Journal of Pharma Research
Available online through
www.jprinfo.com

Research Article
ISSN: 2319-5622

RP-HPLC Method Development and Validation for Simultaneous Estimation of Roxithromycin and Ambroxol Hydrochloride in Tablet Dosage form

Vanteru Ramya*, Syed Mujtaba Ahmed
Department of Analysis, Maheshwara Institute of Pharmacy, JNTU, Hyderabad, Telangana, INDIA.

Received on: 24-07-2015; Revised and Accepted on: 03-08-2015

ABSTRACT

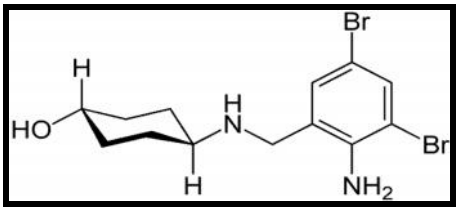
A rapid, precise, and accurate HPLC method for simultaneous estimation of Ambroxol Hcl and Roxithromycin as the bulk drug and in pharmaceutical dosage forms. Chromatographic separation of the drugs was performed on Agilent Zorbax SB C18 (150 x 4.6 mm; 5 µm particle size) analytical column as the stationary phase. The solvent system consisted of 0.1M KH_2PO_4 and methanol in the ratio of 80:20 (v/v) as mobile phase. Evaluation of the separated drugs was performed using a PDA detector covering the range of 200-400 nm. All the two drugs were resolved with the retention time of 2.191min and 4.429 min for ambroxol HCL and roxithromycin respectively. The method was validated with respect to linearity, sensitivity, precision, accuracy and robustness in accordance with ICH guidelines. The validated method was successfully applied to the commercially available pharmaceutical dosage form, yielding good and reproducible result.

Keywords: *Ambroxol HCL, Roxithromycin , HPLC, simultaneous Determination.*

INTRODUCTION

Ambroxol HCL [1, 2] is a mucolytic agent. Chemically, Ambroxol HCL is described as trans-4-(2-Amino-3,5-dibrombenzylamino)-cyclohexanol. Excessive Nitric oxide (NO) is associated with inflammatory and some other disturbances of airways function. NO enhances the activation of soluble guanylate cyclase and cGMP accumulation. Ambroxol HCL has been shown to inhibit the NO-dependent activation of soluble guanylate cyclase. It is also possible that the inhibition of NO-dependent activation of soluble guanylate cyclase can suppress the excessive mucus secretion; therefore it lowers the phlegm viscosity and improves the mucociliary transport of bronchial secretions.

Roxithromycin [3, 4] is a semi-synthetic macrolide antibiotic. Chemically, roxithromycin is known as ((3R,4S,5S, 6R,7R,9R,11S,12R,13S,14R)-6-{[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy}-14-ethyl-7,12,13-trihydroxy-4-{[(2R,4R,5S,6S)-5-hydroxy-4-methoxy4,6-dimethyloxan-2-yl]oxy}-3,5,7,9,11,13-hexa methyl-10-(2,4,7-trioxa-1-azaoctan-1ylidene)-1-oxacyclotetradecan-2one. Roxithromycin prevents bacteria from growing, by interfering with their protein synthesis. Roxithromycin binds to the subunit 50S of the bacterial ribosome, and thus inhibits the translocation of peptides.It is more effective against certain gram-negative bacteria, particularly *Legionella pneumophila*. It can treat respiratory tract, urinary and soft tissue infections [5, 6]. Fig. 1 & 2 shows the chemical structure of Ambroxol Hcl and Roxithromycin respectively.



