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Determination of ethionamide in pharmaceutical preparations by visible spectrophotometry employing two sulphonphthalein dyes

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ABSTRACT

Two simple, sensitive, selective, rapid and reproducible visible spectrophotometric methods are presented for the determination of ethionamide (ETM) in bulk and dosage forms by employing two sulphonthalein dyes: bromophenol blue (BPB method) and bromothymol blue (BTB) as chromogenic agents. . The methods are based on the formation of intensely yellow colored ion-pair complexes upon the reaction of the drug with the cited dyes in chloroform medium. The 1:1 (drug:dye) complexes formed were spectrophotometrically measured at 450 nm with both the dyes without involving the extraction step. Optimization of the experimental conditions is described. Beer's law is obeyed in the concentration ranges of 0.4-10 and 0.5-14 µg mL^{-1} for BPB method and BTB method , respectively, with corresponding molar absorptivity values of 1.86×10^4 and 1.39×10^4 L mol^{-1} cm^{-1}. The calculated limits detection (LOD) and quantification (LOQ) were 0.03and 0.09 µg mL^{-1}(with BPB) and 0.05 and1.15 (with BTB). The suitability of the methods for the quantitative determination of ETM was proved by validation in accordance with the requirements of the ICH guidelines. The coefficients of variation were <1.5 % for intra-day and <2.0 % inter-day analysis, and the accuracy expressed as relative error were< 2.0%. The methods were applied to the determination of ETM in tablets and the results agreed well with the label claim and those obtained by the reference method. The accuracy of the methods was ascertained by recovery study via standard-addition procedure. The possible reaction pathway is also discussed.

Keywords: Ethionamide; Determination; Dyes; Ion-pair; Spectrophotometry.

INTRODUCTION

Ethionamide (ETM), chemically known as 2-ethylthioisonicotinamide), is a traditional second line therapy drug for thetreatment tuberculosis in 250-1000 mg daily dose to avoid rapid development of [1-6]. Reliable analytical methods are required to maintain quality of pharmaceutical products. In line with this requirement, several analytical approaches have been developed for the determination of the drug in bulk and dosage forms and include, visual titrimetry[7], potentiometric titrimetry[8], membrane-electrode based- potentiometry[9] and fluorimetry[10,11].

Because of the speed, sensitivity, accuracy and easy affordability, visible spectrophotometry is still being used in pharmaceutical and biomedical analysis laboratories. Visible spectrophotometric methods based on diverse reaction chemistries and employing sodium nitroprusside[12,13], 2,3-dichloro-1,4-naphaquinone [14,15], 4-(2-pyridylazo)-vanadium [16], iron (III) [17], iron (III)-p-phenylenediamine[18], osmic acid [19], N—bromosuccinimide-celestine blue [20] and permanganate [21] as chromogenic agents have been reported for ETM. A kinetic spectrophotometric method [22] based on the catalytic effect of ETM on the reaction between sodium azide and iodine is also found in the literature.

Extractive spectrophotometric methods employing dyes as ion-pair complexing agents are popular because of their sensitivity and selectivity. Reddy and Sastry[23] have reported a method in which ion-pair complex, formed upon the reaction of the drug with alizarin violet 3B or alizarin brilliant violet R in acidic buffer, was extracted into chloroform and absorbance measured at 560 nm. Through the methods are claimed to be sensitive and selective, the liquid-liquid extraction step is tedious, time-consuming ,labour-intensive and very prone to loss of analyte. The methods also require critical pH adjustment to ensure quantitative complexation between drug and dye.

As an answer to these problems, extraction- free spectrophotometry based on drug-dye ion-pair reaction have received considerable attention in recent years for the analysis of pharmaceutical compounds [24-34].

This paper describes,for the first time, the application of sulphonphthalien acid dyes: bromophenol blue and bromothymol blue for the spectrophotometric determination of ETM. The formed ion-pair complex requires no extraction step and is measured directly in chloroform. The methods were applied to the determination of ETM in tablets with satisfactory results. Thecolour reaction pathway is also discussed.

