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DEVELOPMENT AND VALIDATION OF NOVEL HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF GEMIFLOXACIN MESYLATE AND AMBROXOL HYDROCHLORIDE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

A high performance liquid chromatographic (HPLC) method was developed and validated for the simultaneous determination of gemifloxacin mesylate (GEM) and ambroxol hydrochloride (AMB) in combined tablet dosage form by external standard method. The analysis was carried out using acetonitrile, methanol, 0.1% trifluoroacetic acid in the ratio of 20 : 20 : 60% v/v (pH was adjusted to 3.5 with orthophosphoric acid) as a mobile phase on Zorbax SB C_3 (150 mm × 4.6 mm i.d., 5 µm particle size) pre-packed column, at a flow rate of 1.0 mL/min with UV detection of 252 nm. Retention time was found to be 5.08 min and 3.84 min for GEM and AMB respectively. The method was validated for linearity, accuracy, precision and specificity. The method showed good linearity in the range of 4-426 µg/mL and 1-100 µg/mL for GEM and AMB. The detection limit and quantification limits for GEM and AMB was 0.08 & 0.19 µg/mL and 0.19 & 0.60 µg/mL respectively. The % recovery was within the range between 98.75% and 101.16% for GEM and % recovery was within the range between 99.09% and 101.23% for AMB. The % RSD for precision and accuracy of the method was found to be less than 2. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of GEM and AMB in combined dosage form.

Key words: Gemifloxacin mesylate, Ambroxol hydrochloride, HPLC, Validation.

INTRODUCTION

Gemifloxacin (GEM) chemically (R,S)-7[(4Z)-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid methanesulfonate, is a new fluoroquinolone antibacterial compound (Fig. 1). GEM which is

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widely used in chronic bronchitis, pneumonia and urinary tract infections[1-3]. Ambroxol hydrochloride (AMB), chemically 2-amino-3,5-dibromo-N-(trans-4-hydroxycyclohexyl) benzylamine cyclohexanol hydrochloride is a mucolytic expectorant and used to reduce the viscosity of mucous secretions[4] (Fig. 2). A fixed dose combination of GEM and AMB is available for the treatment of upper and lower respiratory tract infections.

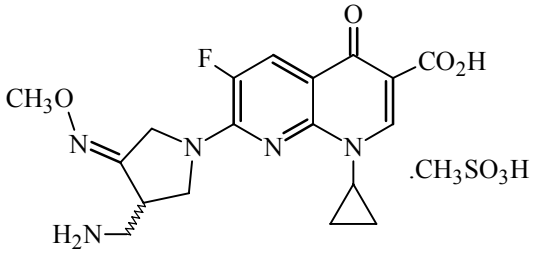

Fig. 1: Chemical structure of gemifloxacin mesylate

Literature survey reveals that few analytical methods are available for estimation of GEM and AMB by spectrophotometry[5-7], LC[8-13] and HPTLC[14] in single or in combined dosage forms. There is no method reported for the estimation of GEM and AMB in combined tablet dosage form. The present study describes the simultaneous estimation of GEM and AMB in tablet dosage form by using HPLC. The proposed method was validated as per International Conference on Harmonization (ICH) guidelines Q2 (R1).

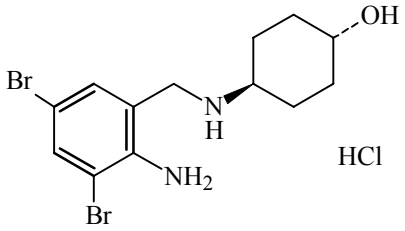

Fig. 2: Chemical structure of ambroxol hydrochloride

EXPERIMENTAL

Material and methods

Chemicals

All the chemicals used were of Analytical Reagent grade and the solvents were of HPLC grade. GEM and AMB, standards were obtained from Zydus Cadila, Ahmedabad,

India. HPLC grade water, methanol and orthophosphoric acid were purchased from S.D. Fine Chemicals, Mumbai, India.

Apparatus

Separation was performed with a waters HPLC equipped with a pump-515, autosampler-2707 and UV detector-2998 operated at 252 nm. Empower software was applied for data collecting and processing. A Systronics-361 pH meter was used for pH measurements.

Chromatographic conditions

Zorbax SB C_3 column (150 mm × 4.6 mm i.d., 5 µm particle size) was used in this study. The mobile phase was acetonitrile : methanol: 0.1% trifluoroacetic acid in the ratio of 20 : 20 : 60% v/v (pH 3.5 was adjusted with orthophosphoric acid). The flow rate was 1.0 mL/min and UV detection was performed at 252 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. The resulting transparent mobile phase was filtered through a 0.45 µm membrane filter (Millipore, Ireland).

