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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF CETRIZINE HYDROCHLORIDE AND AMBROXOL HYDROCHLORIDE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

The present study describes development and validation of a simple, accurate, precise and selective stability-indicating high performance thin layer chromatographic method for simultaneous estimation of Cetrizine hydrochloride and Ambroxol hydrochloride in combined tablet dosage form. Chromatographic separation of both the drugs was carried out by using silica gel $60F_{254}$ as stationary phase and Ethyl acetate: Methanol: Ammonia in the ratio of (8: 1.5: 0.5, v/v/v) as mobile phase. Densitometric detection was carried out at 222 nm. The two drugs were satisfactorily resolved with Rf values of 0.30 ± 0.06 for Cetrizine hydrochloride and 0.71 ± 0.03 for Ambroxol hydrochloride. The developed method was validated in terms of linearity, accuracy, precision and robustness as per ICH guidelines. The drugs were subjected to forced degradation studies under condition of hydrolysis (acid, base), oxidation, photolysis and thermal stress. Results were found to be linear in the concentration range of 25-150 ng $band^{-1}$ for Cetrizine hydrochloride and 300-1800 ng $band^{-1}$ for Ambroxol hydrochloride. The method has been successfully applied for the estimation of drugs in tablet dosage form. The % assay (Mean ± S.D.) was found to be 100.62 ± 1.45 for Cetrizine hydrochloride and 100.37 ± 0.95 for Ambroxol hydrochloride. The developed method can be used efficiently for routine quality control analysis of both the drugs as bulk and in combined tablet dosage form.

KEYWORDS: Cetrizine, Ambroxol hydrochloride, HPTLC, Forced degradation, Tablet dosage form.

INTRODUCTION

Cetrizine hydrochloride (CET), chemically, (±)-[2-[4-[(4-chlorophenyl) phenylmethyl]-1-piperazinyl] ethoxy] acetic acid is second generation histamine H1 anatagonist used in the treatment of allergic rhinitis and chronic urticaria.[1] Ambroxol hydrochloride (AMBH), trans-4-(2-Amino-3, 5-dibrombenzylamino)-cyclohexanol is used in the treatment of respiratory diseases.[2]

Literature survey reveals high performance liquid chromatographic[3,4] and UV Spectrophotometric[5] methods for estimation of CET either as single drug or in combination with other drugs in pharmaceutical dosage form. Analytical methods reported for AMBH includes HPLC[6] and UV[7] either as single or in combination with other drugs. Reports were also available for simultaneous determination of CET and AMBH by UV[8,9], HPLC[10,11] and UPLC[12] method.

To best of our knowledge, no reports were found for stability-indicating high performance thin layer chromatographic method for simultaneous estimation of CET and AMBH in combined tablet dosage form. The present work describes development and validation of stability indicating HPTLC method for simultaneous estimation of CET and AMBH as bulk drugs and in combined tablet dosage form.

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade working standards CET and AMBH were kindly supplied by Sun Pharma (Mumbai, India) and Cipla Pharmaceuticals Ltd. (Mumbai, India), respectively. The tablet dosage form Ambcet (Cipla Pharmaceuticals Ltd., Mumbai, India) labeled to contain 5 mg of CET and 60 mg of AMBH was used for formulation analysis. Ethyl acetate, Methanol, Ammonia (AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Chromatographic separation of drugs was performed on precoated silica gel aluminium plate 60 F_{254} (10 ×10)

with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland). Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using Ethyl acetate: Methanol: Ammonia (8: 1.5: 0.5, v/v/v) as mobile phase with chamber saturation time of 15 min. The length of chromatogram run was 9 cm and development time was approximately 15 min. After development, TLC plates were dried and densitometric detection was performed on CAMAG thin layer chromatography scanner at 222 nm operated by winCATS software version 1.4.2.

Preparation of standard stock solutions

Accurately weighed 5 mg of CET was dissolved in 10 mL of methanol to get 500 mg mL^{-1} from which 0.5 mL was diluted to 10 ml to get the final concentration 25 ng μL^{-1}. For AMBH, 60 mg of drug was dissolved in 10 ml of methanol to get concentration of 6000 mg mL^{-1} from which 0.5 ml was diluted to 10 ml to get 300 ng μL^{-1}.

Selection of Detection Wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that both the drugs showed considerable absorbance at 222 nm. So, 222 nm was selected as the wavelength for detection.

