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PERMANGANOMETRIC DETERMINATION OF ETAMSYLATE IN BULK DRUG AND IN TABLETS

One titrimetric and two spectrophotometric methods which are simple, selective, sensitive, accurate, precise and economical for the determination of etamsylate (ETM) in bulk drug and in tablets employing permanganate as the oxidimetric reagent are described. In titrimetry, ETM is titrated directly with permanganate in sulphuric acid medium. A direct spectrophotometry (method A) involves treating the aqueous solution of the drug with permanganate in alkaline medium and measuring the bluish green product at 610 nm. In indirect spectrophotometry (method B), the drug solution was treated with a fixed concentration of permanganate in H_2SO_4 medium, and after a specified time, the unreacted permanganate was measured at 545 nm. The molar combining ratio in titrimetry and the optimum assay conditions were studied. Titrimetry is applicable over 1-10 mg range and the calculations are based on a 1:4 (ETM:$KMnO_4$) molar ratio. In spectrophotometry, Beer's law is obeyed over 0.5- 5.0 and 1.5--15 μg ml^{-1} for method A and B, respectively. The molar absorptivity values are calculated to be 2.79×10^4 and 4.17×10^4 l mol^{-1} cm^{-1} for method A and B, respectively and the corresponding sandell sensitivity values are 0.0094 and 0.0063 μg cm^{-2}. The limits of detection (LOD) and quantification (LOQ) are also reported for spectrophotometric methods. The applicability of the developed methods was demonstrated by the determination of etamsylate in pure drug as well as in commercial dosage forms.

Key words: etamsylate; assay; titrimetry; spectrophotometry; permanganate; pharmaceuticals.

Etamsylate (ETM), chemically known as 2,5-dihydroxybenzene sulphonic acid with ethylene diamine [1] (Fig. 1), has haemostatic properties. It is used as a systematic haemostatic agent capable of reducing capillary bleeding in the presence of normal number of platelets. ETM is used in the treatment of capillary hemorrhage, hematemesis, hemopthsis, malena, hematuria, epistaxis, menorrhagia and post partum hemorrhage [2]. Owing to its great therapeutical importance and widespread use, several procedures have been reported for the determination of ETM in biological fluids and pharmaceutical formulations. The determination of ETM in rabbit serum [3], human urine [4] and biological fluids [5] has been reported employing UV--spectrophotometry, capillary electrophoresis and kinetic spectrophotometry, respectively. ETM in pharmaceuticals has been determined by a variety of analytical techniques including HPLC [6,7], HPTLC [8,9], UV-spectrophotometry [10,11], irreversible biamperometry [12], adsorptive stripping voltammetry [13], flow injection potentiometry [14] and chemiluminescence spectrophotometry [15,17]. The above-mentioned techniques, of course, are sensitive enough but are expensive. The reported UV spectrophotometric procedures [10,11] have been applied for the assay of ETM when present in combination with mefenamic acid.

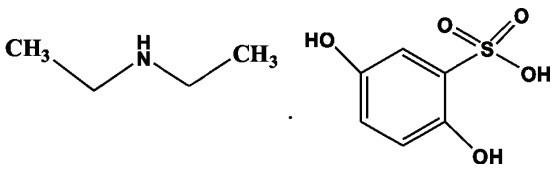

Figure 1. Structure of etamsylate.

As titrimetric and visible spectrophotometric assays offer significant economic advantages over the techniques cited above, the aim of the present investigation was to develop simple and sensitive yet econo-

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mical methods for the determination of ETM in dosage forms.

The literature survey revealed that no titrimetric assay has ever been reported for ETM. The reported visible spectrophotometric methods, although a couple of them sensitive, suffer from one or other disadvantage such as use of costly reagent and poor sensitivity [23], heating step [21,22] or narrow linear range [5] (Table 1). Hence, there is a need to develop simple, selective and sensitive procedures for the determination of ETM in pharmaceuticals.

In this paper, we describe the development and validation of one titrimetric and two spectrophotometric methods for the determination of ETM in bulk drug and in tablets using permanganate as a reagent. Permanganate has earlier been used for the assay of thioxanthines [24], isoniazid [25], methyl thiouracil [26], chloramphenicol [27], amidopyrine [28], valdecoxib [29], nicotine [30], tramodol HCl [31], cefuroxime [32], diloxamide [33] and pentacozine [34], to mention a few and its use for the determination of ETM has not been reported. The methods developed offer the advantages of simplicity, speed, accuracy and precision without the need for costly equipment/chemicals.

