2008 年 5 月 May 2008
色 谱 Chinese Journal of Chromatography
Vol. 26 No. 3 358 ~ 361

Simultaneous determination of gatifloxacin and ambroxol hydrochloride from tablet dosage form using reversed-phase high performance liquid chromatography*

SHAHED Mirza, NANDA Rabindra, DEHGHAN Muhammad Hassan, NASREEN Huda, FEROZ Shaikh

(*Maulana Azad Educational Trust's Y. B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Rauza Bagh, Aurangabad-431001, Maharashtra State, India*)

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 HiQ Sil C_{18} column (250 mm × 4.6 mm, 5 μm), with a mobile phase comprising of a mixture of 0.01 mol/L potassium dihydrogen orthophosphate buffer and acetonitrile (70∶30, v/v), and pH adjusted to 3 with orthophosphoric acid, at a flow rate of 1 mL/min, with detection at 247 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 10 – 60 μg/mL and that for AM was found to be 5 – 30 μg/mL. The correlation coefficients were 0.999 6 and 0.999 3 for GA and AM respectively. The results of the tablet analysis ($n = 5$) were found to be 99.94% with ±0.25% standard deviation (SD) and 99.98% with ±0.36% SD for GA and AM respectively. Percent recovery of GA was found to be 99.92% – 100.02% and that of AM was 99.86% – 100.16%. 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.

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

CLC number: O658 **Document code**: A **Article IC**: 1000-8713(2008)03-0358-04 **Column**: Articles

Chemically gatifloxacin (GA) is 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid sesquihydrate. It is a synthetic broad spectrum 8-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. It acts intravenously by inhibiting topoisomerase II (DNA gyrase) or topoisomerase IV[1,2]. Its structure is given in Fig. 1.

Ambroxol hydrochloride (AM) is a mucolytic agent. Chemically AM is *trans*-4-[(2-amino-3,5-dibromobenzyl) amino] cyclohexanol hydrochloride. The empirical formula for AM is $C_{13}H_{18}Br_2N_2O \cdot HCl$[3]. Its structure is given in Fig. 2.

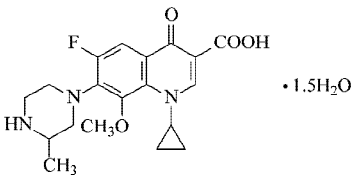

Fig. 1 Structure of gatifloxacin sesquihydrate

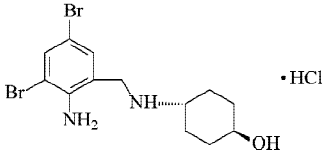

Fig. 2 Structure of ambroxol hydrochloride

Received date: 2008-02-02

Corresponding author: SHAHED Mirza. E-mail: mirzashahed2@yahoo.co.in.

* The electronic edition of the article is published by Elsevier BV on ScienceDirect (http://www.sciencedirect.com/science/journal/18722059).

A literature survey reveals that high performance liquid chromatography (HPLC) methods have already been developed to analyze both the drugs separately[4-6]. 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 ultraviolet-visible (UV-Vis) detector, has been developed for the simultaneous quantitative determination of GA and AM, from the tablet preparation.

1 Experimental

1.1 Chemicals and reagents

Bulk drugs GA and AM were procured from Aristo Pharmaceutical Ltd., Mumbai, India. Acetonitrile (HPLC grade, purity 99.80%), orthophosphoric acid (AR grade, purity 93.00%), and potassium dihydrogen orthophosphate (AR grade, purity 99.50%) were all procured from Qualigens Fine Chemicals (Mumbai, India). A commercial pharmaceutical preparation (Floxigat-M from the Medley Pharmaceuticals Ltd., Mumbai, India) was used. Its labeled content was GA 400 mg and AM 75 mg.

1.2 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 in 25 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 10 – 60 μg/mL for GA and 5 – 30 μg/mL for AM.

1.3 Instruments and chromatographic conditions

Chromatographic separation was performed on a Jasco High Performance Liquid Chromatograph with a PU-2080 HPLC pump equipped with a 20-μL loop and a Jasco UV-2075 variable wavelength detector. A double beam spectrometer, UV-1601, made by Shimadzu, with the scanning range of 190 nm to 1 100 nm, was used for scanning and selecting the detection wavelength. The wavelength of detection chosen was 247 nm. Chromatograms and data were recorded on Borwin Chromatography Version 1.5.

