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Development of RP-HPLC method for simultaneous estimation of gemifloxacin and ambroxol in tablet formulation

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ABSTRACT

A reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of gemifloxacin and ambroxol in tablet dosage forms. Separation of both gemifloxacin and ambroxol was achieved within 7 min with required resolution, accuracy and precision thus enabling the utility of the method for routine analysis. Chromatographic separation was achieved on a BDS Hypersil C_{18} column (250 × 4.6 mm, 3.5μ) using a mobile phase consisting of phosphate buffer pH 5.8 and acetonitrile in the ratio of 40:60 at a flow rate of 1.2 mL per min. The detection was made at 246 nm. The retention time of gemifloxacin and ambroxol were 2.553 and 3.546 min respectively. The method was found linear over the range of 50-90 μg/mL for gemifloxacin and 11.7-21 μg/mL for ambroxol. The proposed method was validated as per the ICH guidelines.
Keywords: Gemifloxacin, Ambroxol, HPLC, Validation.

INTRODUCTION

Gemifloxacin is a quinolone class of antibacterial agent. Chemically, it is known 7-[(4Z)-3-(amino methyl)-4-(methoxyimino)   pyrrolidin-1-yl]-1-cyclo propyl-6-fluoro-4-oxo-1,4-dihydro-1,8 naphthyridine -3-carboxylic acid. The bactericidal action of gemifloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, and recombination.[1-3]

Ambroxol is mucolytic agent. Chemically, it is known as *trans*-4-(2-Amino-3,5-dibrombenzyl amino)-cyclohexanol. Ambroxol is a metabolite of bromhexine and is very potent inhibitor of the neuronal Na+ channels, with 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. It stimulates synthesis and release of surfactant by type II pneumocytes. Surfactant acts as an anti-glue factor by reducing the adhesion of mucus to the bronchial wall, in improving its transport and in providing protection against infection and irritating agents[4-6]

The literature survey revealed that there are two HPLC methods[8,9] and the other methods like HPTLC[10] and UV[12-14] Spectroscopic methods available for the determination of gemifloxacin and ambroxol in their combined dosage forms. Various HPLC methods [15,16] were developed for estimation of drugs in their individual dosage forms. Hence there is a need to develop a method. The present study was aimed to develop a new simple, precise, accurate, robust method for simultaneous estimation of gemifloxacin and ambroxol in combined pharmaceutical dosage form.
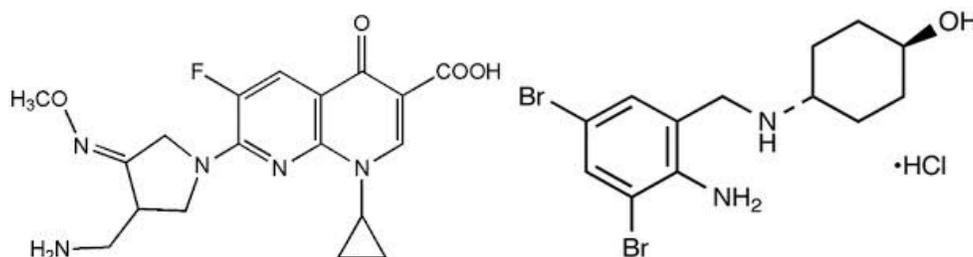
Fig. 1. Structures of gemifloxacin and ambroxol

EXPERIMENTAL

Instrumentation

The Waters HPLC system equipped with auto sampler and UV or DAD was used for method development, and method validation. The output signal was monitored and processed by using Empower software.

Materials

Gemifloxacin and ambroxol bulk drugs were made available from Pharmatrain, Hyderabad, orthophosphoric acid, methanol, acetonitrile were obtained from Merk. Commercially available gemifloxacin and ambroxol tablets were used for the dosage form analysis. All chemicals and reagent used were of HPLC grade, Milli-Q-water was used throughout the experiment. Commercially available gemifloxacin and ambroxol dosage form was used for the study.

Chromatographic conditions

The HPLC system was operated isocratically at flow rate of 1.2 mL/min. The mobile phase found to be most suitable for analysis was phosphate buffer (pH 5.8): acetonitrile in the ratio of 40: 60% v/v, detection was carried out at 246 nm. The separation was carried out on BDS Hypersil C_{18} (4.6 x 250 mm, 3.5 μm).

