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Research Article

## Development and validation of Spectrophotometry methods for estimation of linezolid in bulk and in pharmaceutical Dosage formulation

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### ABSTRACT

A simple, precise and economical, and rapid Spectrophotometric methods for the quantification of Linezolid in bulk material and in tablets. Further, this study is designed to validate the developed methods as per ICH guidelines. **Material & methods:** In Methods A and B, a stock standard solution was prepared by dissolving 10 mg of Linezolid in 100 mL of phosphate buffer pH 7.4 to obtain a concentration of 100 µg/mL. After suitable dilution, 10 µg/mL of Linezolid was prepared and scanned in the UV-visible range 400 -200 nm; In method A zero order spectrum Linezolid showed a maximum absorbance at 251 nm. while in Method B area under curve (AUC) zero-order spectrum was recorded between 245 and 268 nm. For a linearity study, series of dilutions were prepared from stock solutions. **Results:** In Method A and B, Linezolid followed linearity in the concentration range of 3 - 18 µg/mL with ( $r^2 = 0.9978$ ) ( $r^2 = 0.9981$ ). The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 80%, 100% and 120%. The % recovery was found to be in the range 96.15% - 99.32% for method A, while in method B range is 99.16 % - 100.20 %. The low values of % RSD are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % R.S.D. value less than 2 indicate that the method was precise. Ruggedness of the proposed method was studied with the help of two analysts. The proposed method of pharmaceutical formulation the amounts of Linezolid estimated by both these methods (A and B) were found to be 96.25 ± 0.44 and 99.48 ± 1.05, respectively. **Conclusion:** The developed methods are simple, precise, rugged, and economical. Both these methods can be used for routine analysis of Linezolid from its tablet formulation.

**Keywords:** Linezolid, UV-Spectrophotometric methods, area under curve, zero order spectrum

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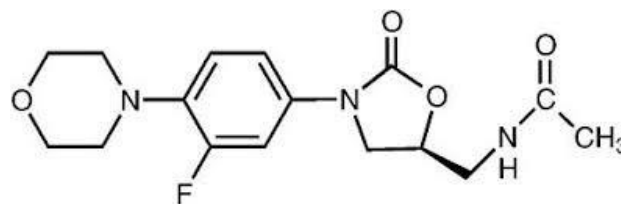
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### INTRODUCTION

Linezolid is a synthetic antibiotic, the first commercially available of the 1, 3-oxazolidinone antibiotic class, used for the treatment of infections caused by multi-resistant bacteria including streptococcus and methicillin-resistant Staphylococcus aureus (MRSA).[1] Chemical name of Linezolid (LIN) is N-{{[(5S)-3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl]methyl}acetamide. It has an empirical formula of C<sub>16</sub>H<sub>20</sub>FN<sub>3</sub>O<sub>4</sub> and a molecular weight of 337.346 g/mol. [2] Linezolid is active after oral or intravenous administration. [3] It inhibits bacterial protein synthesis by acting at an early step and a site different from that of other AMAs. It binds to 23s fraction of the 50s ribosome and interfere with formations of the ternary N-formylmethionine-t-RNA-70s initiation complex. Binding of linezolid distorts the t-RNA binding site overlapping both 50s and 30s ribosomal subunits and stops protein synthesis before it starts. [4] Appropriate use of Linezolid is essential to minimize the risk of resistance development in Gram

positive bacteria. The availability of both parenteral and oral formulations provides the opportunity to transfer appropriate patients to an oral formulation. [5] Linezolid is administered as intermittent intravenous dose of 600mg every 12 h, without loading dose. [6]



Structure of Linezolid

Literature survey reveals that several analytical methods have been reported for the estimation of Linezolid by Spectroscopic methods such as UV/Vis, difference spectrophotometric method combination with other drugs [7] and Apart from above the other methods are Chiral HPLC

Method[8], and HPTLC [9] methods were reported for this compound.

