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Research Article

Validated method for estimation of ethamsylate by UV-spectroscopy

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Abstract

For the estimation of ethamsylate in pharmaceutical tablet dosage forms an accurate, simple, efficient spectrophotometric method was developed and validated. It showed absorption maxima at 300 nm with distilled water. According to ICH guidelines, the method was validated concerning specificity, linearity, accuracy, and precision. Ethamsylate's linearity and range were found to be 5 to 50 µg/ml, with a correlation coefficient of 0.999. The analysis's findings were verified statistically and estimation of ethamsylate was done in marketed formulation.

Introduction

Ethamsylate chemically designated as 2, 5-Dihydroxybenzenesulfonic acid; N-ethylamine is a category of antihemorrhagics / antihemophilic with molecular formula $C_{10}H_{17}NO_5S$ (Figure 1). The molecular mass of Ethamsylate is 263.34 gm/mol [1]. It is a white crystalline powder that is easily soluble in water and ethanol; slightly soluble in acetone and insoluble in chloroform and ether [2]. Ethamsylate stops hemorrhage from small blood vessels by stabilizing the capillary wall and correcting abnormal platelet adhesion. It is also used as prophylaxis and treatment of periventricular hemorrhage in low birth weight infants. It is absorbed from the GI tract (oral) [3,4].

According to the literature review, there are only a few analytical methods available for the separation and estimation of Ethamsylate, such as UV-Spectrometry, RP-HPLC, HPTLC, GC, and LC-MS [5-8]. There is a need to develop and validate a new simple, rapid, reliable, and precise UV spectrophotometric method for the analysis of Ethamsylate [9-11]. Suitable statistical tests were performed on validation data. The goal of this work is to develop a new method for the UV-estimation of Ethamsylate and this is a simple, cost-effective, reproducible, and reliable spectroscopy method.

Materials and methods

Materials

Instruments used- Agilent Tech. provided the Single beam UV-Spectroscopy model no. Cary 60 UV-Vis; Shimadzu Instrument Pvt. Ltd. Provided Digital weighing balance model

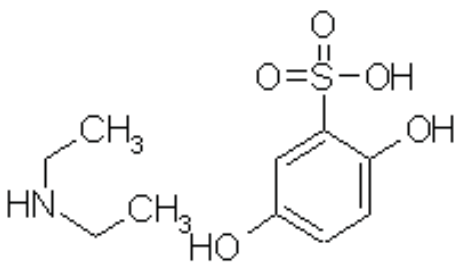

Figure 1: Chemical structure of Ethamsylate.

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no. TX323L; Perkin Elmer provided FT-IR model no. Spectrum 2. Reagents and solutions- Methanol grade A from S.D.Fine Chemical Ltd.; Ethamsylate from Windlas Biotech Ltd (Tables a-c).

Table a: Active Pharmaceutical Ingredient used.

Drug name	Company name	Batch number	Mfg. date	Exp. date
Ethamsylate	Windlas Biotech Ltd.	KM4129	Apr-23	Feb-25

Table b: Instruments used.

S.No.	Name	Model	Suppliers/Manufacturers
1	Single beam UV-Spectroscopy	Cary 60 UV-Vis	Agilent Tech.
2	Digital weighing balance	TX323L	Shimadzu Instrument Pvt. Ltd.
3	FT-IR	Spectrum 2	Perkin Elmer

Table c: Chemicals used.

S.No.	Name	Grade	Suppliers/Manufacturers
1	Distilled Water	Doble distilled	In-house Production
2	Methanol	A	S.D.Fine Chemical Ltd.

Standard stock solution preparation

Weighed accurately 50 mg of Ethamsylate in a 50ml volumetric flask then added 20 ml of distilled water and made the volume up to the mark. 1 ml of this solution was transferred to a 10 ml volumetric flask and diluted up to 10 ml with distilled water. 1 ml of this dilute solution was further diluted with 10 ml of distilled water. This solution contained 10 µg of drug per ml of the solution.