Preparation of standard stock solution

Accurately weigh and transfer 10 mg of Gemifloxacin & 10mg of Ambroxol working standard into two separate 100 mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 7.0 mL of Gemifloxacin & 1.64 mL Ambroxol of the

above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent.

Assay of Pharmaceutical Dosage form: (Sample Preparation)

Twenty tablets were weighed to get the average weight and then ground. An amount of powder equivalent to 10 mg was transferred to a 100 mL volumetric flask, added 70 mL of methanol and sonicated for 10 min with intermediate shaking, followed by making up to volume with methanol to obtain a solution containing 100 mg/mL of gemifloxacin and 100 mg/mL of ambroxol. Further pipetted a 7.0 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent.

RESULTS AND DISCUSSION

Method development

Chromatographic parameters were preliminary optimized to develop a LC method for simultaneous determination of gemifloxacin and ambroxol with short analyses time (< 7 min), and acceptable resolution (> 2). The isoabsortive point of gemifloxacin and ambroxol selected was 246 nm. In order to identify a suitable organic modifier, various compositions of acetonitrile and methanol were tested. Methanol produced a high retention time for Simvastatin and high column pressures due to the high viscosity. Acetonitrile was found to display advantageous separations. Change of percentage of acetonitrile in the mobile phase brought about a great influence on retention time of the two drugs. Effect of mobile phase pH on two drugs was investigated at a pH of 3, 4.5, and 5.8. Different columns like X-terra, Inertsil, Hypersil columns were tried.

Finally separation for simultaneous determination of gemifloxacin and ambroxol was carried out by isocratic elution using 40% phosphate buffer (pH 5.8) and 60% acetonitrile with a flow rate of 1.2 mL/min using BDS Hypersil C$_{18}$ (4.6 x 250 mm, 3.5 μm).The system suitability parameters are summarized in Table-1 and a typical chromatogram is shown in Fig. 2.
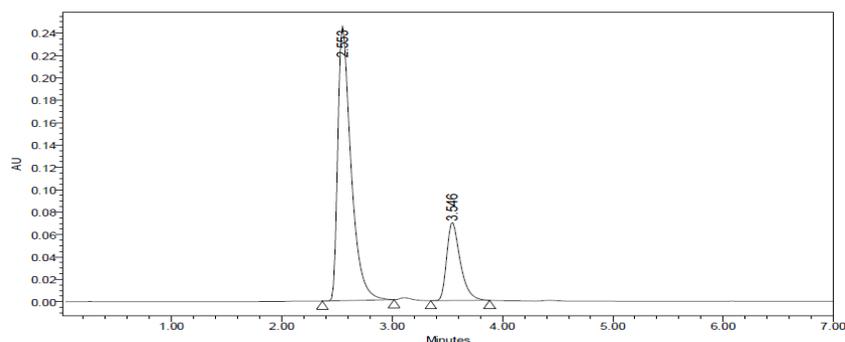

Fig.2. Standard chromatogram of gemifloxacin and ambroxol

Method validation

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[7].

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 (amount) of analyte in the sample. Linearity of detector response for gemifloxacin and ambroxol was established by analyzing serial dilutions of a stock solution of the working standard. Five concentrations ranging from 50-90 μg/mL for Gemifloxacin and 11.7-21 μg/mL for Ambroxol were prepared and analyzed. The linearity graph was plotted using concentration Vs peak area and they were shown in Figs. 3 and 4. Slope, correlation coefficient (R) and intercept were calculated and the results were shown in Table-1.

Precision and Intermediate precision

For the precision study, repeatability study was carried out for short time interval under the same chromatographic conditions. For the intermediate precision study, repeatability study was carried out in different day under the same chromatographic conditions. The sample was injected in five replicate. The peak area for all the five replicate was recorded.

The mean and % relative standard deviation (%RSD) was calculated. From the data obtained the developed RP-HPLC method was found to be precise.

Accuracy

The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of working standard. 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. The results are shown in Table-1.

Robustness

Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like mobile phase ratio, flow rate. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed RP-HPLC method is robust.

