International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 4(6): November-December 2014

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received: 19-10-2014; Revised: 21-10-2014; Accepted: 01-11-2014

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ETHAMSYLATE AND MEFENAMIC ACID IN TABLET FORMULATION

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Keywords:

Ethamsylate, Mefenamic acid, RP-HPLC, Quantification, Tablet formulation

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ABSTRACT

An accurately, sensitive, precise and reproducible RP-HPLC method was developed and subsequent validated for the simultaneous analysis of Ethamsylate and Mefenamic acid in Tablet Formulation. Method development was carried out on C_{18}(ODS)Sunfire, 250 mm X 4.6 mm, 5µ (Particle size) column. The mobile phase was a mixture of Methanol and Water in the ratio of 82:18 v/v. The flow rate was set at 0.8 ml/min and the effluent was monitored by 2998 PDADetector at 256 nm.Calibration curve was linear over the concentration range of 5-30 µg/ml for both the drugs. The retention time of Ethamsylate and Mefenamic acid were found to be 3.3 min and 2.5 min respectively. In the linearity study, the regression equations Ethamsylate and Mefenamic acid were found to be y= 16219.5+121731 and y=45651.06-34804. Correlation coefficient was 0.996 and 0.997 for Ethamsylate and Mefenamic acid respectively. The proposed method was successfully applied for the quantification of pharmaceutical formulation.

INTRODUCTION

Chemically, Ethamsylate (ESL) is 2,5-dihydroxy benzene Sulfonic acid with N-ethylethanamine. It workby increasing capillary endothelial resistance and promoting platelet adhesion. It reduces capillary bleeding when platelets are adequate, probably exerts anti-hyalurodinase action,improves capillary wall stability. It also inhibit PGI 2 production, correct abnormal platelet function, but does not stabilize fibrin.[1-4] Used as a haemostatic agent for prevention and treatment of capillary hemorrhage associated with haemostasis, hemorrhage, hematuria, menorrhagia and post-partum haemorrhage.[4]

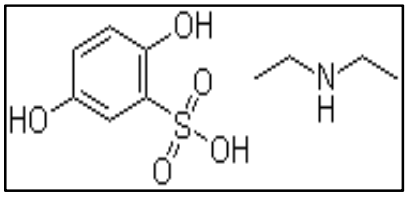

Figure 1: Chemical structure of Ethamsylate

Chemically, Mefenamic acid (MFN) isBenzoic acid 2-(2,3-dimethyl phenyl) amino, N-(2,3-Xylyl) anthranilic acid. It is an Amidobenzoate subclass of analgesic withNon steroidalanti-inflammatory properties. It acts by binds the prostaglandin synthetase receptors COX-1 and COX-2, inhibiting the action of prostaglandin synthatase. It is used for the treatment of rheumatoid arthritis, osteoarthritis, dysmenorrhea, and mild to moderate pain, inflammation and fever.[1-6]

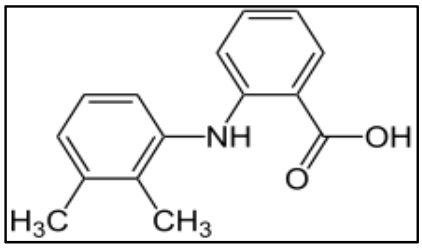

Figure 2: Chemical structure of Mefenamic acid

Literature survey revealed that only two analytical methods have been reported but they are with mobile phase containing acetonitrile and becomes more costly so aim of the present work is to develop simple, effective and economical RP-HPLC method development and validation for simultaneous estimation of Ethamsylate and Mefenamic acid in Pharmaceutical formulation. Therefore here methanol and water are used.

MATERIALS AND METHODS: The Drug samples of Ethamsylate and Mefenamic acid were kindly supplied as gift samples from Indoco Remedies, Aurangabad and Blue Cross Pvt. Ltd, Nashik respectively. HPLC grade methanol and HPLC grade water were procured from Bansal Chemicals, Nashik.

Table 1: HPLC apparatus and conditions

Instrument	ManufacturerandSpcifications
HPLC	Make- Waters Detector :2998 PDA, Software: Empower-2 Pump: 515 dual piston, Column: C18 (ODS) Sunfire.
UV Visible Spectrophotometer	SHIMADZU 2450
Analytical Balance	Shimadzu AUW-120D
Ultrasonicator	Selac DTC-503

Preparations of solutions and reagents:

Preparation of Mobile Phase: Methanol filtered through 0.2µ membrane filter,HPLC grade water was used and thus mobile phase was prepared in the ratio of 82:18 ml and then use for experiment.

