International Journal of Pharmacy and Industrial Research (IJPIR)

IJPIR |Vol.14 | Issue 4 | Oct - Dec -2024

www.ijpir.com

DOI : https://doi.org/10.61096/ijpir.v14.iss4.2024.344-353

ISSN: 2231-3656

Print: 2231-3648

Research

Simultaneous Estimation of Ambroxol And Roxithromycin By Using RP-HPLC In Api & Marketed Formulations

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Published on: 06 Nov 2024

Published by:
DrSriram Publications

Abstract

A Rapid and Precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Roxithromycin and Ambroxol, in its pure form as well as in tablet dosage form. Chromatography was carried out on Altima C18 (4.6 x 150mm, 5µm) column using a mixture of ACN, Methanol and Phosphate buffer pH4.6 (10:25:65 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 215 nm. The retention time of the Roxithromycin and Ambroxolwas 2.344, 3.286±0.02min respectively. The method produce linear responses in the concentration range of 10-50mg/ml of Roxithromycinand 2.5-12.5mg/ml of Ambroxol. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Roxithromycin, Ambroxol, RP-HPLC, validation.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure. Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy.

Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance.[2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.[1,2]

Different methods of analysis

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample. E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.[2]

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture. E.g. Potentiometry, Conductometry, Amperometry.[2]

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics. E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC). [2]

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry).

Introduction to HPLC

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 μm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance[6].

Types of HPLC Techniques

Based on Modes of Chromatography

These distinctions are based on relative polarities of stationary and mobile phases

Reverse phase chromatography: In this the stationary phase is non-polar and mobile phase is polar. In this technique the polar compounds are eluted first and non polar compounds are retained in the column and eluted slowly. Therefore it is widely used technique.

Normal phase chromatography: In this the stationary phase is polar and mobile phase is non-polar. In this technique least polar compounds travel faster and are eluted first where as the polar compounds are retained in the column for longer time and eluted.[4]

Based on Principle of Separation

Liquid/solid chromatography (Adsorption)

LSC, also called adsorption chromatography, the principle involved in this technique is adsorption of the components onto stationary phase when the sample solution is dissolved in mobile phase and passed through a column of stationary phase. The basis for separation is the selective adsorption of polar compounds; analytes that are more polar will be attracted more strongly to the active silica gel sites. The solvent strength of the mobile phase determines the rate at which adsorbed analytes are desorbed and elute. It is widely used for separation of isomers and classes of compounds differing in polarity and number of functional groups. It works best with compounds that have relatively low or intermediate polarity.[3]

Liquid/Liquid chromatography (Partition Chromatography)

LLC, also called partition chromatography, involves a solid support, usually silica gel or kieselguhr, mechanically coated with a film of an organic liquid. A typical system for NP LLC column is coated with ß, ß'-oxy dipropionitrile and a non-polar solvent like hexane as the mobile phase. Analytes are separated by partitioning between the two phases as in solvent extraction. Components more soluble in the stationary liquid move more slowly and elute later.[1,2]

Ion exchange

In this the components are separated by exchange of ions between an ion exchange resin stationary phase and a mobile electrolyte phase. A cation exchange resin is used for the separation of cations and anion exchange resin is used to separate a mixture of anions.[3.16,17]

Size exclusion

In this type, the components of sample are separated according to their molecular sizes by using different gels (polyvinyl acetate gel, agarose gel). ex: separation of proteins, polysaccharides, enzymes and synthetic polymers.[3,15]

Chiral chromatography

In this type of chromatography optical isomers are separated by using chiral stationary phase.

Affinity chromatography

In this type, the components are separated by an equilibrium between a macromolecular and a small molecule for which it has a high biological specificity and hence affinity.

MATERIALS AND METHODS

Roxithromycin (Pure)-Sura labs, Ambroxol (Pure)-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC- Merck.

