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Research Article

RP-HPLC METHOD OF SIMULTANEOUS ESTIMATION OF GEMIFLOXACIN MESYLATE AND AMBROXOL HCL IN COMBINED DOSAGE FORM.

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ABSTRACT

A reversed-phase liquid chromatographic (RP-HPLC) method was developed for the simultaneous determination of Gemifloxacin mesylate(GEM) and Ambroxol HCl(AMB) incombined dosage form. The analysis was carried out using phenomenex C18, pre-packed column. Mobile phase, containing phosphate Buffer:Acetonitrile:methanol(50:25:25) with trietylamine 0.2% adjusted to pH 6.0 with orthophosphoric acid, was pumped at a flow rate of 1.0 mL/min with UV-detection at 246 nm. Retention time was 4.64 min and 7.41 min for Gemifloxacin mesylate(GEM) and Ambroxol HCl(AMB) respectively. The method was validated for linearity, accuracy, precision, and specificity. The method showed good linearity in the range of 32-192 µg/mL for GEM and 7.5-45 µg/mL for AMB. The detection limit of the proposed method was 0.042 and 0.053 µg/mL and the quantification limit was 0.130 and 0.162 µg/mL for Gemifloxacin mesylate(GEM) and Ambroxol HCl (AMB), respectively. The % recovery was within the range between 98.75% and 101.58% for Gemifloxacin mesylate(GEM) and % recovery was within the range between 99.09% and 100.58% for Ambroxol HCl (AMB) . The percentage 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 Gemifloxacin mesylate(GEM) and Ambroxol HCl(AMB) in combined dosae form.

Keywords: Gemifloxacin mesylate, Ambroxol HCL, RP-HPLC.

INTRODUCTION

Gemifloxacin,(R,S)-7-(3-aminomethyl-4-syn-methoxyimino-1-pyrrolidinyl)-1cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylicid methanesulfonate, is a new fluoroquinolone antibacterial compound. Gemifloxacin which is widely used in chronic bronchitis, pneumonia, and urinary tract infections.[1-3] Ambroxol hydrochloride, chemically 4([(2-amino, 3, 5 dibromophenyl)-methyl] amino)-cyclohexyl or N- [(trans-p-hydroxy cyclo hexyl)-(2-amino 3, 5-dibromo benzyl)-amino] cyclohexanol hydrochloride is a mucolytic expectorant and used to reduce the viscosity of mucous secretions.[4] A fixed dose combination of Gemifloxacin Mesylate (GEM) and Ambroxol Hydrochloride (AMB) is available for the treatment of upper and lower respiratory tract infections. Tablet is available commercially as G-CIN A.

On detailed literature survey, it was found that these drugs have been estimated individually and in combinations by various methods.[5-17]To the best of our knowledge, no study has been described for the simultaneous determination of both drugs in combined dosage form by UV spectrophotometric method. The present paper described two simple, rapid, economic methods for simultaneous estimation of Gemi and Ambro by Reverse phase high performance liquid chromarography method. The proposed methods were validated as per International

Conference on Harmonization (ICH) guidelines Q2 (R1). [18-19]

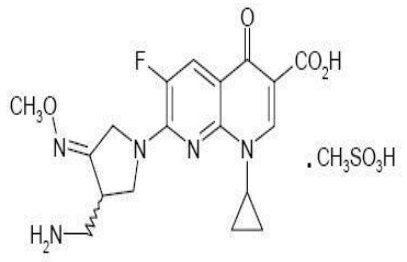

Figure 1(a): Chemical structure of GM

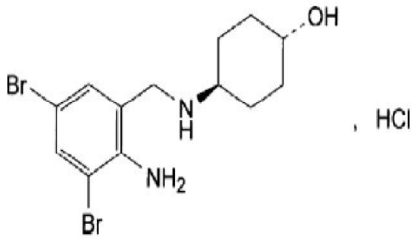

Figure 1(b): Chemical structure of AMB

MATERIALS AND METHODS

Chemicals

All the chemicals used were of Analytical Reagent grade, and the solvents were of HPLC grade. Gemifloxacin mesylate(GEM) and Ambroxol HCl (AMB), standards were obtained from zydus cadila, Ahmedabad, India. HPLC grade water, methanol, orthophosphoric acid, and triethylamine (TEA) were purchased from S.D. Fine Chemicals, Mumbai, India.

Apparatus

Separation was performed with a Shimadzu LC 2010 CHT equipped with a Rheodyne injector valve with a 20.0 µL loop and a UV/ VIS detector operated at 246 nm. LC solution software was applied for data collecting and processing. A Chemiline pH-meter was used for pH measurements.

Chromatographic Conditions

Phenomenex C18 column (250mm x 4.6mm 5µ) was used in this study. The mobile phase was phosphate Buffer: Acetonitrile: methanol (50:25:25) with trietylamine 0.2% adjusted to pH 6.0 with orthophosphoric acid The Flow rate was 1.0 mL/min and UV detection was performed at 246 nm by UV detector and PDA detection at 246 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. The resulting transparent mobile phase was filtered through a 0.45-mm membrane filter (Millipore, Ireland).

