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Der Pharmacia Lettre, 2010, 2(2): 346-349
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Simultaneous estimation and method validation of Gatifloxacin and Ambroxol Hydrochloride in tablet dosage form by RP-HPLC

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Abstract

A simple, selective, rapid, and precise reverse phase HPLC method has been developed for the simultaneous estimation of gatifloxacin and ambroxol hydrochloride in pharmaceutical dosage form. A phenomenex Luna C_{18} column (250x4.6mm, 5µ) was used for the separation. The mobile phase was 0.02M dibasic ammonium phosphate buffer: acetonitrile (60:40%v/v) (Ph7) at a flow rate of 1.5ml/min with detection at 265nm. The retention time of gatifloxacin and ambroxol hydrochloride was 2.870 and 3.607 minutes respectively. The developed method was validated in term of accuracy, precision, specificity, system suitability, linearity, and robustness, limit of detection and limit of quantification. The proposed method is suitable for simultaneous determination of gatifloxacin and ambroxol hydrochloride in pharmaceutical dosage form

Keywords: Gatifloxacin and Ambroxol hydrochloride

INTRODUCTION

Gatifloxacin [1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid sesquihydrate] is a synthetic broad-spectrum antimicrobial fluoroquinolone that is active against both gram-negative and gram-positive bacteria [1]. Ambroxol hydrochloride [Trans-4-(2-amino-3, 5-dibromo benzylamino)-cyclohexanol hydrochloride] is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus [2]. A literature survey reveals that high performance liquid chromatography (HPLC) methods have already been developed to analyze both the drugs separately [3–8]. However, there is no reported analytical HPLC method for estimation of gatifloxacin and ambroxol hydrochloride in the combined tablet dosage form. In the present investigation, a simple, precise, accurate RP-HPLC method has been developed for the simultaneous estimation of gatifloxacin and ambroxol hydrochloride in tablet dosage form [9-10].

MATERIALS AND METHODS

Reagents and chemicals

Reference standards gatifloxacin and ambroxol hydrochloride were obtained as gift samples from East west pharma, beldi. Acetonitrile & methanol were HPLC grade and ammonium phosphate was A R grade from Merk chemicals, Mumbai.

Preparation of standard stock solution:

Accurately weighed quantity of 400mg gatifloxacin and 60 mg ambroxol hydrochloride was transferred to a 100ml volumetric flask, dissolved in 15ml of mobile phase, sonicated for 15 min and the volume was made up with mobile phase. From the standard stock preparation 10ml of solution was taken in 100ml volumetric flask and further diluted with mobile phase

Preparation of sample solution:

For estimating the tablet dosage form, 20 tablets from a batch were randomly selected and powdered. Weigh accurately 679 mg of ground tablet powder (equivalent to 400mg gatifloxacin and 60 mg ambroxol hydrochloride) transfer it in 100 ml of volumetric flask.15ml of mobile phase was added. The mixture was subjected to sonication for 10 min with intermediate shaking for complete extraction of drugs. Cool at room temperature and the solution was made up to the mark with mobile phase. The sample was centrifuged in tight enclosure for 10 min at 3000 RPM. Then 10 ml of clear solution was transferred into a 100 ml volumetric flask and diluted with mobile phase. 20µl of this solution was injected for HPLC analysis.

Table1: Recovery Studies for gatifloxacin and ambroxol hydrochloride

Inj. Sample	Spike level	Amount Present	Amount Recovered	% Recovered
Gatifloxacin	80 %	320.48	320.99	100.15%
	100 %	400.60	399.35	99.68%
	120 %	480.72	480.56	99.96%
Ambroxol HCL	80 %	48.24	48.75	101.05%
	100 %	60.30	60.79	100.81%
	120 %	72.36	73.30	101.29%

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of gatifloxacin and ambroxol hydrochloride was 2.870 and 3.607 minutes respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard solution and sample solution were calculated.

