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R E S E A E C H A R T I C L E

Analytical Method Development and Validation for the Simultaneous Estimation of Cefuroxime and Linezolid by RP-HPLC

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A B S T R A C T

A new method was established for simultaneous estimation of Cefuroxime and Linezolid by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Cefuroxime and Linezolid by using Agilent C185µm (4.6*250mm) column, flow rate was1ml/min, mobile phase ratio was Phosphate buffer pH 4.0:ACN (30:70%v/v), detection wave length was 254nm. The instrument used was Shimadzu, model No. SPD-20MA LC+20AD, Software-LC-20 Solution.Theretentiontimeswerefoundtobe3.503minsand2.577 mins. The % purity of Cefuroxime and Linezolid was found to be 100.3% and 101.1%respectively. The system suitability parameters for Cefuroxime and Linezolid such as theoretical plates and tailing factor were found to be 1.3, 5824.4 and1.2,2936.0 the resolution was found to be 9.4. The analytical method was validated according to ICH guidelines (ICH, Q2(R1)). The linearity study for Cefuroxime and Linezolid was found in concentration range of 20µg-100µg and 20µg-100µg and correlation coefficient (r2) was found to be 0.999 and 0.999,% mean recovery was found to be 102.5% and 101.0%, %RSD for repeatability was 0.6 and 0.5, %RSD for intermediate precision was 0.7and 0.6 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.1and 3.02 & LOQ value was 10.1and10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Cefuroxime and Linezolid in API and Pharmaceutical dosage form.

Keywords: AgilentC18, Cefuroxime and Linezolid, RP-HPLC

A R T I C L E I N F O

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1. Introduction

Cefuroxime is an enteral second-generation cephalosporin antibiotic. As with the other cephalosporins, it is susceptible to beta-lactamase, although as a second-generation variety, it is less so. Hence, it may have greater activity against Haemophilus influenzae, Neisseria gonorrhoeae, and Lyme disease. Unlike most other second-generation cephalosporins, cefuroxime can cross the blood-brain barrier.

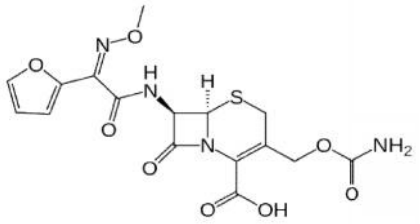

Fig 1: Structure of Cefuroxime

Linezolid is an antibiotic used for the treatment of infections caused by Gram-positive bacteria that are resistant to other antibiotics.Linezolid is active against most Gram-positive bacteria that cause disease, including streptococci, vancomycin-resistant enterococci (VRE), and methicillin-resistant Staphylococcus aureus (MRSA). The main uses are infections of the skin and pneumonia although it may be used for a variety of other infections including drug resistant tuberculosis. It is used either by injection into a vein or by mouth.

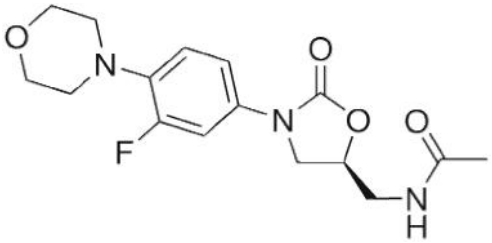

Fig 2: Structure of Linezolid

2. Materials and Methods

Instrumentation

HPLC Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution UV double beam UV 3000 UV Win 5 Lab India Digital weighing pH meter Ultra sonicator Suction pump.

Chemicals

Cefuroxime and Linezolid, Potassium dihydrogen, Acetonitrile, Methanol, Water.

