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Original Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF GATIFLOXACIN AND AMBROXOL HCL IN PHARMACEUTICAL DOSAGE FORM

SAROJ KUMAR RAUL*, BAINAPALLI SHANMUKHA, DURGASI JHANSI

Maharajah's College of Pharmacy, Phool Baugh, Vizianagaram, Andhra Pradesh, India.
Email: saroj.raul@rediffmail.com

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ABSTRACT

Objective: To develop a simple, selective, linear, precise and accurate RP-HPLC method for simultaneous estimation of Gatifloxacin and Ambroxol HCl in tablet dosage form.

Methods: The chromatographic separation was performed using Inerstil ODS-3V (4.6 x 250 mm, 5 µm particle size). Mobile phase was composed of phosphate Buffer pH-5 and methanol (40:60v/v) at a flow rate of 1 ml/min. Detection was carried out using PDA detector at 237 nm. The method was validated as per ICH guidelines.

Results: The retention times for Gatifloxacin and Ambroxol HCl were observed as 2.443 and 4.863 min respectively. Linearity range was observed in concentration of 32-112 µg/ml for Gatifloxacin and 6-21 µg/ml for Ambroxol HCl. The percentage recoveries of Gatifloxacin and Ambroxol HCl are 99.64% and 99.78% respectively. The correlation coefficients for both the components are close to 1.

Conclusion: This method is simple, selective, linear, precise, accurate and sensitive hence can be successfully employed for the routine quality control of dosage forms containing both the drugs in pharmaceutical industries.

Keywords: RP-HPLC, Method development, Gatifloxacin, Ambroxol HCl, Validation.

INTRODUCTION

Gatifloxacin is chemically named as 1-cyclopropyl-6-fluoro-8-methoxy-7-(3 methyl piperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid (fig. 1) an antibiotic of the fourth-generation inhibits the bacterial enzymes DNA gyrase and topoisomerase IV [1]. It has the broader spectrum of antibacterial activity than the older fluoroquinolones and shows good activity against gram+ve and gram –ve microorganisms [2]. Gatifloxacin having selective antimicrobial activity against Streptococcus pneumoniae and penicillin-resistant Pneumococci. It is also active against anaerobic pathogen, bacteriodes fragilis, and mouth anaerobes [3].

Ambroxol HCl is chemically named as trans-4-(2-Amino-3, 5-dibrom benzylamino)-cyclohexanol, (fig. 2). It is a mucoactive drug with several properties including secretolytic and secretomotoric actions that restore the physiological clearance mechanisms of the respiratory tract, which play an important role in the body's natural defence mechanisms [4]. Ambroxol is a metabolite of bromhexine. It is an expectoration improver and mucolytic agent used in the treatment of acute and chronic disorders characterized by the production of excess or thick mucus [5]. It stimulates cellular surfactant production, increases the amount of antibiotic penetration and thus reduces daily dose of Gatifloxacin and exhibits anti-inflammatory properties as well [6].

Literature survey reveals that few spectrophotometric methods [7-13] and HPLC methods [14-16] have been reported for the estimation of Gatifloxacin and Ambroxol HCl. The aim of the present study is to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Gatifloxacin and Ambroxol HCl in pharmaceutical dosage forms as per ICH guidelines [17]. The validation procedure followed the guidelines of USP 30 [18].

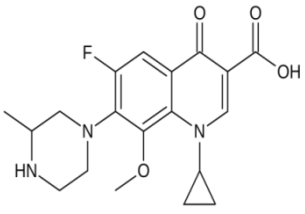

Fig. 1: Structure of Gatifloxacin

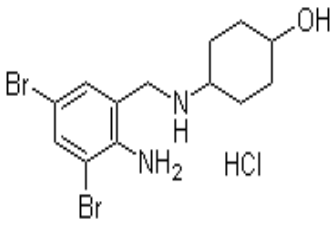

Fig. 2: Structure of Ambroxol HCl

MATERIALS AND METHODS

Instrumental and analytical conditions

The HPLC analysis was carried out on Shimadzu HPLC (10A series) equipped with PDA detector and running on Spin chrome software. The column used is Inertsil ODS C_{18} column (4.6x250 mm, 5 µm particle size) and detection was performed at 237 nm. The injection volume of the sample was 10 µl and the run time was 6 minutes. An isocratic mobile phase consisted of phosphate buffer and methanol in the ratio 40:60v/v at pH 5 was carried out with the flow rate at 1 ml/min. The mobile phase was filtered through 0.45 µm membrane filter and degassed before use.

