ISSN 0976 – 3333

Available Online at www.ijpba.info.

International Journal of Pharmaceutical & Biological Archives 2010; 1(2): 140 - 143

*ORIGINAL RESEARCH ARTICLE*

# A New Stability- Indicating RP-HPLC Method to Determine Etamsylate in Injection Formulation.

Chandrshekhara.S[1*], Prakash Goudanavar[1], Shamrez Ali M [2], Basavaraj K Nanjwade[2], Mahibub M Kanakal[3], Yusrida Darwis[3]

[1]*KLES's College of Pharmacy, Nipani, Karnataka, India*
[2]*KLES's College of Pharmaceutical Sciences , K.L.E. University , Nehru Nagar , Belgaum, Karnataka, India,*
[3]*Department of Pharmaceutical Technology, School of Pharmaceutical Sciences, University Sains, .Pinang, Malaysia.*

Received 19 April 2010; Revised 3 May 2010; Accepted 5 May 2010

## ABSTRACT

A simple and accurate Reverse Phase High-Performance Liquid Chromatography (RPHPLC) method was developed to determine Etamsylate in injection formulations. The drug was chromatographed on a reversed phase C-18 column. Eluents were monitored at a wavelength of 220 nm using a mixture (40:60) Methanol: Buffer (phosphate buffer). Solution concentrations were measured on weight basis to avoid the use of an internal standard. The method was spastically validated for linearity, accuracy, precision and selectivity. Due to its simplicity and accuracy, method will be useful for routine quality control analysis.

**Key Words:** RP-HPLC, Selectivity, Accuracy, Precision, Stability.

## INTRODUCTION

Etamsylate (2,5-di-Hydroxy- Benzene sulphonic acid compound with N-Ethylethanamine) [1] (**Fig.1a**) is belongs to class of synthetic antihaemorrhagic anti-angioprotective drug acting on the first step of haemostasis (endothelium platelet interaction). It exhibits antihemorrhagic properties and also enhances P-selectin membrane expression in human platelets and cultured endothelial cells by improving platelet adhesiveness and restoring capillary resistance, it is able to reduce bleeding time and blood loses [2,3]. In British Pharmacopoeia thin layer chromatographic method has been reported for quantitative estimation of etamsylate (**Fig.1a**) and its known impurity (benzene-1,4-diol) (**Fig.1b**).

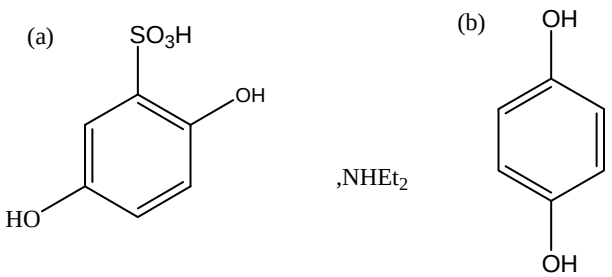


**Fig. 1. (a) Structure of Etamsylate. (b) Structure of Hydroquinone (benzene- 1,4-diol), a known impurity of Etamsylate.**

Very few analytical methods for Etamsylate are being reported in literature, there are few specific methods which is for the determination in tablet formulations [4], and Thin layer chromatographic method has been reported in British Pharmacopoeia for quantitative estimation of Etamsylate and its known impurity (benzene-1,4-diol) , no method was found for Etamsylate determination in injections. Hence there was a need of development and validation of a stability-indicating method for the assay of Etamsylate in injections.

## MATERIALS AND METHODS

### Instrumentation

Analysis was performed on a high performance liquid chromatography consisted of a dual piston reciprocating two LC-10AT VP pumps from Shimadzu Corp, Japan (model HPLC class 10AT), photo iodide array detector from Shimadzu Corp., Japan (model SPD-10M VP) and auto sampler of SIL-10AD series. The HPLC system was equipped with data acquisition and processing software "Class-LC-10 series" Shimadzu Corp., Japan.