Chromatographic Conditions:

Column	**:**Agilent C18 column (4.6×150mm)5µ
Mobile phase ratio	**:**Phosphate buffer pH 4.0: ACN (70:30%v/v)
Detection wavelength	**:**254 nm
Flow rate	**:** 1.0 ml/min
Injection volume	**:**10µl
Column temperature	**:** Ambient

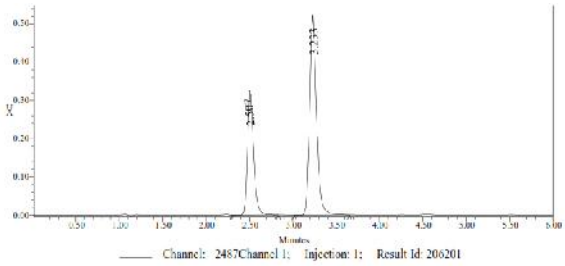

Fig 3: Optimized Chromatogram

Observation: The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

Preparation of the individual Cefuroxime standard preparation:

10mg of Cefuroxime working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of the individual Linezolid standard preparation:

10mg of Linezolid working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of Sample Solution :(Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Linezolid and Cefuroxime (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted up to the mark with diluant.

Method Validation

Accuracy:

Preparation of standard solution (Linezolid and Cefuroxime): Accurately weighed 10 mg of Linezolid and 10mg of Cefuroxime working standard were transferred into a 10mL and 100ml of clean dry volumetric flasks.

Precision

Repeatability:

Preparation of standard stock solution: Accurately 10 mg of Linezolid and 10mg of Cefuroxime working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Intermediate Precision (Ruggedness): To evaluate the intermediate precision (also known as ruggedness) of themethod, precision was performed on different days by using different make column of same dimensions.

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

LOD:

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

LOQ:

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y- intercepts of regression lines.

Linearity:

Preparation of stock solution: Accurately 10 tablets were weighed & crushed in mortar and pestle and weight equivalent to 10 mg of Linezolid and Cefuroxime (marketed formulation) sample were transferred into a 10mL clean dry volumetric flask and about 7mL of Diluant was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Range:

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 1µg-5µg and 100µg- 500µg of Cefuroxime and Linezolid respectively.

Robustness:

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

System suitability:

10 mg of Cefuroxime and 10 mg of Linezolid working standard was accuratel y weighed and transferred into a 10 ml clean dry volumetric flask and add about 20ml of diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further 1 ml of Cefuroxime and Linezolid was pipetted out from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

3. Results and Discussions

Linearity:

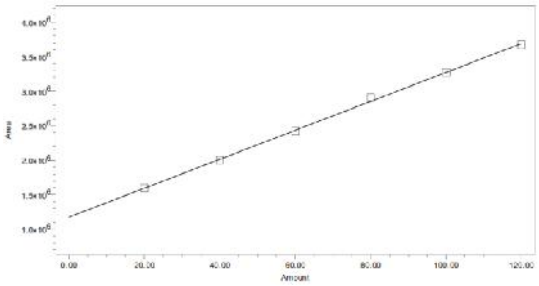

Fig 4: Calibration curve of Linezolid

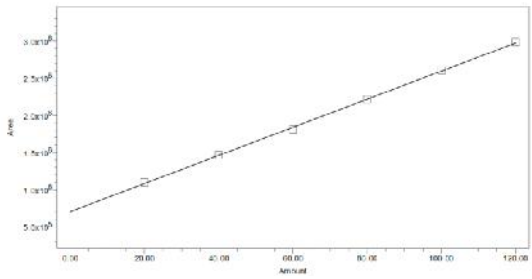

Fig 5: Calibration curve of Cefuroxime

Robustness:

Flow Rate: Analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

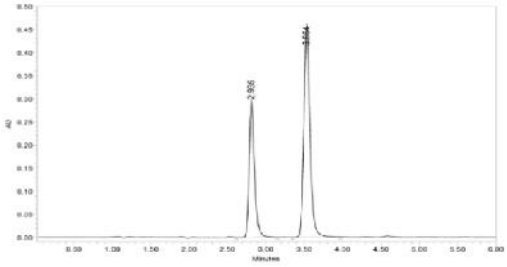

Fig 6: Chromatogram for Robustness Less flow

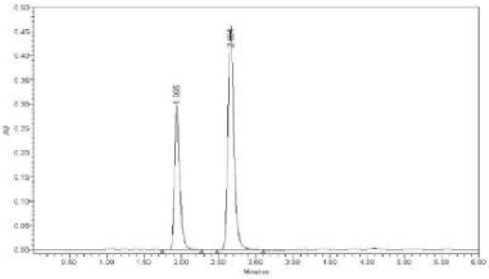

Fig 7: Chromatogram for Robustness More flow

The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2 ml/min.

