alzental (µg/ml)	Conc. found (µg/ml)	Recovery* %	Conc. added form pure drug (µg/ml)	Conc. taken from Alzental (µg/ml)	Conc. found (µg/ml)	Recovery* %
	10	0	10.00	100	0.2	0	0.1986	99.3	0.1	0	0.09960	99.6
	10	10	20.00	100	0.2	0.2	0.4023	100.58	0.1	0.1	0.19920	99.6
	10	20	29.91	99.7	0.2	0.4	0.6044	100.73	0.1	0.2	0.30146	100.49
	10	30	39.75	99.38	0.2	0.6	0.8098	101.23	0.1	0.3	0.40239	100.60
	10	40	50.08	100.16	0.2	0.8	1.0135	101.35	0.1	0.4	0.49801	99.60
Mean*				99.85				100.65				99.98
N				5				5				5
S.D.				0.3111				0.8087				0.5167
R.S.D.				0.3115				0.8035				0.5168
V				0.09679				0.6540				0.2670
S.E.				0.1391				0.3616				0.2311

*Mean of three different experiments

Table No.5: Application of standard addition technique for the determination of Alzental in pharmaceutical dosage forms through reaction with Rhodamine B and Thymol blue

Items	Alzental® tablets							
	Rhodamine B				Thymol blue			
	Conc. added form pure drug (µg/ml)	Conc. taken from Alzental(µg/ml)	Conc. found (µg/ml)	Recovery* %	Conc. added form pure drug (µg/ml)	Conc. taken from Alzental(µg/ml)	Conc. found (µg/ml)	Recovery* %
	1.5	0	1.5197	101.31	2	0	2.0139	100.70
	1.5	0.5	2.0039	100.20	2	2	3.9769	99.42
	1.5	1	2.4825	99.3	2	4	6.0554	100.92
	1.5	1.5	3.0157	100.52	2	6	7.9723	99.65
	1.5	2	3.5118	100.34	2	8	10.0046	100.05
Mean*				100.34				100.89
N				5				5
S.D.				0.697				0.988
R.S.D.				0.694				0.979
V				0.485				0.976
S.E.				0.312				0.443

*Mean of three different experiments.

Table No.6: Statistical data for the determination of pharmaceutical tablets of Alzental through the proposed methods compared with the reference method[8]

S.No	Statistics	Reference method[(8)]	(Alzental® tablet)				
			Vanillin	NQS	TCNE	Rhodamine B	Thymol blue
1	Mean recovery*± SD	100.17±0.537	99.85±0.311	100.65±0.809	99.98±0.517	100.34±0.697	100.89±0.988
2	N	5	5	5	5	5	5
3	Variance	0.288	0.0968	0.6540	0.2670	0.485	0.976
4	S.E.	0.244	0.1391	0.3616	0.2310	0.312	0.443
5	*t-test***	----	1.1531(2.306)[a]	1.106(2.306)[a]	0.571(2.306)[a]	0.4320(2.306)[a]	1.432(2.306)[a]
6	F-ratio**	----	2.982(5.05)[b]	2.269(5.05)[b]	1.08(5.05)[b]	1.685(5.05)[b]	3.389(5.05)[b]

* Average of three experiments. a and b are Theoretical Student *t-test* and F- ratio at p=0.05.

Table No.7: Results of the intraday and interday precision for the determination of Albendazole with Vanillin, NQS, TCNE, Rhodamine B and Thymol blue methods

S.No	Item	conc.ug/ml	Intraday		Interday	
			mean ± SD	RSD	mean± SD	RSD
1	Vanillin	10 µg/ml 70 µg/ml	99.93± 0.441 99.87±0.508	0.442 0.509	100.09±0.644 100.01±0.639	0.643 0.638
2	NQS	0.2 µg/ml 3µg/ml	100.03±0.598 9 100.07±0.479	0.598 0.478	99.89±0.842 100.02±1.264	0.840 1.257
3	TCNE	0.1 µg/ml 0 .4µg/ml	100.05±0.836 100.10±0.559	0.835 0.558	101.01±1.039 99.80±1.455	1.001 1.463
4	Rhodamine B	3 µg/ml 4.5 µg/ml	100.12 ± 0.897 100.03±1.574	0.895 1.568	99.90 ± 0.904 99.74±0.505	0.904 0.506
5	Thymol blue	6 µg/ml 10 µg/ml	100.00± 0.634 100.16± 0.751	0.633 0.744	100.35±1.67 100.17±1.007	1.66 1.008

Table No.8: Results of the robustness for the determination of Albendazole with Vanillin, NQS, TCNE, Rhodamine B and Thymol blue methods

S.No	*Robustness*					
	Recovery %± SD					
	Item	**Vanillin**	**NQS**	**TCNE**	**Rhodamine B**	**Thymol blue**
1	**Reagent + 0.05ml**	99.41±0.524	100.19±0.452	101.66±0.803	98.03±0.375	100.7±0.653
2	**Reagent - 0.05 ml**	98.39± 0.825	98.04± 0.796	100.28± 0.573	100.05±0.589	99.5±0.921
3	**Br_2 + 0.05 ml**	-	-	-	100.13±0.511	101.048±0.763
4	**Br_2 - 0.05 ml**	-	-	-	101.86±0.934	100.32±0.576
5	**Acid + 0.05 ml**	99.93±0.451	-	-	101.19±0.723	100.09±0.588
6	**Acid-0.05 ml**	98.56± 0.767	-	-	100.17±0.518	98.01±0.743
7	**Base+0.05 ml**	-	99.97±0.585	98.20±0.869	-	-
8	**Base-0.05 ml**	-	101.81± 1.0015	101.43± 0.748	-	-
9	**Time+2 min.**	99.24± 0.564	99.48± 0.432	99.35± 0.620	101.46±0.802	99.01±0.673
10	**Time-2 min.**	98.74 ± 0.0.712	98.28 ± 0.0.724	100.51 ± 0.590	99.60±0.523	101.32±0.725

Table No.9: Results of Ruggedness for the determination of Albendazole using Vanillin and TCNE methods

S.No	Item	Recovery% ± SD	
1	Vanillin	50 µg/ml	99.26±0.507
		110 µg/ml	100.78±0.583
2	TCNE	0.1 µg/ml	100.69±0.568
		0.4 µg/ml	100.05±0.548