Preparation of ESL and MFN standard stock solutions: Accurately weighed quantity of ESL (10 mg) and MFN (10 mg) were transferred into two different 10 ml volumetric flask separately, dissolved in Methanol and diluted upto mark with it. This will give a stock solution having strength of 1000 µg/ml of both.

Preparation of ESL and MFN standard solutions: From the standard stock solution 1 ml of ESL and1 ml of MFN solution is taken in two different 10 ml volumetric flask and make upto mark with methanol to get 100µg/ml of ESL and 100µg/ml MFN. This is standard solution.

From the above solution 3 ml of ESL and 1 ml of MFN solution is taken in 10 ml volumetric flask and dilute upto the mark with methanol. This will give a standard solution having strength of 30µg/ml of ESL and 10µg/ml of MFN.

Preparation of sample solution: Twenty tablets were weighed accurately and powdered. Powder equivalent to 10 mg of ESL (containing 10 mg of MFN) was weighed and transferred to 10 ml volumetric flask, dissolved in methanol by ultrasonication of the flask for 15 minutes and volume was made up to the mark with methanol so as to get concentration of (1000 µg/ml). The solution was filtered through whatmann filter paper no. 41. From this solution prepare solutions containing 100µg/ml by transferring 1 ml of solution to 10 ml volumetric flask. From the above solutions prepare solutions having concentrations ESL (30µg/ml) and MFN (10µg/ml) with methanol.

Selection of detection wavelength: In the present study, standard solutions of ESL and MFN were scanned over the range of 200-400 nm wavelengths. ESL showed absorbance maxima at 256nm and MFN showed absorbance maxima at 285nm. The zero-order overlain spectra of both solutions were recorded. Thus 256nm was selected as wavelength maxima.

RESULT

Linearity: Calibration curves were constructed by plotting peak areas vs. concentrations of ESL and MFN, and the regression equations were calculated. The calibration curves are plotted over the concentration range of 5-30µg/ml for both ESL and MFN. Accurately measured standard solutions of ESL and MFN (0.5, 1.0, 1.5 2.0, 2.5, 3.0 ml) were transferred to a series of 10 ml volumetric flask and diluted upto the mark with methanol. Aliquots of each solution were injected under the operating chromatographic conditions described above.

Limit of detection (LOD) and Limit of quantitation (LOQ): LOD and the LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ = Standard deviation of the response

S = Slope of calibration curve.

Precision: The Intraday and Interday precision of the method was determined by analyzing the 3 different concentration levels of ESL and MFN, corresponding responses 3 times on the same day and on 3 different days. For intra-day precision three replicates of the formulation solutions containing 5, 10, 15µg/ml of ESL, 10, 20, 30µg/ml of MFN were carried out three times on the same day and for inter-day precision three replicates of the formulation solutions of same concentrations were carried out for the three consecutive days at the same concentration level. The results were reported in terms of relative standard deviations (RSD)

Accuracy (% Recovery): Accuracy of proposed method was ascertained on the basis of recovery study of ESL and MFN by standard addition method. Recovery studies were carried out by addition of working solution of standardmixture of ESL and MFN to pre-analyzed tablet solution at three different levels, 80%, 100% and 120%. In 100 % recovery study for amount of standard drug solution added was 10 µg/ml and in 80 % and 120 % recovery study the amount of standard drug solution added was 8µg/ml and 12µg/ml of ESL and MFN respectively. The amounts of ESL and MFN were calculated by applying obtained values (n=3) to the regression equation of the calibration curve.

Robustness: In the robustness study, the influence of small variations of the analytical parameters on peak areas of the drugs and retention times were examined. The parameters include variation in flow rate 0.6, 0.7, and 0.8 ml/min and another parameter is change in temp 30°C and 35°C.

System Suitability: The tests to ensure that the method can generate results of acceptable accuracy and precision. Parameter includes theoretical plates, asymmetry, resolution, precision and capacity factor etc.

Solution Stability: The stability of standard solutions was determined by compairing the results of area of analyte. The area values were determined within 48 hours and also assay of sample solution was determined within 48 hours.