***Corresponding Author:** Chandra Shekhar, **Email:** shekhar_bio@yahoo.co.in, **Contact No:** 09341141561.

**Materials, Reagents and Chemicals**

Etamsylate was purchased from the Perfect Protein Pvt Ltd. Raigad, Maharashtra, The methanol (HPLC Grade) used was obtained from E-Merck (India) Ltd. Mumbai; HPLC grade water was prepared using Millipore Purification System (Millipore, Molsheim, France, Model Milli-Q.) and Sodium-di-Hydrogen Phosphate used was AR-grade purchased from S.D fine Chemicals ltd., Mumbai, India.

## EXPERIMENTAL

**Chromatographic Conditions**

The experiment was performed on a 250 mm x 4.6 mm , (ID) x 5 µm particle size Inertsil ODS C $_{18}$ stainless steel column. The mobile phase consisted of 60 % buffer ( Monobasic Sodium Hydrogen phosphate Buffer plus 5 ml Triethanolamine (TEA), 600 ml buffer was used and finally adjusted to pH 2.8 using OrthoPhosphoricAcid (OPA) which was filtered through 0.25µm Nylon membrane filter paper before use and 40% methanol. The chromatography was performed at ambient temperature using a flow rate 1.0 ml/min. Eluents were monitored on photo-doide array detector at a wavelength of 224 nm. The volume of each injection was 20 µl.

**Standard and Sample Solution**

50mg of Etamsylate standard was accurately weighed and dissolved in 100ml of mobile phase as a diluent to get a concentration of 500 µg/mL; 10 ml of the aliquot was further diluted with 50 ml of mobile phase to get a concentration of 50 µg. Etamsylate injection which is in the concentration of 125mg/ml is accurately pipetted and is diluted up to a concentration of 50 µg/mL using the mobile phase as diluent.

**Preparation of Solutions Used for Assay Validation**

For the study of Etamsylate response linearity, different standard solutions were prepared in mobile phase at concentrations ranging from 1 to 100 µg/ mL. System precision was evaluated by performing five consecutive injections of Etamsylate standard solution. Method precision was evaluated by six repeated assays of the same lot of commercial formulation. Assay accuracy was assessed at 50%, 75 %, 100 %, 125 %, and 150% Etamsylate by recovery experiments.

Forced degradation studies were performed to provide an indication of the stability –indicating properties and specificity of the method. Forced degradation was attempted using acid, base and hydrogen peroxide. A degradation sample was prepared by taking 12.5 gm of Etamsylate drug and 100.12 mg of sodium sulphite was taken in 100ml volumetric flask and the solution was made up to the mark by distilled water. Mixed thoroughly till the solute is completely dissolved in the solvent. The solution is then made up to 50 µg/mL. Degradation experiments were performed by taking 5 mL of the above solution in each of three different 50 mL round bottom flasks. To the first flask, 10 mL of 1N HCl was added for acidic degradation. To the second flask, 10 mL of 1 N NaOH was added for basic degradation, To the third flask, 10ml of 30 % $H_20_2$ was added which was then refluxed until 8 hrs for degradation. After completing the degradation treatments. Samples were allowed to cool to room temperature and treated as follows. First, sample was neutralized by adding 1 N NaOH and second one with 1 N HCl. To the third with 1 N sodium bisulfate solution was added. Samples were injected and analyzed against a control sample (lacking degradation treatment).

**Procedure**

All solutions were prepared on a weight basis and solution concentrations were also measured on a weighed a basis to avoid the use of an internal standard. Prior to injecting solutions, the column was equilibrated for at least 30 min with the mobile phase following through the system. Acceptable results for the number of theoretical plates, tailing factor, precision, and detector linearity criteria were assured before sample analysis [5, 6]. Quantification was accomplished using an external standard method. Each solution was injected in triplicate and the relative standard deviation (RSD) was calculated

## RESULTS AND DISCUSSION

**System Suitability**

A suitability test was applied to representative chromatograms to check various parameters such as column efficiency, peak tailing, RSD of peak area, and capacity factor. The results obtained were within acceptable limits (**Table 1**).Thus, the system meets suitability criteria.

