Spectrophotometric Estimation of Ethamsylate in Tablets and Injection

K. CHITRA*, K. SUJATHA, IMAN RABIA AHMED, K. SHALINI, B. LAKSHMI PRIYA AND SHARON S. VARGHESE
Sri Ramachandra College of Pharmacy, Sri Ramachandra Medical College and Research Institute (Deemed University), Porur, Chennai-600 116.

Accepted 27 January 2005
Revised 3 August 2004
Received 8 December 2003

Two simple, accurate, rapid and sensitive methods have been developed for the estimation of ethamsylate in dosage forms. Method A is based on the nitrosation of the drug followed by chelation of the o-nitroso derivative formed with Cu^{++} ions forming a stable purple coloured chromogen, which shows absorption maximum at about 525 nm while method B is based on the reduction of Fe^{+++} to Fe^{++} ions by ethamsylate which then reacts with potassium ferricyanide to produce a blue coloured complex that shows maximum absorption at about 750 nm against reagent blank. In both the methods Beer's law was obeyed in the concentration range of 2-20 μg/ml.

Chemically ethamsylate is diethyl ammonium-2,5-dihydroxy benzene sulphonate and is used as a haemostatic agent[1]. It stops haemorrhage from small blood vessels by stabilizing the capillary wall. It is indicated for prevention and treatment of capillary haemorrhage associated with haematemesis, menorrhagia and post-partum haemorrhage. The drug is official in British Pharmacopoeia and Martindale[1,2]. Literature survey revealed potentiometric[2], IR[2] and UV spectrophotometric[3] methods for the estimation of ethamsylate in tablets and injection.

The present work describes two simple colorimetric methods for the estimation of ethamsylate in pharmaceutical dosage forms. Method A is based on the nitrosation of the drug followed by chelation of the o-nitroso derivative formed with Cu^{++} ions forming a stable purple coloured chromogen showing maximum absorption at about 525 nm against reagent blank. Method B is based on the reduction of Fe^{+++} to Fe^{++} ions by ethamsylate, which then reacts with potassium ferricyanide to produce blue coloured complex showing maximum absorption at about 750 nm against reagent blank. A systronics spectrophotometer 117 with 1 cm matched cuvettes was used for the measurement of absorbance. Sodium nitrite solution (3%), 1M hydrochloric acid, 1% copper acetate, 0.01M ferric chloride and 0.025% potassium ferricyanide were freshly prepared in distilled water.

***For correspondence**
E-mail: chitrasrmc@rediffmail.com

Standard solution of ethamsylate was prepared by dissolving 100 mg in 100 ml and diluting 10 ml of this solution to 100 ml with distilled water (100 μg/ml). Twenty tablets of ethamsylate were weighed and powdered in a glass mortar. Powder equivalent to 100 mg of ethamsylate was accurately weighed and dissolved in distilled water to make 100 ml. The solution was then filtered and 10 ml of the filtrate was diluted to 100 ml with distilled water. Volume equivalent to 100 mg of ethamsylate was transferred into 100 ml volumetric flasks and diluted to volume with distilled water. 10 ml of this solution was diluted to 100 ml with distilled water.

In method A, aliquots of standard solution equivalent to 0.1 to 2.5 ml were transferred to a series of 10 ml volumetric flask. To each flask, 2 ml of 3% sodium nitrite solution was added, and the contents were mixed well. Then 2 ml of 1% copper acetate and 0.3 ml of 1M hydrochloric acid were added and mixed well after each addition. The flasks were heated on a boiling water bath for 25 min and cooled. The volume in each flask was adjusted to 10 ml with distilled water. The absorbance of the solution in the flask was measured at 525 nm against reagent blank prepared in the same manner without the addition of the drug and the calibration curve was plotted. Similarly the absorbance of the sample solution was measured and the amount of ethamsylate was determined by referring to the calibration curve.