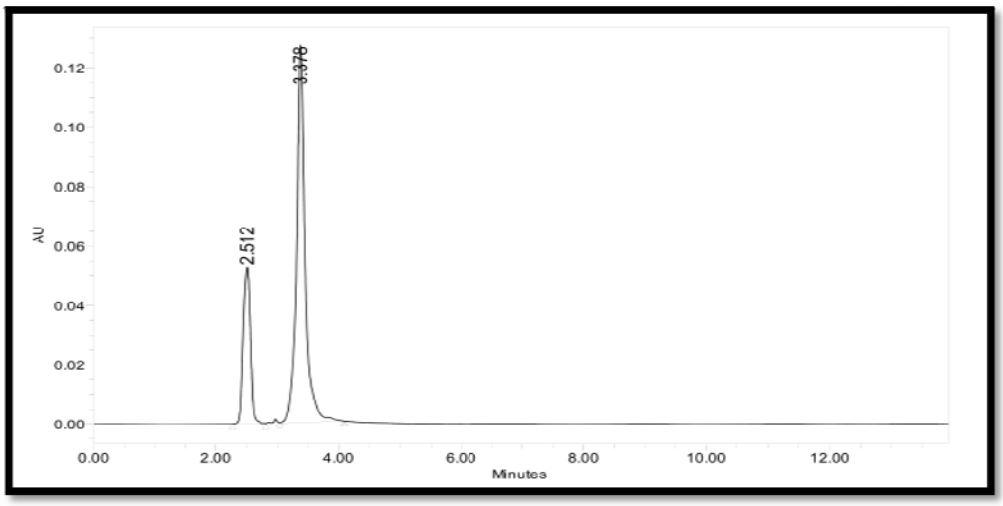

Figure 3: Typical Chromatogram of Mefenamic acid and Ethamsylate

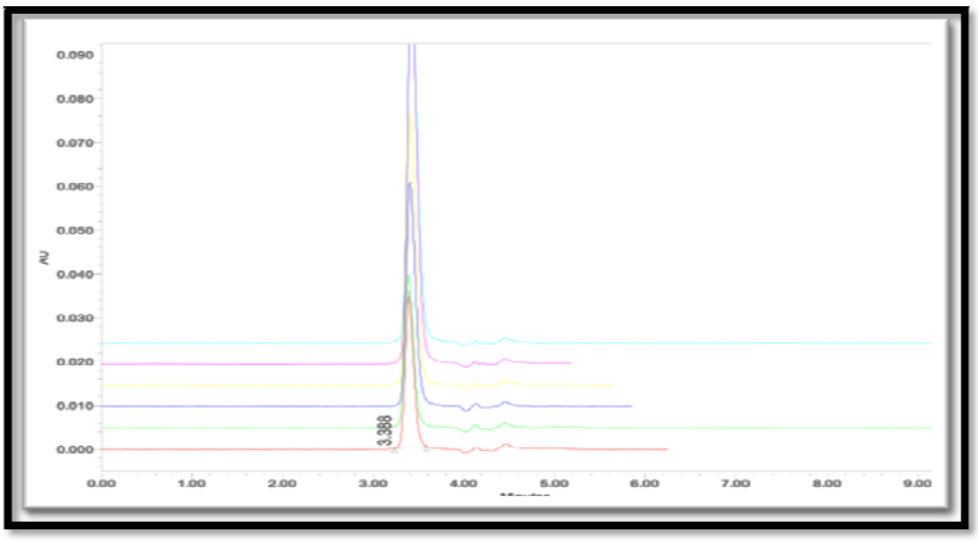

Figure 4: ESL Linearity (5-30 µg/ml)

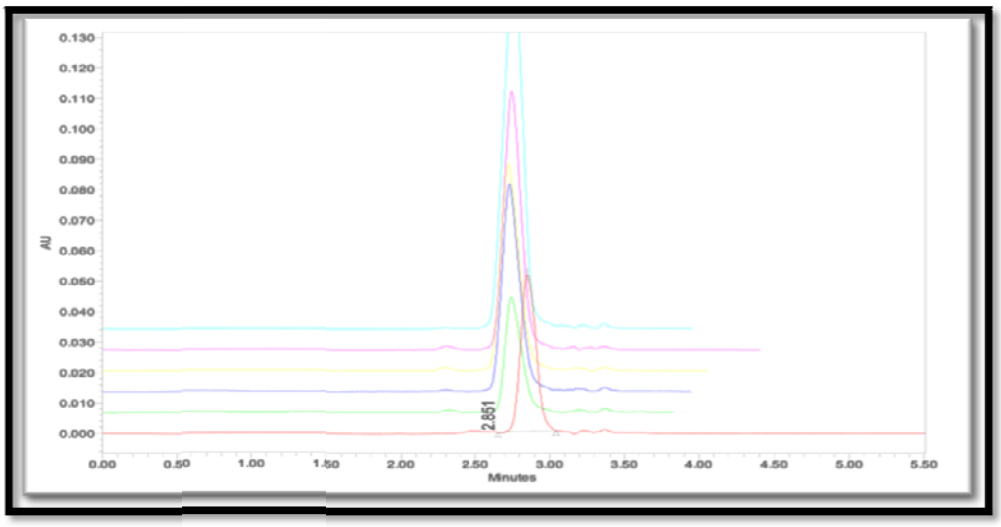

Figure 5: MFN Linearity (5-30 µg/ml)

