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Development and validation of hptlc method for the simultaneous estimation of roxithromycin and ambroxol hydrochloride in combined dosage form

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Abstract*—The simple, accurate and precise method For simultaneous determination of Roxythromycin (ROX) and Ambroxol Hydrochloride (AMB) in tablets by HPTLC methods is developed, In this method, the chromatograms were developed using a mobile phase of Benzene: Diethyl ether: Triethylamine (4 :5: 1) on precoated plate of Silica gel 60 F_{254} and quantified by densitometry absorbance mode at 365 nm for ROX and 255nm for AMB, Rf of ROX and AMB were 0.95 and 0.36 respectively.

Keywords*—Thin layer chromatography, densitometry, validation and quantification, Roxithromycin, Ambroxol hydrochloride.

I. INTRODUCTION

Roxithromycin is chemically {3R,4S,5S,6R,7R,9R,11S,12R,13S,14R)-6[(25,3R,45,6R)-4-dimethylamino-3-hydroxy 6-methyloxan-2-yl]oxy-14- ethyl-7,12,13-trihydroxy- [(2R,4R,5S,6S)-5 hydroxy- 4-methoxy-4,6-dimethyloxan-2-yl]oxy-10-(2-methoxymethoxyimio)-3,5,7,9,11,13-hexamethyt-1-oxacycloTetradecan-2 one. It is a semi-synthetic antibiotic which is used to treat respiratory tract, urinary and soft tissue infections.

A survey of literature revealed a colorimetric method for determination of roxithromycin, spectrophotometric method, LC-Ms method.Ambroxol hydrochloride is a mucolytic agent used in treatment of respiratory disorders associated with viscid or excessive mucus. Chemically it is Trans-4-(2-amino-3,5dibromobenzyl) amino) cyclohexanol.Literature survey revealed few methods have been reported for estimationAmbroxol hydrochloride by spectrophotometry Colorimetric method & HPTLC method.

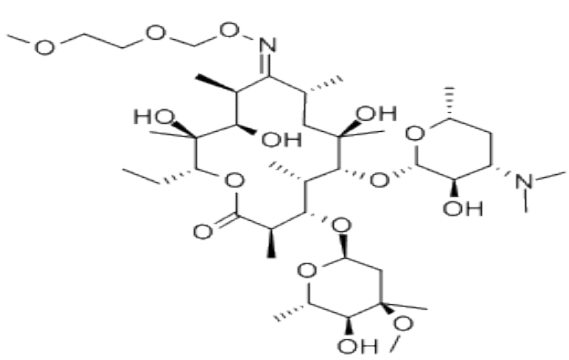


Figure 1 Structure of Roxithromycin

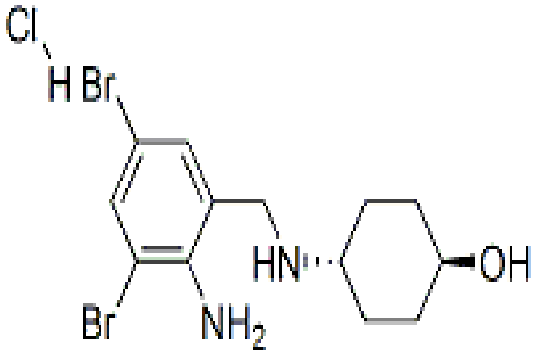


Figure 2 Structure of Ambroxol hydrochloride

II. MATERIALS AND METHODS

Instrumentation

HPTLC system equipped with CAMAG LINOMAT V automatic sampler applicator; CAMAG TLC SCANNER; computer integrator controlled by CATS4 software; CAMAG twin trough glass chamber with stainless steel lid. Precoated silica gel F_{254} on aluminum sheets (20 x 20cm).

Chemicals and reagents

Standard sample of ROX and AMB were provided by central drug Testing Lab. Mumbai. Tablets of combined form were procured from the market. All other reagents were analytical grade. Benzene, Diethyl ether, Triethylamine and Dichloromethane was obtained from science House (Mumbai, India). Stationary phase used is silica gel F_{254} precoated aluminum plates.

