International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.2, pp 928-932, April-June 2011

Development and Validation of RP-HPLC Method for the estimation of Ethamsylate in Bulk drug and Pharmaceutical formulations

Vamshikrishna N, A.Sathish Kumar Shetty*

Department of Pharmaceutical Analysis, National College of Pharmacy, Balarj Urs Road, Shimoga (District), Karnataka, India – 577 201.

Corres.author: drskshetty@rediffmail.com
Cell: 09449100007

Abstract: A new, simple sensitive, rapid, accurate and precise RP-HPLC method was developed for the estimation of Ethamsylate in bulk drug and pharmaceutical formulation. Ethamsylate was chromatographed on a reverse phase C18column (25cm x 4.6 mm i.d; particle size 5 µm) in a mobile phase consisting of methanol and disodiumhydrogen orthophosphate buffer (pH: 3.0) in the ratio of 60:40 % v/v. The mobile phase was pumped at a flow rate of 0.6 ml/min with detection at 290 nm. The detector response was linear in the concentration of 10-50 µg/ml. The limit of detection and limit of quantitation was found to be 0.89 and 2.7 ng/ml, respectively. The intra and inter day variation was found to be less than 2%. The mean recovery of the drug from the solution was 99.83%. The proposed method is simple, fast, accurate, precise and reproducible hence, it can be applied for routine quality control analysis of Ethamsylate in bulk drug and pharmaceutical formulation.
Key words: RP-HPLC, Quantitation, Ethamsylate, chromatographed.

INTRODUCTION:

Ethamsylate[1-5] is an orally administered haemostatic drug. It is used in the treatment of capillary hemorrhage, hematemesis, hemoptysis, malena, hematuria, epistaxis, menorrhagia and post partum hemorrhage. It is believed to work by increasing capillary endothelial resistance and promoting platelet adhesion. It reduces capillary bleeding when platelets are adequate, probably exerts antihyalurodinase action-improves capillary wall stability. It is also claimed to inhibit PGI_2 production and correct abnormal platelet function, but does not stabilize fibrin[6].

Ethamsylate is chemically 2,5-dihydroxy benzene sulfonicacid with N-ethylethanamine. The molecular formula of Ethamsylate is $C_{10}H_{17}NO_5S$. The molecular mass of Ethamsylate is 263.33 g/mol. It is official drug in British Pharmacopoeia. It is completely soluble in water, methanol, and ethanol but partially soluble in methylene chloride.

Literature survey reveals that, only kinetic spectrophotometric determination[7], Spectrophoto - metric simultaneous estimation from a Binary Mixture by Dual Wavelength[8] and Simultaneous Equation Methods[9] and some HPTLC methods[10] have been reported.

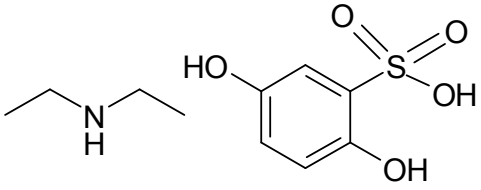

Figure 1. Chemical structure of Ethamsylate

EXPERIMENTAL

MATERIALS AND METHODS:

Quantitative HPLC was performed on a isocratic high pressure liquid chromatography (shimadzu HPLC class VP-Series) with one LC-10 AT VP pump, UV/VIS detector SPD-10A VP, CTO-10 AS VP column oven (shimadzu), SCL-10A VP system controller (shimadzu), a disposable guard column LC-18 (PELLIGUARD)TM, LC-18, 2 cm, supelco, inc., Bellefonte, and a Reverse Phase C-18 Column (150mm x 4.6 mm i.d.particle size 5 µm) was used . The HPLC system was equipped with the software class, N-2000 CHROMTECK (Shimadzu).

REAGENTS AND CHEMICALS

Disodium hydrogen phosphate and orthophosphoric acid of AR grade were obtained from Qualigens Fine Chemicals Ltd., Mumbai. Methanol of HPLC grade was purchased from E.Merck (India) Ltd., Mumbai. Standard Ethamsylate was obtained as a gift sample from Biocon Limited, Karnataka, India. The commercially available Ethamsylate tablets were procured from the local market.

PREPARATION OF BUFFER

DISODIUM HYDROGEN ORTHOPHOSPHATE BUFFER (PH-3.0)

Disodium hydrogen orthophosphate buffer was prepared by dissolving 7.09 gm of disodium hydrogen phosphate in 1000 ml of double distilled water and the pH was adjusted to 3.0 with ortho-phosphoric acid.

CHROMATOGRAPHIC CONDITIONS

The mobile phase consisting of methanol and disodiumhydrogen orthophosphate buffer (pH: 3.0) in the ratio of 60:40 % v/v was filtered through 0.45µ membrane filter before use, degassed and pumped from the solvent reservoir into the column at a flow rate of 0.6 ml/min. The detection was monitored at 290 nm and the run time was 10 minutes. The volume of injection loop was 20 µl and prior to the injection of the drug solution, the column was equilibrated for at least 30 minutes with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.