UV spectrophotometric method was reported for the quantitative determination of Linezolid in pharmaceutical dosage forms. The developed method was simple, precise, specific and accurate analysis proved that method is reproducible and selective for the analysis of Linezolid in bulk drug and tablet.

## EXPERIMENTAL WORK

### Instrument:

A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe with 10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: Wavelength range: 200 – 400 nm; scan speed: Medium; sampling interval: 1.0 nm.

### Material and Methods:

Active Pharmaceutical Ingredient (API) of Linezolid supplied by ALEMBIC Pharmaceutical

Limited, Vadodara, India) As the tablet formulation was available in Indian market, LIDOMAX-600mg tablets were used as pharmaceutical formulation for further analysis. Solvent used for preparation of solution is phosphate buffer pH 6.4, phosphate buffer pH 7.4 and Methanol.

### Preparation of solvent:

- Phosphate buffer pH 6.4 : Dissolve 1.79gm of disodium hydrogen phosphate, 1.36gm of potassium dihydrogen phosphate and 8.0 gm of sodium chloride in sufficient water to produce 1000ml. and adjust the pH, if necessary.
- Phosphate buffer pH 7.4 : Dissolve 2.38 gm of disodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate and 8.0 gm of sodium chloride in sufficient water to produce 1000ml. and adjust the pH, if necessary.[10]

### Preparation of standard stock solution:

A standard stock solution of Linezolid was prepared by dissolving accurately weighed 10mg of the drug in phosphate buffer pH 7.4 in 100ml volumetric flask, sonicate and to obtain the concentration of 100 µg/ml for method A and B.

### Methods:

#### A. Zero order UV spectrophotometric method :

Determination of wavelength:

1. Methanol: From the standard stock solution; 1 ml (100 µg/ml) was diluted with 10 ml methanol to obtain the concentration of 10 µg/ml. and this solution was scanned in the UV-region 400 - 200nm. Linezolid showed  $\lambda_{max}$  at 251nm (Fig.1)
2. Phosphate buffer pH 6.4 : From the standard stock solution; 1 ml (100 µg/ml) was diluted with 10 ml Phosphate buffer pH to obtain the concentration of 10 µg/ml. and this solution was scanned in the UV-region 400 - 200nm. Linezolid showed  $\lambda_{max}$  at 251nm (Fig.2)
3. Phosphate buffer pH 7.4: From the standard stock solution; 1 ml (100 µg/ml) was diluted with 10 ml solvent to obtain the concentration of 10 µg/ml. and this solution was scanned in the UV-region 400 - 200nm. Linezolid showed  $\lambda_{max}$  at 251nm (Fig.3)

#### B. Area under curve UV spectrophotometric method:

Determination of wavelength : From the standard stock solution; 1 ml (100 µg/ml) was diluted with 10 ml Phosphate buffer pH 7.4 to obtain the concentration of 10 µg/ml. and this solution was scanned in the UV-region 400 - 200nm. Linezolid shows maximum absorbance at 251nm. And the AUC of zero order spectrum was recorded between 245 – 268nm. (Fig.4)[11]

### Validation of The Method:

#### 1. Linearity:

Different aliquots of linezolid in range 3 – 18 ml were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with water to get concentrations 3,6,9,12,15, and 18 µg/ml, for method A and method B.

#### 2. Accuracy (% Recovery):

To the pre analyzed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80%, 100% and 120%. The solutions were reanalyzed by proposed method.[12]

#### 3. Precision:

Precision of the method was studied as intraday and inter-day variations. For method A and B Intra-day precision was determined by analyzing the 9,12 and 15 µg/ml of Linezolid solutions for three times in the same day. Inter-day precision was determined by analyzing the 12 , 15 and 18 µg/ml of Linezolid solutions daily for three days over the period of week.[13]

#### 4. LOD and LOQ (Sensitivity):

The sensitivity of measurements of Linezolid by the use of the proposed method was estimated in terms of the Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated using equation  $LOD = 3.3 \times N/B$  and  $LOQ = 10 \times N/B$ , where, 'N' is standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.[14]

#### 5. Repeatability:

Repeatability was determined by analyzing 5 µg/ml for method A, while in method B 8 µg/ml concentration of Linezolid solution for six times.

