Asian Journal of Chemistry Vol. 18, No. 2 (2006), 783–787

Determination of Ethamsylate in Pharmaceutical Preparations by Liquid Chromatography

R. NAGESWARA RAO*, P. NAGARAJU, V. SUMANTH REDDY, P. RADHA KRISHNAMURTHY, S. UDAY BHASKAR and P. GOPAL

Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Berhampur, AT/PO-Mohuda Ganjam-760 002, India

A reverse phase high-pressure liquid chromatographic method (HPLC) has been developed for the estimation of ethamsylate in its tablet dosage forms using RP-C_{18} column. The mobile phase (methanol, acetonitrile and phosphate buffer pH 2.8) was pumped at a flow rate of 1 mL/min in the ratio of 20 : 70 : 10 and the eluents were monitored at 290 nm. The intra- and inter-day variation was found to be less than 2% showing high precision of the assay method. The mean recovery of the drug from the solution containing 20 or 40 µg/mL was 98.72 ± 1.20% indicating high accuracy of the proposed HPLC method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining ethamsylate in bulk drug samples or in pharmaceutical dosage forms.

Key Words: Ethamsylate, High-pressure liquid chromatography, Estimation.

INTRODUCTION

Ethamsylate[1, 2] is used under the category of haemostatics. It is also used in prevention and treatment of capillary haemorrhages associated with haematemeris, haedmoptysis, menorrhagia and post-abortion and post-partum haemorrhages and chemically it is known as 2,5-dihydroxy benzene sulfonic acid compound with diethyl amine (1 : 1). Literature survey reveals that few high-pressure liquid chromatography (HPLC)[3], spectrophotometric methods[4, 5] were reported for its analytical monitoring in either biological fluids or formulations. The present study describes the determination of ethamsylate in bulk drug samples and pharmaceutical dosage forms by using RP-C_{18} column with UV detection. Owing to the widespread use of HPLC in routine analysis, it is important that well validated HPLC methods are to be developed for estimating ethamsylate. The object of this study is to develop a simple, precise, rapid and accurate reverse phase HPLC method for the determination of ethamsylate in bulk drug samples or in pharmaceutical dosage forms.

EXPERIMENTAL

Ethamsylate and butorphanol were gift samples from M/s Aristo Pharmaceutical Industries Ltd., Bhopal, India. Methanol, acetonitrile and water used were of HPLC grade (Qualigens). All other reagents used in this study were of AR grade (Qualigens).

An isocratic HPLC (Waters India, USA) with a single Waters 510 pump, Waters 486 tunable absorbance detector and RP-C_{18} column (Bondapak, 5 μm particle size) was used. The HPLC system was equipped with Millennium 32 software.

Preparation of Stock Solution of Internal Standard: Ethamsylate was used as internal standard for HPLC estimation of butorphanol. About 100 mg of ethamsylate was accurately weighed, transferred to a 100 mL volumetric flask, dissolved in methanol and made up to volume with methanol so as to give a stock solution of 1000 μg/mL (stock-1). 1 mL of Stock-I solution was diluted to 100 mL with methanol to give 10 μg/mL (stock-II). 1 mL of stock-II was added to standard ethamsylate solution.

Preparation of Stock Solution of Ethamsylate: About 100 mg of ethamsylate was accurately weighed and transferred to a 100 mL volumetric flask. It was dissolved in methanol and the solution was made up to volume with methanol. Each mL of this stock solution (stock-1) contained 1000 μg of ethamsylate. 10 mL of stock-I solution was diluted to 100 mL with methanol to give a stock solution containing 100 μg/mL (stock-II).

Chromatographic Conditions: Methanol and phosphate buffer were filtered before use through 0.2 μm membrane filter. The flow rate of the mobile phase was maintained at 1 mL /min in the ratio of 20 : 70 : 10 (methanol : acetonitrile : phosphate buffer, pH 2.8). The column temperature was maintained at 40°C and concentration of drug was detected by UV detector at 290 nm. The data was acquired, stored and analyzed with Millennium 32 software.

Procedure

From stock-I solution of ethamsylate 0.5–1 mL quantities of solution were transferred to 10 mL volumetric flasks. To these solutions 1 mL of butorphanol (internal standard) containing 100 μg/mL was added and volume was made up to 10 mL with methanol to get 0.5, 1, 2, 4, 8, 10, 20, 40, 80, 100 μg/mL. The standard solutions, prepared as above, were filtered through 0.4 μm membrane filter and the filtrate was injected five times into the column at a flow rate of 1 mL/mm. The ratio of drug peak area to that of internal standard for each of the drug concentrations was calculated. The regression of the drug concentration over the ratio of drug peak area to that of internal standard was obtained. This regression equation was used to estimate the amount of ethamsylate in pharmaceutical dosage forms.

