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Development and Validation of a RP-UPLC Method for Determination of Linezolid in Pharmaceutical Formulation

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ABSTRACT

A simple, sensitive and accurate RP-UPLC method has been developed for the determination of Linezolid in Tablet formulation. The mix of the Linezolid was found to be 251nm in Acetonitrile: Buffer [40:60(v/v)]. The method shows high sensitivity with linearity 5 to 30µg/ml (regression r^2 = 0.999). This method was tested and validated for various parameters according to ICH guidelines and USP. The Detection limit and quantitation limit were found to be 50mg ml–1 and 150 mg ml–1 in Acetonitrile: Buffer [40:60(v/v)] respectively. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation < 2%), while being simple, cheap and less time consuming and can be suitably applied for the estimation of Linezolid in Tablet pharmaceutical formulation.

Keywords: linezolid, Acetonitrile: Buffer 40:60 (v/v). RP-UPLC, Methanol.

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INTRODUCTION

Analytical chemistry is a scientific discipline that develops methods, instruments and strategies to obtain information on the composition and nature of matter. Analytical chemistry is concerned with the chemical characterization of matter and thus pharmaceutical analysis covers matter having pharmaceutical applications. Knowledge of chemical composition of many substances is important in our daily life. Analytical chemistry plays an important role in nearly all aspects of chemistry viz. agricultural, clinical, environmental, forensic, manufacturing, metallurgical, and pharmaceutical chemistry.

UPLC refers to Ultra Performance Liquid Chromatography. It improves in three areas: chromatographic resolution, speed and sensitivity analysis. It uses fine particles and saves time and reduces solvent consumption. The UPLC is based on the principal of use of stationary phase consisting of particles less than 2 µm (while HPLC columns are typically filled with particles of 3 to 5 µm). The underlying principles of this evolution are governed by the van Demeter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency) 14. The Van Demeter curve governed by an equation with three components shows that the usable flow range for a good efficiency with small diameter particles is much greater than for larger diameters The advent of UPLC has demanded the development of a new instrumental system for liquid chromatography, which can take advantage of the separation performance (by reducing dead volumes) and consistent with the pressures (about 8000 to 15,000 PSI, compared with 2500 to 5000 PSI in HPLC). Efficiency is proportional to column length and inversely proportional to the particle size18. Therefore, the column can be shortened by the same factor as the particle size without loss of resolution. The application of UPLC resulted in the detection of additional drug metabolites, superior separation and improved spectral quality.

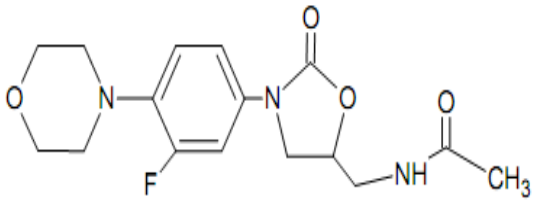


FigURE 1: Structure of Linezold

Molecular Formula : $C_{16}H_{20}FN_3O_4$

Molecular Weight : 337.346 g/mol

IUPAC Name :(S)-N-({3-[3-fluro-4-(morphine-4-yl)phenyl1]-2-oxo-1,3xazolidi-5-yl}methyl)acetamide

Description: white to off white powder

Melting Point: 154°C

Solubility: Linezolid is slightly soluble in acetone, and Insoluble in water, ethanol.

Mode of action: Protein synthesis inhibitors

Dose: 400mg / 600 mg

Therapeutic Category: Synthetic Antibacterial Agent of the oxazolidine class (Antibiotic)

MATERIALS AND METHOD

1. Instrument The liquid chromatographic system consisted of following components UPLC SYSTEM – TL/ARF/UPLC-05, Waters Acquity separation module PDA, Waters Empower Software-3. The chromatographic separation for Linezolid was carried out on a by using column ACQUITY UPLC ® HSS, 1.8µ 2.1×50 mm.

