Development of Stability Indicating RP-HPLC Method for Simultaneous Determination of Azithromycin and Ambroxol HCl (SR) in the Tablet Formulation

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ABSTRACT

A sensitive and specific isocratic RP-HPLC was developed for quantitative estimation of Azithromycin and Ambroxol HCl tablet formulation. The developed method consisting the mobile phase K_2HPO_4 – pH 6.5 : Acetonitrile (68 : 32) with isocratic programming, Hypersil, BDS, C 8, column (150 mm x 4.6 mm i.d., 5µm particle size) as stationary phase with a flow rate of 1.5 mL/minute by using λ_{max} 215nm and PDA detector. Proposed method was found to be linear in the concentration range of 100.0 to 360.0 µg/mL for Azithromycin and 15.0 to 54.0 µg/mL for Ambroxol HCl respectively, the correlation coefficient was found to be 0.999. Precision study showed that the percentage relative standard deviation was within the range of acceptable limits, and the mean recovery was found to be 100.36 % for Azithromycin and 100.24% for Ambroxol HCl. The developed method was validated for specificity by stress studies. Ambroxol HCl and Azithromycin were subjected to stress condition and products were analyzed by using photo diode array detector. It was found to be stable in milder condition of stress (0.1 M HCl, 0.1 M NaOH, 3% H_2O_2, at 60°C/10 minutes). The analyte peaks were well resolved from the degraded impurities.

Key words: RP-HPLC, Isocratic, Azithromycin, Ambroxol HCl, Stress studies, Method validation

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INTRODUCTION

Azithromycin ($C_{38}H_{72}N_2O_{12}$) (Fig 1), is a (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-15-oxo-11-{[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-]oxy}-1-oxa-6-azacyclo pentadec-13-yl 2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranoside[1]), which belongs to a group of selective and very effective Macrolide Antibiotic prevents bacteria from growing by Interfering with their protein synthesis. Azithromycin binds to the 50S subunit of the bacterial ribosome, and thus inhibits translation of mRNA. Nucleic acid synthesis is not affected[2]. In protein binding Conc. dependent, decreasing from 51% at. 0.02 µg/mL to 7% at 2 µg/mL

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Ambroxol Hydrochloride ($C_{13}H_{18}Br_2N_2O \cdot HCL$) (Fig 2) is a *trans*-4-[(2-Amino-3,5dibromobenzyl) amino] cyclohexanol Hydrochloride[3]. This drug, which acts by Acts by inhibiting broncho constriction in the guinea pig and to reduce TNFα,IL2, INFγ production in BAL mono nuclear cells and used as Mucolytic expectorant.

Very few methods have been reported like liquid chromatography and UV spectrophotometric method with some hyphenated techniques on Azithromycin and Ambroxol HCl alone or in combination. But no such stability indicating method using simple solvents like methanol and acetronitrile with low mobile phase ratio was found. This paper deals with the forced degradation[4] of Azithromycin and Ambroxol HCl under stress conditions[5] like hydrolysis, oxidation, thermal and photolysis, finally validation of the developed method for assay in pharmaceutical solid dosage form (Tablets).
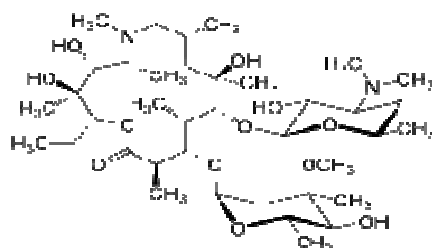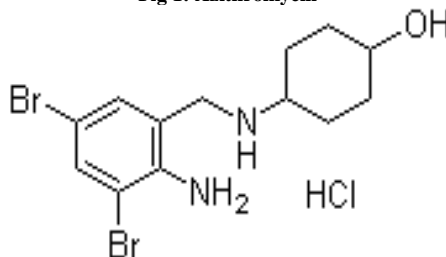

Fig 1: Azithromycin

Fig 2: Ambroxol HCl.

MATERIALS AND METHODS

HPLC instrumentation and conditions
Chromatographic analysis was performed on a binary gradient Waters Alliance-LC HPLC, photo diode array detector and the output signal was monitored and integrated using Empower2 software. Hypersil BDS-C8 (150X4.6mm i.d., 5µm particle size) column was used for separation.

