EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 10
200-206
Year: 2019

A NEW RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFIXIME AND LINEZOLID IN ITS BULK AND TABLET DOSAGE FORM

B. Nagaveni*, S. K. Godasu, M. Sowmya, R. Suneetha, P. Raju

Sri Indu institute of Pharmacy, Sheriguda Telangana, India.

***Corresponding Author: B. Nagaveni**
Sri Indu institute of Pharmacy, Sheriguda Telangana, India.

Article Received on 01/07/2019 **Article Revised on 21/07/2019** **Article Accepted on 11/08/2019**

ABSTRACT
Objective: A New method was established for simultaneous estimation of Cefixime And Linezolid by RP-HPLC method. **Methods**: Chromatographic separations were carried using BDS Ypersil C_{18} column (4.6x250mm,5µm) column with a mobile phase composition of 0.1M KH2PO4: Methanol (55:45) have been delivered at a flow rate of 1ml/min and the detection was carried out using waters HPLC auto sampler, separation module 2695 with PDA detector at wavelength 247 nm. **Results**: The retention time of 3.151 and 5.006 minutes for Cefixime and Linezolid respectively. The method was validated and there sponse was found to be linear in the drug concentration range of 20µg/ml to 60 µg/ml for Cefixime and 60µg/ml to 180µg/ml for and Linezolid. The values of the correlation coefficient were found to0.999for Cefixime and 0.999 for Linezolid respectively. The LOD and LOQ for Cefixime were found to be 0.064 and 0.214 respectivly. The LOD and LOQ for Linezolid were found to be 0.3596 and 1.1988 respectively. **Conclusion**: The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Cefixime And Linezolid in pharmaceutical dosage form.

KEYWORDS: Cefixime, Linezolid, RP-HPLC, Simultaneous estimation.

INTRODUCTION

Cepixime Like all beta-lactam antibiotics, cefixime binds to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, causing the inhibition of the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that cefixime interferes with an autolysin inhibitor.[1] The IUPAC name of cefixime is (6R, 7R)-7-{[2-(2-amino-1, 3-thiazol-4-yl)-2(carboxymethoxyimino) acetyl] amino}-3 ethenyl-8-oxo-5-thia-1azabicyclo-[4.2.0] oct-2-ene-2 carboxylic acid. It is soluble in ethanol and methanol.

Linezolid is a synthetic antibacterial agent of the oxazolidinone class of antibiotics. It has in vitro activity against aerobic Gram positive bacteria, certain Gram negative bacteria and anaerobic microorganisms. It selectively inhibits bacterial protein synthesis through binding to sites on the bacterial ribosome and prevents the formation of a functional 70S-initiation complex. The results of time-kill studies have shown linezolid to be bacteriostatic against enterococci and staphylococci.[2] For streptococci, linezolid was found to be bactericidal for the majority of strains IUPAC name of N-{[(5S)-3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl]methyl}acetamide. It is soluble in water, and methanol.

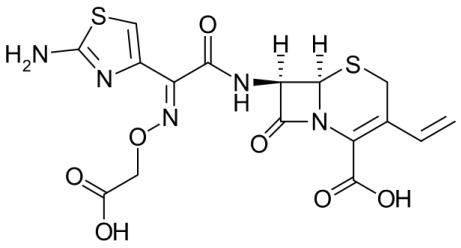

Figure 1: Structure of Cefixime.

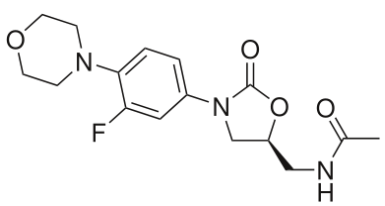

Figure 2: Structure of Linezolid.

Literature survey shows that a number of methods have been reported for estimation of Cefixime And Linezolid individually or in combination with other drugs Those are HPLC Methods[3-18], However, there is only few HPLC methods[19-21] is reported for the simultaneous estimation of these drugs in combined dosage forms.

The aim of the present study was A New Rp-Hplc Method For Simultaneous Estimation Of Cefixime And Linezolid In Its Bulk And Tablet Dosage Form.

MATERIALS AND METHODS

Chemicals and Reagents: Cefixime And Linezolid were obtained as a gift sample from Ranbaxy laboratories limited, Hyderabad. KH_2PO_4 was analytical grade supplied by Dr.Reddys, Mumbai, Orthophosphoric acid (Finar), Acetonitrile (Molychem, HPLC grade) and Water for HPLC (Loba chemi), Methanol (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, PDA detector and Empower 2 software. Analysis was carried out at 247 nm with an BDS YPersil C_{18} column (4.6x250mm,5µm), dimensions at ambient temperature. The optimized mobile phase consists of Potassium dihydrogenphosphate: Methanol (55:45). Flow rate was maintained at 1 ml/min and run time for 30 min.

