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RP-HPLC Method Development and Validation for the Analysis of Pharmaceutical Drugs - LINEZOLID

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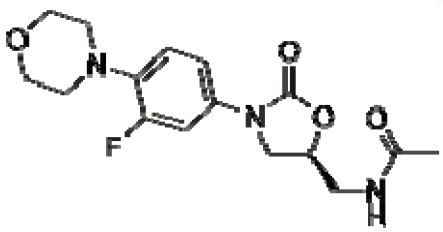
Abstract: ***A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of LINEZOLID. Isocratic elution at a flow rate of 1.2ml /min was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of Acetonitrile: 0.1M Acetic Acid 50:50 (v/v). The UV detection wavelength was at 254 nm. Linearity was observed in concentration range of 100-140 ppm. The retention time for Linezolid was 3.3 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Linezolid.***

Keywords: Linezolid, HPLC Method, Development, 254nm.

1. Introduction

Drugs: LINEZOLID

Structure:



$C_{16}H_{20}FN_3O_4$

Figure 1: Chemical Structure of LINEZOLID

IUPAC Name	(S)-N-({3-[3-fluoro-4-(morpholin-4-yl) phenyl]-2-oxo-1, 3-oxazolidin-5-yl} methyl) acetamide
Formula	$C_{16}H_{20}FN_3O_4$
Molecular Weight	337.346 g/mol
Classification Of Drugs	Oxazolidinone
Antimicrobial Spectrum	Mainly active against gram-positive organisms
Solubility	water-soluble (approximately 3 mg/ mL)

Pharmacokinetic Data

Bioavailability	~100% (oral)
Protein Binding	Low (31%)
Metabolism	Hepatic (50–70%, CYP not involved)
Half-Life	4.2–5.4 hours (shorter in children)
Excretion	Nonrenal, renal, and fec

The oxazolidinones have been known as monoamine oxidase inhibitors since 1950s.[1] Their antimicrobial properties were discovered by researchers at E.I. duPont de Nemours in the 1970s.[1] In 1978, DuPont patented a series of oxazolidinone derivatives as being effective in the treatment of bacterial and fungal plant diseases, and in 1984, another patent described their usefulness in treating bacterial infections in mammals. In 1987, DuPont scientists presented a detailed description of the oxazolidinones as a new class of antibiotics with a novel mechanism of action. [1] Early compounds were found to produce liver toxicity, however, and development was discontinued.

Pharmacia and Upjohn Company was developed a Linezolid – a first synthetic oxazolidinones drugs in 1990s. [1] First approved for use in 2000, [1] linezolid was the first commercially available 1, 3-oxazolidinone antibiotic. Linezolid is quite expensive; a course of treatment may cost one or two thousand U.S. dollars for the drug alone, not to mention other costs. Linezolid is member of oxazolidinones

class of drugs, is active against Gram-positive bacteria that cause disease, including streptococci, vancomycin-resistant enterococci (VRE), and methicillin-resistant Staphylococcus aureus (MRSA). [1] Linezolid is used in infections of the skin and soft tissues and pneumonia although off-label use for a variety of other infections is becoming popular. Linezolid is protein synthesis inhibitor it stops the growth of bacteria by disrupting their production of proteins. Bacterial resistance to linezolid has remained very low since it was first detected in 1999, although it may be increasing. It can be used in patients of all ages and in people with liver disease or poor kidney function.Linezolid is more effective than glycopeptide antibiotics (such as vancomycin and teicoplanin) and beta-lactam antibiotics in the treatment of skin and soft tissue infections (SSTIs) caused by Gram-positive bacteria.[2] Linezolid appears to be cheaper and more effective than vancomycin in the treatment of diabetic foot infections.[3] Linezolid has not only effected on most Gram-negative bacteria. Pseudomonas and the Enterobacteriaceae, for instance, are not susceptible.[1] In vitro, it is active against Pasteurella multocida,[4][1] Fusobacterium, Moraxella catarrhalis, Legionella, Bordetella, and Elizabethkingia meningoseptica, and moderately active against Haemophilus influenzae.[1] It has also been used to great effect as a second-line treatment for Capnocytophaga infections.[5][1] It has poor activity against gram -ve aerobic and anaerobic organisms. Linezolid is bacteriostatic against enterococci and staphylococci and bactericidal against streptococci. It produces its action by inhibiting protein synthesis within the bacterial cell. It acts as an inhibitor of bacterial protein synthesis by blocking the formation of the 70S ribosomal initiation complex. Linezolid has almost 100% bioavailability and the area under the plasma concentration curve is identical after oral and iv. administration.[6]