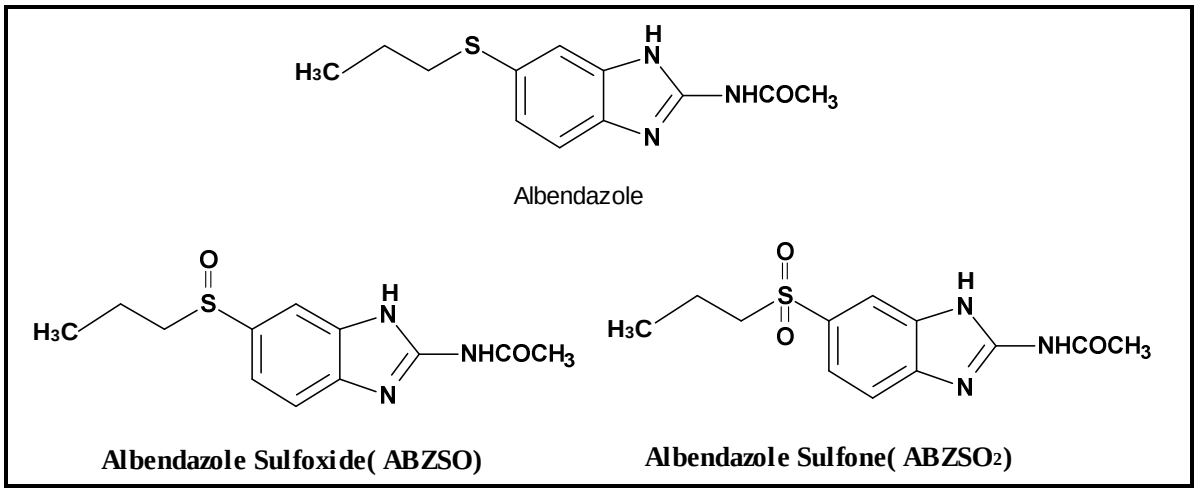
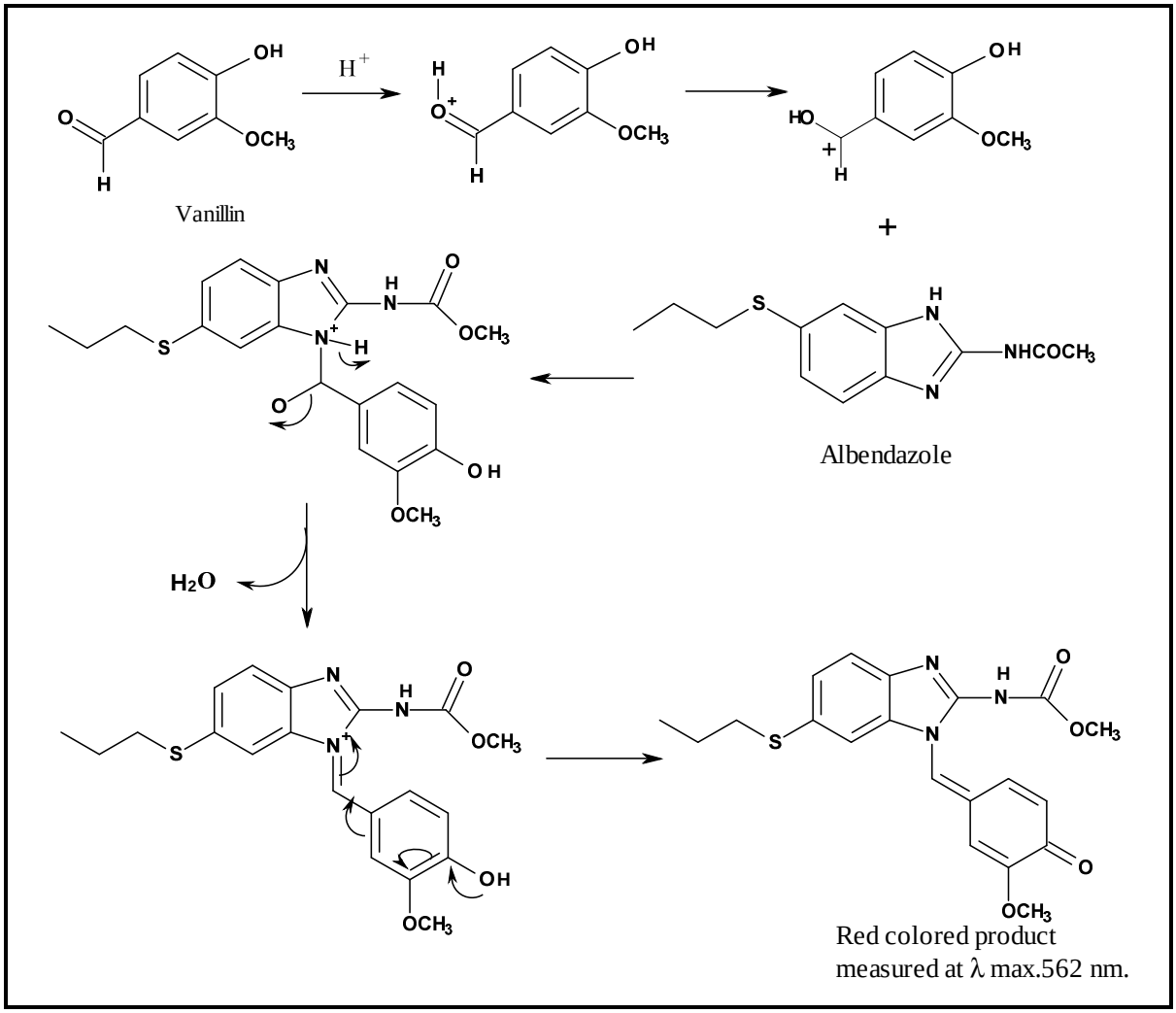
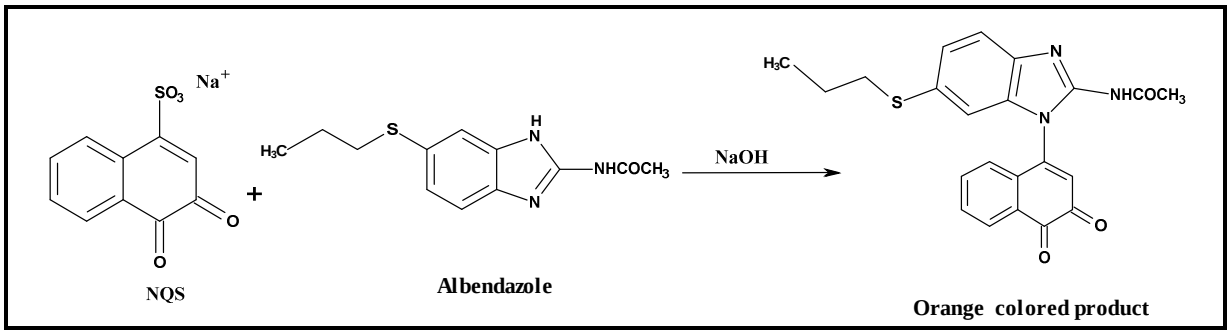


