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Research Article

DEVELOPMENT AND VALIDATION OF A NEW RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF CEFIXIME AND LINEZOLID IN TABLET DOSAGE FORMS

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ABSTRACT

A simple, precise, stability indicating new RP-HPLC method was developed and validated for the Simultaneous determination of Cefiximeand Linezolid. In this method separation of liquid was done by using column Inertsil ODSC18, 150X4.6mm, 5µwith mobile phase of 0.1M Sodium Dihydrogen Ortho Phosphate (pH 4.7) and Acetonitrile were mixed in the ratio of 60:40 ratio. The detection wavelength was found to be 272 nm with a flow rate of 1ml/minute and temperature of 300C.Retention time of Cefixime was 3.161 minutes and Linezolide was 4.774 minutes respectively. The proposed method was validated as per standard guidelines. In the range of 8µg to 45µg the linearity of Cefiximeshows a co-relation coefficient of 0.9991 and Linezolide was 24 to144 µg 0.9991 respectively. System precision was found % RSD 0.31 and 0.27, whereas method precision was found % RSD to be 0.24 and 0.55 of Cefixime and Linezolide respectively. The % mean recovery of Cefiximeand Linezolide was found to be 99.87 and 99.77% respectively. The method was found to be robust even by changing in the flow rate 0.1ml and temperature change of ±5 condition .The developed method can be successfully employed for the routine analysis of Cefiximeand Linezolidein pharmaceutical dosage forms.

Key words: Cefixime(CEF), Linezolide(LIN), RP-HPLC Method development,Validation,Combined dosage Forms.

INTRODUCTION

Cefixime (CEF) is an oral third generation cephalosporin class of antibiotic. Chemically, it is (6R,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] [2].clinically used in the treatment of susceptible infections including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary-tract infections[3]. Linezolid (LIN) is first 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-5-yl]methyl}acetamide and it is also useful as Antibacterial Agents[1][2]. From the literature survey conducted, it was found that there are very few analytical methods reported for the estimation of Cefixime and Linezolid by reverse phase HPLC method. Presently the new formulation Cefixime and Linezolid available in market. There is no specific and economical method determination of Cefixime and Linezolid in combined pharmaceutical dosage form. So it was felt that there is a need to develop a sensitive analytical method for the simultaneous estimation of Cefixime and Linezolid in various pharmaceutical dosage forms available in market.

MATERIALS AND METHODS

Apparatus

Materials and methods

Chromatographic conditions

The mobile phase used was mixture of buffer of 0.1M Sodium Di Hydrogen Ortho Phosphate (pH 4.7) and Acetonitrile were mixed in the ratio of 60:40 v/v employing isocratic elution at a flow rate of 1.0 mL min-1 .The analytical column used was Inertsil ODSC18 (150mmx4.6mm,5µm) at a temperature of 30°C .The detection was carried out at a wavelength of 272nm for a run time of 12 min.

Preparation of standard stock solution:

An accurately weighed quantity of 100mg of Cefixime and 600mg of Linezolid were transferred into a 100 ml volumetric flask. Dissolved with 70 ml of diluent and sonicated for 15minutes and made volume up to the mark with the same solvent.

Preparation of standard solution

From the standard stock solution 1 ml is pipette out into 100 ml volumetric flask and made up the volume with diluent.

Preparation of sample stock solution

Twenty tablets were weighed and ground to a fine powder. An amount of powder equivalent to 100mg of Cefixime 600mg of Linezolid was weighed accurately and transferred into a 100 ml volumetric flask containing 70 ml of diluent and sonicated for 30 min and diluted to 100 ml with the same solvent.

Preparation of sample solution

The sample stock solution was filtered through 0.45 µm membrane filter and 1 ml of the filtrate was taken into 100 ml volumetric flask and made up to the volume with diluent.

Methodology

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for CEF and LIN were obtained with a mobile phase 0.1M Sodium Di Hydrogen Ortho Phosphate (pH 4.7) and Acetonitrile were mixed in the ratio of 60:40v/v at a flow rate 1ml/min to get better reproducibility and repeatability. Quantification was carried out at 272nm based on peak area because both drugs show good absorbance at this wavelength(Figure3). Complete resolution of the peaks with clear baseline was obtained (Figure 6). 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) guidelines.

