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S Rubesh *et* al
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Original Article

RP-HPLC Method Development and Validation for Simultaneous Estimation of Linezolid and Cefixime in API and Pharmaceutical Dosage Form

M Naga, S Rubesh Kumar *, V Uma Maheswara Rao

Department of Pharmaceutical Analysis & Quality Assurance, Teegala Ram Reddy College of Pharmacy, Hyderabad, Telangana, India

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ABSTRACT

A new sensitive, accurate, precise and validated RP-HPLC method was developed for the concurrent assessment of linezolid and cefiximein in API and pharmaceutical dosage form. The chromatographic separation was achieved on WATERS Alliance 2695: Empower 2, 996 PDA detector, Phenomenex Luna C18 column, mobile phase consist of a mixtures of methanol: water pH 3.8 (47:53%v/v) with detection wavelength at 260nm. The retention times for linezolid and cefixime were found to be as 2.4 and 3.9min respectively. The developed method was validated for the various parameters as per ICH (Q2R(1)) guidelines. The system suitability, specificity, accuracy, precession, detection and quantification limits and robustness studies showed the developed method was found to within the limits. Hence the developed method can be used for the routine analysis of linezolid and cefiximein in bulk and pharmaceutical dosage forms.

Keywords: Linezolid, Cefixime, RP-HPLC and Validation.

1. INTRODUCTION

Linezolid, chemically (*S*)-*N*-({3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl}methyl)acetamide, a synthetic antibiotic belong to a new category of antimicrobials called the oxazolidinones. Linezolid disrupts bacterial expansion by inhibiting the initiation process in protein synthesis. Distinctively, linezolid binds to a site on the bacterial 23S ribosomal RNA of the 50S subunit and prevents the bargain of a resourceful 70S initiation

Corresponding author *
Dr. S. Rubesh Kumar
Professor & HOD
Department of Pharmaceutical Analysis & Quality Assurance
Teegala Ram Reddy College of Pharmacy
Meerpet, Saroor Nagar (M), Hyderabad-500 097,
Telangana, India
Email- rubystill@gmail.com

multifaceted, which is an essential section of the bacterial translation process. The results of time-kill studies have shown linezolid to be bacteriostatic against enterococci and staphylococci. For streptococci, linezolid was found to be bactericidal for the majority of strains. Linezolid is also a reversible, nonselective inhibitor of monoamine oxidase [1-2]. Therefore, linezolid has the potential for interaction with adrenergic and serotonergic agents Fig1.

Cefixime, chemically it consists of a dihydrothiazine ring fused to a beta-lactam ring containing a suitable side sequence at 7 positions and as the trihydratean antibiotic. The antibacterial consequence possesses a mechanism of action comparable to penicillins i.e. inhibition of transpeptidation course resulting in the arrangement of inadequate cell wall; osmotic impel from the outside isotonic environment of the host cell to the inside of the hypertonic bacterial cytoplasm and finally activation of the autolysin enzyme leading to the lysis of bacteria [3]. Cefixime is used to treat positive infection caused by bacteria such as bronchitis (infection of the airway tubes leading to the lungs); gonorrhea (a sexually transmitted disease); and infections of the ears, throat, tonsils, and urinary tract Fig 2.

There are few analytical procedures are established for the simultaneous estimation of cefixime and linezolid in spectroscopic and chromatographic methods [4-8]. Hence a new analytical method developed for the simultaneous estimation of cefixime and linezolid in API and pharmaceutical dosage form and the method was validated as per ICH guidelines (Q2R(1)) [9-10].

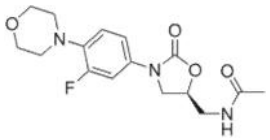


Fig 1: Linezolid

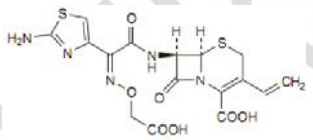


Fig 2: Cefixime

2. METHOD DEVELOPMENT

2.1 Material Used:

Methanol and water (HPLC grade) obtained from SD fine chemicals, linezolid and cefixime (in house), LINCEF marketed Alkem Laboratories Ltd formulation.

2.2 Chromatographic condition:

Method development was optimized with the following chromatographic circumstances mobile phase consist of methanol: water (47:53%v/v), follow rate of 0.9ml/min, C_{18} (4.6×150mm, 5µ) Phenomenex Luna was used for the chromatographic separation of analyte, detection wavelength was at 260nm and injection volume was 10µl. Instrument used was WATERS Alliance 2695: Empower 2, 996 PDA Detector.