HCL

Fig. 1: Chemical structure of ambroxol HCL

***Corresponding author:**
Vanteru Ramya
Department of Analysis,
Maheshwara Institute of Pharmacy, JNTU, Hyderabad,
Telangana, INDIA.
**E-Mail: ramyavanteru@gmail.com*

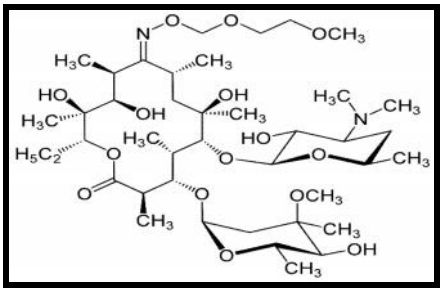


Fig. 2: Chemical structure of roxithromycin

The literature reports, many methods for simultaneous quantitative determination of Ambroxol Hcl and Roxithromycin in bulk, tablet dosage form, capsule dosage form and human plasma. These methods include simultaneous estimation of Ambroxol Hcl and Roxithromycin by UV spectrophotometry [7], HPTLC [8-10], HPLC [11-14], and LC-MS [15]. The summary of reported methods is shown in Table 1.

Few methods have been reported in the literature for the simultaneous determination of Ambroxol Hcl and Roxithromycin, pharmaceutical formulations and biological samples. The aim of the present investigation is to develop and validate a sensitive, precise and accurate RP-HPLC method for the simultaneous quantification of Ambroxol Hcl and Roxithromycin in bulk and in its combined pharmaceutical formulation.

MATERIALS AND METHODS

Apparatus:

A Waters 2695 alliance with binary HPLC pump equipped with Waters 2998 PDA detector and Waters Empower2 software was used in the present investigation.

Mobile phase:

The solvents and chemicals used in the preparation of mobile phase were of HPLC grade and analytical grade, respectively. The mobile phase used was 0.1M KH_2PO_4 and acetonitrile in the ratio of 80:20 *v/v*. Before use, the mobile phase was filtered through millipore membrane filter and degassed for 15 minutes by sonication.

Chromatographic conditions:

Agilent Zorbax SB C18 (150 x 4.6 mm; 5 µm particle size) analytical column was used for separation and simultaneous

analysis of ambroxol HCL and roxithromycin. The column temperature was maintained at 30±1^{0}C. The separation was carried out under isocratic elution. The flow rate was maintained as 1.0 ml/min. The injection volume was 10 µl. The eluents were detected at 238 nm.

Standard solutions:

The standard stock solution was prepared by dissolving 180mg of Ambroxol HCL and 450mg of Roxithromycin in 50 ml mobile phase. Working standard solutions equivalent to 360-1080 µg/ml ambroxol HCL and 900-2700 µg/ml roxithromycin was prepared from stock solution by appropriately diluting the stock standard solution with the mobile phase.

Sample Solution:

Ten tablets were weighed and crushed to a fine powder. The powder equivalent of 60mg of Ambroxol HCL and 150mg of Roxithromycin was taken in a 50 ml volumetric flask containing 20 ml of mobile phase, sonicated for 20 minute and made up to mark with the same solvent. The resultant mixture was filtered through 0.45 µm filter paper. The filtrate was diluted appropriately with the mobile phase to get a final concentration of 180 µg/ml ambroxol HCL and 450µg/ml roxithromycin.

RESULTS AND DISCUSSION

HPLC parameters optimization:

So as to study the simultaneous elution of more than one drug under isocratic conditions, different chromatographic conditions (type of the column, mobile phase composition, flow rate and pH) have been investigated. The objective of the simultaneous HPLC method development was to achieve a peak tailing factor <2, USP plate count ≥ 2000, retention time in between 3 and 5 minutes, along with good resolution. This objective was obtained using mobile phase consisting of 0.1M potassium dihydrogen phosphate – acetonitrile in the proportion of (80/20, *v/v*). The pH of the mobile phase was adjusted to 4.5 with orthophosphoric acid. Under the above described conditions, the analyte peaks were well defined, resolved and free from tailing. The tailing factors were <2 for both the peaks. The elution orders were ambroxol HCL (retention time- 2.191 min) and roxithromycin (retention time- 4.429 min) at a flow rate of 1.0 ml/min (Fig. 3). The optimum wavelength for detection was 238 nm at which much better detector responses for the selected drugs were obtained.

