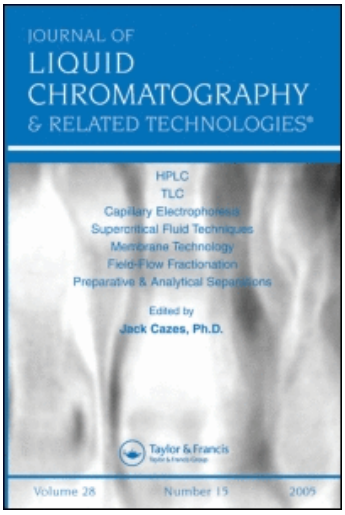

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Application of HPLC for the Simultaneous Determination of Ethamsylate and Mefenamic Acid in Bulk Drugs and Tablets

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To cite this Article Jaiswal, Yogini , Talele, Gokul and Surana, Sanjay(2007) 'Application of HPLC for the Simultaneous Determination of Ethamsylate and Mefenamic Acid in Bulk Drugs and Tablets', Journal of Liquid Chromatography & Related Technologies, 30: 8, 1115 — 1124
To link to this Article: DOI: 10.1080/10826070601128493
URL: http://dx.doi.org/10.1080/10826070601128493

Journal of Liquid Chromatography & Related Technologies®, 30: 1115–1124, 2007

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070601128493

Application of HPLC for the Simultaneous Determination of Ethamsylate and Mefenamic Acid in Bulk Drugs and Tablets

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Abstract: A simple high performance liquid chromatographic (HPLC) method was developed for the analysis of ethamsylate (ET) and mefenamic acid (MA) in bulk drug and tablets. This method showed good resolution for both the drugs. An isocratic reverse-phase HPLC assay was used with PDA detection. Some solvent systems prepared using methanol or acetonitrile and water or buffer systems with different pH values were tested. Best resolution has been determined using a Luna C_8 column with acetonitrile and water (60:40,V/V) as mobile phase adjusted to pH 2.5 with acetic acid. Ibuprofen was used as an internal standard, as use of internal standard makes the analyses more accurate. Samples were eluted isocratically with mobile phase at flow rate 1.0 mL min^{-1} and detected at 300 nm.

Keywords: Ethamsylate, Mefenamic acid, Ibuprofen, Internal Standard, Isocratic elution, Reverse phase HPLC

INTRODUCTION

Mefenamic acid is N-2,3-xylyl anthranilic acid with anti inflammatory and analgesic properties. It is used in relieving symptoms of rheumatic disorders.[1,2] The drug and the capsules are official in IP, EP, BPC, BP and USP.[3–7] The usual dose of mefenamic acid is 250 to 500 mg given thrice a day.[8–11]

Ethamsylate, 2,5-dihydroxy benzene sulphonic acid compound with N-ethyl ethanamine.[12] It reduces capillary bleeding and is also used in

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menorrhagia (excessive menstrual blood loss).[13] It has a usual dose of 500 mg thrice daily for adults and 250 mg thrice a day in children.[14–17]

Literature survey reveals potentiometric IR and UV spectrophotometric methods for the determination of ethamsylate in tablets.[18–21] The BP and IP report titrimetric assay procedure for mefenamic acid whereas USP states a HPLC technique.[22] Various spectrophotometric, polarographic and HPLC techniques for determination of mefenamic acid have been reported.[23–32]

The present work presents a simple and sensitive HPLC method for simultaneous estimation of ethamsylate and mefenamic acid with the use of internal standard which makes the method more accurate and suitable for routine determination of both the drugs.

EXPERIMENTAL

Materials and Chemicals

Acetonitrile and methanol of high performance liquid chromatographic (HPLC) grade and acetic acid were obtained from Merck (Darmstadt, Germany). Water was deionised and then doubly distilled. The filters with pour size of 0.45 and 0.2 μm (Millipore, Bangalore, India) were used for filtration of mobile phase and sample solution, respectively. Tablets containing 250 mg of both the drugs were obtained commercially.