Assay of Ethamsylate Tablets

Twenty tablets (containing 250 or 500 mg) were weighed, finely powdered and an accurately weighed sample of tablets equivalent to 100 mg of ethamsylate was placed in a 100 mL volumetric flask. 70 mL of methanol was added and the flask was allowed to stand for 5 h with intermittent sonication to ensure complete solubility of the drug. The mixture was then made up to 100 mL with methanol, thoroughly mixed and filtered through a 0.2 μm membrane filter. An aliquot of this filtrate was transferred to a volumetric flask along with appropriate volume of butorphanol (internal standard) solution and made up to volume with methanol

to give an expected concentration 100 µg/mL of ethamsylate and 10 µg of butorphanol (internal standard). All determinations were conducted in triplicate.

The precision of the assay was determined in terms of intra- and inter-day variation in the peak area ratio for a set of drug solutions (20 or 40 µg/mL) assayed five times on the same day and on three different days. The intra- and inter-day variation in the peak area ratio of the drug solution to that of internal standard was calculated in terms of coefficient of variation (CV) and obtained by multiplying the ratio of standard deviation to the mean with 100 [CV = (s.d./mean) × 100].

The accuracy of HPLC assay method was assessed by adding known amount (20 or 40 µg) of the drug to drug solution of known concentration (20 µg/mL) along with 10 µg internal standard and subjecting the samples to the proposed HPLC method. Also, known amount of drug solution (20 or 40 µg/mL) was added to the volumetric flask containing the powder sample of the tablet formulation with known amount of the drug and internal standard. The drug was estimated as per the procedure described above for the estimation of ethamsylate in tablet formulations. In both cases, the recovery studies were replicated five times. The accuracy was expressed in terms of the recovery and calculated by multiplying the ratio of measured drug concentration to the expected drug concentration with 100 so as to give the per cent recovery.

RESULTS AND DISCUSSION

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in previous years because of their importance in quality control of drugs and drug products. The goal of this study was to develop a rapid and sensitive HPLC method for the analysis of ethamsylate in bulk drug samples and its tablet formulations using most commonly employed RP-C18 column with UV detection.

The run time was set at 15 min and the retention times for ethamsylate and internal standard (butorphanol) were 2.4 min and 3.6 min respectively (Fig. 1). Each sample was injected 5 times and the retention times of the drug and internal standards were same. The ratios of peak area of ethamsylate to peak area of internal standard for different concentrations set up as above were calculated and the average values for five such determinations are shown in Table-1. The peak areas of both the drug and internal standard were reproducible as indicated by low coefficient variation (3%). When the concentration of ethamsylate and its respective peak area ratios were subjected to regression analysis by least squares method, a good linear relationship ($r = 0.9999$) was observed between the concentration of ethamsylate and the respective peak areas in the range 0.5–100 µg/mL. The regression of ethamsylate concentration over its peak area ratio was found to be $Y = -0.1755 + 1.9105X$ (where Y = ratio of peak area of drug to that of internal standard, X = concentration of ethamsylate). This regression equation was used to estimate the amount of ethamsylate either in tablet formulation or in validation study (precision and accuracy).

