Stability-Indicating HPTLC Determination of Ambroxol Hydrochloride in Bulk Drug and Pharmaceutical Dosage Form

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Abstract

A simple, selective, precise, and stability-indicating high-performance thin-layer chromatographic (HPTLC) method for the analysis of ambroxol hydrochloride both as a bulk drug and in formulations was developed and validated. The method employed HPTLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of methanol–triethylamine (4:6 v/v). The system was found to give a compact spot for ambroxol hydrochloride (R_f value of 0.53 ± 0.02). Densitometric analysis of ambroxol hydrochloride was carried out in the absorbance mode at 254 nm. The linear regression analysis data for the calibration plots showed good linear relationship with r^2 = 0.9966 ± 0.0013 with respect to peak area in the concentration range 100–1000 ng/spot. The mean value ± standard deviation of slope and intercept were 164.85 ± 0.72 and 1168.3 ± 8.26 with respect to peak area. The method was validated for precision, recovery, and robustness. The limits of detection and quantitation were 10 and 30 ng/spot, respectively. Ambroxol hydrochloride was subjected to oxidation and thermal degradation. The drug undergoes degradation under oxidation and heat conditions. This indicates that the drug is susceptible to oxidation and heat. Statistical analysis proves that the method is repeatable, selective, and accurate for the estimation of said drug. Stability indicating of new chemical entities is an important part for the drug development of ambroxol hydrochloride and for its estimation in plasma and other biological fluids; the novel Statistical analysis proves that the method is repeatable and selective for the analysis of ambroxol hydrochloride as bulk drug and in pharmaceutical formulations. The proposed developed HPTLC method can be applied for identification and quantitative determination of ambroxol hydrochloride in bulk drug and dosage forms. This work is to determine the purity of the drug available from the various sources by detecting the related impurities.

Introduction

Ambroxol hydrochloride, chemically 4-{[(2-amino-3,5-dibromophenyl)-methyl]-amino]}-cyclohexanol or N-{[(trans-p-hydroxycyclohexyl),(2-amino-3,5-dibromobenzyl)]}-amine (1), is an active metabolite of bromhexine, a mucolytic expectorant particularly useful in bronchitis with chronic obstructive bronchitis and more effective in silicosis as secretolytic and surfactant stimulant asthma, sinusitis secretory otitis media, or smokers cough if mucus plugs are present (2). It is an expectoration improver and mucolytic agent used in the treatment of acute and chronic disorders characterized by the production of excess or thick mucus. It works to decrease mucus viscosity by altering its structure. It is a white to yellowish crystalline powder; slightly soluble in water and ethanol; soluble in dimethylformamide, methanol; insoluble in chloroform and benzene; melting point 2400°C; administered orally. It is official in Martindale – The Extra Pharmacopoeia (1). Literature survey reveals that high-performance liquid chromatography methods are reported for the estimation of ambroxol hydrochloride in pharmaceutical formulations (3–7). The estimation of ambroxol by colorimetry has been carried out by UV (8,9).

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Experimental

Materials

Ambroxol hydrochloride was a gift sample from Cipla India, (Daman, India). All chemicals and reagents used were of analytical-grade and purchased from Qualigens Fine Chemicals (Mumbai, India).

HPTLC instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag microliter syringe on precoated silica gel aluminium Plate 60F-254 (20 cm × 10 cm with 0.2 mm thickness, E. Merck, Darmstadt, Germany) using a Camag Linomat IV (Muttenz, Switzerland). A constant application rate of 100 nL/s was employed, and the space between two bands was 8 mm. The slit dimension was kept 5 mm × 0.45 mm micro, 5 mm/s scanning speed was employed. The mobile phase consisted of methanol–triethylamine (4:6, v/v). Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature. The length of

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chromatogram run was ~ 70 mm. Subsequent to the development, TLC plate was dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 254 nm. The source of radiation utilized was deuterium lamp.

Calibration curve of ambroxol hydrochloride

A stock solution of ambroxol hydrochloride (1000 µg/mL) was prepared in ethanol. Different concentrations were prepared as 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg/mL solution, respectively. Then, 10.0 µL of each solution were spotted on three replicate on TLC plates to obtain concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 ng/spot of ambroxol hydrochloride, respectively. The data of peak area versus drug concentration were treated by linear least square regression.

