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Research Article

SPECTROPHOTOMETRIC DETERMINATION OF TRIMETAZIDINE USING ACIDIC TRIPHENYL METHANE DYES

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ABSTRACT

Simple and sensitive extractive spectrophotometric methods have been described for the determination of Trimetazidine in pure and pharmaceutical formulations. The developed methods involve formation of coloured chloroform extractable ion-pair complexes of Trimetazidine with bromocresol green (BCG), bromophenol blue (BPB), bromothymol blue (BTB) and bromocresol purple (BCP) in acidic medium. The extracted complexes showed absorbance maxima at 410, 417, 414 and 408nm with use of the cited dyes respectively. The stoichiometry of the complex is found to be 1:1 in each case. Beer's law is obeyed in the concentration ranges 2.5-25µg/ml with all the four dyes. 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 four methods. All the four methods have been validated as per the guidelines of ICH. The methods have been applied to the determination of Trimetazidine in commercial tablets and results of analysis were validated statistically through recovery studies.

Keywords: Trimetazidine, Spectrophotometry, Triphenyl methane dyes and Ion-pair complex.

1.0 INTRODUCTION

Trimetazidine, chemically, 1-[(2,3,4-trimethoxyphenyl)methyl] piperazine dihydrochloride (**Figure 1**) is a clinically effective antianginal agent used in the prophylaxis and management of angina pectoris, and in the treatment of Meniere's disease in which patient feels hearing loss affecting inner ear[1-6]. The antianginal efficacy of Trimetazidine is comparable to propranolol but it does not reduce cardiac rate–pressure product or coronary blood flow[7].

The literature survey revealed that a considerable number of methods were reported for the quantification of Trimetazidine in biological fluids and pharmaceutical preparations. These methods include : HPLC with electrochemical detection[8], GC-MS[9], HPTLC[10], RP-HPLC[11], UV spectrophotometric method[12-14], Slow injection chemiluminescence[15], Voltammetry[16], and by LC-MS[17,18]. Spectrophotometric method for determination of Trimetazidine in formulation using chloranil as chromogenic agent has been reported[19]. Study of pharmacokinetics and bioequivalence of trimetazidine dihydrochloride tablets in healthy population[20] and Stability-indicating determination of trimetazidine dihydrochloride in the presence of two of its related substances using a direct GC/MS method[21] are also available in the literature. Validation of spectrophotometric dissolution method[22] for modified release trimetazidine pharmaceutical dosage form was also reported.

Reviewing the literature also revealed that nothing has been published concerning the spectrophotometric determination of Trimetazidine using triphenyl methane dyes viz., bromocresol green (BCG), bromophenol blue (BPB), bromothymol blue (BTB) and bromocresol purple (BCP). The developed methods are based on the formation of coloured chloroform extractable ion-pair complexes of Trimetazidine with acidic triphenyl methane dyes.

2.0 EXPERIMENTAL

2.1 INSTRUMENTS

For recording UV-Vis spectra of the study, SHIMADZU 140 double beam spectrophotometer and ELICO SL 210 UV-Visible double beam spectrophotometer with quartz cells of 10 mm path length have been used. For *p*H measurements, an Elico model Li-120 *p*H meter was employed.

2.2 MATERIALS

The dyes *viz*., Bromothymol blue (BTB), Bromophenol blue (BPB), Bromocresol green (BCG) and Bromocresol purple (BCP) of analytical grade supplied by SD Fine Chemicals Ltd. Mumbai, were used without any further purification. The solvents, Chloroform HPLC grade and AR grade HCl and Sodium acetate supplied by SD Fine Chemicals, Mumbai were used in the study.

2.3 METHODS

Method A

Method A involves the interaction of the drug with BCG (Bromocresol green) to form ion-pair complex, extractable into chloroform. This ion-pair complex absorbs around 415 nm in the UV region. The absorbance of this band increases with increasing the concentration of the drug from which the calibration curve is constructed for quantification of drug. 0.025% aqueous solution of BCG was used. CH_3COONa-HCl buffer of required *p*H was used and the desired *p*H was maintained with the help of a *p*H meter.