Chemicals and reagents
Azithromycin and Ambroxol HCl (API) were provided by Glenmark pharma pvt. Ltd. Nashik (India). Methanol, Acetonitrile and water of HPLC grade were purchased from Merck, Mumbai (India). Commercial formulation of Azithromycin is 500mg and Ambroxol HCl is 75mg label claim was purchased from a local medical store.

Forced degradation studies
The drug subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage[6]. Specific conditions were described as follows,

i) Acidic conditions
Acid decomposition studies were carried out at drug strength of 2mg/mL for Azithromycin and 0.3mg/mL for Ambroxol HCl, in 0.1N HCl at 60°C for 10 min. The pH of the solution should be neutralized with 0.1N NaOH before injecting in to the column.

ii)Alkaline conditions

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Alkaline decomposition studies were carried out at drug strength of 2mg/mL for Azithromycin and 0.3mg/mL for Ambroxol HCl, in 0.1N NaOH at 60°C for 10 min. The pH of the solution should be neutralized with 0.1N HCl before injecting in to the column.

iii) Oxidative conditions
The oxidative stress studies were done at drug strength of 2mg/mL for Azithromycin and 0.3mg/mL for Ambroxol HCl, in 3% H_2O_2 at 60°C for 10 hours.

iv) Thermal conditions
Thermal degradation studies were carried out in water by exposing 2mg/mL for Azithromycin and 0.3mg/mL for Ambroxol HCl concentration solution to 105°C in sand bath for 24 hours.

v) Humidity conditions
Humidity degradation studies were carried out in water by exposing 2mg/mL for Azithromycin and 0.3mg/mL for Ambroxol HCl to 92% RH for 25°C. Samples were withdrawn and analyzed by HPLC after suitable dilutions.

vi) Photolytic conditions
Photolytic degradation studies were carried out in water by exposing 2mg/mL for Azithromycin and 0.3mg/mL for Ambroxol HCl concentration solution to sunlight for 1.20 million Lux hours. Pure solid drug (in 1mm thick layer in a petriplate) was also exposed to sunlight for 8 hours. Suitable controls were kept under dark conditions. Samples were withdrawn periodically and analyzed by HPLC after suitable dilutions.

Method development
The stressed samples were initially analyzed by HPLC using a Hypersil BDS-C8 column and a mobile phase composed of methanol and acetonitrile (64:36). As the separation and peak shape were not good, therefore, organic modifier concentration was changed. Eventually, a mobile phase composition methanol : acetonitrile (68:32) gave the best results. During these studies, the mobile phase flow rate was constant at 1.5mL/min. The analytical wavelength was 215 nm.

Validation of the developed method
i) Specificity
The evaluation of the specificity of the method was determined against placebo. The interference of the excipients of the claimed placebo present in pharmaceutical dosage form was derived from placebo solution. Further the specificity of the method toward the drug was established by means of checking the interference of the degradation products in the drug quantification for assay during the forced degradation study[7].

ii) Linearity
Linearity was established by triplicate injections of solutions containing drug in the concentration range of 100.0-360.0µg/mL for Azithromycin and 15.0-54.0 µg/mL for Ambroxol HCl. The peak areas versus concentration data were evaluated by linear regression analysis.

iii) Precision
Intraday precision was established by making six injections of three points in the above linear range (100,180 and 250µg/mL for Azithromycin and 20, 30 and 40µg/mL for Ambroxol HCl) on the same day. These studies also repeated on next day to determine inter-day precision.

iv) Accuracy - Recovery studies
Recovery of method was determined by adding 80%, 100% and 120% of test drug to previously analyzed sample of standard Azithromycin and Ambroxol HCl (API). For each concentration level, three sets were prepared and injected in duplicate. The recovery of added drug was determined.

v) Robustness
To determine the robustness of the method, experimental conditions are purposely altered and chromatographic characters are evaluated. Influence of small changes in chromatographic conditions such as changes in flow rate

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(±0.25mL/min), % organic phase (±2%) and column temperature (±5°C) were studied to determine the robustness of the method.

vi) System suitability
Six replicate injections of standard preparations were injected and asymmetries, theoretical plates, tailing factor and % relative standard deviation (%RSD) for peak area were determined.

