Vol.3.Issue.4., 2015
(Oct-Dec.)

Bulletin of Pharmaceutical and Medical Sciences (BOPAMS)
A Peer Reviewed International Journal http://www.bopams.com

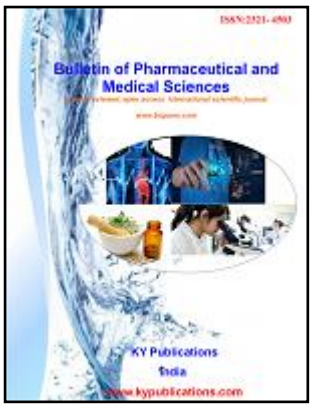
RESEARCH ARTICLE

INTERNATIONAL STANDARD SERIAL NUMBER
E-ISSN 2341-4103

A NEW VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF AMBROXOL AND AZITHROMYCIN IN COMBINED DOSAGE FORM

K.PADMAVATHI[1], Dr.M.SUBBA RAO[2]*

[1]Department of Chemistry,Acharya Nagarjuna Univesity,Guntur,A.P,India
[2]Department of Chemistry,Acharya Nagarjuna Univesity,Guntur,A.P,India
*Corresponding authors:mannamsrao@gmail.com



ABSTRACT

A new sensitive high performance liquid chromatographic method for the estimation of ambroxol and azithromycin in combined dosage form has been developed. Chromatography was carried out on a Hypersil C_{18} column (250×4.6 mm, 5µ) with a flow rate of 1.0mL/min of mobile phase and UV detection at a wavelength of 240nm and ambient column temperature with mobile phase of phosphate buffer(pH-3.5) and acetonitrile in the ratio of 45:55%v/v as the mobile phase. Acyclovir was used as an internal standard for this study. The retention times for ambroxol and azithromycin were found to be 3.326min and .4.472min respectively. The proposed method was found to be linear in the concentration range of concentration range of 10-30µg/ml for ambroxol and 50-150µg/ml for azithromycin respectively. The method was validated as per ICH guidelines and was found to be suitable for bioequivalence and pharmacokinetic studies.

Key Words: Ambroxol, Azithromycin, RP-HPLC and ICH Guidelines.

INTRODUCTION

Ambroxol [1,2] is a mucolytic agent, used in the treatment of respiratory disorders associated with viscid or excessive mucus. Ambroxol Hcl [Figure.1(a)] is a trans-4-[(2-Amino-3,5dibromobenzyl) amino] cyclohexanol Hydrochloride. This drug, 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.

Azithromycin[3,4] [Fig.1(b)] 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 -4hyltetra hydro -2H-Pyran-2-yloxy)-3,5,6,8,10,12,14-heptamethyl-1-oxa 6cyclopentade-can-5-one, is a bacteriostatic drug acts by inhibiting protein synthesis. It binds reversibly to 50S ribosomal subunits of sensitive microorganism.

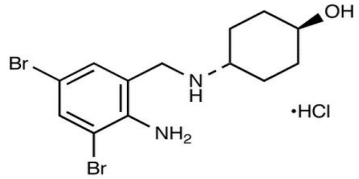


Fig.1(A).Chemical Structure of Ambroxol

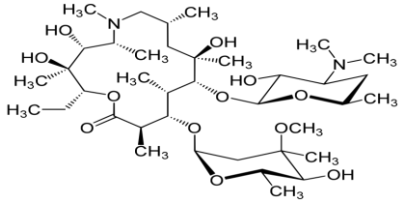


Fig.1(b).Chemical Structure of Azithromycin

Many analytical methods were reported for the determination of ambroxol and azithromycin in singly or in combination with other drugs [5-19].Only three HPLC methods[20-22] were reported so for the above said drugs in combined formulations and basing on the tis accord it made essential to develop a new RP-HPLC method for routine analysis of the above said drugs in combined formulations, and in this accord attempts were made by the author to develop simple, precise and accurate RP-HPLC method for the simultaneous assay of the titled drugs and extended it for their determination in formulations.

