Validated HPTLC Method for Determination of Cefixime Trihydrate and Erdosteine in Bulk and combined Pharmaceutical Dosage Form.

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Abstract

A simple, accurate, precise and rapid high-performance thin-layer chromatographic method for determination of Cefixime Trihydrate and Erdosteine in Bulk and combined Pharmaceutical Dosage Form was developed and validated. The method employed TLC aluminium plates precoated with silica gel $60F_{254}$ as the stationary phase. The solvent system consisted of Ethyl Acetate : Acetone : Methanol : Water (7.5:2.5:2.5:1.5) as mobile phase. Densitometric analysis was carried out at 235 nm. The system was found to give compact spots for Cefixime Trihydrate and Erdosteine at R_f of 0.35 ± 0.05 and 0.56 ± 0.05 respectively. The linear regression analysis data showed good linear relationship in the concentration range 100-500 ng band^{-1} and 150-750 ng band^{-1} for Cefixime Trihydrate and Erdosteine respectively. Percent Recovery for Cefixime Trihydrate was 99.47-101.85 and that for Erdosteine was 98.99-101.51. Method was found to be reproducible with % relative standard deviation (%R.S.D) for intra and interday precision to be <1.5% over the said concentration range. The limits of quantitation for Cefixime Trihydrate and Erdosteine were 11.14 ng band^{-1} and 15.63 ng band^{-1} respectively. The method was validated for precision, accuracy, specificity and robustness. The method has been successfully applied in the analysis of combined capsule dosage form.

Keywords:

Cefixime Trihydrate; Erdosteine; densitometry; Validation; HPTLC

1. Introduction

Cefixime trihydrate, (CEF) is the third generation cephalosporin antibiotic. Cefixime Trihydrate is given orally in the treatment of susceptible infections including respiratory tract infections like acute exacerbations of chronic bronchitis, gonorrhoea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary-tract infections [1]. It official in USP. Chemially it is 5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-corboxylic acid, 7-[[2-amino-4-thiazolyl)][(carboxymethoxy)imino] acetyl]amino]-3-ethenyl-8-oxo-, trihydrate, [6R-[6∝,7β(Z)]]-(6R,7R)-7-[2-(-amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid, 7^2-(Z)-[O-carboxymethyl)oxime] [2]. Erdosteine (ERDO) [[2-Oxo-2-[(tetrahydro-2-oxo-3-thienyl) amino] ethyl]thio]acetic acid is a mucolytic and is official in Martindale[3]. It Modulates mucus production, viscosity and increases mucociliary transport, thereby improving expectoration and thus it shows mucolytic and antitussive activity [4,5]. Investigations have been done to study effect of mucolytic on antibiotic penetration in sputum and it reveals that mucolytics improve the same[6-8]. Hence combination of an antibiotic with a mucolytic is a treatment of choice for acute exacerbations of chronic bronchitis. Also comparative evaluation of CEF plus ERDO and Amoxicillin plus

Bromhexine shows that former gives faster and better symptomatic relief and was also better tolerated than later [9]. As Combination is not available in the market it was developed and optimized. There are several investigations concerning the determination of CEF alone and in combination with other drugs in pharmaceutical preparations and plasma by UV, HPLC, LC-MS, HPTLC methods [10-14]available. Also Stability indicating HPTLC method [15], HPLC using a fluorescent chiral tagging reagent method [16], LC-MS- MS method [17] were available for ERDO alone.

No references have been found for simultaneous quantitative determination of CEF and ERDO in pharmaceutical preparations. Hence attempts were made to develop simultaneous HPTLC method.

In this paper we report simple, accurate, precise and sensitive Reverse phase high performance thin layer chromatography method for simultaneous determination of Cefixime trihydrate and Erdosteine in combined capsule dosage form.The proposed method is optimized and validated according to ICH guidelines.

2. Experimental

2.1. Drugs, Reagents and Chemicals

CEF was kindly provided by Maxim Phramaceuticals, Pune, India and ERDO was obtained from Glenmark Pharmaceuticals, Mumbai, India. Ethyl Acetate, Acetone, Methanol (all AR grade) were purchased from Sisco Research Laboratories Ltd, Mumbai.