EXPERIMENTAL SECTION

1.1. Apparatus

A Systronics model 166 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) with matched 1-cm quartz cells was used for absorbance measurements.

1.2. Reagents and materials

The reagents used were of analytical grade, and water was always double distilled. Organic solvents used were spectroscopic grade and purchased from (Merck, Mumbai, India). Solution of bromophenol blue (BPB) and bromothymol blue (BTB) (both from LobaChemie Ltd., Mumbai, India; 0.1%) were prepared in chloroform.

Pure ethionamide (ETM) sample, certified to be 99.86% pure was provided from Panacea Biotec Ltd. as gift and used as received.

2.3. Preparation of standard drug solution

A stock standard solution equivalent 400 µg mL^{-1}ETM was prepared by dissolving accurately weighed 20 mg of pure drug in chloroform in a 50 mL dry calibrated flask and diluted to the mark with the same solvent and mixed will. The stock solution was diluted to 40 and 20 µg mL^{-1} working concentrations with chloroform to be used in BPB method and BTB method, respectively.

2.4. Procedures

2.4.1. Preparation of calibration graphs using BPB method

Aliquots (0.1 – 1.25 mL) of 40 µg mL^{-1} ETM standard solution were measured accurately into a series of 10 mL calibrated flasks using a micro burette. To each flask was added 1.5 mL of BPB solution (0.1% in chloroform), and the volume was make up to mark with chloroform and mixed well. The absorbance of each solutionwas measured at 450 nm after 5 min against the reagent blank simultaneously prepared.

2.4.2. Preparation of calibration graphs using BTB method

The above procedure was repeated with 0.25 – 3.5 mL of 20 µg mL^{-1} ETM solution and 1.0 mL of 0.1% BTB solution, the absorbance of the above solution being measured at the same wavelength.

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2.4.3. Procedure for tablets

Twenty tablets were weighed accurately and ground into fine powder. A portion of the powder equivalent to 20 mg of ETM was weighed accurately and transferred into a 50 mL calibrated flask, 30 mL of chloroform was added, and the contents were shaken for 20 min. The volume was diluted to the mark with chloroform, mixed well and filtered using Whatman 42 filter paper. The filtrate (400 µg mL^{-1} in ETM) was diluted to 40 and 20 µg mL^{-1} levels for assay by BPB method and BTB method, respectively by taking 1.0 and 2.0 mL in five replicates.

2.4.4. Procedure for placebo and synthetic mixture analyses

A placebo blank of the composition: 20 mg talc, 30 mg starch, 20 mg sucrose, 20 mg lactose, 10 mg gelatin, 20 mg sodium alginate, 30 mg magnesium stearate, 20 mg methyl cellulose, was prepared by homogeneous mixing in a mortar. Twenty mg of placebo was placed in a 50 mL calibration flask and its extract was prepared as described

under procedure for tablets. Two mL of the extract was subject to analysis following the general procedures. To 10 mg of the placebo blank prepared above, 20 mg of pure ETM was added, mixed thoroughly and the mixture was quantitatively transferred into a 50 mL calibrated flask; and then steps described under procedure for tablets were followed.

2.4.5. Procedure for stoichiometric relationship

Job's method of continuous variations [35]of equimolar solutions was employed. ETM and dye solution (BPB and BTB) equivalent to 1.2×10^{-4} M each were prepared in chloroform. ETM and BPB or BTB solutions mixed in complementary ratios keeping the total volume at 5 mL, and the absorbance was measured at 450 nm. A plot of absorbance Vs mole fraction of the drug was prepared in each case.

RESULTS AND DISCUSSION

3.1. Absorption spectra

ETM, being a basic nitrogen-containing compound, reacts instantaneously with BPB and BTB in chloroform, giving characteristic yellow coloured products which exhibit an absorption maximum at 450 nm (Figure 1). The coloured species can be attributed the formation of ion-pair complex between the drug and the dye. The yellow colour is thought to be a result of the proton transfer from the acidic dye to the basic centre of the drug. Subsequently, the dye is converted to an open quinoidal yellow-colouredanion[36]. The latter forms an ion-pair with the drug cation. The possible reaction pathway is shown in (Figure 2 a&b).