#### 6. Ruggedness:

Ruggedness of the proposed method is determined for 12 µg/ml concentration of by analysis of dilution from homogenous slot by two analysts using same operational and environmental conditions. For method A and B.

#### 7. Analysis Of Bulk Material :

From the standard stock solution of Linezolid, an appropriate volume 9 ml was diluted to 10 ml mark to obtain 9 µg/ml for method A , while in method B volume 12ml was diluted to 10 ml mark to obtain 12µg/ml the resulting solution was scanned in UV-spectrophotometer between 200-400 nm. The spectrum was recorded at 251nm.[15]

#### 8. Analysis Of Tablet Formulation:

For the analysis of commercial formulation, LIDOMAX-600 twenty tablets were weight; average 5weight was determined and crushed into fine powder. A quantity of drug equivalent to 10 mg of Linezolid was transferred into 100 mL of volumetric flasks containing 50 mL of Phosphate buffer pH7.4, shaken manually for 25 min and volume was

made up to the mark with same solvent and filtered through Whatmann filter paper no 41. An appropriate volume 9 mL was diluted to 10 mL mark to obtain 9µg/mL for method A, while in method B volume 12 ml was diluted to 10 ml mark to obtain 12µg/ml concentration. and scanned in UV-spectrophotometer between 200-400nm. the analysis reported for six times.[16]

**RESULT AND DISCUSSION**

**Determination of  $\lambda_{max}$  in different solvents or buffers:**

**1. Trial : Methanol**

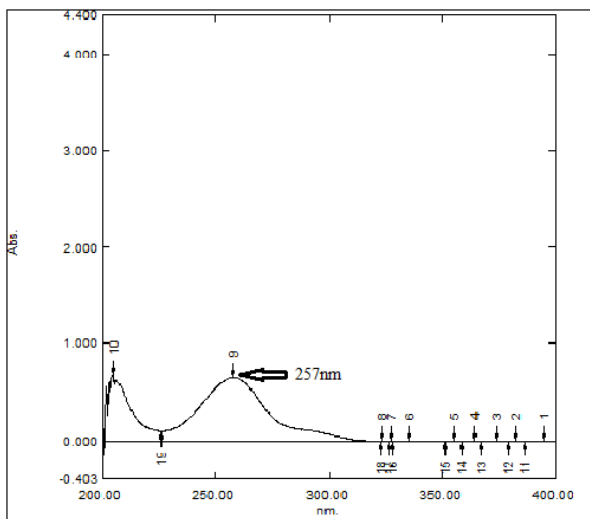


Figure 1 Linezolid scan in Methanol showing  $\lambda_{max}$  257nm.

**2. Trial : Phosphate buffer pH 6.4**

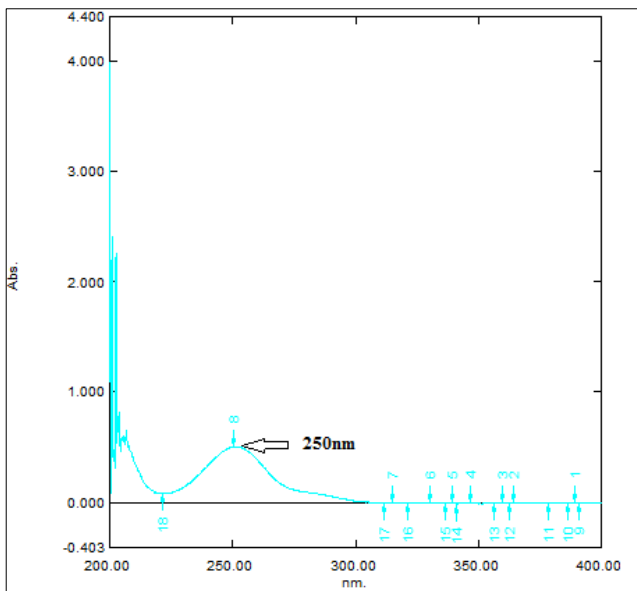


Figure 2 Linezolid scan in Phosphate buffer pH 7 showing  $\lambda_{max}$  250nm.