Preparation of stock solution

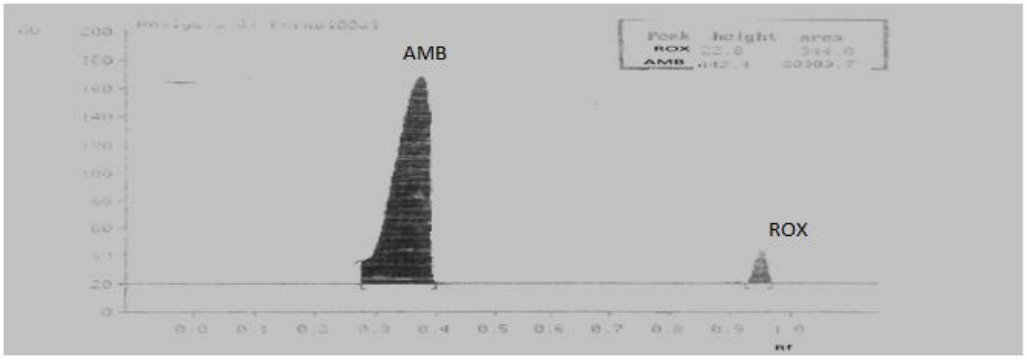
Accurately 10mg of AMB and 10mg of ROX reference std is weighed and transferred in 10ml volumetric flask 5ml dichloromethane (DCM) is added, sonicated for 15 minutes and diluted to prepare stock solution with DCM.

Sample preparation

To determine the content of AMB and ROX in tablet (label claim: 60mg AMB and 150mg ROX) 5 tablets were weighed; average weight is taken and crushed to fine powder. 10mg from it is transferred to 10ml volumetric flask 5ml DCM is added and sonicated and diluted further with DCM.

III. CHROMATOGRAPHY

Linear ascending development was carried out in a 20cm x 10cm twin though glass chamber (CAMAG) using the mobile phase Benzene: Diethyl ether: Triethyl ether (4:5:1) (v/v). The chamber is saturated for 15 minutes. Plates were dried in a current of air with the help of hair dryer. The source of radiation utilized is deuterium lamp emitting a continuous UV spectrum between 200nm and 400nm. Slit dimension were 5mm x .045mm and the scanning speed of $20mms^{-1.}$ For preparation of the calibration curve 1mg/ml of working standard of AMB and ROX in DCM is prepared. Calibration curve from 10-200µg/ml for AMB is prepared and check for reproducibility, linearity and validating the proposed method. Same procedure is repeated for ROX using standard solution in the range of 50-250µg/ml.Sample was spotted on precoated TLC plates by using Linomat 5 automatic sampler. TLC plates were developed up-to 8cm. contents of AMB and ROX were determined by comparing area of the chromatogram of sample with calibration curve of working standard both.



Densitogram of Roxithromycin and ambroxol hydrochloride

IV. RESULT AND DISSCUSSION

Validation of method

Development method is validated in terms of linearity, accuracy, precision, LOD, LOQ, robustness. The linear regression data (n=5) showed a good linear relationship over a concentration range of 10-200µg/ml for AMB and 50-250µg/ml for ROX.