Calibration Curve (Linearity)

Calibration curves were constructed by plotting peak area vs. concentration of CEF and LIN, and a regression equation were calculated. The calibration curves were plotted over the concentration range of 8-48µg/ml for CEF and 24-144µg/ml for LIN. From the standard stock solution of a mixture of CEF and LIN, (0.4, 0.8, 1.2, 1.6, 2, 2.4, ml) aliquots were taken in 100 ml volumetric flask and diluted to mark with the mobile phase(buffer: acetonitrile 60:40, v/v).Aliquots (20µl) of each solution were injected under the operating chromatographic condition described above. The results were shown in Table-2 andTable-3, the calibration curve for Cefixime and Linezolid were shown in Fig-5 and Fig-6.

Method precision (Repeatability)

The precision of the instrument was checked by repeated injected six sample solutions of CEF and LIN 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. The results were shown in Table-4 and 5.

Intermediate precision (Reproducibility)

The intraday and interday precision of the proposed method were determined by analyzing the corresponding responses three times on the same day and on three different days over a period of 1week for three different concentrations of sample solutions of CEF and LIN. The result was reported regarding relative standard deviation (% RSD).The results were shown in Table-6 and 7.

Limit of detection and Limit of quantification

LOD and LOQ of dug calculated using the following equations designated by International Conference on Harmonization (ICH) guidelines31.

LOD = 3.3 × σ/S

LOQ = 10 × σ/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 3.70 µg/ml and 1.21 µg/ml and 11.5 µg/ml and 3.79 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 (80, 100, 120 % 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. The % recovery for Cefixime and Linezolid were calculated by injecting the samples, and the results were shown in Table-8

Robustness

Robustness as a measure of method capability to remain unaffected by small, but deliberate changes in chromatographic conditions was studied by testing influence of small changes in mobile phase pH (±0.2),organic phase composition(90% to 110%), column temperature (±5˚C) and flow rate (± 0.2 mL min-1).System suitability parameters like USP plate count, USP tailing and Resolution were checked and they found to be within limits.

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 the sample was recorded and is shown in (Figure 6).The results were shown in Table 9.

RESULTS AND DISCUSSION

An RP-HPLC method was developed and validated for the determination of Phentermine in tablet dosage forms onInertsil ODSC18 column (C18, 150 X 4.6 mmi.d., 5µm) with variable wavelength detection at 272 nm. The retention time of CEFandLIN was 3.161 min and 4.774 min and respectively.Linear correlation was obtained between area and concentration of CEF and LIN in the concentration range of8-48µg/ml and 24-144 µg/ml respectively. The low RSD value of interday and intraday at 272 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 3.70 µg/ml and 1.21 µg/ml and 11.5 µg/ml and 3.79 µg/ml respectively. These data show thatmethod is sensitive for the determination of CEFand LIN.The recovery experiment was performed by thestandard addition method. The results of recovery studies indicate that the proposed method is ishighly accurate. The proposed validated methodwas successfully applied to determine CEF andLIN in the tablet dosage form. Nointerference of the excipients with the absorbanceof interest appeared; hence the proposed methodis applicable for the routine simultaneousestimation of CEF and LIN in pharmaceuticaldosage form.

CONCLUSION

In this proposed method the linearity is observed in the concentration range of 8-48µg/ml and

24-144 µg/ml with co-efficient of correlation, (r^2)= 0.9991 and (r^2) = 0.9991 for CEF and LIN,respectively at 272 nm. The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and itis 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.

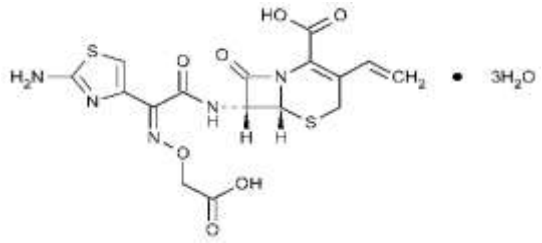

Fig. 1: Chemical structure of cefixime trihydrate (CEF)

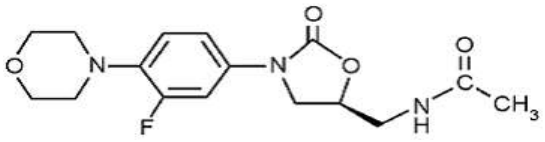

Fig. 2: Chemical structure of Linezolid (LIN)