**Table 1. System Suitability Parameters.**

| S.No. | Parameter | Value | Acceptance Limits |
|---|---|---|---|
| 1 | Theoretical Plates | >3500 | More than 3000 |
| 2 | Tailing Factor | 0.85 | Less than 2 |
| 3 | RSD of peak | 0.675 | Less than 2% |

| | | | |
|---|---|---|---|
| | area | | |
| 4 | Capacity factor | 1.15 | Not Less than 1 |

## Specificity

Specificity of the developed method was assessed by sample degradation studies. The percentage of etamsylate recovered is shown in **Table 2**, and the representative chromatogram of etamsylate without forced degradants in **Fig. 2** and representative chromatogram of the solvent **Fig. 3**.

**Table No. 2. Degradation of Etamsylate**

| Condition | Time (hr) | Recovery (%) of etamsylate | $R_T$ of Degradation Products (min) |
|---|---|---|---|
| Acid 1 N HCl.reflux | **8** | 73.10 | 3.108 |
| Base 1 N NaOH, reflux | **8** | 55.19 | 3.151 |
| $H_2O_2$ 30%, reflux | **8** | 90.5773 | 3.095 |

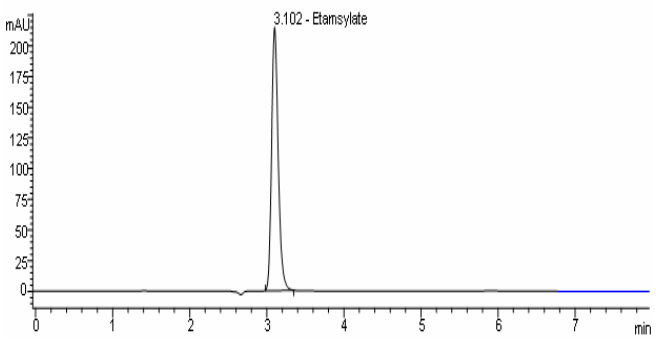


**Fig. 2. HPLC Chromatogram of Standard Etamsylate (Control sample, lacking degradation)**

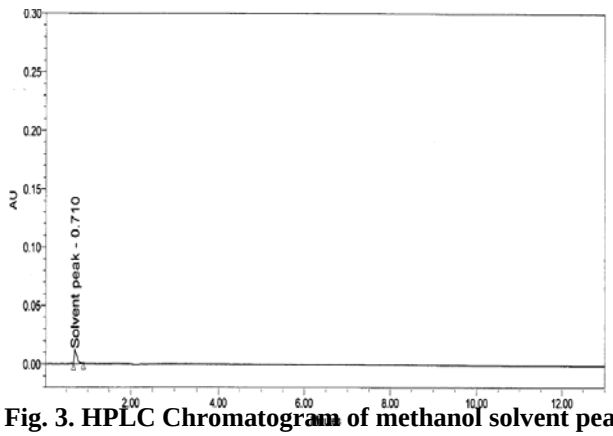


**Fig. 3. HPLC Chromatogram of methanol solvent peak**

## Linearity

Five solutions containing Etamsylate of different concentration were analyzed (**Fig.3** & **4**). The curve of the peak area vs. concentration proved linear, with a correlation coefficient of 0.9986. The plot of the peak area response vs. micrograms injected is shown in Figure 4 the method is linear as the peak response is directly proportional to concentration.

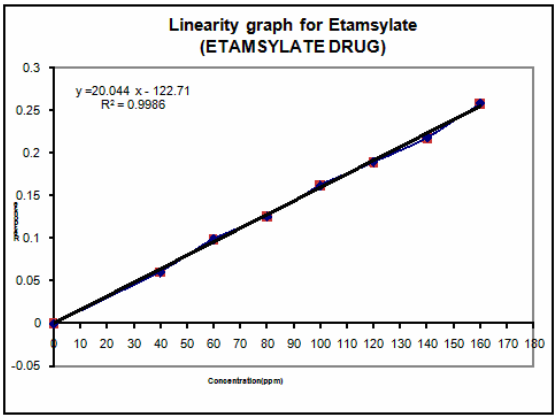


**Fig 4. Linearity Graph of Peak Areas Response against Concentration Injected Assay**

## Assay

The assay results of three different samples were compared with claimed value are listed in **Table 3** retention time was found to be 3.10min and low RSD values (<1%) indicate that the method is precise and accurate