The safety and tolerability of linezolid are advantageous. High-resolution structures of linezolid bound to the 50S ribosomal subunit show that it binds in a deep cleft that is surrounded by 23S rRNA nucleotides.[7] Mutation of 23S rRNA has for some time been established as a linezolid resistance mechanism. Although ribosomal proteins L3 and L4 are located further away from the bound drug, mutations in specific regions of these proteins are increasingly being associated with linezolid resistance [7].

2. Experimental

2.1 Chemicals and Reagents

All HPLC SOLVENTS used like Acetonitrile, Acetic Acid which are of HPLC grade were purchased from E.Merck,

2.2 Instrumentation and Analytical Conditions

The analysis of the drug was carried out on Shimadzu HPLC model (VP series) containing LC-20AT (VP series) pump, variable wave length programmable UV/visible detector SPD-20A and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using phenolex C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing. Isocratic elution with, Acetonitrile, 0.1M Acetic Acid 50:50(v/v) was selected with a flow rate of 1.2 ml /min .The detection wavelength was set at 254 nm with a runtime of 10 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30 min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

2.3 Preparation of Stock, Working Standard Solutions and Sample Solutions

0.03374 gm of Linezolid was weighed and transferred into a 100ml volumetric flask. Water was added and sonicated to dissolve it completely and made up to the mark with the same solvent. Further 10 ml of the above stock solution was pipette into a 100ml volumetric flask and diluted up to the mark with water. The contents were mixed well and filtered through Ultipor N66Nylon 6, 6 membrane sample filter paper. The calibration curve was plotted with the concentrations of the 100 to 140 ppm solutions. Calibration solutions were prepared and analyzed immediately after preparation.

Table 1: Chromatographic Conditions for Linezolid

Sr. No	*Test*	*Result*
	H.P.L.C CONDITIONS	
1	Elution	ISOCRATIC
2	A.P.I CONC	100ppm
3	Mobile Phase	Acetonitrile:0.1M Acetic Acid(50:50)
4	pH	3.0
5	Column	C18
6	Wavelength	254 nm
7	Flow Rate	1.2 ml/min
8	Runtime	10 Min
9	Retention Time	3.350
10	Area	1721.843
11	Th.Plates	9450
12	Tailing Factor	1.489

13	Pump Presure	75 kgf

3. Method Validation Procedure

The objective of the method validation is to demonstrate that the method is suitable for its Intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness and system suitability.

3.1 Linearity

Table 2: Linearity of LINEZOLID

Sr. No	*CONC*	*Area*
1	100 ppm	1721.843
2	110 ppm	1875.565
3	120 ppm	1995.896
4	130 ppm	2145.562
5	140 ppm	2334.364

The developed method has been validated as per ICH guidelines. Solutions of LINEZOLID in the mass concentration range of 100 ppm to 140 ppm was injected in to the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of LINEZOLID was obtained by plotting the peak area ratio versus the applied concentrations of LINEZOLID. The linear correlation coefficient was found to be 0.9733

Table 3: Linear Regression Data for Calibration curve

Drug	LINEZOLID
Concentration range	100-140 ppm
Slope (m)	15.31
Intercept (b)	190.843
Correlation coefficient	0.9972