HPLC method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Roxithromycin and Ambroxol working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Pipette out 0.3 ml of Roxithromycin and 0.75ml of Ambroxol stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Orthophosphoric acid and Phosphoric acid (pH 3): Acetonitrile and Methanol: ACN with varying proportions. Finally, the mobile phase was optimized to Buffer: Methanol: ACN in proportion 65:25:10v/v respectively.

Optimization of Column

The method was performed with various C18 columns like ODS and Zodiac column. Altima C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used : Waters HPLC with auto sampler and PDA detector 996 model.
Temperature : 35ºc
Column : Altima C18 (4.6×150mm, 5µ)
Buffer : Phosphate buffer (pH-4.6)-Dissolve 0.9g of anhydrous dihydrogen phosphate and 1.298g of Citric acid mono hydrate in sufficient water to produce 1000mL. Adjust the pH 4.6 by using ortho phosphoric acid.
pH : 4.6
Mobile phase : Buffer: Methanol: ACN (65:25:10v/v)
Flow rate : 1ml/min
Wavelength : 212 nm
Injection volume : 10 µl
Run time : 14 min

Validation

Preparation of buffer and mobile phase

Preparation of Phosphate buffer (pH-4.6)

Dissolve 0.9g of anhydrous di hydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce 1000mL. Adjust the p H 4.6 by using ortho phosphoric acid.

Preparation of mobile phase

Accurately measured 650 ml (65%) of Buffer and 250 ml of Methanol (25%) and 100ml (10%) of Acetonitrile were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Buffer: Methanol: ACN (65:25:10v/v)
Column : Altima C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 215 nm
Column temp : 38ºC
Injection Volume : 10 µl
Run time : 5 minutes

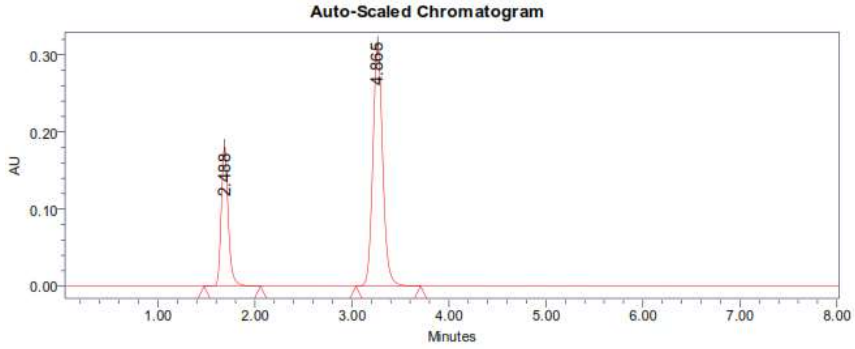

Fig 1: Optimized Chromatogram

Table 1: - peak results for optimised

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ambroxol	2.488	1308595	247456		1.2	5835.5
2	Roxithromycin	4.866	124505	19187	6.0	1.1	5745.2

From the above chromatogram it was observed that the Ambroxol and Roxithromycin peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