EXPERIMENTAL

Apparatus

All absorbance measurements were made on a Systronics model 106 digital spectrophotometer (Ahmedabad, India) provided with 1-cm matched quartz cells.

Materials and reagents

All chemicals and reagents used were of analytical or pharmaceutical grade.

Standard ETM solution. Pharmaceutical grade ETM certified to be 98.97% pure was kindly supplied by Biocon India Ltd., Bangalore, India, and used as the reference standard.

Standard ETM solutions (1 mg ml^{-1}, 10 and 30 μg ml^{-1}) were prepared by dissolving a calculated quantity of pure drug in water.

Pharmaceutical formulations of etamsylate such as Dicynene-250 (Dr. Reddy's Lab.Ltd., H. P., India) and K-Stat-250 (Mercury, Lab. Ltd., H. P., India) were purchased from local markets.

Potassium permanganate (0.02 M, 1000 and 600 μg ml^{-1}). An approximately 0.02 M solution was prepared by dissolving 790 mg of $KMnO_4$ (Merck, Mumbai, India) in water and diluting to 250 ml in a calibrated flask, and standardized using H. A. Bright's procedure [35] and used in titrimetry. The stock standard solution was then diluted appropriately with water to get 1000 and 600 μg ml^{-1} working concentrations for method A and B, respectively.

Sulphuric acid (5 M). Concentrated acid (S. D. Fine Chem, Mumbai, India, sp. gr. 1.84) was appropriately diluted with water to get the required concentrations.

Sodium hydroxide (5 M). Prepared by dissolving 20 g of the chemical (Merck, Mumbai, India) in 100 ml of water.

Table 1. Comparison of the performance characteristics of the existing spectrophotometric methods with the proposed methods

Sl. No.	Reagent(s) used	Methodology	Linear range	Remarks	Ref.
1.	Sodium azide and iodine	The decrease in the absorbance of iodine is measured at 348 nm	0.3-3.0 μg ml^{-1}	Sensitive but narrow linear range and absorbance measured at shorter wavelength	5
2.	Iron (III)-*o*-phenanthroline mixture	Tris-[*o*-phenanthroline-iron (II)] complex measured at 510 nm	0.25-30 μg ml^{-1} ($\varepsilon = 4.8\times10^3$ l $mol^{-1}cm^{-1}$)	Requires heating	21
3.	Ammonium molybdate (Mo^{6+})	Resulting molybdenum blue (Mo^{5+}) posses a characteristic λ_{max} at 695-716 nm	2.0-70 pg ml^{-1} ($\varepsilon = 2.7\times10^3$ l $mol^{-1}cm^{-1}$)	Requires heating in a boiling water bath	22
4.	Ce(IV)-MBTH[a]	The absorbance of the reaction product is measured at 514 nm.	4-30 μg ml^{-1}	Less sensitive, uses costly reagent	23
5.	$KMnO_4$	a) Bluish green color product formed in alkaline medium is measured at 610 nm	0.5-5.0 μg ml^{-1} ($\varepsilon = 2.79\times10^4$ l $mol^{-1}cm^{-1}$)	Highly sensitive, wide linear dynamic ranges, inexpensive instrumental setup, use of ecofriendly chemicals and no heating required	Present methods
		b) Unbleached color of permanganate in H_2SO_4 medium is measured at 545 nm	1.5-15 μg ml^{-1} ($\varepsilon = 4.17\times10^4$ l $mol^{-1}cm^{-1}$)		

[a]3-Methyl-2-benzothiazolinone hydrazone hydrochloride

General procedure

Titrimetry

Different volumes (1–10 ml) of standard solution containing 1 mg ml^{-1} ETM were taken in a 100 ml titration flask and the volume was made up to 10 ml with water. The solution was acidified by adding 1 ml of 5 M H_2SO_4 and titrated against 0.02 M $KMnO_4$. The titration was carried by a drop wise addition of $KMnO_4$ with continuous shaking and the end point is the first appearance of pink color which remains for 5 min.