3.2 Precision

Repeatability of the method was checked by injecting replicate injections of 100 ppm of the solution for five times on the same day as intraday precision study of LINEZOLID and the RSD was found to be 0.0589 for intraday and 0.0520 for interday

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Table 4: Precision parameters of LINEZOLID

Injection	*Concentration*	*Intra Day*	*Inter Day*
1	100 ppm	1629.144	1879.285
2	100 ppm	1626.072	1878.448
3	100 ppm	1630.290	1878.700
4	100 ppm	1625.917	1875.818
5	100 ppm	1627.347	1874.858
	RSD	0.0589	0.0520

3.3 Accuracy

The accuracy of the method was determined by calculating recovery of LINEZOLID by the method of standard addition. Known amount of LINEZOLID (100ppm) was added to a pre quantified sample solution and the amount of LINEZOLID was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of LINEZOLID was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve.

3.4 Specificity

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug.

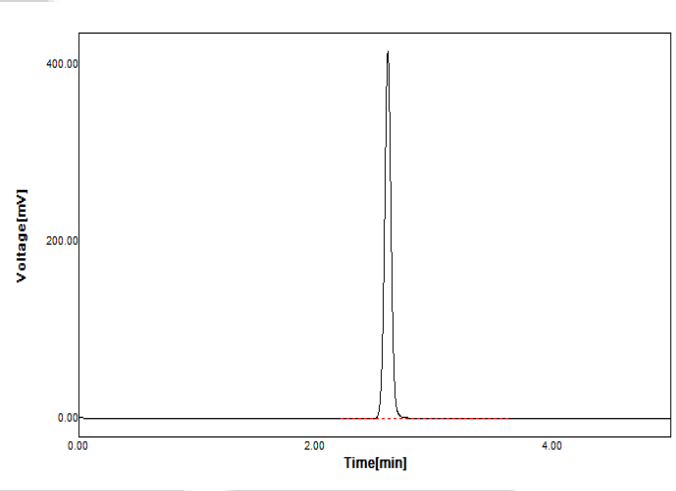

Figure 2: Typical chromatogram of LINEZOLID

3.5 LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 1ppm and 10 ppm respectively as per ICH guide-lines. Results are shown in table 5.

Table 5: Results of LOD and LOQ.

Parameter	*Measured*
LOD	1ppm
LOQ	10ppm

3.6 Robustness

To determine the robustness of the method, two parameters from the optimized chromatographic conditions were varied. First, Instrument and place were changed and second pH was changed 3.0 to 2.8. Results of Robustness are shown in table 6 & 7.

Table 6: Robustness parameters

Parameter	*Modification*
M.Phase	Acetonitrile:0.1M Acetic Acid(50:50)
PH	2.8
Wavelength	254nm
R.T	2.603 Min

Table 7: Robustness results

Accuracy	*Precision*
1636.9031	1607.2660
1636.5038	1606.7297
1636.5115	1609.3283
	1604.2771
	1604.5669
RSD: 0.0098	RSD: 0.0647

3.7 System Suitability Parameter

System suitability tests were carried out on freshly prepared standard stock solutions of LINEZOLID and it was calculated by determining the standard deviation of LINEZOLID standards by injecting standards in five replicates at 5 minutes interval and the values were recorded in Table 8.

Table 8: System suitability parameters of LINEZOLID

Parameters	Values
λ max (nm)	254nm
Correlation coefficient	0.997
Retention time	3.350 min
Theoretical plates	9450
Tailing factor	1.5
Limit of detection	1ppm
Limit of quantification	10ppm

4. Result and Discussion

4.1 Optimization of the Chromatographic Conditions

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug LINEZOLID being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Acetonitrile: 0.1M Acetic Acid (50:50). The retention time of LINEZOLID was found to be 3.350 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise.

5. Conclusion

A validated RP-HPLC method has been developed for the determination of LINEZOLID in bulk form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 3.350 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of LINEZOLID in pharmaceutical analysis.

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