RESULTS AND DISCUSSION

Stress studies
HPLC studies on Azithromycin and Ambroxol HCl under different stress conditions using methanol : acetonitrile (68:32) as the solvent system suggested the following degradation behavior (Fig:5-8).

i) Acid hydrolysis
The Azithromycin was found to be unstable at 0.1N HCl at 60°C for 10min. Ambroxol HCl was found to be stable at 0.1N HCl at 60°C for 10min.

ii)Base hydrolysis
The Azithromycin was found to be unstable at 0.1N NaOH at 60°C for 10min. Ambroxol HCl was found to be stable at 0.1N HCl at 60°C for 10min.

iii) Oxidative condition
The Azithromycin was found to be unstable; no degradation was seen in Ambroxol HCl at 3% oxidation at 60°C for 10hours.

iv) Photolytic condition
No degradation was observed in solid drug and control samples.

v)Thermal conditions
The Azithromycin was found to be unstable; no degradation was seen in Ambroxol HCl at 105°C for 24hours.

vi) Humidity conditions
No degradation was observed in solid drug and control samples.

Validation of the method
The method was validated with respect to parameters like linearity, precision, accuracy, specificity and robustness.

i) Specificity
The method was found to be specific to the drug. The PDA analyses proved that the peak purity value for drug peaks passes acceptance criteria and free from any coeluting peak.

ii)Linearity
The response for the drugs was strictly linear (Fig-9, 10) in the studied concentration range ($R^2 = 0.9999$). The slope and correlation coefficient values were 208.38 and 0.9992 for Azithromycin and 106764.0 and 0.9999 for Ambroxol HCl.

iii) Precision
Table 1 provides data for intra-day and inter-day precision experiments. The R.S.D. values for precision were 1.6 and 0.1%, respectively, there by indicating that the method was sufficiently precise.

iv) Accuracy
The values of % recovery and % relative standard deviations were listed in table 2 indicate that the method is accurate.

v)Robustness
The robustness study for Azithromycin and Ambroxol HCl was studied and the method was found to be robust.

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vi) System suitability

For all system suitability injections, asymmetry was less than 1.3, theoretical plates should not be less than 2,000 for both, and % RSD of peak area should not be more than 2.0% for both. This parameter ensures that the analytical system is working properly and can give accurate and precise results.
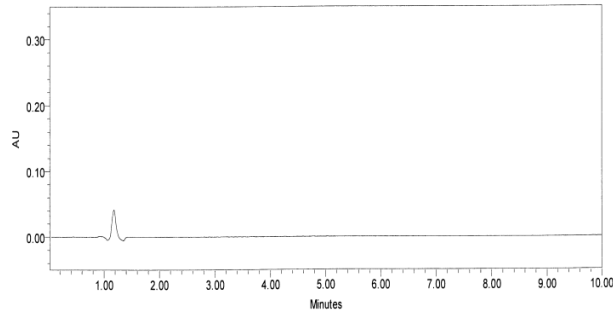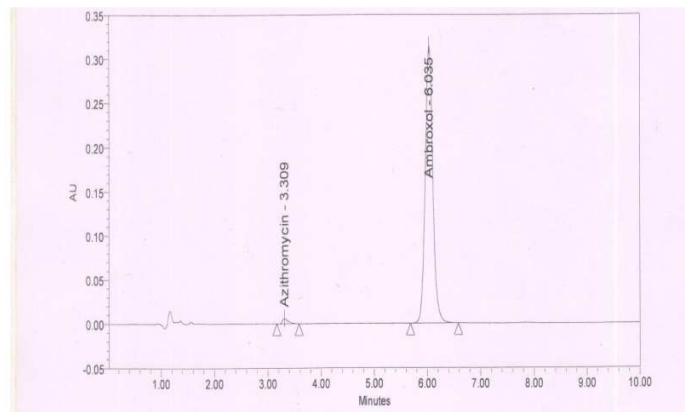

