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Research Article

RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFIXIME TRIHYDRATE AND LINEZOLID IN TABLET DOSAGE FORM

Satish A. Patel and Jinalben V Patel*

Department of Pharmaceutical Analysis, Centre for Health ScienceStudies, Ganpat University, GanpatVidyanagar – 384012, Mehsana, Gujarat, India.

ABSTRACT

A simple and sensitive reversed phase High Performance Liquid Chromatographic method has been developed and validated for the simultaneous analysis of the Cefiximetrihydrate (CEF) and Linezolid (LIN) in tablet dosage form. The separation was carried out using mobile phase consisting of buffer and methanol with pH 2.5 in the ratio of 70:30, v/v. The column used was ACE 5 C18, (150 mm x 4.6 mm i.d., 5 µm) with flow rate 1.2 ml/min using PDA detection at 250 nm. The method was linear over a concentration range of 23.33 – 40 µg/ml and 70 – 120 µg/ml for CEF and LIN, respectively. The retention time of CEF and LIN were found to be 3.30 min and 8.07 min, respectively. Results of analysis were validated statistically and by recovery studies. The mean recovery was 101.9 ± 0.82 and 102.6 ± 1.15 for CEF and LIN, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) for CEF and LIN were found to be 0.78 and 2.37 µg/ml and 2.42 and 7.34 µg/ml, respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate which is useful for the routine determination of CEF and LIN in its pharmaceutical dosage form.

Keywords: Cefiximetrihydrate, Linezolid, RP-HPLC, Validation.

INTRODUCTION

Cefiximetrihydrate (CEF) is an oral third generation cephalosporin class of antibiotic. Chemically, it is (6*R*, 7R)-7-{[2-(2-amino-1,3-thiazol-4-yl)-2(carboxymethoxy-imino)acetyl]amino}-3-ethenyl-8-oxo-5-thia-1 azabicyclo-[4.2.0]oct-2-ene-2 carboxylic acid[1], clinically used in the treatment of susceptible infections including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary-tract infections[2](Figure 1). It is official in Indian Pharmacopoeia (IP)[3], British Pharmacopoeia(BP)[4], United States Pharmacopoeia(USP)[5], European Pharmacopoeia(EP)[6], Japanese Pharmacopoeia (JP)[7]. Literature survey reveals spectrophotometric, TLC, HPTLC, HPLC and HPCE method for estimation of CEF individually[8-14] and in combination with other drugs in bulk drugs and human plasma[15-23]. Linezolid (LIN) is first drug of the oxazolidinone class of antibiotic drug and chemically it is N-{[(5S)-3-[3-fluoro-4(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-yl]methyl}acetamide and it is also useful as antibacterial agents[1,2](Figure 2).Linezolid (LIN) is official in Indian Pharmacopoeia (IP)[3]. Literature survey reveals spectrophotometric, HPLC and HPTLC methods for estimation of LIN individually and in combination with other drugs[24-28]. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of CEF and LIN in their combined dosage forms. Literature survey reveals some simple spectrophotometric method for simultaneous estimation of CEF and LIN in dosage forms[29-30]. The present communication describes simple, sensitive, accurate and precise RP-HPLC method

for simultaneous estimation of both drugs in their combined tablet dosage form.

MATERIALS AND METHODS

Apparatus

RP-HPLC instrument (Shimadzu, LC-2010CHT, Japan) equipped with a UV-Visible detector and a photodiode array detector, auto sampler, ACE 5 C18 column (150 X 4.6mm, 5µ particle size) was used. Chromatograms were automatically obtained by LC-Solution system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India), Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Reagent and materials

CEF and LINbulk powder was kindly gifted by Welable Healthcare, Mehsana, Gujarat, India. The commercial fixed dose combination product was procured from the local market. Methanol AR Grade was procured from Finar Chemicals Ltd., Ahmedabad, India, triethylamine and ortho-phosphoric acid (AR Grade) were procured from Merck Specialities Pvt. Ltd., Worli, Mumbai.

Preparation of buffer solution (pH 2.5)

1 ml of triethylamine(TEA) was transferred to 1000ml volumetric flask and diluted upto mark with HPLC grade water and pH 2.5 was adjusted with dilute ortho- phosphoric acid.

Preparation of mobile phase

Mobile phase was prepared by mixing 70ml of the buffer solution, pH 2.5 and 30ml of methanol.