Preparation of Solutions

Stock solutions containing 1.0 mg/mL of GEM and AMB were prepared in methanol and were used as working solutions (1000 µg/mL). Solutions were protected from light and were found to be stable for at least one week when kept in the refrigerator.

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.

Construction of the Calibration Curve

Aliquots of the standard solutions covering the final working concentration range of 32-192 µg/mL for GEM and 7.5-45 µg/mL for AMB were transferred into a series of 10mL volumetric flasks and diluted with the de-gassed mobile phase to the mark. 20 µl aliquots were injected (n=6) and eluted with the mobile phase under the reported chromatographic conditions. The calibration curves were constructed by plotting the peak area against the final concentration of the drug (µg/mL). Alternatively, the corresponding regression equations were derived.

Analysis of Sample

For analysis, twenty tablets were weighed, powdered and weighed accurately equivalent to 320 mg of GEM and 75 mg 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-mm membrane filter and then final volume of the solution was made upto 100ml with methanol to get the stock solution containing 3200µg/mL of GEM and 750µg/mL AMB. Appropriate aliquots of GEM and ANB were taken within linearity range. The concentration of both drugs was determined using either the calibration curve or the corresponding regression equation.

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 7.5-45µg/mL and 32- 192µg/mL were prepared for AMB and GEM respectively. The chromatograms were recorded and the peak areas were given in Table 1. 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.

Sensitivity

The sensitivity of measurement of GEM and AMB using the proposed method was estimated as the limit of quantification (LOQ) and the lowest concentration detected under these chromatographic conditions as the limit of detection (LOD). The LOD and LOQ were calculated by using the equations LOD = 3.3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the peak areas of the drug (n = 6), and B is the slops of the corresponding calibration plot. The limits of detection and quantification for GEM were 0.042µg/mL and 0.130µg/mL respectively and those for AMB were 0.053µg/mL and 0.162µg/mL respectively.

System suitability

Various system suitability parameters were also calculated. It was observed that all the values are within the limit which is shown in Table 2. The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of GEM and AMB in tablet formulation. The results are furnished in Table 3.

Precision

Precision was measured by analysis of sample solutions six times at three different concentrations. Solutions containing 32, 64 and 96 µg/mL of GEM and 7.5, 15 and 22.5µg/mL 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 and 5.

Accuracy

The accuracy of the method was determined by analysis of standard additions 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 6.

Stability

The stability of GEM and AMB in standard and sample solutions containing determined by storing the solutions at ambient temperature (20±10^{0}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 48hrs, 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 2days at ambient temperature.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

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.phenomenex C18 (250mm x 4.6mm 5µ) column in isocratic mode, with mobile phase phosphate Buffer:Acetonitrile:methanol(50:25:25) with trietylamine 0.2% adjusted to pH 6.0 with orthophosphoric acid. The flow rate was 1mL/min and identical components were measured with PDA Detector at 246 nm. Linearity was assessed by plotting concentration vs. area which is shown in Fig 2 and Fig 3 respectively with is linear in the range of7.5 – 45.0 µg/mL for AMB and 32-192 µg/mL for GEM with correlation coefficient 0.9992 and 0.9998 respectively with good linearity response greater than 0.998. The % recovery was found to be within limits of the acceptance criteria with recovery range 98.75% and 101.58% for GEM and 99.09% and 100.58%

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 0.042 and 0.053 µg/mL, and the quantification limit was 0.130 and 0.162 µg/mL for Gemifloxacin mesylate(GEM) and Ambroxol HCl (AMB), respectively.Typical chromatogram of the sample is shown in Fig 5.The assay procedures were repeated for six times and the results were found to give 99.56% of GEM and 102.06% of AMB.

