



Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com



METHOD DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF LINEZOLID IN PURE AND TABLET DOSAGE FORM

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ABSTRACT

The present research work discusses the development of UV Spectroscopic method for the estimation of Linezolid. Simple, specific, accurate and cost effective spectroscopic method has been developed for the estimation of Linezolid in bulk as well as formulation. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ_{max}) was found to be 251nm. The validation was performed as per ICH guidelines for linearity, accuracy, precision, LOD and LOQ. The method shows high sensitivity with linearity in the range of 1-6 $\mu\text{g/ml}$ and shows a linear relationship between the absorbance and concentration with coefficient of correlation 0.999. The regression of curve was $Y = 0.0563x + 0.0211$. The precision of method was found to be good. The percentage recovery was found to be 99.47 ± 0.33 . The optimized showed good reproducibility and recovery with $RSD < 2\%$. The proposed method will be suitable for analysis of Linezolid in bulk as well as pharmaceutical formulations in quality control purpose. It is thus concluded that the proposed method is new, simple, cost effective, safe, accurate, precise and environmental friendly.

KEYWORDS

Linezolid, UV Spectroscopic method, Sensitive, Validation and ICH guidelines.

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INTRODUCTION

Linezolid (Figure No.1) is used an oxazolidinone antibiotic (anti-bacterial) which inhibits bacterial protein synthesis by acting at an early step and a site different from that of other Antimicrobial agents¹. Chemically it N{[(5S)-3-[3-fluoro-4-(morpholin-4-yl) phenyl] -2-oxo-1,3-oxazolidin-5yl] methyl] acetamide}². It is available as white crystalline powder which is freely soluble in distilled water, ethanol, chloroform, methanol and DMSO³. Fewer methods have been reported for the quantitative determination of Linezolid, which includes UV⁴⁻⁸,

HPLC⁹⁻¹², Electrometric method¹³, Chiral Chromatography¹⁴, HPTLC¹⁵, Microbiological assay¹⁶, bioanalytical method using LC^{17,18}. Several methods have been reported in literature for the determination of Linezolid in the presence of other drugs which includes UV¹⁹⁻²³, HPLC²⁴⁻²⁶, Fluorimetry²⁷. The aim of present work was to develop simple, sensitive, specific spectrophotometric method for detection of Linezolid in bulk as well as pharmaceutical formulation.

MATERIALS AND METHODS

Equipment and reagents

A Labindia model 3000+ double beam UV-Visible Spectrophotometer with two matched cuvette cells of one cm light path were used for the measurement of absorbance. The Linezolid bulk drug was kindly gifted by Hetero Labs Pvt. Ltd. Hyderabad. The pharmaceutical dosage form was procured from market. Distilled water was used for the study.

Preparation of standard stock solution

Accurately weighed 10 mg of Linezolid was transferred into 10ml volumetric flask volume was made up to 10ml with distilled water to get a concentration of 1000µg/ml and filtered through the Whatman filter paper no.41.

Determination of λ_{max}

From the stock solutions, 1.0ml of linezolid was transferred to 100 ml volumetric flask and the volume was adjusted to the mark with distilled water to obtain Strength 10µg/ml. The solution was scanned in the UV range 200-400 nm (Figure No.2).

Construction of calibration curve

Calibration curve was plotted against concentration and absorbance, regression equation was computed. The results tabulated in the Table No.1 and Figure No.3.

Preparation of Sample solution

Twenty tablets were weighed, average weight determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 10mg of Linezolid was transferred into 10ml volumetric flask containing 30ml distilled Water, shaken manually for 10 min., volume was adjusted to mark with same solvent and filtered through

Whatmann filter paper no.45. An appropriate aliquot was transferred to 10 ml volumetric flask, volume was adjusted to the mark and absorbance was recorded at 251nm (Table No.2).

METHOD VALIDATION

The Proposed method was validated as per the ICH Q2 (R1) guidelines for linearity, accuracy, precision, LOD and LOQ (Table No.3).

Accuracy

Accuracy was carried out at 80 %, 100 % and 120 % of target concentration. From the amount found, percentage recovery was calculated (Table No.4).

Precision

Precision of the method was studied by carrying out intraday, interday analysis and expressed as percentage Relative Standard Deviation. For this purpose 1 (LQC), 4 (MQC) and 6µg/ml (HQC) solutions were prepared and the absorbance's of the solutions were measured for six times within the same day and in different days at 251nm against blank (Table No.5).

Limit of Detection (LOD) and Limit of Quantization (LOQ)

LOD and LOQ of the drug were calculated using the following equations according to International Conference on Harmonization (ICH) guidelines

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where σ = the standard deviation of the response and

S = the slope of the regression equation.

