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Research Article

STABILITY INDICATING HPTLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF AMBROXOL HYDROCHLORIDE AND CETIRIZINE DIHYDROCHLORIDE IN COMBINE TABLET DOSAGE FORM

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ABSTRACT
A new simple, sensitive and precise high performance thin layer chromatographic method has been developed for estimation of Ambroxol hydrochloride and Cetirizine dihydrochloride simultaneously from a combined Solid dosage form.In this method pre coated silica gel 60 GF 254 TLC plate was used as stationary phase and the chromatogram was developed using CCl_4: Chloroform: Methanol (3:5:2v/v/v) as mobile phase. Ambroxol hydrochloride and Cetirizine dihydrochloride showed Rf values 0.74 ± 0.2, 0.47 ± 0.2 respectively. The plate was scanned and quantified at 230 nm using Camag TLC Scanner. The linear concentration 240-720ng/band and 20-60 ng/band for Ambroxol hydrochloride and Cetirizine dihydrochloride respectively. The percentage recovery for Ambroxol hydrochloride and Cetirizine dihydrochloride was found to be 100.08 % , 101.21%. As the method could effectively separate the drug from its degradation product it can be employed as stability indicating one. The method is validated as per ICH guidelines
KEYWORDS: Ambroxol hydrochloride ,Cetirizine dihydrochloride, HPTLC, ICH

INTRODUCTION

Stability study is important to conduct for evaluating and ensuring product safety. The investigation of the stability of drugs represents an important issue in the drug quality evaluations. Many environmental conditions such as light, moisture, as well as the chemical susceptibility of substance to hydrolysis or oxidation can play extremely serious role in pharmaceutical stability. A stress testing of drug Substance can help to identify the likely degradation products and to provide important information on drug's inherent stability. Consecutively; it can be a fundamental contribution to development and validation of stability indicating analytical method used in monitoring of quality of pharmaceutical products.

It is important to recognize and be aware of potential for instability in both manufactured and extemporaneous product. The ICH guidelines Q1A on stability testing of new drug substances and products emphasis that the testing of those features which are susceptible to change during storage must be done by validating stability indicating testing method.

Ambroxol is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus. Chemically it is Trans-4-(2-amino-3, 5-dibromobenzylamino) cyclohexanol hydrochloride. Cetirizine is an anti-allergic medicine. Cetirizine is histamine H1-receptor antagonist. Chemically it is (±) -[2- [4- [(4-chlorophenyl) phenylmethyl] -1- piperazinyl] ethoxy] acetic acid, dihydrochloride. Literature survey reveals individual determination and validation of Ambroxol HCl and cetirizine HCl by spectrophotometry[1,2], RPHPLC,[3] methods has been developed. To our Knowledge no article related to stability indicating method development by HPTLC method has been reported in literature.

The aim of present work is to develop an accurate, specific, repeatable, simple method foe determination of Ambroxol HCl and cetirizine HCl in tablet dosage form as well as pure drug. The proposed method was validated as per ICH guidelines.

MATERIALS AND METHODS

Instrument

CAMAG HPTLC instrument was used in this method. CAMAG HPTLC is equipped with CAMAG TLC scanner-3, Linnomate V Automatic sample applicator controlled by WIN CATS software (1.4.3 version). Aluminium packed silica Gel 60 F_{254} HPTLC plates (20

X 10cm, layer thickness 0.2mm, E.MERCK). Reflux condenser is used for degradation study.

Reagent and chemicals

Ambroxol hydrochloride and cetirizine Dihydrochloride were received as gift samples from Westcost pharmaceutical ltd, Ahmedabad. The tablet dosage formulation (CETZINE ® A) was purchased from local pharmacy. Tablet contains CTZ dihydrochloride 5mg and Ambroxol hydrochloride 60mg. Methanol, chloroform, CCl_4, H_2O_2 used were purchased form Merck chemicals Corporation Ltd. Mumbai, India. All the chemicals used were analytical grade.