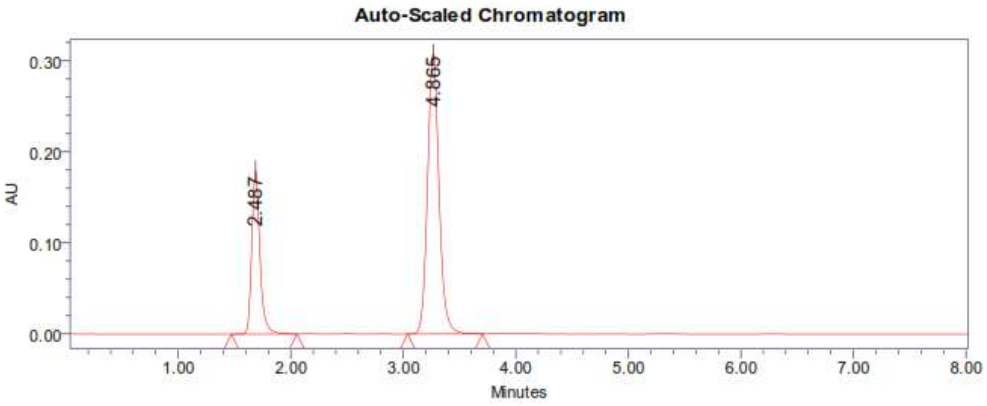

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ambroxol	2.487	1307139	246586		1.2	5565.5
2	Roxithromycin	4.865	124452	19117	6.0	1.1	5355.2

- *Resolution between two drugs must be not less than 2*
- *Theoretical plates must be not less than 2000*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

Assay (Standard)

Table 3: Peak results for assay standard

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Ambroxol	2.488	1308945	247282		1.3	5568.0	1
2	Roxithromycin	4.838	124336	19189	6.0	1.2	5359.2	1
3	Ambroxol	2.492	1309481	247456		1.0	5565.5	2
4	Roxithromycin	4.846	124505	19187	6.0	1.3	5355.2	2
5	Ambroxol	2.493	1317926	247578		1.0	5545.5	3
6	Roxithromycin	4.844	124903	19210	6.0	1.3	5352.1	3

Assay (Sample)

Table 4: Peak results for Assay sample

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Ambroxol	2.494	1307139	246586		1.3	6568.0	1
2	Roxithromycin	4.840	124452	19117	6.0	1.1	5359.2	1
3	Ambroxol	2.491	1308903	248422		1.3	5565.5	2
4	Roxithromycin	4.842	124632	19178	6.0	1.2	5355.2	2
5	Ambroxol	2.491	1325993	248924		1.3	5391.1	3
6	Roxithromycin	4.834	126697	19237	6.0	1.2	5564.0	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Ambroxol and Roxithromycin in pharmaceutical dosage form was found to be 100.3%.

Linearity
Chromatographic data for linearity study
Ambroxol

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	2.5	47510
66.6	5	85701
100	7.5	124802
133.3	10	162731
166.6	12.5	199732

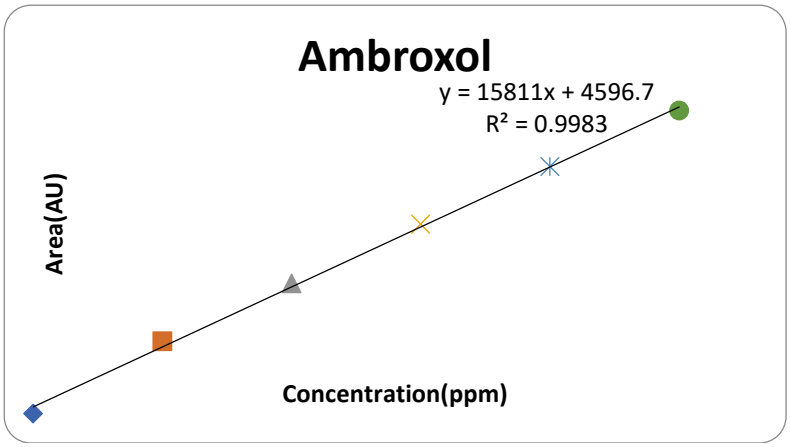

Fig 3: Calibration graph for Ambroxol

Roxithromycin

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	10	518934
66	20	956781
100	30	1413873
133	40	1863458
166	50	2267084