EXPERIMENTAL:

a. INSTRUMENTATION: The present analysis was carried on Water's 2695 HPLC system provided with Hamilton Syringe, Hypersil C_{18} column (250×4.6 mm, 5µ), auto sampler and 2996 Photodiode array detector. Data was acquired and processed with Empower 2 software. Shimazdu (Tokyo, Japan) electronic weighing balance [Model BL 220 H] was used for weighing the samples. Elico pH meter (Hyderabad, India) LI 120 model was used for pH measurements.

b. CHEMICALS AND REAGENTS: Pharmaceutically grade pure sample of ambroxol and azithromycin were obtained from Hetero Drugs Ltd,Hyderabad as gifted samples and commercial tablets of ambroxol and azithromycin in the brand name of Azyxin Plus[Azithromycin IP equivalent to Azithromycin (anhydrous) 250 mg Ambroxol Hydrochloride [in sustained release form] 75mg] were procured from the local pharmacy**.** Milli-Q water, Acetonitrile and methanol (HPLC Grade), Orthophosphoric acid (GR Grade), potassium dihydrogen orthophosphate monohydrate (GR Grade) was obtained from Qualigens Ltd., Mumbai. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard class-A, volumetric glassware.

c. PREPARATION OF PHOSPHATE BUFFER: The buffer was prepared by dissolving 2.72grams of Potassium dihydrogen phosphate in 1000 mL of milli-Q water. The pH of the buffer solution was adjusted to 3.5 + 0.05 with ortho phosphoric acid.

d. MOBILE PHASE PREPARATION: Prepare a filtered and degassed mixture of phosphate buffer and acetonitrile in the ratio of 45:55 v/v respectively.

e.DILUENT PREPARATION: Mobile phase is used as diluent in the present assay.

f.PREPARATION OF STOCK & WORKING STANDARD SOLUTIONS: Standard stock solutions of the present studied drugs was prepared by weighing accurately 50mg of ambroxol and 10mg of azithromycin were transferred into a clean and dry 100ml volumetric flask. To this flask about 70 ml of diluent was added and sonicated for five minutes. Later, the volume of the flask was made unto the mark with the same diluent[Concentrations 500µg/ml for ambroxol and 100µg/ml, for azithromycin]. From the above prepared stock solution pipette out suitable aliquots and transferred into a clean and dry 10ml volumetric flask, the diluent was added up to the mark to get final concentration of 10 - 30µg/ml for ambroxol and 50 - 150µg/ml, for azithromycin respectively.

g. PREPARATION OF SAMPLE SOLUTION: Ten tablets of Azyxin Plus[Azithromycin IP equivalent to Azithromycin (anhydrous) 250 mg Ambroxol Hydrochloride [in sustained release form] 75 mg] procured from the local market were powdered to fine powder. Then sample solution was prepared by weighing and transferring equivalently 100mg of the fine powder of formulation mixture into a 100ml clean and dry volumetric flask containing 70ml of diluent and sonicated to dissolve it completely and the volume made up to the mark with the same solvent. From above prepared stock solution pipette out aliquots of the above solution and transferred into a clean and different dry 10ml volumetric flasks, the diluent was added up to the mark 10ml to get final concentration of 10-30µg/ml for for ambroxol and 50-150µg/ml for azithromycin, respectively. 20µL volumes of these standard and sample solutions were injected five times and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas.

RESULTS AND DISCUSSION

i. HPLC METHOD DEVELOPMENT: In the development of the present method for the selected drugs a number of experimental trials were made by changing the columns and mobile phase by varying its composition as

well as by changing the solvents. All these trials have resulted either in low resolution or asymmetric peaks or peaks with more tailing factors or longer time of elution.

