AMERICAN JOURNAL OF PHARMTECH RESEARCH
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Simultaneous Estimation of Aceclofenac and Esomeprazole Sodium in Bulk By RP-HPLC

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ABSTRACT

A simple, accurate, precise and specific RP-HPLC method has been developed for the simultaneous estimation of Aceclofenac and Esomeprazole Sodium in bulk. Chromatographic analysis was carried out on C18 column (250mm × 4.6mm, 5µm). Mobile phase used is a homogenous mixture of ACN: Methanol in the ratio of 50:50 v/v. The detection was carried out at 285nm. The retention times were found to be 3.00 and 4.41 min for Aceclofenac and Esomeprazole Na respectively. Both the drugs showed linearity in the range of 30 – 70mcg/ml. The correlation coefficient was found to be 0.99 and 0.992 for Aceclofenac and Esomeprazole Na respectively. The developed method was validated as per ICH guidelines.

Keywords: Aceclofenac, Esomeprazole Sodium, RP-HPLC, Validation, Simultaneous estimation.

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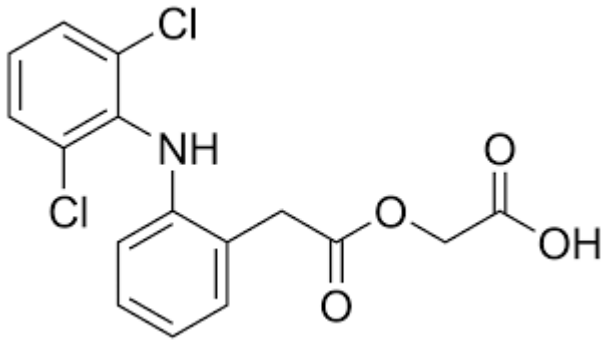
Received 17 March 2016, Accepted 22 March 2016

Please cite this article as: Narla D *et al*., Simultaneous Estimation of Aceclofenac and Esomeprazole Sodium in Bulk By RP-HPLC. American Journal of PharmTech Research 2016.

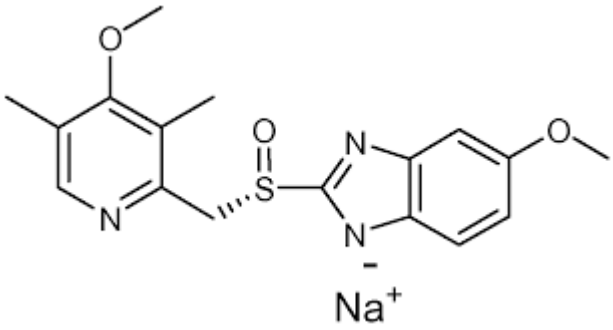
INTRODUCTION

Aceclofenac, (2-[2-[2-[(2,6-dichloropheyl)amino]phenyl]oxyacetic acid)[1,3] , is a non-steriodal anti inflammatory (NSAID) drug used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. It is official in I.P and B.P.



Chemical structure of Aceclofenac

Esomeprazole sodium[2,3], (S)-5-Methoxy-2-[(4-methoxy-3,5-dimethyipyridin-2-yl)methylsulfinyl]-3H-benzoimidazole, is a proton pump inhibitor which reduces stomach acid secretion through inhibition of H^+/K^+ ATPase in the parietal cells of stomach. It is used in the treatment of dyspepsia, peptic ulcer disease, gastroesophageal reflux disease and Zollinger – Ellison disease. Esomeprazole is the S – enantiomer of omeprazole.



Chemical structure of Esomeprazole Sodium:

Since introduction of these drugs there is need for reliable, sensitive and fast methods for their determination in bulk samples.

Literature survey:

Literature survey revealed various methods have been already developed for estimation of Aceclofenac and Esomeprazole separately in bulk and dosage forms. Aceclofenac has been estimated by UV-Spectroscopy[4-9], RP-HPLC[10-15] and HPTLC[16,17] methods. Esomeprazole Sodium was estimated by UV Spectrophotometry[18-20], RP-HPLC[21,22] and HPTLC[23] in bulk and dosage forms in combination with other drugs. There is no method developed for simultaneous estimation of these both drugs in bulk. Hence an attempt has been made to develop a method for the

determination of both drugs in synthetic mixture and has been duly validated as per ICH guidelines.