Analysis of marketed tablet formulation

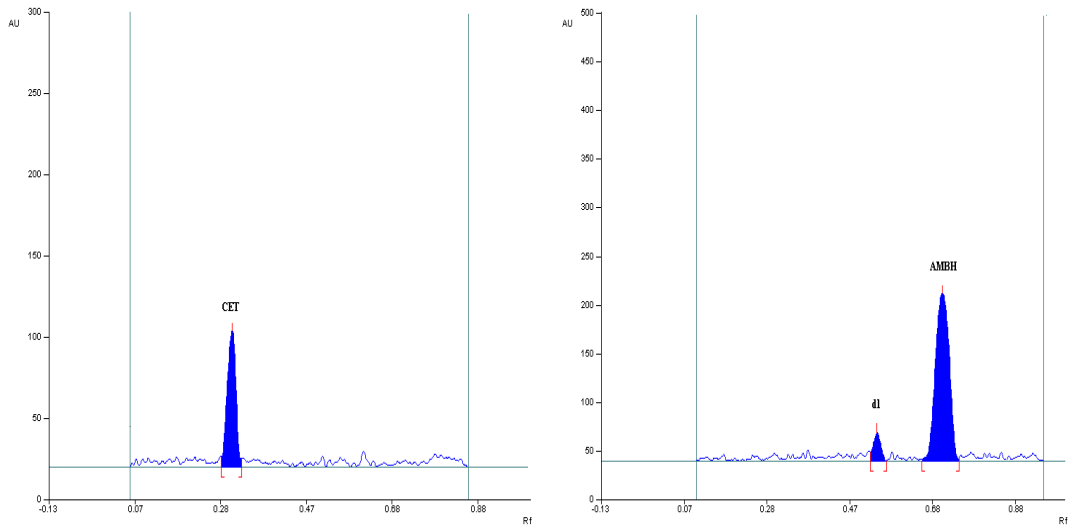
Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 5 mg of CET (60 mg of AMBH) was weighed and transferred to a 10 mL volumetric flask containing 6 mL of methanol and the content was sonicated for 15 min. The solution was filtered and the volume was made up to the mark with methanol. From the above solution, 0.5 mL of solution was diluted with methanol to obtain final concentration of 25 ng $band^{-1}$ for CET and 300 ng $band^{-1}$ for AMBH. Two µL volume of this solution was applied on TLC plate. After development, peak areas of the bands were measured at 222 nm and the amount of each drug present in each sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies of bulk drug

The forced degradation studies were carried out on bulk drug substance in order to prove the stability-indicating property and selectivity of the developed method. The degradation was carried out under hydrolytic, oxidative, photolytic and thermal stress conditions as per ICH guidelines.

Acid treatment

1 mL working standard solution of CET (500 ng μL^{-1}) was mixed with 1 mL of 0.1 N HCl and 8 mL of methanol. The solution was kept at room temperature for 4 h. 2 µL volume of resulting solution was applied on TLC plate to get concentration 100 ng $band^{-1}$ and analyzed under optimized chromatographic conditions. Same procedure was repeated for AMBH (6000 ng μL^{-1}) to get final concentration 1200 ng $band^{-1}$ applied on the TLC plate. After acid hydrolysis, CET showed 8.62 % of degradation with reduction in peak area and AMBH showed 13.23 % of degradation with appearance of additional product at Rf 0.53. The densitogram of CET and AMBH obtained after acid degradation is shown in Fig. 2.