In method B, aliquots of standard solution equiva-

TABLE 1: OPTICAL CHARACTERISTICS AND PRECISION

Observation	Method A	Method B
Absorption maxima (nm)	525	750
Beer's law limit (μg/ml)	2-20	2-20
Correlation coefficient	0.9999	0.9999
Molar extinction coefficient (l^{-1} $mole^{-1}$ cm^{-1})	1.194×10^5	1.760×10^4
Sandell's sensitivity (μg/cm^2/0.001 Absorbance unit)	0.00294	0.01996
Regression equation (y=mx+c)		
Slope (m)	3.39×10^{-2}	5×10^{-2}
Intercept (c)	0.0035	-0.000225
% Range of error		
(Confidence 95%)	±0.5648	±0.6543

lent to 0.1 to 2.5 ml were transferred to a series of 10 ml volumetric flask. To each flask, 05 ml of ferric chloride 0.5 ml of 0.025% potassium ferricyanide reagent were added. The solution was kept for 5 min to complete the reaction and the volume in each flask was adjusted to 10 ml with distilled water. The absorbance of the solution in each flask was measured at 750 nm against reagent blank prepared in the same manner without the addition of the drug and the calibration curve was plotted. Similarly the absorbance of the sample solution was measured and the amount of ethamsylate was determined by referring to the calibration curve.

To test the accuracy and reproducibility of the proposed method, recovery experiments were carried out by additional amounts of the drug to the reanalyzed formulation and reanalyzing the mixture by proposed method. The results are shown in Table 1. Method A involves nitrosation of the drug followed by chelation of the o-nitroso derivative formed with Cu^{++} ions, forming a stable purple coloured chromogen, which shows absorption maximum at about 525 nm. Method B is based on the reduction of Fe^{+++} ions to Fe^{++} ions by ethamsylate which then reacts with potassium ferricyanide to produce blue coloured complex showing maximum absorption at about 750nm against reagent blank.

Stability study of the chromogen was carried out by measuring the absorbance values at time intervals of 0 min to 4 h and it was found to be stable for 4 h for both methods. The optical characteristics such as absorption maxima, Beer's law limits, correlation coefficient (r), slope (m), y-intercept (c), molar absorptivity, Sandell's sensitivity were calculated from 5 replicate readings are incorporated in Table 1. The molar absorptivity and Sandell's sensitivity values show the sensitivity of both the methods, until the precision is confirmed by % COV (coefficient of variance) values included in Table 2, which are less than 2%. The analysis results of marketed formulations are in good agreement with the reported method 3 which is also a spectrophotometric method for the estimation of ethamsylate. The reproducibility, repeatability and accuracy of these methods were found to be good, which is evident by low standard deviation. The percent recovery obtained (were 99.1 % for method A and 100 % for method B) indicates non-interference from the common excipients used in the formulation. Thus these methods developed in the present investigation are simple, sensitive, accurate and precise and can be successfully applied for the routine estimation of ethamsylate in pharmaceutical dosage form.

ACKNOWLEDGEMENTS

The authors wish to thank the management and the principal, Sri Ramachandra Medical College and RI (DU) for providing laboratory facilities. The authors are also grateful to ATOZ Pharmaceuticals, Ambatur, for providing a gift sample of ethamsylate.

TABLE 2: ANALYSIS DATA OF TABLET/INJECTION FORMULATION.

Formulation	Label claim (mg/tablet or mg/ml)	Method	% label claim* ±SD	% COV	S.E of mean	% Recovery	Reported method[2]
Tablet 1		M1	99.1±1.50	1.51	1.86	98.5	
Alstat	250						98.9
(Albert David)		M2	99.7±1.67	1.97	0.83	99.7	
Tablet 2		M1	99.1±1.27	1.29	1.58	99.9	
Dicynene	250						99.6
(Dr. Reddy's Lab)		M2	100±0.43	0.93	0.47	99.8	
Tablet 3		M1	98.8±1.59	1.62	1.98	98.9	98.8
Medistat	250						
(Comed)		M2	99.9±1.42	1.42	0.07	99.9	
Injection 1		M1	101±1.5	1.13	1.48	101	
Alstat	125						98.4
(Albert David)		M2	100±1.48	0.98	0.49	100	
Injection 2		M1	102±1.37	1.24	1.51	102	
Dicynene	125						99.6
(Dr. Reddy's Lab)		M2	100±1.28	1.41	0.70	100	
Injection 3		M1	101±1.42	1.11	1.28	101	
Medistat	125						99.1
(Comed)		M2	100±1.59	1.28	0.64	100	

*Mean of five determinations. M1 – method A, M2 – method B.

REFERENCES

1. Parfitt, K., Eds., In; Martindale: The Complete Drug Reference, 32nd Edn., The Pharmaceutical Press, London, 1999, 720.
2. British Pharmacopoeia, Vol. I, The H.M. Stationary Office, UK, 1998, 547.
3. Shenoy, K.R.P., **Eastern Pharmacist,** 2000, 512, 135.