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Simultaneous Estimation of Azithromycin and Ambroxol Hydrochloride in Combined Dosage form by RP-HPLC Method

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ABSTRACT

A simple, accurate and precise RP-HPLC method was developed and subsequently validated for simultaneous determination of Azithromycin and Ambroxol Hydrochloride in combined dosage form. The Proposed HPLC method utilizes C18 (250 mm × 4.6 mm × 5 µm) column with mobile phase comprising of KH_2PO_4 (pH 5.0 adjusted with 1% Orthophosphoric acid: Methanol (60:40% v/v) at a flow rate of 1.0 ml/min. Selection of wavelength for determination of Azithromycin (50 µg/ml) and Ambroxol Hydrochloride (15 µg/ml) show reasonably good response at 215 nm. Quantitation was achieved based on peak area with linear calibration curves at concentration ranges 25 - 75 µg/ml for Azithromycin and 7.5 - 22.5 µg/ml for Ambroxol Hydrochloride with Correlation Coefficient of 0.999.The Retention times of Azithromycin and Ambroxol Hydrochloride were found to be 6.73 min and 4.17 min respectively. The limit of detection (LOD) was 0.36 and 0.18 for Azithromycin and Ambroxol Hydrochloride respectively. The limit of quantification (LOQ) was 1.09 and 0.56 for Azithromycin and Ambroxol Hydrochloride respectively. The accuracy was found within the limit. The precision (inter - day, intra - day and repeatability) was found within the limit. The method was validated as per ICH guideline. The method can be successfully employed for simultaneous estimation of Azithromycin and Ambroxol Hydrochloride in combined dosage form.

Keywords: Azithromycin; Ambroxol Hydrochloride; RP-HPLC; Method Validation; ICH guideline

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INTRODUCTION

Azithromycin (AZM) acts by inhibiting the protein synthesis by binding to the 50s ribosomal subunit. Depending upon its concentration, the antibiotic can be Bacteriostatic or Bactericidal [1-3]. It is chemically represent as (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-11-[(2S,3R,4S,6R)-4-(dimethyl amino)-3-hydroxy-6-methyl oxan-2-yl] oxy-2-ethyl-3,4,10-trihydroxy-13-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyl oxan-2-yl] oxy-3,5,6,8,10,12,14-heptamethyl-1-oxa-6-azacyclopentadecan-15-one [4,5] (Figure 1).

Ambroxol (AMB) depolymerizes mucopolysaccharides directly as well as by liberating lysosomal enzyme. Network of fibers in tenacious sputum is broken. It is particularly useful if mucus plugs are present. Ambroxol is the active metabolite of bromhexine. Ambroxol causes an increase in secretion in the respiratory tract. It promotes surfactant production and stimulates ciliary activity [1-3]. It is chemically represent as trans-4-(2-Amino-3,5-dibrombenzylamino)-cyclohexanol [4,5] (Figure 2).

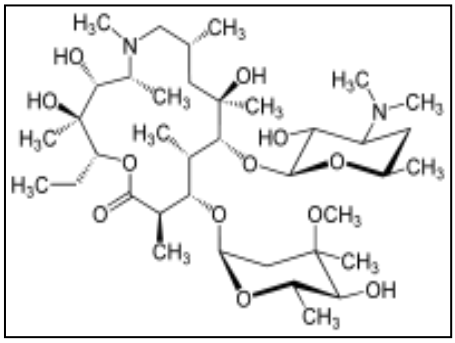

Figure 1: Structure of Azithromycin

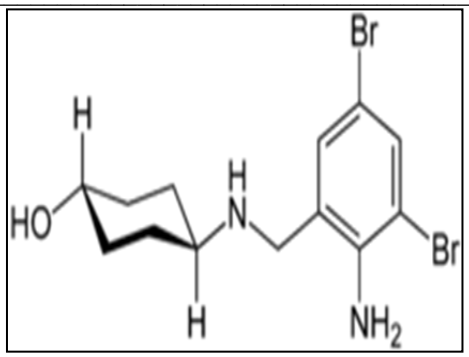

Figure 2: Structure of Ambroxol

Based on literature review, it can be concluded that number of UV spectrophotometric and chromatographic methods are available for estimation of both the drugs either alone or in combination with other drugs [6-17] so, there is a need to develop RP-HPLC method for the simultaneous estimation of both the drugs and to validate the developed methods (Table 1).