Preparation of standard stock solutions(166.6µg/ml CEF and 500µg/ml LIN)

The stock solutions of CEF and LIN were prepared by accurately weighing 16.66 mg CEF and 50 mgLIN (according to ratio of combination CEF:LIN, 1:3) in same 100 ml volumetric flasks and dissolve it in 30ml methanol and it was sonicated for 5 min and diluted to 100 ml with buffer, pH 2.5.

Preparation of working standard solutions

The working standard solutions of CEF and LIN were prepared by accurately transferring (7,8, 9, 10,11,12ml) aliquots to 50ml volumetric flask and were made upto mark with buffer:methanol (70:30).

Preparation of sample solution

Twenty tablets were weighed and powdered. The powder equivalent to 16.66 mg of CEF and 50 mg of LIN was transferred to 100ml volumetric flask. Methanol (30 ml) was added and sonicated for 20 min. The solution was filtered through whatman filter paper No. 41 and the volume was adjusted up to the mark with buffer pH 2.5 to prepare 166.6µg/ml of CEF and 500 µg/ml of LIN. Above solution is further diluted to obtain solution having 33.33µg/ml CEF and 100 µg/ml of LIN.

Methodology

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for CEF and LIN was obtained with a mobile phase buffer:methanol (70:30, v/v) at a flow rate 1.2 ml/min to get better reproducibility and repeatability. Quantification was carried out at 250 nm based on peak area because both drugs shows good absorbance at this wavelength (Figure 3). Complete resolution of the peaks with clear baseline was obtained (Figure 4). System suitability test parameters for CEF and LIN for the proposed method are reported in Table 1.

Validation of proposed method

The proposed method was validated according to the International Conference on Harmonization(ICH) guidlines[31].

Calibration Curve (Linearity)

Calibration curves were constructed by plotting peak area vs. concentration of CEF and LIN, and regression equation were calculated (Figure 5, 6). The calibration curves were plotted over the concentration range of 23.33–40 µg/ml for CEF and 70-120 µg/ml for LIN. From standard stock solution of mixture of CEF and LIN, (7, 8, 9, 10, 11, 12 ml) aliquots were taken in 50 ml volumetric flask and diluted upto mark with the mobile phase(buffer: methanol, 70:30, v/v).Aliquots (20µl) of each solution were injected under the operating chromatographic condition described above.

Method precision (Repeatability)

The precision of the instrument was checked by repeated injected six sample solutions of CEF (26.66 µg/ml) and LIN (80µg/ml) under the same chromatographic condition and measurement of peak area, retention time and tailing factor. The low %RSD values(less than 2%) indicates that proposed method is repeatable.

Intermediate precision (Reproducibility)
The intraday and interdayprecision 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 sample solutions of CEF(26.66, 30, 33.33 µg/ml) and LIN (80, 90, 100µg/ml). The result was reported in terms of relative standard deviation (% RSD).

Limit of detection and Limit of quantification
LOD and LOQ of dug calculated using the following equations designated by International Conference on Harmonization (ICH) guidelines[31].

$$LOD = 3.3 \times \sigma/S$$
$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

The limit of detection (LOD) and limit of quantification (LOQ) for CEF and LIN were found to be 0.78 µg/ml and 2.37 µg/ml and 2.42 µg/ml and 7.34 µg/ml,respectively. These data show that method is sensitive for the determination of CEF and LIN.

Accuracy (Recovery study)
The accuracy of the method was determined by calculating recovery of CEF and LIN by the standard addition method. Known amounts of standard solutions of CEF (50, 100, 150 % level) were added to pre quantified sample solutions of CEF and LIN. The amounts of CFE and LIN were estimated by applying obtained values to the regression equation of the calibration curve. The closeness of obtained value to the true value indicates that the proposed method is accurate.

Assay of pharmaceutical formulation
The proposed validated method was successfully applied to determine CEF and LIN in their tablet dosage form. The result obtained for CEF and LIN was comparable with the corresponding labeled amounts. The RP-HPLC chromatogram for CEF and LIN in sample was recorded and is shown in (Figure 7).

RESULTS AND DISCUSSION
A RP-HPLC method was developed and validated for the determination of CEF and LIN in tablet dosage forms on ACE 5 C18 column (C18, 150 X 4.6 mmi.d., 5µm) with variable wavelength detection at 250 nm. The retention time of CEFand LIN was 3.30 min and 8.07 min and respectively. Linear correlation was obtained between area and concentration of CEF and LIN in the concentration range of 23.33-40 µg/ml and 70-120 µg/ml respectively. The low RSD value of interday(0.73-0.90% for CEF and 1.15-1.45 % for LIN) and intraday (0.52-0.81% for CEF and 0.81-1.07% for LIN)at 250 nm, reveal that proposed method is precise. The limit of detection (LOD) and limit of quantification (LOQ) for CEF and LIN were found to be 0.78µg/mland2.37 µg/ml and 2.42µg/ml and 7.34 µg/ml respectively. These data show that method is sensitive for the determination of CEF and LIN.
The recovery experiment was performed by the standard addition method. The mean recoveries were101.9 ± 0.82 and 102.6 ± 1.15 for CEF and LIN, respectively(Table 2). The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated method was successfully applied to determine CEF and LIN in the tablet dosage form. The results obtained for CEF and LIN were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of CEF and LIN in pharmaceutical dosage form.