Figure No.1: Structure of Albendazole



Scheme 1: Proposed reaction mechanism between Albendazole and Vanillin



Scheme 2: Proposed reaction mechanism between Albendazole and NQS (24)

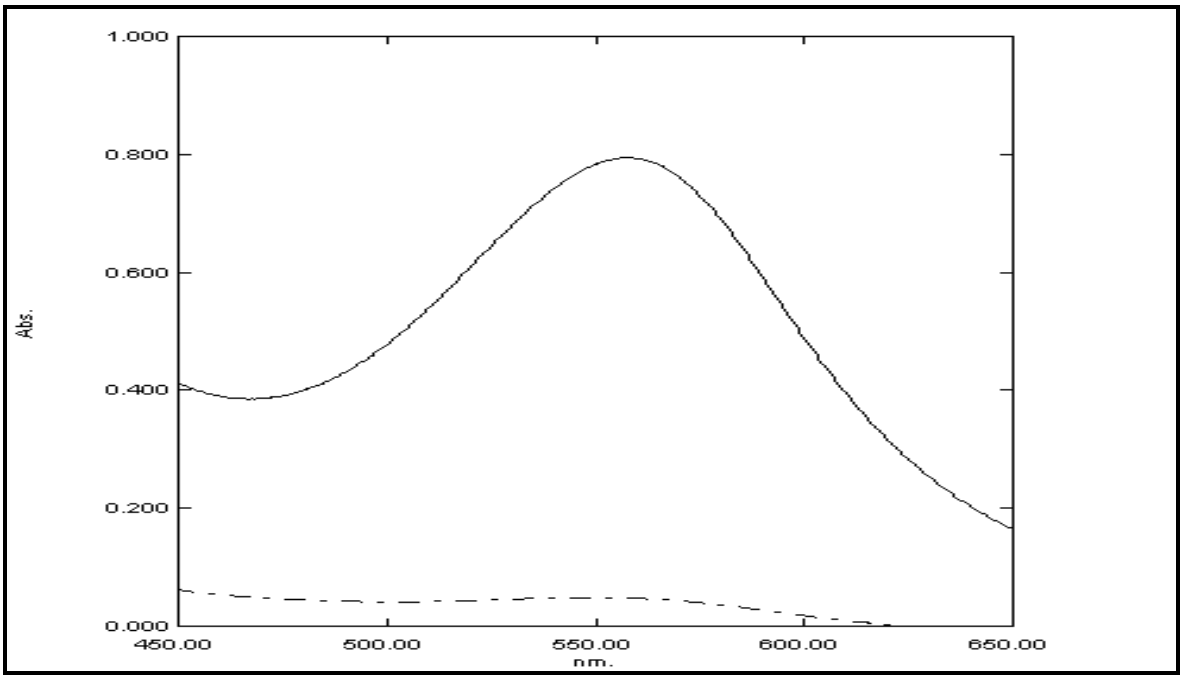


Figure No.2: Absorption spectra for the reaction between Vanillin and Albendazole (110 µgml^{-1}) at showing λ_{max}562 nm against reagent blank

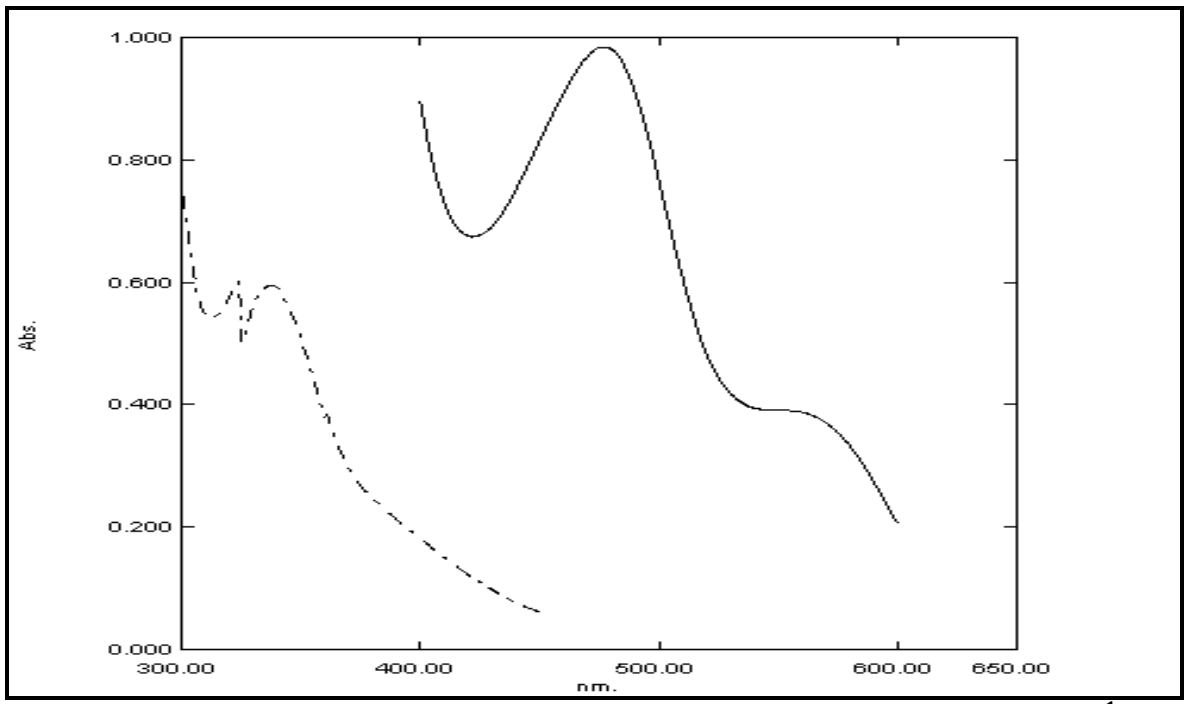


Figure No.3: Absorptions spectra for the reaction between NQS and Albendazole (3 µgml^{-1}) at showing λ_{max}482 nm against reagent blank

