Research Article

Development of New Analytical Method and Its Validation for the Determination of Ethamsylate in Bulk and Marketed Formulations

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ABSTRACT

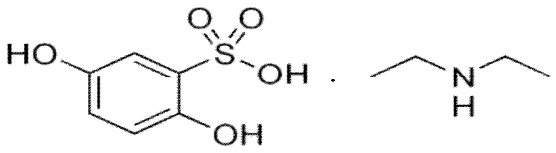
Method is based on the oxidation of Ethamsylate with ferric ammonium sulphate followed by complex formation of resulting ferrous ion (Fe^{2+}) with 1, 10- phenanthroline to form orange red coloured chromogen exhibiting absorption maximum at 509nm and obeyed Beer's law in the concentration range of 2-10 μg/ml. The correlation coefficient was found 0.9998. The method has been validated according to ICH Guidelines.

Keywords: Ethamsylate, Ferric ammonium sulphate, 1, 10- Phenanthroline.

INTRODUCTION

A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge for chemical substances came from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample[1].

Ethamsylate is chemically Diethylammonium 2, 5-dihydroxybenzenesulphonate.Ethamsylate is haemostatic drug. It is believed to work by increasing capillary endothelial resistance and promoting platelet adhesion. It also inhibits biosynthesis and action of those prostaglandins which cause platelet disaggregation, vasodilation and increased capillary permeability. It hasbeen used in the prevention and treatment of capillary bleeding in menorrhagia especially IUD induced bleeding, bleeding after abortion, PPH, malena, hematuria and bleeding after tooth extraction. It is easily soluble in water, soluble in ethanol and slightly soluble in acetone, insoluble in chloroform and ether[2-4].



The literature survey reveals that few analytical methods for this drug are reported, which include chromatographic and spectrophotometric methods[13-17].

The validation defines eight steps which are Accuracy, Precision, Specificity, Limit of detection, Limitof quantitation, Linearity and range, Ruggedness which are described in validation part.

EXPERIMENTAL

INSTRUMENTS

(1) The instrument used for the present study was PC based Jasco V-630 UV-Visible double beam Spectrophotometer with 1 cm matched pair quartz cell and spectral bandwidth of 1.5 nm.

(2) SHIMADZU UV-Visible 1700spectrophotometer.

MATERIALS

Pure drug of Ethamsylate was obtained from Yarrow chem Ltd, and commercial formulations were procured from local market. All the chemicals used were of analytical grade.

REAGENTS

- Ferric ammonium sulphate (1%w/v)
- 1, 10- Phenanthroline (0.5%w/v)
- Distilled water
- Dilute Nitric acid

PREPARATION OF REAGENTS AND SOLUTIONS

Preparation of 0.5 % w/v 1, 10 Phenanthroline: The solution was prepared by dissolving 500mg of 1, 10 - Phenanthroline in 100ml distilled alcohol.

Preparation of 1.0 % w/v Ferric ammonium sulphate: The solution was prepared by dissolving 1gm of Ferric ammonium sulphate in 50ml of distilled water and adding 6ml of dilute Nitric acid. Finally make up the volume up to 100ml with distilled water.

Preparation of standard stock solution of Ethamsylate: Standard stock solution was prepared by accurately weighing 100 mg of Ethamsylate in 100 ml calibrated volumetric flask and made up the volume with distilled water up to 100 ml to obtain 1000µg/ml.

Preparation of working standard solution of Ethamsylate: Working standard was prepared by transferring of 10ml standard stock solution into 100 ml calibrated volumetric flask and made up the volume with distilled water for getting concentration of 100µg/ml.