MATERIALS AND METHOD

All analytical runs were carried out using LC 1120 chromatograph equipped with AB solvent system, Rheodyne loop injector of capacity 20µl and UV Visible (VWD) detector set at 285nm. The instrument is equipped with Agilent TC- C18 column (250mm × 4.6mm, 5µm). An isocratic elution was adopted using mixture Acetonitrile and Methanol in the ratio of 50:50 v/v. the flow rate was set at 0.8ml/min and the injection volume is 7.5µl at ambient temperature. The HPLC instrument was controlled by a PC with EZchrome software.

Aceclofenac and Esomeprazole Na bulk drugs were obtained as gift samples from Medchem private ltd. HPLC grade Methanol and Acetonitrile were obtained from Rankem laboratories, India. Milli – Q water was used throughout the experiment. All other reagents used were of analytical grade.

METHODS

Preparation of standard stock solution (1000mcg/ml):

10mg of Aceclofenac and 10mg of Esomeprazole Na working standards were accurately weighed and transferred into 10ml volumetric flask. 7.5ml of methanol was added to the flask and is sonicated for 15mins to ensure complete dissolution. Finally the volume was made upto the mark with methanol.

Preparation of working standard solutions:

From the standard stock solution further dilutions were made to obtain the concentrations of 30mcg/ml, 40mcg/ml, 50mcg/ml, 60mcg/ml and 70mcg/ml.

Preparation of test solution:

Aceclofenac (10mg), Esomeprazole Na (10mg) and placebo (100mg) were thoroughly mixed and transferred into a 100ml volumetric flask. Add 75ml of methanol and sonicate for 20 mins. The solution is filtered through Whatman filter paper No.41. The retained residue was washed with methanol. The washings and filtrate were combined and the volume is made up to the mark with methanol to obtain the final concentration of 100mcg/ml.

Method development:

After performing several trial and error methods, the following method was optimized based on the asymmetry and theoretical plates values.

Optimized method:

Column : C18 columnn (250mm X 4.6mm, 5µm)

Mobile Phase –A : Acetonitrile

Mobile Phase – B : Methanol

Diluent : Mobile Phase

Mode : Isocratic

Flow Rate : 0.8ml/min

Column temperature : 30°C

Injection volume : 7.5µl

Wavelength : 285nm

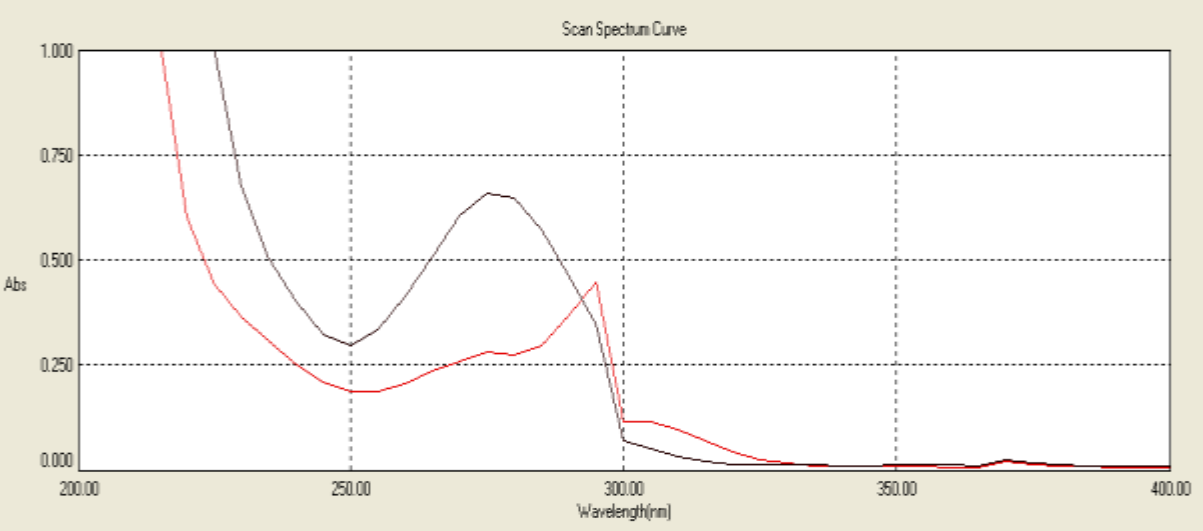


Figure 1: Overlay UV spectrum of Aceclofenac and Esomeprazole Sodium.