**3. Trial : Phosphate buffer pH 7.4**

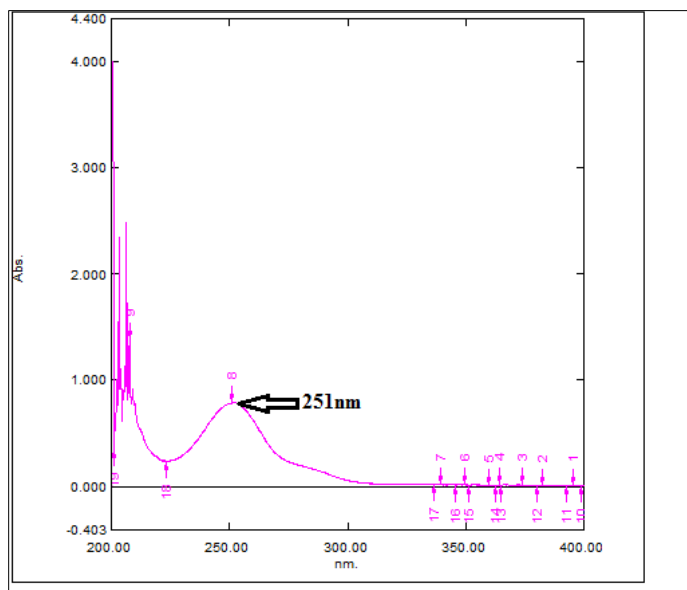


Figure 3 Linezolid scan in phosphate buffer pH 7.4 for determination of  $\lambda_{max}$  251nm

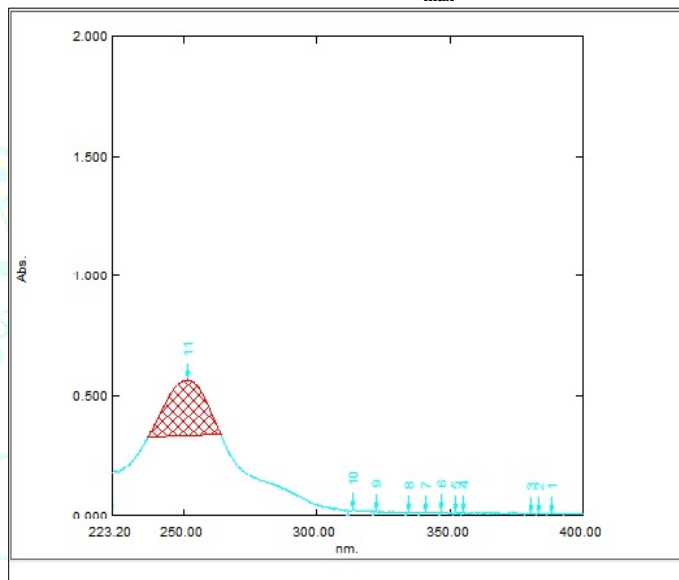


Figure 4 AUC Zero Order Spectrum of Linezolid showing absorbance maximum at 245-268 nm

**METHOD VALIDATION:**

Phosphate buffer pH 7.4 was selected for Method Validation of Linezolid by Zero order derivative and AUC Zero order Derivative.

**1. Linearity:**

The linear regression data for the calibration curves showed good linear relationship over the concentration range 3-18µg/ml for Linezolid (Fig.5,6). Linear regression equation was found to be  $Y=0.0543x + 0.0243$  ( $r^2 = 0.9978$ )  $Y=0.0543x + 0.0205$  ( $r^2 = 0.9981$ ) for method A and B .The result is expressed in Table 1.