EXPERIMENTAL

Materials and Method

Azithromycin and Ambroxol Hydrochloride were procured as a Gift samples from West Coast Pharmaceutical Works Limited., Ahmedabad, Gujarat, India. All Chemicals and Reagents used were of HPLC grade. Orthophosphoric acid was of AR grade. The Pharmaceutical formulation used in this study was AZITHRAL – A (Azithromycin 500 mg and Ambroxol Hydrochloride 75 mg) Tablet procured from the local market.

Instruments

RP- HPLC System: Analytical Technology, alchrome A 2000 Software
pH meter: Systronic, model no. 335
Weighing balance: Swisser
Sonicator: Toshcon Toshniwal process instruments.

Table 1: Chromatographic condition

Parameters	Optimized Condition
Column	C18 (250 mm × 4.6 mm × 5 µm)
Wavelength detection	215 nm
Mobile phase	KH_2PO_4 (pH 5.0 adjusted with 1% Orthophosphoric acid: Methanol (60:40% v/v)
Temperature	Ambient
Injection volume	20 µl
Flow rate	1.0 ml/min

Preparation of Standard Stock and Working Standard Solution

Accurately weighed about 250 mg of AZM was transferred in 100 ml volumetric flask. Dissolved and diluted up to marked with methanol to get concentration 2500 µg/ml solution. Take 10 ml of this solution in 100 ml of volumetric flask diluted with mobile phase to get concentration 250 µg/ml of AZM.
Accurately weighed about 75 mg of AMB was transferred in 100 ml volumetric flask. Dissolved and diluted up to marked with methanol to get concentration 750 µg/ml solution. Take 10 ml of this solution in 100 ml of volumetric flask diluted with mobile phase to get concentration 75 µg/ml of AMB.

Selection of Wavelength for Determination

Standard solution of AZM (50 µg/ml) and AMB (15 µg/ml) were prepared using their working standard solution using Methanol as a solvent. Each solution was scanned between 200 - 400 nm using Methanol as a blank. The point at which both drug shows absorbance, was selected as wavelength for determination.

Method Validation

Specificity

Specificity is ability to measure specifically the analyte of interest without any interference from excipient and mobile phase component. For the determination of specificity 250 µg/ml solution of the standard AZM and 75 µg/ml solution of the standard AMB was injected. Marketed formulation of same concentration was also injected. Both chromatograms were compared.

Linearity

Linearity was evaluated by analysis of working standard solutions of Azithromycin and Ambroxol Hydrochloride of five different concentrations. Linearity was evaluated by analysis of working standards of Azithromycin and Ambroxol Hydrochloride of five different concentrations. The ranges of linearity were from 25 µg/ml to 75 µg/ml for Azithromycin and 7.5 µg/ml to 22.5 µg/ml for Ambroxol Hydrochloride. The peak area and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients.

Accuracy

The Standard was spiked with formulation at these concentration levels of 80%, 100%, 120% and the mixture were analyzed by the proposed method. The experiment was conducted in triplicate.

Precision

Pure samples of AZM and AMB were analyzed over different days to obtain inter-day (intermediate precision, n=3) and within the same day to obtain intra-day precision (repeatability, n=3), then the% RSD values were calculated.

Robustness

Method robustness was evaluated by changing the flow rate, pH and mobile phase composition to evaluate the impact on the performance of the method and the results will be expressed in terms of% RSD.

LOD (Limit of Detection) and LOQ (Limit of Quantification):

The Limit of Detection and Limit of Quantification of the developed method was assessed by analysing five replicates of each solution. The LOD and LOQ may be calculated as:

LOD=3.3 × SD/Slope

LOQ=10 × SD/Slope

Where, SD=five replicates of absorbance; Slope=the mean slope of the 5 calibration curves.