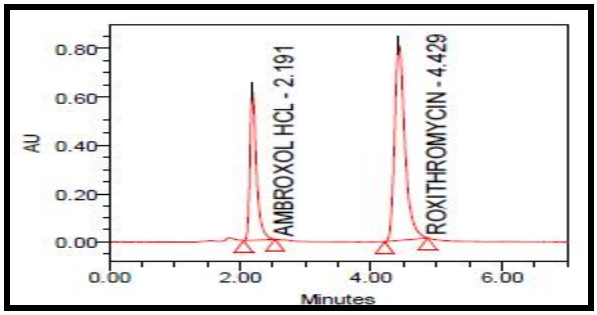


Fig. 3: Typical chromatogram of ambroxol HCL and roxithromycin

Method validation:

The optimized RP-HPLC method for simultaneous assay of Ambroxol Hcl and Roxithromycin was validated according to ICH guidelines [19] with respect to system suitability, linearity, sensitivity, accuracy, precision and robustness.

System suitability:

In relation to U.S. Pharmacopeia, system suitability tests are an integral part of a liquid chromatographic method. System suitability tests are used to confirm that the column efficiency, resolution and reproducibility of the chromatographic system are sufficient for the analysis. System suitability test was assessed from five replicate injections of the standard solution containing 180µg/ml ambroxol HCL and 450 µg/ml roxithromycin. The results of system suitability in comparison with the required limits are shown in Table 1. The results are found to be suitable and are within the accepted limits.

Table No. 1: System suitability test of the HPLC method

Parameters	Results		Recommended limits
	Ambroxol HCL	Roxithromycin	
Retention time	2.191	4.429	-
Peak area	4294100 (%RSD – 0.5)	8881136 (%RSD – 0.4)	RSD ≤1
USP resolution	-	9.545	> 1.5
USP plate count	3547	4091	> 2000
USP tailing factor	1.270	1.295	≤ 2

Linearity and range:

The linearity of the method was determined by analyzing five different concentrations of each drug. The calibration curve was plotted by area under the peak responses of the drugs against their corresponding concentrations. Calibration curves were linear over the concentration range of 360-1080 µg/ml for ambroxol HCL and 900-2700 µg/ml for roxithromycin. The parameters such as a regression equation and regression coefficient are given in Fig. 4 & 5. The results show a good correlation between the peak areas of the drugs and their corresponding concentrations.

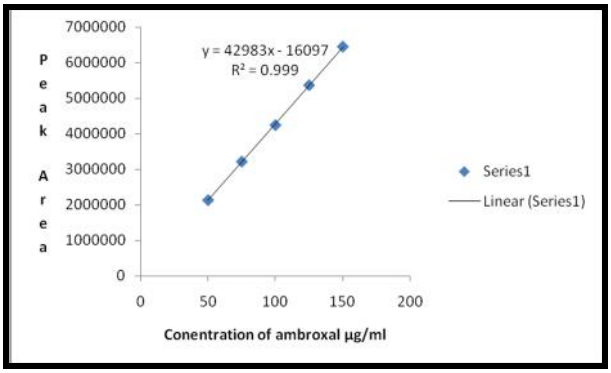


Fig. 4: Linearity curve of ambroxol HCL

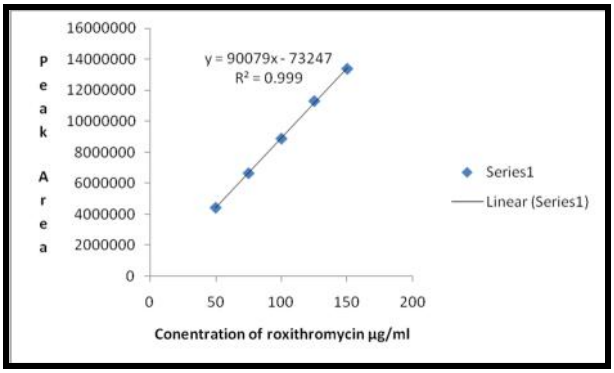


Fig. 5: Linearity curve of roxithromycin

Sensitivity:

The sensitivity of the method was assessed by calculating limit of detection (LOD) and limit of quantification (LOQ) according to ICH guidelines. The results are summarized in Table 2. The low values of LOD and LOQ demonstrate the sufficient sensitivity of the method. The chromatograms of ambroxol HCL and roxithromycin at LOD and LOQ levels are presented in Fig. 6 & 7.

Table No. 2: Sensitivity of the HPLC method

Parameters	Results	
	Ambroxol HCL	Roxithromycin
LOD	1.875	2.1054
LOQ	6.249	7.0180

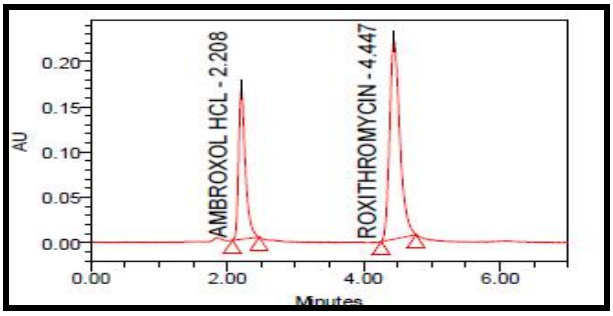


Fig. 6: Chromatogram of ambroxol HCL and roxithromycin at LOD level

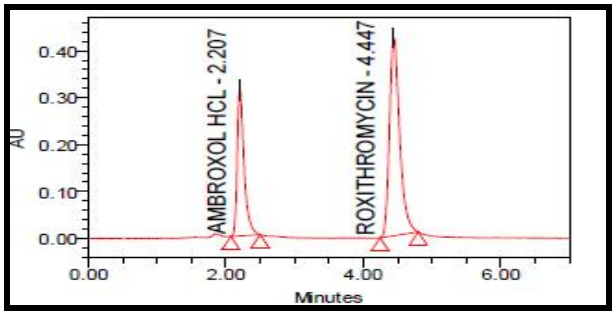


Fig. 7: Chromatogram of ambroxol HCL and roxithromycin at LOQ level

Precision:

Precision was determined by injecting six standard solutions of ambroxol HCL (180µg/ml) and roxithromycin (450µg/ml). The peak areas were determined. Relative standard deviation of peak areas of the two drugs was then calculated to represent precision. The results are summarized in Table 3. The low % RSD values indicated that the method was precise.

Table No. 3: Precision of the HPLC method

Ambroxol HCL		Roxithromycin	
Peak area	%RSD	Peak area	%RSD
4294452	0.06	8888034	0.03
4293751		8883298	
4299290		8882692	
4293097		8881404	
4297314		8886576	
4297203		8880015	

Accuracy:

Accuracy of the method was evaluated by recovery studies at three concentration (50%, 100%, and 150%) levels by standard addition method. The mean percentage recoveries obtained were shown in Table 4. The good % recovery values showed that the method was highly accurate.