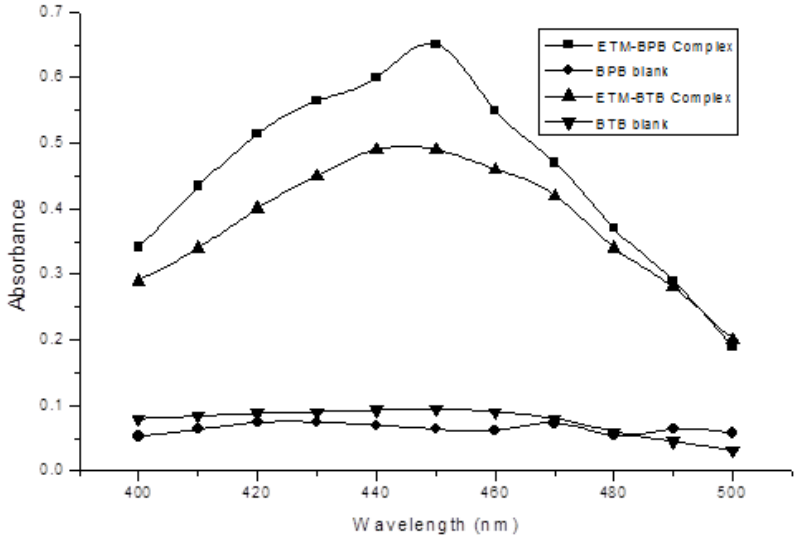

Fig. 1 – Absorption spectra of ETM-BPB and ETM-BTB complexes and their blanks.

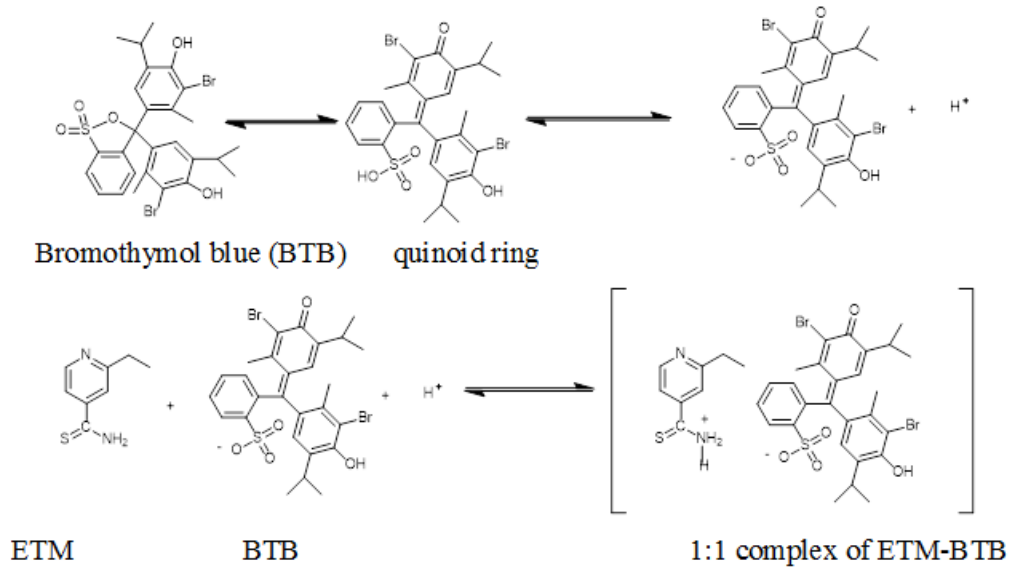

Fig. 2a – The possible reaction pathway for the formation of ETM-BTB ion-pair complex.

