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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF LINEZOLID IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise, accurate sensitive and specific RP-HPLC method for the determination of Linezolid in pharmaceutical dosage form. Chromatogram was run through Sunfire C18 150 x 4.6 mm, 5m. Mobile phase containing 0.01N Potassium dihydrogen phosphate: Methanol taken in the ratio 65:35 was pumped through column at a flow rate of 1.2ml/min. Buffer used in this method was 0.01N Potassium dihydrogen phosphate (4.8ph) buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 252.0nm. Retention time of Linezolid was found to be 2.469 min. % RSD of the Linezolid were and found to be 0.9 % RSD of Method precision of Linezolid was found to be 0.5. % Recovery was obtained as 100.29% for Linezolid. LOD, LOQ values obtained from regression equation of Linezolid were 0.10, 0.31. Regression equation of Linezolid is y = 39003x+14938. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

INTRODUCTION

Linezolid: It is indicated in adults and children for the treatment of infections caused by susceptible Gram-positive bacteria, including nosocomial pneumonia, community-acquired pneumonia, skin and skin structure infections, and vancomycin-resistant Enterococcus faecium infections, Examples of susceptible bacteria include Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, and Streptococcus agalactiae.

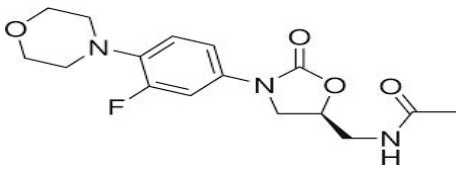

Fig.1. Linezolid

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IUPAC Name:	N-{[(5S)-3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3- oxazolidin-5-yl] methyl} acetamide
Molecular Weight:	337.3
Molecular Formula:	C16H20FN3O4
Appearance:	Crystalline, white-to off-white powder
Physical State:	Solid
Solubility:	Slightly soluble in ethanol, ethyl acetate & water
Storage:	Store at 25°C (77°F)
PKA:	The calculated pKa is 1.8 and was determined from solubility versus pH data and confirmed linezolid using 1H-NMR. This pKa indicates that linezolid is unionized in aqueous media above pH 4
Log P:	3.5 (logPC = 0.55) in aqueous buffers (I = 0.1 M) and n-octanol and is independent of pH in the range of pH 3 to 9

Mechanism of action: Linezolid exerts its antibacterial effects by interfering with bacterial protein translation. It binds to a

site on the bacterial 23S ribosomal RNA of the 50S subunit and prevents the formation of a functional 70S initiation complex, which is essential for bacterial reproduction, thereby preventing bacteria from dividing **Pharmacokinetics:** Linezolid is an oxazolidinone antibacterial agent effective against most strains of aerobic Gram-positive bacteria and mycobacteria. It appears to be bacteriostatic against both staphylococci and enterococci and bactericidal against most isolates of streptococci. Linezolid has shown some in vitro activity against Gram-negative and anaerobic bacteria but is not considered efficacious against these organisms, Linezolid is a reversible and nonselective inhibitor of monoamine oxidase (MAO) enzymes and can therefore contribute to the development of serotonin syndrome when administered alongside serotonergic agents such as selective serotonin re-uptake inhibitors (SSRIs) or tricyclic antidepressants (TCAs). Linezolid should not be used for the treatment of catheter-related bloodstream infections or catheter-site infections, as the risk of therapy appears to outweigh its benefits under these circumstances. **Absorption:** Linezolid is extensively absorbed following oral administration and has an absolute bioavailability of approximately 100%. Maximum plasma concentrations are reached within approximately 1 to 2 hours after dosing (Tmax) and range from 8.1-
12. mcg/mL after single doses and 11.0-21.2 mcg/mL after multiple dosing.

MATERIALS AND METHODS

Materials

- Linezolid pure drug (API), Linezolid Tablets (Linospan), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instruments

- Electronics Balance-Denver
- p^H meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbance of Linezolid solution.

Preparation of buffer:	
0.01N KH2PO4 Buffer:	Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 4.8 with dil. Orthophosphoric acid solution.
0.1%OPA Buffer:	1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Metabolism and Elimination

Linezolid is primarily metabolized to two inactive metabolites: an aminoethoxyacetic acid metabolite (PNU-142300) and a hydroxyethyl glycine metabolite (PNU-142586), both of which are the result of morpholine ring oxidation. The hydroxyethyl glycine metabolite - the most abundant of the two metabolites - is likely generated via non-enzymatic processes, though further detail has not been elucidated.