PROCEDURE

Stock solution of Ethamsylate was prepared by dissolving 10 mg of Ethamsylate in 10 ml standard volumetric flask containing 2.5 ml of mobile phase and the solution was sonicated for 20 min. and then made upto the mark with mobile phase to get a concentration of 1000 µg/ml. Subsequent dilutions of this solution were made with mobile phase to get concentration of 10-50 µg/ml. The standard solutions prepared as above were injected into the 20 µl loop and the chromatogram was recorded as shown in **Figure 2.**

The retention time of Ethamsylate was found to be 4.222 min. The calibration curve was constructed by plotting concentration versus peak area ratio. The amount of Ethamsylate present in sample was calculated through the standard calibration curve. The linearity experiment was carried out in triplicate to ascertain accuracy and precision of the method. The peak area ratios of the drug versus concentration were found to be linear and the results are furnished in **Table 1.**

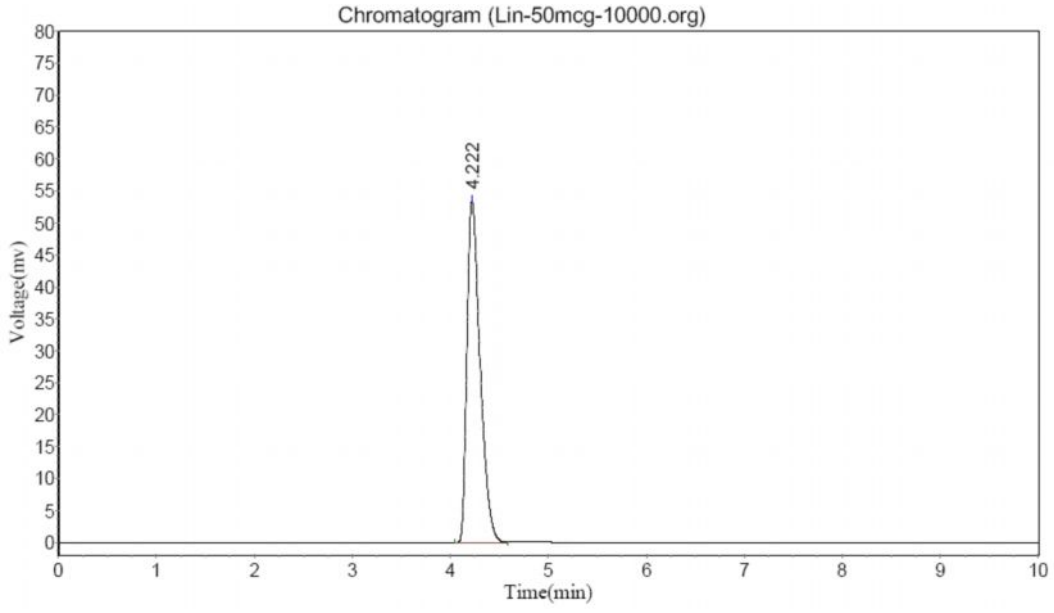

Figure 2. Typical chromatogram of Ethamsylate

Table 1. Calibration data of the method

Sl. No	Concentration (µg/ml)	Area
1	0	0
2	10	109123.00
3	20	200925.906
4	30	300568.250
5	40	407998.344
6	50	498156.094

ASSAY

Twenty tablets each containing 250 mg were weighed accurately and powdered. A quantity equivalent to100 mg of Ethamsylate was weighed accurately and transferred to 100 ml volumetric flask containing 30 ml of mobile phase. The contents were sonicated for 20 min. and made up to the mark with the mobile phase. The resulting solution is filtered through a membrane filter. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined for the pure drug. The sample solution was injected under the chromatographic conditions and the chromatogram was recorded. The amount of Ethamsylate present in tablet formulation was determined by comparing the peak area from the standard. The results were furnished in Table 2.

VALIDATION OF PROPOSED METHOD

Selectivity of the method was assessed on the basis of elution of Ethamsylate using the above mentioned chromatographic conditions. The linearity, precision, accuracy, limit of detection, limit of quantitation and robustness has been validated for the determination of Ethamsylate. The results are furnished in Table 3**.**

LINEARITY

The standard curve was obtained in the concentration range of 10-50 µg/ml. The linearity was evaluated by linear regression analysis using the least square method. It was found that correlation coefficient and regression analysis are within the limits.

PRECISION

The precision of the assay was determined in terms of intra-day and inter-day precision. The intra-day and inter-day variation in the peak area of drug solution was calculated in terms of coefficient of variation (C.V.) obtained by multiplying the ratio of standard deviation to mean with 100. The results are furnished in **Table 4.**

Table 2. Assay of Ethamsylate

Components	Label Claim (mg)	Amount	% Found
Brand 1	250	249.7	99.88
Brand 2	250	249.4	99.76

Table 3. Optical Characteristics of Ethamsylate by RP-HPLC method.