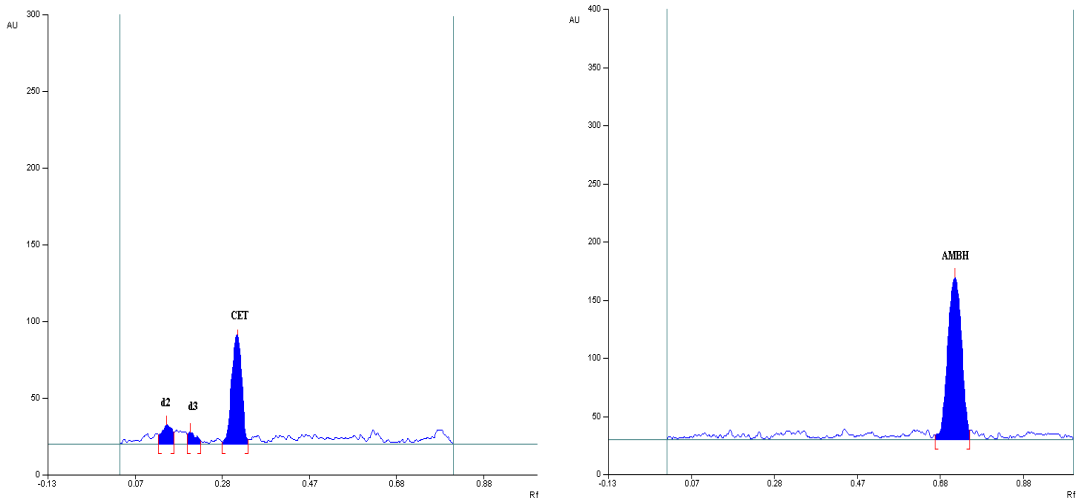
(a) **(b)**

Fig. 2: Representative densitogram of (a) CET (b) AMBH with degradation product d1(Rf =0.53)

Alkali treatment

1 mL working standard solution of CET (500 ng μL^{-1}) was mixed with 1 mL of 0.1 N NaOH and 8 mL of methanol. The solution was keep at room temperature for 4 hr. 2 µL of resulting solution was applied on TLC plate to get concentration 100 ng $band^{-1}$. Same procedure was

repeated for CET (6000 ng μL^{-1}) to get final concentration 1200 ng $band^{-1}$ applied on the TLC plate. Both the drugs were prone to alkali induced degradation with % degradation of 15.68 and 9.67 for CET and AMBH, respectively. The densitogram obtained after alkali degradation is shown in Fig. 3.

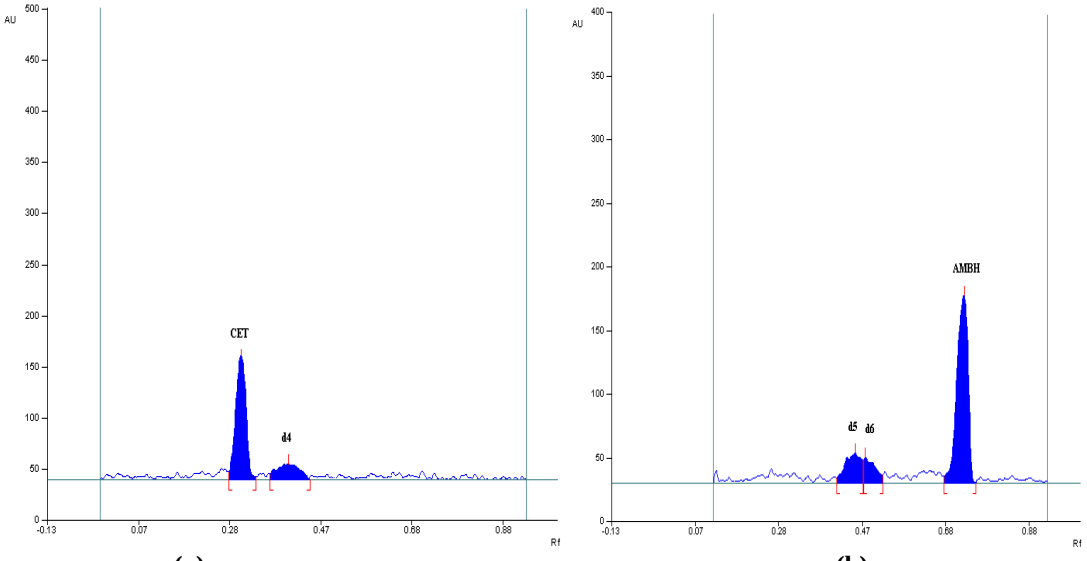


(a) **(b)**

Fig. 3: Representative densitogram of (a) CET with degradation product d2 (Rf= 0.13) and d3 (Rf= 0.19) (b) AMBH

Oxidative degradation

1 mL working standard solution of CET (500 ng μL^{-1}) was mixed with 1 mL of 6 % solution of H_2O_2 and 8 mL of methanol. The solution was keep at room temperature for 6 hr. 2 µL of resulting solution was applied on TLC plate to get concentration 100 ng $band^{-1}$. Same procedure was repeated for CET (6000 ng μL^{-1}) to get final concentration 1200 ng $band^{-1}$ applied on the TLC plate. About 83.01 % CET was recovered with one additional product and 79.63 % AMBH was recovered with two degradation peaks at Rf values of 0.45 and 0.49.