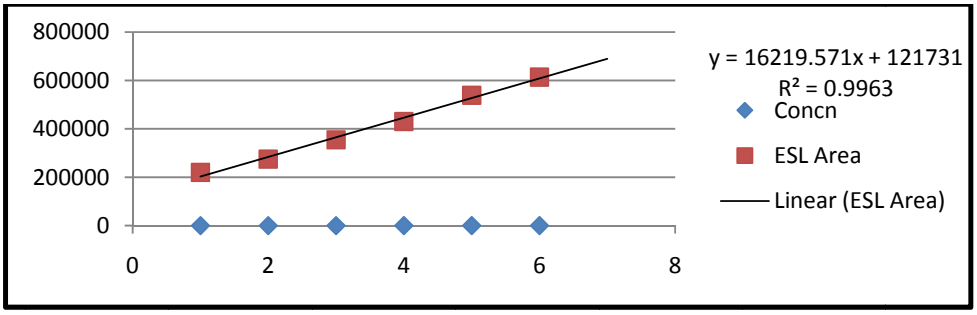

Figure 6: Linearity curve of Ethamsylate (5-30µg/ml)

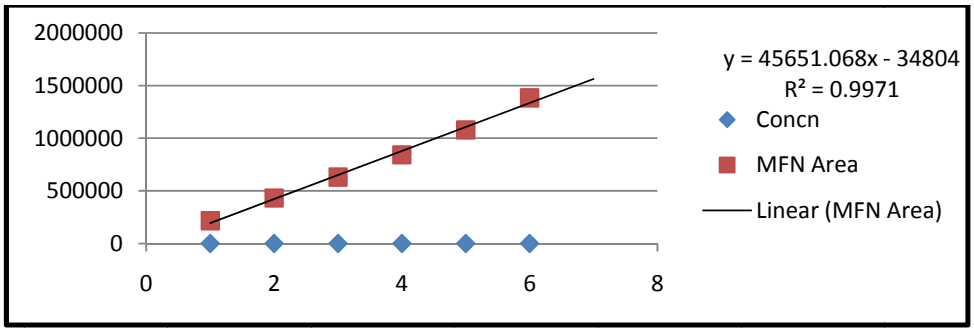

Figure 7: Linearity curve of Mefenamic acid (5-30µg/ml)

Table 2: Summery of Validation Parameters

Parameter	Result	
	Ethamsylate	**Mefenamic acid**
Linearity Range (µg/ml)	5-30	5-30
Correlation Coefficient (r^2)	0.996	0.997
Regression Equation	y= 16219.5+121731r^2=0.996	y=45651.06-34804r^2=0.997
Accuracy (% Recovery) (n=3)	101.06	100.6
Precision (%RSD)		
Intraday (%RSD)	1.21	0.792
Interday (%RSD)	1.301	0.381
Limit of detection(LOD) (µg/ml)	28.28	28.26
Limit of qantitation(LOQ) (µg/ml)	85.72	85.64

Table 3: Robustness data

Parameters	ESL	MFN
	R.T.	**R.T.**
Flow rate (0.6ml)	3.6	2.6
Flow rate (0.7ml)	3.6	2.6
Flow rate (0.8ml)	3.6	2.8
Change in temp (30.4ºC)	2.7	2.6
Change in temp (33ºC)	2.7	2.6
Change in temp (35ºC)	3.3	2.4

Table 4: System Suitability Parameters

Parameters	ESL	MFN
Retention time	3.3 min	2.5 min
Tailing Factor	1.1	1.0
Theorotical Plates	3983	2161
Resolution	4	
Area	614104	436528

DISCUSSIONS

The objective of the proposed work was to develop new analytical method for the determination of ESL and MFN and to validate the methods according to ICH guidelines. An economical, simple, sensitive, precise and accurate method has been developed for the simultaneous determination of Ethamsylate and Mefenamic acid in Tablet Formulation and it can thereby be easily adopted for routine quality control analysis.

Results of this analysis confirmed that the proposed method was suitable for the simultaneous determination of these drugs in pharmaceutical formulations with virtually no interference of the additives. Hence, the proposed method can be successfully applied in simultaneous estimation of ESL and MFN in tablet formulation.

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