Parameters	RESULT
Linear Range (µg/ml) (C)	10-50
Correlation co-efficient (r^2)	0.9994
Retention Time, min	4.222
LOD (µg/ml)	0.418
LOQ (µg/ml)	1.268
Tailing factor	1.399
Theoretical plates	4593.646

Table 4. Precision of the proposed HPLC method

Concentration of Ethamsylate (µg/ml)	Intra-day Precision		Inter-day Precision	
	Mean area (n=3)	%C.V.	Mean area (n=3)	%C.V.
80	234454.10	1.65	224507.47	0.43
100	300881.90	0.89	297270.26	0.25
120	365528.10	0.70	349106.97	0.26

Table 5. Recovery studies of the proposed HPLC method.

Level of % recovery	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
80%	99.68%	0.3251	0.3261	0.1877
100%	99.96%	0.4645	0.4647	0.2682
120%	99.87%	0.5014	0.5020	0.2895

*n = 3

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ*)*

The LOD and LOQ for Ethamsylate were predicted basing on the parameters of standard error of estimate and slope, calculated from linearity of the response data of Ethamsylate.

ROBUSTNESS

The robustness was checked by changing the flow rate to 0.5 and 0.7 ml/min and the wavelength at 288 and 292nm, the method suits best.

ACCURACY

The accuracy of the HPLC method was assessed by adding known amount of drug solution to a solution of known concentration and subjecting the samples to the proposed HPLC method. The recovery studies were replicated 3 times. The accuracy was expressed in terms of recovery and calculated by multiplying the ratio of measured drug concentration to the expected drug concentration with 30 µg/ml so as to give the percentage recovery. The results are furnished in **Table 5.**

RESULTS AND DISCUSSION:

By applying the proposed method, the run time of the method was set at 10 min and Ethamsylate appeared on the typical chromatogram at 4.222 min, which indicates a good base line. When the same drug solution was injected 3 times, the retention time of the drug was same. Linearity range was observed in the concentration range of 10-50 µg/ml. The regression equation of Ethamsylate concentration over its peak area ratio was found to be Y=9963+3720x (r = 0.9994) where Y is the peak area ratio and X is the concentration of Ethamsylate (µg/ml). The proposed HPLC method was also validated for intra-day and inter-day variation. The coefficient of variation in the peak area of the drug for 3 replicate injections was found to be less than 1%. The tailing factor was found to be 1.399, which indicates good shape of peak. The number of theoretical plates were found to be 4593.646, which indicates efficient performance of the column. The limit of detection and limit of quantitation was found to be 0.89 ng/ml and 2.7 ng/ml, indicates the sensitivity of the method. To optimize the chromatographic conditions, various combinations of disodiumhydrogen orthophosphate buffer and methanol were tested. The use of methanol and disodiumhydrogen orthophosphate buffer (pH: 3.0) in the ratio of 60:40 % v/v resulted in peak with good shape and resolution. The high percentage of recovery of Ethamsylate ranging from 99.44 to 100.45 indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by proposed HPLC method.

CONCLUSION:

The proposed HPLC method was found to be simple, rapid, sensitive, precise and accurate for the estimation of Ethamsylate in pharmaceutical formulations. Hence, this method can be easily and conveniently adopted for routine quality control analysis of Ethamsylate in bulk drug and it's pharmaceutical formulations.

ACKNOWLEDGEMENT:

The authors are highly thankful to the National education society and Principal, National college of Pharmacy, Shimoga for providing all the facilities to carry out the research work.

REFERENCES

1. British Pharmacopoeia, Her Majesty's stationery office, London, Ph Eur Monograph 1204; 2005 vol-1, 762.
2. www.cemolink.com/msds/2624-44-4_130.doc.
3. http://en.wikipedia.org/wiki/Ethamsylate.
4. ONeil M.J, The Merk Index- an encyclopedia of chemicals, Drugs and Biologicals. 13th edNew Jersey, Merk and co., 3757:664.
5. Sweetmann S C, Martindale – The Complete Drug Reference, 33rd ed London (UK), Pharmaceutical Press., 2002:732.
6. EL-ENANY Nahed., BELAL Fathalla., RIZK Mohamed., "Kinetic spectrophotometric determination of Ethamsylate in dosage forms". Journal of AOAC International 2007; 90(3):679-85.
7. Anju Goyal., Singhvi.I., "Spectrophotometric Estimation of simultaneous estimation of Etamsylate and Mefanamic acid from a Binary Mixture by Dual Wavelength and Simultaneous Equation Methods". 2008, 70(1), 105-108.
8. Roshan I., Kaushik KV., Diptish KN., Spectrophotometric methods for Simultaneous estimation of Ethamsylate and Tranexamic acid from combined tablet dosage form. Int J Chem Tech Res. 2010, 2(1),74-8.
9. Roshan I., Kaushik KV., Diptish KN., Spectrophotometric methods for Simultaneous estimation of Ethamsylate and Tranexamic acid from combined tablet dosage form. Int J Chem Tech Res. 2010, 2(1),74-8.
10. Yogini SJ., Gokl ST., Sanjay JS., A simple and sensitive HPTLC method for quantitative analysis of Ethamsylate in tablets. J Planar Chrom. sep/oct;2005, 18, 380-3.

*****