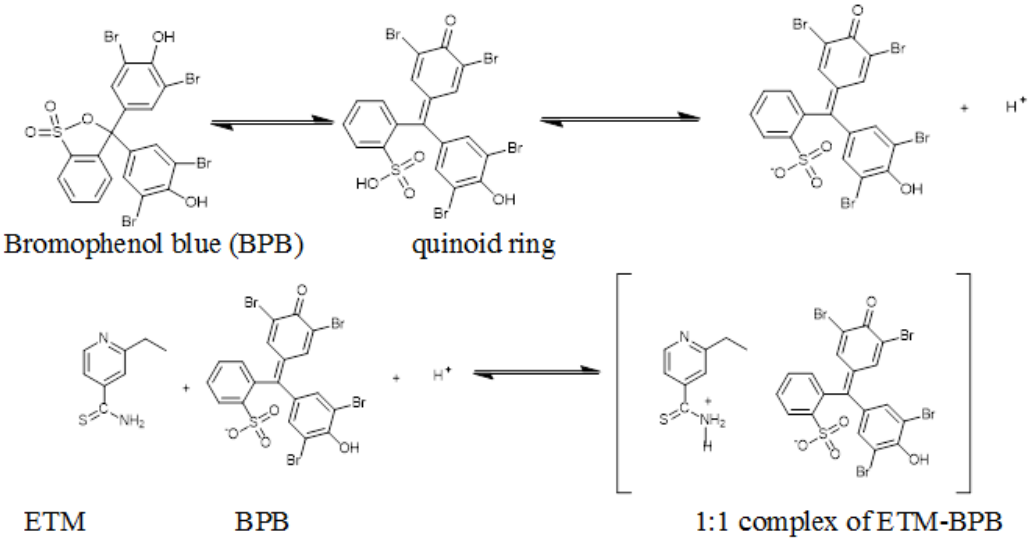

Fig. 2b – The possible reaction pathway for the formation of ETM-BPB ion-pair complex.

3.2. Method development

3.2.1. Optimization of reaction conditions

The influence of some variable on the ion-pair complexation reaction was tested to establish the most favorable conditions to achieve maximum analytical sensitivity and obedience to Beer's law. In this respect, the reagent concentration/volume, standingtime and reaction medium were optimized in accordance with the procedures given in the experimental section.

3.2.2. Reaction medium

The effect of several organic solvents viz., chloroform, methylene chloride, dichloroethane, carbon tetrachloride, xylene, hexane, etc. was tried as the reaction medium. Chloroform was found ideal of all the solvents, yielding maximum sample absorbance and minimum blank absorbance.

3.2.3. Standing time and stability of coloured species

The optimum reaction time was investigated from 0.5 to 5 min after mixing the reactants at room temperature (30 ± 2.0^0c). Fullcolour development was attained in 2 min, and the absorbance remained stable for at least 60 min both the methods.

3.2.4. Effect of reagent concentration

The effect of reagent concentration was studies by measuring the absorbance of solution containing a fixed concentration of ETM (8 µg mL^{-1} in both the methods) and varying amount of respective dye. Maximum colour intensity of the ion-pair complex was achieved with 1.5 mL in BPB method and 1.0 mL in BTB method. Although longer volumes of reagent has no pronounced effects on the ion-pair complex formation, slight increase on blank absorbance was observed in both the cases. (Figure 3).