Reagents and chemicals

Gatifloxacin and Ambroxol HCl were obtained as gift sample from Aristo Pharma Limited. Tablets were procured from local pharmacy containing 400mg of Gatifloxacin and 75mg of Ambroxol HCl (Gatiwiz AM). Ultra pure water was obtained from a Millipore system. HPLC grade methanol was obtained from Qualigens fine chemicals limited. All other chemicals used were AR grade.

Preparation of mobile phase

Accurately weighed 2.72 gm of sodium dihydrogen orthophosphate was transferred into a 1000 ml volumetric flask and about 900 ml of milli-Q water was added and sonicated to degas and finally volume adjusted with water. Then pH adjusted to 5 with dilute ortho phosphoric acid solution. Buffer and Methanol taken in the ratio of 40:60 into a mobile phase bottle and mixed. Then filtered through 0.45µ nylon membrane filter and degassed. The mobile phase was used as diluent.

Preparation of standard stock solution

Standard stock solutions were prepared by dissolving 80mg of Gatifloxacin and 15mg of Ambroxol HCl into 100 ml volumetric flasks separately with diluents. Sonicated for 30 minutes and volume was adjusted with diluents. The working standard solution of Gatifloxacin (80µg/ml) and Ambroxol HCl (15µg/ml) were prepared by diluting the stock solution with mobile phase.

Preparation of sample solution

Twenty tablets of Gatiwiz-AM containing 400mg of Gatifloxacin and 75mg of Ambroxol HCl were weighed and crushed into a fine powder. The quantity of powder equivalent to 80 mg of Gatifloxacin and 15 mg of Ambroxol HCL was accurately weighed and dissolved in sufficient mobile phase in a 100 ml volumetric flask. The solution was sonicated for 15 min, filtered through 0.45µ nylon membrane filter, and diluted to 100 ml with mobile phase. Further dilution was made with mobile phase to give a final concentration of Gatifloxacin (80µg/ml) and Ambroxol HCl (15µg/ml).

Method development

Various mobile phase combinations were tried initially to separate Gatifloxacin and Ambroxol HCl on C18 column. In order to achieve acceptable peak shapes and perform the separation on a suitable run time, various buffer systems were also tried systematically. Mobile phase composed of phosphate buffer and methanol indicated that the resolution between Gatifloxacin and Ambroxol HCl increased. Therefore phosphate buffer (pH5) and methanol in the ratio 40:60v/v at a flow rate of 1 ml/min was selected as optimised mobile phase. Inertsil ODS C18 (4.6x250 mm, 5 µm particle size) was used as the stationary phase to improve resolution. To analyze both the drugs, detection was tried at various wavelengths but 237 nm was selected as the detection wavelength as both the drugs showed maximum absorption. The retention time was found to be 2.443 and 4.863 min for Gatifloxacin and Ambroxol HCl respectively. The chromatogram obtained was shown in fig. 3. The system suitability parameters were shown in table 1.