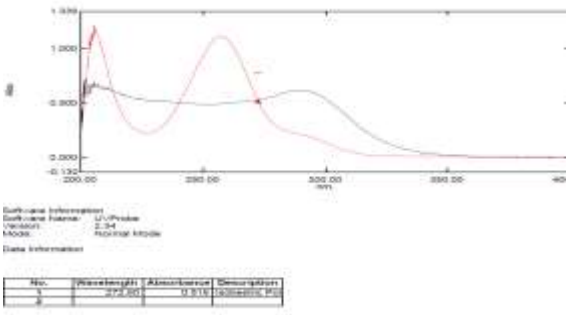

Fig. 3: UV Spectra for determination of wavelength

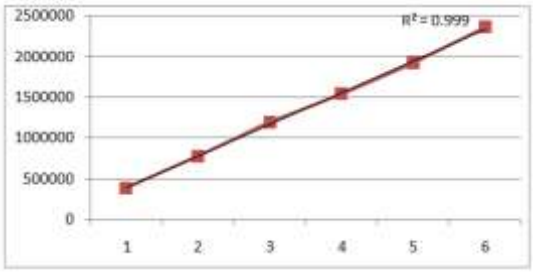

Fig. 5: Calibration curve of CEF at 272 nm

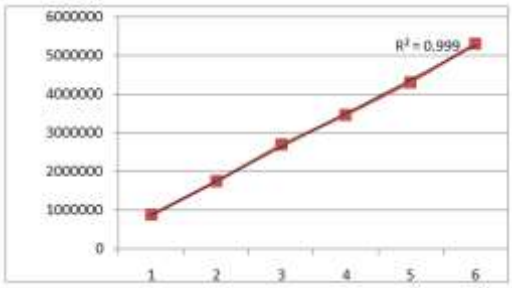

Fig. 6: Calibration curve of LIN at 272 nm

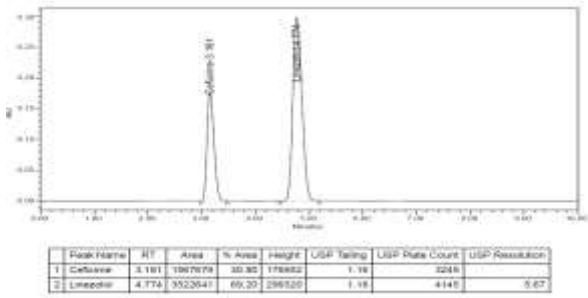

Fig. 4: Chromatogram of mixed standard solution of CEF: LIN at 272 nm

Table 1: System suitability parameters

System Suitability Parameters	Cefixime	Linezolid	Acceptable range
Resolution	5.72		4-14
Tailing factor	1.16	1.6	NMT 2
Number of theoretical Plates	3245	4188	NLT2000
Retention time(min)	3.16	4.75	---

Table 2: Linearity of Cefixime

S.NO	Concentration(µg/ml)	Area
1	8	392223
2	16	776631
3	24	1196110
4	32	1540307
5	40	1919429
6	48	2359462
	Slope	1553
	R^2	0.999
	y-intercept	3148

Table 3: Linearity for Linezolid.

S.NO	Concentration (µg/ml)	Area
1	24	886767
2	48	1747722
3	72	2690799
4	96	3471358
5	120	4311463
6	144	5303881
	Slope	347923
	R^2	0.999
	y-intercept	12930

Table 4: Precision for Cefixime.

S.NO	RT	Area
1	3.161	1568635
2	3.163	1562152
3	3.166	1571845
4	3.157	1567716
5	3.159	1569116
6	3.156	1558825
Avg	3.160	1566382
SD	0.0038	4882.13
%RSD	0.120	0.312

Table 5: Precision for Linezolid.

S.NO	RT	Area
1	4.774	3522641
2	4.772	3518625
3	4.776	3541655
4	4.754	3524169
5	4.752	3535145
6	4.758	3540122
Avg	4.764	3530393
SD	0.0108	9812.09
%RSD	0.227	0.278

Table 6: Method Precision for Cefixime.

S.NO	RT	Area
1	3.155	1566537
2	3.160	1567574
3	3.158	1565025
4	3.155	1570015
5	3.161	1564241
6	3.159	1558732
Avg	**3.158**	**1565354**
SD	**0.0025**	**3827.55**
%RSD	**0.080**	**0.245**

Table 7: Method Precision for Linezolid.

S.NO	RT	Area
1	4.754	3524169
2	4.756	3557145
3	4.758	3521845
4	4.756	3550862
5	4.758	3562875
6	4.757	3566895
Avg	**4.757**	**3547299**
SD	**0.0015**	**19591.61**
%RSD	**0.032**	**0.552**

Accuracy

Table 8: Accuracy results.

