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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF AZITHROMYCIN AND AMBROXOL HYDROCHLORIDE IN TABLETS

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ABSTRACT: A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Azithromycin and Ambroxol Hydrochloride in combined dosage form. The separation was carried out using a mobile phase consisting of acetonitrile and mono basic potassium phosphate buffer of pH 8.5 in the ratio of 65:35 v/v. The column used was C_{18} phenomenex Gemini 5µ, 250cm x 4.6mm id with flow rate of 2ml/min using PDA detection at 220nm. The described method was linear over a concentration range of 96-145µg/ml and 80-125µg/ml for the assay of Azithromycin and Ambroxol Hydrochloride respectively. The retention times of Ambroxol and Azithromycin were found to be 3.7min and 6.1min respectively. Results of analysis were validated statistically and by recovery studies. The limit of quantification (LOQ) for Azithromycin and Ambroxol Hydrochloride were found to be 96.7µg/ml and 8.35µg/ml respectively. Then the limit of detection (LOD) for Azithromycin and Ambroxol Hydrochloride were found to be 31.91 µg/ml and 2.75 µg/ml respectively.
The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate which is useful for the routine determination of Azithromycin and Ambroxol Hydrochloride bulk drug and in its pharmaceutical dosage form.
KEYWORDS: Ambroxol, Azithromycin and Acetonitrile.

INTRODUCTION

Azithromycin is a macrolide antibiotic belonging to the azalide group. Chemically it is (2R, 3S, 4R, 5R, 8R, 10R, 11R, 12S, 13S,14S)-11-((2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2Hpyran-2-yloxy)-2-ethyl-3,4,10-trihydroxy-13-((2S,4R,5S)-5-hydroxy-4-methoxy-4hyltetrahydro-2H-Pyran-2-yloxy)-3,5,6,8,10,12,14-heptamethyl-1-oxa 6cyclopentade-can-5-one[1], used as antibiotic and antibacterial. Ambroxol is a mucolytic agent, used in the treatment of respiratory disorders associated with viscid or excessive mucus. Chemically it is Trans-4-((2-amino-3, 5 dibromobenzyl) amino) cyclohexanol. The literature survey revealed that few methods have been reported for the estimation of Azithromycin and Ambroxol. Hence we attempted to develop a simple, accurate, and economical analytical method. This paper describes validated RP-HPLC for simultaneous estimation of Azithromycin and Ambroxol Hydrochloride in combination using acetonitrile and monobasic potassium phosphate at pH 8.5 in the ratio of 65:35. The column used was C_{18} phenomenex Gemini, 5µ, 250cm x 4.6mm, with flow rate of 2ml/min using PDA detection at 220nm.

MATERIALS AND METHODS

Standard bulk drug sample Azithromycin and Ambroxol were provided by Novel Therapeutics Pvt. Ltd., Chennai. Tablets of combined form were procured from the local market. All other reagents used were of HPLC grade. HPLC (Shimadzu, Prominence) method was developed using C_{18} Phenomenex Gemini 5µ 250cm x 4.6mm id. Mobile phase selected for this method was acetonitrile: monobasic potassium phosphate buffer pH: 8.5 at the ratio of 65:35v/v. pH was adjusted with diluted

potassium hydroxide[2]. Flow rate employed was 2ml/min. Detection of eluent was carried out at 220nm using PDA detector. Standard stock solutions of pure drugs were made separately in mobile phase containing 80-125µg/ml of Azithromycin and 96-145 µg/ml of Ambroxol and filtered through a 0.2 µ Nylon membrane syringe filter. Each solution was injected and a chromatogram was recorded. Mean retention times Ambroxol and Azithromycin were found to be 3.7 and 6.1 respectively.