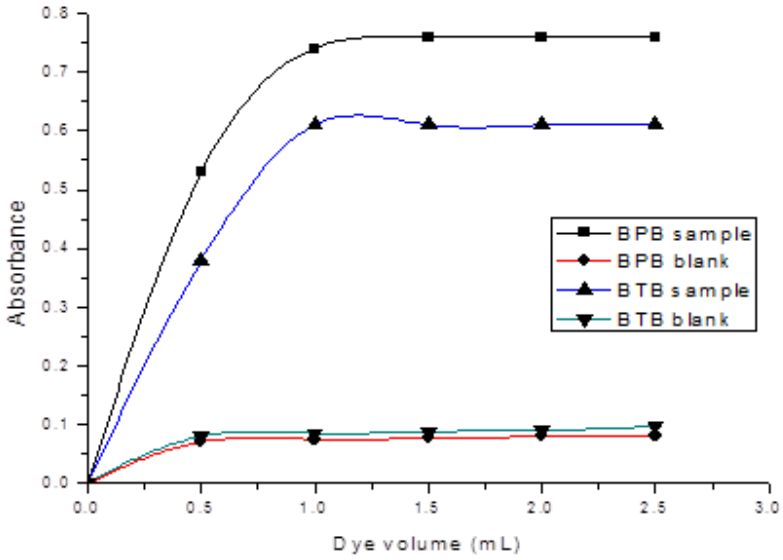

Fig. 3 – Effect of dye concentration.

3.2.5.Stoichiometry

Job's method of continuous variations of eqimolar solutions was employed to study the combining ratio, and the study indicated a 1:1 (drug:dye) stoichiometric relationship as shown in Figure 4.

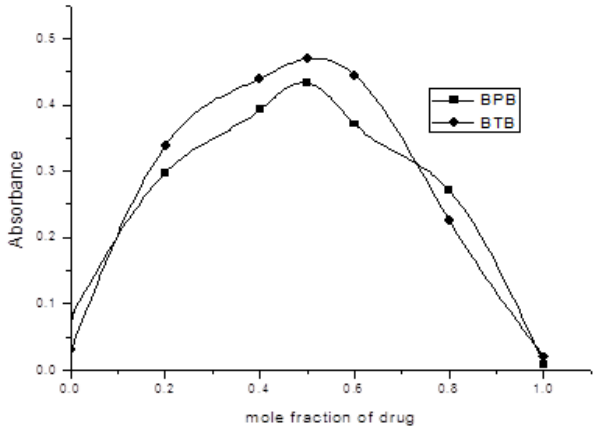

Fig. 4 – Job's plots.

The conditional stability constant (K_f) of the ion-pair complexes was calculated from the continuous variations data using the following equation [36]:

$$K_f = \frac{A/A_m}{[1-A/A_m]^{n+2} C_M(n)^n}$$

where A and A_m are the observed maximum absorbance and the absorbance value when all the drug present is associated, respectively. C_m is the concentration of the drug at the maximum absorbance and n is the stoichiometry with which the dye is associated with drug. The log K_f values for ETM-BPB and ETM-BTB complexes were calculated to be 6.12 and 6.32 respectively.

3.3. Method validation

3.3.1. Linearity and sensitivity

At described experimental conditions, calibration curve constructed by plotting absorbance Vs concentration (Figures 5a and 5b). The linear regression equations, standard deviation of slopes and intercepts, correlation coefficients and linear ranges are given in Table 1. The molar absorptivity value of each method was calculated and presented in Table 1, from which it is clear that BPB method is slightly more sensitive than BTB method.

Table 1: Sensitivity and regression parameters

Parameter	BPB method	BTB method
λ_{max}, nm	450	450
Colour stability	1h	1h
Linear range, µg mL^{-1}	0.4-10	0.5-14
Molar absorptivity (ε), L mol^{-1} cm^{-1}	1.86×10^4	1.4×10^4
Sandell sensitivity*, µg cm^{-2}	0.0091	0.0123
Limit of detection (LOD), µg mL^{-1}	0.03	0.05
Limit of quantification (LOQ), µg mL^{-1}	0.09	0.15
Regression equation, Y**		
Intercept (a)	0.0286	0.09981
Slope (b)	0.01058	0.00817
Standard deviation of a (S_a)	9.98×10^{-2}	6.09×10^{-2}
Standard deviation of b (S_b)	9.84×10^{-2}	6.74×10^{-2}
Regression coefficient (r)	0.9991	0.9979

**Limit of determination as the weight in µg mL^{-1} of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm. **Y=a+bX, where Y is the absorbance, X is concentration in µg mL^{-1}, a is intercept and b is slope.*