The amount of ETM in the aliquot was computed from the following formula:

Amount (mg) = VM_rS/n

where V is volume of $KMnO_4$ solution consumed, M_r is relative molecular mass of drug, S is strength of $KMnO_4$ (mol/L) and n is amount of $KMnO_4$ (mol) reacting with 1 mol of ETM.

Spectrophotometry

Method A. Different aliquots of 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ml of standard ETM solution (10 μg ml^{-1}) were transferred into a series of 10 ml standard volumetric flasks and the total volume in each flask was adjusted to 5 ml with water. To each flask, 1 ml of 5 M NaOH followed by 1 ml of 1000 μg ml^{-1} $KMnO_4$ were added. The contents of each flask were mixed well and kept aside for 15 min with occasional shaking. The volume was made up to the mark with distilled water and the absorbance was measured at 610 nm *vs* reagent blank prepared in a similar manner.

Method B. Different aliquots (0.0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0) ml of standard 30 μg ml^{-1} ETM solution were accurately measured and transferred into a series of 10 ml calibrated flasks by means of a microburette and the total volume was adjusted to 5 ml with water. To each flask 2 ml of 5 M H_2SO_4 and 1 ml of 600 μg ml^{-1} $KMnO_4$ were added using microburette. The contents of each flask were mixed well and kept aside for 15 min. The volume was made up to the mark with distilled water and the absorbance was measured at 545 nm *vs* water.

Calibration graphs were prepared by plotting either the increasing absorbance values in method A or decreasing absorbance values in method B *versus* concentrations of ETM. The concentration of the unknown was read from the respective calibration graph or deduced from the regression equation derived using the Beer's law data.

Twenty tablets, each containing 250 mg of ETM, were weighed accurately and finely powdered. The quantity of the powdered tablet containing 100 mg of ETM was weighed accurately and transferred into a 100 ml calibrated flask, 60 ml of water was added and the content shaken thoroughly for 15–20 min to extract the drug into the liquid phase; the volume was finally diluted to the mark with water, mixed well and filtered using a whatman No. 42 filter paper. First 10 ml of the filtrate was discarded, and a subsequent amount of the tablet extract was subjected to analysis by titrimetry. The tablet extract containing ETM at a concentration of 1 mg ml^{-1} was then diluted stepwise with water to obtain working concentrations of 10 and 30 μg ml^{-1} in ETM for method A and B, respectively. A convenient aliquot was then subjected to analysis by spectrophotometric procedures described above.

Placebo blank analysis

A placebo blank of the composition: talc (20 mg), starch (10 mg), acacia (15 mg), methyl cellulose (10 mg), sodium citrate (10 mg), magnesium stearate (15 mg) and sodium alginate (10 mg) was made and its solution was prepared as described under "tablets", and then subjected to analysis.

Procedure for the determination of etamsylate in a synthetic mixture

To the placebo blank of the composition described above, 100 mg of ETM was added and homogenized, transferred to a 100 ml standard flask and the solution prepared as described under tablets. The solution was mixed well and filtered using a whatman No. 42 filter paper. The resulting solution was assayed (n = 5) by titrimetry according to the same procedure described above. The synthetic mixture solution (1 mg ml^{-1} in ETM) was then diluted stepwise with water to obtain working concentrations of 10 and 30 μg ml^{-1} in ETM for method A and B, respectively. A convenient aliquot was then subjected to analysis by either method described above. The analysis was used to study the interferences of excipients such as talc, starch, acacia, methyl cellulose, sodium citrate, magnesium stearate and sodium alginate.

RESULTS AND DISCUSSION

Method development

Numerous inorganic and organic substances have been determined using $KMnO_4$ as the oxidimetric reagent [36,37]. A close examination of the literature survey presented in the introduction part reveals that $KMnO_4$ has not been used for the spectrophotometric determination of ETM. Hence, the reaction between ETM and $KMnO_4$ was investigated based on which one titrimetric and two spectrophotometric methods were developed. In titrimetry, ETM is directly titrated against $KMnO_4$ in sulphuric acid medium. The

end point was detected by the first appearance of pink color which remained for 5 min. The reaction stoichiometry was found to be 1:4 (ETM:$KMnO_4$). Both spectrophotometric methods are based on the oxidation of ETM by $KMnO_4$ either in basic medium (method A) or in acid medium (method B). In method A, an excess of $KMnO_4$ was added to ETM in NaOH medium followed by the determination of manganate (K_2MnO_4) which is bluish-green in color, resulting from the reduction of $KMnO_4$ by ETM. The increase in the absorbance was measured at 610 nm (Fig. 2a). In method B, the residual oxidant after the complete oxidation of ETM by a measured excess of $KMnO_4$ in H_2SO_4 medium was determined by measuring its decrease in absorbance at 545 nm (Fig. 2b). In titrimetric method and spectrophotometric method B, the amount of $KMnO_4$ reacted corresponds to the amount of drug which served as the basis of the assay.