System Suitability

System suitability is the checking of a system to ensure system performance before or during the analysis of unknown. Parameters such as Theoretical Plates, Tailing factors, Resolution and Retention time were determined and compared against the specifications set for the method.

Estimation of Marketed formulation:

Weight twenty tablets and powdered. An accurately weighed powder equivalent to about 50 mg of AZM and 15 mg of AMB was transferred to 100 ml volumetric flask and the volume was made up to the mark using Methanol as a solvent. The solution was sonicated for 20 min. The solution was filtered through whattman filter paper no.42. First few ml of filtrate were discarded. 1 ml of the solution from the above filtrate was diluted to 10 ml with water to produce 50 µg/ml of AZM and 15 µg/ml of AMB. The absorbance of resulting solution was measured at selected wavelength. Chromatogram of this solution was taken and amount of AZM and AMB was calculated using regression equation.

RESULTS AND DISCUSSION

Method Development

Standard solution of AZM (50 µg/ml) and AMB (15 µg/ml) were prepared using their working standard solution using Methanol as a solvent. The overlain spectra of the drugs suggested that both the components show reasonably good response at 215 nm (Figure 3).

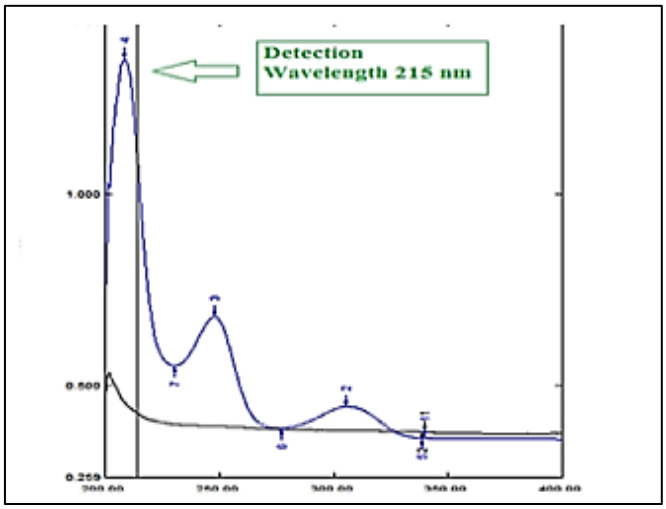

Figure 3: Overlain spectra of Azithromycin 50 µg/ml and Ambroxol HCl 15 µg/ml

VALIDATION

Linearity

The calibration curves of AZM and ABZ were linear in the range of 2-10 µg/ml and 16-80 µg/ml respectively. The regression equations of calibration curves showed in Figures 4, 5 and 6. The calibration curve data shown in Table 2.

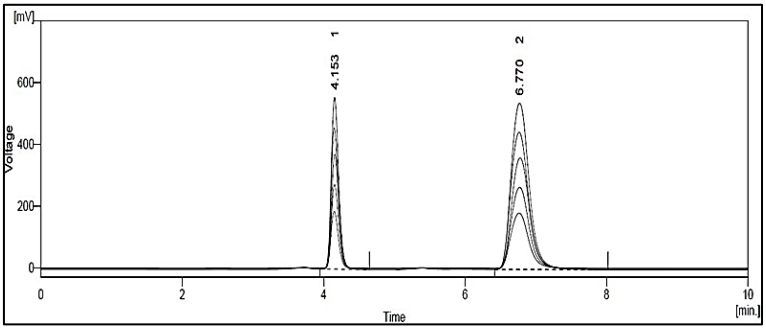

Figure 4: Linearity graph of AZM and AMB

Table 2: Calibration curve data of AZM and AMB

Calibration curve data of AZM			Calibration data of AMB		
Conc. (µg/ml)	**Mean area ± SD (n=5)**	**% RSD**	**Conc. (µg/ml)**	**Mean area ± SD (n=5)**	**% RSD**
25	3128.46 ± 26.86	0.86	25	3128.46 ± 26.86	0.86
37.5	4625.33 ± 37.35	0.81	37.5	4625.33 ± 37.35	0.81
50	6323.37 ± 44.38	0.70	50	6323.37 ± 44.38	0.7
62.5	7780.67 ± 66.00	0.85	62.5	7780.67 ± 66.00	0.85
75	9466.63 ± 87.35	0.92	75	9466.63 ± 87.35	0.92