CONCLUSION
In this proposed method the linearity is observed in the concentration range of 23.33-40 µg/ml and 70-120 µg/ml with co-efficient of correlation, (r^2) = 0.9991 and (r^2) = 0.9976 for CEF and LIN, respectively at 250 nm. The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the CEF and LIN in combined dosage form without any interference of excipients.

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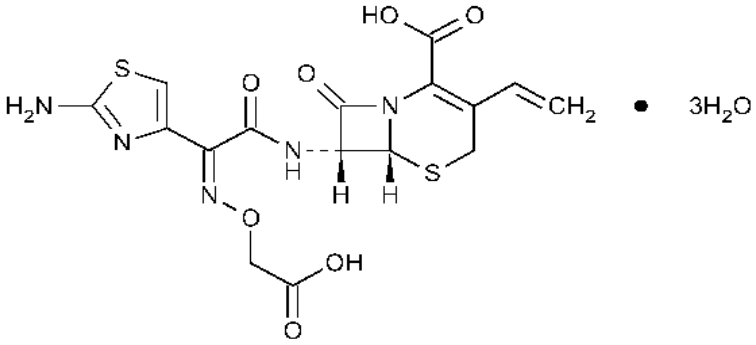

Fig. 1: Chemical structure of CefiximeTrihydrate (CEF)

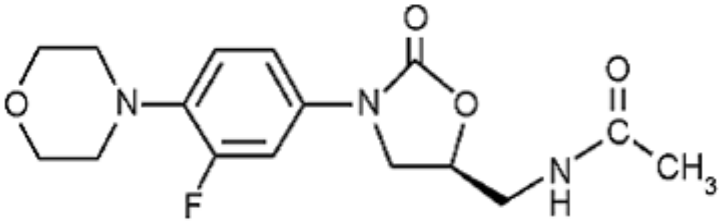

Fig. 2: Chemical structure of Linezolid (LIN)