Preparation of solutions

Preparation of buffer

Accurately measured 1ml of OPA (orthophosphoric acid) was transferred to 1000ml volumetric flask and 250 ml of HPLC Grade water added initially, after sonicating for 30 min volume is makeup with HPLC Grade water, to give in 0.1% solution

Preparation of mobile phase

Transfer 1000ml of HPLC water into 1000 ml of beaker add 13.608 gm KH_2PO_4. Transfer the above solution 550ml of 0.1% KH2PO4, 450ml of Methanol is used as mobile phase. They are mixed and sonicated for 20min.

The diluents

The Mobile phase was used as the diluent.

Preparation of standard stock solution

Accurately weigh and transfer 200mg of Cefixime and 600mg Linezolid into 100ml of volumetric flask and add 10ml of Methanol and sonicate 10min (or) shake 5min and make with water.

Transfers the above solution into 2.0ml into 100ml volumetric flask dilute to volume with water.

Preparation of Sample stock solution

Commercially available 20 tablets ware weighed and powdered the powdered equivalent to the 1079.10mg of Cefixime and Linezolidof active ingredients were transfer into a 100ml of volumetric flask and add 10ml of Methanol and sonicate 20min (or) shake 10min and makeup with water.

Transfers above solution 2.0ml into 100ml of the volumetric flask dilute the volume with Methanol. And the solution was filtered through 0.45µm filter before injecting into HPLC system.

Procedure: 10 ml of the standard and sample solutions were injected into chromatographic system and areas for the Cefixime and Linezolid peaks were measured**.**

METHOD

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 10 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into BDS YPersil C_{18} column (4.6x250mm,5µm), the mobile phase of composition Potassium dihydrogenphosphate: Methanol (55:45) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Cefixime and Linezolid in their tablet dosage form. The result obtained for Cefixime And Linezolid was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method

Linearity and Range: Stock solution was prepared by dissolving the appropriate amount of Cefixime and Linezolid in 10 ml of diluent and further diluted to the required concentrations with diluent. The solution was prepared at five concentration levels ranging from 50µg/ml to 150µg/ml of Cefixime and 50µg/ml to 150 µg/ml of Linezolid. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The resulte are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Cefixime and Linezolid and calculate the individual recovery and mean recovery values. The resulte are shown in table 4,5.

Precision Studies: precision was caliculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 7.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2ml/min. The Organic composition in the Mobile phase was varied from 50% to 50%. The results are shown in table 8,9.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines.
LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

The results are shown in table 10,11.

RESULTS AND DISCUSSION

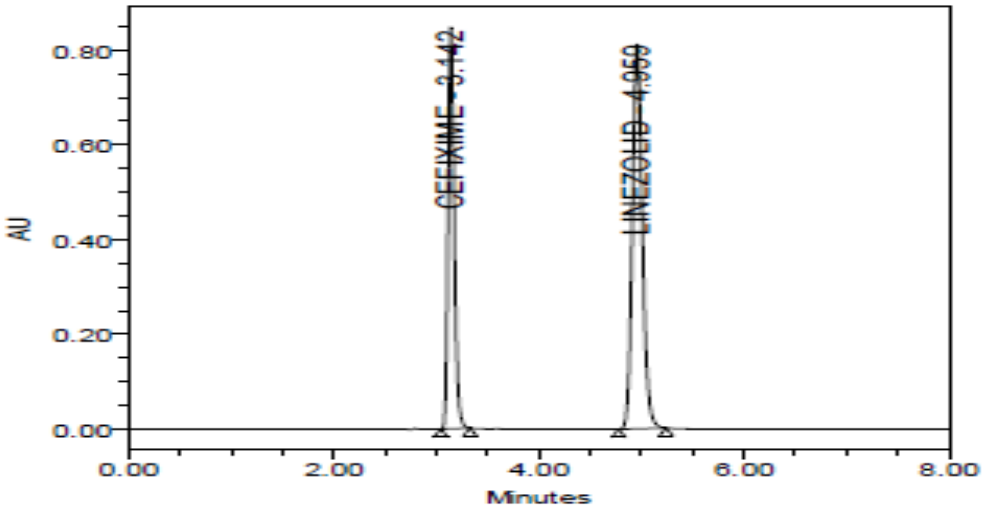

Figure 3: Standard chromatogram.