HPLC Apparatus

A LC-10 AT VP HPLC pump (Shimadzu) equipped with a Rheodyne injection system with 100 μL loop. Detection was accomplished with a SPD M-10 photo diode array detector at 300 nm. Integration and system parameters were controlled by Shimadzu SPD M-10 software system. Chromatographic analyses were carried out at room temperature on a 5 μm C_8 Luna column (250 × 4.6 mm i.d, Phenomenex) packed with the same material. The separations were achieved by isocratic elution with a flow rate of 1.0 mL min^{-1}. The mobile phase used was acetonitrile and water (60:40,V/V) adjusted to pH 2.5 by acetic acid. Working standard solutions of various dugs were analyzed for internal standard selection with same chromatographic conditions. Ibuprofen was then selected as the internal standard as it satisfied all the necessary requirements of an internal standard.

Solutions

1.0 mg each of ethamsylate and mefenamic acid were accurately weighed and transferred to 10 mL volumetric flasks separately; 1.0 mL of mobile

phase consisting of acetonitrile and water (60:40,V/V) was added into each flask and shaken for 5 min in an ultrasonic bath. The mixtures were made up to volume with the mobile phase. The internal standard solution was prepared by accurately weighing 0.5 mg of ibuprofen and transferring it to a 100 mL volumetric flask; 1.0 mL of mobile phase was added and the flask was shaken for 5 min in an ultrasonic bath. The volume was made up with the mobile phase. Internal standard solution was prepared by accurately weighing 500 μg/mL.

Sample Preparation

To determine the content of ethamsylate and mefenamic acid simultaneously in conventional tablets (label claim 250 mg of both the drugs, combination tablet containing both the analytes). Twenty tablets were weighed, their mean weight determined, and they were finely powdered. Powder equivalent to 250 mg of ethamsylate and 250 mg of mefenamic acid was weighed and transferred in a 100 mL volumetric flask containing 50 mL of mobile phase sonicated for 30 min and dilute to 100 mL with the mobile phase. The resulting solution was centrifuged at 300 r.p.m for 5 min. Supernatant containing 100 μg/mL of ethamsylate and mefenamic acid was taken and filtered using 0.45 μm filter. A 20 μL volume of sample solution (100 μg/mL of ethamsylate and mefenamic acid respectively) was injected into HPLC six times, under the same conditions described above. The peak areas were measured at 300 nm for ethamsylate and mefenamic acid.

Method Validation

The method was validated as per the ICH guidelines.[33–35] The parameters validated are listed below.[36–42]

Precision

Precision of the method was determined with the product. An amount of the product powder equivalent to 100% of the label clam of ethamsylate and mefenamic acid was accurately weighed and assayed. System repeatability was assayed by six replicate injections and six times measurement of the sample solution at the analytical concentration. The repeatability of the method was obtained from the RSD value by repeating the assay six times in the same day for intra-day precision. Intermediate precision was assessed by the assay of two, six sample seta on different days (inter-day precision). The inter-day and intra-day variation for determination of ethamsylate and mefenamic acid was carried out at three different levels 50, 100 and 150 μg/mL.

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were separately determined at a signal to noise ratio (S/N) of 3 and 10. LOD and LOQ were experimentally verified by diluting known concentrations of ethamsylate and mefenamic acid until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate determinations.

Specificity

The specificity of the HPLC method was determined by the complete separation of ethamsylate and mefenamic acid along with other parameters like retention time (t_r), capacity factor (k), tailing or asymmetrical factor (T) etc.

Recovery Studies

Recovery studies were carried out by adding 80, 100 and 120% of label claim to the known amount of drug sample (standard addition method). At each level of the amount six determinations were performed and the results obtained were compared with the expected results.

RESULTS AND DISCUSSION

Optimization of Procedures

Initially methanol and water were tried in the ratio of 80:20 (v/v) for each drug individually. Mefenamic acid showed good peak resolution but ethamsylate peak did not show good peak nature. Then methanol was replaced by acetonitrile and the mobile phase was tried in different ratios along with the change in pH from 2 to 5 with acetic acid. Sticking of both the peaks was observed at pH 6.0. At pH 2.5 both the peaks showed good separation along with internal standard. Acetonitrile:water in the proportion of 50:50 (v/v) showed tailing in mefenamic acid. In the proportion of 65:35 (v/v) acetonitrile: water did not show good peak nature for ibuprofen used as internal standard. To improve the resolution of three peaks acetonitrile:water 60:40 (v/v) was tried at pH 2.5 and this ratio was selected for validation.