Precision

The intraday and inter day precision of the method were estimated by performing six determination of drugs solution at two different concentrations; the results obtained as shown in table 1&2;

Table 1
n=6 (Ambroxol)

Amount µg/band	Intra-day precision		Inter-day precision	
	Mean area (AU)	RSD (%)	Mean area (AU)	RSD (%)
50	6200	0.18	5800	0.90
100	13800	0.38	12800	1.62

Table 2
n=6 (Roxithromycin)

Amount µg/band	Intra-day precision		Inter-day precision	
	Mean area (AU)	RSD (%)	Mean area (AU)	RSD (%)
50	290	0.17	300	0.98
100	604	0.40	625	1.49

Robustness

Robustness was measured by analysis of the sample solution by making small changes to mobile phase composition. Benzene: Diethyl ether: triethyl amine in the ratio 4.0:5.0:1.0 (v/v) and Benzene: Diethyl ether: triethyl amine in the ratio of 3.5:4.5:2.0 (v/v) were selected with different distances 8 & 9cm for different amount of AMB & ROX in the concentration of 50 to 100mcg per band; the low values of RSD obtained after introduction of small changes in mobile phase shown in table 3 were indicative of the robustness;

Table 3
Ambroxol 50 µg n=2(bands)

Condition	Recovery %	RSD in %
Benzene: diethyl ether:triethyl amine in the ratio 4:5:1(v/v)	100.5	0.78
Benzene: diethyl ether:triethyl amine in the ratio 3.5:4.5:2(v/v)	99.19	1.21
Development distance		
8cm	101.29	0.89
9cm	99.99	1.18

Table 4
Roxithromycin 100 µg n=2(bands)

Condition	Recovery %	RSD in %
Benzene: diethyl ether:triethyl amine in the ratio 4:5:1(v/v)	100.80	1.08
Benzene: diethyl ether:triethyl amine in the ratio 3.5:4.5:2(v/v)	99.02	1.12
Development distance		
8cm	102.9	1.02
9cm	99.98	1.23

Limit of detection & Limit of quantization

The limits of detection (LOD) and limit of quantitation (LOQ) were calculated from slopes of the calibration plots and the standard deviation (SD) of the response by the use of the equations LOD 3.0 XSD/S and LOQ 10 XSD/S. the limit of detection and limit of quantitation obtained by this method for ambroxol 0.5mcg and 480mcg; roxithromycin 10mcg and 450mcg respectively.

Specificity

Specificity of the method is ascertained by analyzing reference standard and samples. The bands for ambroxol and roxithromycin formulations were confirmed by comparing R_f values and U.V spectra of these separated bands with those from standard the peak purity of ambroxol and roxithromycin accessed by comparing the spectra acquired at the peak start(S) peak apex(N) and peak end(E) of a band. It was found that r(S, M) = 0.999 and r (M, E) = 0.999, it was in good correlation(r=0.999) was also obtained between standard and spectra of samples containing AMB and ROX.

Recovery

The analyzed samples was spiked with an additional 25,50,75,100 mcg of Ambroxol & Roxithromycin standard 7 mixture were analyzed again, in triplicate by proposed method, to check different amounts if the drug from the formulation, Recovery was 99.98-101.09% which is shown in table 5 & 6.

Table 5
(Ambroxol)

Amount of Drug added (%)	Theoretical Content (mcg)	Recovery (%)	RSD
25	125	100.80	1.08
50	150	101.45	1.39
75	175	101.98	1.34
100	200	99.06	1.29

Table 6
(Roxithromycin)

Amount of Drug added (%)	Theoretical Content (mcg)	Recovery (%)	RSD
25	125	99.989	1.02
50	150	100.34	1.32
75	175	101.01	1.08
100	200	99.01	1.19

Ruggedness

Ruggedness is measure of reproducibility of a test results under normal, expected operating Condition from instrument & from analyst to analyst, ruggedness was tested by analysis of 50,100mcg Ambroxol, and roxithromycin for per band were listed in table 7 & table 8 respectively.

Table 7
(Ambroxol)

Variable	Recovery	RSD
Analyst I	98.98	1.12
Analyst II	100.98	1.21

Table 8
(Roxithromycin)

Variable	Recovery	RSD
Analyst I	100.02	1.01
Analyst II	99.922	0.999

V. CONCLUSION

The proposed method HPTLC method is simple, economic, accurate, & reproducible & can be used in routine analysis for simultaneous determination of AMB & ROX in combined dosage form.

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