A reversed-phase HiQ Sil C_{18} column (250 mm × 4.6 mm, 5 μm) was used for the analysis. The mobile phase comprised of a mixture of 0.01 mol/L potassium dihydrogen orthophosphate buffer and acetonitrile (70∶30, 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.

2 Results and discussion

2.1 Method validation[7]

Every 20 μL of the working standard solution of GA in the mass concentration range of 10 to 60 μg/mL, and that for AM in the mass concentration range of 5 to 30 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 0.999 6 and 0.999 3 for GA and AM, respectively.

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 LODs were 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 de-

viations (RSDs) of the peak area were found to be 1.42% and 1.78% for GA and AM, respectively.

A repeatability test 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 the method 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 precision (RSDs) were 1.12% and 0.95%, respectively, at $n = 3$, for both GA and AM. The inter-day precisions (RSDs) were 0.72% and 0.45%, 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 amounts recovered and the values of percent recovery were calculated, which are listed in Table 1.

Table 1 Results of recovery studies

Amounts present/(mg/tab)		Amounts of standard added/mg		Total amounts recovered/mg		Recoveries/%	
GA	AM	GA	AM	GA	AM	GA	AM
400	75	320	60	719.64	134.81	99.95	99.86
400	75	320	60	719.78	135.21	99.97	100.16
400	75	320	60	720.14	134.97	100.02	99.98
400	75	400	75	799.12	149.91	99.89	99.94
400	75	400	75	799.60	149.79	99.95	99.86
400	75	400	75	799.36	149.89	99.92	99.93
400	75	480	90	879.82	164.78	99.98	99.87
400	75	480	90	879.29	165.00	99.92	100.00
400	75	480	90	878.68	164.90	99.85	99.94

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 there was 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, pH of the mobile phase, and flow rate of the mobile phase were altered and the chromatographic characteristics were evaluated. No significant change was observed.

2.2 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 ultrasonicated for 30 min for complete dissolution. The solution was then filtered through a 0.2 μm Nylon 6,6 (N66) 47 mm 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 99.94% with ± 0.25% standard deviation (SD) and 99.98% 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 4.0 min and AM was 5.4 min, which were well-resolved peaks with a resolution factor of 4.

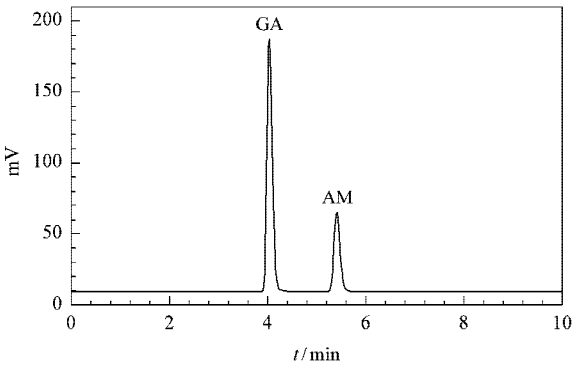

Fig. 3 Chromatogram of tablet analysis

3 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 10 – 60 μg/mL and 5 – 30 μg/mL respectively. The correlation coefficient for GA was found to be 0.999 6 and that for AM was 0.999 3. 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.

Acknowledgments

The authors are thankful to Aristo Pharmaceutical Ltd., Andheri (W), Mumbai, India, for supplying free samples of both gatifloxacin and ambroxol hydrochloride.

References:

[1] Zhao X L, Xu C, Domagala J, et al. Proc Natl Acad Sci USA, 1997, 94:13 991
[2] Fukuda H, Hiramatsu K. Antimicrob Agents Chemother, 1999, 43:410
[3] British Pharmacopoeia 2004
[4] Heinanen M, Barbas C. J Pharm Biomed Anal, 2001, 24(5/6):1 005
[5] Zarzuelo A, Sayalora M L, Lopez F G, et al. J Liq Chromatogr Relat Technol, 2001, 24(7):1 007
[6] Borner K, Hartuig H, Lode H. Chromatographia, 2000, 52(Suppl):S105
[7] Sethi P D. HPLC quantitative analysis of pharmaceutical formulations. New Delhi: CBS Publication and Distributors, 2001:3