2.2. Instrumentation

Chromatographic separation was performed on a Merck TLC plates precoated with silica gel 60 F_{254} (10 cm ×10 cm with 250 μm thickness, E. Merck, Darmstadt, Germany, purchased by Anchrom Technologies, Mumbai, India). The samples were applied onto the plates using Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe as a band with 6 mm width using a Camag Linomat 5 applicator (Camag, Muttenz, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (20cm x 10 cm, 10 x 10 cm). Densitometric scanning was performed on Camag TLC scanner 3 at 235 nm for all measurements and operated by winCATS software (V 1.4.2, Camag).

2.3. Preparation of Standard Stock Solution

10 mg of each of CEF and ERDO were weighed separately and dissolved in 5 mL of AR grade methanol and then volume was made up to 10 mL so as to get the concentration 1000 μg mL^{-1}.

2.4. Selection of analytical wavelength

From the standard stock solution further dilutions were done using mobile phase and scanned over the range of 200- 400 nm and the spectra were overlain. It was observed that both drugs showed considerable absorbance at 235nm as shown in Fig.1 and therefore 235 nm was selected as detection wavelength.
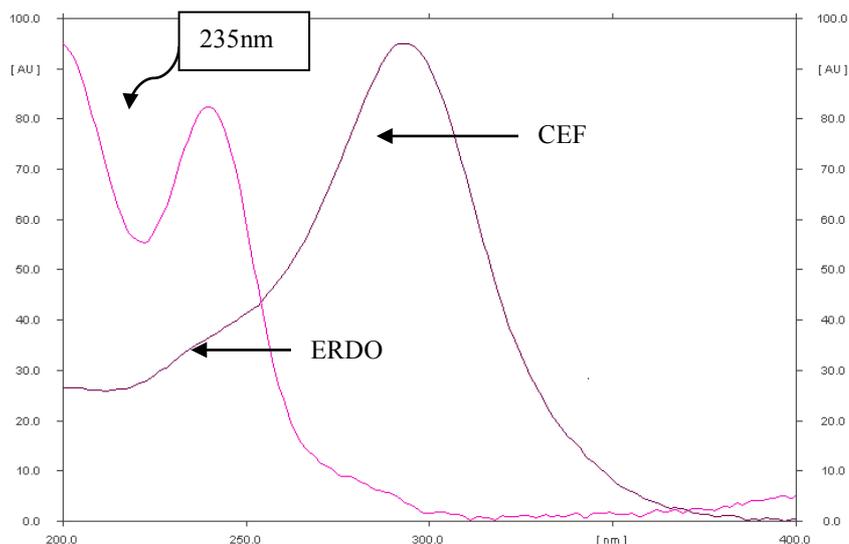
Fig.1 Overlain Spectra of Cefixime Trihydrate (CEF) and Erdosteine (ERDO)

2.5. Preparation of calibration curves

The standard solutions of each drug were prepared by dilution of the stock solution with methanol to get a concentration of 100 µg mL^{-1}. From standard solution of CEF   1- 5 µL and from ERDO standard solution 1.5-7.5 µL were spotted on the TLC plate to obtain final concentration of 100-500 ng band^{-1} for CEF and 150-750 ng band^{-1} for ERDO . The plate was developed in ascending vertical manner using solvent system Ethyl Acetate:Acetone: Methanol:Water (7.5:2.5:2.5:1.5) (v/v/v/v) after 15 min of chamber saturation. Linear ascending development was carried out in a twin trough glass chamber (20cm x 10 cm, 10 x 10 cm). The length of chromatogram run was 90 mm. The developed plates were dried and densitometric scanning was performed in the absorbance mode at 235 nm. The slit dimension was kept at 5 x 0.45 mm. After completion of chromatographic analysis, peak areas of both drugs were noted and plotted against corresponding concentrations and least square regression analysis was performed to generate the calibration equation.

The equations of the regression line for CEF was

Y=4.970 x+43.59   r^2=0.996

and that for ERDO

Y=5.731x-221.3   r^2=0.995

2.6. Analysis of Capsule formulation

As formulation is not availabale in the market till date, it was developed and optimised. Each hard gelatin capsule contains 200 mg of Cefixime Trihydrate USP equivalent to Cefixime   and 300 mg of Erdosteine. The contents of 20 capsules were emptied and powdered. A quantity of powder equivalent to 10 mg of CEF was weighed and transferred to 10 mL volumetric flask. Methanol was added to the same flask and sonicated for 10 minutes. The volume was made up to 10 mL with methanol. The solution was filtered using whatmann filter paper No.41. The stock solution was spotted with the help of applicator to get final concentration of 200 ng band^{-1} for CEF and 300 ng band^{-1} for ERDO. The solutions were spotted keeping 10 mm distance between bands. The amount of each drug present per capsule was estimated from the respective calibration curves.