The various experimental parameters were optimized and used throughout the experiment.

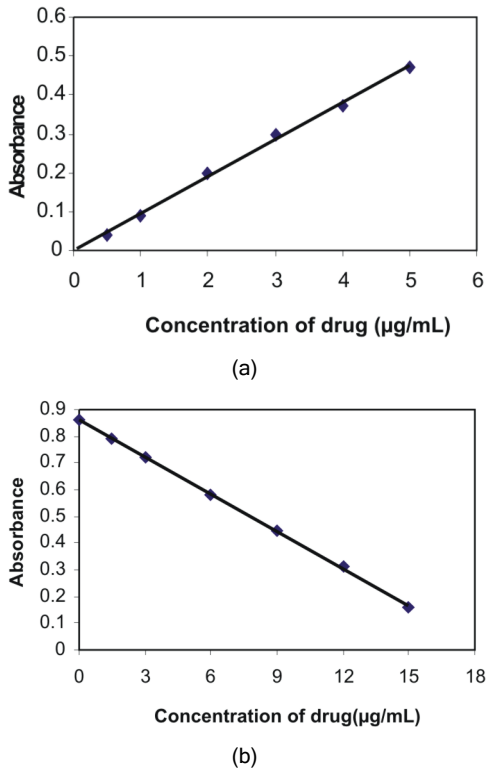


Figure 2. Calibration curve for method A (a) and B (b).

Optimization of variables

Efforts were made to increase the sensitivity of the methods by finding the optimum concentration of each reactant.

In titrimetry, the reaction was found to be stoichiometric in H_2SO_4 medium. Reproducible and stoichiometric results were obtained when 0.45–2.27 M H_2SO_4 was maintained. Hence, 1 ml of 5 M H_2SO_4 solution in a total volume of 11 ml was found to be the most suitable concentration for a quantitative reaction between ETM and $KMnO_4$. The reaction stoichiometry was calculated to be 1:4 (ETM:$KMnO_4$) in the 1–10 mg range. Below and above these limits, irregular stoichiometries were obtained. As the reaction between ETM and $KMnO_4$ was a little bit slow, the titration was carried out by the dropwise addition of $KMnO_4$ solution and a continuous shaking during the titration, to the first appearance of pink color which remained for 5 min.

Spectrophotometry

Method A

Potassium permanganate quantitatively oxidizes ETM in the NaOH medium and reduces itself to manganate ion – the bluish green colored chromogen [38,39] which absorbs maximally at 610 nm (Fig. 3). The experimental variables for the formation of the stable and sensitive colored product were optimized. Even though a higher concentration of the $KMnO_4$ resulted in increased sensitivity of the method, blank absorbance also increased concomitantly. Hence, 1 ml of 1000 µg ml^{-1} $KMnO_4$ in a total volume of 10 ml was fixed which showed the maximum absorbance associated with a minimum blank reading (Fig. 4a). The absorbance readings were maximum and almost constant with an overall NaOH concentration of 0.5–1.2 M was maintained at a drug concentration of 4 µg ml^{-1} (Fig. 4b). 1 ml of 5 M NaOH in a total volume of 10 ml was determined to be optimum for the reaction. The reaction was complete in 15 min and any delay up to 35 min had no effect on the absorbance (Fig. 4c).