Wavelength selection

1ml of standard stock solution was pipette out and transferred to a 10 ml of the volumetric flask, then the volume was made up to the mark with distilled water. This solution contained 1µg/ml of the drug. The absorbance of this solution was scanned in the UV range of 200 to 400 nm against distilled water as blank [12,13]. Ethamsylate was detected at 300 nm as shown in Figure 2.

Melting point determination

The melting point was determined by the melting point apparatus. The compound was placed in one-end-sealed capillary. The capillary and thermometer were placed in the caves made for the capillary and thermometer respectively [14]. The temperature at which the compound melted was measured by the thermometer and the melting range of Ethamsylate was found to be 127 °C – 131 °C.

Method validation

The devised analytical technique was validated following ICH for the following criteria: system suitability; specificity; accuracy; precision; linearity and range; Limit of Detection (LOD) and Limit of Quantification (LOQ) [15-17].

System suitability

1gm of Ethamsyltae was dissolved in 50 ml of water in a stoppered vessel at room temperature and the vessel was shaken for a few minutes and examined visually for a few more minutes and examined visually for the presence of any residue. No residue was present in the solution interfering with the free solubility of Ethamsylate in water.

Specificity

The system's appropriateness for specificity was tested to see if any contaminants interfered with the analytical peak's retention time. Blank injections were used to conduct the trial [18].

About 1ml of the stock solution was taken in six 10 ml volumetric flasks and about 1ml of a 10 µg/ml solution of each excipient (magnesium stearate, starch, talc, lactose) was added to them and the volume was made up to mark with distilled water. The concentrations of the solutions were determined and % interference was calculated. The results are shown in Table 1.

Accuracy

For Ethamsylate, the accuracy study was carried out at 80%, 100%, and 120%. Each level's area was used to calculate the recovery percentage. 10 µg/ml Ethamsylate standard solution was spiked with 8, 10, and 12 µg/ml sample solution of Ethamsylate tablets. The absorbance was measured and % recovery results are shown in Tables 2,3.

Acceptance Criteria: Each level of concentration should have a recovery percentage of 98% - 102%.

Precision

0.5 ml of the standard stock solution was taken in a 10ml

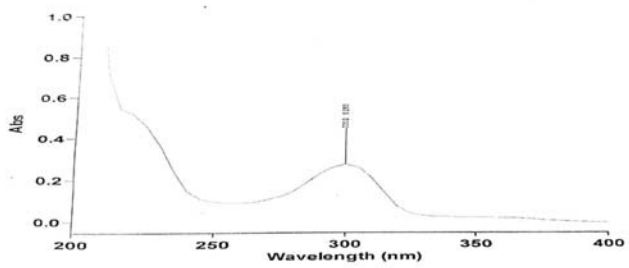

Figure 2: Scan of Ethamsylate in the range of 200 to 400 nm.

Table 1: Specificity study for Ethamsylate.

No.	Conc. (µg/ml)	Before the addition of excipients		After the addition of excipients		% interference
		Abs.	Conc.	Abs.	Conc.	
1.	10	0.1761	10.07	0.1769	10.06	0.099
2.	10	0.1777	10.00	0.1800	9.87	1.3
3.	10	0.1742	10.21	0.1744	10.17	0.197
4.	10	0.1775	10.01	0.1773	10.02	-0.1
5.	10	0.1755	10.13	0.1751	10.15	-0.198
6.	10	0.1753	10.10	0.1763	10.07	0.296
Mean						0.2656

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volumetric flask and the volume was made up to the mark with distilled water. This solution contains 5 µg/ml of the drug. The absorbance of this solution was measured six times and recorded. The results are shown in Table 4.

Acceptance Criteria: The RSD percentage should not be greater than 2% for the area of six standard injection results.

Intra-day precision

2 ml, 3 ml, and 4 ml of the standard stock solution was taken in a 10 ml volumetric flask and the volume was made up to the mark with distilled water to obtain 20, 30, and 40 µg/ml concentrations. The absorbance of these solutions individually was measured thrice within a day and recorded. The results are shown in Table 5.

Inter-day precision

2 ml, 3 ml, and 4 ml of the standard stock solution was taken in a 10 ml volumetric flask and the volume was made up to the mark with distilled water to obtain 20, 30, and 40 µg/ml concentrations. The absorbance of these solutions individually was measured thrice in three days and recorded. The results are shown in Table 6.