METHOD VALIDATION:

System suitability:

System suitability tests are integral part of liquid chromatographic method. Standard solution was analyzed 6 times and the theoretical plates, asymmetry, resolution and % RSD were calculated. Results were given in Table: 1

Linearity:

The linearity of the proposed method was constructed for Aceclofenac and Esomeprazole Na by injecting five levels of solution (60, 80, 100, 120 & 140%) into the chromatographic system. Linearity curve was obtained by plotting the concentrations of compound versus peak area response. The linearity was evaluated by linear regression analysis. Linearity data and graph were given in figure.2 & 3

Accuracy:

The accuracy of the developed method was evaluated by recovery experiments. The recovery studies for selected drugs were carried at three different concentration levels (80%, 100%, 120%). The percentage recovery and %RSD were calculated.

Precision:

Precision of the method was demonstrated by Repeatability or intra-day precision. Six repeated injections of sample solutions were made and the %RSD was calculated.

Sensitivity (LOD & LOQ):

LOD and LOQ were calculated based on the standard deviation of the response of drug solutions and slope of calibration curve as per ICH guidelines using following equations

$$LOD = 3.3\frac{\sigma}{S}$$

$$LOD = 10\frac{\sigma}{S}$$

Where σ = standard deviation of response and S = slope of calibration curve.

Robustness:

The robustness of the developed method was determined by changing the experimental conditions such as change in flow rate (±0.1ml/min), ratio of mobile phase (45:55/55:45) and detection wavelength (±5nm). The results of robustness in terms of % RSD were calculated.

RESULTS AND DISCUSSION

System suitability:

System suitability studies for theoretical plates and resolution was determined. % RSD of peak areas of six replicates of Aceclofenac and Esomeprazole sodium mixture was found to be within limits.

Table 1: System suitability data

parameter	Aceclofenac	Esomeprazole sodium
Theoretical plates (N)	*4247*	7144
%RSD	0.36415	0.1586
Resolution (R)	0	5.25

six replicates

Linearity:

A series of standard solutions in the range of 60% to 140% of the target assay were prepared and injected. A plot of average peak area versus concentration in µg/ml is made. Regression equation for Aceclofenac is y = 18353x + 24673 and correlation coefficient was found to be 0.990. Regression equation for Esomeprazole sodium is y = 17202x – 36449 and correlation coefficient was found to be 0.992. Linearity graphs were shown in the figures 2 & 3.