RESULTS AND DISCUSSION

The proposed method for determination of Linezolid in marketed formulation (tablet) showed Sandell's sensitivity of 0.0121µg/cm²/0.001 absorbance units. Linear regression of absorbance on concentration gave the equation $y = 0.0563x + 0.0211$ with a regression co-efficient (R^2) of 0.999 (Figure No.3) and the linearity range was 1- 6µg/ml. The higher percentage recovery value (95-105%) indicates that there is no interference of the excipients present in the formulation. Thus the method is useful for the determination of Linezolid in bulk and pharmaceutical formulations.

Table No.1: Calibration of proposed method

S.No	Conc. (mcg / ml)	Absorbance at 251nm
1	1	0.082
2	2	0.129
3	3	0.187
4	4	0.248
5	5	0.304
6	6	0.359

Table No.2: Assay of Linezolid formulation

S.No	Formulation	Label claim (mg/tab)	Amount found (mg) (n=4) Mean ± SD	Assay	% RSD
1	Lizomac	600mg	592.8 ± 0.00115	98.80 %	1.402

Table No.3: Optimum conditions, Optical characteristics and Statistical data of the Regression equation in UV method

S.No	Parameter	UV method
1	λ_{max} (nm)	251
2	Beer's law limits (mcg / ml)	1-6
3	Sandell's sensitivity (mcg / cm ² -0.001 absorbance units)	0.0121
4	Regression equation (Y*)	y = 0.0563x + 0.0211
5	Slope (b)	0.0563
6	Intercept (a)	0.0211
7	Correlation coefficient(r ²)	0.999
8	% RSD**	< 2%
9	Limit of detection (mcg / ml)	0.603
10	Limit of quantitation (mcg / ml)	0.830

*Y=bX + a where X is the concentration of Linezolid in mcg/ml and Y is the absorbance at the respective λ_{max} .

**Average of six determinations

Table No.4: Determination of Accuracy results for Linezolid at 251nm

S.No	Brand name	Amount of sample (mcg/ml)	% of Spiked sample	Amount of drug added (mcg/ml)	Amount Recovered	% Recovery ± SD
1	Lizomac	2	80	1.6	3.508	97.44 ± 0.18
2	Lizomac	2	100	2	4.101	102.52 ± 0.54
3	Lizomac	2	120	2.4	4.332	98.45 ± 0.27

**Average of six determinations.

Table No.5: Determination of Precision results for Linezolid at 251 nm

S.No	Concentration mcg/ml	Inter-day Absorbance Mean ± SD **	% RSD	Intra-day Absorbance Mean ± SD **	% RSD
1	LQC (1mcg/ml)	0.084 ± 0.0015	1.817	0.082 ± 0.000577	0.704
2	MQC (4mcg/ml)	0.249 ± 0.001	0.401	0.248 ± 0.001154	0.465
3	HQC (6mcg/ml)	0.358 ± 0.0015	0.418	0.360 ± 0.000577	0.160