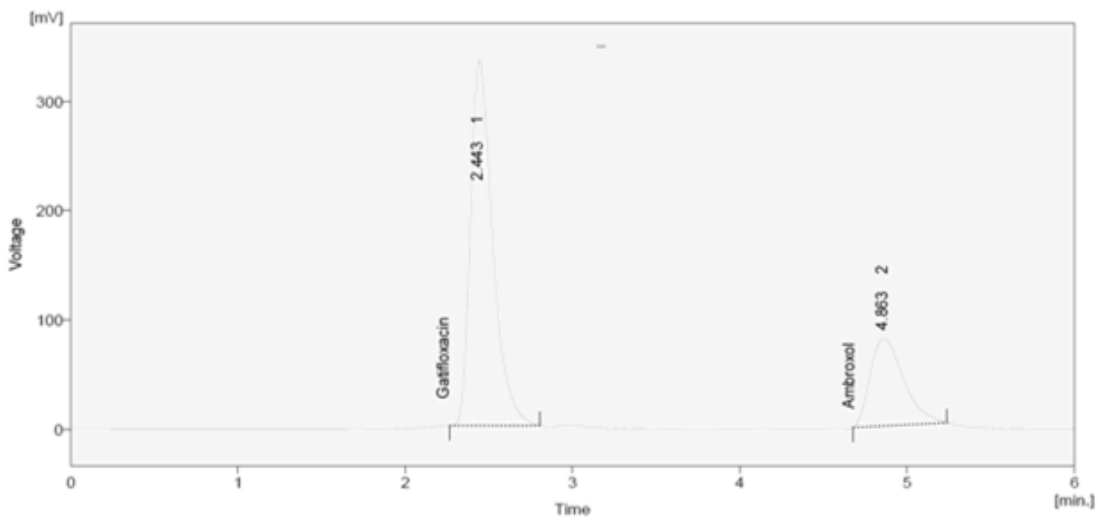

Fig. 3: Standard chromatogram

Table 1: System suitability parameters

Parameter	Gatifloxacin	Ambroxol HCl
Retention time	2.443	4.863
USP Plate count	5562	3491
USP Tailing	1.07	1.21
USP Resolution		8.52

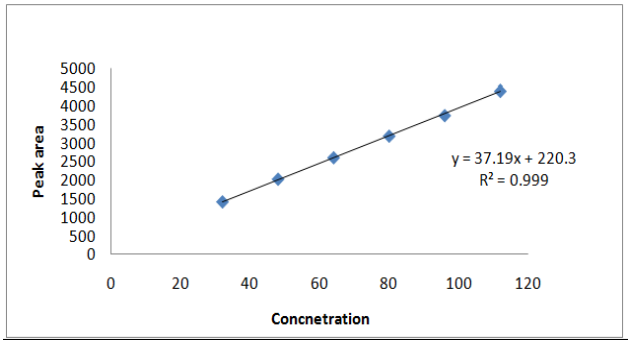

Fig. 4: Linearity graph of Gatifloxacin

Method validation

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The above method was validated according to ICH guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method. They were tested using the optimized chromatographic conditions and instruments.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample. Linearity of detector

response for Gatifloxacin and Ambroxol HCl was established by analyzing serial dilutions of a stock solution of the working standard. Six concentrations such as 32, 48, 64, 80, 96 and 112 µg/ml for Gatifloxacin and 6, 9, 12, 15, 18 and 21 for Ambroxol HCl were prepared and analyzed. The linearity graph was plotted using concentration verses peak area and shown in fig. 4 and fig. 5.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. Repeatability of the method was checked by injecting replicate injections of 80µg/ml of the Gatifloxacin solution and 15 µg/ml of Ambroxol HCl for six times on the same day as intraday precision study of Gatifloxacin and Ambroxol HCl and the chromatogram was recorded. The mean area and % relative standard deviation was calculated. From the data obtained, the developed RP-HPLC method was found to be precise. The result was shown in table 2.

Accuracy

The accuracy of the method was determined by recovery experiments. A known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet sample. Percent recovery was calculated by comparing the area with pre analyzed sample. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated by subtracting the total area from pre analyzed sample area. The results were shown table 3.

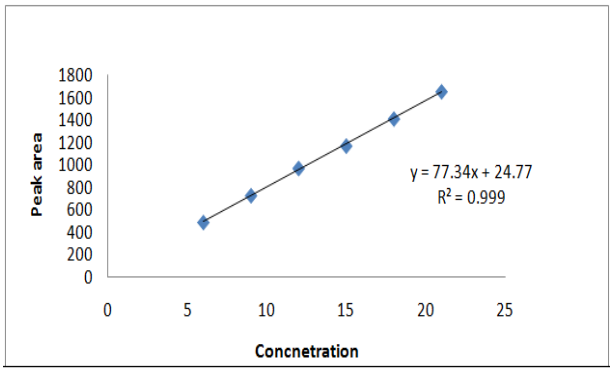

Fig. 5: Linearity graph of Ambroxol HCl

Table 2: Precision result for Gatifloxacin and Ambroxol HCl

Injections	Gatifloxacin	Ambroxol HCl
1	3051.330	1162.913
2	3081.182	1127.772
3	3057.557	1138.675
4	3049.975	1149.400
5	3058.355	1157.508
6	3057.427	1162.930
Mean	3059.304	1149.866
Standard deviation	11.285	14.233
%RSD	**0.37**	**1.24**