Optimized Chromatographic Condition

Stationary phase: Pre-coated silica gel 60 F_{254} Aluminium plates (20x10cm)
Mobile phase: CCl_4: Chloroform: Methanol (3:5:2v/v/v)
Chamber saturation: 25 minutes
Development distance: 70mm
Development time: 15 minutes
Relative temperature: 25 ±2˚C
Relative Humidity: 44-49%
Band width: 6mm
Detection wavelength (mm): 230nm
Distance between two tracks: 10 mm

Preparation of calibration curve

Analysis was performed on 10 x 10 cm HPTLC silica Gel G60 F_{254} aluminium plate. Calibration curve were plotted over a concentration range of 240-720ng/band and 20-60 ng/band for Ambroxol hydrochloride and Cetirizine dihydrochloride respectively. For the calibration curve accurately measured solution of AMB(8, 12, 16, 20 and 22 µl), and solution of CTZ (8, 12, 16, 20 and 22 µl) were spotted on pre-coated TLC plate under nitrogen stream using Linomat 5 automatic spotter. The plate was dried in air and developed using mixture of CCl_4: chloroform: Methanol (3:5:2, v/v/v) as mobile phase in a Camag twin through chamber. and scanned using WIN CAT software. The analysis was repeated in triplicate.

Analysis of commercial formulation

Content of twenty tablets of a formulation were weighed and finely powdered. Powder equivalent to 30 mg of AMB and 2.5 mg of CTZ was transferred into 50 ml volumetric flask and dissolved in methanol with vigorous shaking. The solution was sonicated for 20 minutes and the volume was made up to mark with the same solvent1 ml of filtrate was transferred in to 10 ml volumetric flask and volume was made up to mark with methanol to get the concentration of 300 µg/ml & 25 µg/ml AMB and CTZ respectively. Take 1 ml of above stock solution, transferred in to 10 ml volumetric flask and volume was made up to mark with methanol to get the concentration of 30µg/ml and 2.5 µg/ml of AMB and CTZ respectively.

Application of Sample

The TLC plates were pre-washed with methanol and activated by keeping at 115°C for about 30 minutes. Aliquots were applied on the precoated silica gel G60 F_{254} TLC plate. Samples were spotted in the form of bands of width 8 mm with Hamilton microlitre syringe on the pre-coated silica gel 60 F_{254} plate (20X10 cm). Slit dimension was kept at 6 x 0.45mm. The distance between bands was 10mm. From the peak area the amount of AMB and CTZ in formulation was simultaneously calculated using the respective calibration graph.

Degradation Study using HPTLC Technique

Forced degradation studies are an important part of the drug development process. Although the concept of stress testing is not new to the pharmaceutical industry, they are regularly been performed to determine stability and half-life of the drug products. The purpose of stability testing is to provide evidence on how quality of a drug substance varies with time under the influence of verity of environmental factors like temperature, humidity, light, and other storage conditions.

Acid Degradation

Accurately weighted AMB (50 mg) and CTZ (50 mg) in mixture and individually was transferred in 50ml volumetric flask and dissolved in methanol (25ml). Hydrochloric acid solution (25ml, 2N) was added. The final solution was transferred in 100 ml of the round bottom flask and refluxed at 90 ± 2^0 for five hours. At time intervals of 0, 15, 30, 60, 90, 120, 180, 240, 300 minutes 2.5 ml of the solution was transferred in a series of 25 ml of volumetric flasks and diluted up to the mark with methanol to stop further degradation. One ml of each solution was transferred in a series of 10 ml volumetric flasks and diluted to the mark with methanol. 200 ng/ band of mixture, 200 ng/ band of AMB, 100 ng/ band of CTZ was analyzed employing HPTLC method.

Oxidative Degradation

Accurately weighted AMB (50 mg) and CTZ (50 mg) in mixture and individually was transferred in 50ml volumetric flask and dissolved in methanol (25ml). Hydrogen peroxide solution (25ml, 3%v/v) was added. The final solution was transferred in 100 ml of the round bottom flask and refluxed at 90 ± 2^0 for five hours. At time intervals of 0, 15, 30, 60, 90, 120, 180, 240, 300 minutes. 2.5 ml of the solution was transferred in a series of 25 ml of volumetric flasks and diluted up to the mark with methanol to stop further degradation. One ml of each solution was transferred in a series of 10 ml volumetric flasks and diluted to the mark with methanol.

200 ng/band of mixture, 200 ng/band of AMB, 100 ng/band of CTZ was analyzed employing HPTLC method.