However, finally the Hypersil C_{18} column (250×4.6 mm, 5µ) with a flow rate of 1.0mL/min of mobile phase and UV detection at a wavelength of 240nm and ambient column temperature with mobile phase of phosphate buffer(pH-3.5) and acetonitrile in the ratio of 45:55 v/v resulted in excellent elution of the two drugs with low retention and run times. The same buffer was used as diluent in the preparation of standard and sample solutions. With the above optimized conditions ambroxol and azithromycin gave acceptable retention time (3.326min and .4.472min for ambroxol and azithromycin respectively), plates and good resolution at 240nm respectively [Figure.2]. This developed method was further validated in pharmaceutical dosage forms with satisfactory precision and accuracy at lower concentration of both the drugs in its solid combined dosage forms.

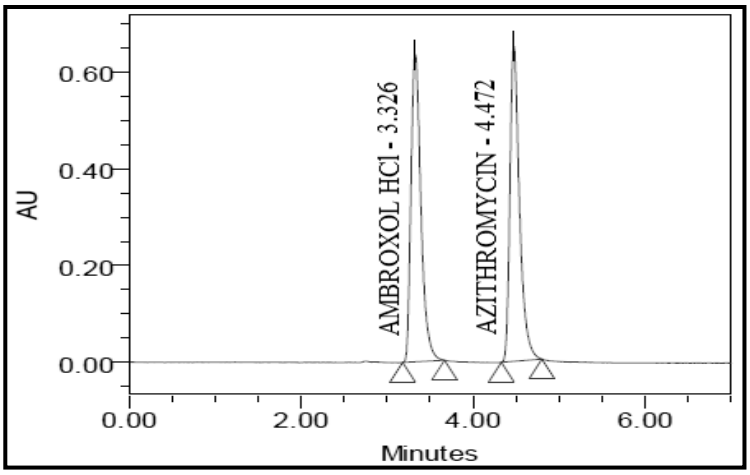


FIG 2. STANDARD CHROMATOGRAM OF AMBROXOL AND AZITHROMYCIN

ii. METHOD VALIDATION: The developed RP-HPLC method was validated in accordance with ICH guidelines[23] using the following parameters.

a.SYSTEM SUITABILITY: System suitability parameters like number of theoretical plates, HETP and peak tailing were determined for both the drugs with the proposed method and their values were tabulated in **Table.1**. It was found from above data; that all the system suitability parameters for developed method for ambroxol and azithromycin were within the limit.

b. SPECIFICITY:

i. BLANK AND PLACEBO INTERFERENCE: The specificity of the proposed method was established by injecting blank and placebo using the above chromatographic conditions. The chromatogram of blank showed no peaks at the retention time of ambroxol and azithromycin peak revealing that the diluent solution used in sample preparation do not interfere in estimation of ambroxol and azithromycin in tablets. Similarly the chromatogram of placebo solution showed no peaks at the retention time of ambroxol and azithromycin peak indicating that the placebo used in sample preparation do not interfere in estimation of ambroxol and azithromycin in their formulations.

c. LINEARITY OF DETECTOR RESPONSE: The linearity was performed with UV- detector at 220nm and the respective peak areas were recorded**.** Further, standard curves were plotted for ambroxol and azithromycin [**Figures.4(a) &4(b)]** and linear regression analysis was calculated respectively. Two standard curves were obtained in the concentration range of 10-30µg/ml for ambroxol and 50-150µg/ml for azithromycin respectively [**Table.3(a)&(b)]**. The slope and intercept value for calibration curve were **y = 12446.66.x+ 63894.47(r^2 = 0.9997)** for ambroxol and **y= 50333.67.x-7311 (r^2 = 0.9996)** for azithromycin respectively. From the data obtained it is revealed that an excellent correlation exists between response factor and concentration of cited drugs within the concentration range indicated as above respectively.

