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RP-HPLC Method for Simultaneous Estimation of Azithromycin and Ambroxol Hydrochloride in Tablets

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An isocratic RP-HPLC method has been developed and validated for simultaneous analysis of azithromycin with ambroxol hydrochloride in combined solid dosage forms. It is simple, accurate, economical and reproducible for simultaneous estimation of two component drug mixture. Chromatography was performed on a 250 mm × 4.6 mm, 5 µm particle size, C_{18} (ODS) column with a methanol:acetonitrile:phosphate buffer in ratio of 50:20:30 pH 5.0 as mobile phase at the flow rate of 1 mL/min. The detection wavelength was 260 nm and analysis was performed at room temperature. Linearity was observed in concentration range of 25-125 µg/mL of azithromycin and 5-25 µg/mL of ambroxol hydrochloride. Different analytical parameters such as linearity, precision, accuracy and robustness were determined according to International Conference on Harmonization ICH Q2B guidelines. Results of analysis were validated statistically and by recovery studies.

Key Words: Simultaneous, HPLC, Azithromycin, Ambroxol.

INTRODUCTION

The azithromycin (AZM) is the first member of a class of macrolide antibiotics[1]. Chemically it is 4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2*H*-pyran 2-yloxy)-2-ethyl-3,4,10-trihydroxy-13-((2S,4R,5S)-5-hydroxy-4-methoxy-4-methyltetra hydro-2*H*-pyran-2-yloxy)-3,5,6,8,10,12,14-heptamethyl-1-oxa-6-azacyclopentadecan-15-one. It is used primarily to treat various bacterial infections caused by respiratory pathogens, such as aerobic gram-positive microorganisms and aerobic gram-negative microorganisms. Azithromycin is rapidly absorbed and is widely distributed to tissues and becomes concentrated in cells. Peak plasma concentrations are achieved[2] within 2 to 3 h. Since azithromycin is obtained from erythromycin, impurities present will undergo the same modifications and the azithromycin analogues of these impurities can be found in azithromycin bulk samples. In addition, degradation products of azithromycin as well as intermediate compounds of the semi-synthesis may be present[3]. It is very difficult to determine small amounts of degradation products in a vast excess of parent drug and even more so when the compounds do not present a chromophore as this makes their detection more difficult[4]. Azithromycin has been analyzed by HPLC using electrochemical[5,6], fluorescence[7], mass spectrometry[8] and UV[9,10] for detection in bulk material and pharmaceutical forms. The USP method[11] describes the use of a high pH mobile phase (pH 1.0) which requires the use of specific column which is expensive and difficult to obtain commercially. USP method also employs amperometric electrochemical detection which is not available in many laboratories.

Ambroxol hydrochloride (ambroxol hydrochloride) is an expectorant and mucolytic agent. Chemically it is *trans*-4-(2-amino-3,5-dibromobenzyl)aminocyclohexanol hydrochloride[12]. Ambroxol chemically *trans*-4-(2-amino-3,5-dibromobenzyl amino)cyclohexanol is one of the most popular medicines used to relieve the symptoms of coughs, asthma and colds. Methods available for the determination of ambroxol hydrochloride include capillary electrophoresis[13-15], spectrophotometry[16], gas chromatography[17,18], L.C. with potentiometric detection[19], MS detection[20] and UV detection[21-24].

However no references have been found for quantitative determination of azithromycin and ambroxol hydrochloride in pharmaceutical preparations. A comprehensive, validated and simple analysis method for azithromycin and ambroxol hydrochloride is, therefore, crucial. The major advantage of the proposed method is that azithromycin and ambroxol hydrochloride can be determined on a single chromatographic system.

EXPERIMENTAL

All chemicals and reagents used were of HPLC grade. Sodium dihydrogen phosphate, disodium hydrogen phosphate,

methanol-procured from Merck, India. High pure water was prepared by using Millipore Milli Q plus purification system.

Chromatographic conditions: A high performance liquid chromatograph system, with class VP data handling system (Shimadzu-LC 2010) was used for the analysis. The purity determination performed on a stainless steel column 250 mm long, 4.6 mm internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5 mm diameter (inertsil C_{18}, 5m, 250 mm × 4.6 mm, make: Shimadzu Ltd., Japan) with the mobile phase containing methanol:acetonitrile: phosphate buffer in the ratio of 50:20:30 (v/v/v pH 5.0) at ambient temperature. Flow rate was kept at 1 mL/min and the elution was monitored at 260 nm.