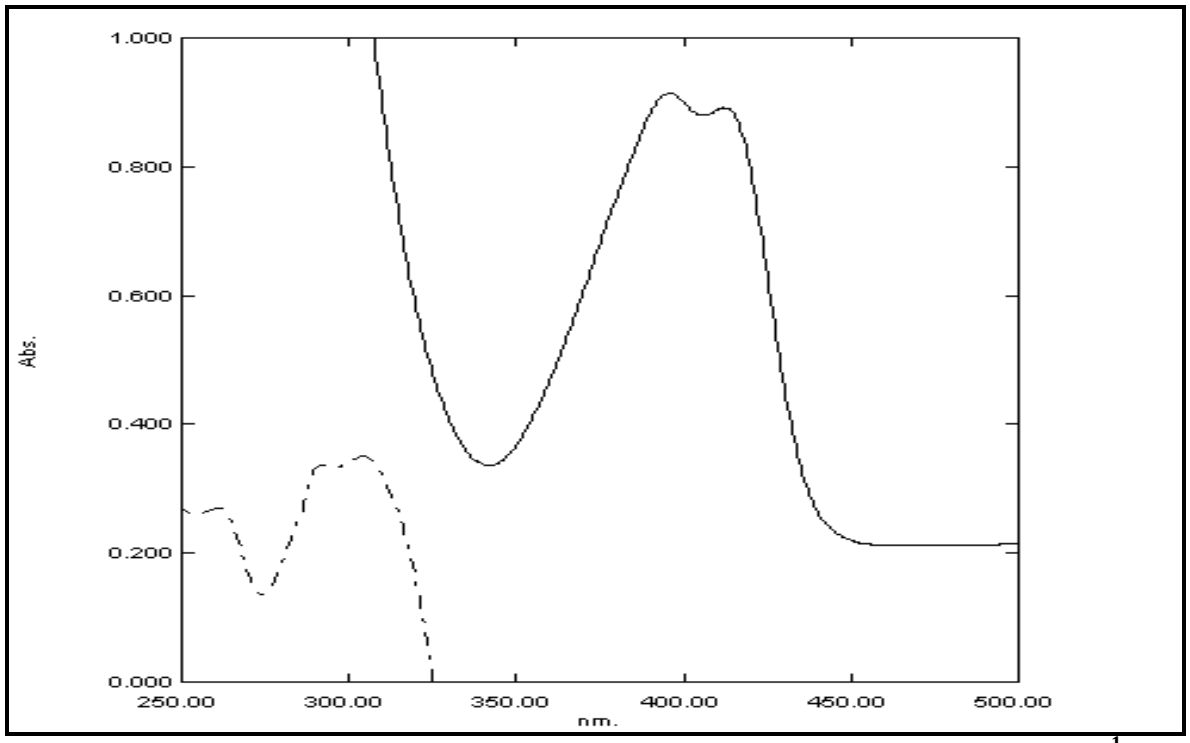


Figure No.4: Absorption spectra for the reaction between TCNE and Albendazole (1 µgml^{-1}) at showing λ_{max}395 nm against reagent blank

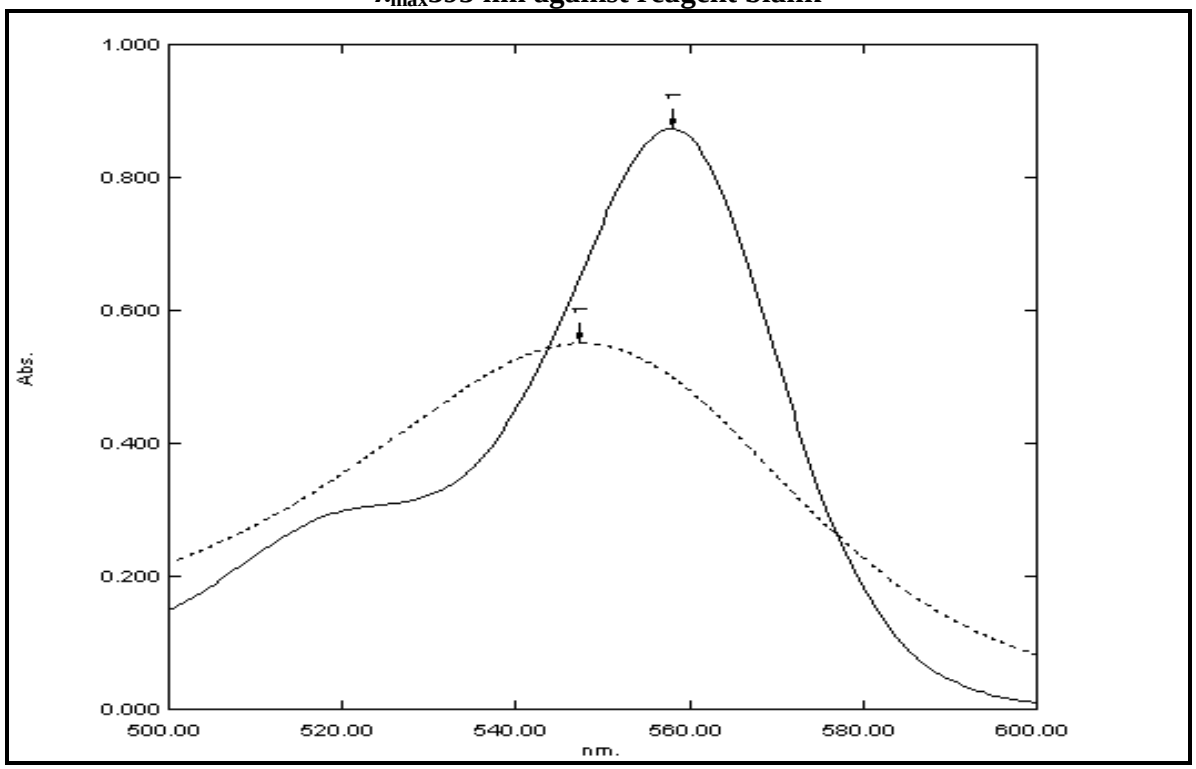


Figure No.5: Absorption spectra for the reaction between Rhodamine B with Albendazole (4.5 µgml-1) at λ max555 nm and Thymol blue with Albendazole (12 µgml-1) at λ max545 nm

