

Simultaneous Determination of Gatifloxacin and Ambroxol Hydrochloride in a Tablet Formulation by Liquid Chromatography

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Abstract

A rapid and accurate liquid chromatographic method has been developed for the simultaneous determination of gatifloxacin (GFC) and ambroxol hydrochloride (AMB) in a tablet formulation. Chromatographic separation of the two drugs was achieved on a Phenomenex column (200mm×4.6 mm, 5µm). The mobile phase consisting of a mixture of 0.1 M phosphate buffer adjusted to pH 5.5 and acetonitrile in the ratio of 55:45 was delivered at a flow rate of 1.0 ml/min. Detection was performed at 254 nm using UV detector. The retention time for GFC was around 2.2 and AMB was around 4.5 min; separation was complete in less than 10 min. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range of 10 – 200 µg/ml and 10 – 100 µg/ml and correlation coefficient was found to be 0.9992 and 0.9983 for GFC and AMB respectively. The method was validated for accuracy, precision and recovery studies. Statistical analysis proved the method was precise, reproducible, selective, specific, and accurate for analysis of GFC and AMB. The wide linearity range, sensitivity, accuracy, short retention time, and simple mobile phase imply the method is suitable for routine quality control of formulation products.

Keywords: Gatifloxacin; Ambroxol Hydrochloride; Simultaneous Determination; Chromatography; Pharmaceuticals

Introduction

Gatifloxacin (GFC) is chemically 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid [1] has broader spectrum of antibacterial activity than the older fluoroquinolones and shows good activity against gram +ve and gram -ve microorganisms [2]. Ambroxol Hydrochloride (AMB) is chemically trans-4-(2-amino 3,5-dibromobenzyl)amino-cyclohexanol, as hydrochloride, is used to reduce bronchial hyper-reactivity, stimulates cellular surfactant production, increases the amount of antibiotic penetration and thus reduces daily dose of gatifloxacin and exhibits anti-inflammatory properties as well [3]. These two drugs are not official in any pharmacopoeia; GFC and AMB are used in dual drug therapy for the treatment of upper respiratory tract infection for adults. Some methods can be found for the individual determination of roxithromycin and ambroxol hydrochloride. High performance liquid chromatography [4] and LC/ESI-MS/MS [5] methods have been reported for the estimation of gatifloxacin in dosage forms and from human plasma. Methods available for the determination of ambroxol hydrochloride include capillary electrophoresis [6-8], spectrometry [9], gas chromatography [10,11] and LC with potentiometric detection [12], MS detection [13] and UV detection [14-18] methods have been reported for the estimation of ambroxol HCl.

In recent years pharmaceutical preparations containing both these drugs have been available commercially. However, no references have been found for simultaneous determination of GFC and AMB in pharmaceutical preparations. Liquid chromatography with UV detection is often preferred in ordinary laboratories because of its wide suitability and availability. The reported methods for the individual determination of the drugs cannot be easily applied for the simultaneous determination of both drugs in the formulation owing to their large differences in physico-chemical properties. The present paper describes a rapid and accurate LC method for the simultaneous determination of GFC and AMB in the tablet formulation.

Materials and Methods

Chemicals

GFC and AMB were obtained as the gift samples from Aristo Pharma Ltd., India. HPLC grade methanol, acetonitrile and water (triple distilled) were procured from Qualigens Fine Chemicals, Mumbai, India.

Chromatographic conditions

Chromatography was performed on a (Shimadzu HPLC Class 10A Series) equipped with two LC-10AT pumps with a variable UV-Vis detector SPD-10A. Samples (20µl) were injected by means of a Rheodyne injector fitted with a 20µl loop. Class LC-10AT series, version 5.03 were employed for data collecting and processing. A Phenomenex RP-C18 column (250 mm × 4.6 mm i.d.; Particle size 5µ) was used for separation. The mobile phase consisting of 0.1 M phosphate buffer adjusted to pH 5.5, acetonitrile (55:45 % v/v) was delivered at a flow rate of 1.0 ml/min. The mobile phase was filtered through a 0.45µm membrane and degassed for 30 min in an ultrasonic bath. Analysis was performed at ambient temperature and detection was performed at 254 nm. The injection volume was 20µl.