METHOD

From the standard working solution ranging from 0.2 to 1.0ml (1ml=100µg/ml) were transferred in to a series of 10 ml volumetric flasks. To each flask 1.5 ml of Ferric ammonium sulphate was added followed by 1.5 ml of 1,10-phenanthroline. The reaction mixtures were kept a side for 10 minutes for the completion of reaction and volume was made up to 10ml with distilled water. The absorbance of orange red colored chromogen was measured at 509 nm against a reagent blank. The results are given in **Table no. 1** and spectra is given in **Figure no.2**

METHOD OF VALIDATION

A. Linearity

Linearity was determined over the range of 2 to 10µg/ml. 5 different 10ml volumetric flasks were taken. To each flask 0.2, 0.4, 0.6, 0.8 and 1.0ml of working standard of Ethamsylate were added. To this 1.5 ml of 1.0 % Ferric ammonium sulphate was added followed by 1.5 ml of 0.5 % of 1, 10 Phenanthroline. The reaction mixtures were kept a side for 10 minutes for the completion of reaction and volume was made up to 10 ml with distilled water. The blank was also prepared simultaneously in the same way omitting the drug. The absorbance of the resulting solutions was measured at 509nm against reagent blank. The results are given in **table no.1** and graph is given in **figure no.3**

B. Accuracy(%Recovery)

The accuracy of the methods was determined by calculating % recovery of Ethamsylate by standard addition method. Known volumes of standard solutions of Ethamsylate were taken for recovery studies in 3 different levels 80, 100, 120% and recovery study was carried out. The results are given in **table no.3**

C. Method precision (% Repeatability)

The precision of the methods was checked by repeated measurement of the absorbance of standard solutions (n = 5) of 2, 4, 6, 8, and 10µg/ml without changing the parameters for the method. The repeatability was expressed in terms of relative standard deviation (RSD). The result is given in **table no.3**

D. Intermediate precision

The intraday and inter day precision of the proposed methods were performed by analyzing the corresponding responses three times on the same day and on three different days over a period of one week for three different concentrations of standard solutions of Ethamsylate (2, 4, 6 µg/ml). The results are given in **table no.3**

E. Reproducibility

The absorbance readings of 4µg/ ml were measured at different laboratory using different spectrophotometer by another analyst and the %RSD values obtained to verify their reproducibility. The results are given in **table no.3**

F. LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations

designated by International Conference on Harmonization (ICH) guideline:

LOD = 3.3 X σ/S and LOQ = 10 X σ/S

Where, σ = the standard deviation of the response,
S = slope of the calibration curve.
The results are given in **table no.3**

G. ANALYSIS OF FORMULATION

Ethamsylate is marketed as Ethamcip-250mg tablet manufactured by Cipla Limited were taken for analysis.

Preparation of sample solution: Tablet powder equivalent to 100mg was weighed accurately and transferred into 100ml volumetric flask and made up the volume with distilled water to get 1000µg/ml concentration (Stock solution-A). Stock solution-A was further diluted to get concentration of 100µg/ml (Stock solution-B).

Procedure: From Stock solution B 0.2ml was pipetted out into a 10ml volumetric flask. To this 1.5 ml of 1.0 % Ferric ammonium sulphate was added followed by 1.5 ml of 0.5 % of 1, 10 Phenanthroline was added. The reaction mixtures were kept a side for 10 minutes for the completion of reaction and volume was made up to 10ml with distilled water. The blank was also prepared simultaneously in the same way omitting the drug. The absorbance of the resulting solutions was measured at 509nm against reagent blank in UV Spectrophotometer. Amount of Ethamsylate obtained was calculated from calibration curve and the result is recorded in **table No.2**

RESULTS AND DISCUSSION

The proposed method involve the oxidation of Ethamsylate with ferric ammonium sulphate and subsequent complexation of resulting Fe2+ with 1, 10-phenanthroline. The probable reaction mechanism is shown in Figure 1. Fe^{2+}oxidizes Ethamsylate and the produced Fe^{2+}forms Orange red colored complex. The absorbance of the colored solution increases linearly with an increasing concentration of the Ethamsylate. The method obeys Beer-Lambert's law in the concentration range of 2-10 µg/ml of ethamsylate. Themethod was validated for various parameters like linearity, accuracy, precision and recovery, LOD, LOQ and Reproducibility.

