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Pharmaceutical Sciences
Volume 4 Issue 2, 2024
eISSN No.: 2582-8371

A Novel RP-HPLC Method Development and Validation for the Simultaneous Estimation of Ethamsylate and Mefenamic Acid in Pure Drugs and Formulation

V. Bhaskara Raju, K.S.Sumanth,* K.Kalyani, B.Mohan Gandhi, A.Pavani Gayatri, A. Swapna, P. Brahma Teja, K. Ramanjaneyulu

Sri Vasavi Institute of Pharmaceutical Sciences, Pedatadepalli, Tadepalligudem, Andhra Pradesh, India.

****Corresponding Author***
E mail id: sumanth.kamatham222@gmail.com

ABSTRACT

A new, simple and rapid RP-HPLC method was developed and validated for the estimation of ETAMSYLATE and MEFENEMIC ACID in pure drugs and formulation.The chromatographic separation was achieved on SHISEIDO C18 column (250 x 4.6 mm i.d, 5µ) using Acetonitrile : water (pH 3.0±0.1) in the ratio of 75:25 v/v, with a flow rate of 1 ml/min and detection at 220 nm. The retention times for ETS and MFA were found to be 4.15 and 6.8 min respectively. Linearity was established in the range of 10-50 µg/ml for both ETS and MFA respectively. The method was precise with %RSD < 2 for both intraday and interday precision. The accuracy of the method was performed over three levels of concentration and the recovery was in the range of 98-102%.

Keywords*: Etamsylate, Mefenamic acid, RP-HPLC, Method development, Validation*

INTRODUCTION

Etamsylate is an oral antihemorrhagic agent. It is used in the treatment of capillary hemorrhage, hematuria, menorrhagia and post-partum hemorrhage. It works by increasing resistance in capillary endothelium and promoting platelet adhesion. In addition, etamsylate is effective in the prevention and treatment of pre and postsurgical capillary hemorrhages in all delicate operations and in those affecting highly vascularized tissues such as E.N.T., gynecology, obstetrics, urology, ophthalmology, plastic and reconstructive surgery. Etamsylate is chemically benzene sulfonic acid derivative (fig.1). The molecular formula of etamsylate is $C_{10}H_{17}NO_5S$ and the molecular weight is 263.311 g/mol. It is completely soluble in water, methanol, ethanol and acetonitrile. It is official in BP and EP. Mefenamic acid is a non-steroidal anti-inflammatory drug, and is used to treat mild to moderate pain such as headache, tooth pain, postoperative and postpartum pain and dysmenorrhoea. It is also useful in treatment of musculoskeletal and joint disorders such as osteoarthritis and rheumatoid arthritis, and in menorrhagia. Mefenamic acid binds the prostaglandin synthetase receptors COX-1 and COX-2, inhibiting the action of prostaglandin synthetase. As these receptors have a role as a major mediator of inflammation and/or a role for proteinoid signaling in activity-dependent plasticity, the symptoms of pain are temporarily reduced. Chemically mefenamic acid is an anthranilic acid derivative (fig.2). The molecular formula of mefenamic acid is $C_{15}H_{15}NO_2$ and the molecular weight is 241.28. It is white crystalline powder, insoluble in water, soluble in methanol, dil.solutions of alkali hydroxides. It is official in IP, BP, USP and EP [1-3].

Pharmaceutical Sciences
Volume 4 Issue 2, 2024
eISSN No.: 2582-8371

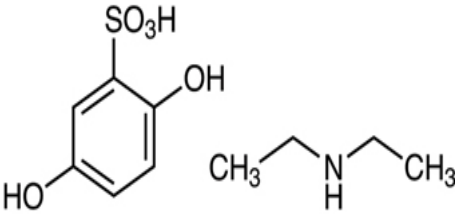

Fig.1 Chemical structure of Etamsylate

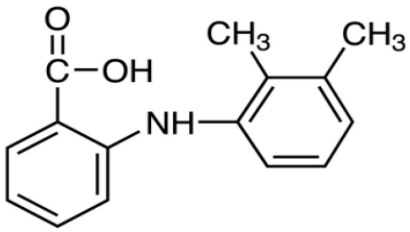

Fig.2 Chemical structure of Mefenemic acid

The present study is to develop a simple and rapid RP-HPLC method for ETS and MFA. A Literature survey reports that analytical methods for the estimation of ETS and MFA based on UV [4-8], HPTLC [9], HPLC [10-17], Stability indicating HPLC [18-20], related substances [21-24], LC-MS [25] were reported.