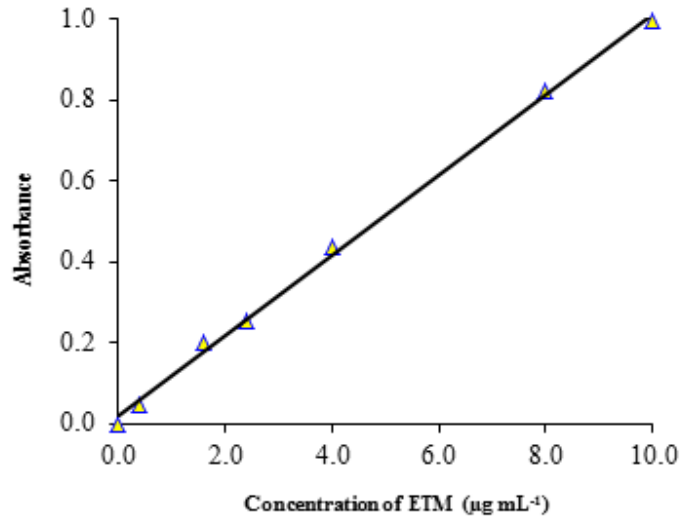

Fig. 5a – Calibration curve for BPB method.

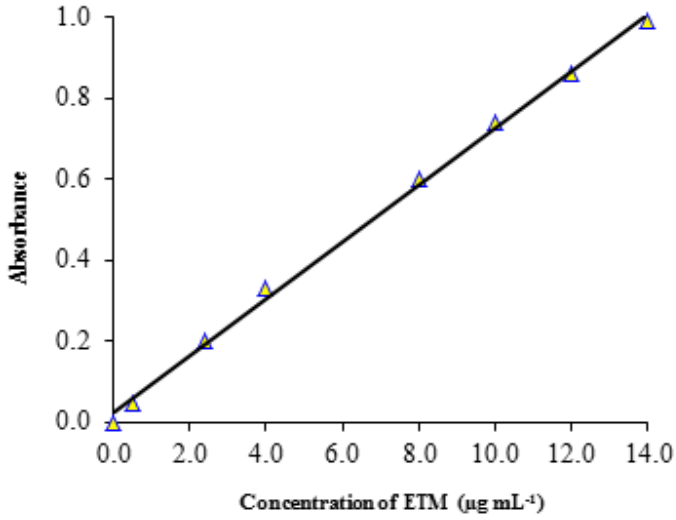

Fig. 5b – Calibration curve for BTB method.

The limits of detection (LOD) and quantification (LOQ), calculated using the equations(Miller and Miller, 1993):

$$\text{LOD} = \frac{3S}{k} \qquad \text{and} \qquad \text{LOQ} = \frac{10S}{k}$$

where S is the standard deviation of replicate blank absorbance values, and *k* is the sensitivity, that is the slope of the calibration graph. The calculated LOD and LOQ values also presented in Table 1and indicate the high sensitivity of the described methods.

3.3.2. Precision and Accuracy

Percent relative standard deviation (%RSD) as an indicator precision and percent relative error (%RE) as a measure of accuracy of the suggested methods were evaluated by replicate determination at three concentration levels. The inter-day and intra-day precision and accuracy results are presented in Table 2 indicating good repeatability and reproducibility besides reasonably high accuracy.

Table 2: Evaluation of intra-day and inter-day accuracy and precision

Method	ETM taken ($\mu g\ mL^{-1}$)	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=7)		
		ETM found[a] ($\mu g\ mL^{-1}$)	RSD[b] %	RE[c] %	ETM found ($\mu g\ mL^{-1}$)	RSD[b] %	RE[c] %
BPB	2.0	2.03	0.28	1.50	1.97	0.64	1.50
	4.0	4.05	0.33	1.25	3.95	1.25	1.25
	6.0	5.91	0.25	1.51	5.98	1.89	1.47
BTB	3.0	2.99	0.59	0.33	3.02	0.84	0.66
	6.0	6.10	1.46	1.67	5.91	1.36	1.51
	9.0	9.17	0.47	1.89	9.12	0.44	1.33

[a]Mean value of seven determinations; [b] Relative standard deviation (%); [c] Relative error (%).