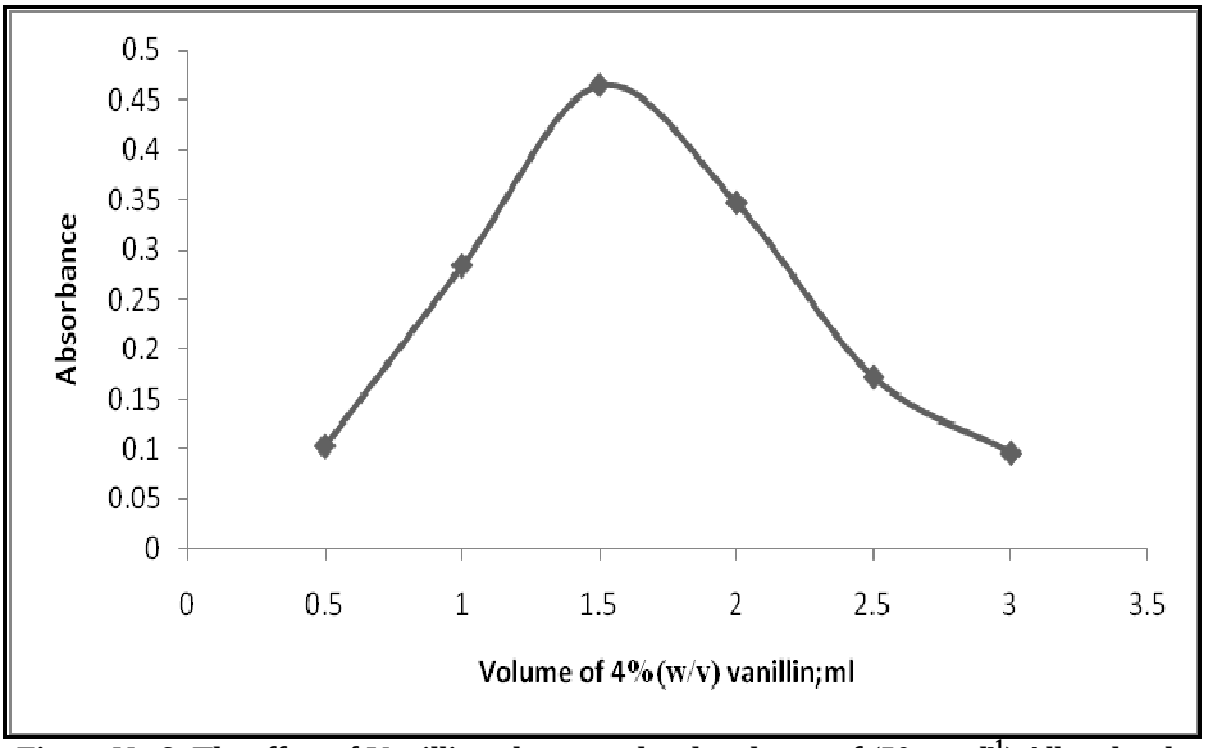


Figure No.6: The effect of Vanillin volume on the absorbance of (50 µgml^{-1}) Albendazole

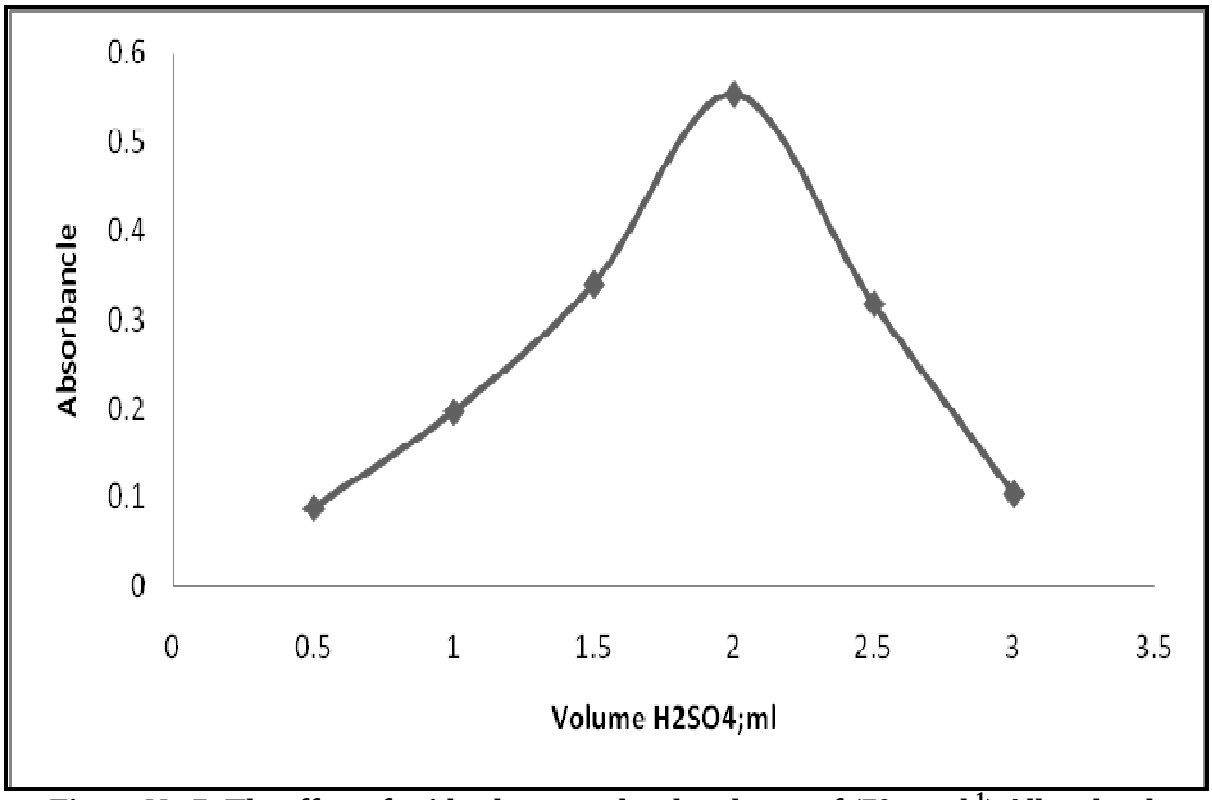


Figure No.7: The effect of acid volume on the absorbance of (70 µgml^{-1}) Albendazole