Although different analytical methods are available, there are very few methods developed on simultaneous estimation of etamsylate and mefenemic acid in pure drugs. The main objective of the study is to develop and validate a cost effective, accurate and precise method for the estimation of ETS and MFA in bulk and pharmaceutical formulations.

MATERIALS AND METHODS

A. Chemicals and Reagents

Etamsylate (ETS) and Mefenemic acid (MFA) working standards were procured from Yarrow Chemicals Pvt. Ltd, Mumbai. Commercially available as tablets sylate-M were procured as gift samples from Gilead sciences Pvt. Ltd. HPLC grade water was purchased from Thermo Fisher Scientifics Ltd., Mumbai. HPLC grade methanol, Acetonitrile, Orthophosphoric acid, Acetic acid, Triethyl amines, Potassium hydroxide of AR grade were procured from Merck specialties Pvt. Ltd., Mumbai.

B. Instrumentation and Analytical Conditions

RP-HPLC method was performed on the HPLC system (Shimadzu) consisting of binary gradient pump with UV detector (LC-20AD). Rheodyne injector with 20 µl fixed loop was used for injecting sample on SHISEIDO C18 column (250 x 4.6 mm i.d, 5µ) in the present study.

C. Preparation of Solutions

- **Preparation of standard stock solutions:**
  Standard stock solutions were prepared by transferring accurately weighed 100 mg of ETS, MFA into separate 100 ml volumetric flask and make up to required volume with HPLC grade water for ETS and Acetonitrile for MFA. From this take 1ml and make up to 10ml this gives the conc. 100ug/ml. Finally dissolved in diluent (mobile phase) to obtain a standard solution of ETS (10 µg/ml), and MFA (10 µg/ml).

- **Preparation of the mobile phase:**
  The mobile phase is a mixture of acetonitrile and water, p^H is adjusted to 3.0 using ortho phosphoric acid. The prepared mobile phase was filtered through 0.45 µm membrane filter (Millipore) and sonicated before use. Mobile phase is pumped in the ratio of 75: 25 %v/v (acetonitrile: water).

Pharmaceutical Sciences
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RESULTS AND DISCUSSION

Method development and optimization

The choice of the detection wavelength was based on the scanned absorption spectrum of Etamsylate and mefenemic acid. 10 mg of Etamsylate and mefenemic acid were dissolved in 10 ml of methanol.The UV-spectrum of Etamsylate and mefenemic acid was separately scanned in the wavelength range 200-400 nm against blank. After correlation of the spectrums 220 nm wavelength was selected for the analysis (Fig. 3). Trails were performed using different columns (Hypersil BDS C18, Symmetry C18, Phenomenex C18 and Shiseido C18), buffers (Acetate, Phosphate, Ortho phosphoric acid), pH (3-6), organic phases (Acetonitrile, Methanol). Shiseido C18 column (250mm X 4.6 mm, 5 µ) produced good separation with efficient resolution and more theoretical plates. The drugs were eluted with Shiseido C18 column at a flow rate of 1.0 ml/min using a mobile phase consisting of acetoniitrile: water (pH 3.0) in the ratio of 75:25 v/v respectively. The retention times for ETS and MFA were found to be 4.15 and 6.8 min respectively.

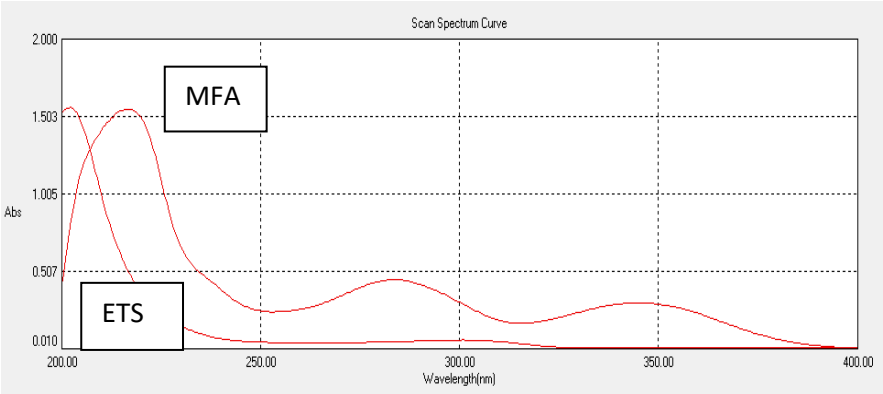

Fig.3: UV Overlay spectrum of ETS and MFA

System Suitability

Under optimized chromatographic conditions 20 µl of solution containing 30 µg/ml of ETS and 30 µg/ml of MFA was injected into the system in six replicates. Chromatograms were recorded and studied for different system suitability parameters like retention time, peak area, number of theoretical plates, tailing factor and resolution. The results were shown in table 1.