Method validation

Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (500 ng/spot of ambroxol hydrochloride). The intra- and inter-day variation for the determination of ambroxol hydrochloride was carried out at three different concentration levels of 300, 500, and 700 ng/spot.

Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition of methanol–triethylamine (4.5:5.5 and 3.5:6.5, v/v) were tried, and chromatograms were run. The amount of mobile phase, temperature, and relative humidity was varied in the range of ± 5%. The plates were prewashed by methanol and activated at 60 ± 5°C for 2, 5, and 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40, and 60 min. Robustness of the method was done at three different concentration levels: 300, 500, and 700 ng/spot.

Limit of detection and limit of quantification

In order to determine detection and quantification limit, ambroxol hydrochloride concentrations in the lower part of the linear range of the calibration curve were used. Ambroxol hydrochloride solutions of 10, 12, 14, 16, 18, and 20 µg/mL were prepared and applied in triplicate (10.0 µL each). The amount of ambroxol hydrochloride by spot versus average response (peak area) was graphed, and the equation for this was determined. The standard deviations (SD) of responses were calculated. The average of standard deviations was calculated (ASD). Detection limit was calculated by $(3.3 \times \text{ASD})/b$ and quantification limit was calculated by $(10 \times \text{ASD})/b$, where b corresponds to the slope obtained in the linearity study of method.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for ambroxol hydrochloride in sample was confirmed by comparing the R_f values and spectra of the spot with that of standard. The peak purity of ambroxol hydrochloride was assessed by comparing the spectra at three different levels [i.e., peak start (S), peak apex (M), and peak end (E) positions of the spot].

Recovery studies

The analyzed samples were spiked with extra 50%, 100%, and 150% of the standard ambroxol hydrochloride, and the mixture was analyzed by the proposed method. At each level of the amount, six determinations were performed. This was done to check the recovery of the drug at different levels in the formulations (Table IV).

Analysis of ambroxol hydrochloride in prepared formulation

To determine the concentration of ambroxol hydrochloride in tablets (labeled claim: 30 mg/tablet), the contents of 20 tablets were weighed, their mean weight determined, and they were finely powdered. The powder equivalent to 10 mg of ambroxol hydrochloride was weighed. The drug from the powder was extracted with ethanol. To ensure complete extraction of the drug, it was sonicated for 30 min, and the volume was made up to 10.0 mL. The resulting solution was centrifuged at 3000 rpm for 5 min, and supernatant was analyzed for drug content. The 0.5 mL was taken and volume made up to 10.0 mL by ethanol, to make a 50 µg/mL solution. Then, 10.0 µL of the described solution (500 ng/spot) was applied on TLC plate followed by development and scanning as described in the "HPTLC instrumentation" section. The analysis was repeated in triplicate. The possibility of excipient interferences in the analysis was studied.

Forced degradation of ambroxol hydrochloride

Hydrogen peroxide-induced degradation

10 mg of ambroxol hydrochloride was separately dissolved in 10.0 mL of methanolic solution of hydrogen peroxide (3.0%, v/v). The solution was stored for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. 1.0 mL of the solution was taken and diluted up to 10.0 mL with methanol. The resultant solution was applied on the TLC plate in triplicate (10.0 µL each, i.e. 1000 ng/spot). The chromatograms were run as described in the "HPTLC instrumentation" section.

Dry heat degradation product

The powdered drug that was stored at 550°C for ~ 3 h under dry heat condition showed significant degradation. The degraded products were resolved from the standard. In all degradation studies, the average peak areas of ambroxol hydrochloride is measured after application (1000 ng/spot) of three replicates (Please revise this sentence).

Results and Discussion

Development of optimum mobile phase

TLC procedure was optimized with a view to developing a stability-indicating assay method. Initially, methanol–triethylamine (3:7, v/v) gave good resolution with R_f value of 0.53 for ambroxol hydrochloride, but typical peak nature was missing. Finally, the mobile phase consisting of methanol–triethylamine (4:6, v/v) gave a sharp and well-defined peak at R_f value of 0.53

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(Figure 1). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature.

Calibration curves

The linear regression data for the calibration curves (n = 3) (Table I) showed a good linear relationship over the concentration range 100–1000 ng/spot with respect to peak area. No significant difference was observed in the slopes of standard curves (ANOVA, P > 0.05).

Validation of the method

Precision

The repeatability of sample application and measurement of peak area were expressed in the terms of %RSD, and the results are depicted in Table II, which reveal intra- and inter-day variation of ambroxol hydrochloride at three different concentration levels of 300, 500, and 700 ng/spot.

