RESEARCH ARTICLE

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DEVELOPMENT AND VALIDATION OF HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF AMBROXOL HYDROCHLORIDE AND CEFPODOXIME PROXETILE IN THEIR COMBINED TABLET DOSAGE FORM

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Abstract: The present manuscript describes new, simple, accurate, and precise high performance thin layer chromatography method for the simultaneous determination of Ambroxol Hydrochloride and Cefpodoxime Proxetile in combined tablet dosage form. Chromatographic separation of the drugs was performed on aluminium plates precoated with silica gel 60 F_{254} as the stationary phase and the solvent system consisted of Chloroform: Methanol: Hexane: Glacial acetic acid (6: 2: 4: 0.2 v/v/v/v). Densitometric evaluation of the separated zones was performed at 245 nm. The two drugs were satisfactorily resolved with R_f values 0.22 and 0.64 for Ambroxol Hydrochloride and Cefpodoxime Proxetile, respectively. The linear regression data for the calibration plots showed good relationship with $r^2 = 0.99925$ from 120-720 ng/spot for Ambroxol Hydrochloride and $r^2 = 0.99897$ from 200-1200 ng/spot for Cefpodoxime Proxetile. The methods were validated for precision, accuracy, and recovery. The percentage recovery for Ambroxol Hydrochloride was found to be 99.75 – 100.19 % and 99.86 – 100.02% for Cefpodoxime Proxetile. The limits of detection and quantification were 21.16 and 64.11 ng/spot per spot for Ambroxol Hydrochloride and 41.16 and 124.72 ng/spot per spot for Cefpodoxime Proxetile, respectively.

Keywords: Ambroxol Hydrochloride, Cefpodoxime Proxetile, High Performance Thin Layer Chromatography Method.

INTRODUCTION

Ambroxol Hydrochloride (AMB) is chemically Trans–4–(2–Amino-3, 5–dibromobenzylamino) - cyclohexanol[1] is a secretolytic agent used in the treatment of tracheobronchitis, emphysema with bronchitis pneumoconiosis, chronic inflammatory pulmonary conditions, bronchiectasis, bronchitis with bronchospasm asthma[2]. It is official in Indian Pharmacopoeia (IP) and British Pharmacopoeia (BP). IP[1] describes High Performance Liquid Chromatography (HPLC) method and BP[3] describes HPLC, Spectrophotometric and Thin Layer Chromatography (TLC) method. Literature survey also reveals Spectrophotometric[4-5], HPLC[6-7], Ultra Performance Liquid Chromatography (UPLC) [8] and HPTLC[9] methods for determination of AMB with other drugs.
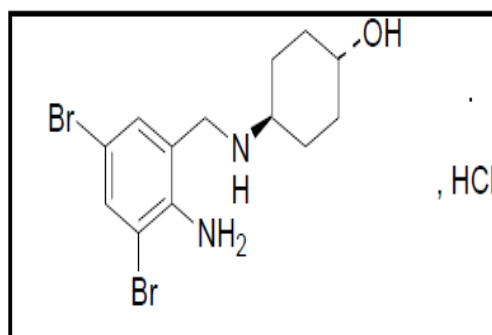

Figure 1. Structure of Ambroxol hydrochloride

Cefpodoxime Proxetile (CEFPO) is chemically 1-(isopropoxy carbonyloxy) ethyl (6R, 7R)-7-[2-(2-amino-4-thiazolyl)-(z)-2-(methoxyimino) acetamido]-3-methoxymethyl-3-cephem-4-carboxylate[10], is a third generation cephalosporin antibiotic. It is used for infections of the respiratory tract, urinary tract and skin and soft tissues. It has greater activity against staphylococcus aureus[11]. Cefpodoxime Proxetile is official in IP and USP. IP[12] and USP[13] describe liquid chromatography method for its estimation. Literature survey reveals Spectrophotometric[14-15], RP-HPLC[16] and HPTLC[17] methods for determination of CEFPO with other drugs.
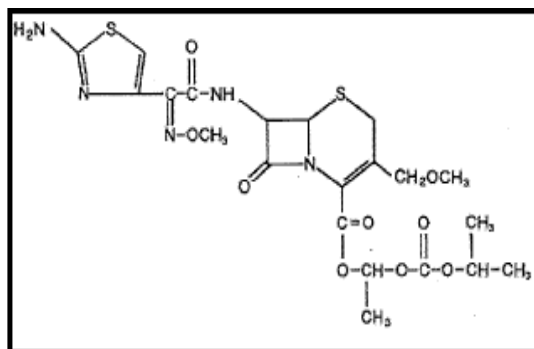
Figure 2. Structure of Cefpodoxime Proxetile

