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Research Article

Simultaneous determination of gatifloxacin and ambroxol hydrochloride from tablet dosage form using RP-HPLC

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ABSTRACT

A reversed-phase high performance liquid chromatography (HPLC) method was developed, validated, and used for the quantitative determination of gatifloxacin (GA) and ambroxol hydrochloride (AM), from its tablet dosage form. Chromatographic separation was performed on a Thermo Hypersil Keystone ODS C_{18} column (250 mm × 4.6 mm, 5 µm), with a mobile phase comprising of a mixture of phosphate buffer and acetonitrile (60:40, v/v), and pH adjusted to 3 with ortho phosphoric acid, at a flow rate of 1 mL/min, with detection at 250 nm. Separation was completed in less than 10 min. As per International Conference on Harmonisation (ICH) guidelines the method was validated for linearity, accuracy, precision, limit of quantitation,limit of detection, and robustness. Linearity of GA was found to be in the range of 20–100 µg/mL and that for AM was found to be 5–15 µg/mL. The correlation coefficients were 0.9989 and 0.9966 for GA and AM respectively. The results of the tablet analysis (*n*= 5) were found to be 99.86% and 100.09% for GA and AM respectively. Percent recovery of GA was found to be 98.2%–101.02% and that of AM was 98.86%–102.05%. The assay experiment shows that the method is free from interference of excipients. This demonstrates that the developed HPLC method is simple, linear, precise, and accurate, and can be conveniently adopted for the routine quality control analysis of the tablet.

Keywords: reversed-phase high performance liquid chromatography (RP-HPLC); gatifloxacin (GA); ambroxol hydrochloride (AM); pharmaceutical tablet dosage form.

INTRODUCTION

Chemically gatifloxacin (GA) is 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quino linecarboxylic acid sesquihydrate. It is a synthetic broad spectrum8-methoxyfluoroquinolone antibacterial drug, used in the treatment of community-acquired pneumonia, acute bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis and complicated and uncomplicated urinary tract infections. Its acts intravenously by inhibiting topoisomerase II (DNA gyrase) or topoisomerase IV (Zhao X Let al., 1997; Fukuda H et al., 1999). Its empirical formula for GA is $C_{19}H_{22}FN_3O4$. Its structure is given in Fig.1.

Ambroxol hydrochloride (AM) is a mucolytic agent. Chemically AM is trans-4-((2-amino-3,5-dibrom obenzyl) amino) cyclohexanol hydrochloride. The empirical formula for AM is $C_{13}H_{18}Br_2N_2O.HCl$ (British Pharmacopoeia., 2003). Its structure is given in Fig.2.

A literature survey reveals that high performance liquid chromatography (HPLC) methods have already been developed to analyze both the drugs separately (Heinanen M et al., 2001, Zarzuelo A et al., 2001, Borner K et al., 2000). However, there is no reported analytical HPLC method for estimation of GA and AM in the combined tablet dosage form. In the present investigation, an economical, precise, accurate reversed-phase HPLC method, using an photo diode array (PDA) detector, has been developed for the simultaneous quantitative determination of GA and AM, from the tablet preparation.

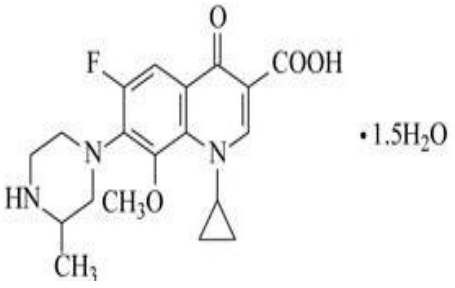

Figure 1: Structure of gatifloxacin sesquihydrate

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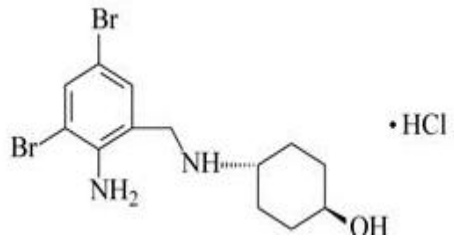

Figure 2: Structure of ambroxol hydrochloride

EXPERIMENTAL

Chemicals and reagents

Bulk drugs GA and AM were procured from Aristo Pharmaceutical Ltd., Mumbai, India. Acetonitrile (HPLCgrade, purity 99.80%), orthophosphoric acid (AR grade, purity 93.00%), and disodium hydrogen orthophosphate (AR grade, purity 99.50%) were all procured from Qualigens Fine Chemicals (Mumbai, India). A commercial pharmaceutical preparation (ECOGAT A) was used. Its labeled content was GA 400 mg and AM 75 mg.

Preparation of stock, working standard solutions and sample solution

A stock solution of GA and AM (100 µg/mL) was prepared, by taking 10 mg of each drug, accurately weighed, in separate 100-mL volumetric flasks. They were dissolved in25 mL of mobile phase and then the volume was made up to the mark to get 100 µg/mL.

For each drug, appropriate aliquots were pipetted out from the standard stock solution into a series of 10-mL volumetric flasks. The volumes were made up to the mark, with the mobile phase, to get a set of solutions for each drug. The concentration range over which the drug obeyed Beer's law was chosen. The range was found to be 20–100 µg/mL for GA and 5–15 µg/mL for AM.

Instruments and chromatographic conditions

Chromatographic separation was performed on a shimadzu HPLC pump equipped with a 20-µL loop and a LC20ATvp PDA detector. The wavelength of detection chosen was 250 nm. A reversed-phase thermo Hypersil Keystone C_{18} column (250 mm × 4.6 mm,5 µm) was used for the analysis. The mobile phase comprised of a mixture of disodium hydrogen orthophosphate buffer and acetonitrile (60:40, v/v), and pH adjusted to 3 with orthophosphoric acid, at a flow rate of 1.0 mL/min. The injection volume was 20 µL.