Linearity

Both the drugs showed good correlation coefficient in the concentration range of 50–250 μg/mL with their correlation coefficients ($r = 0.998 \pm 0.011$) and ($r = 0.998 \pm 0.010$) respectively. The results of linearity studies are shown in Table 1.

Table 1. Linear regression data for calibration curves (n = 6)

Parameters	Ethamsylate	Mefenamic acid
Linearity range	50–250 μg/mL	50–250 μg/mL
r ± S.D.	0.998 ± 0.020	0.998 ± 0.073
Slope ± S.D.	4.251 ± 0.011	1.77 ± 0.010
Intercept ± S.D	50.736 ± 0.046	40.36 ± 0.030

Precision

The within-run precision and between-run precision of the proposed HPLC method were determined by the assay of the tablets, six times per day for consecutive six days expressed as %RSD. The intra-day and inter-day precisions have been depicted in Table 2.

LOD and LOQ

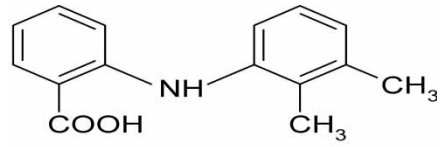
The LOD and LOQ were found to be 0.5 μ and 1.9 μ for ethamsylate and 1.5 μ and 5.9 μ for mefenamic acid respectively (Figure 1).

Specificity

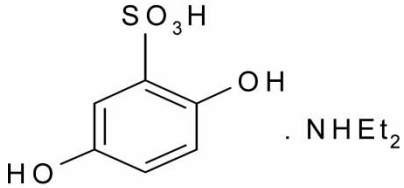
The specificity of the proposed method is illustrated in Figure 2 where complete separation of ethamsylate and mefenamic acid was noticed in the presence of tablet excepients. The average retention time for ethamsyalte and mefenamic acid were found to be 2.20 ± 0.05, 10.05 ± 0.01 and for ibuprofen 7.49 ± 0.02 respectively, for six replicates. The peaks obtained were sharp and have clear base line correction.

Table 2. Intra-day and inter-day precision of ethamsylate (a) and mefenamic acid (b) (n = 6)

	Intra-day precision			Inter-day precision		
	S.D. of areas	R.S.D (%)	S.E.	S.D. of areas	R.S.D (%)	S.E.
Ethamsylate[a]	1.390	0.020	0.010	1.860	0.050	0.012
Mefenamic acid[b]	1.640	0.090	0.021	1.37	0.050	0.023



Mefenamic Acid



Ethamsylate

Figure 1. Structures of analytes to be analysed.

Recovery Studies

The proposed method when used for extraction and then subsequent estimation of ethamsylate and mefenamic acid from pharmaceutical dosage form after spiking with additional drug afforded recovery of 99.89% and 99.61% respectively. The mean recoveries of ethamsylate and mefenamic acid from marketed formulation are depicted in Table 3.

The data of summary of validation parameters are listed in Table 4.

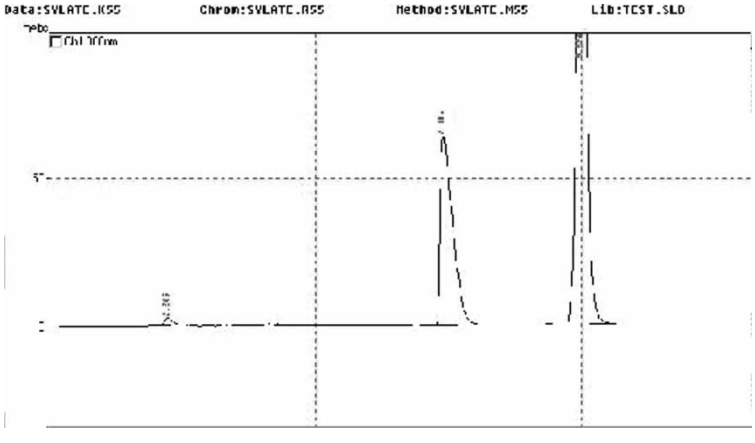


Figure 2. Chromatogram of standard ethamsylate (Rt = 2.24) and mefenamic acid (Rt = 10.05) along with internal standard ibuprofen (Rt = 7.49) with acetonitrile:water (40:60, v/v) at pH 2.5 at 300 nm.

