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Simultaneous high performance thin-layer chromatography determination of gatifloxacin and ambroxol hydrochloride in pharmaceutical formulation

M.M.Baing*, V.V.Vaidya, N.J.Rane, G.R.Singh, W.Shah

Department of Chemistry, Ramnarain Ruia College, Matunga, Mumbai - 400 019, (INDIA)

E-mail : manisha_baing@yahoo.com; vikas_vaidya@yahoo.com

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ABSTRACT

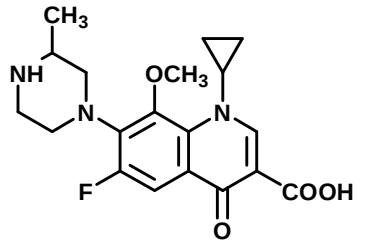
A simple, economic, fast and precise High performance thin layer chromatographic method has been developed for the simultaneous determination of Gatifloxacin and Ambroxol HCl from the tablet formulation. Ciprofloxacin is used as internal standard. Gatifloxacin is antibiotic drug and Ambroxol is a mucolytic expectorant, and this combination is used as antibiotic drug. The separation was performed on Silica gel $60F_{254}$ HPTLC plates with n-Butanol: GAA: Distilled water: methanol in the proportion (4:1:1:0.3) v/v, as a solvent system. The determination was carried out using the densitometric absorbance mode at 235 nm. The linearity range for Gatifloxacin and Ambroxol hydrochloride 480 μg mL^{-1} to 1120 μg mL^{-1} and 72 μg mL^{-1} to 168 μg mL^{-1} respectively. The HPTLC method was evaluated in terms of sensitivity linearity, accuracy, precision and reproducibility.

KEYWORDS

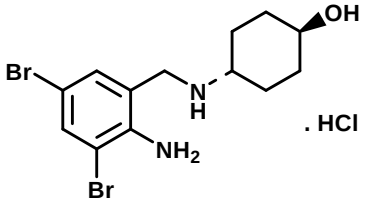
Thin layer chromatography;
Pharmaceutical formulation.

INTRODUCTION

Gatifloxacin is chemically 1-cyclopropyl-6-fluoro-1-4 dihydro-8-methoxy-7-(3-methyl-1-piperazenyl)-4-oxo-3-quinoline carboxylic acid which due to its extremly high activity is used as antibiotic for treating infections with gram positive and gram negative bacteria . Ambroxol hydrochloride is chemically trans-(2-amino3,5-dibromobenzyl) amino cyclohexanol hydrochloride. It is a metabolite of bromgexine and acts to reduce the viscosity of tenacious mucous secretions via fragmentation of long mucopolysaccharide chains, it also enhances penetration power of antibiotic. The structures of these two drugs are shown in Figure 1[1]. This combination is used as antibiotic drug. The literature revealed no method was available for simultaneous de-



Gatifloxacin



Ambroxol hydrochloride

Figure 1 : The structure of gatifloxacin and ambroxol hydrochloride

termination Gatifloxacin and Ambroxol hydrochloride in such pharmaceutical formulation by HPTLC[2-4]. The method described is economic, simple, fast, precise and accurate for simultaneous determination of Gatifloxacin and Ambroxol hydrochloride from pharmaceutical formulation.

EXPERIMENTAL

Chemical and reagents

Standards were from reputed research centre. Gatrich tablets manufactured by Cipla were procured from the market. Methanol, n-butanol, and glacial acetic acid used were analytical grade and distilled water. All dilutions were performed in standard volumetric flasks

Instrumentation and chromatographic conditions

A camag, Linomat IV sample applicator was used. Camag Twin trough glass chamber (20x10cm) was used for development of plates. And Camag TLC scanner II equipped with cats 3 Version software was used for interpretation of data.

Preparation of working standard solutions

The stock solution of Gatifloxacin was prepared by dissolving 160mg Gatifloxacin(99.99%) and 24 mg Ambroxol hydrochloride (99.92%) in methanol in standard 50ml volumetric flask (3200µg mL^{-1} & 480µg mL^{-1} respectively) (solution A).

The stock solution of Ciprofloxacin was prepared by dissolving 200 mg of Ciprofloxacin (internal standard) (99.95%) in methanol in 100 ml volumetric flask (2,000 µg mL^{-1}) Solution B.

Sample preparation

Twenty tablets were weighed and their average weight was calculated. The tablets were crushed in to a homogeneous powder and a quantity equivalent to one tablet (1218.5 mg) was weighed in a 100mL volumetric flask, dissolved in methanol, and filtered through Whatman no. 41 filter paper. The filtrate (2ml) was quantitatively transferred to a 10mL volumetric flask; 2mL of internal standard solution was added to it, and diluted up to the mark with methanol.

Chromatographic condition

The experiment was performed on silica gel $60F_{254}$ HPTLC plates using mobile phase comprising of n-butanol: water: glacial acetic acid: methanol in the volume ratio (4:1:1:0.3)v/v. The plate was prewashed by methanol and activated in an oven at 110^0C for 1 hour before use. The sample solutions were applied on the HPTLC plate as sharp bands of 7 mm width with the help of Camag Linomat IV sample applicator at the distance of 15 mm from the edge of the HPTLC plate with the speed of 10 sec/µl. Ascending development to distance of 8cm was performed in saturated 20cm x 10cm camag twin trough chamber for 15min at room temperature. The developed TLC plate was air dried and then scanned between 200 and 400 nm using Camag TLC Scanner II with Cats 3 version of the software. The wavelength chosen for further quantification was 235 nm. The overlain spectra and HPTLC chromatogram for Gatifloxacin and Ambroxol hydrochloride are shown in Figure 2 and Figure 3 respectively.

