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Simultaneous RP-HPLC determination of gatifloxacin and ambroxol hydrochloride in pharmaceutical preparations

Manisha M.Baing*, V.V.Vaidya, N.J.Rane, P.A.Champanerkar
Department of Chemistry, Ramnarain Ruia College, Matunga, Mumbai-400 019, (INDIA)
E-mail : manisha_baing@yahoo.com
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ABSTRACT

A rapid and accurate reversed phase high performance liquid chromatographic method has been developed for the simultaneous determination of Gatifloxacin and Ambroxol hydrochloride. Ciprofloxacin was used as internal standard. Chromatographic separation of the two drugs was performed on a BDS Hypersil C_{18} column (150mm × 4.6 mm, 5μm) with a 65:15:20 (v/v) mixture of 0.05M potassium dihydrogen phosphate, acetonitrile, and methanol (0.1%triethyl amine pH adjusted to 3.0 with orthophosphoric acid) at a flow rate of 1.0 ml/min and detection at 220nm. The separation was complete in less than 10 mins. The method was validated for linearity, accuracy, precision, limit of quantitation and robustness[2,3]. Linearity, accuracy and precision were found to be acceptable over the ranges of 120 - 280μg mL^{-1} for Gatifloxacin and 18- 42μg mL^{-1} for Ambroxol hydrochloride.

KEYWORDS

Column liquid chromatography;
Gatifloxacin;
Ambroxol hydrochloride.

INTRODUCTION

Gatifloxacin is chemically 1-cyclopropyl-6-fluoro-1-4 dihydro-8-methoxy-7-(3-methyl-1-piperazenyl)-4-oxo-3-quinoline carboxylic acid which due to its extremely high activity is used as antibiotic for treating infections with gram positive and gram negative bacteria . Ambroxol hydrochloride is chemically trans-(2-amino3,5-dibromobenzyl) amino cyclohexanol hydrochloride. It is a metabolite of bromgexine and acts to reduce the viscosity of tenacious mucous secretions via fragmentation of long mucopolysaccharide chains, it also enhances penetration power of antibiotic. The structures of these two drugs are shown in figure 1[1]. This combination is used as antibiotic drug. The literature survey revealed that simultaneous determination of Gatifloxacin and Ambroxol hydrochloride with internal standard by HPLC is not reported. However, references are available for simultaneous determination of Gatifloxacin and Ambroxol hydrochloride by HPLC[4,5]. The objective of the present work is to develop a simple, fast and accurate HPLC method for simultaneous determination of Gatifloxacin and Ambroxol hydrochloride from pharmaceutical formulation.

EXPERIMENTAL

Chemicals and reagents

Standards were obtained from reputed research centres. Gatrich tablets manufactured by Cipla were procured from the market. Acetonitrile, Methanol, Triethyl amine used were HPLC grade and Orthophosphoric acid was GR grade. Double distilled water was used throughout the work. All dilutions were performed

in standard volumetric flasks.

Preparation of stock and working standard solutions

A stock solution of Gatifloxacin (2000μg mL^{-1}) was prepared by dissolving 100mg Gatifloxacin(99.99%) in methanol in standard 50ml volumetric flask(solution A). A stock solution of Ambroxol hydrochloride (600μg mL^{-1}) was prepared by dissolving 60mg Ambroxol hydrochloride (99.92%) in methanol in standard 100ml volumetric flask(solution B). Internal standard (IS-Ciprofloxacin) stock solution (2500μg mL^{-1}) was prepared by 250 mg Ciprofloxacin in methanol in a 100ml standard volumetric flask. Solutions containing mixture of Gatifloxacin, Ambroxol hydrochloride at five different concentrations were prepared in the mobile phase and Ciprofloxacin was added to each as internal standard (100μg mL^{-1}).The concentration ranges for each of two drugs in the working standard solutions were 120μg mL^{-1} - 280μg mL^{-1} for Gatifloxacin and 18μg mL^{-1} - 42μg mL^{-1} for Ambroxol hydrochloride.

Sample preparation

Twenty tablets were weighed and their average weight was calculated. The tablets were crushed in to a homogeneous powder and a quantity equivalent to one tablet (1218.5 mg) was weighed in a 100mL volumetric flask, dissolved in methanol, and filtered through Whatman no. 41 filter paper. The filtrate (5ml) was quantitatively transferred to a 100mL volumetric flask, 4mL of internal standard solution was added to it, and diluted up to the mark with mobile phase.