3.3.3. Robustness and Ruggedness

For the evaluation of the method robustness,three optimized experimental variables, namely, reagent volume, contact time and measurement wavelength, were slightly altered, and their impact on the performance of the methods assessed. To study ruggedness, analysis was performed by three different analysts using the same procedure, and also by a single analyst using three different cuvettes. The results of these studies remained unaffected relative to those obtained under optimum conditions as shown by lower %RSD values in Table 3.

Table 3: Method robustness and ruggedness expressed as intermediate precision (% RSD)

Method	ETM taken ($\mu g\ mL^{-1}$)	Robustness (%RSD)			Ruggedness (%RSD)	
		Parameters altered			Inter-analysts (n=3)	Inter-cuvettes (n=3)
		Volume of dye[*]	Contact time[**]	λmax, nm[ψ]		
BPB	2	0.86	0.67	2.6	1.26	2.18
	4	0.92	0.88	1.3	1.62	1.88
	6	1.12	0.58	1.8	0.98	2.04
BTB	3	0.96	0.83	1.7	1.26	2.65
	6	1.35	1.07	1.2	0.77	2.11
	9	1.04	0.76	1.6	1.18	2.38

[*]Volume of dye were 1.3,1.5 and 1.7 mL in BPB method, 0.8, 1.0 and 1.2 mL in BTB method.
[**]Contact time used 4, 5 and 6 min .[ψ]Wavelengths used 448, 450, 452 .

3.3.4. Selectivity

To determine the selectivity of the described methods, placebo and synthetic mixture analysis were performed. Replicate analyses of placebo blank showed absorbance almost equal to that of reagent blank. When the synthetic mixture was subjected to analysis, at three concentration levels by the proposed methods, the percent recoveries of pure drug ranged from 97.42±0.86 to 103.7±1.14 indicating non-interference from the inactive ingredients.

3.4. Application to Tablets

The proposed methods were applied for the determination of ETM in three brands of tablets each containing 250 mg of active component and the results are presented in Table4. The same batch tablet powder was subjected to the assay by the reference method, and results were statistically evaluated by applying Student's t- and variance ratio F-test. The evaluated t- and F-values did not exceed the tabulated values at the 95% confidence level for four degrees of freedom, indicating agreeing accuracy and precision between the proposed methods and the reference method.

Table 4: Results of analysis of tablets by the proposed methods and statistical comparison of the results with the official method

Tablet brand name	Nominal amount	Found* (% of nominal amount ± SD)		
		Official method	Proposed methods	
			BPB Method	BTB Method
Ethide	250	99.33±1.05	100.3±0.85 t = 1.6 F= 1.53	98.86±0.76 t = 0.81 F= 1.91
Ethiokox	250	98.88 ± 1.14	97.37±0.94 t = 2.30 F= 1.47	97.28±0.79 t = 2.58 F= 2.08
Myobid	250	97.94 ± 1.06	98.67±0.95 t = 1.14 F= 1.24	99.57±0.82 t = 2.71 F= 1.67

**Mean value of five determinations.*
(Tabulated t-value at the 95% confidence level and for four degrees of freedom is 2.77).
(Tabulated F-value at the 95% confidence level and for four degrees of freedom is 6.39).

3.4.1. Accuracy by recovery test

Pre-analyzed tablet powder was spiked with pure ETM at three levels and the total was found by the proposed methods. The determination each level was replicated thrice. The results of percent recovery of drug which are an indication of accuracy are summarized in Table 5, and demonstrate the methods' freedom from interference by the co-formulated substances in the tablets.

Table 5: Results of recovery experiment throughstandard-addition method.