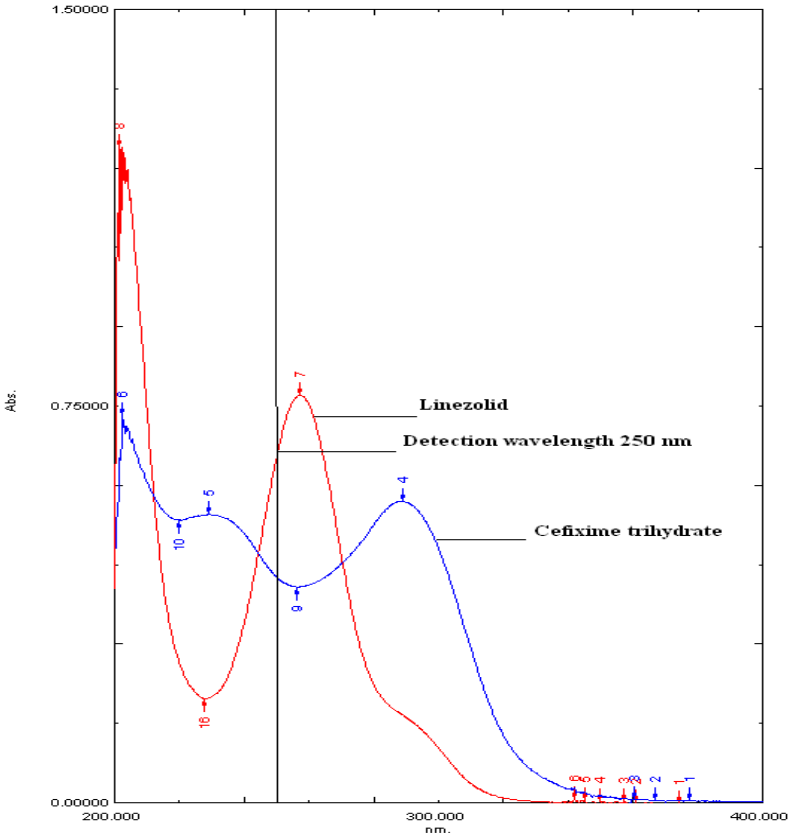

Fig. 3: UV Spectra for determination of wavelength

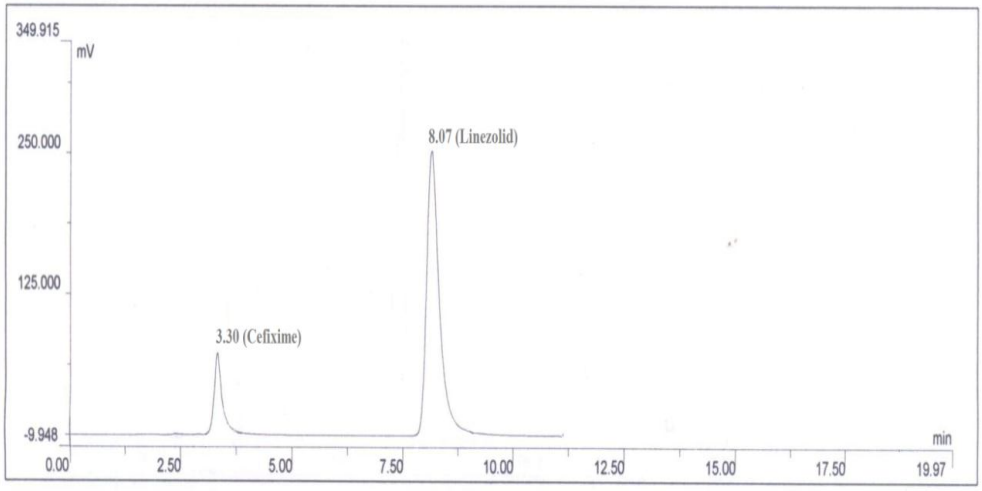

Fig. 4: Chromatogram of mixed standard solution of CEF: LIN (33.33:100 µg/ml) at 250 nm