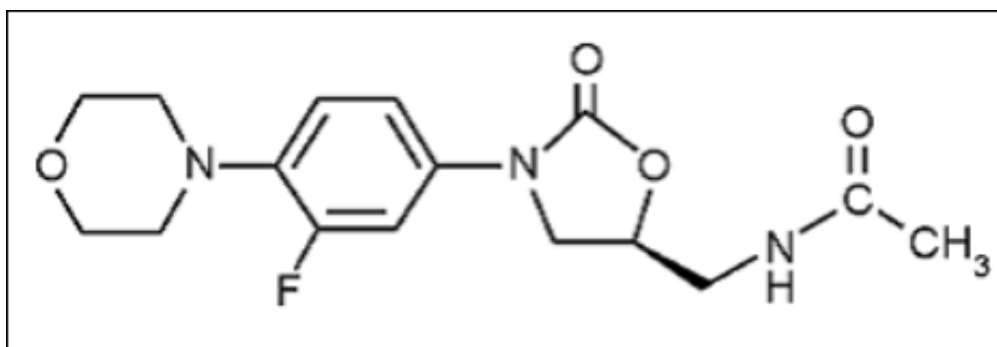


Figure No.1: Structure of Linezolid

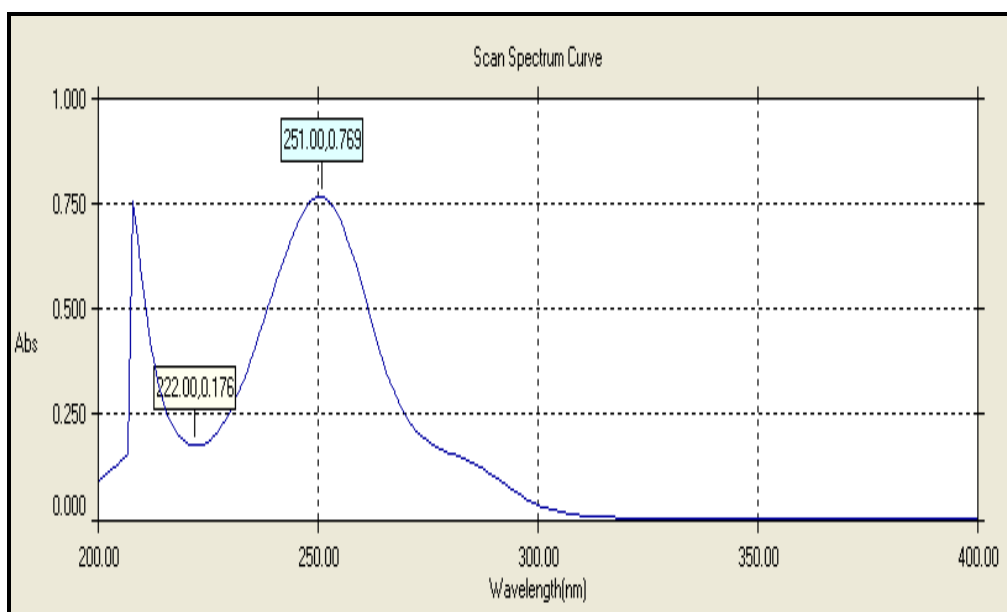


Figure No.2: Calibration curve of Linezolid at 251nm

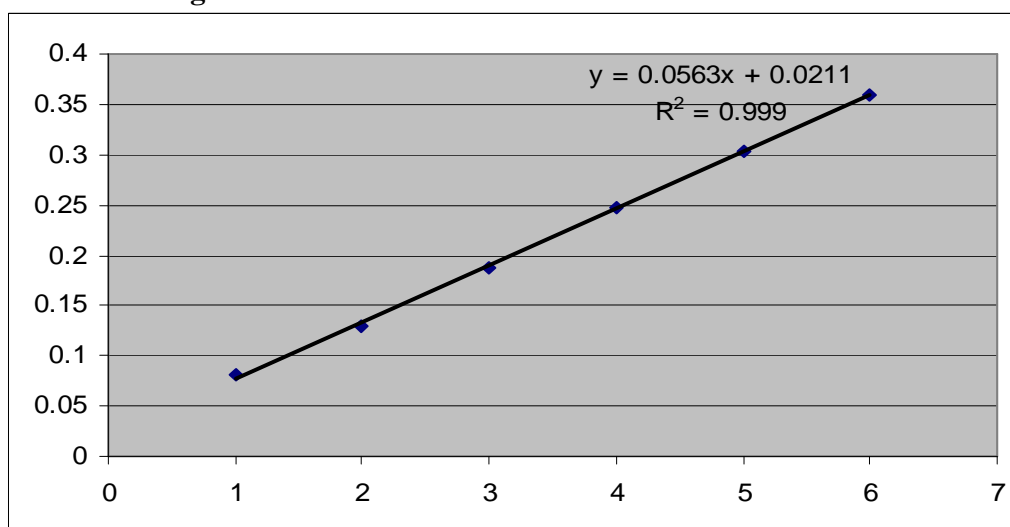


Figure No.3: Construction of calibration curve

CONCLUSION

A simple, sensitive, accurate and precise UV spectrophotometric method has been developed for quantitative determination of Linezolid in bulk and Pharmaceutical dosage form (tablet). The UV spectrum was scanned between 200 to 400 nm and 251 nm was selected as maximum wavelength for absorption. Beer's law was obeyed in the concentration range of 1-6µg/ml. % Recovery was calculated, was found to be 97.44 - 102.52 and the method was successfully applied to the pharmaceutical dosage form containing the Linezolid drug without any interference by the excipients. The method was fast and economical and it was also selective and sensitive for the desirable range. Results of the analysis were validated as per ICH guidelines and by recovery studies.

ACKNOWLEDGEMENT

We are highly thankful to Hetero Labs Pvt. Ltd. for providing the gift sample of Linezolid which made us for the fulfilment of this work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Sushma S and Pushpa Latha E. Method Development and Validation of Spectrophotometric Method for the Estimation of Linezolid in Pure and Tablet Dosage Form, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*, 3(2), 2015, 82 - 87.