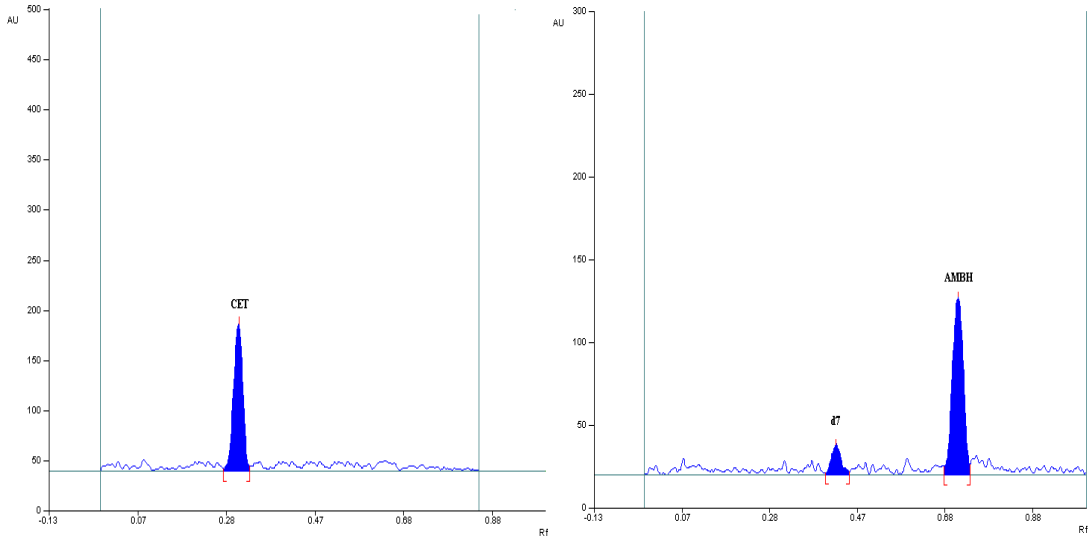


(a) **(b)**

Fig. 4: Representative densitogram of (a) CET with degradation product d4 (Rf= 0.40) (b) AMBH with degradation product d5 (Rf= 0.45) and d6 (Rf= 0.49)

Dry heat Degradation

Dry heat degradation was performed by keeping drugs in solid state individually in oven at 60ºC for period of 24 h. A sample was withdrawn at appropriate times, weighed and dissolved in methanol to get solution of 50 ng μL^{-1} for CET and 600 ng μL^{-1} for AMBH. 2 µl of the resulting solution was applied to HPTLC. Upon exposure to dry heat, CET showed 8.66 % of degradation whereas AMBH showed 13.75 % degradation with one degradation peak at Rf 0.43. The representative densitogram of samples subjected to dry heat degradation is shown in Fig. 5.

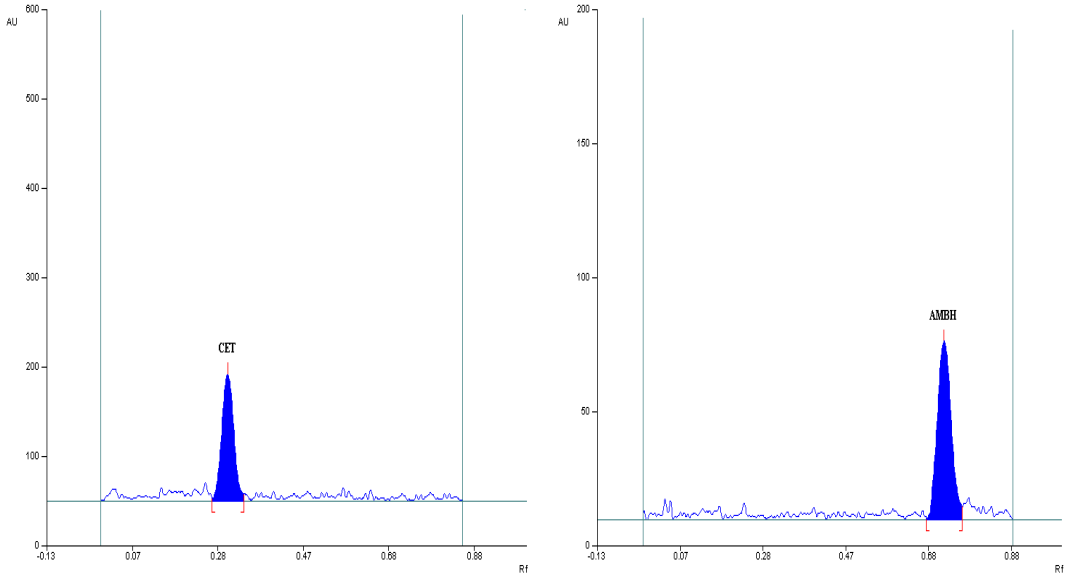


(a) **(b)**

Fig. 5: Representative densitogram of (a) CET (b) AMBH with degradation product d7 (Rf= 0.43)