Fig 3: Chromatogram of Diluent

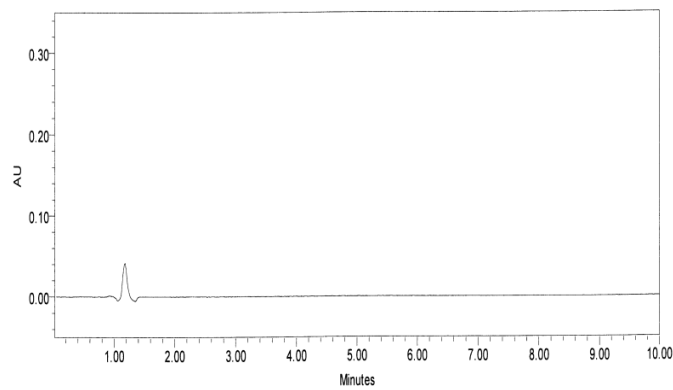**Fig 4: Chromatogram of Azithromycin and Ambroxol HCl standard**

Fig 5: Chromatogram of degradation diluent
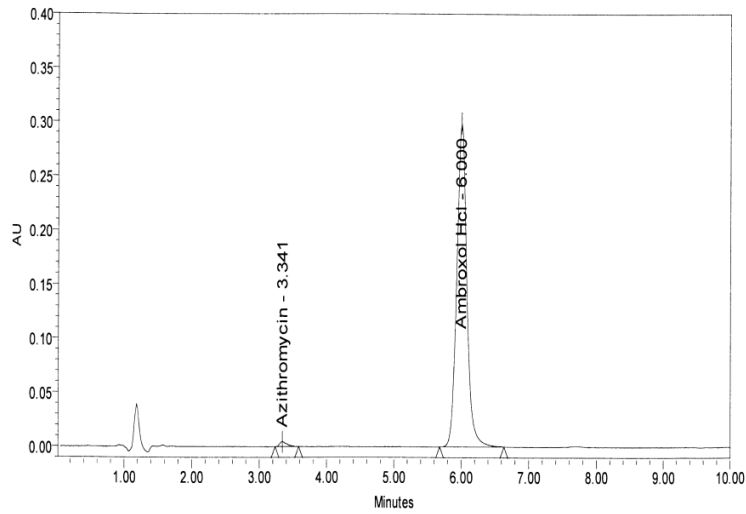
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Fig 6: Chromatogram of Azithromycin and Ambroxol HCl degradation standard
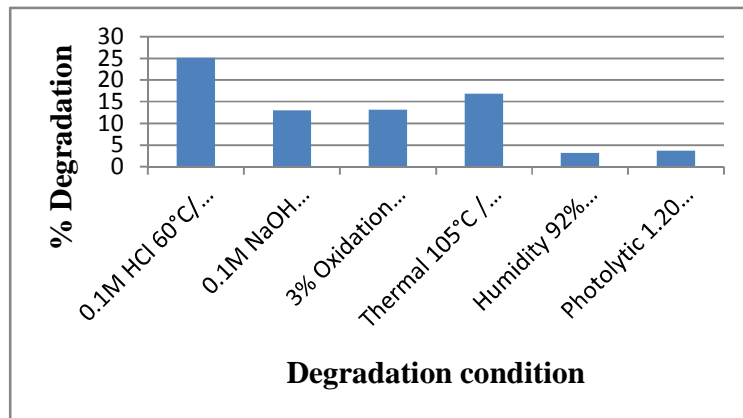

Fig 7: Forced degradation study of Azithromycin
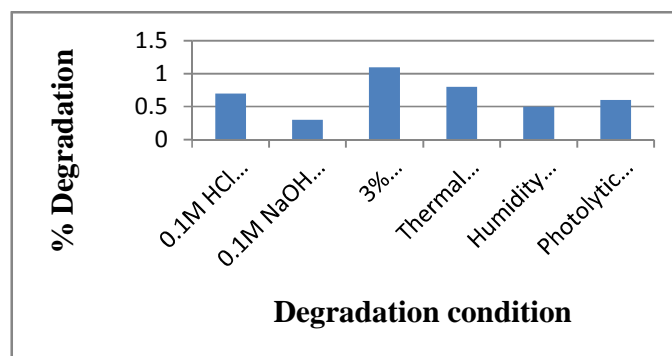