Diluent:	Based up on the solubility of the drugs, diluent was selected, ACN and Water taken in the ratio of 50:50
Preparation of standard stock solution:	Accurately weighed 30mg of Linezolid is transferred to 50ml volumetric flask. 3/4th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (600µg/ml of Linezolid)
Preparation of standard working solution[100% solution]	1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (60µg/ml of Linezolid).
Preparation of sample stock solutions:	10 Tablets were weighed and the average weight of each Tablet was calculated, then theweight equivalent to 1 Tablet (600mg Tablet) was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (6000µg/ml of Linezolid)
Preparation of sample working solutions: [100% solution]	0.5ml of filtered sample stock solution was transferred to 50ml volumetric flask and made up with diluent. (60µg/ml of Linezolid)

Method Validation: According to ICH Guidelines Method Validation can be defined as "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics". Method validation study include system suitability, linearity, precision, accuracy, Specificity, robustness, limit of detection, limit of quantification and stability of samples, reagents, instruments.

Optimised Method:

Chromatographic condition:

Mobile Phase:65% 0.01N Kh2po4 : 35% Methanol

Flow Rate: 1.2 ml/min

Column: Sunfire C18[4.6 x 150mm, 5um]

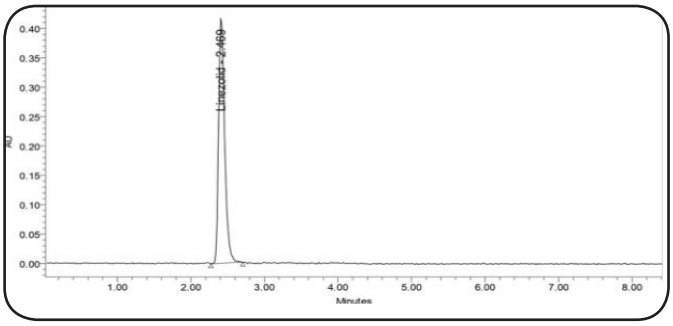
Detector Wavelength: 240nm

Column Temperature: 30^0C

Injection Volume: 10ul

Run Time: 10 min

Diluent: water and ACN [50:50]



Optimised chromatogram

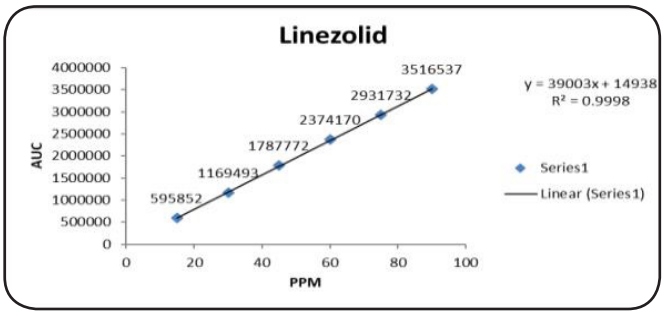
System suitability studies

Table. 3. System suitability parameters for Linezolid			
Sr. No.			
Inj	RT [min]	USP plate count	Tailing
1	2.438	3836	1.33
2	2.441	3790	1.35
3	2.443	3883	1.33
4	2.446	3907	1.33
5	2.449	3981	1.33
6	2.463	3902	1.34

All the system suitability parameters were within the range and satisfactory as per ICH guidelines. The system suitability parameters were determined by preparing standard solution of Linezolid (60ppm) and the solution were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Linearity

Table 2. Linearity table for Linezolid	
Conc[ug/ml]	Peak Area
0	0
15	595852
30	1169493
45	1787772
60	2374170
75	2931732
90	3516537



Six linear concentrations of Linezolid (15-90µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Linezolid was y = 39003x + 14938. Correlation coefficient obtained was 0.999 for the two drugs.

Accuracy

Accuracy Table of Linezolid

% Level	Amount spiked [ug/ml]	Amount Recovered [ug/ml]	% Recovery	Mean % Recovery
50%	30	29.73	99.0	100.29%
			.0	
			9	
	30	30.05	10	
			0.	
			17	
	30	30.26	10	
			0.	
			88	
100%	60	60.77	10	
			1.	
			28	
	60	60.36	10	
			0.	
			59	
	60	60.33	10	
			0.	
			55	
150%	90	88.79	98	
			.6	
			5	
	90	90.67	10	
			0.	
			75	
	90	9.58	10	
			0.	
			64	

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 100.29% for Linezolid**.**

3.4 Precision

System Precision

Table 4. System precision table of Linezolid	
Sr. No.	Area of Linezolid
1	2357236
2	2392683
3	2361491
4	2371441
5	2341875
6	2333634
Mean	2359727

S.D.	21129.0
%RSD	0.9

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated % RSD obtained as 0.9% for Linezolid. As the limit of Precision was less than 2 the system precision was passed in this method.