Photo degradation studies

Photolytic studies were carried out by exposure of drug individually to UV light up to 200 watt h square meter^{-1} for a period of 24 h. Samples were weighed, dissolved in methanol to get concentration of 50 ng µL^{-1} for CET and 600 ng µL^{-1} for AMBH. 2 µL volume of the resulting solution was applied to HPTLC and analyzed. Both the drugs were found to be stable after exposure to UV light.



(a) **(b)**

Fig. 6: Representative densitogram of (a) CET (b) AMBH

RESULTS AND DISCUSSION

Optimization of chromatographic method

The purpose of developing this stability indicating HPTLC method is to achieve the separation of CET and AMBH and its degradation products. The chromatographic separation was achieved by linear ascending development in 10 cm × 10 cm twin trough glass chamber using Ethyl acetate: Methanol: Ammonia (8: 1.5: 0.5, v/v/v) as mobile phase and detection was carried out at 222 nm. The retention factors were found to be 0.30 ± 0.06 for CET and 0.71 ± 0.03 for AMBH, respectively. Representative densitogram of mixed standard solution of CET and AMBH is shown in Fig. 7.

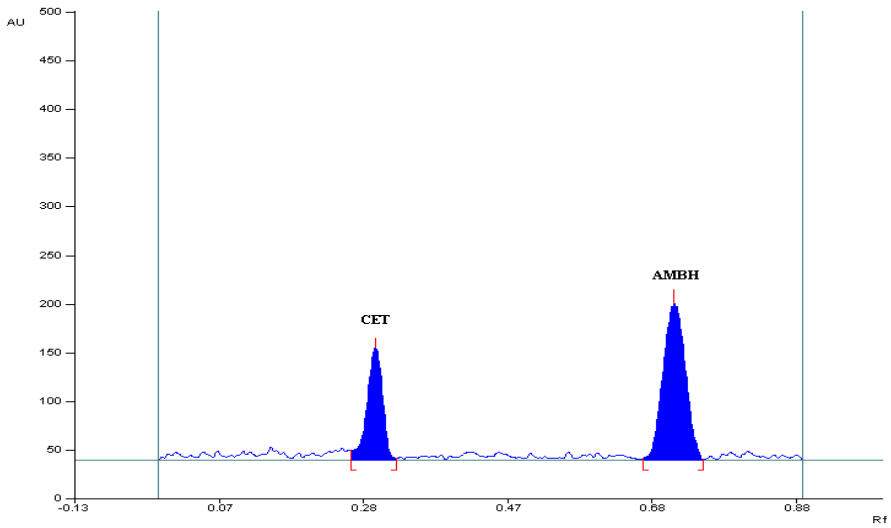


Fig. 7: Representative densitogram of mixed standard solution of CET (100 ng band^{-1}, Rf= 0.30 ± 0.06) and AMBH (1200 ng band^{-1}, Rf = 0.71 ± 0.03)

Stress degradation studies showed no interference of degradation products at retention time of drug. The degradation products were well resolved from both the drugs indicating specificity of the method. The results obtained after stress degradation studies are summarized in Table 1.

Table 1: Data of forced degradation studies of CET and AMBH

Stress conditions/ duration	CET		AMBH	
	% Assay of active substance	% degradation	% Assay of active substance	% degradation
Acidic / 0.1 N HCl/ Kept at RT for 4 h	91.38	8.62	86.76	13.23
Alkaline/0.1 N NaOH/ Kept at RT for 4 h	84.32	15.68	90.33	9.67
Oxidative/6 % H_2O_2/ Kept at RT for 6 h	83.01	16.99	79.63	20.36
Dry Heat 60º C for 24 h	91.34	8.66	86.25	13.75
Photolysis UV Light 24 h	100.34	stable	99.80	stable

Method Validation

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness as per ICH guidelines.[13, 14]

Linearity

Aliquots 1, 2, 3, 4, 5 and 6 µL of standard stock solutions of CET (25 µL^{-1}) and AMBH (300 ng µL^{-1}) were applied by over spotting on TLC plate. Linear response was obtained in the concentration range 25-150 ng band^{-1} for CET and 300-1800 ng band^{-1} for AMBH with correlation coefficient values of 0.999 and 0.996, respectively. The calibration curves obtained for CET and AMBH are depicted in Fig. 8 and Fig. 9.