The combined dosage forms of AMB and CEFPO are available in the market for the Treatment of lower Respiratory Tract Infection in adults. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of AMB and CEFPO in their combined dosage forms. Literature survey does not reveal any simple HPTLC method for simultaneous estimation of AMB and CEFPO in combined dosage forms. The present communication describes simple, specific, rapid, accurate and precise chromatographic method based on High Performance Thin Layer Chromatographic method for simultaneous estimation of both drugs in their combined tablet dosage forms.

MATERIALS AND METHODS

Reagents and Materials

AMB and CEFPO bulk powder was kindly gifted by Cadila Pharmaceuticals Ltd. Ahmedabad, Gujarat, India and Baroque Pharmaceutical Ltd., Khambhat, Anand, Gujarat, India respectively. The commercial fixed dose combination product FINECEF- AM (AMB – 60 mg, CEFPO – 100 mg) was procured from the local market which is manufactured by Abott Healthcare Private Limited (AHPL). All chemicals and reagents were of analytical grade and were purchased from Thermo fisher scientific Pvt. ltd, Mumbai, India.

Instrumentation

CAMAG HPTLC instrument (Camag Muttenz, Switzerland) 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 (100 X 100 mm, layer thickness 0.2mm, E.MERCK). Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag Muttenz, Switzerland). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 to 400 nm.

Optimized chromatographic condition

Stationary phase: Pre-coated silica gel 60 F_{254} Aluminium Plates (10x10cm)

Mobile phase: Chloroform: Methanol: Hexane: Glacial acetic acid (6:2:4:0.2 v/v/v/v)

Chamber saturation: 20 minutes

Development distance: 70mm

Development time: 15 minutes

Relative temperature: 25 ±2°C

Scanning Speed: 20 mm/sec

Detection wavelength: 245 nm

AMB R_f: 0.22

CEFPO R_f: 0.64

Preparation of standard stock solutions

An accurately weighed quantity of AMB (100 mg) and CEFPO (100 mg) were transferred to a separate 100 ml volumetric flask and 50 ml methanol is added to both volumetric flask and sonicated for 10 minutes. Volume was adjusted up to the mark with methanol to obtain standard solution having concentration of AMB (1000 ng/μl) and CEFPO (1000 ng/μl). 12 ml of AMB (1000 ng/μl) and 20 ml of CEFPO (1000 ng/μl) aliquot were transferred to a separate 100 ml volumetric flask and diluted up to concentration of AMB (120 ng/μl) and CEFPO (200 ng/μl) with methanol.
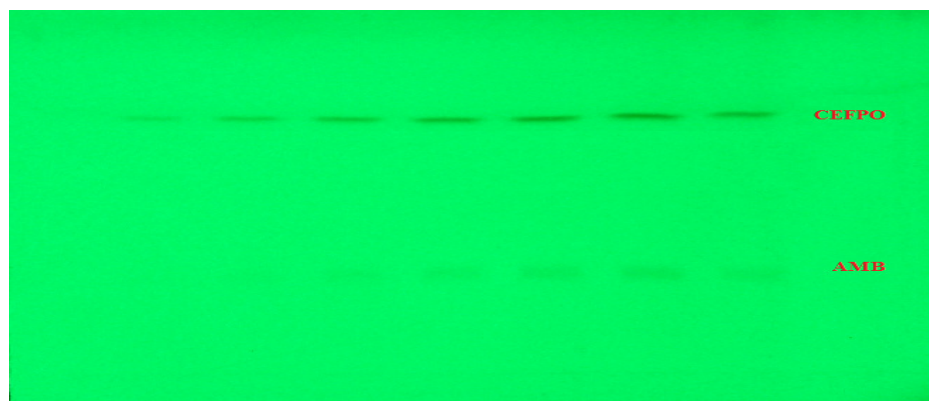