RESULTS AND DISCUSSION

Method validation

Every 20 µL of the working standard solution of GA in the mass concentration range of 20 to 100 µg/mL, and that for AM in the mass concentration range of 5 to 15 µg/mL, was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curves of GA and AM were obtained by plotting the peak areas versus the applied concentrations of GA and AM. The linear regression coefficients were found to be 0.9989 and 0.9966 for GA and AM, respectively (Sethi P D., 2001, ICH., 1994,1996).

The limits of detection (LODs) (signal to noise ratio = 4) and the limits of quantitation (LOQs) (signal to noise ratio =11) for GA and AM were determined. The results of LODswere found to be 0.3 µg/mL and 0.6 µg/mL for GA and AM, respectively. The LOQ results were found to be 0.5 µg/mL and 0.9 µg/mL for GA and AM, respectively.

The instrument precision was performed by injecting 20 µL of both GA and AM (10 µg/mL), in six replicates, into the chromatographic system, under optimized chromatographic conditions. Parameters evaluated were repeatability of peak response of drugs. The relative standard deviations (RSDs) of the peak area were found to be 0.56% and 1.24% for GA and AM, respectively.

Repeatability of the method was checked by injecting replicate injections of the combined solution (40 µg/mL and 7.5 µg/mL of GA and AM respectively). Variability of themethod was studied by analyzing the solution on the same day (intra-day precision) and on three different days (inter-day precision). The results obtained for intra-day preci-sion (RSDs) were 0.308% and 0.541%, respectively, at *n* = 3, for both GA and AM. The inter-day precisions (RSDs) were 0.325% and 0.721%, respectively, at *n* = 3, for both GA and AM.

Accuracy of the method was tested by carrying out recovery studies at three different spiked levels (80%, 100%, and 120%) on the basis of the label claim. The estimation was carried out as described earlier. At each level, three determinations were performed and results obtained. The results from validation and system suitability studies are listed in Table 1.

Table 1: Results from validation and system suitability studies

Method parameters	Gatifloxacin	Ambroxol HCl
Theoretical plate	5513.05	4958.21
Resolution	2.63	0
Linearity range (µg/ml)	20-100	5-15
Percentage Recovery(%)	99.86	100.09
Correlation co-efficient(R^2)	0.9989	0.9966
Accuracy (%RSD)	0.56	1.24
Intraday precision (%RSD)	0.308	0.541
Interday precision	0.325	0.74
LOD (µg/ml)	0.3	0.6
LOQ (µg/ml)	0.5	0.9

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of

20 µg/mL was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both GA and AM from any of the impurities, if present. As therewas no interference of impurities and also no change in the retention time, the method was found to be specific.

To determine the robustness of the method, experimental conditions such as the composition of the mobile phase, pHof the mobile phase, and flow rate of the mobile phase were altered and the chromatographic characteristics were evaluated. No significant change was observed.

Tablet analysis

Twenty tablets of GA and AM in combination were weighed, their average weight was determined, and finally they were crushed to a fine powder. The tablet powder equivalent to 40 mg of GA and 7.5 mg of AM was weighed and transferred to a 100 mL volumetric flask, first dissolved in 50 mL of mobile phase, and then the volume was made up to the mark with the mobile phase. The content was ultra sonicated for 30 min for complete dissolution. The solution was then filtered through a 0.2 µm Nylon 6,6 (N66) 47mm membrane. The selection of the mixed sample solution for analysis was carried out by the optimization of various dilutions of the tablet dosage form, considering the label claim. The mixed sample solution of 40 µg/mL of GA and 7.5 µg/mL of AM, which was falling in the Beer's-Lamberts range, showed good results and was selected for the entire analysis. The results of tablet analysis (n = 5) were found to be 99.86% with ±0.25% standard deviation (SD) and100.06% with ± 0.36% SD for GA and AM respectively.

From the typical chromatogram of GA and AM (Fig. 3), it was found that the retention time of GA was 2.8 min and AM was 3.2 min, which were well-resolved peaks with a resolution factor of 2.63.

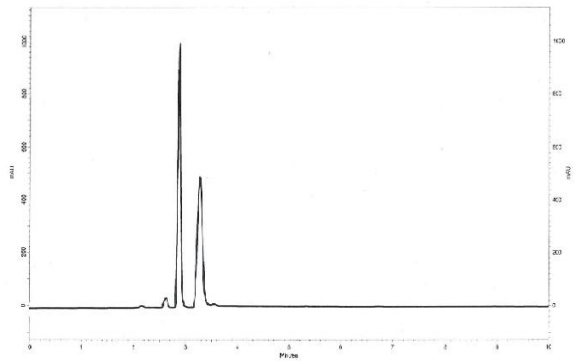

Figure 3: Chromatogram of tablet analysis

CONCLUSIONS

The developed method was validated in terms of accuracy, repeatability, and precision. A good linear relationship was observed for GA and AM in the concentration ranges of 20–100 µg/mL and 5–15 µg/mL respectively. The correlation coefficient for GA was found to be 0.9989 and that for AM was 0.9966. The inter-day and intra-day precision results were good enough to indicate that the proposed method was precise and reproducible. The assay experiment showed that the contents of gatifloxacin and ambroxol hydrochloride estimated in the tablet dosage form were free from the interference of excipients. This demonstrated that the developed HPLC method was simple, linear, precise, and accurate, and could be conveniently adopted for the routine quality control analysis of GA and AM, simultaneously, from its pharmaceutical formulation and bulk drug.

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