**Table 1: Linearity studies of Linezolid**

Method A		Method B	
Conc. (µg/ml)	Absorbance	Conc. (µg/ml)	Absorbance, Mean ± SD
0	0	0	0
3	0.198	3	0.2043 ± 0.0012
6	0.357	6	0.3379 ± 0.0056
9	0.595	9	0.5299 ± 0.0039
12	0.674	12	0.668 ± 0.0089
15	0.846	15	0.8357 ± 0.0021
18	0.984	18	0.9901 ± 0.0060

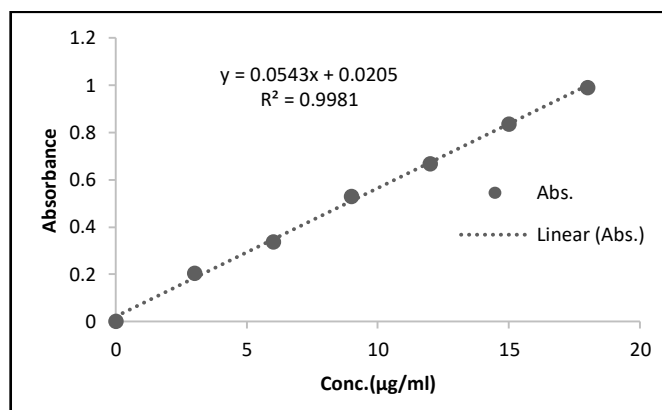


Figure 6 Linearity curve of Linezolid by AUC Zero order derivatives

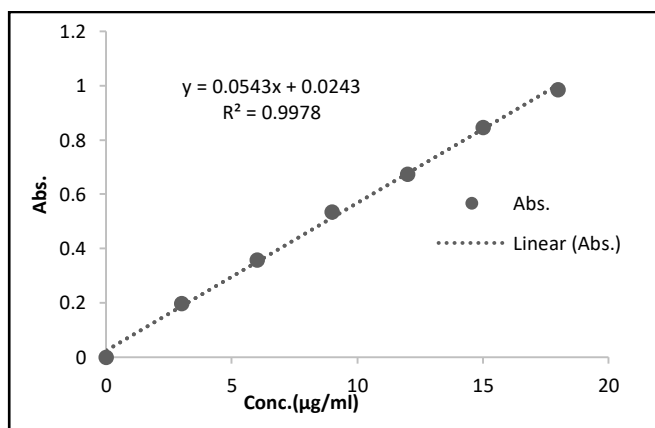


Figure 5 Linearity curve of Linezolid by Zero order derivatives

**2. Accuracy:**

The solutions were reanalyzed by proposed method; results of recovery studies are reported in Table 2, In method A, the mean % recovery was found to be in the range of 96.15 – 99.32 %, while in method B, it was found to be in the range of 99.16 – 100.20 %, for all three methods with %R.S.D.>2 indicate that the methods were precise.

**Table 2: Accuracy studies of Linezolid**

% value	Method A		Method B	
	% recovery ±SD	%RSD	% recovery ±SD	%RSD
80%	96.15 ± 0.69	0.72	99.16 ± 0.71	0.72
100%	99.05 ± 0.43	0.44	99.26 ± 1.22	1.24
120%	99.32 ± 0.53	0.56	100.20 ± 0.60	0.60

\*(n=3)

**3. PRECISION:**

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These result shows reproducibility of the assay. The % R.S.D. values found to be less than 2, so that indicate this method precise for the determination of both the drugs in formulation (Table 3)

**Table 3: Precision studies of Linezolid**

Methods	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		Amount found*	% RSD	Amount found*	% RSD
A	9	8.94	0.57	8.91	0.70
	12	11.97	0.84	11.96	0.93
	15	14.94	0.82	14.86	0.43
B	12	11.99	0.62	11.89	0.77
	15	14.91	0.69	14.88	0.49
	18	17.84	0.70	17.87	0.77

**4. SENSITIVITY:**

The linearity equation was found to be  $Y=0.1353x - 0.0558$  ( $r^2 = 0.9982$ ). The LOQ and LOD for Linezolid were found to

be 0.28 µg and 0.85 µg, for method A. while in method B linearity equation was found to be  $y = 0.1382x - 0.0812$  ( $r^2 = 0.9962$ ). The LOQ and LOD for Linezolid were found to be 0.48 µg and 1.47 µg respectively (Table 4)