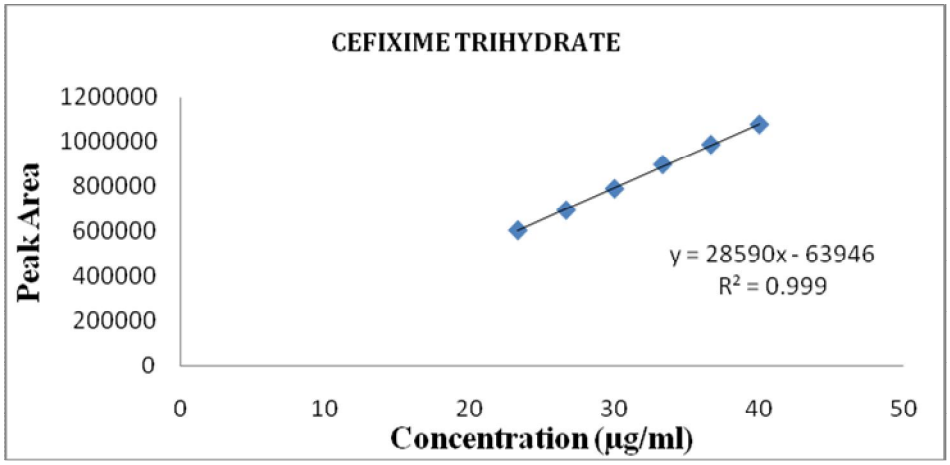

Fig. 5: Calibration curve of CEF at 250 nm

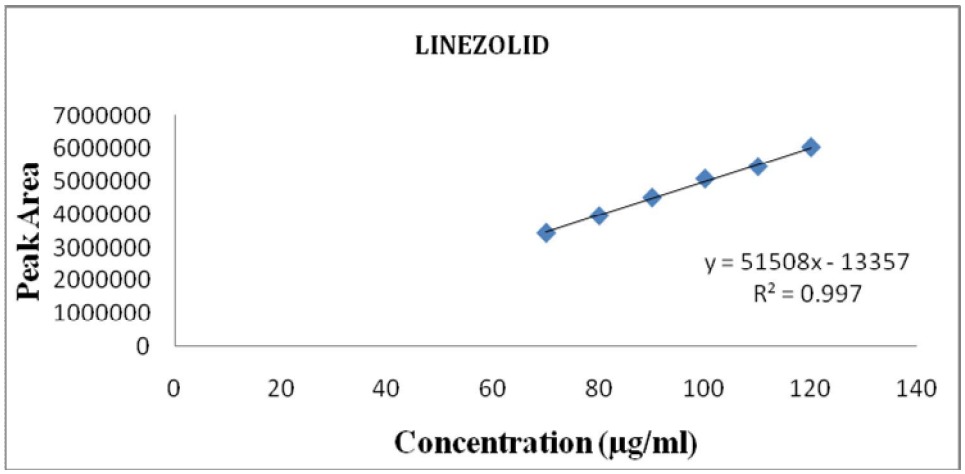

Fig. 6: Calibration curve of LIN at 250 nm

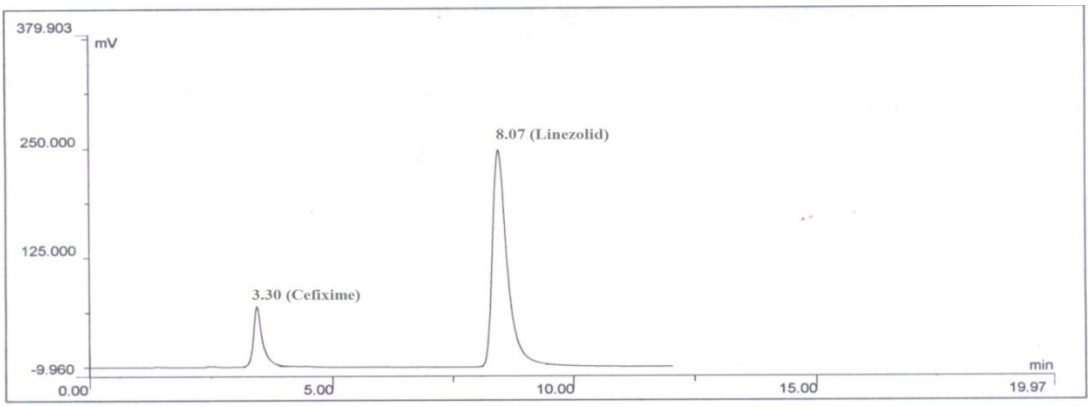

Fig. 7: Chromatogram of sample solution of CEF: LIN (33.33:100 µg/ml) at 250nm

Table 1: System suitability parameters

Parameters	CEF ± RSD (n = 6)	LIN ± RSD (n = 6)
Retention time (min)	3.305± 0.87	8.073 ± 0.76
Tailing factor	1.484 ± 0.57	1.488 ± 0.67
Theoretical plates	2303 ± 0.92	4566 ± 0.95
Resolution	15.27 ± 0.67	

Table 2: Determination of Recovery

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % Recovery ± RSD
CEF	I	8	50 %	101.3 ± 0.72
	II	8	100 %	101.7 ± 0.30
	III	8	150 %	102.9 ± 0.29
LIN	I	1	50 %	101.4 ± 0.54
	II	1	100 %	102.6 ± 0.19
	III	1	150 %	103.8 ± 0.32

Table 3: Analysis of formulation of CEF and LIN by proposed method (n=6)

Sample No.	Label Claim		Amount Found		% Label Claim	
	CEF (mg/tab)	LIN (mg/tab)	CEF (mg/tab)	LIN (mg/tab)	CEF (%)	LIN (%)
1	200	600	200.6	603.0	100.3	100.5
2	200	600	201.9	601.9	100.9	100.3
3	200	600	200.6	600.0	100.3	100.0
4	200	600	200.9	601.9	100.4	100.3
5	200	600	201.6	601.7	100.8	100.3
6	200	600	201.5	602.1	100.8	100.4
Mean			201.2	601.8	100.6	100.3
S.D.			0.554	0.99	0.276	0.164
RSD%			1.65	0.99	0.83	0.16

Table 4: Regression analysis data and summary of validated parameters for the proposed method

Parameters		RP-HPLC method	
		CEF	LIN
Detection wavelength(nm)		250	250
Beer's law limit		23.33 to 40	70 to 120
Regression equation y = mx + c		y = 28590x - 63946	y = 51508x – 133571
Slope		28590	51508
Intercept		63946	133571
Correlation coefficient		0.9991	0.9976
Repeatability (% RSD, n = 6)		0.92	1.13
Precision (%RSD)	Intraday(%RSD)	0.52 - 0.81	0.81 - 1.07
	Interday(%RSD)	0.73 - 0.90	1.15 - 1.45
LOD (µg/ml)		0.78	2.42
LOQ (µg/ml)		2.37	7.34
% Recovery (Accuracy, n = 6)		101.9 ± 0.82	102.6 ± 1.15
% Assay		100.6 ± 0.83	100.3 ± 0.16

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