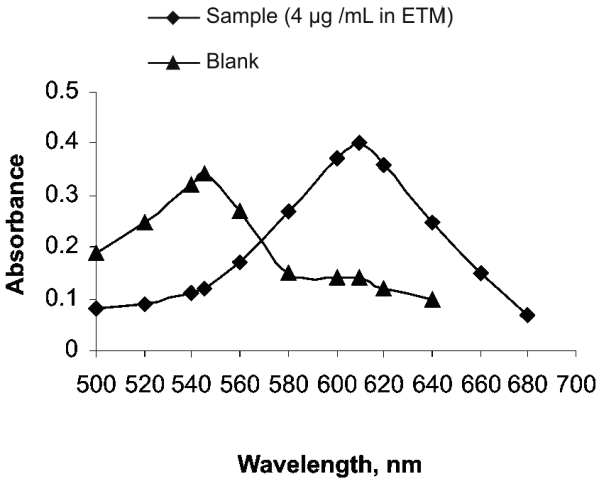
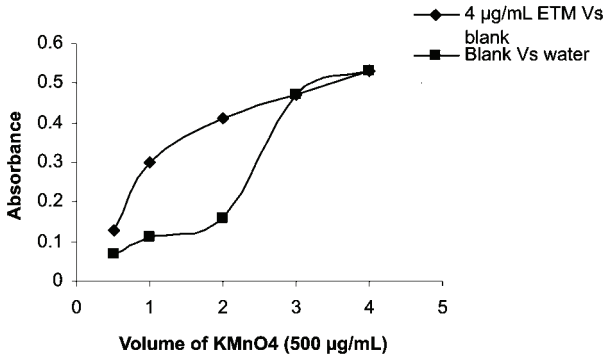
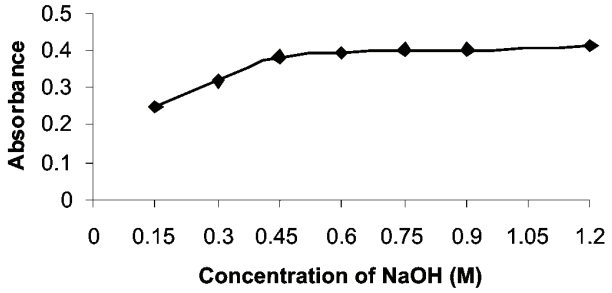


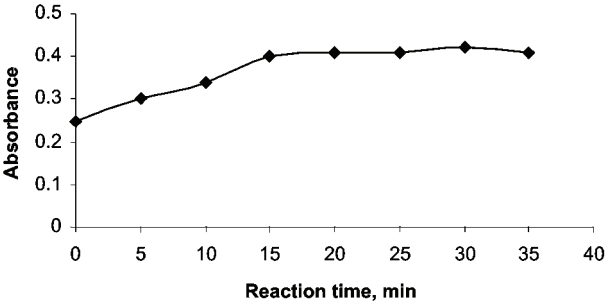
Figure 3. Absorption spectra of method A (Bluish green color produced for 4 µg ml^{-1}).



(a)



(b)



(c)

Figure 4. Effect of a) $KMnO_4$, b) NaOH and c) reaction time (Method A); 4 µg ml^{-1} ETM.

Method B

Preliminary experiments were performed to determine the maximum concentration of $KMnO_4$ which gave the maximum absorbance at 545 nm in the acid medium employed and this was found to be 60 µg ml^{-1} (Fig. 5). Since the oxidation reactions with permanganate are usually performed in H_2SO_4 medium [40], the reaction of the oxidant with the drug was carried out in H_2SO_4 medium. When a fixed concentration of permanganate was reacted with increasing concentrations of ETM in H_2SO_4 medium, a concomitant fall in the concentration of permanganate occurred, as shown by the decreasing absorbance at 545 nm (Fig. 6). Hence, different concentrations of ETM were reacted with 1 ml of 600 µg ml^{-1} $KMnO_4$ to determine the concentration range over which ETM could be determined. To check the effect of H_2SO_4 concentration on the reaction, 1-4 ml of 5 M H_2SO_4 was added to a fixed concentration of ETM (9 µg ml^{-1}) and $KMnO_4$ (60 µg ml^{-1}), and it was observed that constant absorbance readings were obtained when 2-4 ml of 5 M H_2SO_4 in a total volume of 10 ml was used (Fig. 7a). Hence, 2 ml of 5 M H_2SO_4 was fixed. The reaction was found to be complete and quantitative when the reaction mixture was allowed to stand for 15 min, and beyond this standing time up to 30 min the absorbance remain constant (Fig. 7b).