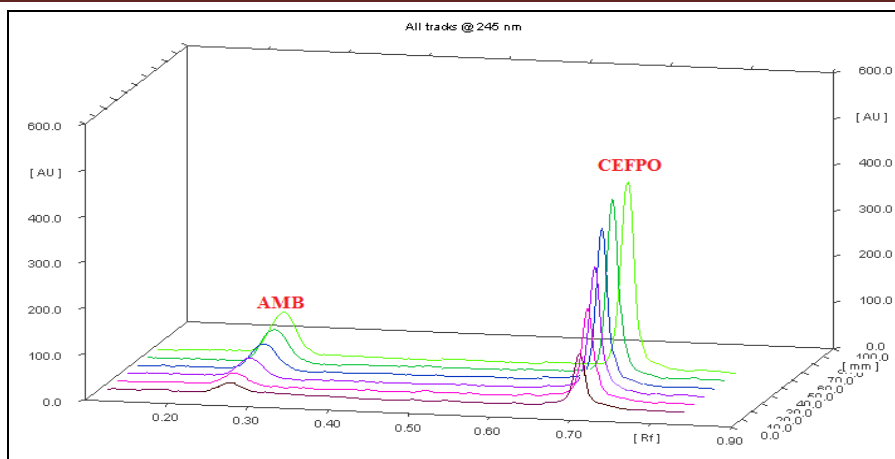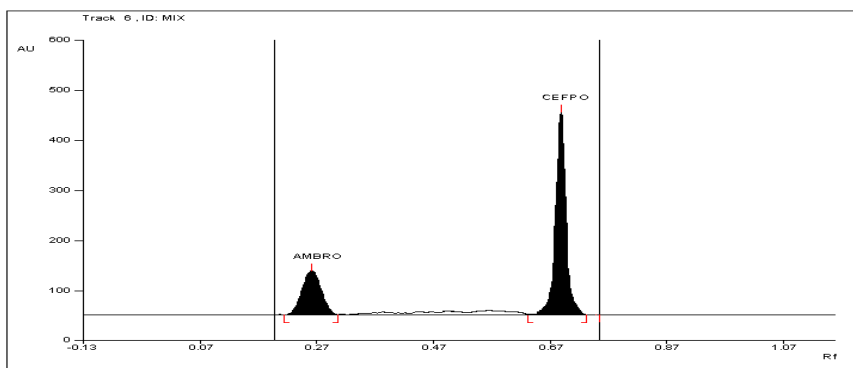Figure 3. Photograph of developed HPTLC plate of AMB and CEFPO

Figure 4. Overlain view of all tracks of AMB and CEFPO at 245nm

Figure 5. Densitogram of marketed formulation containing
420 ng/spot AMB and 700 ng/spot CEFPO

VALIDATION OF THE PROPOSED METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[18].

Linearity and range

From the mixed standard stock solution 120 ng/µl of AMB and 200 ng/µl of CEFPO, 1 to 6 µl solution spotted on HPTLC plate to obtain final concentration 120-720 ng/spot for AMB and 200-1200 ng/spot for CEFPO. Each concentration was applied six times to the HPTLC plate. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

Precision

The precision of the method was verified by repeatability and intermediate precision studies.

a) Repeatability

Repeatability studies were performed by analysis of all concentrations (120, 240, 360, 480, 600 and 720 ng/spot for AMB and 200, 400, 600, 800, 1000 and 1200 ng/spot for CEFPO) of the drug in six times on the same day.

b) Intermediate precision

The intermediate precision of the method was checked by intraday and inter day study. The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of AMB and CEFPO (360, 480, 600 ng/spot for AMB and 600, 800, 1000 ng/spot for CEFPO). The result was reported in terms of relative standard deviation (% RSD).

Specificity

The specificity of the method was determined by analyzing standard drug and test samples. The spot for AMB and CEFPO in the samples was confirmed by comparing the R_f and spectrum of the spot with that of a standard. The peak purity of AMB and CEFPO was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

Accuracy

Accuracy of the method was carried out by applying the method to drug sample (AMB and CEFPO combination tablet) to which know amount of AMB and CEFPO standard powder corresponding to 50, 100 and 150% of label claim had been added (standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

ANALYSIS OF AMB AND CEFPO IN COMBINED TABLET DOSAGE FORM

Twenty Tablets were weighed and powdered. The powder equivalent to 60 mg of AMB and 100 mg of CEFPO was transferred to a 100 ml volumetric flask. Methanol (50 ml) was added to it and sonicated for 20 min. The solution was filtered through Whatman filter paper (0.45μ) and the volume was adjusted up to the mark with methanol. This solution is expected to contain 600 ng/μl of AMB and 1000 ng/μl of CEFPO. This solution (20 ml) was taken in to a 100 ml volumetric flask and the volume was adjusted up to mark with methanol to get a concentration of AMB (60 ng/μl) and CEFPO (100 ng/μl). 3.5 µl of the prepared sample was applied on pre-washed TLC plate, developed in the above mobile phase,

dried in air and photo metrically analyzed by running chromatogram in optimized mobile phase. From the peak area obtained in the chromatogram, the amounts of both the drugs were calculated.