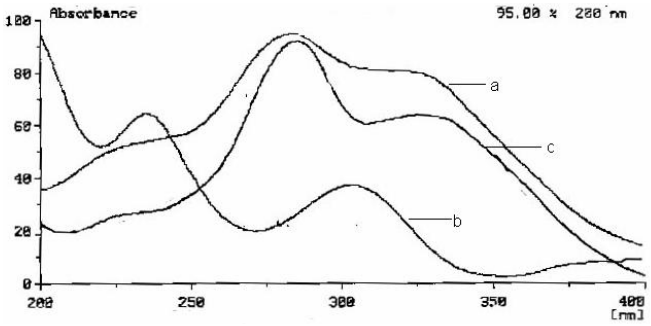


Figure 2 : UV spectra of a) Gatifloxacin b) Ambroxol hydrochloride c) Ciprofloxacine in methanol

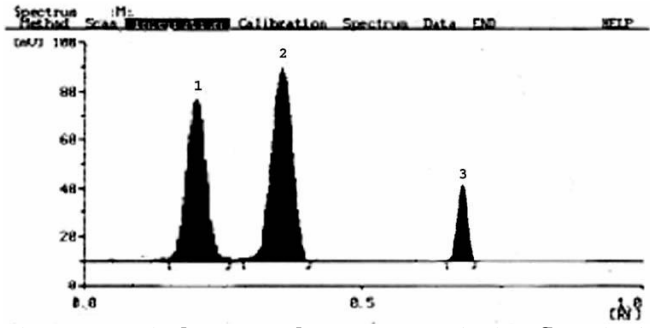


Figure 3 : Typical HPTLC chromatogram 1) Ciprofloxacin 2) Gatifloxacin, 3) Ambroxol hydrochloride

RESULT AND DISCUSSION

Linearity

Linearity was evaluated by analysis of working standard solutions of Gatifloxacin and Ambroxol hydrochloride of five different concentrations[5]. The con-

centration range for each of the two pharmaceuticals in the working standard solutions was 480 μg mL^{-1} to 1120 μg mL^{-1} for Gatifloxacin and 72 μg mL^{-1} to 168 μg mL^{-1} for Ambroxol hydrochloride. The peak area and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression data obtained for the two pharmaceuticals were represent in TABLE 1. The result shows that with-in the concentration range mentioned above there was an excellent correlation between peak area ratio and concentration of each drug.

TABLE 1 : Results of linearity

Analyte	Slope (mean)	Intercept (mean)	Correlation coefficient (n = 5)
Gatifloxacin	0.0021	0.0252	0.9998
Ambroxol hydrochloride	0.0026	0.0063	0.9998

Limit of detection and limits of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively. The LOD and LOQ of Gatifloxacin and Ambroxol hydrochloride were experimentally determined by six injections of each drug. The LOD of Gatifloxacin and Ambroxol hydrochloride were found to be 25μg mL^{-1} and 11μg mL^{-1} respectively. The LOQ of Gatifloxacin and Ambroxol hydrochloride were found to be 75μg $mL^{-1}$and 33μg mL^{-1}

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the mixed standard solutions[5]. The relative standard deviations were less than 2% for the three drugs. Method precision was determined from results from six independent determinations at 100% of the test concentrations of Gatifloxacin and Ambroxol hydrochloride in the product. The RSD were 0.78 and 0.22 respectively.

Assay

From the above sample solution 10μl was spotted in triplicate along with same concentration of standard solution on to the plate under the optimized chromatographic conditions. The peak area values of Gatifloxacin, Ambroxol hydrochloride were calculated. The amount of Gatifloxacin, Ambroxol hydrochloride present in this solution were then estimated using calibration curve method. Results of assay are tabulated in TABLE 2.

TABLE 2 : Result for assay

Drug	Labeled claim (mg)	Drug found in mg(n = 6)	% RSD	% Assay
Gatifloxacin	400	400.83	0.78	100.21
Ambroxol hydrochloride	60	59.81	0.22	99.69

Recovery studies

Recovery experiments were carried out to check for the presence of positive or negative interferences from excipients present in the formulation, and to study the accuracy and precision of the method. Recovery experiment was performed by the standard addition method. The recovery of the added standard was studied at three different levels viz 110%, 120% and 130% of the estimated amount of the drug[5]. Each set of recovery of added standard was calculated. The results of recovery experiment are tabulated in TABLE 3.

TABLE 3 : Result for recovery

	Initial Concentration In mg	Amount added in mg	Total Concentration In mg	Amount of drug found in (mg)	Recovery (%)
Gatifloxacin	400	00	400	400.99	100.25
	400	40	440	441.05	100.24
	400	80	480	482.15	100.45
	400	120	520	522.42	100.46
Ambroxol hydrochloride	60	0	60	59.96	99.94
	60	6	66	65.80	99.70
	60	12	72	72.09	100.13
	60	18	78	78.15	100.20

Robustness

The robustness of the method was studied, during method development, by determining the effects of small variation, of mobile phase composition(±2%), chamber saturation period, development distance and scanning time (10% variation of each). No significant change of Rf or response to drugs was observed, indicating the robustness of the method.

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CONCLUSION

The high performance thin layer chromatographic method for the determination of Gatifloxacin, Ambroxol hydrochloride from their fixed dosage form was found to be accurate and precise. Thus, the proposed HPTLC method can be successfully applied for the routine quality control analysis of Gatifloxacin, Ambroxol hydrochloride from their fixed dosage form.

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