Thermal Degradation

AMB (1gm) and CTZ (1gm) in mixture and individually was transferred in to petridishes. Than these petridishes were placed in to oven without disturbances for 24 hrs. At time intervals of 2, 4, 6, 8, 10, 12, 15, 18, 21, 24 hr 25 mg of sample was transferred in a series of 25 ml of volumetric flasks and diluted up to the mark with methanol to stop further degradation. One ml of each solution was transferred in a series of 10 ml volumetric flasks and diluted to the mark with methanol. 200 ng/band of mixture, 200ng/band of AMB, 100 ng/band of CTZ was analyzed employing HPTLC method

RESULT AND DISCUSSION

The method was validated by establishing linearity, accuracy, inter day and intra day precision of measurement of sample application. The limit of detection and limit of quantification were also determined. Peaks of AMB and CTZ are shown in figure no 1.

Linearity Calibration Curve

Calibration curve was found to be linear in the range of 240-720ng/band and 20-60 ng/band for AMB and CTZ. Five concentration points were assayed in triplicate. Lineaity curve is shown in firure no 2. All the drugs showed good linearity in the tested range. The regression co-efficient (R2) value for AMB and CTZ was found to be 0.997and 0.998 respectively as shown in figure no 3, 4 for each. Data for calibration curve are shown in table no 1,2 for AMB and CTZ respectively.

Accuracy

Recovery studies were carried out for the accuracy parameter. The study was carried out at two levels. To the powdered formulation, the standard drugs of AMB and CTZ were added 50% and 100% levels, dilutions were made, and analyzed by the method. The % recovery and % RSD were calculated, and found to be within the limit. Data for recovery study are shown in table 2.

Specificity

There was no interference from sample placebo and peak purity of AMB and CTZ. It showed that developed method was specific for the analysis of AMB and CTZ in solid dosage form as shown in figure no 5, 6

Precision

Intra-day assay precision was found by analysis of standard drug at three times on the same day. Inter-day assay precision was carried out using the standard drug at two different days, and % RSD was calculated. The RSD was found to be less than 2 for both inter-day and intra-day assay precision.

Degradation study

Result of degradation study are shown in Table no 4 and graphs of degradation study are shown in figures 7,8,9.

Summary of all the Validation parameter are shown in Table no 5 the determined validation parameters are in the acceptable ranges. And result of Marketed formulation is shown in table no 6.

CONCLUSION

The proposed method is simple, accurate, cost effective, less time consuming and the statistical analysis proved that the method is reproducible and efficient for the simultaneous estimation of AMB and CTZ as bulk drugs and in combined pharmaceutical dosage forms without any interference from the excipients.

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Table 1: Data for calibration curve for AMB

Sr. No.	Concentration (ng/band)	Area	% CV
		Mean ± S.D (n=3)	
1	240	4185.99 ±9.67	0.1845
2	360	4658.33± 13.46	0.1615
3	480	5130.87± 13.13	0.1151
4	600	5603.31± 18.31	0.1328
5	720	6075.75± 34.01	0.2029

Table 2: Data for calibration curve for CTZ

Sr. No.	Concentration (ng/band)	Area	% CV
		Mean ± S.D (n=3)	
1	20	2913.124 ±10.88	1.88
2	30	2943.514± 18.88	1.56
3	40	2973.90± 27.39	1.57
4	50	3004.299 ± 27.67	1.23
5	60	3034.24± 17.75	0.62

Table 3: Data for recovery study

Drug	Amount taken (ng/band)	Amount added (ng/band)	Total Area	Recovered concentration (ng/band)	% Recovery ± SD (n = 3)
AMB	480	384	6641.23	383.6	99.89 ± 1.54
	480	480	7024.43	480.96	100.02 ± 0.88
	480	576	7399.21	576.15	100.02 ± 1.41
CTZ	40	32	3072.12	32.47	101.46 ± 1.11
	40	40	3095.46	39.9	99.97± 0.72
	40	48	3118.92	47.71	99.3 ± 0.29

Table 4: Data for degradation study

Stress condition	% Degraded (AMB)		% Degraded (CTZ)	
	Standard	Formulation	Standard	Formulation
Acid	10.47	10.98	12.01	11.89
Oxidative	17.04	16.56	24.17	24.10
Thermal	13.05	12.73	14.78	14.34