RESULTS AND DISCUSSION

The results of validation studies on simultaneous estimation method developed for AMB and CEFPO in the current study involving Chloroform: Methanol: Hexane: Glacial acetic acid (6: 2: 4: 0.2 v/v/v/v) as the mobile phase for HPTLC are given below.

The proposed method was found to be simple, specific, accurate, and precise for the routine simultaneous estimation of two drugs. The linearity range for AMB and CEFPO were found to be 120 – 720 ng/spot and 200-1200 ng/spot respectively. Regression analysis data and summary of all validation parameters is given in Table 1. Precision was calculated as repeatability (% RSD) and intra and inter day variation (% RSD) for both the drugs. Accuracy was determined by calculating the recovery and the mean was determined. The LOD and LOQ were found to be 21.15 and 64.11 ng/spot respectively for AMB and 41.16 and 124.73 ng/spot respectively for CEFPO indicates sensitivity of the proposed method. The peak purity of

AMB and CEFPO was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot. The peak purity was found to be 0.9985 and 0.9994 for AMB and CEFPO respectively. The method was successfully used to determine the amounts of AMB and CEFPO present in tablets. The results obtained are in good agreement with the corresponding labelled amount. By observing the validation parameters, the method was found to be specific, accurate and precise. Hence the method can be employed for the routine analysis of these drugs in combinations.

CONCLUSION

Introducing HPTLC into pharmaceutical analysis represents a major step in terms of quality assurance. Today HPTLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC - thus reducing the analysis time and cost per analysis.

The developed HPTLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable

for the analysis of AMB and CEFPO in pharmaceutical formulation without any interference from the excipients. The common excipients and other additives are usually present in the tablet dosage form do not interfere in the analysis of AMB and CEFPO in method, hence it can be conveniently adopted for routine quality control.

Table 1.

Regression analysis data and summary of validation parameters for the proposed method

Parameters	High Performance Thin Layer Chromatography method	
	AMB	**CEFPO**
Concentration Range (ng/spot)	120 – 720	200 – 1200
Slope (m)	2.961430	3.569898
Intercept (c)	-24.97130	1602.241
Correlation Coefficient (r^2)	0.99925	0.99897
Accuracy (% recovery) (n = 3)	99.75 – 100.19 %	99.86 – 100.02%
Repeatability (%RSD) (n = 6)	0.32 %	0.10 %
Intraday (n = 3) (%RSD)	0.45 – 0.51 %	0.10 – 0.13 %
Interday(n = 3) (%RSD)	1.10 – 1.37 %	0.55 – 1.35 %
LOD (ng/spot)	21.16	41.16
LOQ (ng/spot)	64.11	124.72

Table 2.

Recovery data of proposed method

Drug	Level	Amount taken (ng/spot)	Amount added (ng/spot)	Amount Recovered (ng/spot) (n=3)	% Recovery (n=3)
AMB	0 %	360	0	360.7	100.19 ±0.045
	50 %	360	180	539.6	99.925 ±0.017
	100 %	360	360	720.2	100.02 ±0.035
	150 %	360	540	897.8	99.755 ±0.070
CEFPO	0 %	600	0	599.4	99.90 ± 0.029
	50 %	600	300	900.2	100.02 ±0.060
	100 %	600	600	1198.4	99.86 ± 0.080
	150 %	600	900	1499.1	99.94 ± 0.051

Table 3.

Analysis of AMB and CEFPO by proposed method

	Label claim (mg)		Amount taken (ng/spot)		Amount Recovered (ng/spot) (n=3)		% Label claim	
Tablet	AMB	CEFPO	AMB	CEFPO	AMB	CEFPO	AMB	CEFPO
FINECEF - AM	60	100	420	700	419.7	700.4	99.92 %	100.05 %

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