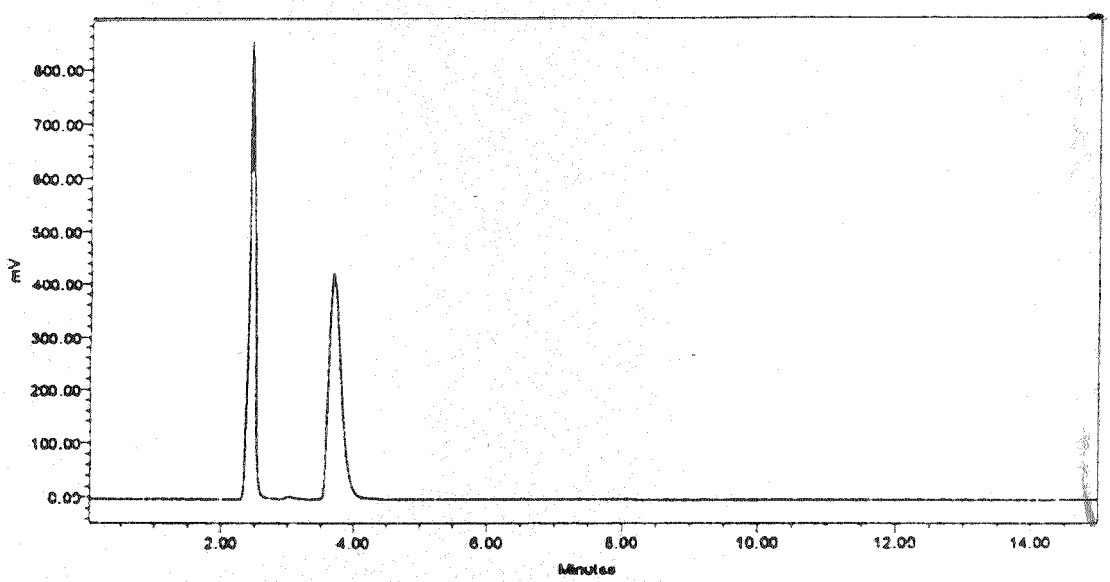

Fig. 1. Typical chromatogram of ethamsylate

TABLE-1
CALIBRATION OF THE HPLC METHOD FOR THE ESTIMATION OF ETHAMSYLATE

Concentration of ethamsylate (μg/mL)	Mean (±s.d) peak area ratio (n = 5)	CV (%)
0.5	0.956	1.16
1	1.813	1.52
2	3.793	2.32
4	7.482	0.78
8	14.983	1.28
10	19.121	0.98
20	37.934	2.31
40	75.492	1.61
80	152.971	0.67
100	190.932	1.52

Regression equation (from 0.5 to 100 μg/mL): $Y = -0.1755 + 1.9105X$ ($r = 0.9999$).

The proposed HPLC method was also validated for intra- and inter-day variation. When the solutions containing 20 or 40 μg/mL of ethamsylate along with 10 μg/mL of butorphanol were repeatedly injected on the same day, the coefficient of variation (CV) in the peak area ratio for five replicate injections was found to be less than 2% (Table-2). The results show that the proposed HPLC method is highly reproducible. When a known amount of drug solution (20 or 40 μg) was added to a known amount of drug solution (20 μg), there was a high recovery (99.72 ± 1.20%) of ethamsylate (Table-3) indicating that the proposed method is highly accurate.

The HPLC method, developed in the present study, has also been used to quantify ethamsylate in tablet dosage forms. Ethamsylate (containing 250 or 500 mg of drug) was analyzed by procedure described above. The average drug content was found to be 99.3% of the labelled amount (Table-4). No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulations did not interfere with the estimation of the drug by the proposed HPLC

method. A known amount of the drug solution was added to the powder sample of the tablet dosage form and subjected to the estimation of the drug by the proposed method. There was high recovery of ethamsylate (98.32 ± 1.20%) indicating that the proposed procedure for the determination of ethamsylate in the tablet dosage forms is highly accurate.

TABLE-2
PRECISION OF THE PROPOSED HPLC METHOD

Ethamsylate concentration (μg/mL)	Concentration of Ethamsylate (μg/mL) found on			
	Intra-day		Inter-day	
	Mean (n = 5)	CV (%)	Mean (n = 5)	CV (%)
20	20.09	1.89	20.14	1.92
40	40.12	1.25	40.09	1.61

TABLE-3
RECOVERY OF ETHAMSYLATE

Amount of drug added (M)	Mean (±s.d.) amount (μg) recovered (n = 5)	Mean (±s.d.) % of recovery (n = 5)
20	20.03 ± 0.06	100.15 ± 0.3
40	39.89 ± 0.08	99.72 ± 1.20

TABLE-4
MEAN (±S.D.) AMOUNT OF ETHAMSYLATE IN TABLET DOSAGE FORMS BY PROPOSED HPLC METHOD

Brand of the tablet	Labelled amount (mg)	Observed amount (mg)	Purity (%)
AAA	250	248.3 ± 1.04	99.32 ± 0.86
BBB	500	491.3 ± 0.81	98.30 ± 1.01

ACKNOWLEDGEMENTS

The authors thankful to the management of College of pharmaceutical sciences, Mohuda, for providing facilities for developing HPLC method. The authors also thanks to M/s Aristo Pharma Ltd., Bhopal, India for providing gift samples of ethamsylate and butorphanol.

REFERENCES

1. The Merck Index, 11th Edn., p. 587 (1989).
2. Martindale: The Extra Pharmacopoeia, 31st Edn., p. 763.3 (1996).
3. J. Man and Y. Liu, *Yaowu Fenxi Zazhi*, **4**, 209 (1984).
4. Z.C. Xu, X.Y. Li and A.Z. He, *Fenxi Huaxue*, **22**, 420 (1994).
5. EthHuang and Q. Bi, *Yaowu Fenxi Zazhi*, **8**, 150 (1998).

(*Received*: 13 May 2004; *Accepted*: 7 November 2005) AJC-4488