Accuracy Standard	Cefixime 1575541	Linezolid 3551384
80% spike	1246115	2825851
	1228125	2852745
	1276325	2801425
Avg	1250188	2826674
Amount recovered	**79.55**	**79.59**
% recovery	**99.44%**	**99.49%**
100% spike	1571845	3557145
	1567716	3521845

	1569116	3550862
Avg	1569559	3543284
Amount recovered	**99.87**	**99.77**
% recovery	**99.87%**	**99.77%**
120% spike	1845435	4281475
	1908545	4254842
	1878452	4185661
Avg	1877477	4240659
Amount recovered	**119.47**	**119.41**
%recovery	**99.56%**	**99.51%**

Assay

Table 9: Assay Results.

	Cefixime	**Linezolid**
Standard	1562445	3560335
	1565232	3554251
	1563839	3557293
Avg	1562445	3560335
Sample	1565025	3521835
	1562432	3544866
Avg	1563729	3533351
LC	**200**	**600mg**
Standard weight	**200**	**600**
Sample weight	**1102.1**	**1102.1**
Standard purity	**99.99**	**99.8**
Avg weight	**1102.1**	**1102.1**
Amount/tablet	**199.79mg**	**594.77mg**
%assay	**99.89%**	**99.13%**

CONCLUSION

Based on the results which have been obtained from the analysis using the proposed method, it can be concluded that the method has a linear response in the range 8 to 46µg/ml for Cefixime and 24 to144 µg/ml for Linezolid. The result of the analysis of marketed tablet dosage form by the proposed method is highly reproducible, reliable, as well as in agreement with a label claim of the drugs. The additive present in the synthetic mixture did not interfere in the analysis. So that, the method can be used for the routine analysis of drugs in combination.

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REFERENCES

1. Indian pharmacopeia, Govt. of India, Ministry of Health and family welfare, published by the Indian Pharmacopoeial commission, 2, 2010, 1590-1592.
2. The United State Pharmacopoeia, 28th ed, The United States Pharmacopoeial Convention Inc., Rockville, 2005; 1413-15,1834.
3. Rang HP, Dale MM, Ritter JM and Flower RJ, Book of Pharmacology, 6thed, Elsevier's Publication, 2006, 666-672.
4. Patel NS, Tandel FB, Patel YD, Thakkar KB. Development and validation of stability-indicating HPLC method for simultaneous estimation of cefixime and linezolid. Indian J Pharm Sci 2014; 76:535-40
5. Satish A. Patel and Jinalben V Patel. Rp-HPLC method for simultaneous estimation of cefixime trihydrate and linezolid in tablet dosage form. IJPCBS 2013, 3(2), 372-379.
6. Dolly t.Gadhiya, Hina l. Bagada.Simultaneous equation method for the estimation of cefixime trihydrate and linezolid in their combined tablet dosage form by UV-visible spectrophotometry.International Bulletin of Drug Research.,3(5): 29-38, 2013.
7. Patel DP, Goswami K, Patel M.Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Cefixime and Linezolid in Combined Dosage Form.Int J Pharm Res Scholars.V-1, I-4, 2012.
8. Dharuman J, RavichandranV, PillaiKM, Spectrophotometric method for estimation of linezolid in tablets, Indian J. PharmSci, 2004;235-236.
9. Virypaxappa BS, Shivaprasad KH, Latha MS, A Simple Method for Spectrophotometric Determination of Cefixime in Pharmaceuticals; IntJ.Chem Res; 2010; 2(1):23-30.
10. Dhoka MV, VaidyaPravin D, PandeAmol V, AroraAseem A, Development and Validation of Analytical Method for Estimation of Cefixime in Swab Samples, IntJ.ChemTech Res, 2011;2(4):1918-1923.
11. SatyanarayanaBattu, VasudevPottbathini. A new stability indicating hplc method for simultaneous estimation of cefixime and linezolid in pharmaceutical dosage form and plasma sample. Int J Pharm 2014; 4(1):321-329.
12. Dishathakkar, Rajshreemashru. Simultaneous estimation of cefiximetrihydrate and linezolid in combine pharmaceutical formulation.Int J Pharm PharmSci, Vol 5, Issue 1, 76-81,2013.
13. ICH guidelines, Validation of Analytical Procedures: Text and Methodology, Q2(R1) Nov 2005.