Fig 8: Forced degradation study of Ambroxol HCl
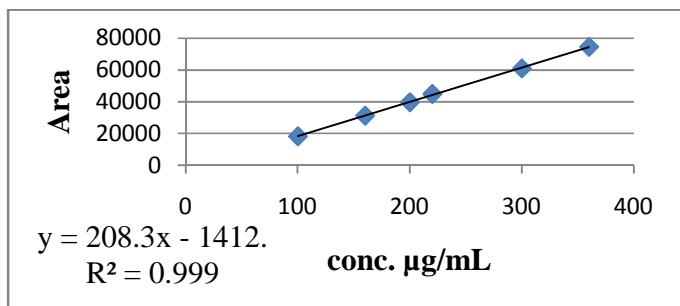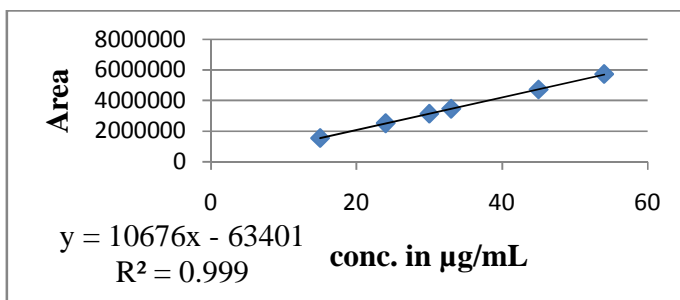
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Fig 9: Linearity Graph of Azithromycin

Fig 10: Linearity Graph of Ambroxol HCl.

Table 1: Reproducibility and precision data evaluated through intra-day and inter-day studies (*n*=6)

Drug	Conc.(µg/mL)	Intraday		Interday	
		Amt. found ± S.D	RSD	Amt. found ± S.D	RSD
Azithromycin	100	100.08 ± 0.31	0.31	100.11 ± 0.35	0.34
	180	180.52 ±0.43	0.23	180.49 ±0.54	0.29
	250	250.23 ±0.49	0.19	250.26 ±0.51	0.20

Drug	Conc.(µg/mL)	Intraday		Interday	
		Amt.found ± S.D	RSD	Amt. found ± S.D	RSD
Ambroxol HCl	20	20.21 ± 0.29	1.43	20.21 ± 0.32	1.58
	30	30.64 ± 0.32	1.04	30.64 ± 0.45	1.46
	40	40.54 ± 0.53	1.30	40.54 ± 0.56	1.38

Table 2: Recovery studies (*n* = 3)

Sample No.	For Azithromycin			For Ambroxol HCl		
	Amount Added (mg)	Amount Recovered (mg)	% Recovery	Amount Added (mg)	Amount Recovered (mg)	% Recovery
Accuracy-80% Set-1	232.29	99.40	99.4	37.24	37.29	100.1
Accuracy-80% Set-2	235.58	100.34	100.3	37.24	37.36	100.3
Accuracy-80% Set-3	233.61	100.24	100.2	37.24	37.29	100.1
Accuracy-100% Set-1	477.02	101.68	101.7	74.48	73.63	98.9
Accuracy-100% Set-2	470.70	100.94	100.9	74.48	73.78	99.1
Accuracy-100% Set-3	476.14	101.77	101.8	74.48	73.93	99.3
Accuracy-120% Set-1	703.93	99.25	99.2	111.71	113.41	101.5
Accuracy-120% Set-2	704.87	100.07	100.1	111.71	113.42	101.5
Accuracy-120% Set-3	704.59	99.56	99.6	111.71	113.29	101.4
Mean			100.36			100.24
SD			0.94			1.03
% RSD			0.94			1.03

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CONCLUSION

A validated RP-HPLC method for the determination of Azithromycin and Ambroxol HCl in marketed formulation was developed, and this method was applied to stress induced studies of Azithromycin and Ambroxol HCl. The developed method is simple, accurate, precise, and specific and could separate drug from degradation products. It is suggested for routine analysis of Azithromycin and Ambroxol HCl in its marketed formulation.

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