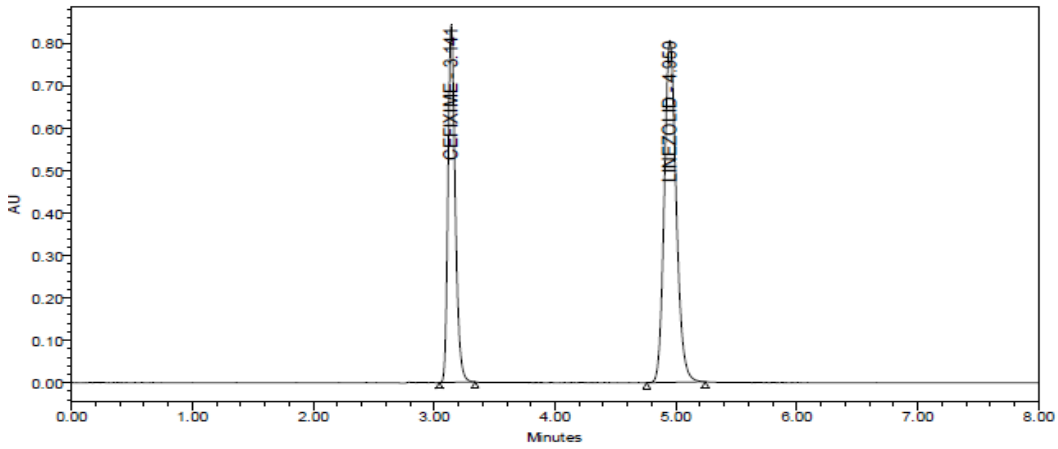

Figure 4: Sample chromatogram.

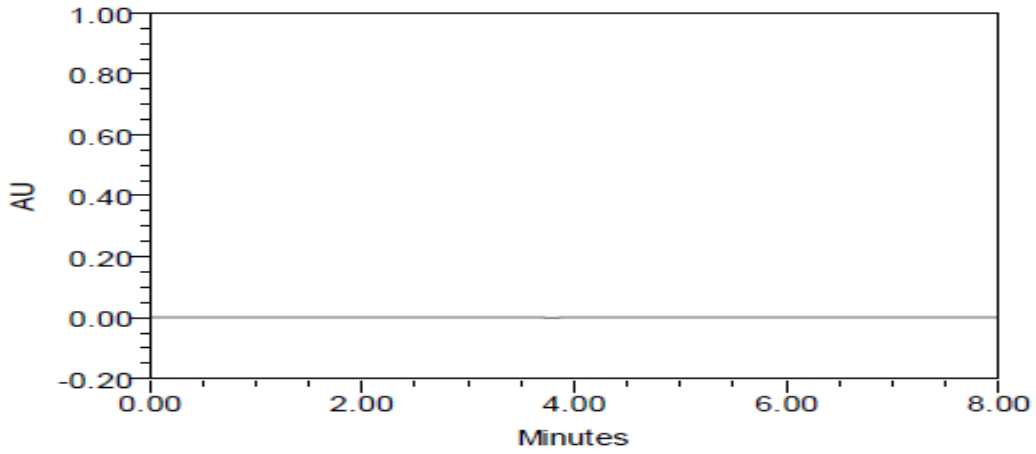

Figure 5: Blank chromatogram.

Table 1: System suitability parameters.

Parameters	Cefixime	Linezolid
Retention time	3.151	5.006
USP Plate count	13834	12410
USP Tailing	1.25	1.14

Table 2: Assay results for Cefixime and Linezolid.

	Label Claim (mg)	% Assay
Cefixime	200	99.4
Linezolid	600	99.8

Table 3: Linearity results for Cefixime and Linezolid.

Cefixime		Linezolid	
Concentration(µg/ml)	**Area**	**Concentration(µg/ml)**	**Sample Absorbance**
50	1748741	50	2762855
75	2622321	75	4154630
100	3496968	100	5534392
125	4377378	125	6927136
150	5246715	150	8307779
Correlation coefficient	0.999	Correlation coefficient	0.999