2. Reagents and materials Linezolid pure drug were obtained from Glenmark Generics Research Center, Taloja (Navi Mumbai) as a gift sample with 99.6% (w/w) assay value and was used without further purification. All chemicals and reagents used were of analytical grade (MERCK, RANKEM India). Linezolid tablets were purchased from local market and used with in self-life period. Each tablet was labeled contain 400mg of Linezolid.

3. Chromatographic Conditions the mobile phase was prepared by dissolving 2.72gm of K2HPO4 and 2ml TEA in 1000 ml water adjust pH 3.0 with OPA. From the previous solution, 600 ml was mixed with 400 ml of acetonitrile. Prior to use the mobile phase was filtered through 0.45 µm membrane filters and degassed by sonication for 10 min. the analysis was carried out on an UPLC Water Acquity system. The analytes were conducted on an analytical column ACQUITY UPLC ® HSS, 1.8µ 2.1×50 mm with a detection wavelength of 251 nm. The operating temperature of the column was set at 30°C. the injection volume was 0.5 µL, and the flow rate was maintained at 0.4 mL/min. the run time was 1.2 min. Column temperature 40°C0.

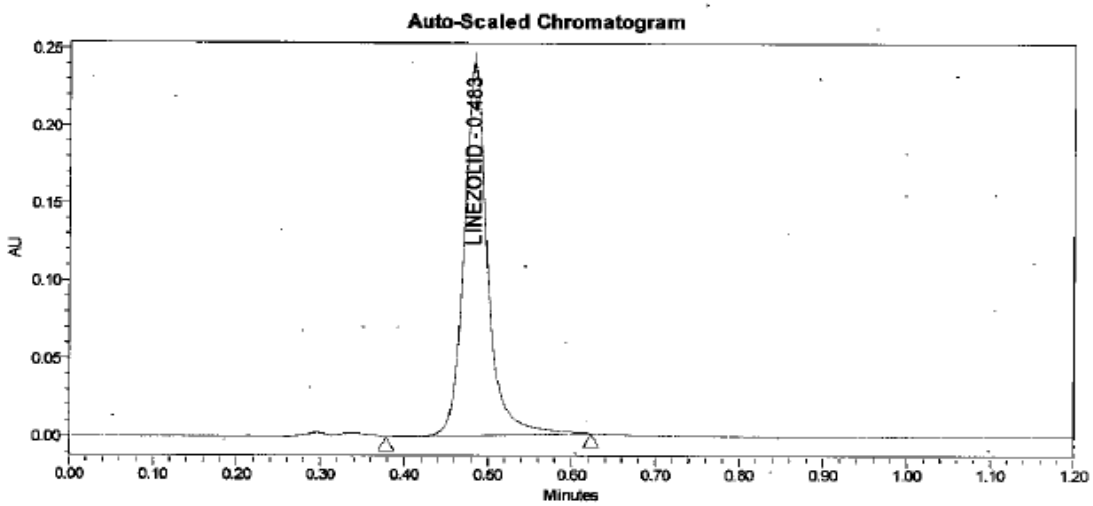
4. Preparation of Linezolid standard solution:

Weigh and transfer 30 mg of Linezolid working Standard into 50 mL volumetric flask, add 30mL diluent and sonicate to dissolve. Dilute to volume with diluent and mix well. Further dilute 5.0 ml of the solution into 50ml volumetric flask (60ppm), dilute to volume with diluent and mix well.

5. Preparation of sample solution:

Take a 5 tablet and crush. Weighed and transfer powder equivalent to 600 mg Linezolid in to 250mL volumetric flask, add 175mL diluent and sonicate this solution for about 15 min. with intermediate shaking. Allow to stand this solution for 10 min. Makeup volume with diluent. Pipette out 5mL of this solution in to 200mL volumetric flask and make up to volume with diluents and mix. Filter the solution through 0.45µ membrane filter and inject

6. Preparation of Placebo Solution: Weighed and transfer placebo powder equivalent to 600 mg Linezolid in to 250mL volumetric flask, add 175mL diluent and sonicate this solution for about 15 min. with intermediate shaking. Allow to stand this solution for 10 min. Makeup volume with diluent. Pipette out 5mL of this solution in to 200mL volumetric flask and make up to volume with diluents and mix. Filter the solution through 0.45µ membrane filter and inject.