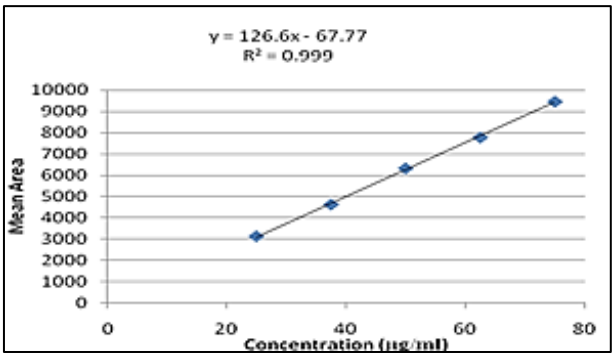

Figure 5: Calibration curve of AZM

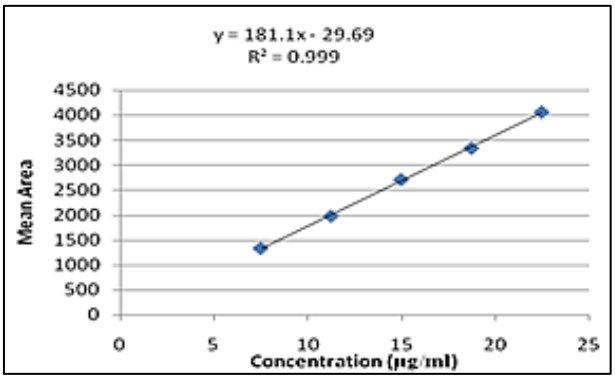

Figure 6: Calibration curve of AMB

Accuracy

The percentage recoveries of drugs from marketed formulation were determined by standard addition of pure drugs at three known concentrations and excellent recoveries were obtained at each level. The percentage recoveries for AZM and AMB at three levels were found within limits. The results of accuracy studies are shown in Tables 3 and 4.

Table 3: Accuracy data of AZM

Conc. (µg/ml)	% of STD Added	Total Conc. (µg/ml)	Amount Found (µg/ml)	% Recovery ± SD	%RSD
25	80	45	45.03	100.08 ± 1.32	1.32
25	100	50	49.76	99.53 ± 1.17	1.18
25	120	55	54.57	99.24 ± 0.37	0.38

Table 4: Accuracy data of AMB

Conc. (µg/ml)	% of STD Added	Total Conc. (µg/ml	Amount Found (µg/ml)	% Recovery ± SD	% RSD
7.5	80	13.5	13.56	100.52 ± 1.71	1.7
7.5	100	15	14.96	99.81 ± 1.52	1.53
7.5	120	16.5	16.6	100.67 ± 1.35	1.35

Precision

Relative Standard Deviations (% R.S.D.) for repeatability were found to be 0.87% and 0.76% for AZM and AMB, respectively. The intraday precision showed% R.S.D. for AZM and AMB was in limit. The results of repeatability, intraday and inter- day precision of method is illustrated in Tables 5, 6 and 7.

Table 5: Repeatability data of AZM and AMB

AZM		AMB	
Conc. (µg/ml)	**Area**	**Conc. (µg/ml)**	**Area**
50	6295.47	15	2699.22
	6308.13		2659.52
	6320.79		2710.14
	6177.49		2715.68
	6314.41		2707.34
	6314.45		2707.53
Mean Area ± SD	6288.46 ± 55.03	Mean Area ± SD	2699.90 ± 20.48
%RSD	0.87	%RSD	0.76

Table 6: Precision data of AZM and AMB (Intra – day)