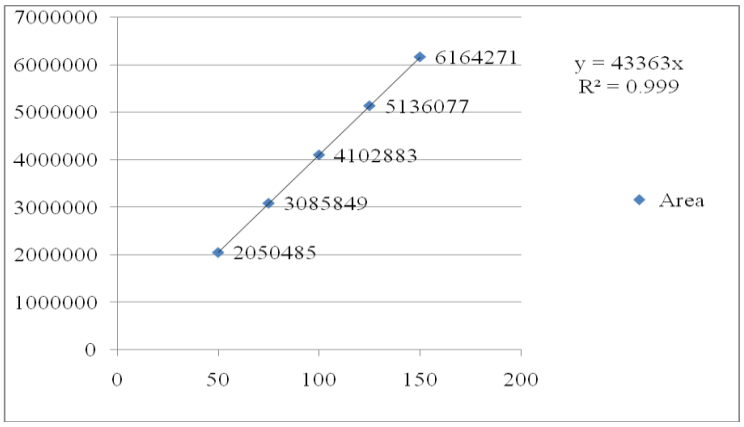

Figure 4: Linearity graph for Cefixime.

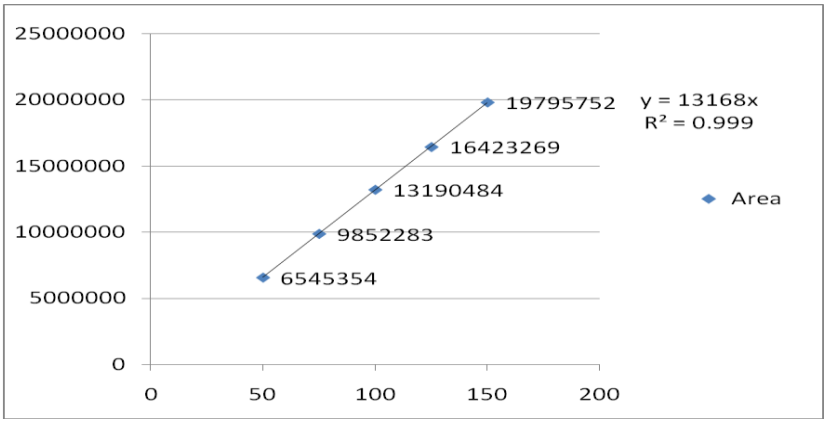

Figure 5: Linearity graph for Linezolid.

Table 4: Accuracy results for Cefixime.

Cefixime					
Spiked level	**Sample weight**	**µg/ml added**	**µg/ml founded**	**%Recovery**	**%Mean**
50%	539.50	19.798	19.81	100	100
100%	1079.10	39.75	39.75	100	
150%	1618.50	59.58	159.58	100	

Table 5: Accuracy results for Linezolid.

Linezolid					
Spiked level	**Sample weight**	**µg/ml added**	**µg/ml founded**	**%Recovery**	**%Mean**
50%	539.50	59.994	95.84	100	100
100%	1079.10	120.00	192.85	100	
150%	1618.50	179.98	287.30	100	

Table 6: Precision results for Cefixime and Linezolid.

Injection	**Cefixime Area**	**Linezolid Area**
Injection-1	3497992	5530638
Injection-2	3494166	5539119
Injection-3	3496916	5538527
Injection-4	3490921	5537198
Injection-5	3494856	5539719
Average	3495012	5538526
%RSD	0.08	0.06

Table 7: Intermediate precision resultes for Cefixime and Linezolid.

S.NO	**Sample Weight**	**Sample Absorbance-1**	**Sample Absorbance-2**	**% Assay**	**% Assay**
1	1079.10	0.3618	0.2874	99	100
2	1079.10	0.3617	0.2881	99	100
3	1079.10	0.3610	0.2869	99	100
4	1079.10	0.3607	0.2885	99	100
5	1079.10	0.3604	0.2887	99	100
6	1079.10	0.3612	0.2891	99	100
Average assay				99	100
STD				0.15	0.29
%RSD				0.15	0.29

Table 8: Robustness data for Cefixime.

parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate(0.8ml/min)	3.503	13634	13126
Increased flow rate(1.2ml/min)	2.865	13657	12790
Decreased temperature(20^0c)	3.148	1.29	1.27
Increased temperature(30^0c)	3.148	1.27	1.29

Table 9: Robustness data for Linezolid.

parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate (0.8ml/min)	5.533	12.45	12.26
Increased flow rate (1.2ml/min)	4.538	12.25	12.14
Decreased temperature(20^0c)	4.967	1.16	1.15
Increased temperature(30^0c)	4.984	1.16	1.16

Table 10: LOD data for Cefixime and Linezolid.

S.no	Sample name	RT	Area
1	Cefixime	3.144	1900016
2	Linezolid	4.953	4620746

Table 11: LOQ data for Cefixime and Linezolid.

S.no	Sample name	RT	Area
1	Cefixime	3.124	1926075
2	Linezolid	4.954	5091221

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Cefixime and Linezolid in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Cefixime and Linezolid in pure and its pharmaceutical dosage forms.