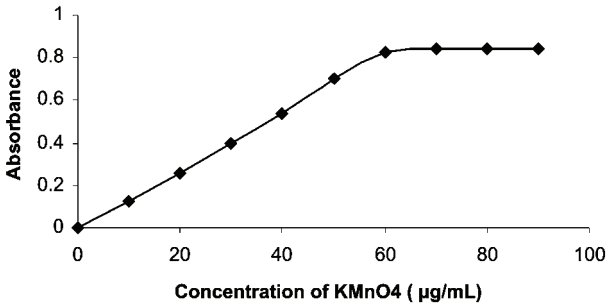


Figure 5. Effect of $KMnO_4$ (Method B).

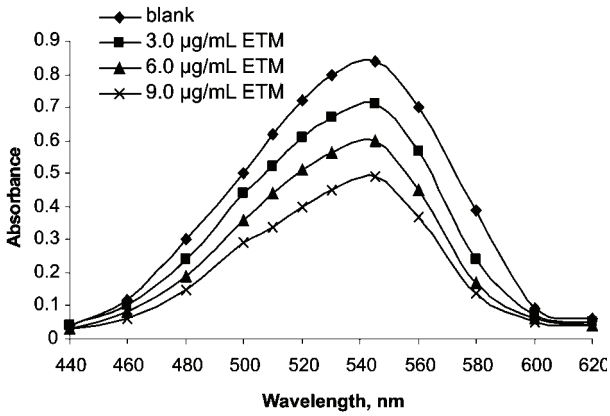


Figure 6. Effect of ETM concentration on the absorbance of 60 µg ml^{-1} $KMnO_4$ (Method B).

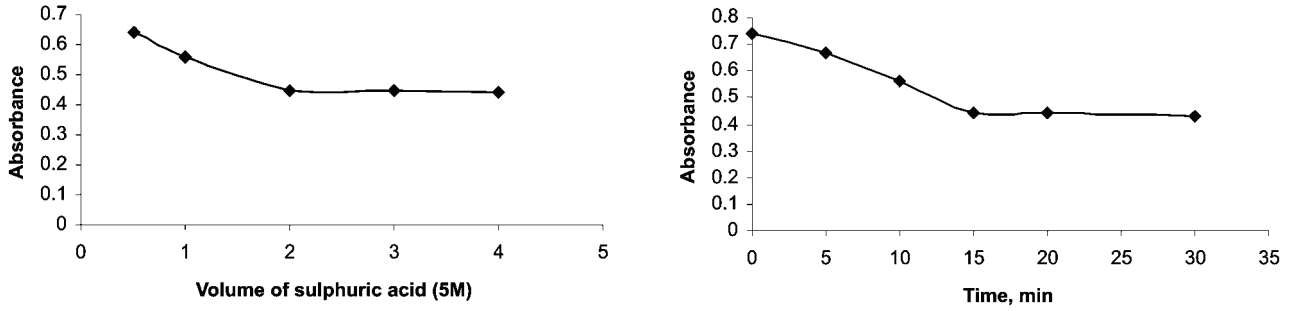
Method validation procedures

The proposed method has been validated for linearity, sensitivity, precision, accuracy, selectivity and recovery.

Linearity and sensitivity

Over the range investigated (1-10 mg), a fixed stoichiometry of 1:4 (ETM:$KMnO_4$) was obtained in titrimetry which served as the basis for calculations. In spectrophotometry, under optimum conditions a linear relation was obtained between absorbance and concentration of ETM in the range 0.5-5.0 µg ml^{-1} in method A and 1.5-15µg ml^{-1} in method B. The calibration graph is described by the equation:

$y = a + bx$



(a) *(b)*

Figure 7. Effect of a) H_2SO_4 and b) the reaction time (Method B; 9 μg ml^{-1} ETM).

where y is absorbance, a is intercept, b is slope and x is concentration) obtained by the method of least squares. The correlation coefficient, intercept and slope for the calibration data are summarized in Table 2. Sensitivity parameters such as apparent molar absorptivity and sandell sensitivity values, the limits of detection and quantification are calculated as per the current ICH guidelines [41] are compiled in Table 2 speak of the excellent sensitivity of the proposed methods. The limits of detection (*LOD*) and quantification (*LOQ*) were calculated according to the same guidelines using the formula:

$LOD = 3.3\sigma/s$

and

$LOQ = 10\sigma/s$

where σ is the standard deviation of five reagent blank determinations and s is the slope of the calibration curve.