Table 3: Accuracy result for Gatifloxacin and Ambroxol HCl

Analyte	% Conc	Amount added (µg)	Amount found(µg)	% recovery	Mean% recovery
	50%	64	62.82	98.16	
Gatifloxacin	100%	80	80.55	100.69	99.64
	150%	96	96.10	100.10	
	50%	12	12.07	100.58	
Ambroxol HCl	100%	15	14.88	99.2	99.78
	150%	18	17.92	99.56	

Specificity

Spectral purities of Gatifloxacin and Ambroxol HCl chromatographic peaks were evaluated for the interference of the tablet excipients, degradation components or due to the presence of impurities as per the methodology. In the work, a solution containing a mixture of the tablet excipients were prepared using the sample preparation procedure to evaluate possible interfering peaks. The representative chromatogram did not show any other peaks, which confirmed the specificity of the method.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was observed that the variations like flow rate of mobile phase, wavelength etc. does not have any significant effect on the method performance, which demonstrated that the developed RP-HPLC method is robust. The results were shown in table 4.

Table 4: Robustness results for Gatifloxacin and Ambroxol HCl

Parameters	Gatifloxacin		Ambroxol HCl	
	Retention time (Rt)	USP tailing	Retention time (Rt)	USP tailing
Flow rate 1 (0.8/min)	2.497	1.72	5.052	1.87
Flow rate 2 (1.2 ml/min)	2.405	1.80	4.807	1.76
Wavelength 1 (235 nm)	2.447	1.52	4.863	1.64
Wavelength 2 (239 nm)	2.450	1.54	4.877	1.78

Table 5: Assay Results

Sample	Batch no	Label claim (mg)	% Amount found	Average
	1	400	99.7	
Gatifloxacin	2	400	99.8	99.7
	3	400	99.6	
	1	75	98.9	
Ambroxol HCl	2	75	99.6	99.37
	3	75	99.6	

Ruggedness

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analysts on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results obtained employing different analyst.

Detection and quantitation limits

The LOD and LOQ of Gatifloxacin and Ambroxol HCl were determined by using the signal to noise ratio approach as defined in ICH guidelines. According to the determined signal to noise ratio, the LOD and LOQ for Gatifloxacin were 2µg/ml and 6µg/ml respectively. For Ambroxol HCl, LOD and LOQ were 0.20µg/ml and 0.6µg/ml respectively.

Assay of pharmaceutical formulation

Three different batches of Gatiwiz AM were analyzed using the validated method. For the analysis, six replicates of each batch were assayed. The mean peak area of the drug was calculated and the drug content in the tablet was quantified. The result found was comparable with the corresponding labelled amounts and they were shown in table 5.

CONCLUSION

In this present work a new simple, selective, linear, precise, accurate and robust HPLC method was developed and validated for the estimation of Gatifloxacin and Ambroxol HCl in pharmaceutical dosage form in accordance with the ICH guidelines. This method gives good resolution between both the compounds with a short analysis time. Linearity was observed in the concentration range of 32-112µg/ml for Gatifloxacin and 6-21µg/ml Ambroxol HCl for both the drugs at 237 nm. The system suitability tests revealed that numbers of theoretical plate were above 2000 and the tailing factor is less than 2. The percentage recoveries of Gatifloxacin and Ambroxol HCl were 99.64% and 99.78% respectively which shows the accuracy of the method. Precision values were within the acceptability limit which indicates that the method is precise. Specificity experiment shows that there is no interference of excipients with the main peaks which confirmed the specificity of the method. The lowest values of LOD and LOQ as obtained by the method indicate the sensitivity of the method. The assay results of the pharmaceutical formulation by this method are highly reproducible, reliable and are in good agreement with the label claim of the drug. Thus this method can be useful for the routine analysis of Gatifloxacin and Ambroxol HCl in combined dosage form.

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CONFLICT OF INTERESTS

Declared none

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