Linearity and range

A linear relationship was observed between absorbance and concentration in the working range of 5 to 50 µg/ml of drug in the solution at 300 nm [19]. Take distilled water as blank and also plot a graph between the absorbance obtained and the concentration of the solution. The correlation coefficient of 0.999 was calculated using the area of each level which is shown in Table 7, Figure 3.

Acceptance Criteria: The correlation coefficient for the results of the area of five standard injections must be greater than or equal to (> =) 0.999.

Table 2: Recovery Study of Ethamsylate Tablets.

S.No.	Conc. of reference std. solution (µg/ml)	Conc. of sample solution added (µg/ml)	Conc. after spiking (µg/ml)	% Recovery
80% - Rec-1	10	8	17.9	99.44
80% - Rec-2	10	8	18.1	100.54
80% - Rec-3	10	8	18.1	100.55
100% - Rec-1	10	10	20.5	102.50
100% - Rec-2	10	10	19.7	99.00
100% - Rec-3	10	10	19.7	98.51
120% - Rec-1	10	12	22.2	100.90
120% - Rec-2	10	12	22.3	101.37
120% - Rec-3	10	12	22.1	100.45
Mean				100.36

Table 3: Results of Statistical Analysis of Ethamsylate Tablets.

S.No.	Parameter	Results
1.	Mean ± SD	100.36 ± 1.1598
2.	% Coefficient of variation	1.156

Table 4: Readings of Repeatability Studies for Ethamsylate.

Theoretical conc. (µg/ml)	Absorbance	Observed conc. (µg/ml)	Mean conc. (µg/ml)	SD	RSD
5	0.0979	5.0	5.03333	0.09428	1.873
	0.0954	4.9			
	0.0974	5.0			
	0.1009	5.2			
	0.0970	5.0			
	0.1000	5.1			

Table 5: Intraday Precision.

Conc. (µg/ml)	Absorbance			Conc. Found (µg/ml)			Mean Conc. (µg/ml) ± SD	RSD
	1	2	3	1	2	3		
20	0.3528	0.3483	0.3483	20.4	20.0	19.9	20.13 ± 0.2624	1.304
30	0.5140	0.5154	0.51343	30.0	30.3	29.6	30 ± 0.2449	0.816
40	0.6829	0.6936	0.6959	40.0	40.3	40.5	40.23 ± 0.16996	0.422
Mean RSD 0.847								

Table 6: Interday Precision.

Conc. (µg/ml)	Absorbance			Conc. Found (µg/ml)			Mean Conc. (µg/ml) ± SD	RSD
	1	2	3	1	2	3		
20	0.3485	0.3528	0.3468	20.0	20.5	20.1	20.2 ± 0.21602	1.069
30	0.5155	0.5171	0.5144	30.0	30.2	29.7	29.96 ± 0.20548	0.685
40	0.6834	0.6839	0.6937	39.7	40.0	40.3	40.0 ± 0.24494	0.61237
Mean RSD 0.7887								

Table 7: Results of Co-relation Coefficient.

S. No.	Concentration (µg/ml)	Absorbance (nm)
1	5	0.1027
2	10	0.1778
3	15	0.2672
4	20	0.3484
5	25	0.4299
6	30	0.5141
7	35	0.6197
8	40	0.6828
9	45	0.7812
10	50	0.8534

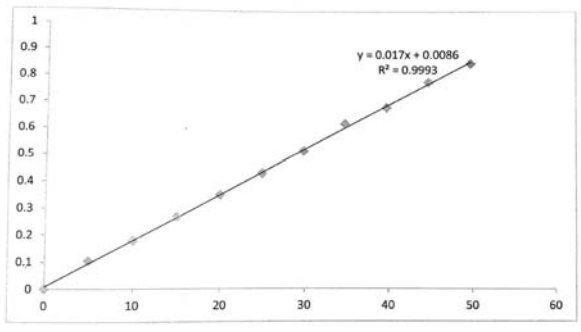

Figure 3: Calibration curve of Ethamsylate.