The LOD values for ambroxol and azithromycin were found to be 1.47µg/mL and 4.92µg/mL, respectively and the LOQ values for ambroxol and azithromycin were found to be 4.49µg/mL and 14.98µg/mL respectively revealing good sensitivity of the proposed method[**Table.3**]

d. PRECISION: The precision of the developed method was evaluated by carrying out inter-day and intra-day analysis by injecting six replicate injections of 100% test concentration of the above mentioned drugs and the results were expressed in terms of standard deviation and %RSD. The results were summarized in **Table.4**. From the results [%RSD of 0.967& 0.699 for ambroxol and 0.736&1.44 for azithromycin] that revealed that the developed method was found to be precise as the %RSD values were < 2 %, respectively.

e. ACCURACY: The accuracy of the method was determined at three concentration levels(50,100 and 150%) by recovery experiments that was carried out in triplicate preparations on composite blend collected from 10 tablets of ambroxol and azithromycin, analyzed as per the proposed method. The percentage recoveries ranged from 99.93-99.96% for ambroxol and 99.96-99.97% for azithromycin respectively. From the data reported in **Table.5(a) &(b),** revealed that the developed RP-HPLC method was found to be accurate for ambroxol and azithromycin assay.

f. ROBUSTNESS STUDIES: The robustness study of the developed assay method for ambroxol and azithromycin were established in all variance conditions. Assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence, the analytical method would be concluded as robust [**Table.6]**.

g. SOLUTION STABILITY STUDY: The stability studies at 100% test concentration of the above mentioned drugs in mobile phase were carried out for 24hrs at 35ºC. From the above studies the analytes were stable in mobile phase for 24hrs that indicated reliability of analysis in the proposed procedure [**Table.7]**.

h. ANALYSIS OF MARKETED FORMULATION: Analysis of marketed tablets {Azyxin Plus[Azithromycin IP equivalent to Azithromycin (anhydrous) 250 mg Ambroxol Hydrochloride [in sustained release form] 75 mg]} was carried out using the above said optimized mobile phase and HPLC conditions. The % drug content of tablets obtained by the proposed method for ambroxol and azithromycin was found to be 99.44 and 99.91%, respectively [**Table.8]**.