Instrumentation and chromatographic conditions

Chromatography was performed with a Merck Hitachi pumpL-7100, a Merck autosampler L-7250, and a Merck Diode array detector L-7455. Chromatograms and data were recorded by means of HSM software. Compounds were separated on a 150mm x 4.6mm, i.d., 5μm particle, BDS C_{18} column. The mobile phase was 0.05M potassium dihydrogen phosphate: acetonitrile: methanol, 65:15:20 (v/v). containing 0.1% triethyl amine pH adjusted to 3.0 with orthophosphoric acid. The flow rate was 1.0mL min^{-1}. Twenty microlitres of sample was injected and detection wavelength was 220nm.for determination of both the drugs. The overlain spectra Gatifloxacin and Ambroxol hydrochloride

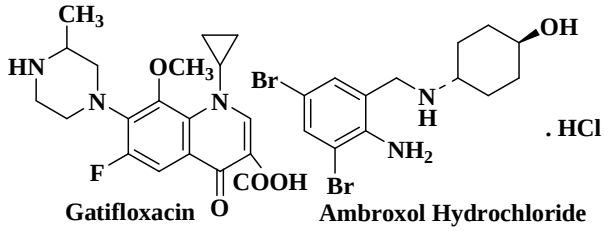

Figure 1: The structure of Gatifloxacin and Ambroxol hydrochloride

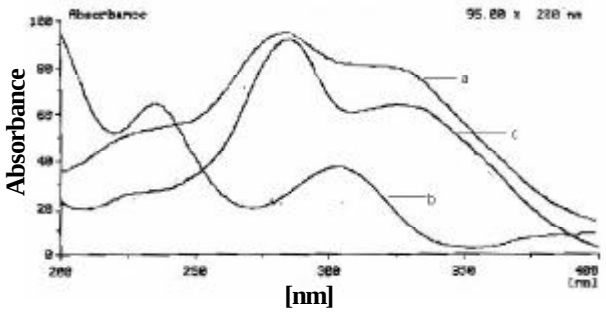

Figure 2: UV spectra of (a) Gatifloxacin (b) Ambroxol hydrochloride (c) Ciprofloxacin in methanol

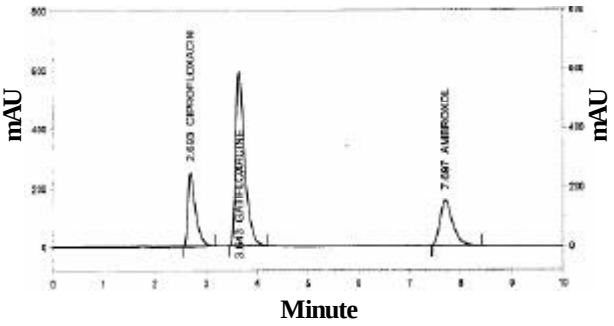

Figure 3: HPLC Chromatogram obtained during Simultaneous determination of Gatifloxacin[2], Ambroxol hydrochloride[3] with Ciprofloxacin [1] as internal standard

with internal standard Ciprofloxacin are shown in figure 2 and typical HPLC chromatograms obtained from simultaneous determination of Gatifloxacin and Ambroxol hydrochloride from pharmaceutical formulation are shown in figure 3.

RESULT AND DISCUSSION

Method validation

The chromatographic conditions were optimized to obtain good baseline separation and peak shape.

Linearity

Linearity was evaluated by analysis of working stan-

dard solutions of Gatifloxacin and Ambroxol hydrochloride of five different concentrations[3]. The linear ranges from 120 to 280μg mL^{-1} for Gatifloxacin and 18 - 42μg mL^{-1} for Ambroxol hydrochloride. The drug to internal standard peak area ratio and concentration of each drug were subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression data obtained for the two pharmaceuticals are listed in TABLE 1. The results shows that within these concentration ranges there was excellent correlation between peak area ratio and concentration of each drug.