2.7. Method Validation

As per ICH guidelines, method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation, robustness and specificity.

2.8. Linearity

Linearity of the method was studied by spotting five concentrations of each drug prepared in the methanol, in the range of 100-500 ng band^{-1} for CEF and 150-750 ng band^{-1} for ERDO and noting the peak areas.

2.9. Accuracy

For accuracy of method, recovery study was carried out by applying the method to drug sample to which known amount of both drugs were added separately at level of 80, 100 and 120% of label claim (standard addition method). At each level of the amount, three determinations were performed and the results obtained were compared with expected results.

2.10. Precision

The precision of the method was demonstrated by system precision and repeatability. In System precision 6 repeated measurements of standard solutions of both drugs were made and percentage RSD was calculated. Repeatability was demonstrated by intra-day and inter-day variation studies. In the intra day studies, 3 repeated measurements of standard and sample solutions were made in a day and percentage RSD were calculated. In the inter day variation studies, 3 repeated measurements of standard and sample solutions were made on 3 consecutive days and percentage RSD were calculated.

2.11. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD was calculated using the following formula

$$LOD = \frac{3.3x\ Standard\ Deviation\ of\ the\ response}{Slope\ of\ calibration\ curve}$$

LOQ was calculated using the following formula

$$LOQ = \frac{10x\ Standard\ Deviation\ of\ the\ response}{Slope\ of\ calibration\ curve}$$

2.12. Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which time of spotting to development, time of development to scanning were altered and the changes in the area values were noted by calculating % RSD values.

2.13. Specificity

The specificity of the method was ascertained by comparing R$_f$ values and spectra of Standard and sample.

3. Result and Discussion

3.1. Optimization of Solvent System and Chromatographic Conditions

Chromatographic separation studies were carried out on the stock solution of CEF and ERDO. Initially the plates were spotted with 10μL of stock solution and developed by linear

ascending development method using solvents like toluene, hexane, methanol, chloroform, dichloromethane, ethyl acetate, acetone, acetonitrile, etc. Based on the results of these initial chromatograms, binary and ternary mixtures of solvents were tried to achieve optimum resolution and acceaptable peak parameter. The final mobile phase consisting of Ethyl Acetate:Acetone:Methanol:Water in the ratio of (7.5:2.5:2.5:1.5) was selected since optimum resolution and good peaks for both the drugs were obtained as shown in Fig. 6.2.2. The samples were applied in form of bands of width 6 mm on precoated aluminum sheets of silica gel 60 F_{254}. The application position (X) and (Y) were kept at 10 mm and 10 mm respectively to avoid edge effect. Linear ascending development was carried out in a twin trough glass chamber (20cm x 10 cm, 10 x 10 cm), using 15 mins of chamber saturation. The length of chromatogram run was 90 mm. The plate was dried and scanned at 235nm over 90 mm distance.
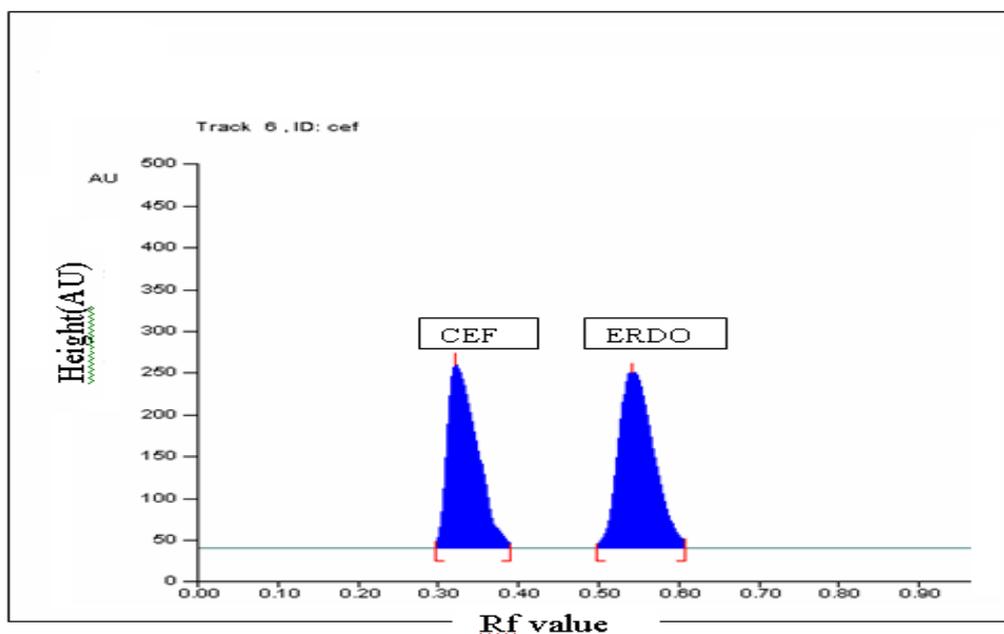
Fig.2 Densitogram of ERDO and CEF