2.3 Preparation of standard solution:

Accurately weigh and transfer 10 mg of linezolid and cefixime working standard into a 10ml of clean dry volumetric flasks individually add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with methanol. Further pipette 0.15ml of cefixime and 0.6ml the above linezolid stock solutions into a 10ml volumetric flask and dilute up to the mark with methanol.

2.4 Preparation of mobile phase:

Accurately measured 470ml (47%) of methanol and 530ml of Water (53%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration. The mobile phase was used as the diluent.

2.5 Preparation of Sample Solution:

Take average weight of 10 tablets and crush to make powder in a mortar by using pestle and weight 10 mg equivalent weight of linezolid and cefixime sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further the solution was diluted to get a concentration of 10 µg/ml with the diluent and the resulting solution was filtered through 0.45 µ filter under vacuum filtration.

3 Method validation parameters:

3.1 Method validation[9-11]

The method validation was done as per the ICH guidelines Q2R(1), and accordingly the parameters evaluated were Specificity, precision, accuracy, linearity, ruggedness, robustness and system suitability studies.

3.2 **Specificity:** Specificity of an analytical method is its ability to measure accurately and specifically the concentration of analyte without interference from other API, diluents, mobile phase. Solutions of mobile phase, sample solution, standard solution were injected into liquid chromatography. Retention times of sample and standard were compared.

3.3 Linearity and Range: The linearity of an analytical procedure is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within given range, was studied by analyzing five analyte concentrations of drug ranging from 20-100µg/ml for linezolid and 5 to 25µg/ml cefixime.

3.4 Accuracy: Accuracy refers to the closeness of a measured value to a standard or known value. The percentage recovery was studied for 50%, 100% and 150%, each level was injected three times.

3.5 Precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of same homogenous sample under prescribed conditions. This experiment was conducted to prove the repeatability of the assay results obtained by quantification methodology.

System precision, method precision and intermediate precision was performed.

Repeatability: 20µl of standard solution was injected for six times and measured the peak area for all six injections in HPLC and % RSD for the area of six replicate injections was calculated.

Intermediate precision: The ruggedness of an analytical method is the degree of reproducibility of test results obtained by same samples under different conditions. The freshly prepared standard solutions was injected on inter and intraday.

3.6 LOD and LOQ: The Detection and quantification limits for the linezolid and cefixime were performed and calculated using S/N ratio method.

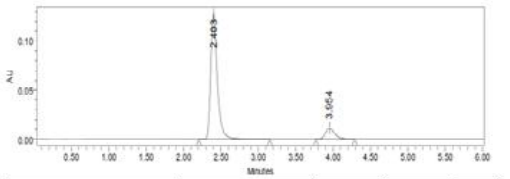
3.7 Robustness of an analytical method is measure of its capacity to remain unaffected small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness measures the lack of internal influences on the test results. As part of the robustness, deliberate change in the flow rate and mobile phase composition was made to evaluate the impact on the method.

Change in flow rate: The flow rate was varied at 0.8 ml/min to 1.0ml/min. Standard solution of linezolid and cefixime was used for analysing the varied flow rates along with method flow rate.

Change in Organic composition: The Organic composition was varied up to ±5%, was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition for the standard API solutions.

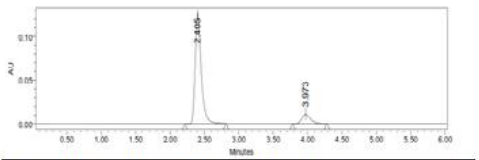
3. RESULT AND DISCUSSION

The developed method clearly symptomatic that the linezolid and cefixime were evidently separated by using the mobile phase consist of methanol: water (47:53%v/v) on an Phenomenex Luna C18 (4.6×150mm, 5µ), detection wavelength at 260nm, method was found to be system suitable with a retention time 2.403 and 3.954 min, resolution of the both the compound was 8.1, tailing factors was 1.4 and 1.3, theoretical plates 8807 and 5066 for linezolid and cefixime. The assay of market formulation was found to be 100.3% w/v. The chromatogram is shown in Fig 3-4.