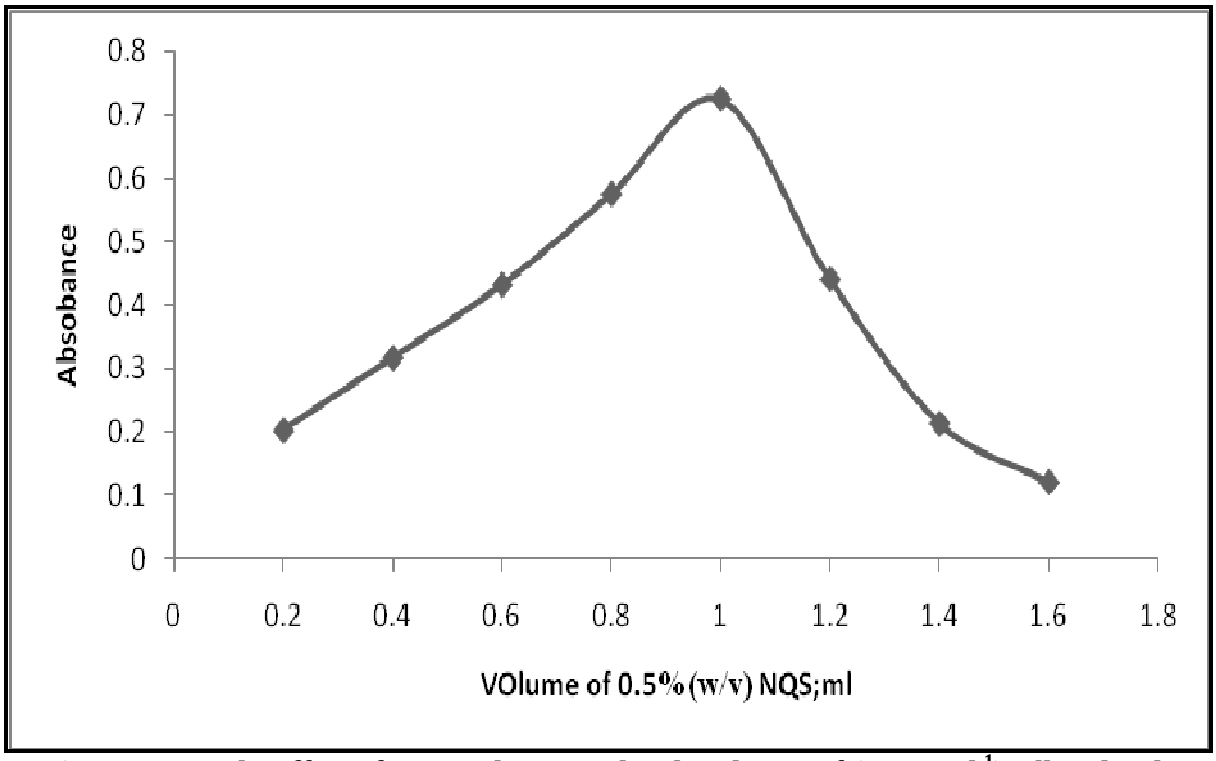


Figure No.8: The effect of NQS volume on the absorbance of (2.2 µgml^{-1}) Albendazole

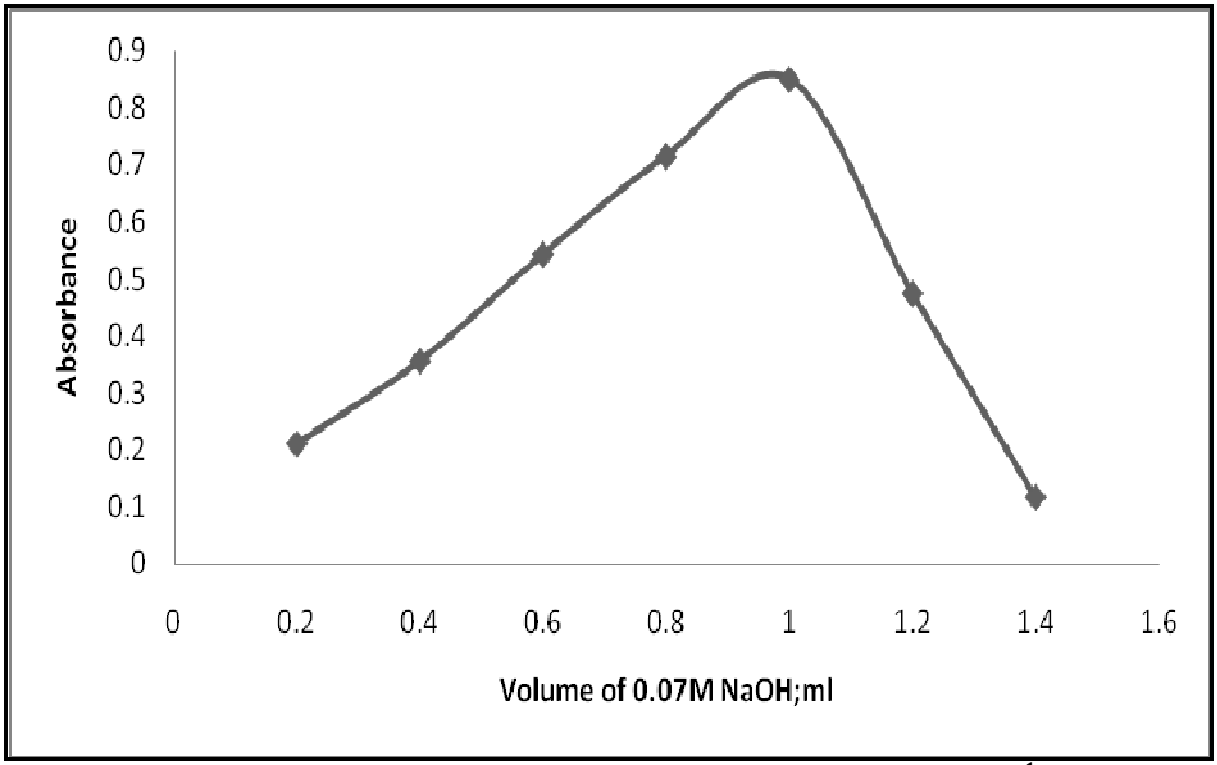


Figure No.9: The effect of base volume on the reaction of NQS with(2.6 µgml^{-1}) Albendazole