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Limit of detection and limit of quantification

The smallest concentration of analyte that can be accurately detected by an analytical process is referred to as the Limit of Detection (LOD) and Limit of Quantification (LOQ) [20].

The LOQ might be substantially higher in concentration or it might be equal to the LOD.

Equations were used to calculate the LOD and LOQ.

$LOD = 3.3 \times \sigma / S$ and

$LOQ = 10 \times \sigma / S$

Where,

σ is the standard deviation of the Calibration curve.

S is the average of the slope of the corresponding Calibration curve.

Estimation of ethamsyltae

The powder of twenty tablets of Ethamcip (250 mg) from Cipla Ltd. was taken and accurately weighed. Accurately about 92 mg powder (equivalent to 50 mg of Ethamsylate) was dissolved in about 30ml water and ultra sonicate for up to 15 minutes and filtered through Whatman Filter paper 40 into 50 ml volumetric flak. The residue was washed with more water into the flask. The volume was made up to the mark with water. 2.5 ml of the resultant solution was transferred to 25 ml volumetric flask and the volume was made up to the mark with water to obtain a solution of 10 µg/ml of the drug [21]. The absorbance of this solution was measured in quadruplets and recorded. The concentration was then determined from the calibration curve. The results are shown in Tables 8 and 9.

Result and discussion

Specificity

The specificity test for Ethamsylate was conducted. It was concluded that the developed method is specific and there are no interferences of excipients since the % interference was negligible (0.2656). The results are shown in Table 1.

Readings of accuracy (Tables 2,3)

Precision

Keeping the concentration of the drug the same, the procedure was repeated 6 times. The calculated RSD for the repeatability study is 1.873, which is acceptable; this shows good repeatability of the method.

The absorbance readings were taken in triplicate for each concentration at 0 hr, 3 hr, and 6 hr within a day. The calculated mean RSD was 0.847.

The absorbance readings were taken in triplicate for each concentration at 0 hr, 24 hr, and 48 hr intervals. The calculated mean RSD was 0.7877.

Linearity and range

With a correlation value (R^2) of 0.999, the calibration curve for anastrozole (API) demonstrated good linearity in the range of 5-50 µg/ml. For anastrozole, the standard calibration curve has the regression equation y = 0.017x + 0.0086.

LOD (Limit of Detection) and LOQ (Limit of Quantification)

Ethamsylate's minimum concentration levels at which it can be reliably detected (LOD) and quantified (LOQ) were found to be 0.16 µg/ml and 1.64 µg/ml, respectively, indicating that the method's sensitivity is high.

Estimation of ethamsyltae (Tables 8,9)

Table 8: Results of estimation of Ethamsylate in Ethamcip tablet.

Brand	Label claim (mg)	Theoretical conc. (µg/ml)	Calculated conc. (µg/ml)	Amount found (mg/tab)	% Assay	Mean % Assay ± SD
Ethamcip	250	10	10.10	252.50	101.00	101.1 ± 0.59
			10.03	250.74	100.30	
			10.12	253.00	101.20	
			10.19	254.76	101.90	

Table 9: Results of statistical analysis of Ethamcip tablet.

S. No.	Parameter	Results
1	Mean ± SD	101.1 ± 0.59
2	% Coefficient of variation	0.563

Conclusion

The findings of this investigation demonstrate that the proposed method is simple, rapid, accurate, precise, economical, and reproducible for UV spectrophotometric estimation of Etamsylate from pharmaceutical formulation. The absorbance of this solution was scanned in the UV range of 200 to 400 nm and it was detected at 300 nm. The results of statistical analysis for marketed formulations were studied and are shown in Table 9. Investigation of some new economical analytical methods is needed for the quantitative estimation of Ethamsylate in bulk and pharmaceutical dosage forms with high sensitivity, accuracy, and precision. The spectroscopic method is used because of its simplicity; high specificity and low cost can also effectively analyze the compound.