ANALYSIS OF FORMULATION

20 tablets of the formulation were weighed and the average weight per tablet was calculated. Twenty tablets were crushed and ground to a fine powder. A quantity of powder equivalent to 1452.6ml of Azithromycin and Amboroxol[3-7] was weighted and transferred to 100ml standard flask. The powder was dissolved in the mobile phase and filtered through 0.2 µ Nylon membrane syringe filter. The sample solution was suitably diluted and used for the analysis. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was loaded in the 20 µl fixed-sample loop of the injection port. The solution was injected and a chromatogram was recorded. The injections were repeated six times and peak areas were recorded. A representative chromatogram has been given in **Figure -1.** The peak area ratios of each of the drugs were calculated and the amount of each drug present per tablet was estimated from the respective calibration curves. The result of analysis reported in **(Table-1**). The stability of the sample in mobile phase was analyzed after 24 hrs; it was found no change in analytical parameters[8-9].

RECOVERY STUDIES

To study the accuracy, repeatability and precision of the above methods, were carried out by addition of standard drug solution to pre-analyzed sample at different levels. Results of recovery studies were found to be satisfactory and are reported in **(Table-1).**

RESULTS AND DISCUSSION

The developed RP-HPLC method for simultaneous estimation of Azithromycin and Ambroxol from combined dosage form utilizing C_{18} column and acetonitrile: monobasic potassium phosphate as mobile phase. Detection of eluent carried out using PDA detector at 220nm. The run time per sample is just 7 mins. The excipients in the formulation did not interfere in the accurate estimation of Azithromycin and Ambroxol. The method was validated as per ICH guidelines in terms of linearity, accuracy, specificity, precision, repeatability of measurement of peak area as well as repeatability of sample application and the results are shown in **Table -2**. Since this developed method can be used for routine analysis of two components in formulation.

Table 1: Analysis of formulation and recovery studies

Drugs	Labelled amount (mg)	Amount taken for assay (µg/ml)	*Amount found (mg)	% Label claim	*% Recovery	*Method precision (%RSD)
Azithromycin	500	500	496.94±0.742	99.38	99.58±0.895	0.318
Ambroxol	60	60	59.74±0.570	99.56	99.63±0.823	0.287

* Each value is a mean of six observations.

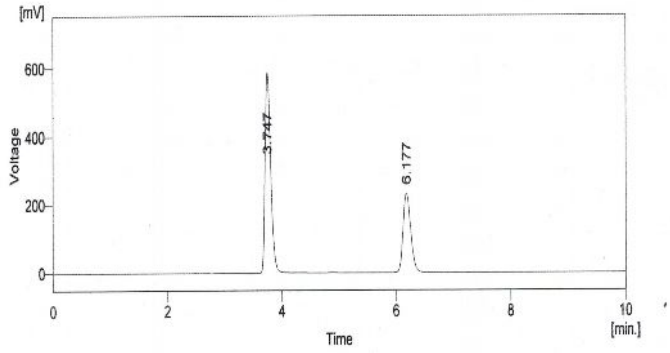
Table 2:

Validation parameters	Azithromycin	Ambroxol
Linearity range (µg/ml)	80-125	96-145
Correlation Co-efficient (r)	0.9998	0.9993
LOD (µg/ml)	31.91	2.75
LOQ (µg/ml)	96.7	8.35
Repeatability (% RSD)	0.318	0.286
*Accuracy	99.58±0.895	99.63±0.823
System precision (%RSD)	0.807	0.447
Robustness (%RSD)		
Effect of variation in pH of buffer		
pH 8.3	0.577	0.276
pH 8.7	0.756	0.453
Effect variation in flow rate		
1.8ml/min	0.298	0.280
2.2ml/min	0.749	0.116
Peak purity index	1.0000	1.0000
Resolution factor (R_S)	11.314	
No.of theoretical plates (N)	10100	6726
Tailing factor	1.314	1.609
Capacity factor (K)	0.433	
Asymmetry factor (A_S)	0.96	
Slope (m)	9.52	14.62
Intercept (c)	20.42	22.16

*Each value is a mean of six observations.

Figure-1 Chromatogram for formulation

SAMPLE NAME : AMBROXOL + AZITHROMYCIN (ASSAY)
SYSTEM : HPLC
DETECTOR : UV - VIS
TYPE OF ANALYSIS : PERCENT ON AREA



Result Table (Uncal - C:\Clarity\PROJ\Calib\AMBROX+AZITHRO-019)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	3.74	4027.912	65.5
2	6.17	2124.350	34.5
	Total	6152.262	100.0

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