**Table 4: Sensitivity studies of Linezolid**

Methods	Linear regression equation	LOD (µg/ml)	LOQ(µg/ml)
A	$y = 0.1353x - 0.0558$ ( $r^2 = 0.9982$ )	0.28	0.85
B	$y = 0.1382x - 0.0812$ ( $r^2 = 0.9962$ )	0.48	1.47

## 5. REPEATABILITY:

Repeatability was determined for selected concentrations of Linezolid solution for six times and the % amount determined with % RSD less than 2 for all the two methods. The results are expressed in Table 5.

**Table 5: Repeatability studies of Linezolid**

Methods	Amount taken ( $\mu\text{g/ml}$ )	Amount found*	% Amount found* $\pm$ SD	% RSD
A	5	4.91	98.35 $\pm$ 1.54	1.57
B	8	7.94	99.31 $\pm$ 1.30	1.32

## 6. Ruggedness

Peak area was measured for same concentration solutions, six times. The results are in the acceptable range for the drugs. The results are given in Table \*. The result showed that the % R.S.D. was less than 2 %.( Table 6)

**Table 6: Ruggedness studies of Linezolid**

Methods	Conc. ( $\mu\text{g/ml}$ )	Analyst I		Analyst II	
		% Amount found* $\pm$ SD	% RSD	% Amount found* $\pm$ SD	% RSD
A	12	99.24 $\pm$ 1.42	1.43	99.32 $\pm$ 0.92	0.94
B	12	99.45 $\pm$ 1.37	1.38	99.01 $\pm$ 1.22	1.23

## 7. Determination of Linezolid in bulk:

The concentrations of the drug were calculated from linear regression equations. The % amount found was between % to % (Table 7).

**Table 7: Analysis of Linezolid in bulk**

Methods	Amount taken ( $\mu\text{g/ml}$ )	Amount found*	% Amount found* $\pm$ SD	% RSD
A	9	8.72	96.98 $\pm$ 0.31	0.32
B	12	11.98	99.88 $\pm$ 0.93	0.93

## 8. Analysis of Linezolid in tablet formulation:

The spectrum was recorded at 251nm. The concentrations of the drug were calculated from linear regression equation. The % amount was found between % to % (Table 8).

**Table 8: Analysis of Linezolid in tablet formulation**

Methods	Amount taken ( $\mu\text{g/ml}$ )	Amount found*	% Amount found* $\pm$ SD	% RSD
A	9	8.66	96.25 $\pm$ 0.44	0.45
B	12	11.93	99.48 $\pm$ 1.05	1.06

## CONCLUSION

The spectrophotometric both developed methods are economical, simple, accurate, precise and rugged, and can be used for the usual study of Linezolid from its pharmaceutical formulations. The methods are developed for quantification on Linezolid in tablets. It is also used in routine quality control of the formulations containing Linezolid.

Parameter	Method A	Method B
Beer's-Lamberts's range( $\mu\text{g/ml}$ )	3 - 18 $\mu\text{g/ml}$	3 - 18 $\mu\text{g/ml}$
Regression equation ( $y=mx + c$ )	0.0543x + 0.0243	0.0543x + 0.0205
Correlation coefficient( $r^2$ )	0.9978	0.9981
Slope(m)	0.0543	0.0543
Intercept(C)	0.0243	0.0205
Repeatability(n=6)	98.35 $\pm$ 1.54	99.31 $\pm$ 1.30
Ruggedness : Analyst I	99.24 $\pm$ 1.42	99.45 $\pm$ 1.37
Analyst II	99.32 $\pm$ 0.92	99.01 $\pm$ 1.22
LOD( $\mu\text{g/ml}$ )	0.28	0.85
LOQ( $\mu\text{g/ml}$ )	0.48	1.47

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