Robustness of the method

The standard deviation of peak areas was calculated for each parameter, and %RSD was found to be less than 2%. The low %RSD values (Table III) indicated the robustness of the method.

LOD and LOQ

The calibration curve in this study was plotted between the amount of analyte versus average response (peak area), and the regression equation was obtained ($y = 154.27x + 1313.9$) with a regression coefficient of 0.9964. Detection limit and quantification limit was calculated by the method as described in the "LOD and LOQ" section and found to be 6.51 and 19.72 ng, respectively. However, by experiment, the LOD and LOQ were 10 and 30 ng, respectively. This indicates the adequate sensitivity of the method.

Specificity

The peak purity of ambroxol hydrochloride was assessed by comparing the spectra at peak start, peak apex, and peak end positions of the spot [i.e., r^2 (S, M) = 0.9998 and r^2 (M, E) = 0.9988]. Good correlation (r^2 = 0.9989) was also obtained between standard and sample spectra of ambroxol hydrochloride.

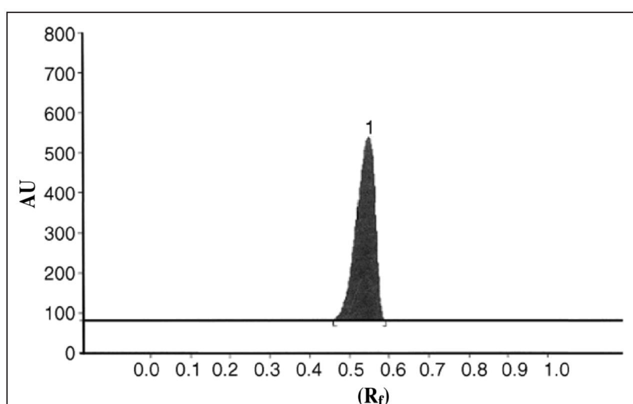

Figure 1. A typical HPTLC chromatogram of ambroxol hydrochloride (R_f = 0.53) in methanol–triethylamine (4:6, v/v) at 254 nm.

Table I. Linear Regression Data for the Calibration Curves*

Linearity range (ng/spot)	100–1000
r^2 ± SD	0.9966 ± 0.0013
Slope ± SD	164.85 ± 0.72
Confidence limit of slope†	164.03–165.66
Intercept ± SD	1168.3 ± 8.26
Confidence limit of intercept†	1158.95–1177.64

* n = 3. † 95% confidence limit.

Table II. Intra- and Inter-Day Precision of HPTLC Method*

Amount	Intra-day precision				Inter-day precision			
(ng/spot)	MA†	SD	%RSD	SE‡	MA†	SD	% RSD	SE‡
300	5962.08	35.20	0.58	14.27	5880.26	54.11	0.91	22.10
500	9520.15	42.52	0.41	17.07	9481.12	62.20	0.64	25.43
700	13314.30	54.21	0.46	22.18	13190.09	84.83	0.66	34.61

* n = 6; † ME = Mean area; ‡ Standard error.

Table III. Robustness of the Method*

Parameter	SD† of peak area	%RSD†
Mobile phase composition	1.21	0.97
Amount of mobile phase	1.07	0.82
Temperature	0.91	0.67
Relative humidity	0.89	0.54
Plate pretreatment	0.53	0.40
Time from spotting to chromatography	0.44	0.38
Time from chromatography to scanning	0.38	0.33

* n = 6. † Average of three concentrations: 300, 500, and 700 ng per spot.

Table IV. Recovery Studies*

Excess drug added to the analyte (%)†	Amount recovered (mg)	Recovery (%)	%RSD	SE
0	101.20	101.19	0.41	0.32
50	150.92	100.60	0.69	0.56
100	198.67	99.31	0.71	0.55
150	247.12	98.88	0.46	0.37

* n = 6. † Matrix containing 100 mg drug.

Table V. Summary of Validation Parameters

Parameter	Data
Linearity range (ng per spot)	100–1000
Correlation coefficient	0.9966 ± 0.0013
Limit of detection (ng per spot)	10
Limit of quantitation (ng per spot)	30
Recovery (n = 6)	100.01±1.32
Precision (% RSD)	
Repeatability of application (n = 6)	0.33
Repeatability of measurement (n = 6)	0.17
Inter-day (n = 6)	0.71
Intra-day (n = 6)	0.47
Robustness	Robust
Specificity	Specific

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Recovery studies

The proposed method, when used for extraction and subsequent estimation of ambroxol hydrochloride from pharmaceutical dosage forms after spiking with 50, 100, and 150% of additional drug, afforded recovery of 98–102% (Table IV). The data summary of validation parameters are listed in Table V.