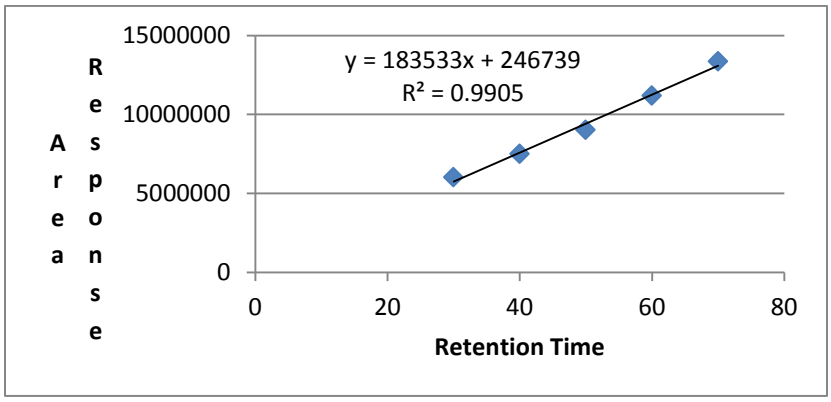


Figure 2: Calibration curve of Aceclofenac

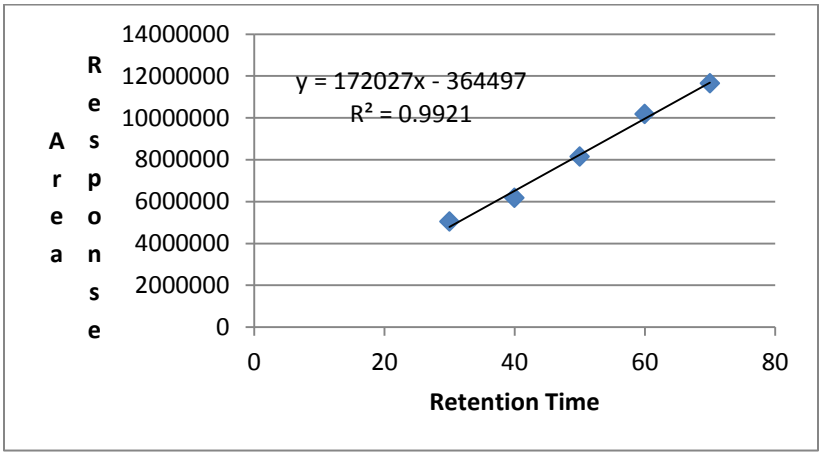


Figure 3: Calibration curve of Esomeprazole Sodium

Precision:

Repeatability: Six replicates of sample solutions of 100% level were prepared and injected. %RSD were found to be 1.86 and 1.32 for Aceclofenac and Esomeprazole sodium respectively.

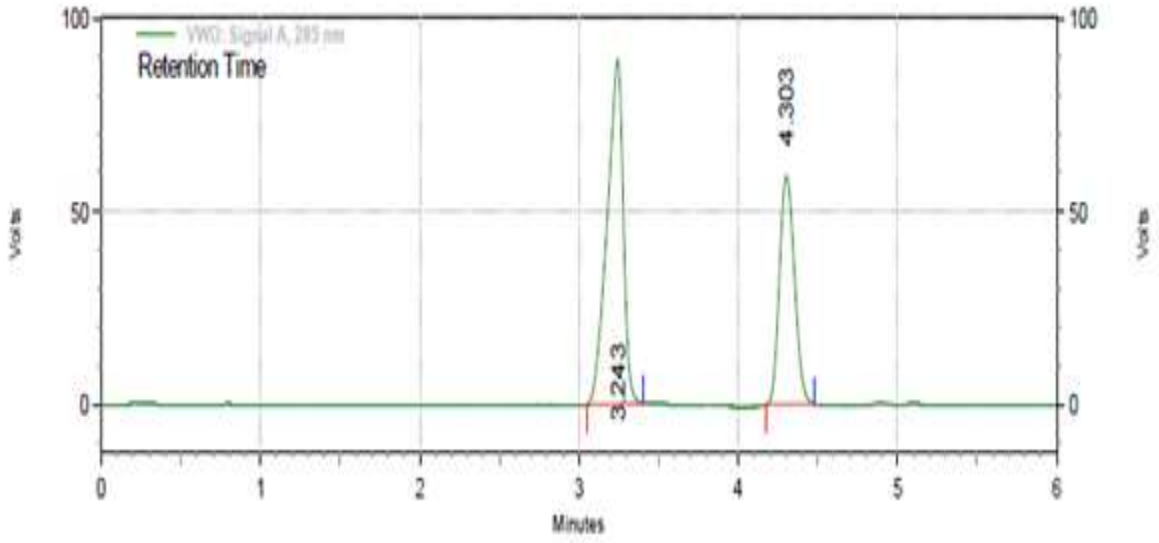


Figure 4: Precision chromatogram.

Accuracy:

Three replicates of each level of 80%, 100% and 120% were injected and the amount recovered, % recovery was calculated. The results were given in the table 2.