Table No. 4: Accuracy of the HPLC method

Drug	Spiked Level	µg/ml added	µg/ml found	% Recovery	% Mean
Ambroxol HCL	50%	356.760	356.59	100	100
	50%	356.760	355.55	100	
	50%	356.760	356.18	100	
	50%	356.760	356.06	100	
	50%	356.760	356.03	100	
	50%	356.760	356.44	100	
	100%	713.520	712.76	100	100
	100%	713.520	712.90	100	
	100%	713.520	711.83	100	
	150%	1070.362	1069.53	100	100
	150%	1070.362	1068.80	100	
	150%	1070.362	1068.93	100	
	150%	1070.362	1068.77	100	
	150%	1070.362	1069.01	100	
	150%	1070.362	1068.91	100	
Roxithromycin	50%	897.300	898.75	100	100
	50%	897.300	898.73	100	
	50%	897.300	897.72	100	
	50%	897.300	898.09	100	
	50%	897.300	898.67	100	
	50%	897.300	897.87	100	
	100%	1794.600	1795.69	100	100
	100%	1794.600	1795.17	100	
	100%	1794.600	1796.13	100	
	150%	2692.106	2691.61	100	100
	150%	2692.106	2701.44	100	
	150%	2692.106	2695.25	100	
	150%	2692.106	2697.31	100	
	150%	2692.106	2700.68	100	
	150%	2692.106	2699.39	100	

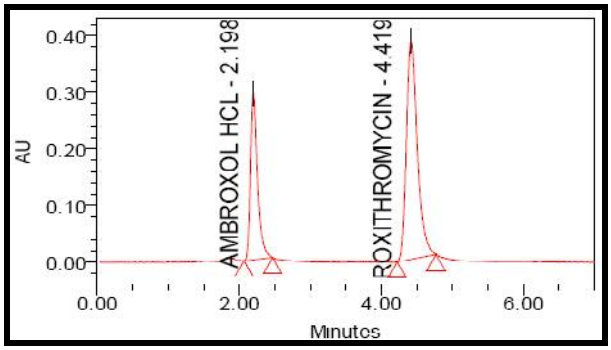


Fig. 8: Chromatogram of Ambroxol Hcl and Roxithromycin at 50% level

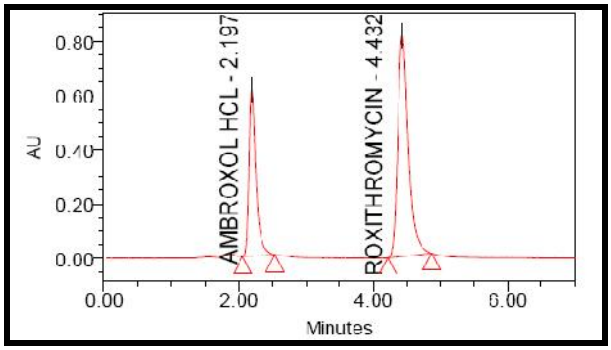


Fig. 9: Chromatogram of ambroxol HCL and roxithromycin at 100% level

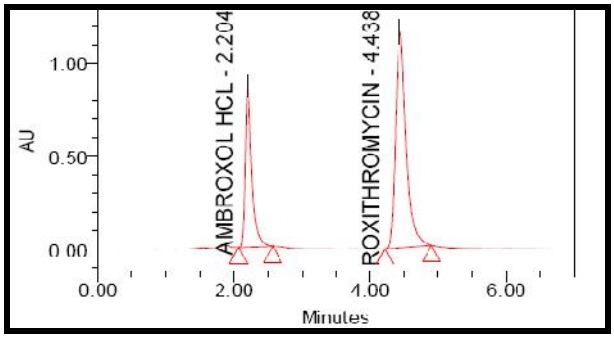


Fig. 10: Chromatogram of ambroxol HCL and roxithromycin at 150% level

Robustness:

In order to show the robustness of the method, system suitability parameters were evaluated at different flow rate and different column temperature. The parameters used to define robustness are retention time, USP tailing factor and USP plate count. The results showed (Table 5) that slight variations in method parameters had a negligible effect on the analysis.