Recovery studies

To study the accuracy, reproducibility and precision of the above methods, were carried out by addition of standard drug solution to pre-analyzed sample at different levels. Result of

recovery studies were found to be satisfactory and are reported in [Table- 1]

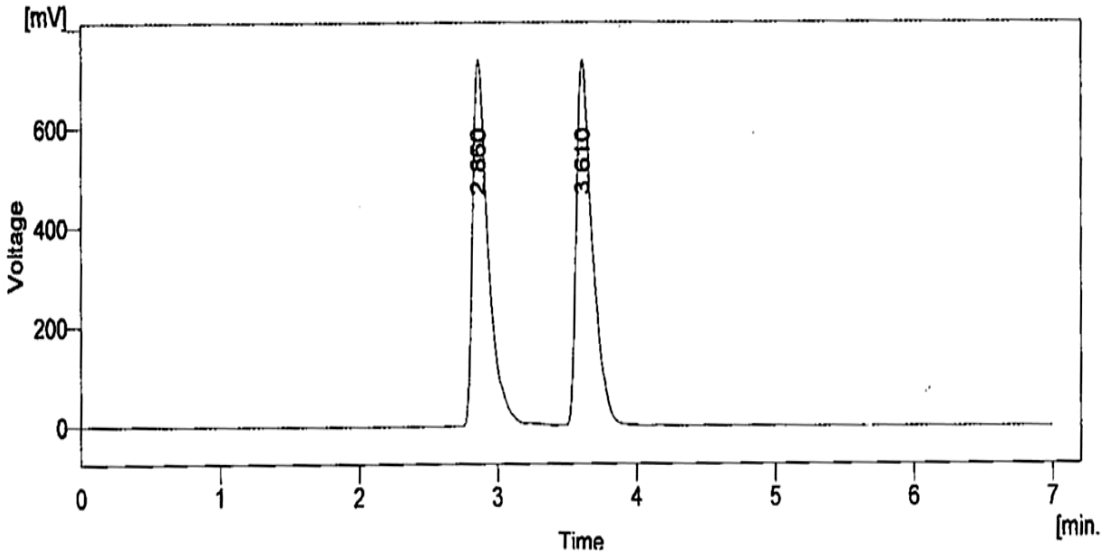

Fig. 1. Typical Chromatogram of Sample Solution

RESULTS AND DISCUSSION

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Gatifloxacin and Ambroxol hydrochloride in combined tablet dosage form.

S.No	Parameters	Gatifloxacin	Ambroxol HCL
1	System suitability		
	a)Tailing factor	1.750	1.625
	b)Theoretical plates	3147	5014
2.	Specificity	Specific	Specific
3	Precision:		
	a)System Precision	0.15%	0.15%
	b)Method Precision	0.13%	0.14%
4	Linearity	0.9999	0.9999
5	Accuracy	99.68%-100.15%	101.05%-100.81%
6	Robustness	Robustted	Robustted
7	LOD	0.42	0.09
8	LOQ	1.28	0.29

The separation was carried out by using a mobile phase consisting of 0.02M Ammonium phosphate buffer and acetonitrile (pH 7.0) in the ratio of 60: 40v/v. The column used was C_{18}

phenomenex luna(250cm x 4.6mm) with flow rate of 1.5ml/min and u-v detection was carried out at 265nm. The method was validated as per ICH guidelines in terms of linearity, accuracy, specificity, precision, repeatability of measurement of peak area as well as repeatability of sample application and the results are shown in above table.

CONCLUSION

The evaluation of obtained values suggests that the proposed HPLC methods provide simple, precise, rapid and robust quantitative analytical method for determination of Gatifloxacin and Ambroxol hydrochloride in combined dosage form. Hence method can be easily conveniently adopted for routine estimation of Gatifloxacin and Ambroxol hydrochloride in combined dosage form.

Acknowledgements

The authors are thankful to the secretary and correspondent Smt. N. Sendamaraai**,** J. K. K. Rangammal charitable trust and Dr. P. Perumal, Principal, J. K. K. Nataraja College of Pharmacy, Komarapalayam, for the support rendered.

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