S.No	Name	Rt (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP Plate count
1	Linezolid	2.403	781341	126836		1.4	8807
2	Cefixime	3.954	94770	11034	8.1	1.3	5066

Fig 3: Optimized Chromatogram (Standard)



S.No	Name	Rt (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP Plate count
1	Linezolid	2.405	766162	126265		1.4	8204
2	Cefixime	3.973	92250	10838	8.0	1.3	7403

Fig 4: Optimized Chromatogram (Sample)

The developed method was validated as per the ICH guidelines (Q2R(1)) manual, Specificity studies showed that there was no interferences of impurity peaks on the developed method and the analyte peaks were well resolved. Linearity studies were performed for the concentration 20-100µg/ml for linezolid and 5 to 25µg/ml cefixime and the correlation coefficient was found to be 0.999 and 0.9998**.** The table is tabulated in the Table 1 and linearity graph is shown in Fig 5 and 6.

Table 1: Linearity results for Linezolid and Cefixime

SI.NO	Concentration «g/ml	Average Peak Area	Concentration «g/ml	Average Peak area
1	20	241842	5	26755
2	40	483922	10	49833
3	60	696853	15	72257
4	80	918474	20	96655
5	100	1184829	25	116854
Correlation Coefficient		**0.999**		**0.9998**

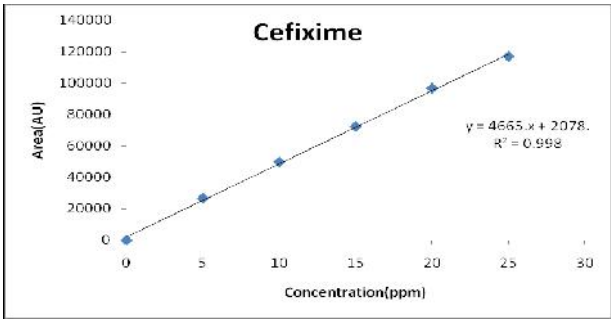


Fig 5: Linearity plot for cefixime

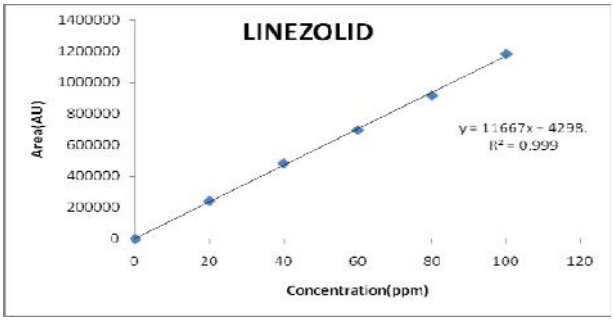


Fig 6: Linearity plot for Linezolid

The repeatability for linezolid and cefixime the % RSD was 0.291 and 0.370. Intermediate precision was performed for the different days the %RSD for day 1was found to be 0.395 and 0.324 and %RSD for day 2 was found to be 0.361 and 0.200. Precision studies were within the accepted limits results are shown in the Table 2-7.

Table 2: Results of repeatability for Linezolid

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Linezolid	2.493	760183	12398	5200	1.18
2	Linezolid	2.497	765471	12472	5213	1.16
3	Linezolid	2.497	761940	12948	5208	1.17
4	Linezolid	2.496	764822	12218	5202	1.18
5	Linezolid	2.497	761833	12309	5193	1.16
Mean			762849.8			
Std.dev			2221.132			
%RSD			0.291162			

Table 3: Results of repeatability for Cefixime

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Cefixime	3.925	92842	1038	7639	1.28
2	Cefixime	3.952	92103	1033	6718	1.22
3	Cefixime	3.977	92481	1094	6762	1.21
4	Cefixime	3.940	92810	1084	6748	1.23
5	Cefixime	3.952	92183	1029	6878	1.21
Mean			**92483.8**			
Std.dev			**342.8596**			
%RSD			**0.370724**			

Table 4: Results of Intermediate precision Day 1 for Cefixime

S.No	PeakName	RT	Area (µV*sec)	Height (µV)	USPPlate count	USPTailing	Resolution
1	Cefixime	3.976	92441	1138	6281	1.2	4.9
2	Cefixime	3.966	92218	1093	6392	1.2	4.9
3	Cefixime	3.989	92011	1176	6293	1.2	4.9
4	Cefixime	3.987	92847	1065	6039	1.2	4.9
5	Cefixime	3.985	92381	1075	6153	1.2	4.9
6	Cefixime	3.962	92103	1167	6293	1.2	4.9
Mean			**92333.5**				
Std.De			**299.3231**				
%RS D			**0.324176**				