References

1. British Pharmacopoeia, Her Majesty's Stationery Office. London, Ph Eur Monograph. 1204; 2005; 1:762.
2. Mendham J. Vogel's. Textbook of Quantitative Chemical Analysis. 6th ed. Pearson Education (Singapore) Pvt. Ltd., Indian branch, Delhi. 2003; 3-8:630.
3. O'Neil MJ. Royal Society of Chemistry. Cambridge, UK. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 13th ed New Jersey, Merck and Co. 3757:664.
4. Sweetman SC. Martindale C. The Complete Drug Reference. 33rd ed London (UK), Pharmaceutical Press. 2002:732.

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DOI: https://dx.doi.org/10.17352/ijpsdr.000050

5. Hema SRG. A review on new analytical method development and validation by RP-HPLC. Int Res J Pharm Biosci, 2017; 4:41-50.

6. Nagaraju P, Krishna chaithanya K, Srinivas VDN. Reverse Phase HPLC Method for the Determination of Ethamsylate in Bulk and Tablet Dosage Forms. Asian Journal Research Chem. 2008; 1(2):0974-4169.

7. Vamshikrishan N, Shetty ASK. Development and validation of RP-HPLC Method for the estimation of Ethamsylate in bulk drug and its Pharmaceutical Formulations. Inetrnational Journal ChemTech Res. 2011; 3(2):928-32.

8. Neeraj K, Himani A, Abhijit K, Dhaneshwar SR, Bharat P. Stress degradation studies on etamsylate using stability-indicating chromatographic methods. Analytica Chimica Acta. 2005; 536(1-2):49-70.

9. Yogini SJ, Gokul ST, Sanjay JS. Quantitative Analysis of Ethamsylate and Mefenamic Acid in Tablets by Use of Planar Chromatography. Journal of Planar Chromatography. 2005; 18:460-4.

10. Roshan I, Kaushik KV, Diptish KN. Spectrophotometric methods for Simultaneous estimation of Ethamsylate and Tranexamic acid from combined tablet dosage form. International Journal ChemTech Res. 2010; 2(1):74-8.

11. Pavia D, Lampman G, Krix G. Introduction to Spectroscopy. Bloomington, IN: Indiana University. 2001.

12. Arshad Md, Karadi AB, Raju SA, Venugopal D. Spectrophotometric estimation of Ethamsylate in bulk drug and its Pharmaceutical Formulations, Pharma Science Monitor. An International Journal of Pharmaceutical Sciences. 2011; 2(4):0976-7908.

13. Rao RN, Nagaraju P, Shrinivasulu C, Kumar PJ. Comparative study of UV Spectrophotometric Methods for the Determination of Ethamsylate in Bulk and Pharmaceutical Formulations. Asian Journal of Chemistry. 2004; 16(3): 1956-1958.

14. Indian Pharmacopoeia, Ministry of Health and Family Welfare India. Vol II (A), 1996; 144-147.

15. International Conference on Harmonization, Q2B: Guideline on the Validation of Analytical Procedures: Methodology and Availability. Federal Register. 1997; 62(96):27463–27467.

16. Patil R, Deshmukh T, Patil V, Khandelwal K. Review on analytical method development and validation. Res Rev J Pharm Anal. 2014; 3:1-10.

17. Vamshikrishna N, Shetty ASK. Development and Validation of RP-HPLC Method for the estimation of Ethamsylate in Bulk drug and Pharmaceutical formulations. Int J ChemTech Res. 2011; 3(2):928-32.

18. Pathuri R, Muthukumaran M, Krishnamoorthy B, Nishat A. A review of analytical method development and validation of the pharmaceutical technology. Curr Pharm Res. 2013; 3:855-70.

19. Chitra K, Sujatha K, Ahmed IR, Shalini K, Priya BL, Varghese SS. Spectrophotometric Estimation of Ethamsylate in Tablets and Injection. Indian J Pharm Sci. 2005; 67:98–100.

20. Shivani Sharma, SwapnilGoyal, KalindiChauhan. A review of analytical method development and validation. International Journal of Applied Pharmaceutics. 2018; 10(6):8-15.

21. Arshad MD, Karadi B. A., et al, Development and Validation of Visible Spectrophotometric methods for the Estimation of Ethamsylate in Pharmaceutical Dosage Forms, Scholars Research Library Der Pharma Chemica. 2011; 3(2): 347- 351.

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