Preparation of standard solution

An accurately weighed quantity of GEM (100 mg) and AMB (25 mg) was transferred in to a 100 mL volumetric flask, dissolved in a sufficient quantity of HPLC grade methanol. The volume was made up to the mark with methanol. Serial dilutions for calibration curve were prepared from the above stock solution.

Study of experimental parameters

Different experimental parameters including, mobile phase composition, detection wavelength and flow rate were intensively studied in order to specify the optimum conditions for the assay procedure. Variables were optimized by changing each, in turn, while keeping all others constant.

Analysis of sample

For analysis, twenty tablets were weighed, powdered and weighed accurately equivalent to 320 mg of GEM and 75 mg of AMB were transferred to a 100 mL volumetric flask and dissolved in 50 mL of methanol by ultra-sonication for 20 min. Then solution was filtered through a 0.45 µm membrane filter and then final volume of the solution was made upto 100 mL with methanol to get the stock solution containing 3200 µg/mL of GEM and 750 µg/mL of AMB. Appropriate aliquots of GEM and AMB were taken within linearity

range. The concentration of both drugs was determined using either the calibration curve or the corresponding regression equation.

System suitability

According to USP 2007, system suitability tests are an integral part of liquid chromatographic methods in the course of optimizing the conditions of the proposed method. System suitability tests were used to verify that the resolution and reproducibility were adequate for the analysis performed. The parameters of these tests are column efficiency (number of theoretical plates), tailing of chromatographic peak, peak resolution factor and repeatability as % R.S.D. of peak area for six injections and reproducibility of retention as % …..S.D of retention time. The results of the test for proposed method was listed in Table 1

Table 1: System suitability parameters for GEM and AMB

Parameter	GEM	AMB
λ_{max} (nm)	252	252
Linearity range (µg/mL)	4-426	1-100
Correlation coefficient (R^2)	0.9969	0.9997
Retention time (RT) (min)	5.08	3.84
Theoretical plates	8745	14578
Capacity factor	0.77	1.82
Tailing factor	1.32	1.14
Resolution	5.44	5.062
Slope	24171	40765
Intercept	-449914	-30838
LOD (µg/mL)	0.08	0.19
LOQ (µg/mL)	0.19	0.60

Validation

The method was validated for assay of GEM and AMB in accordance with ICH guidelines.

Linearity

In order to check the linearity for the developed method, solutions of six different concentrations ranging from 4-426 µg/mL and 1-100 µg/mL were prepared for GEM and AMB respectively. The chromatograms were recorded and the peak areas were given in Table 2. A linear relationship between areas versus concentrations was observed in above linearity range. This range was selected as linear range for analytical method development for estimation of GEM and AMB.

Table 2: Linearity curve for GEM and AMB

Concentration of GEM (µg/mL)	Concentration of AMB (µg/mL)	Mean peak area of GEM*	Mean peak area of AMB*
4	1	50149	14973
21	5	497088	93385
42	10	1209106	205105
106	25	3323229	550880
213	50	7189621	1172639
426	100	16176033	12394578

*Average of six determinations

Accuracy

The accuracy of the method was determined by analysis of standard addition at three levels i.e. multiple-level recovery studies. Reference standard at three different concentrations (50, 100 and 150%) was added to a fixed amount of pre-analyzed sample and the amounts of the drug were analyzed by the proposed method. Results from the recovery studies are given in Table 3.

Precision

Precision was estimated by repeatability. The repeatability was assessed by analyzing sample solutions six times at three different concentrations. Solutions containing 4, 40 and 200 µg/mL of GEM and 1, 10 and 50 µg/mL of AMB were subjected to the proposed

HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table 4.

Table 3: Recovery studies data of GEM and AMB

Spike level [%]	Mean recovery [%] (n = 6)		RSD [%]	
	GEM	AMB	GEM	AMB
50	98.75	99.09	0.3	0.82
100	101.16	101.23	0.43	0.37
150	100.85	100.58	0.6	0.17

Table 4: Precision data of GEM and AMB

Drug	Concentration (µg/mL)	Intra-day		Inter-day	
		Mean	% RSD	Mean	% RSD
GEM	4	4.024	0.03	3.982	0.70
	40	39.812	0.02	39.76	0.32
	200	198.96	0.50	199.16	0.80
AMB	1	1.082	1.81	1.02	0.03
	10	9.892	0.02	10.02	0.03
	50	50.132	0.19	50.024	0.70

Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including degradation products and related substances. In the present work, the chromatograms of the samples were checked for the appearance of any extra peaks. No chromatographic interference from any of the excipients was found at the retention times of the examined drugs. In addition, the chromatogram of each drug in the sample solution was found identical to the chromatogram received by the standard solution at the wavelengths applied. These results demonstrate the absence of interference from other materials in the pharmaceutical formulations and therefore confirm the specificity of the proposed method.