**Table 3 Assay of Etamsylate**

| Injection | Area | % of Label claim |
|---|---|---|
| 1 | 1380.4 | 104.2 |
| 2 | 1378.2 | 103.8 |
| 3 | 1378.9 | 104.3 |
| Mean | | 104.1 |
| SD | | 0.264 |
| %RSD | | 0.253 |

## Precision

Precision was studied to find out the variation in the estimation of etamsylate. For which five replicate injections of etamsylate standard solution was injected and their area, Standard Deviation, and Relative Standard Deviation were compared the result hence observed are recorded in **Table 4.**

**Table 4 System Precision Data.**

| Injection | Area | % of Label claim |
|---|---|---|
| 1 | 1280.4 | 104.2 |
| 2 | 1278.2 | 103.8 |
| 3 | 1278.9 | 104.3 |
| 4 | 1278.9 | 104.5 |
| 5 | 1280.5 | 103.8 |
| Mean | | 104.12 |
| SD | | 0.311 |
| %RSD | | 0.298 |

## Accuracy

Accuracy was determined by recovery study of etamsylate; known amount of standard etamsylate added into the sample and subjected them it to HPLC analysis. Results of recovery studies using the said method are shown in **Table 5.**

**Table 5. Accuracy Data**

| S.No | % Label claim | Amount spiked | Amount found | % Recovery |
|---|---|---|---|---|
| 1 | 50% | 62.5 mg/ml | 67.4986 mg/ml | 107.99 |
| 2 | 50% | 62.5 mg/ml | 66.5582 mg/ml | 106.53 |
| 3 | 50% | 62.5 mg/ml | 67.4986 mg/ml | 107.99 |
| | Mean | | | 107.50 |
| | SD | | | 0.842 |
| | %RSD | | | 0.783 |
| 1 | 100% | 125mg/ml | 130.372 mg/ml | 104.30 |
| 2 | 100% | 125mg/ml | 130.8970mg/ml | 104.72 |
| 3 | 100% | 125mg/ml | 130.2464mg/ml | 104.20 |
| | Mean | | | 104.40 |
| | SD | | | 0.276 |
| | %RSD | | | 0.264 |
| 1 | 150% | 187.5mg/ml | 187.2691mg/ml | 99.87 |
| 2 | 150% | 187.5mg/ml | 187.7936mg/ml | 100.15 |
| 3 | 150% | 187.5mg/ml | 188.4106mg/ml | 100.70 |
| | Mean | | | 100.24 |
| | SD | | | 0.422 |
| | %RSD | | | 0.420 |

**Stability of Sample Solution at Room Temperature**

Stability of reagent, mobile phase, standard and sample solution was studied for 48 hours and compared with freshly prepared solution and found to be stable.

## REFERENCES


1. British Pharmacopoeia , British Pharmacopoeia Commission, UK, 2003; 729.
2. Hernandez MR, Alvarez-Guerra, M, Escolar G, Chiavaroli C, Hannaer P, Garay RP. The hemostatic agent etamsylate promotes platelet/leukocyte aggregate formation in a model of vascular injury. *Fundam. Clin. Pharmacol. 18(4); 423-30 (2004)*
3. Alvarez-Guerra M, Hernandez MR, Escolar G, Chiavaroli C, Garay RP, Hannaert P. The hemostatic agent etamsylate enhances P-selectin membrane expression in human platelets and cultured endothelial cells. Thromb Res., 2002; 107(6), 329-35.
4. Neeraj K, Agrawal N, Kakad A, Dhaneshwar SR., Patil B. Stress degradation studies on etamsylate using stability-indicating chromatographic methods. Anal. Chim. Acta, 2005 ; 536, 49–70.
5. USP 27/NF 22 . The United States Pharmacopeia ,Rockville, MD: 2004; 2622-2625.
6. http://www.ich.org/LOB/media/MEDIA417.pdf