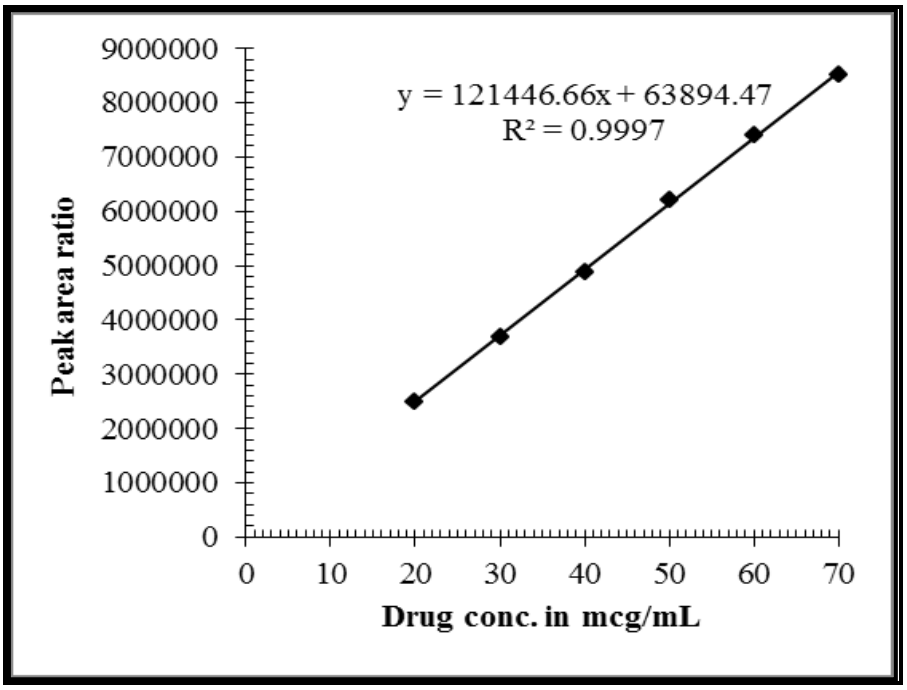


FIG 3.(a). CALIBRATION CURVE FOR AMBROXOL

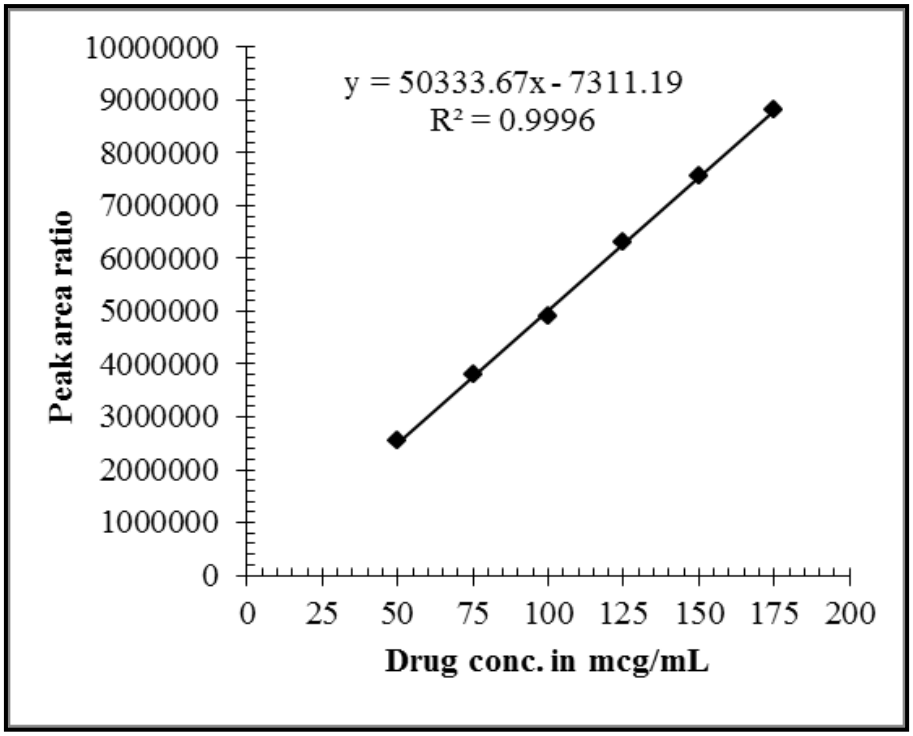


FIG 3.(b). CALIBRATION CURVE FOR AZITHROMYCIN

TABLE.1. SYSTEM SUITABILITY OF AMBROXOL AND AZITHROMYCIN

PARAMETERS	AMBROXOL	AZITHROMYCIN
No. of theoretical plates	8314	4072
Tailing factor	1.416	1.490
Area	4879673	4917564
Retention Time	3.326	4.472

TABLE.2(a). RESULTS OF LINEARITY OF AMBROXOL

µg/mL	PEAK AREA RATIO
20	2494594
30	3696875
40	4876347
50	6198676
60	7394884
70	8512589
Slope,b	**12446.66**
Intercept,a	**63894.47**
Correlation, r^2	**0.9997**

TABLE.2(b).RESULTS OF LINEARITY OF AZITHROMYCIN

µg/mL	PEAK AREA RATIO
50	2542643
75	3796376
100	4907639
125	6310627
150	7564535
175	8809543
Slope,b	**50333.67**
Intercept,a	**-7311**
Correlation, r^2	**0.9996**

Table.3. LOD & LOQ VALUES OF AMBROXOL AND AZITHROMYCIN

	AMBROXOL	AZITHROMYCIN
LOD(µg/mL)	1.47	4.49
LOQ(µg/mL)	4.92	14.98

TABLE.4. RESULTS OF PRECISION OF AMBROXOL AND AZITHROMYCIN

	REPEATABILITY (%ASSAY)		DAY TO DAY (%ASSAY)	
	AMBROXOL	AZITHROMYCIN	AMBROXOL	AZITHROMYCIN
SAMPLE 1	98.48	99.3	99.19	98.79
SAMPLE 2	99.4	99.19	100.76	101.9
SAMPLE 3	100.53	100.07	99.76	99.2
SAMPLE 4	100.1	100.79	99.49	98.3
SAMPLE 5	101.3	99.7	100.93	101.21
SAMPLE 6	100.16	100.92	99.83	100.57
%MEAN*	99.99	99.99	99.99	99.99
SD*	0.967	0.736	0.699	1.44
%RSD*	0.9671	0.736	0.699	1.44