Table 5: Summary of validation parameter

Parameters	AMB (ng/band)	CTZ(ng/band)
Beer's Law limit (ng/band)	240-720 ng/band	20-60 ng/band
Regression equation Y*=mx + c Slope(m) Intercept(c)	 3.9371 3241.1	 3.039 2852.3
Correlation coefficient (r^2)	0.99707	0.998
LOD(µg/ml)	17.032	1.89
LOQ(µg/ml)	51.61	5.6
Precision* Inter-day Precision Intra-day Precision	 100.011 & 0.048 99.98 & 0.26	 99.75 & 0.73 99.55 & 0.30
Accuracy*	99.97& 1.27	100.22 & 0.70

Table 6: Data for assay of marketed formulation

CETZINE ® A	AMB			CTZ		
	Amt taken (ng)	Amt found (ng)	% Amount found (n=3)	Amt taken (ng)	Amt found (ng)	% Amount found (n=3)
Tablet	480	479.89	99.95	40	39.76	99.40

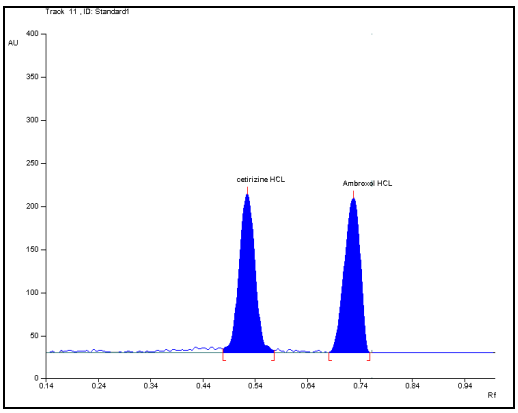

Figure 1: HPTLC chromatogram for Mixture

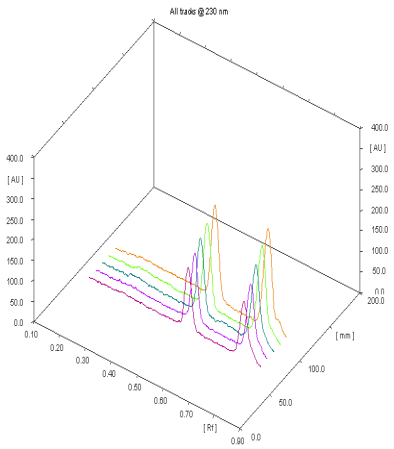