Robustness

Robustness was performed by deliberately changing the chromatographic conditions. The most important parameter to be studied was the resolution factor between two peaks of GEM and AMB. The flow rate of the mobile phase was changed from 1 mL/min to 0.8 mL/min and 1.2 mL/min, where resolution factors obtained were (6.53, 7.22), (6.38, 7.01) and (6.40, 7.61), respectively. The ratio of methanol was changed from 20% to 22% and 18%, where resolution factors obtained were (6.53, 7.22), (6.43, 7.35) and (6.74, 7.52), respectively. Besides the ratio of acetonitrile was changed from 20% to 22% and 18%, where resolution factors obtained were (6.53, 7.22), (5.92, 6.89) and (6.12, 6.73), respectively. Finally the value of pH of the orthophosphoric acid was varied from 3.5 to 3.4 and 3.6, where resolution factors obtained were (6.53, 7.22), (6.21, 6.78) and (6.11, 6.69) respectively. As can be seen from these results, good values of the resolution factor were obtained for all these variations, indicating good robustness of the proposed HPLC method.

Limit of detection and limit of quantification

Limit of detection (LOD) which represents the concentration of analyte at S/N ratio of 3 and limit of quantification (LOQ) at which S/N is 10 were determined experimentally for the proposed method. The detection limit and quantification limts for GEM and AMB was 0.08, 0.19 µg/mL and 0.19, 0.60 µg/mL.

Stability

The stability of GEM and AMB in standard and sample solutions containing determined by storing the solutions at ambient temperature (20 ± 10^{o}C). The solutions were checked in triplicate after three successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48 hrs, as during this time the results did not decrease below 98%. This denotes that GEM and AMB are stable in standard and sample solutions for at least 2 days at ambient temperature.

RESULTS AND DISCUSSION

The RP-HPLC procedure was optimized with a view to develop accurate and stable assay method with the pure drugs GEM and AMB in a combined dosage form. Zorbabx SB C_3 column in isocratic mode, with mobile phase acetonitrile, methanol, 0.1% trifluoroacetic acid (20 : 20 : 60 v/v/v) (pH was adjusted to 3.5 with orthophosphoric acid). The flow rate was 1 mL/min and identical components were measured with UV detector at 252 nm. Linearity was assessed by plotting concentration vs area which is shown in Fig. 3 and Fig. 4