Mobile Phase:

The Organic composition in the Mobile phase was varied from 70% to 60%. Standard solution 60 µg/ml of Linezolid & 60µg/ml of Cefuroxime was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

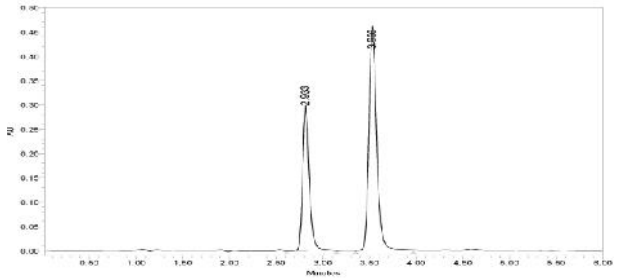

Fig 8: Chromatogram for Robustness less organic

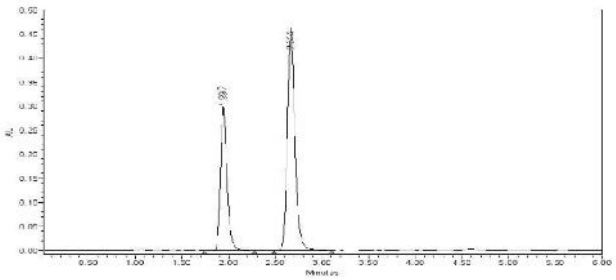

Fig 9: Chromatogram for Robustness more organic

Table No 1: Accuracy results of Cefuroxime

%Concentration (at specificationLevel)	Area	Amount added(m)	Amount found(m)	% Recovery	Mean Recovery
50%	1426646	5	4.9	101.8%	102.5%
100%	2551005	10	9.98	99.9%	
150%	2139845	15	15.0	100.0%	

Table No 2: Accuracy results of Linezolid

%Concentration (at specificationlevel)	**Area**	**Amount Added(mg)**	**Amount Found(mg)**	**%Recover y**	**Mean Recovery**
50%	975578	5	5.0	101.3%	101.0%
100%	1718370	10	9.96	99.6%	
150%	1465857	11	14.9	99.3%	

Table No 3: Repeatability results of Cefuroxime and Linezolid

Name: cefuroxime

	Name	RT	Area	Height (µV)	USP Plate Count	USP Tailing
1	cefuroxime	2.506	1553631	316525	6346.5	1.3
2	cefuroxime	2.516	1508002	296974	6197.1	1.2
3	cefuroxime	2.519	1545624	307327	6184.0	1.3
4	cefuroxime	2.531	1542374	302327	6176.0	1.2
5	cefuroxime	2.544	1561368	302525	6382.1	1.3
Mean			1542200			
Std. Dev.			20490.0			
% RSD			1.33			

Name: Linezolid

	Name	RT	Area	Height (µV)	USP Plate Count	USP Tailing
1	Linezoli	3.230	2790868	497608	7950.1	1.2
2	Linezolid	3.239	2661482	468477	8046.5	1.2
3	Linezolid	3.246	2706096	474632	8054.1	1.2
4	Linezolid	3.257	2703419	473234	8171.8	1.2
5	Linezolid	3.271	2695932	474830	8068.3	1.2
Mean			2711560			
Std. Dev.			47796.3			
% RSD			1.76			