TABLE 1: Results of linearity

Analyte	Slope (mean)	Intercept (mean)	Correlation coefficient (r) n=5
Gatifloxacin	0.014	0.016	0.9999
Ambroxol hydrochloride	0.033	0.002	0.9999

TABLE 2: Results for system suitability

Parameters	Ciprofloxacin	Gatifloxacin	Ambroxol hydrochloride
RSD (n=6)	0.22	0.16	0.11
Theoretical plates	1632	2180	4703
Tailing factor	1.08	1.02	1.12
Resolution	-	3.26	10.68

TABLE 3: Result for recovery

	Initial Concentration in mg	Amount added in mg	Total Concentration mg	Amount of drug found in (mg)	Recovery (%)
Gatifloxacine	400	00	200	400.62	100.16
	400	40	440	440.03	100.01
	400	80	480	479.77	99.95
	400	120	520	519.95	99.99
Ambroxol hydrochloride	60	0	60	59.82	99.70
	60	6	66	66.24	100.36
	60	12	72	72.14	100.20
	60	18	78	78.19	100.24

Limit of detection and quantification

The limit of detection (LOD) and quantification (LOQ) were established at signal to noise ratios of 3:1 and 10:1, respectively. The LOD and LOQ of Gatifloxacin and Ambroxol hydrochloride were determined experimentally by injecting each drug six times. The LOD for Gatifloxacin and Ambroxol hydrochloride were 0.04μg mL^{-1} and 0.03μg mL^{-1} respectively. The LOQ for Gatifloxacin and Ambroxol hydrochloride were 0.12g mL^{-1} and 0.09μg mL^{-1} respectively.

Precision

Repeatability was studied by determination of system precision for six replicate injections of the mixed standard solutions. The relative standard deviations were less than 2% for the two drugs. Method precision was determined from results from six independent determinations at 100% of the test concentrations of Gatifloxacin and Ambroxol hydrochloride in the formulation. The RSD were 0.28 and 0.56 respectively.

System suitability

System suitability tests were performed in accordance with USP 24/NF 19 to confirm the reproducibility of the equipment was adequate for the analysis to be performed. The test was performed by injecting 20μLof mixed solution of drug and internal standard. The RSD values were found to be satisfactory and meeting the requirements of USP24 NF19 (RSD less than 2 %). Theoretical plates, resolution, tailing factor were determined and presented in TABLE 2.

Accuracy

The accuracy of the method was determined by measuring the recovery of the drugs by the method of standard additions. Known amounts of each drug corresponding to 100,110,120 and 130% of the label claim for each drug were added to preanalysed sample, to determine whether the excipients present in the formulation led to positive or negative interferences[3]. Each set of additions was repeated three times. The accuracy was expressed as the percentage of the analytes recovered by the assay; the results obtained are listed in TABLE 3. the results indicate the method enables highly accurate for simultaneous determination of Gatifloxacin and Ambroxol hydrochloride.

Assay

The validated HPLC method was used for simultaneous determination of Gatifloxacin and Ambroxol hydrochloride in their combined dosage form. In the assay experiment, six samples were weighed separately and analysed. The mean assay results in mg and expressed as a percentage of the label claim. The results are given in TABLE 4. The results indicate that the amount of each drug in the tablets is within the require-

TABLE 4: Results for assay

	Gatifloxacin	Ambroxol hydrochloride
Mean amount of drug found (mg per tablet)	400.39	60.04
% Assay	100.10	100.06
RSD	0.28	0.57

ments of 90-110% of the label claim.

Robustness

The robustness of a method is a measure of its capacity to remain unaffected by small variations in method conditions; it provides an indication of the reliability of the method during normal application[6]. The robustness of the proposed method was evaluated by altering the pH of the mobile phase and different columns from different manufacturers- the experimental conditions were changed and the chromatographic characteristics were evaluated.

Mobile phase pH was changed by ±0.2 units from 2.8 to 3.2. At both the ends of the pH range variations in the resolution of Ciprofloxacin (IS), Gatifloxacin and Ambroxol hydrochloride and the retention time of the two drugs with internal standard were within 2%. Column to column variation was also determined using different C_{18} column (Cosmosil C_{18}) the results indicated there were no significant differences between the two columns, and that separation of the two active substances is achievable under the given conditions using the method developed which is satisfactory for the simultaneous determination of Gatifloxacin and Ambroxol hydrochloride in the formulation.

CONCLUSION

We have developed a specific, rapid, sensitive and inexpensive HPLC method for simultaneous determination of Gatifloxacin and Ambroxol hydrochloride in their combined dosage form. The method involves separation on revered phase column with an internal standard, and UV detection. The linearity, precision, accuracy of the method proves that the method is easily reproducible in any quality control setup provides all the parameters are followed accurately.

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