Method	Tablet studied	ETM in Tablet, µg mL^{-1}	Pure ETM added, µg mL^{-1}	Total found, µg mL^{-1}	Pure ETM recovered Percent±SD*
BPB	Ethide 250	2.01	1.0	2.97	99.00±0.74
		2.01	2.0	3.95	98.75±0.32
		2.01	3.0	4.88	97.60±0.44
	Ethiokox 250	1.95	1.0	3.04	102.33±0.49
		1.95	2.0	3.94	98.50±1.12
		1.95	3.0	5.09	103.80±1.09
	Myobid 250	1.97	1.0	3.11	103.66±0.84
		1.97	2.0	4.13	103.25±0.68
		1.97	3.0	4.94	98.80±1.43
BTB	Ethide 250	3.95	2.0	6.11	102.83±1.71
		3.95	4.0	7.93	99.13±0.52
		3.95	6.0	10.22	102.2±0.61
	Ethiokox 250	3.89	2.0	5.97	101.4±1.23
		3.89	4.0	7.88	99.75±0.47
		3.89	6.0	9.88	99.80±1.25
	Myobid 250	3.98	2.0	6.05	100.83±0.84
		3.98	4.0	8.08	101.01±1.44
		3.98	6.0	10.21	102.10±0.46

**Mean value of three determinations.*

CONCLUSION

Unlike the chromatographic methods, the proposed spectrophotometric methods are simple since they use an inexpensive instrument. and easy to perform. The reagents use of are cheap, easily available and the procedures do not involve any critical reaction condition or tedious sample preparation unlike the reported spectrophotometric methods[23]. The results are unaffected by slight deliberate variations in optimized experimental conditions and experimental setup. The methods have demonstrated to be both accurate and precise and free from interference from common additives and excipients. As shown in Table 6, the methods are not only facile but also more sensitive than the available methods

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Table 6: Comparison of the proposed and the existing methods

S.No.	Reagent/s	methodology	Linear range (μgmL^{-1})	LOD /LOQ (μgmL^{-1})	Remark	Ref.No
1	Sodium nitoprusside	Orange colored product in basic medium measured at 510 nm	–	–	–	12
2	Sodium nitroprusside	Orange-red complex measured at 510 nm	5-32	–	–	13
3	DCNQ	Orange colored product in ethanol measured at 440 nm	–	–	–	14
4	DCNQ	Red colored product formed in the presence of ammonia in alcohol medium measured at 530 nm	–	–	–	15
5	PAR-V(V)	Ternary complex (1:1:1) extracted into chloroform and measured at 560 nm.	0.2-20	–	30 min contact time, extraction step	16
6	Iron (III)	Purple-violet color complex in acid medium measured at 510 nm	0-36	–	Longer contact time	17
7	Iron (III)-PPD	Thionine compound measured at 600 nm	–	–	Multiple step reaction involved	18
8	Osmic acid	Light yellow colored formed at pH4 measured at 375 nm	0.25-40	–	60 min contact time , pH adjustment required	19
9	NBS-CB	Unbleached dye color measured in acid medium at 540 nm	0.2-5.0	–	Critical acid conc.; insoluble reagent used	20
10	$KMnO_4$	Blue colored manganite in alkaline medium measured at 610 nm (direct method) Absorbance at a fixed time of 20 min measured (kinetic method)	1-10 1-10	–	Critical NaOH conc. Reaction rate precariously dependent on experimental variables	21
11	Sodium azide- iodine	Decreases in absorbance at the 5^{th} min measured at 348 nm (kinetic method	10-100	0.7/-	Reaction rate precariously dependent on experimental condition	22
12	**BPB/BTB**	**Yellow ion-pair complex formed in chloroform measured at 450 nm**	**0.4-10 0.5-14**	**0.03/0.09 0.05/0.15**	**No drastic experimental conditions, no extraction step, instantaneous reaction, more sensitive.**	**Present methods**

DCNQ: Dichloronaphthoquinone; PAR:Pyridylazo resorcinol; PPD: p-phenylenediamine; NBS: N-bromosuccinimide; CB: Celestine blue.

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