Figure 2: Linearity graph for AMB and CTZ

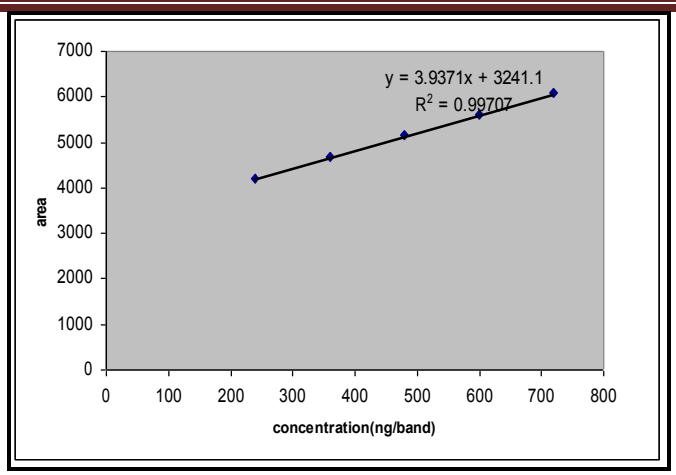

Figure 3: Calibration graph for AMB

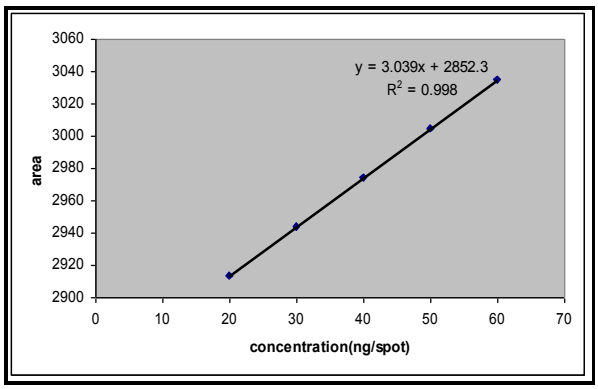

Figure 4: Calibration graph for CTZ

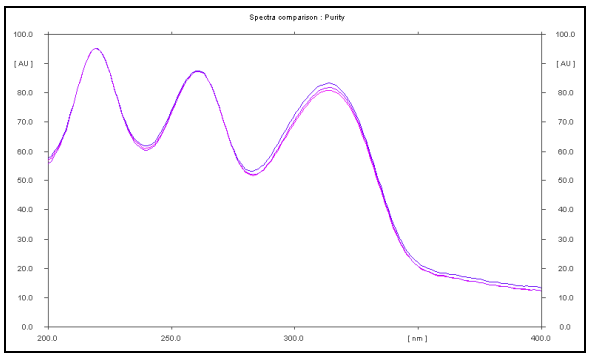

Figure 5: Overlaid spectra of standard and Test for AMB

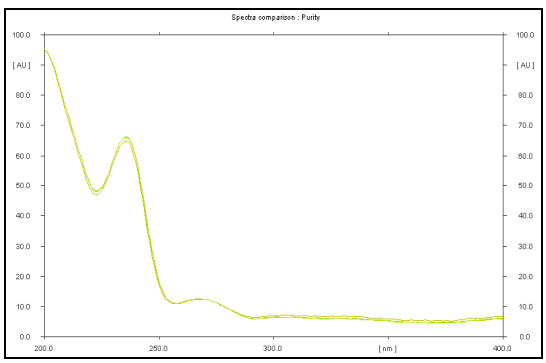

Figure 6: Overlaid spectra showing peak purity of CTZ Std. and Test

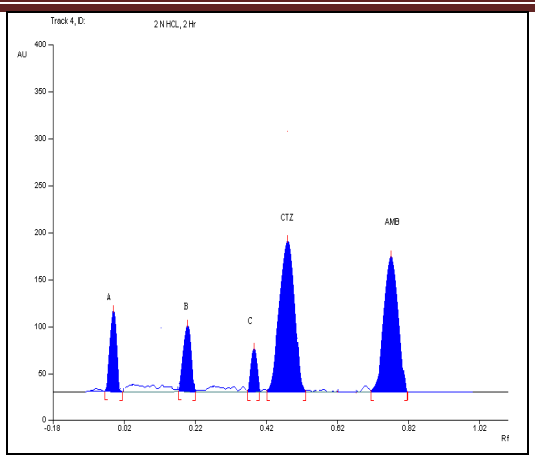

Figure 7: Chromatogram of Degradation of Mixture in 2N HCL, 2hr

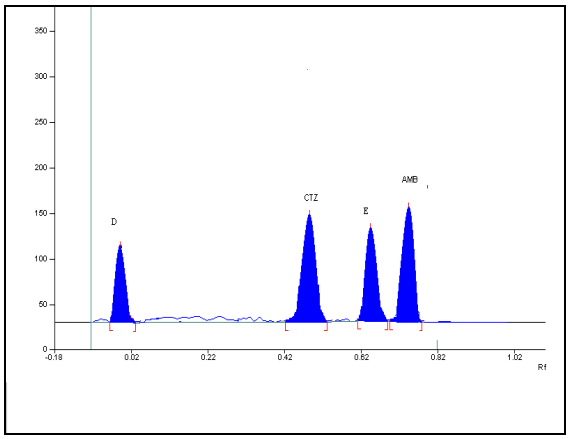

Figure 8: Chromatogram of Degradation of Mixture in 3% H_2O_2 at 1Hr

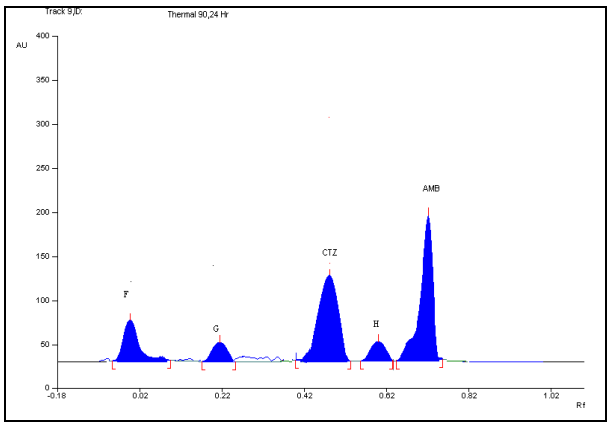

Figure 9: Chromatogram of Degradation of Mixture in 90 ^{0}C, 24 hr

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