*Average of six determinations

TABLE.5(a).RESULTS OF ACCURACY OF AMBROXOL

RECOVERY LEVEL	AMBROXOL			
	AMOUNT ADDED		AMOUNT FOUND	%RECOVERY
	STANDARD	TEST		
50%	**30**	**10.0**	**39.98**	**99.95**
100%	**50**	**10.0**	**59.96**	**99.93**
150%	**70**	**10.0**	**79.97**	**99.96**
MEAN RECOVERY*	**99.94%**			

*Average of three determinations

TABLE.5(b).RESULTS OF ACCURACY OF AZITHROMYCIN

RECOVERY LEVEL	AZITHROMYCIN			
	AMOUNT ADDED		AMOUNT FOUND	%RECOVERY
	STANDARD	TEST		
50%	**75**	**5.0**	**79.98**	**99.97**
100%	**125**	**5.0**	**139.97**	**99.97**
150%	**150**	**5.0**	**154.94**	**99.96**
MEAN RECOVERY*	**99.96%**			

*Average of three determinations

TABLE.6.RESULTS OF ROBUSTNESS STUDIES OF AMBROXOL AND AZITHROMYCIN

	Changed value	Retention time		Tailing factor	
		ABX	AZTM	ABX	AZTM
COLUMN TEMPERATURE	33°C	3.347	4,45	1.360	1.493
	37°C	3.328	4.45	1.370	1.519
FLOW RATE	1.2mL/Min	2.691	3.608	1.375	1.517
	0.8mL/Min	4.449	5.926	1.39	1.571

TABLE.7.STABILITY DATA OF AMBROXOL AND AZITHROMYCIN

DRUG	% ASSAY AT 0 hr	% ASSAY AT 24 hr	% DEVIATION
AMBROXOL	99.40	99.73	0.99
AZITHROMYCIN	99.91	100.17	0.99

TABLE.8. RESULTS FOR HPLC ANALYSIS OF TABLETS

Sample No.	PEAK AREA	
	AMBROXOL	AZITHROMYCIN
1	99.25	99.67
2	99.44	100.43
3	99.57	99.44
4	99.49	99.24
5	99.39	100.01
6	99.52	100.69
AVG*	99.44	99.91
SD*	0.1135	0.567
%RSD*	0.114	0.568

*Average of six determinations

CONCLUSIONS:

A new simple, precise, accurate, fast, and economical RP-HPLC method was developed and validated for the assay of ambroxol and azithromycin in tablet formulations. The proposed method showed high recoveries with good linearity and precision. It can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of ambroxol and azithromycin in combined formulations in quality control labs.

ACKNOWLEDGEMENTS: The authors are thankful to Hetero Labs Pvt, Hyderabad, for providing the gift sample of ambroxol and azithromycin and Department of Chemistry, Acharya Nagarjuna University,Guntur for providing the technical support during the research.

REFERENCES

[1]. The Merck Index. 12 th ed. 1996.67:877

[2]. Budavari S. The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals. Merck Research Laboratories Division of Merck and Co., Inc. Whitehouse Station. Merck Index: 13th ed., 2001: 67–68.

[3]. United States Pharmacopoeia, USP22-NF17, 2006, 1710-1712.

[4]. J.E.F. Reynolds (Ed.), Martindale: The Extra Pharmacopoeia, 32nd edn, The Pharmaceutical Press, London, 1999, 155.

[5]. Kuchekar BS, Shinde GS, Naikwadi IT, Todkar KJ, Kharade SV. Spectrophotometric Estimation of Ambroxol Hydrochloride in Tablets. Indian J Pharm Sci. 2003;30:193–5.

[6]. Reddy MN, Rao KV, Swapna M, Sankar DG. Spectrophotometric Determination of Ambroxol. East Pharmacist. 1998:125–26.