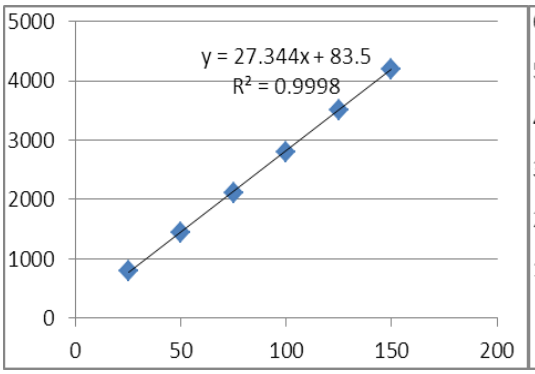


Fig. 8: Calibration curve for CET

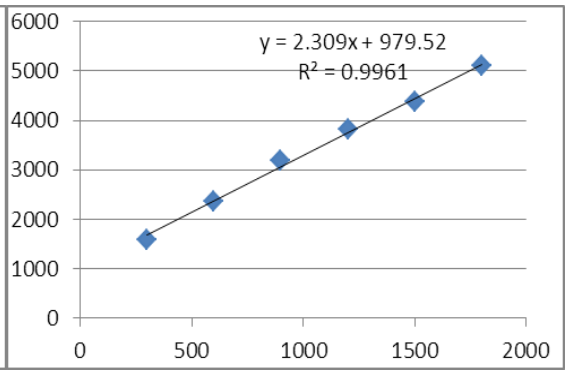


Fig. 9: Calibration curve for AMBH

Precision

Set of three different concentrations (75, 100, 125 ng band^{-1} for CET and 900, 1200, 1500 ng band^{-1} for AMBH) of standard solutions were prepared. All the solutions were analyzed on the same day in order to record any intra day variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.44 to 1.66 for CET and 0.29-0.71 for AMBH. For Inter day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. Inter day variation, as RSD (%) was found to be in the range of 0.49-1.61 for CET and to 0.29-0.50 for AMBH. The smaller values of % R.S.D. (< 2) indicated that method was found to be precise.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD values were found to be found to be 4.20 ng band^{-1} and 68.93 ng band^{-1}for CET and AMBH, respectively. LOQ values were found to be 12.73 ng band^{-1} and 208.88 ng band^{-1} for CET and AMBH, respectively.

Recovery Studies

Recovery studies were carried out by addition of standard drug to pre-analysed sample solution at three different levels 80, 100 and 120 % to check the accuracy of the method. Basic concentration of sample chosen was 50 ng band^{-1} for CET and 600 ng band^{-1}for AMBH. The drug concentrations were calculated from respective linearity equation. The % mean recovery was found to be 100.63 ± 1.23 for CET and 100.04 ± 0.53 for AMBH. The results obtained are shown in Table 2.

Table 2: Recovery Studies of CET and AMBH

Drug	Amount taken (ng band^{-1})	Amount added (ng band^{-1})	Total amount found (ng band^{-1})	% Recovery	% RSD
CET	50	40	91.30	101.53	1.48
	50	50	100.59	100.59	1.21
	50	60	109.75	99.78	0.96
AMBH	600	480	1077.7	99.79	0.54
	600	600	1198.92	99.91	0.50
	600	720	1325.67	100.43	0.55

*Average of three determinations

Robustness Studies

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, chamber saturation time was altered and the effect on the area of drug was noted. The areas of peaks of interest remained unaffected by small changes of the operational parameters (% R.S.D. < 2) indicated robustness of the method. The results are given in Table 3.

Table 3: Robustness Data in Terms of Peak Area (% RSD)

Sr. No.	Parameter	(% RSD)	
		CET	AMBH
1	Mobile phase composition (± 2 % methanol)	0.94	0.89
2	Chamber saturation time (± 5 %)	1.54	1.25

*Average of three determinations

CONCLUSIONS

The developed HPTLC method is simple, precise, specific, accurate, reproducible, and stability-indicating, without interference from the excipients or from degradation products resulting from treatment with acid, alkali, oxidizing agent, UV light and can be used for quantitative analysis of CET and AMBH in pharmaceutical dosage form.

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