AZM			AMB		
Conc. (µg/ml)	**Mean area ± SD**	**% RSD**	**Conc. (µg/ml)**	**Mean area ± SD**	**% RSD**
25	3045.55 ± 18.55	0.61	7.5	1329.75 ± 8.89	0.66
50	6254.06 ± 36.48	0.58	15	2689.12 ± 20.55	0.76
75	9369.16 ± 73.84	0.79	22.5	4044.24 ± 42.93	1.06

Table 7: Precision data of AMB and AMB (Inter – day)

AZM			AMB		
Conc. (µg/ml)	**Mean area ± SD**	**% RSD**	**Conc. (µg/ml)**	**Mean area ± SD**	**% RSD**
25	3103.83 ± 25.51	0.82	7.5	1332.35 ± 10.35	0.77
50	6254.06 ± 36.48	1.08	15	2689.12 ± 20.55	0.76
75	9379.25 ± 84.34	0.89	22.5	4044.24 ± 42.93	1.06

LOD and LOQ

LOD and LOQ for AZM in this method were found to be 0.36 and 1.09 µg/ml and for AMB were found to be 0.18 and 0.56 µg/ml respectively.

Robustness

The results of robustness were illustrated in Table 8.

Table 8: Robustness data of AZM and AMB

AZM			AMB		
Flow rate (ml/min)	**Mean area ± SD**	**% RSD**	**Conc. (µg/ml)**	**Mean area ± SD**	**% RSD**
0.95	6511.98 ± 59.71	0.92	0.95	2799.62 ± 28.90	1.03
1.05	6138.12 ± 98.74	1.61	1.05	2637.57 ± 23.90	0.9
M.P. (Ratio)	**Mean area ± SD**	**% RSD**	**Conc. (µg/ml)**	**Mean area ± SD**	**% RSD**
58.8 : 41.2	6440.32 ± 90.39	1.4	58.8 : 41.2	2764.31 ± 30.34	1.09
61.2 : 38.8	6121.71 ± 86.77	1.41	61.2 : 38.8	2635.29 ± 30.17	1.14
pH	**Mean area ± SD**	**% RSD**	**Conc.(µg/ml)**	**Mean area ± SD**	**% RSD**
4.9	6451.12 ± 93.06	1.4	4.9	2768.92 ± 27.43	0.99
5.1	6007.13 ± 87.99	1.41	5.1	2583.45 ± 31.64	1.22

System Suitability

Parameters such as Theoretical plates, Tailing factors, Resolution and Retention time are illustrated in Table 9.

Table 9: System suitability parameters of AZM and AMB (Inter – day)

Parameters (n=5)	AZM (Mean ± SD)	AMB (Mean ± SD)
Retention time (min.)	6.757 ± 0.007	4.146 ± 0.005
Tailing factor (Tf)	1.330 ± 0.015	1.381 ± 0.035
Number of Theoretical plates (N)	3386.5 ± 7.79	7205.33 ± 21.4
Resolution (Rs)	7.915	

Analysis of Marketed Formulation

Data of marketed formulation were illustrated in Table 10.

Table 10: Analysis of marketed formulation data of AZM and AMB

AZM			AMB		
Amount taken (µg/ml)	**Amount found (µg/ml)**	**% Purity**	**Amount taken (µg/ml)**	**Amount found (µg/ml)**	**% Purity**
50	48.877	97.75	15	14.79	98.64
	50.126	100.25		14.85	99.02
	49.652	99.3		14.76	98.46
	49.549	99.09		14.58	97.25
	49.628	99.25		14.85	99.01
Mean	49.567	99.134	Mean	14.77	98.47
SD	0.44	0.89	SD	0.1	0.72
%RSD	0.87	0.902	%RSD	0.76	0.73

CONCLUSION

All the parameters are validated as per ICH guidelines for the method validation and found to be suitable for routine quantitative analysis in pharmaceutical dosage forms. The result of linearity, precision, Robustness proved to be within limit. Assay results obtained by proposed method are in fair agreement. Recovery studies were satisfactory showed that there is overage of vitamins present in marketed formulation.

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