[7]. Zafer D, Hasan B, Nilgun GG. Quantitative Determination of Ambroxol in Tablets by Derivative UV Spectrophotometric Method and HPLC. J Pharm Biomed Anal. 2003;31:867–72.

[8]. Gunawan I, Ratna H. Quantitative Determination of Ambroxol Hydrochloride in Tablets. J Pharm Biomed Anal. 1993;11:781–4.

[9]. Maarit H, Coral B. Validation of an HPLC Method for The Quantification of Ambroxol Hydrochloride and Benzoic Acid in A Syrup as Pharmaceutical Form Stress Test for Stability Evaluation. J Pharm Biomed Anal. 2001;24:1005–10.

[10]. Botterbolm MHA. Rapid and Sensitive Determination of Ambroxol in Human Plasma and Urine by High-Performance Liquid Chromatography. J Chrom B. 1987;421:211–5.

[11]. Flores-Murrieta FJ, Hoyo-Vadillo C, Hong E, Castaneda-Hernandez G. Assay of Ambroxol in Human Plasma by High-Performance Liquid Chromatography with Amperometric Detection. J Chrom B.1989;490:464–9.

[12]. Koundrourellis JE, Eleftheria TM, Theodora AB. High Performance Liquid Chromatographic Determination of Ambroxol in the Presence of Different Preservatives in Pharmaceutical Formulations. J Pharm Biomed Anal. 2000;23:469–75.

[13]. Colombo L, Marcucci F, Marini MG, Pierfederici P, Mussini E. Determination of Ambroxol in Biological Material by Gas Chromatography with Electron Capture Detection. J Chrom B. 1990;530:141–7.

[14]. Schmid J. Assay of Ambroxol in Biological Fluids by Capillary Gas-Liquid Chromatography. J Chrom B. 1987;414:65–75.

[15]. Hohyun K, Jeong-Yeon Y, Sang BH, Hee JL, Kyung RL. Determination of Ambroxol in Human Plasma by LC-MS/MS. J Pharm Biomed Anal. 2003;32:209–16.

[16]. Pospisilova M, Polasek M, Jokl V. Determination of Ambroxol and Bromhexine in Pharmaceuticals by Capillary Isotachophoresis. J Pharm Biomed Anal. 2001;24:421–8.

[17]. Kamau F.N, Naugi. J.K, Roets. E. and Chepkwony, H.K. "Isocratic liquid chromatographic method for the analysis of Azithromycin in Bulk sample". Journal of Chromatographic science. 2002;24(4): 529-533.

[18]. Biljana Nigovic and Branimir Simunic."Voltammetric assay of Azithromycin in pharmaceutical dosage forms". Journal of pharm Biomed Anal,2003;27(6):115-120.

[19]. Anna Kwiecien, Jan Krzek and Lukasz Biniek; TLC-densitometric determination of Azithromycin in pharmaceutical preparations. In Journal of planer chromatography –Modern TLC, 2008, 177-181

[20]. Shaikh KA, Patil SD, Devkhile AB. Development and validation of a reversed-phase HPLC method for simultaneous estimation of ambroxol hydrochloride and azithromycin in tablet dosage form, J Pharm Biomed Anal. 2008 Dec 15;48(5):1481-4

[21]. M. Senthil Raja, Shan. S.H,, P. Perumal,M.T.S. Moorthy, RP-HPLC Method development and validation for the simultaneous estimation of azithromycin and ambroxol hydrochloride in tabletS, International Journal of PharmTech Research,2(1),2010,36-39.

[22]. M. Sudheer, A. B. N. Nageswara Rao , D. Hari Hara Theja , M. Siva Prakash , P. Ramalingam and A. Madhan Mohan Development of Stability Indicating RP-HPLC Method for Simultaneous Determination of Azithromycin and Ambroxol HCl (SR) in the Tablet Formulation, Der Pharmacia Letters, 2012, 4 (3):803-810.

[23]. ICH Harmonized Tripartite Guideline International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures: Text and Methodology, **2005**, 2(R1).