Table 1. System suitability results for ETS and MFA

INJECTION	ETS peak area	MFA peak area
Injection1	323031	1777231
Injection2	326038	1795831
Injection3	324704	1806119
Injection4	325965	1777868
Injection5	325789	1777139
Injection6	326235	1797125
Average	325293.7	1789289
Standard deviation	1233.171	1203.79
%RSD	0.379095	0.672434
Theoretical Plates	2037	6598
Tailing factor	1.1	1.2

Specificity: The HPLC chromatograms were recorded for blank (Fig. 4a) and standard (Fig. 4b) under optimized analytical conditions and compared for additional peaks, however no additional peaks were found. The two peaks were completely separated in HPLC chromatogram and the resolution was found to be more than 2.

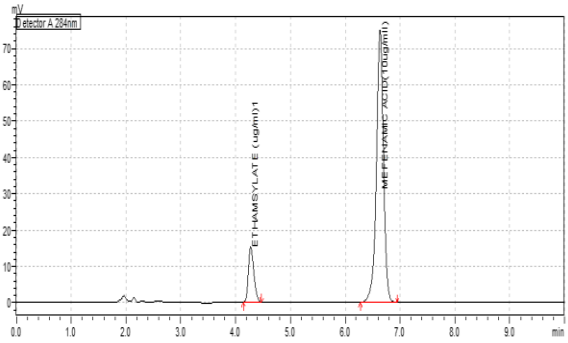

Fig. 4a: Chromatograms for specificity of ETS and MFA

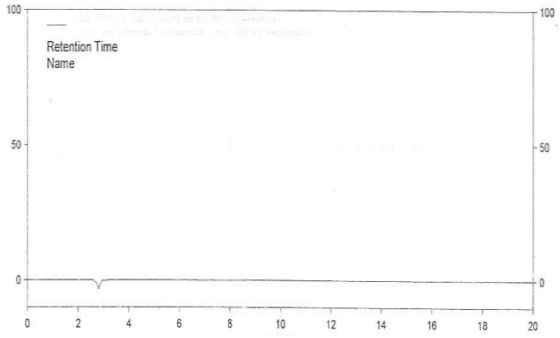

Fig 4b. Blank chromatogram

Linearity: Linearity was established over the range of 10µg/ml to 50 µg/ml for both ETS and MFA by constructing calibration graph between the tested concentration level and corresponding peak areas for six determinations and the results were shown in table 2 and linearity graphs were shown as fig 5a, 5b. The correlation coefficients were >0.99 for two drugs, which meet the method validation acceptance criteria, and hence, the method is said to be linear for the drugs.

Table 2. Linearity results for ETS and MFA

ETAMSYLATE		MEFENAMIC ACID	
Concentration(ug/ml)	Peak Areas*	Concentration(ug/ml)	Peak Areas*
10	110665	10	701393
20	213409	20	1232485
30	321031	30	1777139
40	421588	40	2382627
50	490943	50	2785479
Correlation coeffecient	**0.9945**	Correlation coeffecient	**0.9969**

**Mean of 6 determinations*

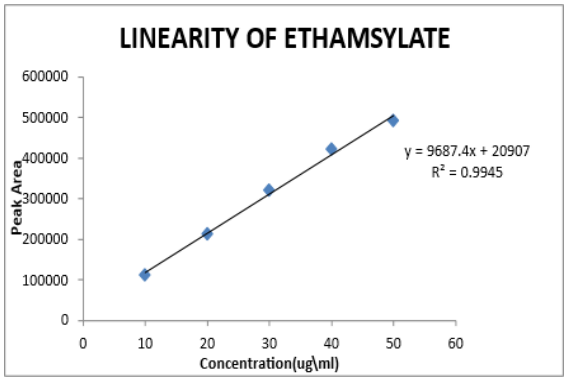

Fig. 5a: Linearity plot of ETS

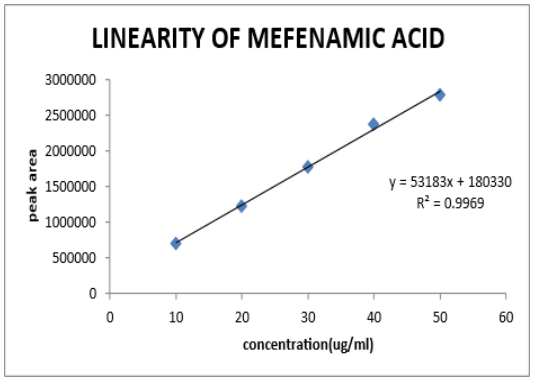

Fig. 5b: Linearity plot of MFA

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Volume 4 Issue 2, 2024
eISSN No.: 2582-8371

Accuracy: The accuracy for proposed method was determined, recovery studies were performed in mentioned levels and recorded (Table 3), Obtained results were found to be within the limits of 98-102%, indicating an agreement between the true value and found value.