Table No. 5: Robustness of the method

Drug	Parameter	Retention time	Peak area	USP Plate Count	USP Tailing
Ambroxol HCL	Flow 1	2.730	5639037	3610	1.219
	Flow 2	2.198	4335584	3541	1.202
	Temperature1	2.211	4334741	3521	1.208
	Temperature2	2.190	4355809	3740	1.286
Roxithromycin	Flow 1	5.515	11451995	4443	1.298
	Flow 2	4.458	8880153	4283	1.284
	Temperature1	4.456	8912867	4107	1.283
	Temperature2	4.300	8935384	4819	1.271

CONCLUSION

A RP-HPLC method has been reported for simultaneous estimation ambroxol HCL and roxithromycin. The proposed method gives good resolution of the two drugs. The validation of developed method was done as per ICH guidelines and proved that method to be simple, sensitive, precise, accurate, selective and robust. The validated method was successfully applied to the determination of commercially available tablet dosage. The method can be used for the routine quality control analysis of tablet dosage forms containing ambroxol HCL and roxithromycin.

ACKNOWLEDGEMENT

The authors thank Department of Analysis, Maheshwara Institute of Pharmacy, JNTU-H, and Hyderabad for providing necessary facilities.

REFERENCES:

1. Abd-Elbary, M. Haider, and S. Sayed. *Pharmaceutical Development and Technology*, **2011**; 1-12.
2. Hang TJ, Zhang M, Song M, Shen JP, Zhang YD. Clin Chim Acta, **2007**; 382(1-2): 20-4.
3. Avinash V, Deosarkar et al., Int J Pharm & Pharm Sci., **2012**; (4): pp 125-129.
4. S.Budavari.Merck Index:An Encyclopedia of chemicals, drugs, & biological. 12th editon Merck research division. **1966**; 67.
5. European Pharmacopoeia,Council of Europe, Strasbourg: 3rd edn, **1996**; p.1448.
6. P.D. Sethi. HPLC: Quantitative Analysis Pharmaceutical Formulations, *CBS Publishers and distributors*, New Delhi (India), **2001**; P. 3-137.
7. Klaus Florey. Analysis Profile of Drugs Substances, *Academic Press*, New York, **2005**; P. 406-435.
8. www.drugbank.ca/drugs/DB06742.
9. www.drugbank.ca/drugs/DB00778.
10. FDA Issues Safety Labeling Changes for AMBROXIT.
11. Esther Roset Shobana and Lakshmi Sivasubramanian. *Asian J. Chem.,* **2008**; 20(6): pp 4159-4162.
12. Yogini Jaiswal, Mohd. Farooq Shaikh, Rakesh Kenjale and Gokul Talele, *Eurasian J. Anal. Chem.,* **2012**; 7(3): pp 168-179.
13. Saira mulla, Anand patil, Sachin morge, Suhas kulkarni. *IOSR Journal of Pharmacy.,* **2012**; 2(5): Pp. 4-7.
14. Ashvin Dhudhrejiya, Pradip chovatiya, Navin Sheth. *Inventi Journals Pvt. Ltd.,* **2012**.
15. Prabhavathi Gutla,Dhanalakshmi K, Nagarjuna Reddy G and Anil kumar P. *Int. J. Pharma. Sci.,* **2013**; 3(5): pp. 356-359.
16. Esther Roset Shobana and Lakshmi Sivasubramanian. *Asian J. Chem.,* **2008**; 20(6): pp. 4159-4162.

17. Meiling Qi, Peng Wang, Ruihua Cong, Jianjun Yang. *J. Pharm. Biomed. Anal.,* **2004**; 35(5): pp. 1287-91.
18. Tai-jun Hang,Meng Zhang·Min Song·Jian-ping Shen·Yin-di Zhang. *Clinica. Chimica. Acta.,* **2007**; 382(1-2): pp. 20-24.
19. International Conference on Harmonization, ICH Guidelines, and Validation of Analytical Procedures Technical Requirements for Registration of Pharmaceuticals for Human Use: Text and Methodology Q 2 (R1), International Conference on Harmonization, Geneva, Switzerland, and November **2005**.

How to cite this article:

Vanteru Ramya, Syed Mujtaba Ahmed: RP-HPLC Method Development and Validation for Simultaneous Estimation of Roxithromycin and Ambroxol Hydrochloride in Tablet Dosage form, J. Pharm. Res., 2015; 4(7): 263-267.

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil