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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. Chromatographi separation was achieved by using a reverse phase C18 coloum (Hypersil BDS 250X 4.6X5µm). 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 µg /ml and 15 -45 µg /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-thaizol-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]

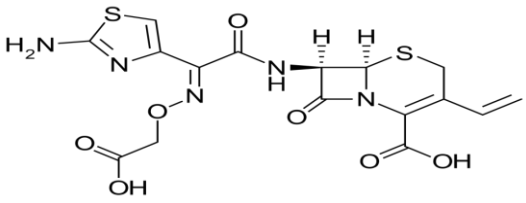

Fig. 1: Cefixime Trihydrate (CEF)

Linezolid (LNZ) is chemically N-{[(5S)-3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl]methyl} acetamideis oxazolidinone antibiotic. It inhibits bacterial protein synthesis by actingat an early step and a site different from that of other AMAs. It binds to 23s fraction of the50s ribosome and interfere with formatiossn of the ternary N-formylmethionine-t-RNA-70sinitiation complex. Binding of linezolid distorts the t-RNA binding site overlapping both 50sand 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]

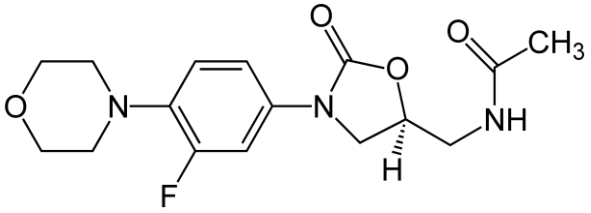

Fig. 2: Linezolid (LNZ)

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Literature survey reveals Spectrophotometric of cefixime [5-9], spectrophptometric for linezolide(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 dihyderogen 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 Linezolide 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

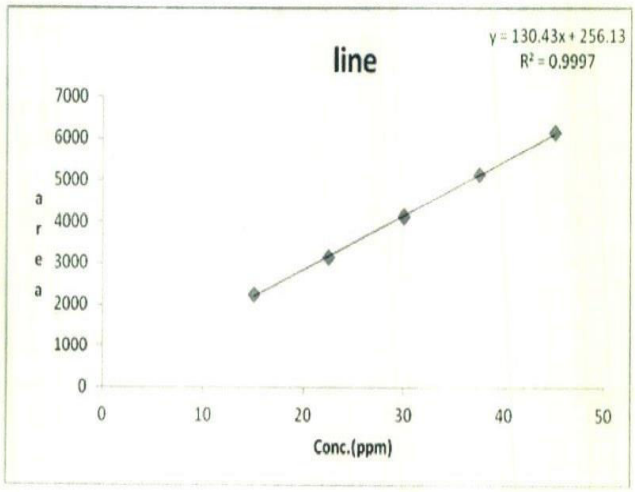

Fig. 3: Standerd caliberation curve Linezolide (LNZ)

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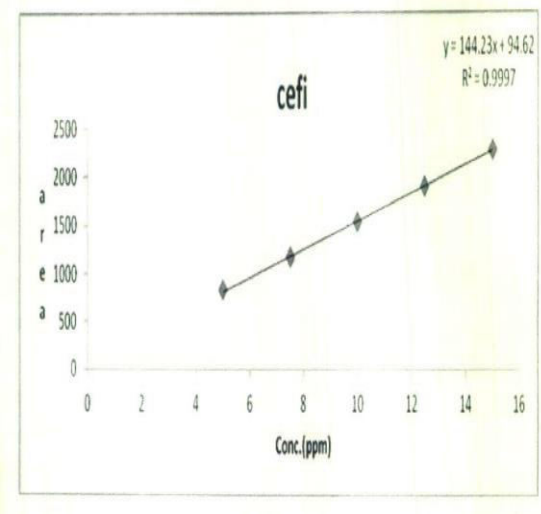

Fig. 4: Standerd caliberation curve Cefixim (CEFI)

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 0.84-1.73for 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	lS.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

References

1. Indian Pharmacopoeia, Volume 2, Ghaziabad: Govt. of Indian Ministry of Health and Family Welfare, the Controller of Publication, 2010, The Indian Pharmacopoeial Commission, pp.1012-14, 1590-92.
2. The United States Pharmacopoeia, 2ndedition, Rockville, MD: The US Pharmacopoeial Convention, Inc., 2009, pp. 909-911.
3. Tripathi KD. Antimicrobial drugs: Essentials of medical pharmacology. New Delhi, ND. J P Brothers medical publishers (p) ltd. 2008:733.
4. Shah V, Raj H. Development and validation of derivative spectroscopic method for simultaneous estimation of cefixime trihydrate and azithromycin dihydrate in combined dosage form.Int J Phar Sci Re 2012;3(6):1753-1760.
5. Khaja pasha, Dr pthil, c.S., Dr. K. Vijaykumar, Dr. Ali Sadath, Dr. V.B.Chimkod Reverse phase Hplc Method for the Determination of Cefixime in Pharmaceutical Dosage Froms. Research Journal of pharmaceutical, Biological and Chemical Scinences. July - September 2010 Rjpbcs Volume 1 (issue 3) page no. 226.
6. Ashok Kumar* , Lalitkishore, Nair Anroop, Kaur Navpreet, Kineticspectrophotometric Method For the estimation of Cefixime in pharmaceutical Formulations. Der pharma chemical, 2011, 3 (4), Page No: 279-291.
7. Khandagle kapil s., Gandhi santosh v. *, Deshpande Padmanbh b., And Gaikwad Nilesh v. A simple and sensitive RpHplc Method of simultaneous estimation of Cefixime and Ofloxacin in combined Tablet Dosage Form. International Journal of Pharmacy and Pharmaceutical Sciences. Vol 3, (issue 1), 2011.
8. Kathiresan K., Murugan R., Hameed, M.S.Inimai, K.G. And Kanyadhara T. Analytical Method Development and validation o9f Cerfixime and Dicoloxacillin Tablets by Rp-Hplc; Rasayan Journal of Chemical, 2009, Vol.2(3), 588-592.
9. Magar S. D, Tupe A. P, Pawar P. Y, Mane B.Y. ;Simultaneous Spertrophotometric EStimatiojn of Cefixime and Azithrohomycin in Tablet Dosage Form;Current Pharma Research, 2012, vol.2(3), 535-538.
10. P.Vanitha, Amala Mateti, P.Prashanthi, Manish kumar Thimmaraju, N. Reghunandan Method Development And Vilidation of Linezolid in Bulk And Formulation using UV Spectro-photometric Method., Journal of Scientific Research in Pharmacy, ISSN 2247-9469, Received on 9-8-2012
11. K. Jayaprasanti,B.Syamasundar, A Validated RP-HPLC Method For The Determination of Linezolid In Pharmaceutical Dosage Forms. International Journal of Pharmacy and Bio sciences, ISSN 0975-6299.
12. Garva Gaur, Vipin Kukkar, Ramaniranjan Singh. A Validated RP-HPLC Method For Estimation of Linezolide Infusion. International journal of pharmaceutical research and development, vol 4 (1) march 2012 page no. 132-135.
13. Shah Helly, Patel Payal, Patel Khushbu, Solanki Sagar. Method development and validation of spectrophotometric methods for simultaneous estimation of cefixime thrihyrate and linezolid in their combined tablet dosage form. International journal of pharmacy and bio sciences, vol 1 (5), phage no. 516-519.
14. Prashanthi P, Amala M, Vanitha P, et al. Development and validation of UV spectrophotometric method for the Estimation of Linezolid in bulk and pharmaceutical formulation. International Journal of Natural Products Research 2012 2(3):57-60.
15. Lories IB. Stability-indicating methods for the determination of linezolid in the presence of its alkaline-induced degradation products. Talanta 2003;60:945-953.
16. Prasanti KJ, SyamaSundar B. A validated RP-HPLC method for the determination of linezolid in pharmaceutical dosage forms. International Journal of Pharma & Bio Sciences 2012; 3(3):44-51.
17. Mallapur S, Panghal S, Mubeen G, et al. A Novel HPLC-PDA Method Development and Validation for theSimultaneous Estimation of Cefixime and Ambroxol HCl in Tablets.Int J Ph Sci 2011;3(2):1279-1287.
18. Trivedi KJ, Chokshi PV, Patel NS. Development and validation Of RP-HPLC method for analysis of Cefixime Trihydrate and Sulbactam Sodium in their combination tablet dosage form. Int J Chem Tech Res 2012;4(4):1628-1632.
19. Khandagle KS, Gandhi SV, Deshpande PB, et al. A simple and sensitive RP-HPLC method for simultaneous estimation of Cefixime and Ofloxacin in combined tablet dosage form. Int J Pharm Pharm Sci 2011;3(1):46-48.

20. Gaur G, Kukkar V, Singh R. A validated RP-HPLC method for estimation of linezolid in linezolid infusion. International Journal of Pharmaceytical Resarch & Development 2012;4(1):132-135.
21. Attimarad M, Nair AB. Simulataneous determination of ofloxacin and cefixime by first and ratio first derivative UV spectrophotometry. Chronicles of young scientists 2011;2(3):144-149.
22. Deshpande MM, Kasture VS, Gosavi SA. Application of HPLC and HPTLC for the Simultaneous Determination of Cefixime Trihydrate and Ambroxol Hydrochloride in Pharmaceutical Dosage Form. Eurasian J Analy Chem 2010;5(3):227-238.
23. Dhoka MV, Gawande VT, Joshi PP. Simultaneous estimation of cefixime trihydrate and erdosteine in pharmaceutical dosage form by using reverse phase - high performance liquid chromatography.Int J Chem Tech Res 2010;2(1):79-87.
24. Patel J, JoshiS. Simultaneous estimation of cefixime trihydrate and ornidazole in combined tablet dosage form by RP-HPLC. J Chem Pharm Res 2014;4(4):2167-2172.

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