Table 5: Results of Intermediate precision Day 1 for Linezolid

S.No	PeakName	RT	Area (µV*sec)	Height (µV)	USPPlate	USPTailing
1	Linezolid	2.406	761058	12401	5192	1.1
2	Linezolid	2.404	762758	12486	5202	1.1
3	Linezolid	2.407	764928	12391	5198	1.1
4	Linezolid	2.406	769383	12482	5213	1.1
5	Linezolid	2.404	761646	12301	5213	1.1
6	Linezolid	2.404	763944	12484	5217	1.1
Mean			**763952.8**			
Std.Dev.			**3018.838**			
%RSD			**0.39516**			

Table 6: Results of Intermediate precision Day 2 for Cefixime:

S.No	PeakName	RT	Area (µV*sec)	Height (µV)	USPPlate count	USPTailing	Resolution
1	Cefixime	3.933	92552	1086	6173	1.2	8.0
2	Cefixime	3.929	92165	1076	6183	1.2	8.0
3	Cefixime	3.973	92087	1092	6103	1.2	8.0
4	Cefixime	3.974	92108	1063	6482	1.2	8.0
5	Cefixime	3.987	92751	1107	6831	1.2	8.0
6	Cefixime	3.962	92817	1083	6153	1.2	8.0
Mean			**92413.33**				
Std.Dev.			**333.9417**				
%RSD			**0.361357**				

Table 7: Results of Intermediate precision Day 2 for Linezolid

S.No	PeakName	RT	Area (µV*sec)	Height (µV)	USPPlate count	USPTailing
1	Linezolid	2.401	762004	12103	5237	1.1
2	Linezolid	2.402	762948	12398	5023	1.1
3	Linezolid	2.406	764803	12402	5983	1.1
4	Linezolid	2.404	761048	12294	5294	1.1
5	Linezolid	2.406	762987	12492	5819	1.1
6	Linezolid	2.404	764933	12358	5183	1.1
Mean			**763120.5**			
Std.Dev.			**1530.057**			
%RSD			**0.2005**			

Accuracy studies was performed for the concentration 50%, 100% and 150%, the mean percentage recovery was found to be 99.1% and 100.3% was within the acceptance criteria Table 8 and 9. The LOD and LOQ studies were determined by slope of intercept S/N ratio method and the LOD and LOQ was found to be with the limits.

Table 8: Accuracy results for Linezolid

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	356422	30	30	100	99.1%
100%	692213	60	58.9	98.2	
150%	1045645	90	89.2	99.1	

Table 9: Accuracy results for Cefixime

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	37165	7.5	7.5	100	100.3%
100%	72393	15	15.0	100	
150%	108416	22.5	22.6	101.1	

Robustness studies were performed for the change in Organic phase ratio and change in the flow rate and method was found to be robust Table 10 and 11. Hence the developed method was found to be adequately resolving the separation of linezolid and cefixime and method was validated as per ICH Q2R (1) manual was found to be within the acceptance criteria.

Table 10: Results for Robustness studies of Linezolid

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	781541	2.403	4242	1.1
Less Flow rate of 0.8mL/min	781047	2.984	5405	1.1
More Flow rate of 1.0mL/min	780183	2.011	5365	1.2
Less organic phase	782932	2.429	4393	1.2
More Organic phase	783192	2.384	4358	1.1

Table 11: Results for Robustness studies of cefixime

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	93779	3.954	6515	1.1
Less Flow rate of 0.8mL/min	91374	4.945	4698	1.1
More Flow rate of 1.0mL/min	92846	3.260	7934	1.2
Less organic phase	91388	4.803	4368	1.3
More Organic phase	92472	3.431	5371	1.1

4. CONCLUSION

A new method was developed for the simultaneous determination of linezolid and cefixime in API and pharmaceutical dosage by RP HPLC. This method was found to be narrative, effortless, precise, accurate and diminutive run time of analysis. The developed method was validated as per the ICH Q2R (1) guidelines and the results obtained were well within the limits. Hence this method can be adopted for the routine analysis of linezolid and cefixime in API and pharmaceutical dosage.

5. ACKNOWLEDGEMENT

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