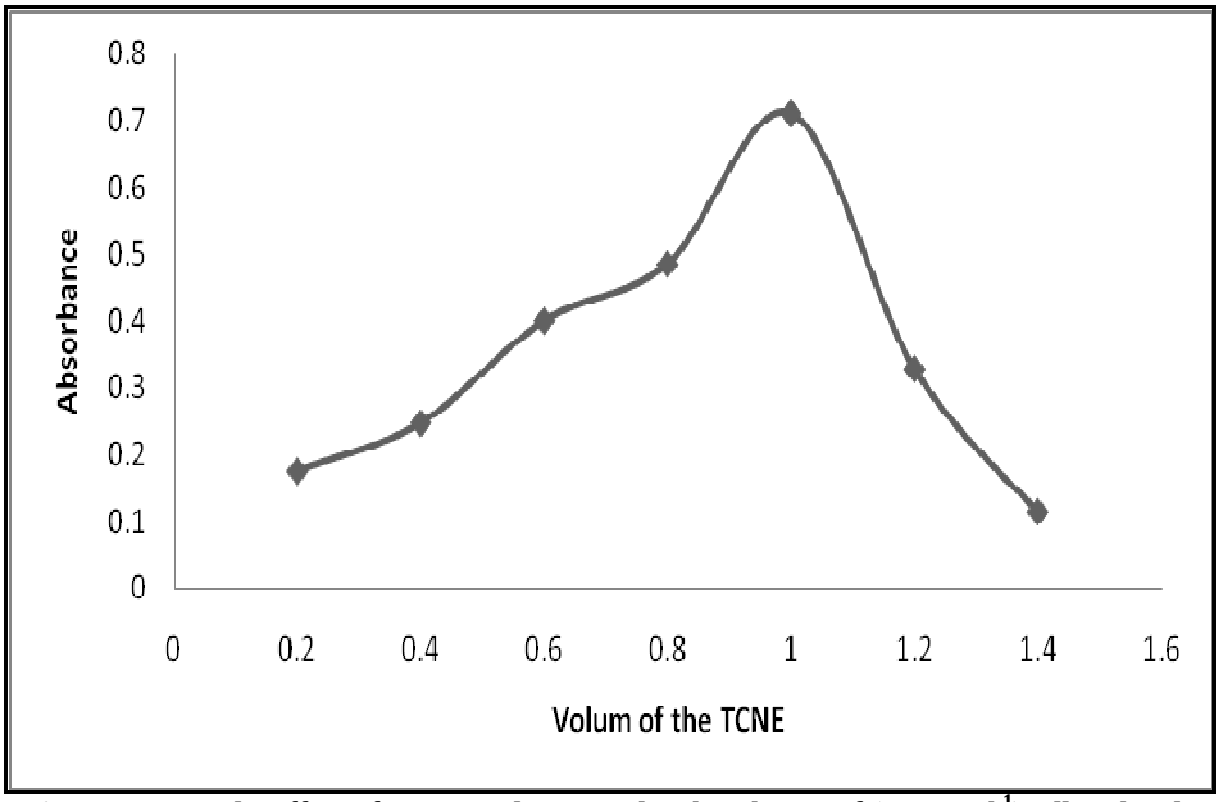


Figure No.10: The effect of TCNE volume on the absorbance of (0.8 µgml^{-1}) Albendazole

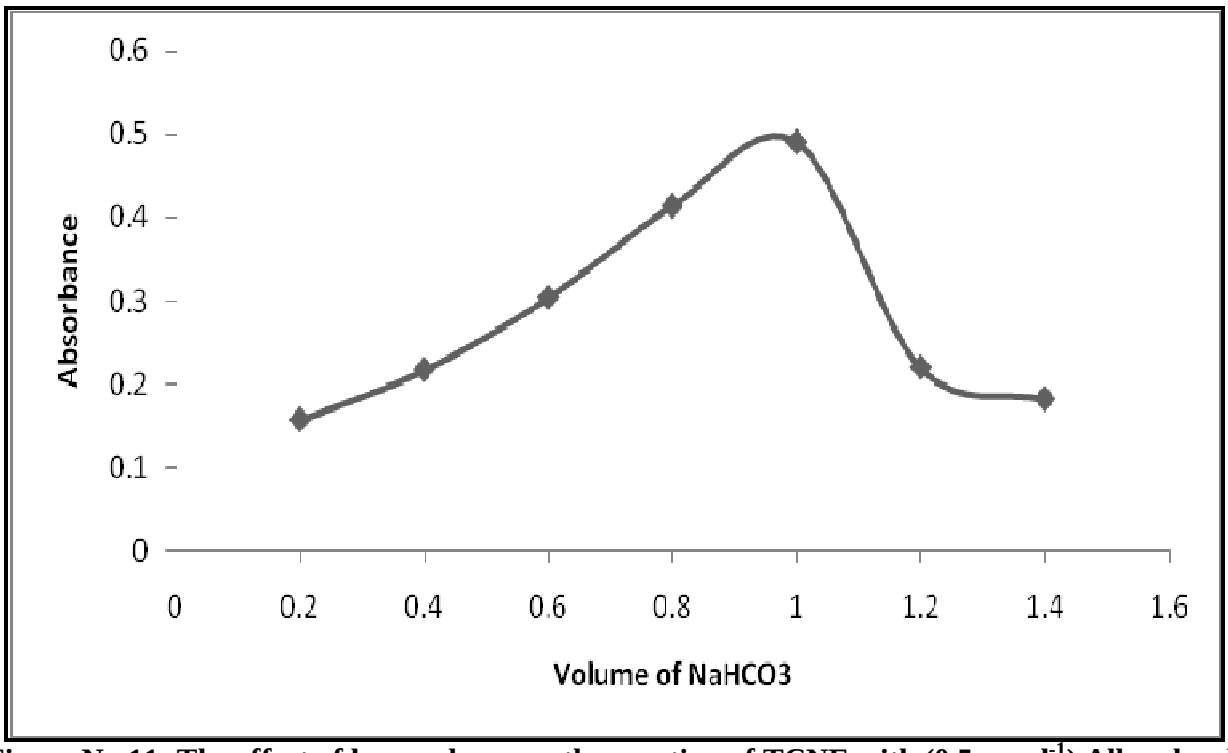


Figure No.11: The effect of base volume on the reaction of TCNE with (0.5 µgml^{-1}) Albendazole