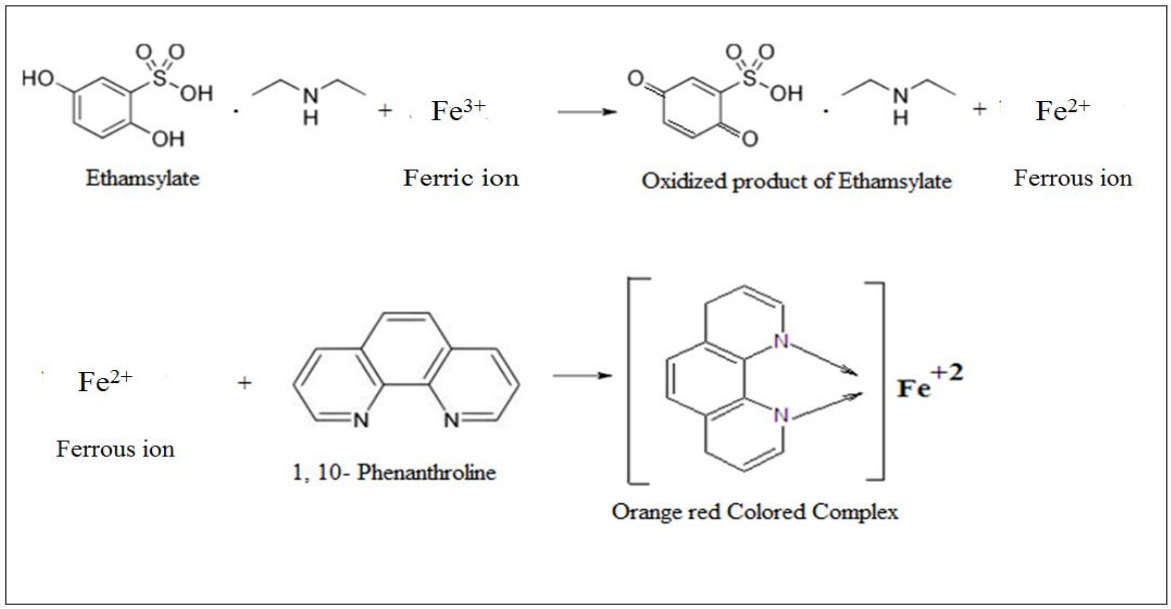


Fig. 1

Table 1: Absorbance of Different concentration of Ethamsylate Obeying beer's law at 509nm

Sr. No.	Volume of working standard of drug (ml)	Concentration of drug taken (µg/ml)	Absorbance at 509nm
1.	0.2 ml	2 µg	0.1762
2.	0.4 ml	4 µg	0.3465
3.	0.6 ml	6 µg	0.5087
4.	0.8 ml	8 µg	0.6821
5.	1.0 ml	10 µg	0.8618

Table 2: Assay result of Marketed formulation of Ethamsylate at 509nm

Formulation	Actual concentration of Ethamsylate(µg/ml)	Amount obtained of Ethamsylate (µg/ml)	% Ethamsylate
Ethamcip-250 tablet	4µg/ml	3.8982 µg/ml	97.456%

Table 3: Statistical data for Ethamsylate at 509nm

Parameter	Result
λmax	509nm
Linear Range (µg/ml)	2-10 µg/ml
Molar Absorptivity (1/mol.cm)	8.55×10^{-2}
Regression Equation (y=bx+a) Slope (b) Intercet (a)	 b = 0.0855 a = 0.0019
Correlation coefficient (R^2)	0.9998
Standard Deviation of Slope	0.0001169
Standard Deviation of Intercept	0.00031411
% recovery	1) At level-1 (80%) = 99.032% 2) At level-2 (100%) = 98.525% 3) At level-3 (120%) = 98.811%
Repeatability Data(%RSD)	0.11-0.695
Reproducibility:- Instrument 1 (%RSD) Instrument 2 (%RSD)	 0.1199 0.15755
Precision (n=3) Inter day precision (%RSD) Intra day precision (%RSD)	 0.0577-0.2566 0.2251-1.225
Limit of Detection (µg/ml)	0.0045128
Limit of Quantitation(µg/ml)	0.013675