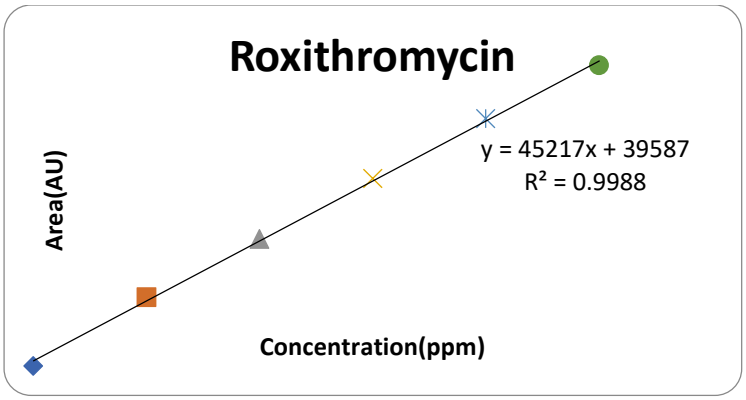

Fig 4: Calibration graph for Roxithromycin

Intermediate precision
Day 1

Table 5: Results of Intermediate precision for Ambroxol

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ambroxol	2.487	1300148	247140	5568.0	1.0
2	Ambroxol	2.489	1304520	245696	5359.2	1.1
3	Ambroxol	2.489	1305937	247870	5565.5	1.0
Mean			1305604			
Std. Dev			3034.47			
% RSD			0.23			

- *%RSD of different sample solutions should not more than 2*

Table 6: Results of Intermediate precision for Roxithromycin

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Roxithromycin	4.882	122487	19115	5568.0	1.0	2.5
2	Roxithromycin	4.877	122626	19003	5359.2	1.1	2.5
3	Roxithromycin	4.877	122632	19073	5565.5	1.0	2.5
Mean			122728.5				
Std. Dev			193.8492				
% RSD			0.15				

- *%RSD of different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Table 7: Results of Intermediate precision Day 2 for Ambroxol

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ambroxol	2.490	1305937	247870	5568.0	1.0
2	Ambroxol	2.489	1306476	246764	5359.2	1.1
3	Ambroxol	2.493	1308271	247280	5565.5	1.0
Mean			1305604			
Std. Dev			3034.47			
% RSD			0.23			

- *%RSD of different sample solutions should not more than 2*

Table 8: Results of Intermediate precision for Roxithromycin

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Roxithromycin	4.874	122702	19123	5568.0	1.0	2.5
2	Roxithromycin	4.873	122962	19165	5359.2	1.1	2.5
3	Roxithromycin	4.878	122962	19165	5565.5	1.0	2.5
Mean			122740.2				
Std. Dev			188.9237				
% RSD			0.15				

- *%RSD of different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Accuracy
The accuracy results for Ambroxol

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	716072.7	15	14. 9	99.3	99.6%
100%	1404258	30	30.1	100.3	
150%	2064609	45	44.7	99.3	

The accuracy results for Roxithromycin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	63467	3.75	3.72	100.8	100.4%
100%	124353.3	7.5	7.57	100. 9	
150%	178607.7	11.25	11.20	99.5	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness
Ambroxol

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1308495	2.344	5568.2	1.3
Less Flow rate of 0.9 mL/min	1300148	2. 244	5922.2	1.2
More Flow rate of 1.1 mL/min	1306476	2.243	5868.8	1.2
Less organic phase	1304520	2.345	5836.2	1.2
More organic phase	1207845	2.344	5282.6	1.1

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Roxithromycin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	124505	3.286	6098.1	1.2
Less Flow rate of 0.9 mL/min	156550	3.181	5999.1	1.2
More Flow rate of 1.1 mL/min	122702	3.181	5989.2	1.1
Less organic phase	122626	3.278	6387.2	1.1
More organic phase	1207845	3.015	4417	1.1

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Roxithromycin and Ambroxol in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Roxithromycin and Ambroxol was freely soluble in ethanol, methanol and sparingly soluble in water. Buffer: Methanol: ACN (65:25:10v/v)was chosen as the mobile phase. The solvent system used in this method waseconomical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Roxithromycin and Ambroxol in bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, Department of Pharmacy, Princeton college of pharmacy in Narapally, Ghatkesar, Telangana, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

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