Method B

Method B involves the interaction of the drug with BPB (Bromophenol blue) to form ion-pair complex, extractable into chloroform. This ion-pair complex absorbs around 415 nm in the UV region. The absorbance of this band increases with increasing the concentration of the drug from which the calibration curve is constructed for quantification of drug. 0.025% aqueous solution of BPB was used. The required pH of reaction mixture was maintained at 2.5 using CH_3COONa-HCl buffer and other experimental conditions are similar as mentioned in Method A.

Method C

Method C involves the interaction of the drug with BTB (Bromothymol blue) to form ion-pair complex, extractable into chloroform. This ion-pair complex absorbs around 415 nm in the UV region. The absorbance of this band increases with increasing the concentration of the drug from which the calibration curve is constructed for quantification of drug. 0.025% aqueous solution of BTB was used. The required pH of reaction mixture was maintained at 3.5 using CH_3COONa-HCl buffer and other experimental conditions are similar as mentioned in Method A.

Method D

Method D involves the interaction of the drug with BCP (Bromocresol purple) to form ion-pair complex, extractable into chloroform. This ion-pair complex absorbs around 407 nm in the UV region. The absorbance of this band increases with increasing the concentration of the drug from which the calibration curve is constructed for quantification of drug. 0.025% aqueous solution of BCP was used. The required pH of reaction mixture was maintained at 2.5 using CH_3COONa-HCl buffer and other experimental conditions are similar as mentioned in Method A.

3.0 RESULTS AND DISCUSSIONS

3.1 Formation of Ion-pair complexes

Trimetazidine has heterocyclic piperazine ring linked to aromatic ring. The secondary type amino group of piperazine is more reactive and the protonation takes place at this group when Trimetazidine reacts with dye stuffs to form ion-pair complexes. The formation of these complexes is presented in **Scheme 1**. The developed methods are based on the interaction of Trimetazidine to form ion-pair complexes with dyes, BCG, BPB, BTB and BCP. The ion-pair complexes of Trimetazidine, quantitatively extracted into chloroform, absorbed maximally at 410, 417, 414 and 408nm with use of the cited dyes respectively (**Figures 2a, 2b, 2c and 2d**) where the reagent blank under similar experimental conditions showed no absorption. The developed methods can be applied for the quantification of Trimetazidine in pharmaceutical industries. 0.025% aqueous solutions of dye stuffs and CH_3COONa-HCl acid buffers of *p*H 3.5, 2.5, 2.8 and 2.5 were used to get stable ion-pair complexes of Trimetazidine with the mentioned dyes. Appropriate pH values are maintained in all the experiments with the help of a *p*H meter.

3.2 Calibration curves for the methods

Different aliquots of solution of Trimetazidine were taken into separating funnels. 5 ml of CH_3COONa-HCl buffer (of *p*H 3.5, 2.5, 2.8 and 2.5) and 5 ml of 0.025% aqueous solution of dye were added. The total volume of the contents in the flask was made up to 20 ml with distilled water. To this, 10 ml of chloroform was added and the contents were thoroughly shaken for 5 min in order to form a stable ion-pair complex. The flask was kept aside for 5 min to allow the organic and aqueous layers to separate. The absorbance of stable colored solution was recorded around 417 nm against blank similarly prepared. The determinations of pure Trimetazidine and its pharmaceutical forms were carried out using the same procedure developed. The calibration curves (**Figure 3**) are constructed which are linear over the concentration ranges which are in permissible range.

The statistical data for the regression equations for the developed methods and optical characteristics of ion-pair complexes of Trimetazidine with dyes are presented in **Table 1**.

3.3 Procedure for the assay of pure drug

Five different solutions of pure Trimetazidine drug in the range of calibration curve were chosen for conducting recovery experiments, the results of which are presented in **Table 2** along with relative standard deviations for the methods developed.

3.4 Procedure for the assay of dosage forms

Ten tablets of Carvidon 30mg were taken and grounded to powder and dissolved in doubly distilled water. The solution was stirred thoroughly, filtered through a Whatman No. 42 filter paper, and taken into a 100 ml standard flask and diluted with required doubly distilled water. The recovery experiments were carried out by selecting different aliquots of this solution which come in the range of calibration curve for the determination of drug in its dosage form. **Table 3** represents the results of the recovery experiments for the assay of dosage forms.

3.5 Stoichiometry

The Job's continuous variation method for the determination of molar ratio between Trimetazidine and dye stuffs[23] was followed. The solutions of Trimetazidine and dye stuffs (BCG, BPB, BTB and BCP) with same concentrations of 8 x 10^{-5}*M* each were mixed in varying the volume ratios such that the total volume of each mixtue was maintained constant. The absorbance of each mixtue solution was measured and plotted against the mole fraction of the drug (**Figure 4).** It is confirmed that 1:1 drug & dye molar ratio exists in all the complexes formed between Trimetazidine and each BCG, BPB, BTB and BCP. The formation constants[24,25] were also determined and found to be 9.34 x 10^5, 9.67 x 10^5, 1.01 x 10^6 and 1.05 x 10^6 K M^{-1} for complexes with BCG, BPB, BTB and BCP respectively.

3.6 Optimization of the factors effecting the absorbance

The effect of pH on the absorbance of ion-pair complexes of Trimetazidine with BCG, BPB, BTB and BCP was studied using CH_3COONa-HCl buffer. It is evident from the **Figure 5** that the absorbance of complexes with BCG, BPB, BTB and BCP was found to be constant within the *p*H ranges 2.2-3.8, 2.0-3.0, 2.0-3.0 and 2.0-3.0 respectively. Thus, all the absorbance measurements were made at *p*H 3.5, 2.5, 2.8 and 2.5 with BCG, BPB, BTB and BCP respectively.

Different volumes of BCG, BPB, BTB and BCP were added separately to a constant volume (8 µg ml^{-1}) of Trimetazidine for studying the effect of concentration of dye on the absorbance of ion-pair complex. It is evident from **Figure 6** that the absorbance gradually increases with the volume of dye upto 3.0 ml, beyond which no change in the absorbance was observed. Hence, in all the experiments carried out with Trimetazidine for its determination, 5 ml of dye was used.

The effect of the presence of foreign substances (excipients) along with Trimetazidine has been studied choosing the concentration level at 8 µg ml^{-1}. Experiments on systems with 10 ml of sample and known amount of foreign substance were carried out adopting the procedures of proposed methods. The results of these experiments and tolerance limits were tabulated in **Table 4**. It is appropriate to mention that any interference by the common excipients found in tablets is completely ignored as the drug content from the powdered tablets was extracted into chloroform.

3.7 Validation of the proposed method

The methods developed for the quantification of Trimetazidine using dye stuffs viz., BCG, BPB, BTB and BCP have been validated in terms of guidelines prescribed by ICH[26] for method validation. The terms mentioned in ICH *viz*. selectivity, specificity, accuracy, precision, limits of calibration curve, LOD, LOQ, robustness, ruggedness and regression equation for the proposed methods were

studied. For comparison with a reference method, the student t-test and variance F-test were performed. The results of Beer's law limits, molar absorptivity, regression equation, correlation coefficients, relative standard deviation and recoveries are presented in **Table 1.** Six replicate determinations were carried out to test the precision of the proposed methods. It is found that the coefficient of variation was less than 1.2% for all the procedures.

The performance order of the developed methods is found to be BCP > BTB > BPB > BCG. The results of the developed methods presented in **Table 2** and **Table 3** were compared to those achieved by reference method in terms of *t*-test at 95% confidence level. It is observed, in all the cases, that the results achieved by developed methods and those by reference methods were identical in terms of statistical data. The results obtained by the proposed methods proved that these methods can be considered as standard methods. These methods are simple and sensitive with high precision and accuracy. Comparative t- and F-tests develop the confidence on the applicability of the methods in pharmaceutical formulations. The results obtained are satisfactorily accurate and precise as indicated by the excellent percent recovery. The optical parameters and statistical comparison validate these methods for application in routine analysis of Trimetazidine in pure and dosage forms.

4.0 CONCLUSIONS

Trimetazidine forms ion-pair complexes with acidic triphenylmethane dyes *viz.,* bromocresol green (BCG), bromophenol blue (BPB), bromothymol blue (BTB) and bromocresol purple (BCP) in acidic medium in 1:1 proportion. These complexes are extractable into chloroform and offer a basis for assay of the drug. The developed methods are simple, sensitive, and reproducible and can be used for routine analysis of Trimetazidine in pure and formulation forms.

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Table 1: Optical characteristics and statistical analysis for the regression equation of the proposed methods for the estimation of Trimetazidine

Parameters	Extraction methods with[b]			
	BCG	BPB	BTB	BCP
λ_{max} (nm)	410	417	414	408
Beer's law limit (µg ml^{-1})	2.5-25	2.5-25	2.5-25	2.2-25
Molar absorptivity (L mol^{-1} cm^{-1})	20853	14732	18570	10151
Formation constant, K, M^{-1}	$9.34x10^5$	$9.67x10^5$	$1.01x10^6$	$1.65x10^6$
Sandell sensitivity (µg cm^{-2})	0.0159	0.0232	0.0185	0.0344
Slope (specific absorptivity), b	0.063	0.043	0.054	0.029
Intercept (a)	0.188	0.010	0.177	0.084
Correlation coefficient (r)	0.996	0.0998	0.982	0.997
Standard deviation of intercepts (% n=6)	0.0092	0.0066	0.0072	0.0039
Limit of detection, µgml^{-1}	0.481	0.512	0.045	0.0449
Limit of quantification, µgml^{-1}	1.461	1.548	1.347	1.361
Regression equation[a]	Y=0.063C 0.188	Y=0.043C±0.0 10	Y=0.054C±0.1 77	Y=0.029C±0.0 84

[a]Withrespect to *Y=bc+a*, where C is the concentration (µg ml^{-1}) and *Y* is absorbance, [b]Six replicate samples

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

Taken (µg ml^{-1})	Proposed method								Reference method Recovery (%)
	Found (µg ml^{-1})				Recovery (%)				
	BCG	BPB	BTB	BCP	BCG	BPB	BTB	BCP	
4	4.01	4.02	3.98	4.05	100.25	100.50	99.50	101.25	99.96
8	8.02	8.02	7.96	8.09	100.31	100.25	99.50	101.13	101.52
12	12.03	12.02	11.97	12.18	100.25	100.16	99.75	101.50	101.56
16	16.1	16.04	15.94	16.19	100.62	100.25	99.64	101.19	100.23
									101.12
									99.72
									101.22
									99.98
RSD (%)					0.179	0.143	0.122	0.162	0.7598
Mean±SD					100.36 ±0.1795	100.292 ±0.144	99.59 ±0.1218	101.125 ±0.164	100.66 ±0.7649
t-test					0.988	1.21	1.376	1.079	
F-test					2.6633	1.74	1.227	2.232	

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

Taken (µg ml^{-1}) Carividon 30mg	Proposed method								Reference method Recovery (%)
	Found (µg ml^{-1})				Recovery (%)				
	BCG	BPB	BTB	BCP	BCG	BPB	BTB	BCP	
4	3.98	4.01	4.05	3.96	99.50	100.25	101.25	99.00	101.12
8	7.99	8.12	8.12	8.07	99.98	101.50	101.50	100.88	99.96
12	11.96	11.98	12.16	11.87	99.67	99.83	101.33	98.92	101.52
16	15.99	15.95	16.24	16.04	99.94	99.68	101.50	100.25	101.25
									99.98
									101.02
									101.10
									99.92
RSD (%)					0.2011	0.821	0.1235	0.971	0.6361
Mean±SD					99.74 ±0.200	100.31 ±0.823	101.39 ±0.125	99.76 ±0.961	100.787 ±0.6412
t-test					1.918	0.465	2.062	0.7600	
F-test					0.150	1.947	0.044	2.654	

Table 4: Interference study in the estimation of Trimetazidine

Sl. No	Excipients	Tolerance limit (µg ml^{-1})
1	Microcrystalline cellulose	101
2	Starch	157
3	Lactose	131
4	Povidone	58
5	Silicon dioxide	71
6	Titanium dioxide	50

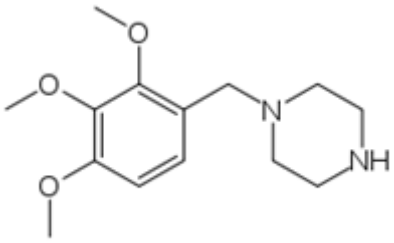
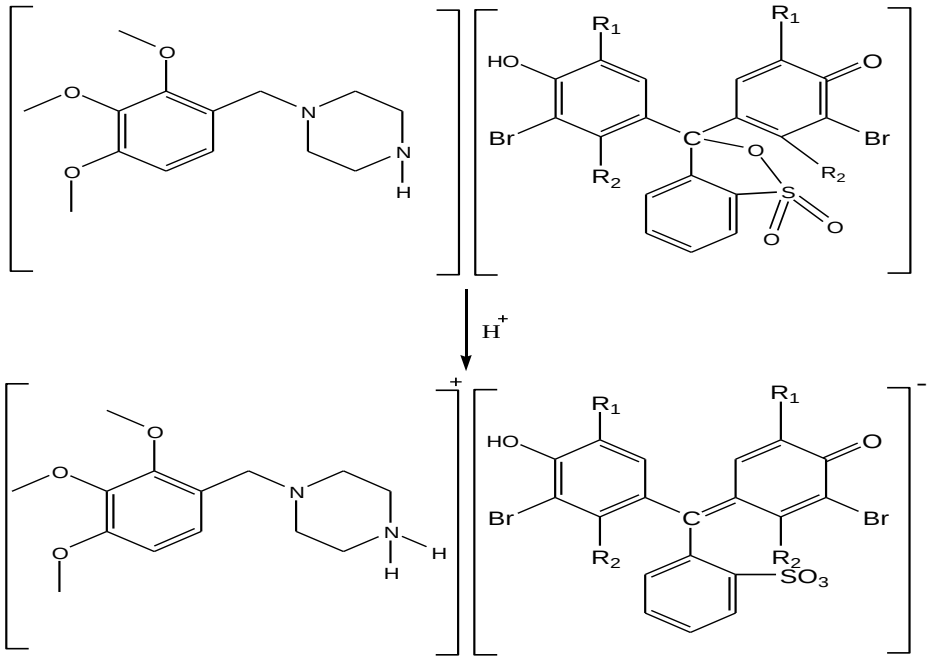


Fig. 1: Structure of Trimetazidine



Scheme 1: Trimetazidine-dye ion pair complex

Bromothymol blue : R_1 = isoporopyl, R_2 = -CH_3

Bromophenol blue : R_1 = -Br, R_2 = -H

Bromocresol green : R_1 = -Br, R_2 = -CH_3

Bromocresol purple: R_1= -CH_3, R_2= -H

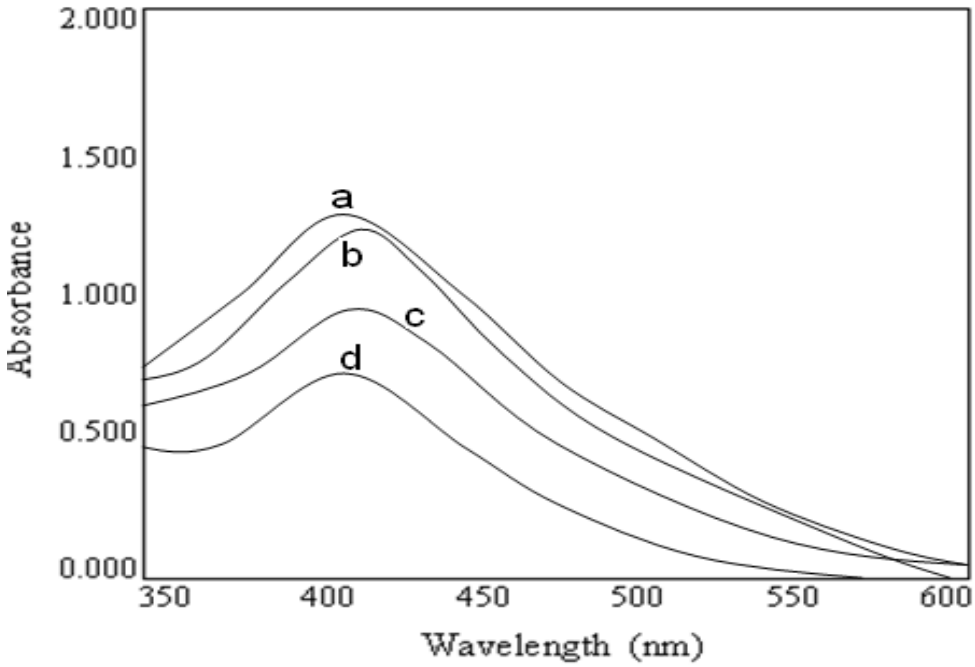


Fig. 2: Absorption spectra of Trimetazidine-dye complex extracted into 10 ml chloroform

a. drug = 25.0 µg ml^{-1} + 5 ml of 0.025% BCG + 5 ml of pH 3.5 buffer
b. drug = 22.5 µg ml^{-1} + 5 ml of 0.025% BPB + 5 ml of pH 2.5 buffer
c. drug = 20.0 µg ml^{-1} + 5 ml of 0.025% BTB + 5 ml of pH 2.8 buffer
d. drug = 17.5 µg ml^{-1} + 5 ml of 0.025% BCP + 5 ml of pH 2.5 buffer

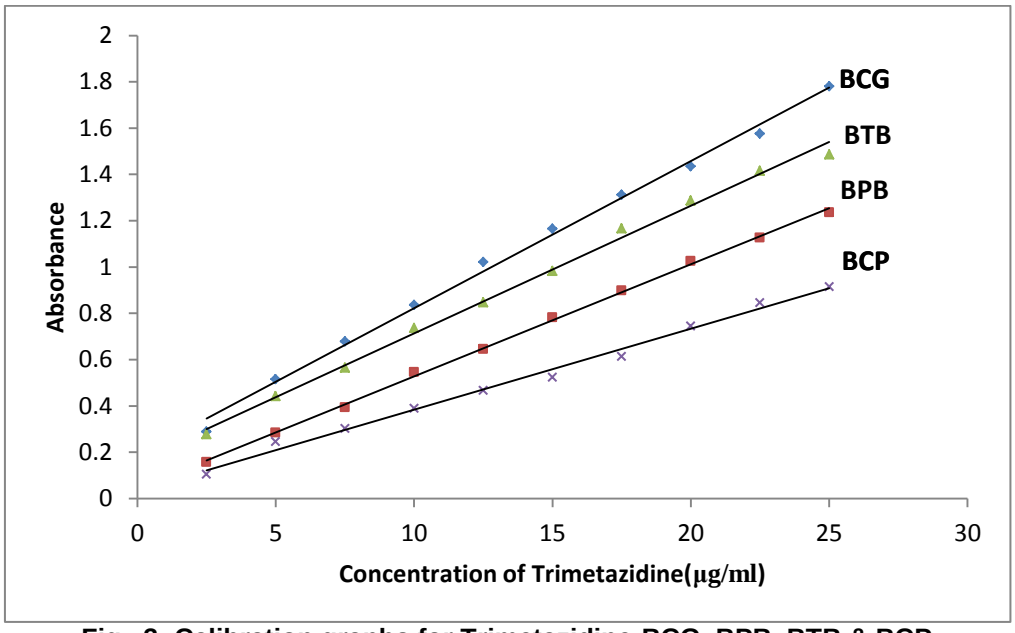


Fig. 3: Calibration graphs for Trimetazidine-BCG, BPB, BTB & BCP ion-pair complexes

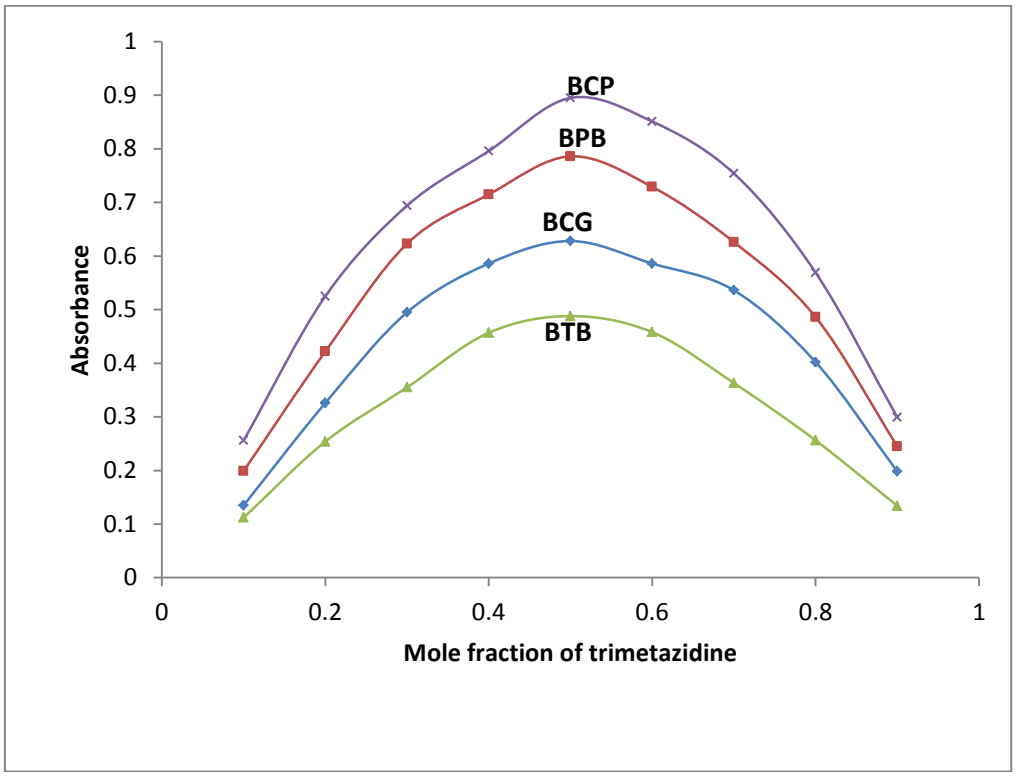


Fig. 4: Continuous-variations study of drug-dye systems
[Trimetazidine] = [Dye] = $8x10^{-5}$M

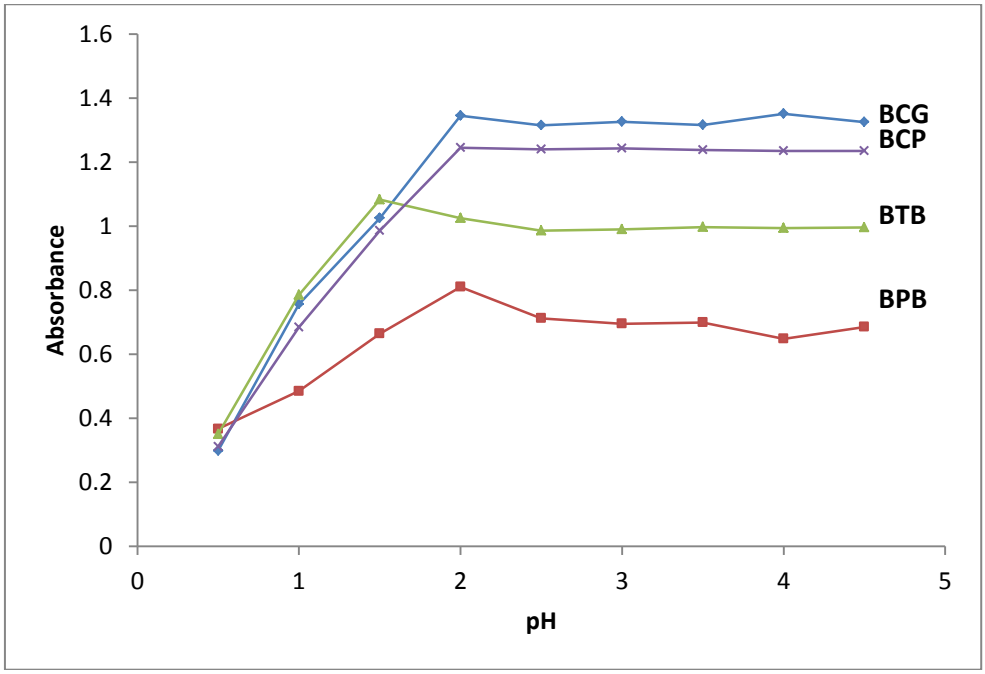


Fig. 5: Effect of pH
[Trimetazidine] = 8µg ml^{-1}, [Dye] = 5ml of 0.025%

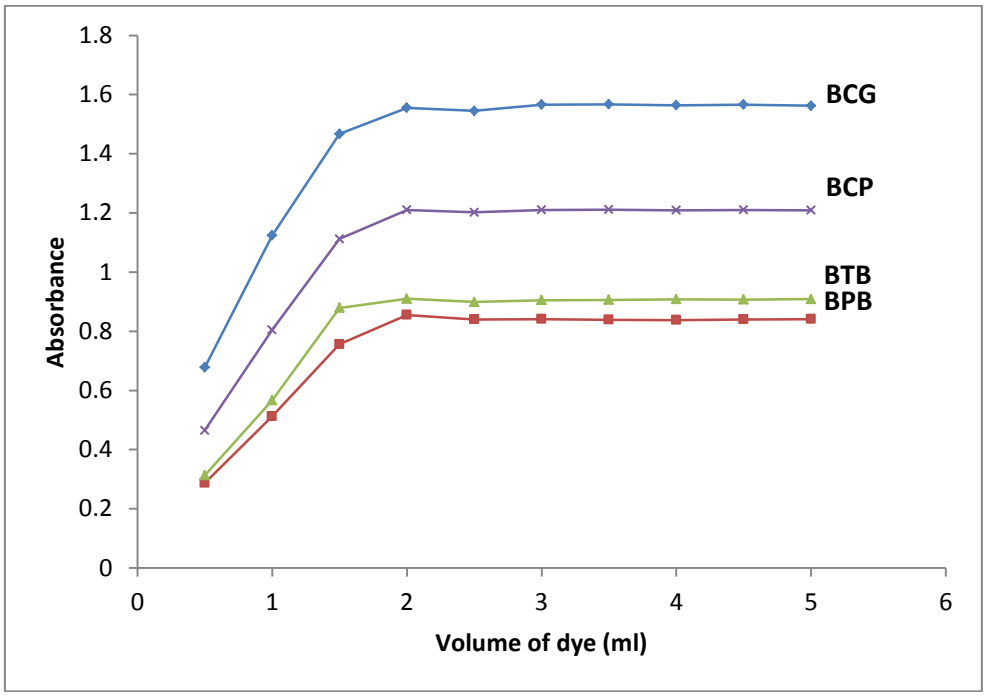


Fig. 6: Influence of the volume of 0.025% dye
[Trimetazidine] = [8µg ml^{-1}]

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