Table 3. Standard addition technique for determination of ethamsylate and mefenamic acid (n = 6)

	Excess drug added in the analytes (%)	Theoretical content (μg)	Recovery (%)	%R.S.D.	S.E.
(a) Ethamsylate	0	1000	99.98	0.023	0.001
	80	1800	99.61	0.073	0.002
	100	2000	99.99	0.018	0.001
	120	2200	99.98	0.023	0.002
(b) Mefenamic acid	0	1000	99.29	0.080	0.002
	80	1800	99.83	0.012	0.003
	100	2000	99.60	0.040	0.005
	120	2200	99.75	0.030	0.004

Stability Studies

Two different solutions of concentrations (150 μg/mL) of both the drugs were prepared and stored at room temperature for 3 days. They were the injected into the HPLC system and no additional peak was found in the chromatogram indicating stability of drugs in sample solution.

Analysis of Marketed Formulation

The peaks at t_r mefenamic acid were found to be 2.20 ± 0.05, for ethamsylate and 10.05 ± 0.01 for mefenamic acid were observed in the chromatogram of the drug samples extracted from tablets (Figure 3). Experimental results of the amount of both the drugs in tablets, expressed as percentage of label claim

Table 4. Summary of validation parameters: statistical data for the calibration graphs of ethamsylate and mefenamic acid (n = 6)

	Ethamsylate	Mefenamic acid
Linearity range	50–250 μg/mL	50–250 μg/mL
Correlation coefficient	0.998	0.998
LOD (μg)	0.500	1.700
LOQ (μg)	1.900	5.900
Recovery (n = 6)	99.890	99.610
Precision (% R.S.D)		
Inter-day	0.050	0.050
Intra-day	0.020	0.090
Specificity	0.999	0.998

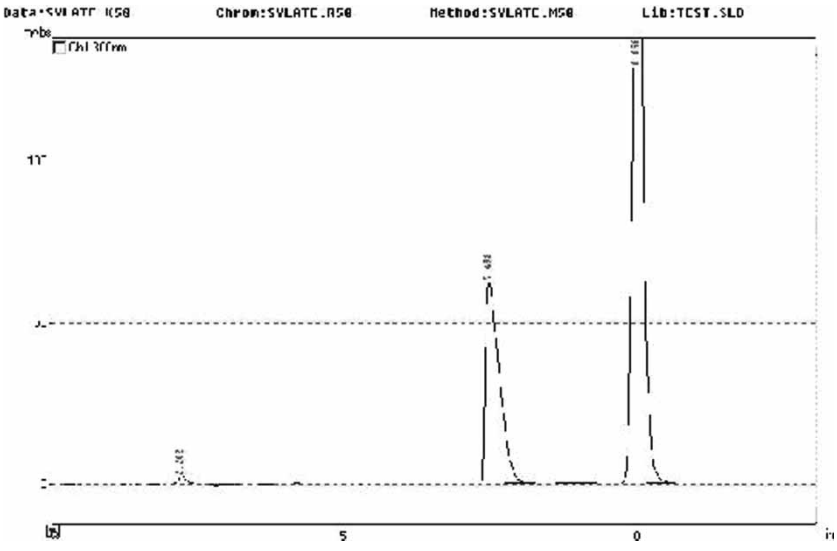


Figure 3. Chromatogram of sample ethamsylate (Rt = 2.24) and mefenamic acid (Rt = 10.05) and internal standard ibuprofen (Rt = 7.49) with acetonitrile:water (40:60, v/v) at 300 nm.

Table 5. Applicability of the proposed method for the determination of ethamsylate and mefenamic acid in commercial tablets (n = 6)

	Ethamsylate	Mefenamic acid
Label claim	250 mg	250 mg
Drug content (%) ± S.D.	99.40 ± 0.030	99.80 ± 0.210
%R.S.D	0.060	0.030

were in good agreement with the label claims, thereby suggesting that there is no interference from any excepients, which are normally present in tablets. The drug content was found to be 99.40% (%RSD = 0.06) and 99.80% (%RSD = 0.03) for ethamsylate and mefenamic acid respectively. The results are shown in Table 5.

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Received October 27, 2006
Accepted December 1, 2006
Manuscript 6973