



Development and Validation of RP-HPLC method for Simultaneous Estimation of Cefexime and Linezolid

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Abstract

This work is concerned with application of simple, accurate, precise and highly selective reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of cefexime and linezolid in combined dosage form. Chromatographic separation was achieved by using a reverse phase C18 column (Hypersil BDS 250X 4.6X5 μ m). The mobile phase composed methanol: potassium dihydrogen phosphate buffer in ratio 40:60 at flow rate of 1ml/min. The pH was adjusted to 3.5 with TEA. Detection was carried out using a UV-vis detector at 277 nm. The mean retention time of cefexime and linezolid was found to be 3.76 min 6.55 min respectively. The method was found to be linear in the range of 5 - 15 μ g /ml and 15 -45 μ g /ml for cefexime and linezolid respectively, with mean recovery of 99.81-100.14 % cefexime and 99.99-100.47 % for linezolid the correlation coefficient for both cefexime and linezolid were closed to one. The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribe values thus proposed method was successfully applied for simultaneous determination of cefexime and linezolid in routine analysis of formulation.

Key-Words: RP-HPLC, Cefexime, Linezolid

Introduction

Cefixime Trihydrate (CEF) is chemically (6R, 7R)-7-[2-(2-amino-1, 3-thiazol-4-yl)-2-[(carboxymethoxy) imido] acetamido] -3-ethyl-8-oxo-5-thia-1-azabicyclo [4.2.0.] -oct-2-ene-2- carboxylic acid is a third generation cephalosporin antibiotic used in the treatment of respiratory tract infection, typhoid fever, complicated and uncomplicated Urinary Tract Infection. It is official in Indian pharmacopoeia (IP,)United State Pharmacopoeia (USP) and British Pharmacopoeia (BP). USP and BP describes High Performance Liquid Chromatography (HPLC) method.[1,2]

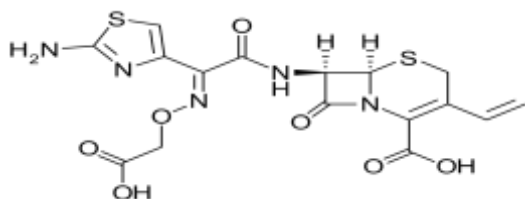


Fig. 1: Cefixime Trihydrate (CEF)

Linezolid (LNZ) is chemically N-{{[(5S)-3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl]methyl} acetamide is oxazolidinone antibiotic. It inhibits bacterial protein synthesis by acting at an early step and a site different from that of other AMAs. It binds to 23s fraction of the 50s ribosome and interfere with formation of the ternary N-formylmethionine-t-RNA-70s initiation complex. Binding of linezolid distorts the t-RNA binding site overlapping both 50s and 30s ribosomal subunits and stops protein synthesis before it starts (Tripathi, 2008). Linezolid is official in IP describe liquid chromatography method for its estimation. [3,4]

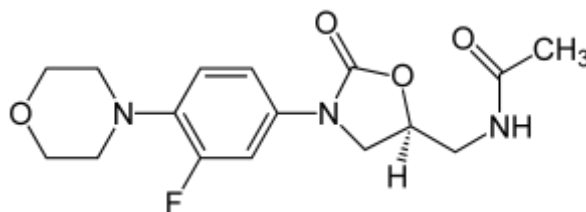


Fig. 2: Linezolid (LNZ)

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Literature survey reveals Spectrophotometric of cefixime [5-9], spectrophotometric for linezolid(10,12) spectrophotometric methods for determination of CEF and LNZ in single and in combination with other drugs have been carried out. (13).

Material and Methods

Chemicals & Reagents

Linezolid Active Pharmaceutical Ingredient (API) was supplied by Windlas Biotech as gift sample . Cefixime Active Pharmaceutical Ingredient (API) was supplied by Arvinod Pharma as gift sample. All chemicals and reagents were of analytical grade. HPLC grade methanol, water from Loba chem. Combined tablet formulation (ZIFITURBO) contain cefixime 200 mg and linezolid 600 mg was procured from local market.

Instrumentation and chromatographic conditions:

The HPLC instrument system consisted of analytical CXTH-30007 equipped with prominence diode array detector (SPD-M20A)with auto sampler. Stationary phase used was a reverse-phase C18 column (Hypersil BDS 250X 4.6X5µm). The data was acquired by a chromatography module connected to a personal computer and processing was performed LC solution software. The mobile phase consisted of 0.05 M potassium dihydrogen phosphate buffer: methanol (40:60v/v) . pH of mobile phase was adjusted 3.5 with TEA. The flow rate was 1ml/min. The mean retention time for CEFI and LINE was 3.76 and 6.55 min respectively.

Preparation of Standard Solution

Accurately weighed quantity of Linezolid 100 mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with methanol. This will give a stock solution having strength of 1000 µg/ml.. Accurately weighed quantity of Cefixime 100 mg (equivalent to 100 mg of CPO) was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with methanol. This will give a stock solution having strength of 1000 µg/ml. Preparation of Working Standard Solution of LINEZOLID 100 µg/ml solution was prepared by diluting 1 ml of stock solution to 10 ml with methanol. Preparation of Working Standard Solution of Cefixime 100 µg/ml, solution was prepared by diluting 1 ml of stock solution in 10 ml with methanol.