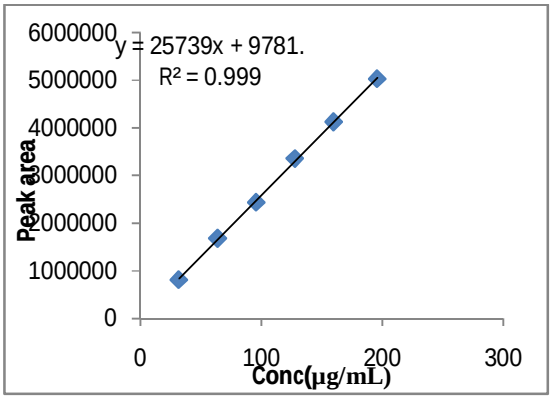

Fig. 2: Calibration Curve of Gemifloxacin mesylate

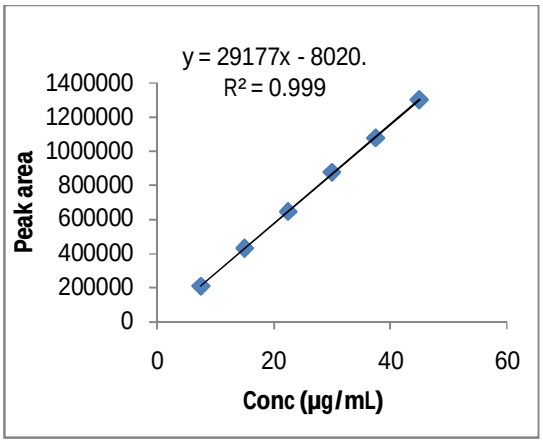

Fig. 3: Calibration Curve of Ambroxol HCl

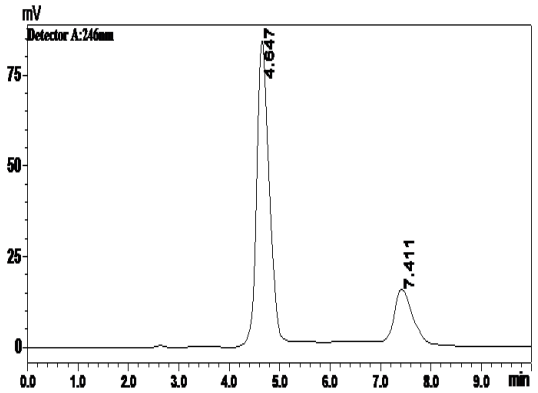

Fig. 4: Chromatogram obtained from Gemifloxacin and Ambroxol standard

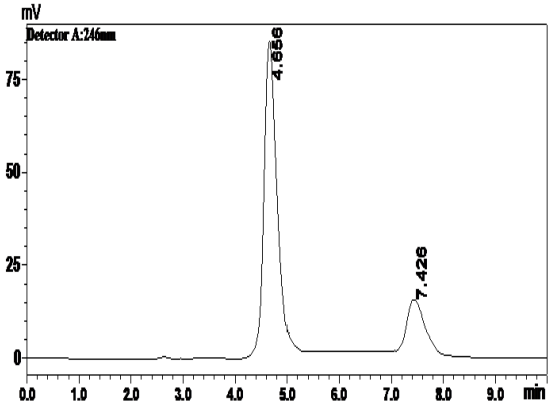

Fig. 5: Chromatogram obtained from Gemifloxacin and Ambroxol in tablet

CONCLUSION

The Proposed study describes new and simple RP-HPLC method for the estimation of Gemifloxacin mesylate and Ambroxol HCl 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 Gemifloxacin mesylate and Ambroxol HCl in combined dosage form as well as for routine analysis in quality control.

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Table 1: Linearity data for GEM and AMB

S. No.	Concn of GEM (µg/ml)	Concn of AMB (µg/ml)	Mean * Peak Area of GEM	Mean* Peak Area of AMB
1	32	7.5	815701	207945
2	64	15	1682499	430888
3	96	22.5	2439999	646537
4	128	30	3356846	877909
5	160	37.5	4129846	1079858
6	192	45	5033529	1304064

*Average of six determinations

Table 2: System Suitability parameters for EM and LH

Parameter	GEM	AMB
λ_{max}	246 nm	246 nm
Linearity range(µg / mL)	32-196	7.5-45
Correlation coefficient (R^2)	0.9992	0.9998
Retention time (RT)	4.56 min	7.41 min
Theoritical plates	1975	2141
Capacity factor	0.77	1.82
Tailing factor	1.22	1.14
Resolution	5.44	5.062
Slope	25739	29177
Intercept	9781	- 8020
LOD(µg / mL)	0.042	0.053
LOQ(µg / mL)	0.130	0.162

Table 3: Results of analysis of sample

Sample	(Mean ±% R.S.D.)	
	GEM	AMB
%Conc. Estimated*	99.56±0.76	102.06±0.65

*Average of six determinations; R.S.D.; Relative Standard Deviation.

Table 4: Intraday precision data for estimation of GEM and AMB

Conc. (µg/mL)	GEM Peak Area		Conc. (µg/mL)	AMB Peak Area	
	Mean ± S.D. (n=6)	%RSD		Mean ± S.D. (n=6)	%RSD
32	815285.3±6855.48	0.84	7.5	202093.3±3133.69	1.88
64	1663438±21712.71	1.3	15	425756±4724.572	1.109
96	2427691±28246.66	1.16	22.5	648478±8846.48	1.36

Table 5: Interday precision data for estimation of GEM and AMB

Conc. (µg/mL)	GEM Peak Area		Conc. (µg/mL)	AMB Peak Area	
	Mean ± S.D. (n=6)	%RSD		Mean ± S.D. (n=6)	%RSD
32	815414.7±8815.5	1.08	7.5	213010±3133.69	1.47
64	1653429±29548.31	1.75	15	436690±7134.11	1.63
96	2441307±35150.11	1.41	22.5	651137±10817.76	1.66

Table 6: Result from recovery studies

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

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