Analysis of prepared formulation

A single spot of R_f 0.53 was observed in a chromatogram of ambroxol hydrochloride samples extracted from capsules. There was no interference from the excipients commonly present in the tablets. The ambroxol hydrochloride content was found to be 99.2% with a %RSD of 0.63. It may be inferred that degradation of ambroxol hydrochloride had not occurred in the formulation that was analyzed by this method. The low %RSD value indicated the suitability of this method for routine analysis of ambroxol hydrochloride in pharmaceutical dosage forms.

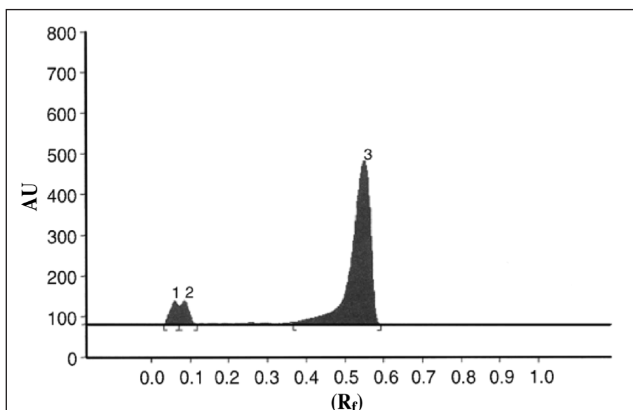

Figure 2. HPTLC chromatogram of ambroxol hydrochloride after treatment with hydrogen peroxide for 8 h.

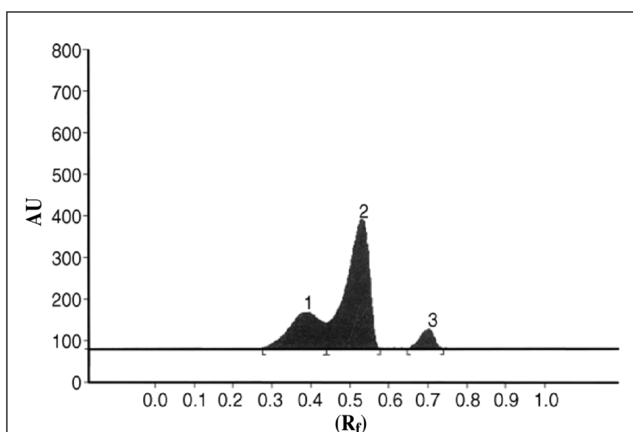

Figure 3. HPTLC chromatogram of ambroxol hydrochloride after heat degradation at 55°C for 3 h.

Table VI. Forced Degradation of Ambroxol Hydrochloride

Serial Number	Sample exposure condition	No. of degradation prods. (R_f value)	AH* Remained (ng/1000 ng) (± SD, n = 3)	S.E.	Recovery (%)
1	3% H_2O_2, 8 h, RT	2 (0.5, 0.7)	812.44 (± 3.91)	2.28	81.12
2	Heat, 3 h, 55°C	2 (0.36, 0.5)	776.25 (± 5.98)	3.46	77.50

* AH = Ambroxol hydrochloride.

Stability-indicating property

The chromatogram of samples degraded with hydrogen peroxide and heat showed well separated spots of pure ambroxol hydrochloride as well as some additional peaks at different R_f values. The spots of degraded product were well resolved from the drug spot as shown in Figures 2–5. The conditions of degradation, number of degradation products with their R_f values, content of ambroxol hydrochloride determined, and percentage recovery were calculated and listed in Table VI.

Conclusion

The developed HPTLC technique is precise, specific, accurate, and stability indicating. The developed method was validated based on ICH Guidelines 10. Statistical analysis proves that the method is repeatable and selective for the analysis of ambroxol hydrochloride as bulk drug and in pharmaceutical formulations.

The method can be used to determine the purity of the drug available from the various sources by detecting the related impurities. It may be extended to study the degradation kinetics of ambroxol hydrochloride and for its estimation in plasma and other biological fluids. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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