Table 2: Recovery studies

sample	Level	Conc(µg/ml)	Amount found (µg/ml)	% recovery	% RSD
	80%	40	39.79	99.48	0.65
Aceclofenac	100%	50	49.94	99.89	0.766
	120%	60	59.26	98.81	0.489
	80%	40	39.71	99.36	1.49
Esomeprazole sodium	100%	50	49.67	99.44	0.46
	120%	60	60.71	101.28	0.644

Sensitivity:

The LOD values for Aceclofenac and Esomeprazole sodium were found to be 0.095µg/ml and 0.090 µg/ml respectively. The LOQ data for Aceclofenac and Esomeprazole sodium were 0.290 µg/ml and 0.273 µg/ml respectively. The data represents the high sensitivity of the proposed validated method.

Robustness:

The standard deviation of peak areas for each parameter is calculated and %RSD was calculated. %RSD values were less than 2% indicating robustness of the developed method.

Assay:

The validated method was applied to the synthetic mixture of Aceclofenac and Esomeprazole sodium. Chromatogram of synthetic mixture of both the drugs was recorded and shown in figure 5.

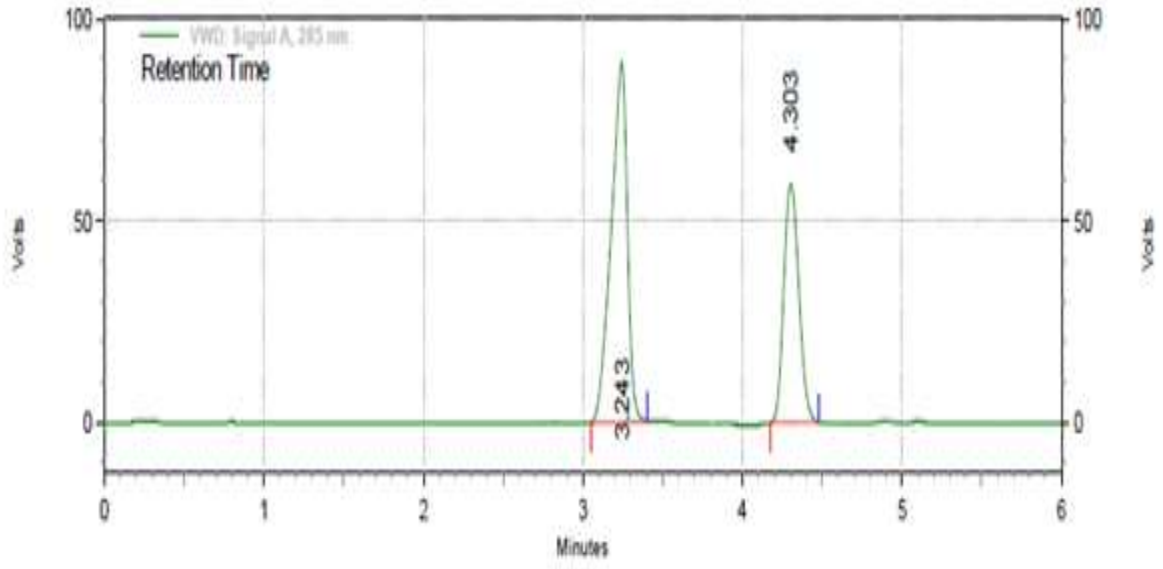


Figure 5: chromatogram of synthetic mixture of Aceclofenac and Esomeprazole sodium.

Summary of validation parameters:

parameters	Aceclofenac	Esomeprazole sodium
Linearity range	*30-70µg/ml*	*30-70µg/ml*
Slope	183532.5	172026.7
Intercept	246739.3	-364497
Correlation coefficient	0.99	0.992
Accuracy(%recovery)	99.39 ± 0.448	100.02 ± 0.887
Repeatability(%RSD, n=6)	1.86	1.32
LOD (*µg/ml)*	*0.095*	0.090
LOQ(*µg/ml)*	0.290	0.273

CONCLUSION

The proposed RP-HPLC method was validated as per ICH guidelines. The method was found to be accurate, precise, specific and suitable for routine quantitative analysis of determination of Aceclofenac and Esomeprazole sodium in bulk.

ACKNOWLEDGEMENT

The authors are highly thankful to the principal Aditya College of Pharmacy, East Godavari district, Andhra Pradesh for providing all the necessary facilities and moral support for carrying out this research work. The authors also extend their gratitude to Medchem.pvt. Ltd for providing drug samples.

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