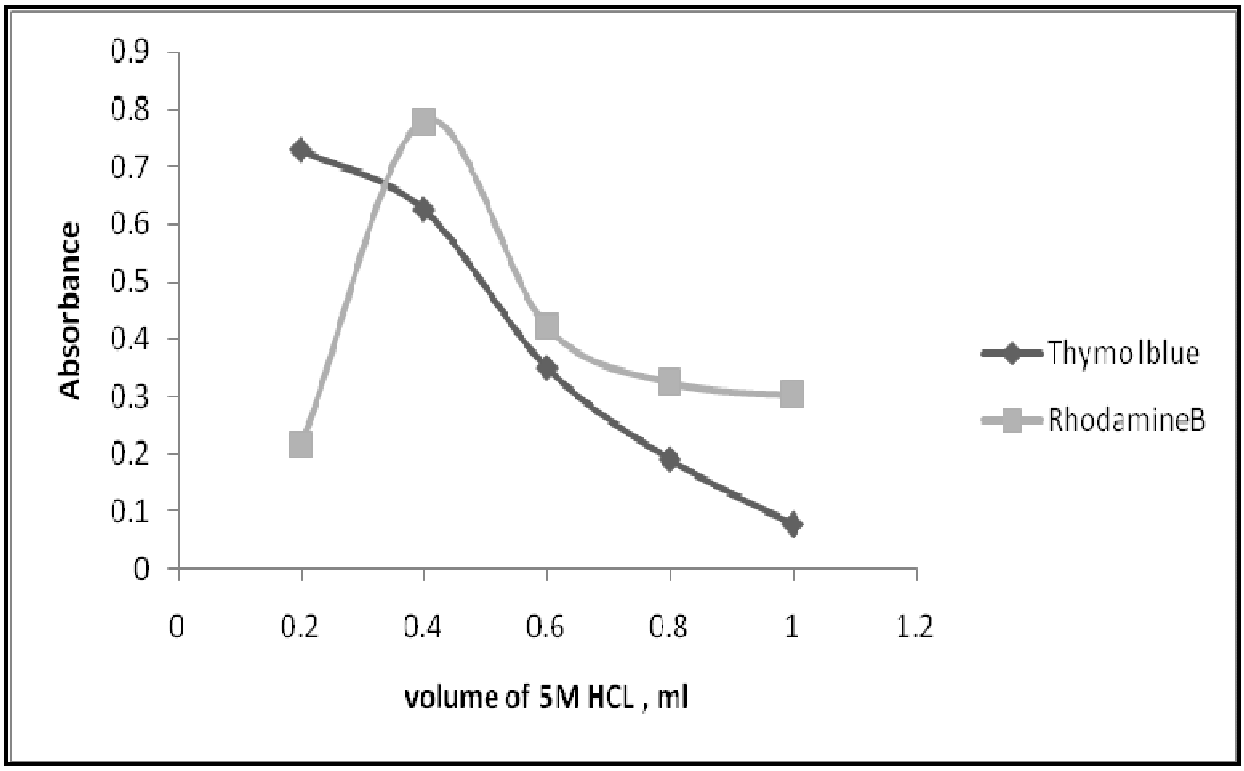


Figure No.12: Effect of volume 5M of HCl on absorbance of Thymol blue Rhodamine B with Albendazole (16 and 4 µg/ml) respectively

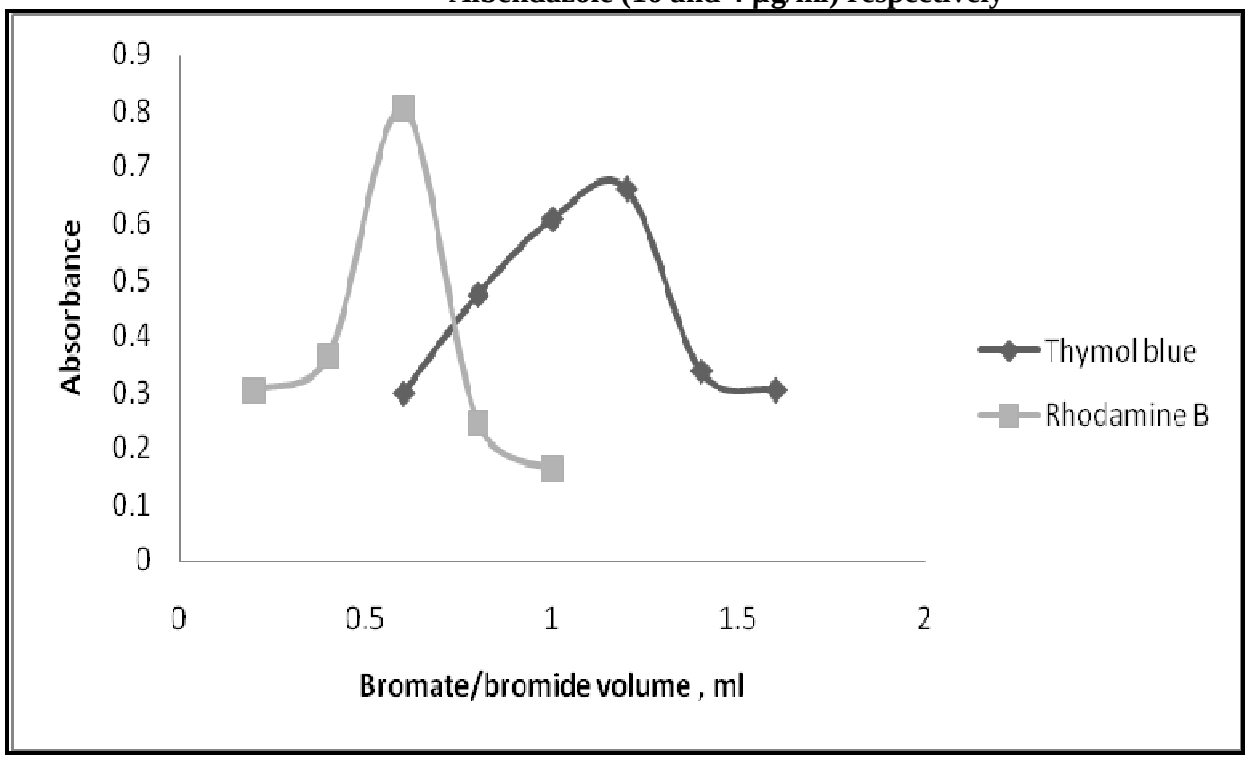


Figure No.13: Effect of volume Bromate-Bromide mixture (35 and 50µg/ml) on absorbance of Thymol blue and Rhodamine B with Albendazole (14 and4 µg/m) respectively

CONCLUSION

The proposed spectrophotometric method provided simple, sensitive, specific and analytical procedures for determination of the cited drug 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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