Peak Results

	Name	RT	Area
1	LINEZOLID	0.483	470638

Figure 2: Chromatogram of Linezolid

Method Validation

1. System suitability:

The standard solution was injected five times into chromatograph & measured the area counts of Linezolid.

The tailing factor of the main peak should be NMT 2.0.

The theoretical plates of the main peak should be NLT 2000.

The %RSD of the main peak area for five standard injections should be NMT 2.0%.

2. Specificity:

The specificity of the method was determined by checking for interference with the drug from placebo components. This method was proven by the no change in absorbance of the drug with and without excipients at respective wavelength. Therefore, proposed method was specific and selective for the drug.

3. Linearity and Range:

A series of Standard preparations of Linezolid to be prepared over a range of 50% to 150% of the working concentration of Linezolid in Linezolid Tablet. (Minimum Five points should be in the range 80-120% of standard/ sample concentration for Assay). Since the working concentration is 100 µg per ml, of Linezolid, the range proposed is about 50 µg per ml to 150 µg per ml of Linezolid.

4. Accuracy (Recovery):

Placebo of Linezolid will be spiked Linezolid with drug substance at three different levels: 80%, 100% and 120% of the label claim in triplicate (total nine determinations) and then to be prepared as described under Appendix A. Each of the sample preparations will be injected in duplicate and the average area count to be taken for calculation. Transfer accurately API and Placebo as given in the table below in volumetric flasks.

5. Precision:

Method Precision: Six sample preparations of Linezolid Injections are to be prepared and injected into the UPLC using the method as described under Appendix A.

Ruggedness (Intermediate Precision) Six sample preparations of the same lot of Linezolid Injections is made by a different analyst, using different column on a different day and injected in duplicate into a different UPLC than using the method as described under Appendix A, along with Standard preparation.

6. Robustness

Robustness of the method was carried out by deliberately made small change in the Flow rate by ±10% (1.35 and 1.65 ml/min), Column temperature by ±50C, Mobile phase composition by ±5% of ACN: Phosphate buffer (81:19, v/v and 79:21, v/v) and change in pH of Phosphate Buffer 6.5±0.2 units had no significant effect on the retention time and chromatographic response of linezolid.

Assay Method Validation Report

UPLC method has been developed for the Assay of in Linezolid Tablet. This report is intended for the validation of UPLC method for the Assay of Linezolid in Linezolid Tablet Specificity

Linearity and Range

A series of Standard preparations of Linezolid to be prepared over a range of 50% to 150% of the working concentration of Linezolid in Linezolid Tablet. (Minimum Five points should be in the range 80-120% of standard/ sample concentration for Assay). Since the working concentration is 100 µg per ml, of Linezolid, the range proposed is about 50 µg per ml to 150 µg per ml of Linezolid.

Table 1: Linearity and Range

% Concentration	Concentration (µg per mL)	Response (Area)	Acceptance Criteria	Statistical Analysis	
50%	30	228080	Correlation coefficient should not be less than 0.999	**Slope**	**7515**
80%	48	364017		**Intercept**	**+2891**
100%	60	454183		**Correlation Coefficient**	**1.000**
120%	72	543428			
150%	90	679393			

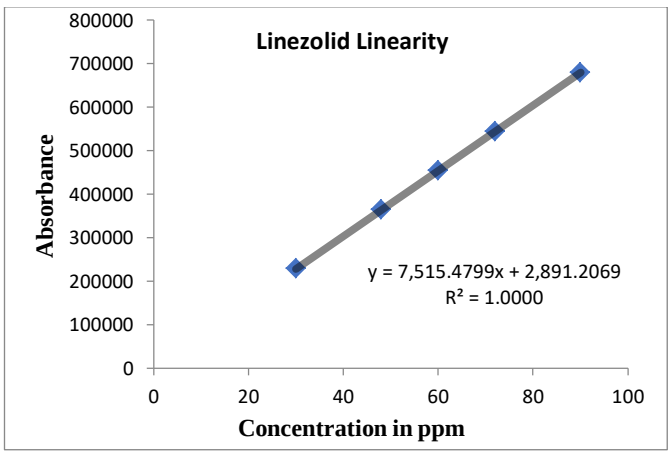


Table 2: Calibration curve of Linezolid

Conclusion: Correlation coefficient is 1.000 Therefore, the UPLC method for the determination of Assay of Linezolid in Linezolid Tablet is linear.