Method development

Preparation of stock solution from the bulk drug: Standard stock

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solution of 500µg/ml of GFC and AMB were prepared by dissolving separately in 50 ml of HPLC methanol in 100 ml volumetric flask, the volume was made up to mark with the same. Standard solutions were prepared by dilution of the stock solution with mobile phase to give the final concentration range of 10-200µg/ml and 10-100µg/ml for GFC and AMB respectively.

Sample preparation: Twenty tablets were weighed accurately. The average weight was determined and then ground to a fine powder. A quantity equivalent to 75 mg of AMB and 400 mg of GFC were transferred to a 100 ml volumetric flask. The contents were ultrasonicated for 15 min with 50 ml of HPLC methanol and made up to the mark with same. The resulting solution was allowed to settle for about an hour, and the supernatant was suitably diluted to give the desired concentration with mobile phase. The solution was filtered using 0.2µm membrane filter. The area under the curve and the drug content per tablet (on an average weight basis) was calculated.

Precision: Intra-day and inter-day accuracy and precision of the assay samples containing (80, 100 and 120µg/ml) for GFC and (40, 60 and 80µg/ml) for AMB were analyzed six times in the same day (intra-day) and for three consecutive days by different analysts (inter-day).

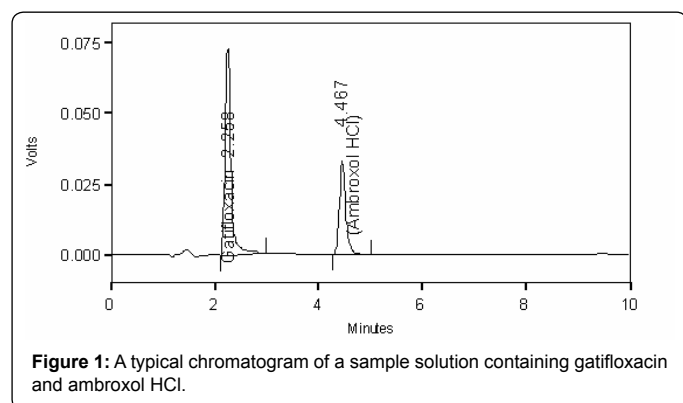
Specificity: The specificity of the method was assessed by analyzing standard drug, pharmaceutical product and placebo and comparing the Retention time of the standard with that of the sample to determine whether the pharmaceutical product and placebo led to interfere.

Accuracy as Recovery studies: Recovery studies were done at three different levels. The pre-analyzed samples were spiked with 80, 100, 120µg/ml and 40, 60, 80µg/ml of pure GFC and AMB respectively, and the mixtures were reanalyzed by the proposed method. Percentage recovery was calculated from the amount of drug found in the solution.

Robustness: By introducing small but deliberate changes in the mobile phase pH (0.1), mobile phase composition ($\pm 2.0\%$), detection wavelength (± 5.0 nm), flow rate (± 10.0 % of absolute value) robustness of the described method was studied.

Results

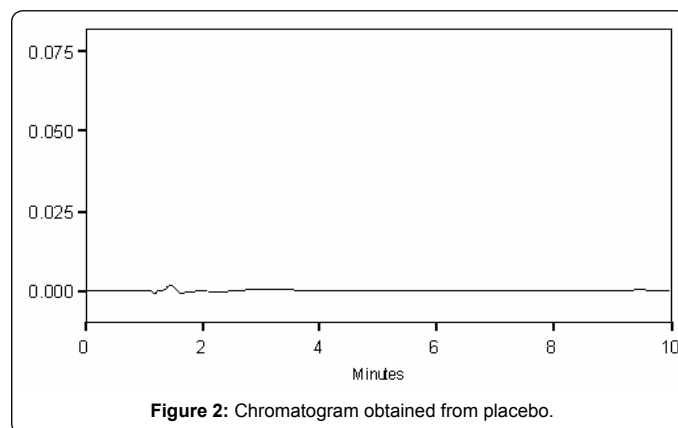
The suitability of the mobile phase was decided on the basis of the various trials. The composition of the mobile phase for development of chromatographic method was optimized by testing different solvent mixtures of varying polarity. After several trials, finally a mobile phase consisting of a mixture of phosphate buffer pH 5.5 and acetonitrile, in the ratio of (55:45 % v/v) was adopted, which



Actual conc. (µg/ml)	Intra-day precision		Intra-day precision	
	SD	%RSD	SD	%RSD
GFC				
80	0.72	0.91	0.85	1.08
100	1.31	1.34	1.29	1.33
120	1.25	1.06	1.30	1.11
AMB				
40	0.39	1.02	0.51	1.33
60	0.59	1.02	0.74	1.29
80	0.65	0.83	0.94	1.21

^an=6

Table 1: Intra and inter day precision of HPLC method^a.



Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery (%)
GFC		
80	79.16	98.95
100	98.79	98.79
120	118.66	98.88
AMB		
40	39.51	98.79
60	59.49	99.15
80	79.27	99.08

^an=6

Table 2: Recovery studies^a.

produces good resolution, reasonable retention and acceptable peak shape for both drugs. The retention time for GFC was around 2.2 and AMB was around 4.5 min. Separation was complete in less than 10 min which is shown in (Figure 1).

A set of six solutions of GFC and AMB at concentrations ranging from 10 to 200µg/ml and 10 to 100µg/ml were prepared. Each sample was analyzed in triplicate; calibration curve was constructed by plotting the peak area against concentration using linear regression analysis. The correlation coefficient was found to be 0.9992 and 0.9980 for GFC and AMB respectively.

The intra-day precision of the developed LC method was determined by preparing the tablet samples of the same batch in six determinations with three concentrations. The R.S.D. of the assay results, expressed as a percentage of the label claim, was used to evaluate the method precision. The obtained R.S.D. values were 1.10% for GFC and 1.17% for AMB. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days, which was found to be 0.96 and 1.28% for GFC and AMB, respectively. The results are shown in (Table 1).

The specificity of the method was confirmed by comparing the Retention time of standard with that of GFC and AMB in the marketed formulation. The blank chromatogram obtained from the placebo is shown in (Figure 2).

The developed method was used to quantify GFC and AMB in



tablet dosage; tablets of 400 mg of GFC and 75 mg of AMB label claim were analyzed and the average drug content was found to be 99.26% and 98.86 % for GFC and AMB respectively for labeled amount. It may therefore be inferred that degradation of GFC and AMB had not occurred in the formulation that were analyzed by this method. The recovery results are shown in (Table 2). The mean recoveries were found to be 98.88 ± 0.08 and 99.01 ± 0.19 for GFC and AMB respectively.

The standard deviation of peak areas was calculated for each parameter such as small changes in the variations of pH of the mobile phase (± 0.1), mobile phase composition ($\pm 2.0\%$), wavelength of detection (± 5.0 nm), flow rate ($\pm 10.0\%$ of absolute value). The % R.S.D. was found to be less than 2%.

Discussion

The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and drug products. The primary goal in developing this LC method is to achieve simultaneous determination of GFC and AMB in the compound formulation under common conditions that are applicable for the routine quality control of this product in ordinary laboratories. consisting of a mixture of phosphate buffer pH 5.5 and acetonitrile, in the ratio of (55:45 % v/v) was adopted, which produces good resolution, reasonable retention and acceptable peak shape for both drugs.

The results show that an excellent correlation existed between peak area and concentration range between 10 to 200 μ g/ml and 10 to 100 μ g/ml for GFC and AMB respectively. Repeatability study was carried out at three different concentration levels of 80, 100 and 120 μ g/ml and 40, 60 and 80 μ g/ml for GFC and AMB respectively. The repeatability results revealed that the developed method is having good repeatability.

The specificity of the method was confirmed by the absence of interfering peaks from the excipients commonly present in the tablets and therefore be interred that degradation of GFC and AMB had not occurred in the formulation that were analyzed by this method. Hence the developed method is specific and selective.

Accuracy and recovery study results revealed that the developed method can be used for determination of GFC and AMB in pharmaceutical formulation. The low % RSD value for analysis of GFC and AMB in marketed formulation indicated the suitability of this method for routine analysis of GFC and AMB in pharmaceutical dosage forms.

Small deliberate changes in the chromatographic condition did not affect the method and the low values of % RSD results revealed that the robustness of the method.

Conclusion

The developed HPTLC method combined with densitometry was found suitable for determination of duloxetine hydrochloride in bulk drug and marketed solid dosage formulation without any

interference from the excipients. Statistical analysis proves that the method is repeatable and selective for the analysis of duloxetine hydrochloride. Its advantages are low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

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