3.2. Linearity

When peak area was plotted Vs Concentration (ng band^{-1}) both CEF and ERDO showed good correlation coefficient in concentration range of 100–500 ng band^{-1} and 150-750 ng band^{-1}, respectively. Linearity was evaluated by determining five standard working solutions.

3.3. Precision

The proposed method was found to be precise as indicated by percent RSD (% Relative Standard Deviation) for system precision and repeatability which was not more than 1.5.

3.4. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD was found to be 5.29 ng band^{-1} and 5.15 ng band^{-1} for CEF and ERDO respectively. LOQ was found to be 11.14 ng band^{-1} and 15.63 ng band^{-1} for CEF and ERDO, respectively.

Table1 summarizes Beer's law limit, linear regression equation, correlation coefficient, LOD, and LOQ for the method .

Table 1. Summary of linearity, LOD and LOQ

Parameters	For Cefixime Trihydrate	For Erdosteine
Wavelength (nm)	235	
Beer's Law Limit (ng band^{-1})	100-500	150-750
Correlation coefficient (r)	0.996	0.995
Linear regression Equation (y = mx + c), (r^2)	4.970 x+43.59 r^2=0.996	Y=5.731x-221.3 r^2=0.995
Slope (m)	4.970	5.731
Intercept (c)	43.59	221.3
Limit of detection (ng band^{-1})	5.29	5.15
Limit of quantitation (ng band^{-1})	11.44	15.63
Precision indicated by %RSD	< 1.5%	< 1.5%

3.5. Analysis of capsule formulation

The proposed method was also evaluated in terms of assay of formulated and optimised CEF and ERDO capsules. Six replicate determinations were performed on the accurately weighed amounts of capsules. The results obtained are shown in Table 2.

Table. 2 Analysis of capsule formulation

Sample No.	Amount present (ng band^{-1})		Amount Found (ng band^{-1})		Label Claim, %	
	CEF	ERDO	CEF	ERDO	CEF	ERDO
1	200	300	203.17	304.01	101.58	101.33
2	200	300	199.51	297.56	99.75	99.18
3	200	300	201.80	297.97	100.90	99.32
4	200	300	199.73	300.17	99.86	100.05
5	200	300	200.15	302.28	100.07	100.76
6	200	300	200.99	299.42	100.49	99.80

*Average of six determinations.

3.6. Accuracy

The proposed method when used for estimation of CEF and ERDO from capsule dosage form after spiking with working standard. Results of Recovery studies are shown in Table 3

Table 3. Recovery Studies of Cefixime Trihydrate and Erdosteine

Level of Recovery	Amount spotted		Amount recovered		Mean Recovery, %		RSD, %	
	CEF	ERDO	CEF	ERDO	CEF	ERDO	CEF	ERDO
80	160	240	160.16	240.60	80.08	80.20	0.68	1.25
100	200	300	202.07	301.38	101.03	100.46	1.13	1.04
120	240	360	241.96	360.86	120.98	120.28	1.09	0.94

3.7. Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which time of spotting to development, time of development to scanning were altered and the changes in the area were noted by calculating % RSD values. The result obtained is shown in Table.4

Table 4. Robustness Study for Cefixime Trihydrate and Erdosteine

Parameters varied	RSD of Peak Area, %	
	CEF	ERDO
Time from Spotting to development	1.17	0.98
Time from development to scanning	1.35	1.24

3.8. Specificity

The method was found to be specific since no interfering spots were seen when R_f values of standard and sample were compared. There is no difference in the spectra of sample and standard solution which indicate the specificity of the method.

4. Conclusion

The validated HPTLC method employed here proved to be simple, fast, accurate, precise and sensitive, thus can be used for routine analysis of CEF and ERDO in combined solid oral dosage forms.

Acknowledgement

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