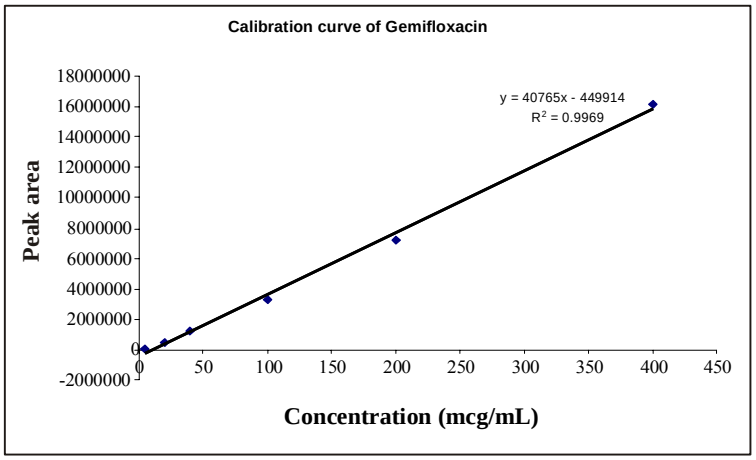

Fig. 3: Calibration curve for GEM

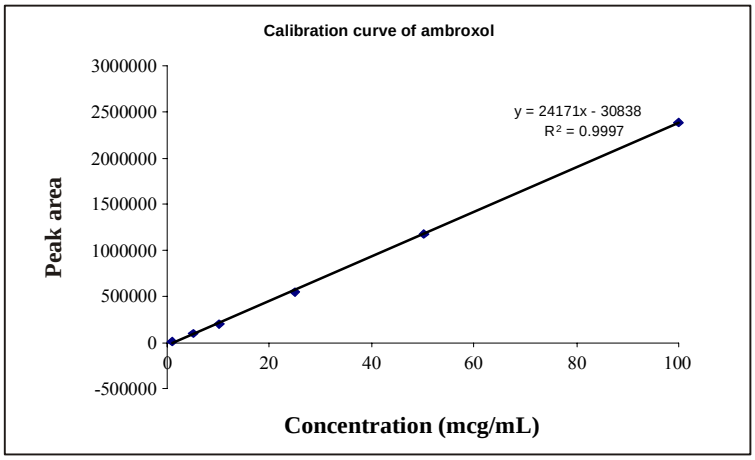

Fig. 4: Calibration curve for AMB

respectively within the range of 4-426 µg/mL for GEM and 1-100 µg/mL for AMB with correlation coefficient 0.9969 and 0.9997 respectively with good linearity response greater than 0.995. The % recovery was found to be within limits of the acceptance criteria with recovery range 98.75% and 101.16% for GEM and 99.09% and 101.23% for AMB. The % RSD for intra-day and inter-day precision is less than 2% for GEM and AMB. The detection limit of the proposed method was 0.08 and 0.19 µg/mL and the quantification limit was 0.19 and 0.60 µg/mL for GEM and AMB respectively. Typical chromatogram of the standard and sample is shown in Fig. 5, 6. The assay procedures were repeated for six times and the results were found to give 99.56% of GEM and 102.06% of AMB results are furnished in Table 5.

Table: 5 Assay and recovery studies of GEM and AMB in formulation

Formulation	Drug name	Label claim (mg)	Amount found (mg)	% Recovery
	GEM	320.0	319.4	99.81
Brand 1	AMB	75.0	76.7	102.26

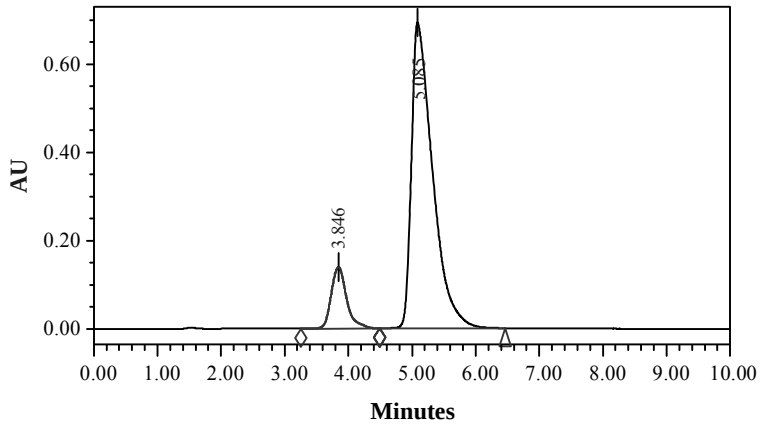

Fig. 5: Typical chromatogram of Gemifloxacin and Ambroxol standard

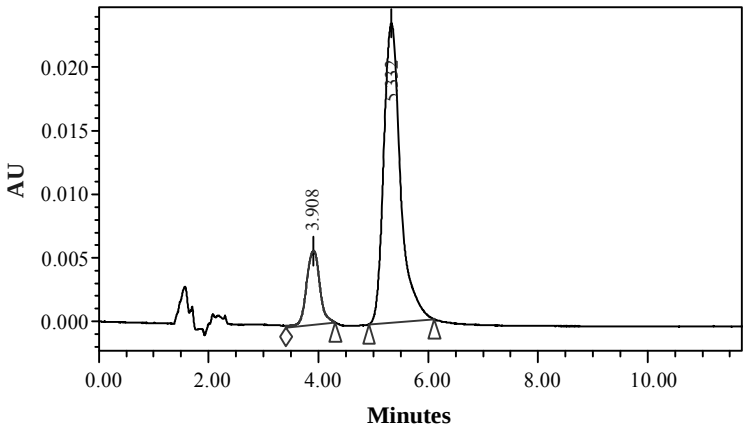

Fig. 6: Typical chromatogram of gemifloxacin and ambroxol in tablet

CONCLUSION

The proposed study describes new and simple RP-HPLC method for the estimation of GEM and AMB in combined dosage form. The method was validated and found to be simple, sensitive, accurate and precise. Therefore the proposed method can be used for quantification of GEM and AMB in combined dosage form as well as for routine analysis in quality control.

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