Table 2. Sensitivity and regression parameters

Parameter	Method A	Method B
λ_{max}, nm	610	545
Linear range, μg ml^{-1}	0.5-5.0	1.5-15
Molar absorptivity (ε), l mol^{-1} cm^{-1}	2.79×10^4	4.2×10^4
Sandell sensitivity[a], μg cm^{-2}	0.0094	0.0063
Limit of detection (*LOD*), μg ml^{-1}	0.06	0.14
Limit of quantification (*LOQ*), μg ml^{-1}	0.18	0.42
Regression equation, y^b		
Intercept (a)	0.0000	+0.860
Slope (b)	0.095	-0.046
Standard deviation of a (S_a)	0.012	0.145
Standard deviation of b (S_b)	0.003	0.011
Regression coefficient (r)	0.998	-0.999

[a]Limit of determination as the weight in μg per ml 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; [b]$y = a + bx$, where y is the absorbance, x is concentration, a is intercept, b is slope.

Precision and accuracy

Intra-day precision and accuracy of the proposed methods were evaluated by the replicate analysis ($n = 5$) of calibration standards at three different concentration levels on the same day. Inter-day precision and accuracy were determined by assaying the calibration standards at the same concentration levels on five consecutive days. The precision and accuracy were based on five consecutive days. The precision and accuracy were based on the calculated relative standard deviation (*RSD*, %) and relative error (*RE*, %) of the found concentration compared to the theoretical one, respectively (Table 3).

Selectivity

The proposed methods were tested for selectivity by placebo blank and synthetic mixture analysis. A convenient aliquot of the placebo blank solution prepared as described earlier was subjected to analysis by titrimetry and spectrophotometry (methods A and B) according to the recommended procedures. In all the cases, there was no interference by the inactive ingredients.

A separate experiment was performed with the synthetic mixture. The analysis of the synthetic mixture solution prepared above yielded percent recoveries ranged between 93.8 and 112.8 with standard deviation of 0.82-1.94 in all the cases. The results of this study are presented in Table 4 indicating that the inactive ingredients did not interfere in the assay. These results further demonstrate the accuracy as well as the precision of the proposed methods.

Application to formulations

In order to evaluate the analytical applicability of the proposed method to the quantification of ETM in commercial tablets, the results obtained by the proposed methods were compared to those of the reference method [42] by applying student's *t*-test for accuracy and *F*-test for precision. The results (Table 5) show that the Student's *t*- and *F*-values at 95% con-

Table 3. Evaluation of intra-day and inter-day accuracy and precision

Method	ETM taken[a]	Intra-day accuracy and precision			Inter-day accuracy and precision		
		ETM found	*RE*[b], %	*RSD*[c], %	ETM found	*RE*, %	*RSD*, %
Titrimetry	2.0	1.99	0.5	1.05	1.97	1.50	2.76
	4.0	3.98	0.5	1.46	3.90	2.50	2.58
	6.0	6.11	1.8	1.17	5.89	1.83	2.28
Spectrophotometric method A	1.0	0.98	2.00	1.58	0.97	3.00	3.66
	2.0	1.96	2.00	1.76	1.95	2.50	4.05
	3.0	2.95	1.67	2.14	2.90	3.33	3.78
Spectrophotometric method B	3.0	3.07	2.33	1.17	2.91	3.00	3.32
	6.0	6.05	0.83	0.22	5.84	2.67	2.96
	9.0	8.95	0.55	1.23	8.67	3.67	2.76

[a]In titrimetry, ETM taken/found are in mg and they are μg ml^{-1} in spectrophotometry; [b]relative error; [c] relative standard deviation

fidence level are less than the theoretical values, which confirmed that there is a good agreement between the results obtained by the proposed methods and the reference method with respect to accuracy and precision.

Table 4. Recovery of the drug from the synthetic mixture

Method	ETM in synthetic mixture taken[a]	ETM recoverd[b] (±*SD*) %
Titrimetry	3.0	99.74±0.98
	6.0	101.2±0.82
	9.0	102.7±1.16
Spectrophotometric method A	1.0	97.06±1.05
	2.0	95.75±0.94
	3.0	93.82±1.11
Spectrophotometric method B	3.0	108.1±1.67
	6.0	112.8±1.94
	9.0	96.83±1.08

[a]mg in titrimetry and μg ml^{-1} in spectrophotometry; [b]mean value of five determinations

Recovery studies

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure ETM at three concentration levels (50, 100 and 150% of that in tablet powder) and the total was found by the proposed methods. In all cases, the added ETM recovery percentage values ranged between 98.7 and 126% with standard deviation of 0.54--1.78 (Table 6) indicating that the recovery was good, and that the co-formulated substance did not interfere in the determination.