LOD and LOQ

The LOD and LOQ of gemifloxacin and ambroxol were determined by using the signal to noise approach as defined in ICH guidelines. The

concentration with signal to noise ratio of at least 3 was taken as LOD and concentration with signal to noise ratio of at least 10 was taken as LOQ. The results are shown in Table-1.

Assay of pharmaceutical formulation

The proposed validated method was successfully applied to determine gemifloxacin and ambroxol in their tablet dosage form. The assay of gemifloxacin and ambroxol was found to be 99.75 ±0.83 and 101.52 ±0.49 respectively. The result obtained for gemifloxacin and ambroxol was comparable with the corresponding labeled amounts and they were shown in Table-1.

CONCLUSIONS

The proposed RP-HPLC method allows for accurate, precise, and reliable measurement of gemifloxacin and ambroxol simultaneously in combined dosage form. The developed RP-HPLC method was found to be simple, rapid, accurate and precise for the concurrent estimation of drugs in respective two-component tablet dosage form of gemifloxacin and ambroxol. The RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of gemifloxacin and ambroxol in multicomponent pharmaceutical preparation.

Table 1: Results for gemifloxacin and ambroxol

System Suitability Parameters	Gemifloxacin	Ambroxol
Retention Time (min)	2.553	3.546
USP Plate Count	2265	4067
USP Tailing Factor	1.71	1.41
USP Resolution		4.55
Validation Parameters		
Assay (%)	101.22	101.92
Linearity Range (µg/mL)	50-90	11.7-21
Correlation coefficient	0.999	0.998
Precision (%RSD)	0.19	0.2
ID precision (%RSD)	0.14	0.2
Accuracy (% Recovery)	100.08	100.59
LOD (µg/mL)	0.033	0.028
LOQ (µg/mL)	0.112	0.098
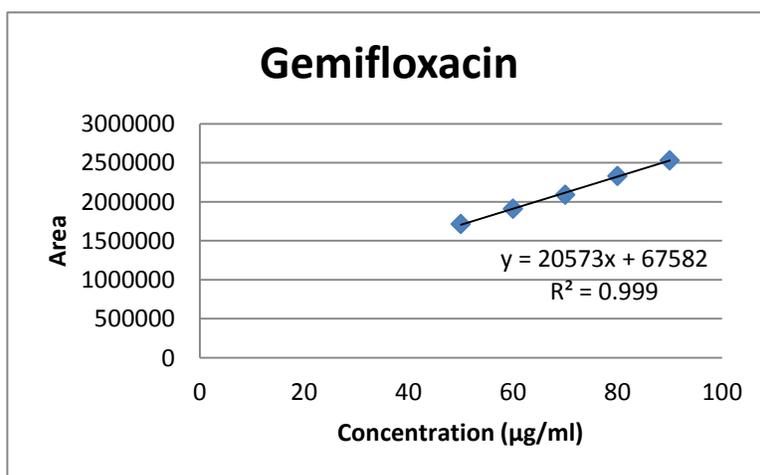
Fig. 3. Calibration curve for gemifloxacin
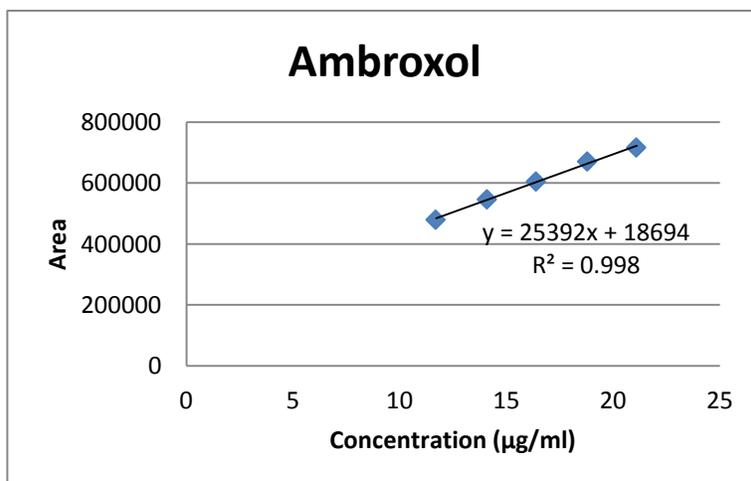
Fig. 4. Calibration curve for ambroxol

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