Table No 4: Intermediate precision results of Cefuroxime

	Peak name	RT	Area
1	Cefuroxime	2.506	1763951
2	Cefuroxime	2.516	1794350
3	Cefuroxime	2.519	1792044
4	Cefuroxime	2.531	1792044
5	Cefuroxime	2.544	1783951
Mean			1786782
Std.dev			10795.03
%RSD			0.60416

Table No 5: Intermediate precision results of Linezolid

	Peak name	RT	Area
1	Linezolid	3.230	2575632
2	Linezolid	3.230	2570930
3	Linezolid	3.246	2613729

4	Linezolid	3.227	2613729
5	Linezolid	3.271	2575632
Mean			2586764
Std.dev			19163.75
%RSD			0.740839

Table No 6: Linearity Results Linezolid

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	471543
2	II	40 ppm	656277
3	III	60 ppm	794999
4	IV	80 ppm	946124
5	V	100 ppm	1002139
Correlation Coefficient			0.999

Table No 7: Linearity Results Cefuroxime

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	471543
2	II	40 ppm	656277
3	III	60 ppm	794999
4	IV	80 ppm	946124
5	V	100 ppm	1002139
Correlation Coefficient			0.999

Table No 8: System suitability results For Linezolid (Flow rate)

S.No	FlowRate(ml/min)	Systemsuitabilityresults	
		USPPlatecount	USPTailing
1	0.8	3483	1.26
2	1.0	2936	1.3
3	1.2	2832	1.1

* Results for actual flow (1.0 ml/min) have been considered from Assay standard

Table No 9: System suitability results for Cefuroxime (Flow rate)

S.No	FlowRate(ml/min)	Systemsuitabilityresults	
		USPPlatecount	USPTailing
1	0.8	6645	1.3
2	1.0	5824.4	1.3
3	1.2	6059.0	1.2

* Results for actual flow (1.0ml/min) have been considered from Assay standard

Table No 10: System suitability results for Linezolid (Mobile phase)

S.No	Changein Organic Compositionin the MobilePhase	Systemsuitabilityresults	
		USPPlatecount	USPTailing
1	10%Less	3254.5	1.1
2	Actual	3516	1.2
3	10%More	3215	1.2

* Results for actual Mobile phase composition (55:45Water : Methanol) have been considered from Accuracy standard

Table No 11: System suitability results for Cefuroxime (Mobile phase)

S.No	Changein Organic Compositionin the MobilePhase	Systemsuitabilityresults	
		USPPlatecount	USPTailing
1	10%Less	6691	1.3
2	Actual	6532.1	1.2
3	10%More	6557	1.3

4. Conclusion

A new method was established for simultaneous estimation of Cefuroxime and Linezolid by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Cefuroxime and Linezolid by using agilentC185µm (4.6*250mm) column, flow rate was1ml/min, mobile phase ratio was Phosphate buffer ph 4.0: ACN (30:70%v/v), detection wavelength was 254nm. The instrument used was Shimadzu, model No. SPD-20MA LC+20AD, Software-LC-20 Solution. The retention times were found to be 3.503mins and 2.577mins. The% purity of Cefuroxime and Linezolid was found tobe100.3% and 101.1% respectively. The system suitability parameters for Cefuroxime and Linezolid such as theoretical plates and tailing factor were found to be 1.3, 5824.4 and 1.2,2936.0 the resolution was found to be 9.4. The analytical method was validated according to ICH guidelines (ICH,Q2(R1)). The linearity study for Cefuroxime and Linezolid was found in concentration range of 20µg-100µg and 20µg-100µg and correlation coefficient(r2)was found to be 0.999 and 0.999, %mean recovery was found to be 102.5% and 101.0%, %RSD for repeatability was 0.6 and 0.5, %RSD for intermediate precision was 0.7 and 0.6 respectively. The precisionstudywasprecise, robust, and repeatable. LOD value was 3.1and 3.02 and LOQ value was 10.1and10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Cefuroxime and Linezolid in API and Pharmaceutical dosage form.

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