Standard solution and calibration curve: Standard stock solution (1 mg/mL) of azithromycin and ambroxol hydrochloride were prepared by dissolving 25 mg of drug in 25 mL of mobile phase, separately. The solutions were suitably diluted with mobile phase to get mixed standard solution containing 50 µg/mL of azithromycin and 25 µg/mL of ambroxol hydrochloride.

Assay: Twenty tablets were weighed accurately and finely powdered. The powder equivalent to 100 mg of azithromycin and 100 mg of ambroxol hydrochloride was weighed accurately and dissolved in 100 mL mobile phase. The solution was sonicated 0.5 h and filtered through a 0.45 µm membrane filter. From this solution, further dilutions were made using mobile phase to get final concentration of 50 µg/mL of azithromycin and 25 µg/mL of ambroxol hydrochloride.

With the optimized chromatographic conditions, a steady baseline was recorded. The retention time of azithromycin and ambroxol hydrochloride was found to be 2.60 and 6.86 min, respectively. A typical chromatogram of sample solution is given in Fig. 1. Detection was done at 260 nm. The assay procedure was repeated for six times and mean peak area was calculated. The concentration of the drugs was calculated and presented in Table-1.

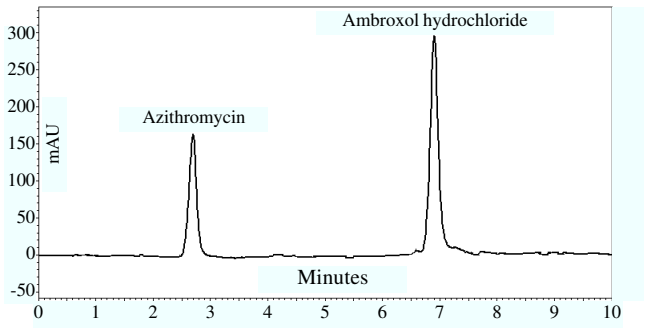

Fig. 1. Typical chromatogram of sample solution

The method was validated as per ICH[25] guidelines. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery were calculated and presented in Table-1. From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated. From the data obtained, the developed HPLC method was found to be precise.

TABLE-1
VALIDATION AND SYSTEM SUITABILITY STUDIES

Parameters	AZM	AMB
Linearity range (µg/mL)	25-125	5-25
Regression equation Y = mx +c	Y = 29731x + 13022	Y = 240831x + 14328
Correlation coefficient	0.9992	0.9978
Intra-day (% RSD)	0.718	0.915
Inter-day (% RSD)	0.318	0.261
Repeatability (% RSD)	0.435	0.293
% Label claim*	100.34	100.58
% Recovery*	100.58	100.76
Theoretical plate/meter	26458	28764
Resolution factor	–	1.60
Asymmetric factor	0.90	1.01
Tailing factor	1.2	1.0

*Y = mx+c, m: slope, c: y axis intercept

The linearity of the method was determined at five concentration levels ranging from 25 to 125 µg/mL for azithromycin and 5 to 25 µg/mL for ambroxol hydrochloride. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 29731x + 13022 (r^2 = 0.9992) for azithromycin and y = 240831x + 14328 (r^2 = 0.9978) for ambroxol hydrochloride. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC-2010), Agilent HPLC by different operators using different columns of similar type intersil C_{18}, hypersil C_{18}. Robustness of the method was determined by making slight changes in the chromatographic conditions. No marked changes in the chromatograms demonstrated that the HPLC method developed is rugged and robust.

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. The results show that for both solutions, the retention time and peak area of azithromycin and ambroxol hydrochloride remained almost unchanged (%RSD < 2) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 h, which was sufficient to complete the whole analytical process.

The system suitability studies were carried out to determine theoretical plate/meter, resolution factor, asymmetric factor and tailing factor. The results were given in the Table-1. The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method.

RESULTS AND DISCUSSION

The developed RP-HPLC method for the simultaneous estimation of azithromycin and ambroxol hydrochloride in combined dosage form utilizing methanol:acetonitrile: phosphate buffer in ratio of 50:20:30 pH 5.0 as mobile phase at a flow rate of 1 mL/min. The detection of eluent was carried out at 260 nm and analysis was performed at room temperature. The run time per sample is just 10 min. The excipients in the formulation did not interfere in the accurate estimation. The method was validated as per ICH guidelines. Thus the proposed RP-HPLC method for the simultaneous estimation of azithromycin and ambroxol hydrochloride in combined dosage forms is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies.

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