Sample Preparation

Accurately 20 tablets were weighed and determine average weight of tablets. Then tablets were finely crushed and tablet powder equivalent to 100 mcg/ml CEFI & 300 mcg/ml LINE was transferred into 100 ml volumetric flask and mobile Phase was added. It was shaken vigorously for 5 to 10 minutes and

sonicate. Make up volume up to 100ml. than solution was filtered and the final concentration of test sample solution was made up to 10 mcg/ml of CEFI and 30 mcg/ml of LINE.

Results and Discussion

Method development and optimization of chromatographic conditions

Chromatographic separation was achieved on C18 (10 mm x4.6 mm, 2.6 µm particle size). Phase by varying concentration of organic phase and water simultaneously Ph was varied. The process of optimization success was achived by making use of 0.05M Potassium dihydrogen Phosphate buffer: Methanol (40:60 v/v) . pH of mobile phase was adjusted 3.5 with TEA . Flow rate was 1 ml/min . both CEFI and LINE were well separated from each other with mean retention time for CEFI and LINE 3.76 min 6.55 min respectively

Method validation:

Linearity:

The linearity of Cefixime and Linezolid was in the range of 5-15 µg/ml, and 15-45µg/ml respectively.

Table 1: Linear Regression Data for Calibration Curve

Parameter	CEFI	LINE
Linearity range (µg/ml)	5-15µg/ml	15-45µg/ml
Correlation coefficient	0.9997	0.9997
Intercept	94.62	256.13

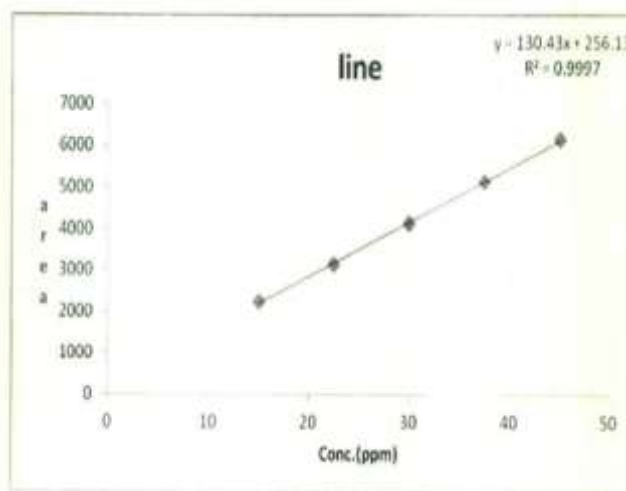


Fig. 3: Standerd calibration curve Linezolid (LNZ)

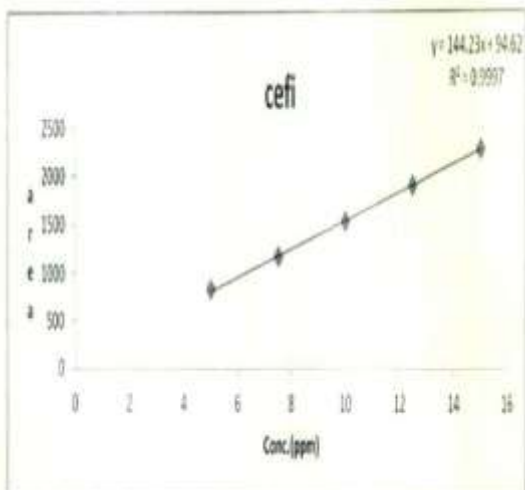


Fig. 4: Standard calibration curve Cefixim (CEFI)

Precision

Intraday precision

The data for intraday precision for Cefixime and Linezolid is shown in table 5.10. The % R.S.D. for Intraday precision was found to be 0.32-0.50 for Cefixime and 0.84-1.73 for Linezolid.

Interday precision

The data for inter day precision for Cefixime and Linezolid is shown in table 5.10. The % R.S.D. for interday precision was found to be 1.06-1.63 for Cefixime and 0.63-1.60 for Linezolid.

Table 2: Result of Intraday precision (RP-HPLC Method)

Cefixime			Linezolid		
Cone.	Amount Found ± S.D	% RSD	Cone.	Amount Foun ± S.D.	%RSD
5	4.86±0.023	0.47	15	14.45±0.2514	1.73
10	9.78±0.0321	0.32	30	29.1S±0.4707	1.61
15	1S.10±0.0763	0.50	45	4S.02±0.3781	0.84

Table 3 -Result of Inter day precision (RP-HPLC Method)

Cefixime			Linezolid		
Cone.	Amount Found ± S.D	% RSD	Cone,	Amount Foun ± S.D.	%RSD
5	4.88±0.0519	1.06	15	14.42±0.2311	1.60
10	9.93±0.1300	1.30	30	29.69±0.1882	0.63
15	15.16±0.2400	1.63	45	45.29±0.3637	0.80

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