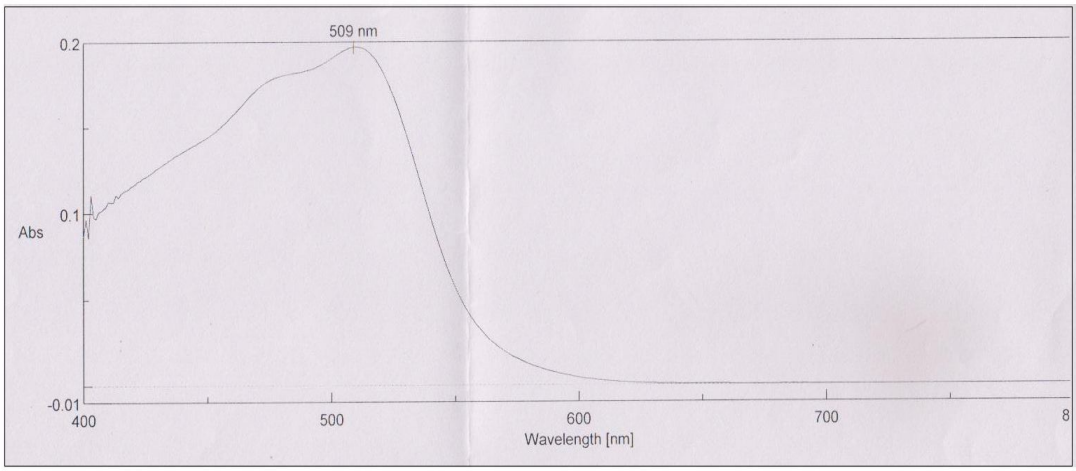


Fig. 2: λ_{max} of Orange red colored complex

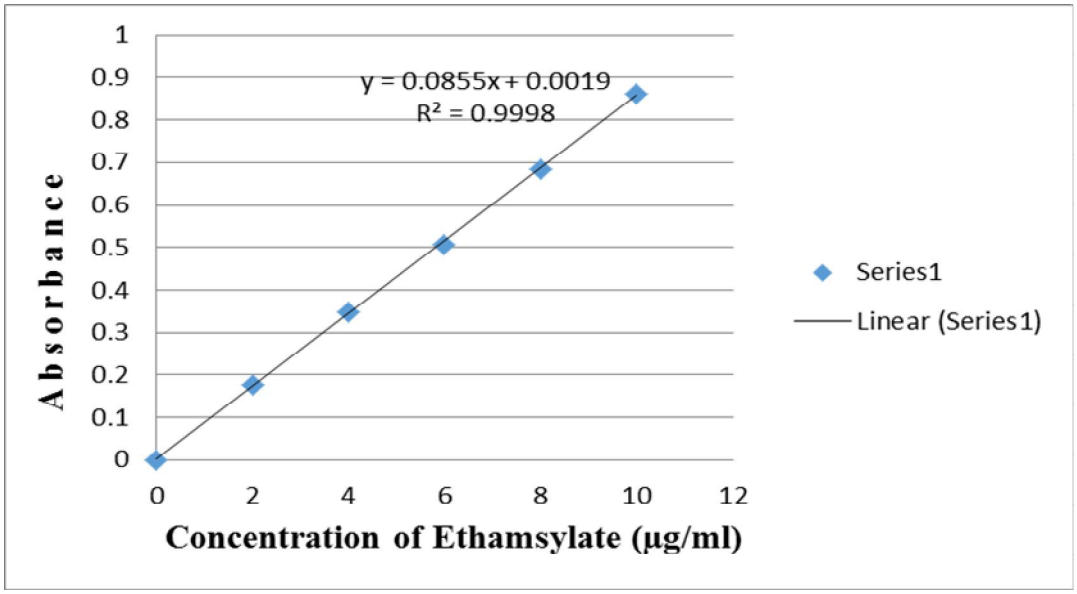


Fig. 3: Calibration Curve of Ethamsylate at 509nm

CONCLUSION

The developed visible spectrophotometric method gives sensitive, accurate, precise and economical results for determination of Ethamsylate in bulk as well as in pharmaceutical formulation. The most striking feature of these methods is its simplicity and low cost.

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