REFERENCES

1. 1.M. Claire mcmanus Anti-infective agents; Enterococcus species; Mechanism of action; Resistance; Staphylococcus species Am J Health-Syst Pharm., 1997; 54: 1420-331.
2. Prabhavathi pernandes, Antibacterial discovery and development the failure of success, Nature Biotechnologyvolume. 2006; 24: 1497–1503
3. Khaja P, Patil CS, Vijaykumar K, Ali S, Chimkod VM. Reverse phase HPLC method for the determination of cefixime in pharmaceutical dosage forms. Res J Pharm Bio Chem Sci., 2010; 1: 226–30.
4. Dragica Z, Traje S, Petar M. High-performance liquid chromatographic method for determination of cefixime and cefotaxime in human plasma. Bulletin Chemists Techno Macedonia. 2003; 22: 39–45.
5. Azhagesh RK, Divya Y, Deepthi Y, Prabu C, Manikantan S. Determination of cefixime trihydrate and cefuroxime axetil in bulk drug and pharmaceutical dosage forms by HPLC. Int J Chem Tech Res., 2010; 2: 334–6.
6. Kathiresan K, Murugan R, Shahul HM, Gokula IK, Taranath K. Analytical method development and validation of cefixime and dicloxacillin tablets by RP-HPLC. Rasayan J Chem., 2009; 2: 588–92.
7. Kapil SK, Santosh VG, Padmanbh BD, Nilesh VG. 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: 46–8.
8. Khan IU, Sharif S, Ashfaq M, Asghar MN. Simultaneous determination of potassium clavulanate and cefixime in synthetic mixtures by high-performance liquid chromatography. Asian J Anal Chem., 2008; 91: 744–9.
9. Sudhakar M, Venkateshwara RJ, Devika GS, Ramesh PR. A validated RP-HPLC method for simultaneous estimation of cefixime trihydrate and ornidazole in tablet dosage forms. Int J Chem Pharm Sci., 2010; 1: 34–9.
10. Shah C, Umalkar D, Rajesh KS. Development of an RP HPLC and force degradation of cefixime and moxifloxacin in bulk and pharmaceutical dosage form. Int J Pharm Res Bio Sci., 2012; 1: 128–47.
11. 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: 79–87.

12. Gandhi SP, Rajput SJ. Study of degradation profile and development of stability indicating methods for cefixime trihydrate. Indian J Pharm Sci., 2009; 71: 438–42.
13. Sutar SV, Warjurkar RP, Pishwikar SA. Spectrophotometric method for degradation study of cefixime trihydrate. Asian J Biomed Pharm Sci., 2012; 2: 50–4.
14. Elsadig HK, Ahmed EM, Izzeldin EB. Study of degradation of cefixime trihydrate under stress conditions using stability indicating reverse phase-high performance liquid chromatography method. Der Pharm Chemica., 2011; 3: 197–207.
15. Jaya PK, Syama SB. A validated RP-HPLC method for the determination of linezolid in pharmaceutical dosage forms. Int J Pharm Bio Sci., 2012; 3: 44–51.
16. Abdel KM, Weshahy SA, Dina SS. Validated stability indicating assay of linezolid by spectrophotometric and high performance liquid chromatographic methods. Aust J Basic Appl Sci., 2012; 6: 767–78.
17. Agrawal H, Mahadik KR, Paradkar AR, Kaul N. Stability indicating HPTLC determination of linezolid as bulk drug and in pharmaceutical dosage form. Drug Dev Ind Pharm. 2003; 29: 1119–26.
18. Khasia VD, Khasia HV, Desai D, Bhakhar DN, Parmar AR. Development and validation of stability indicating RP-HPLC method for linezolid immediate release tablet dosage form. J Pharm Res., 2012; 5: 4115–8.
19. Helly S, Payal P, Khushbu P, Sagar S. Method development and validation of spectrophotometric methods for simultaneous estimation of cefixime trihydrate and Linezolid in their combined tablet dosage form. Int J Pharm Res Bio Sci., 2012; 1: 516–29.
20. Patel DP, Goswami K, Patel M. Development and validation of UV spectrophotometric method for simultaneous estimation of cefixime and linezolid in combined dosage. Int J Pharm Res Scholars. 2012; 1: 112–8.
21. Thakkar D, Mashru R. Simultaneous estimation of cefixime trihydrate and linezolid in combine pharmaceutical formulation. Int J Pharm Pharm Sci., 2013; 5: 76–81.