CONCLUSIONS

The proposed titrimetric method is the first report on the application of titrimetry for the assay of etamsylate. The titrimetric method is straightforward and fast when compared to the other methods reported earlier. The spectrophotometric methods employ

Table 5. Results of analysis of tablets by the proposed methods

Tablet brand name	Label claim mg/tablet	Found[a] (±*SD*), % (label claim)			
		Reference method	Titrimetry	Spectrophotometry	
				Method A	Method B
K-Stat[b] 250	250	98.66±1.14	99.32±0.66	100.1±1.41	98.74±1.52
			t^f = 1.12	*t* = 1.78	*t* = 0.09
			F^g = 2.98	*F* = 1.53	*F* = 1.78
Dicynene[c] 250	250	101.3±0.85	102.1±1.14	100.4±1.26	99.67±1.74
			t = 1.26	*t* = 1.33	*t* = 1.89
			F = 1.79	*F* = 2.19	*F* = 4.19
Hemsyl[d] 250	250	97.16±0.92	96.57±1.42	97.67±1.06	96.76±1.24
			t = 0.78	*t* = 0.81	*t* = 0.58
			F = 2.38	*F* = 1.33	*F* = 1.82
Sylate[e] 250	250	93.45±1.32	92.86±1.04	93.06±1.11	92.84±1.36
			t = 0.78	*t* = 0.51	*t* = 0.72
			F = 1.61	*F* = 1.41	*F* = 1.06

[a]Mean value of five determinations; [b]Mercury, Lab. Ltd., H. P., India; [c]Dr. Reddy's Lab. Ltd., H. P., India; [d]Indoco Rem. Ltd., Mumbai, India; [e]Emcure Pharm. Ltd., Pune, India; [f]the value at 95% confidence level and for four degrees of freedom is 2.77; [g]the value at 95% confidence level and for four degrees of freedom is 6.39

Table 6. Accuracy assessment by recovery experiments

Method	Tablet studied	ETM in tablet[a]	Pure ETM added[a]	Total found[a]	Pure ETM recovered[b] (±*SD*), %
Titrimetry	K-Stat 250	3.97	2.0	6.00	101.7±0.63
		3.97	4.0	8.23	106.6±1.17
		3.97	6.0	10.19	103.7±0.85
	Dicynene 250	4.08	2.0	6.13	102.7±1.05
		4.08	4.0	8.03	98.7±0.93
		4.08	6.0	10.20	102.0±0.64
Spectrophotometric method A	K-Stat 250	2.00	1.0	3.06	106.2±1.54
		2.00	2.0	4.11	105.4±1.78
		2.00	3.0	5.09	103.3±1.62
	Dicynene 250	2.01	1.0	3.03	102.4±1.56
		2.01	2.0	4.18	108.3±1.43
		2.01	3.0	5.18	105.5±1.05
Spectrophotometric method B	K-Stat 250	4.94	2.5	7.52	103.3±0.54
		4.94	5.0	10.04	102.0±1.07
		4.94	7.5	12.58	101.8±0.73
	Dicynene 250	4.98	2.5	7.45	98.7±0.96
		4.98	5.0	10.69	114.3±1.03
		4.98	7.5	12.87	105.2±0.74

[a]mg in titrimetry and μg ml^{-1} in spectrophotometry; [b]mean value of three measurments

mild working conditions without heating or extraction. The methods are based on well established and characterized redox reactions and use cheaper and readily available chemicals. These methods are good alternative to a few reported methods with respect to speed, simplicity, sensitivity, selectivity, cost-effectiveness and the stability of the measured species. Though the sodium azide method [5] looks sensitive, the absorbance is measured at 348 nm where the interference from the co-formulated substances is far more than at longer wavelengths used in the present methods. The methods have been demonstrated to be free from common tablet excipients and additives. These merits coupled with the use of a simple and inexpensive instrument, recommend the use of the methods in routine quality control Laboratories.

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