Table 3. Recovery Studies of ETS and MFA

Drug	Conc. Standard (µg/ml)	Conc. Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery*	%RSD
ETS	10	10	19.88	99.04	0.49
	10	30	39.99	99.99	0.35
	10	50	60.01	101.06	0.41
MFA	10	10	19.77	98.80	1.20
	10	30	39.63	99.00	0.90
	10	50	59.99	99.98	0.34

*****Mean of six determinations*

Precision: Precision was calculated as intra-day and inter-day variations for the drugs. Percent relative standard deviations for estimation of ETS and MFA under intra-day and inter-day variations were found to be less than 2. Results were showed in Table 4.

Table 4. Precision values of ETS and MFA

Drug	Conc. (µg/ml)	Intra-Day		Inter-Day	
		Mean Area*±S.D.	%RSD	Mean Area*±S.D.	%RSD
	10	106809.7±1216.477	1.13	107280±408.20	0.38
	30	324704±1387.85	0.45	325195.7±849.86	0.26
	50	564953± 3863.05	0.6	559816.7±4562.19	0.81
MFA	10	676992±3402.3	0.5	681783±7225.19	1.059
	30	1877868±15800	0.84	185255.7±2510.9	1.35
	50	3118585±3614.7	0.11	3199721±49804.13	1.2

*****Average of 6 determinations*

Sensitivity: It is expressed as Limit of detection and Limit of quantitation. LOD is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) with a stated confidence level (generally 99%). LOQ is the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met.

Table 5. LOD and LOQ of ETS and MFA

Parameter	ETS	MFA
LOD (µg/mL)	0.05	0.015
LOQ (µg/mL)	1.5	0.1

Robustness: Robustness of the method was studied by injecting the standard solutions with slight variations in the optimized conditions namely, ± 1% in the ratio of acetonitrile in the mobile phase, varying wavelength and ± 0.1 ml of the flow rate.

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Table 6. Robustness Parameters of ETS and MFA

Sample	ETS			MFA		
	R_t	Area*	Tailing Factor	R_t	Area*	Tailing Factor
Standard(1ml/min)	4.1	346563	1.58	6.80	295683	1.14
0.8 ml/min	4.3	342541	1.5	6.92	245347	1.03
1.2 (ml/min)	4.2	325261	1.3	6.81	321456	1.16
Org. Phase (+5%)	4.5	394710	1.41	6.40	331586	1.20
Org.Phase (-5%)	4.2	282031	1.3	7.2	282588	1.30
222 nm	4.1	383157	1.10	6.2	296583	1.17
218 nm	3.9	372956	1.10	6.5	285398	1.16

**Average of 3 determinations*

Ruggedness: Ruggedness of the method was studied by changing the experimental conditions such as operators, instruments, source of reagents, solvents and column of similar type.

Table 7. Results for Ruggedness of ETS and MFA

Drug	Analyst	Retention time (min)	Peak Area	RSD (%)	System suitability results	
					Plate count	Tailing factor
ETS	Analyst 1	4.1	352687	0.7	2451	1.63
	Analyst 2	4.2	342896	0.6	2289	1.3
MFA	Analyst 1	6.7	296853	0.5	2983	1.2
	Analyst 2	6.6	286574	0.8	2698	1.1

Assay: The marketed formulation used was sylate-M, consists of 500 mg of Etamsylate and 500 mg Mefenamic acid

Table 8. Assay of ETS and MFA in pharmaceutical formulation

Drug	Label claim	Amount found	Mean* %Recovery ± S.D.	%RSD*
ETS	500 mg	495mg	99.25±0.254	0.457
MFA	500 mg	492mg	99.46±0.692	0.693

**Values are expressed as mean ±SD (n= 3)*

CONCLUSION

In the present work a new, simple, accurate and rapid method was developed by using RP HPLC-UV to determine simultaneous estimation of Etamsylate and Mefenamic acid. The method was validated according to ICH guidelines. Method validation was done by testing its linearity, accuracy, precision, values of LOD and LOQ. Compared to other methods, this RP HPLC method is simple as well as economic for the detection.

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