Repeatability

Table 5. Repeatability Table of Linezolid	
Sr. No.	Area of Linezolid
1.	2370789
2.	2382032
3.	2358667
4.	2392168
5.	2372771
6.	2379914
Mean	2376057
S.D.	11411.4
% RSD	0.5

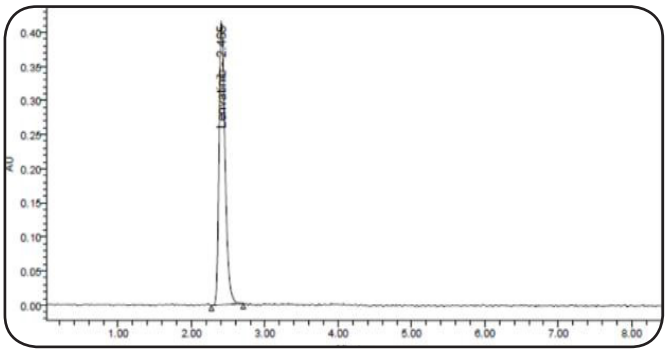
Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated and obtained as 0.5% for Linezolid. As the limit of Precision was less than "2" the system precision was passed in this method.

Intermediate precision (Intra Day-Inter day)

Table 6. Intermediate precision table of Linezolid	
Sr. No.	Area of Linezolid
1	2397235
2	2360721
3	2341756
4	2344683
5	2368505
6	2360669
Mean	2362262
S.D.	19982.6
%RSD	0.8

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated and obtained as 0.8% for Linezolid. As the limit of Precision was less than "2" the system precision was passed in this method.

Specificity



Retention time of Linezolid were 2.465 min. We did not find and interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific**.**

Robustness

Table 6. Robustness Data for Linezolid		
Sr. No.	Condition	% RSD of Linezolid
1	Flow rate(-) 1.1ml/min	0.7
2	Flow rate(+) 1.3ml/min	1.5
3	Mobile phase(-)60B:40A	1.0
4	Mobile phase(+)70B:30A	0.8
5	Temperature(-)27^{0}C	0.3
6	Temperature(+)33^{0}C	0.7

Robustness conditions like Flow minus (1.1ml/min), Flow plus (1.3ml/min), mobile phase minus (60:40A), mobile phase plus (70B:30A), temperature minus (27°C) and temperature plus (33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Sensitivity

Table 7. Sensitivity table of Linezolid		
Molecule	LOD	LOQ
Linezolid	0.10	0.31

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.3ml each of Linezolid, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.9ml each of Linezolid, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

4. Summary and Conclusion

CONCLUSION

A simple, precise, accurate sensitive and specific RP-HPLC

method for the determination of Linezolid in pharmaceutical dosage form. Retention time of Linezolid was found to be 2.469min. % RSD of the Linezolid were and found to be 0.9. % RSD of Method precision of Linezolid was found to be 0.5. % Recovery was obtained as 100.29% for Linezolid. LOD, LOQ values obtained from regression equation of Linezolid were 0.10, 0.31. Regression equation of Linezolid is y = 39003x + 14938. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Table 4. Summary

Parameters		Linezolid	Limit
Linearity Range[ug/ml]		15-90ug/ml	R<1
Regression Coefficient		0.999	
Slope[m]		39003	
Intercept[c]		14938	
Regression Equation[y=mx+c]		Y=39003x+14938	
Assay[%mean]		100.39%	90-110%
Specificity		Specific	No interference of an Peak.
System % RSD precision		0.9	NMT 2.0%
Method % RSD precision		0.5	NMT 2.0%
Accuracy% Recovery		100.29%	98-102%
LOD		0.10	NMT 3
LOQ		0.31	NMT 10
Robustness	FM	0.7	% RSD NMT 2.0
	FP	1.5	
	MM	1	
	MP	0.8	
	TM	0.3	
	TP	0.7	

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