Accuracy (Recovery):

Placebo of Linezolid were spiked with Linezolid drug substance at three different levels: 80%, 100%, 120% of the label claim in triplicate (total nine determinations) and then to be prepared as described under **Methodology.** Each of the sample preparation was injected in duplicate and average area count to be taken for calculation.

Table 3: Accuracy data of the developed method

Sample No.	Amount Added (mg)	Acceptance Criteria	Amount Recovered (mg)	% Recovery
Acc. 80% -1	48.2	Mean recovery should be in the range of 98.0% to 102.0%. The RSD should not more than 2.0%	47.45	98.81
Acc. 80% -2	48.2		47.45	98.81
Acc. 100% -1	60.3		59.45	99.04
Acc. 100% -2	60.3		59.45	99.04
Acc. 120% -1	72.1		71.15	98.80
Acc. 120% -2	72.1		71.15	98.80
			Mean	**98.88**
			SD	**0.134**
			% RSD	**0.135**

Conclusion: Mean recovery is 98.88% & RSD is 0.135%. Therefore, the UPLC method for the determination of Assay of Linezolid in Linezolid Tablet is accurate.

Precision:

Method Precision:

Six sample preparation of Linezolid were prepared and injected into the UPLC using the method as described under **Methodology.**

Table 4: Method Precision data

Sample	% Assay	Acceptance Criteria	Conclusion
1	97.4	RSD should not more than 2.0%	The RSD of method precision is 0.52%. Therefore, the UPLC method for the determination of Assay of Linezolid Injection is reproducible.
2	98.6		
3	98.2		
4	97.9		
5	98.6		
6	97.5		
Mean	**98**		
SD	**0.51**		
%RSD	**0.52**		

Sample	% Assay	Acceptance Criteria	Conclusion
1	97.4	RSD should not more than 2.0%	The RSD of method precision is 0.75 %. Therefore, the UPLC method for the determination of Assay of Linezolid Injection is reproducible.
2	98.6		
3	97.0		
4	98.2		
5	98.7		
6	97.8		
Mean	**98.0**		
SD	**0.738**		
%RSD	**0.75**		

Intermediate Precision:

Six sample preparation of Linezolid were prepared and injected into the UPLC using the method as described under **Methodology.**

Robustness:

Robustness of the method was verified by applying minor and deliberate changes in the experimental parameters, for example:

(i) Column temperature: ±5°C
(ii) Flow rate: ±0.2 mL/min
(iii) pH Variation (±0.2 units)
(iv) Mobile phase composition, organic composition ±5% Change was made to evaluate its effect on the method. Obtained data for each case was evaluated by calculating % RSD and percent of recovery.

Table 5: Robustness data of the Developed Method Changed parameter

Robustness data of the Developed Method Changed parameter	Peak area / %RSD	Rt	Theoretical plates	Tailing factor
Flow rate (±0.2%)				
0.6 ml/min	454183± 0.3	0.452	28445	1.04
0.2 ml/min	452552± 0.5	0.483	24210	1.06
Column temp (± 5°C)				
43°C	455692± 0.4	0.483	24432	1.03
38°C	487455± 0.7	0.447	31268	1.08
Mobile Phase (± 5%)				
65:35	465454± 0.8	0.486	28413	1.12
63:37	454154± 0.7	0.451	24265	1.09
pH Variation (±0.2 units)				
6.3	477682 ± 0.5	0.483	27478	1.04
6.7	465683 ± 0.4	0.489	26410	1.18

CONCLUSION

Linezolid is Synthetic Antibacterial Agent of the oxazolidine class. It is official in United States Pharmacopoeia. The study was undertaken to develop and validate analytical methods to estimate Linezolid.

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