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Original Article

DEVELOPMENT AND VALIDATION OF A SIMPLE AND COST-EFFECTIVE UV SPECTROPHOTOMETRIC METHOD FOR QUANTIFYING LINEZOLID

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ABSTRACT

Objective: This study focuses on the development and validation of a sensitive, simple, accurate, precise and cost-effective Ultraviolet-Visible (UV) spectrophotometric method for the quantification of Linezolid, a widely used antibiotic in pharmaceutical formulations.

Methods: The analysis utilized a solvent system comprising 80% water and 20% methanol (v/v). The absorbance of standard solutions was measured and a calibration curve was constructed. Various analytical performance parameters, including linearity, range, precision, accuracy, Limit of Detection (LOD), Limit of Quantification (LOQ) and ruggedness, were determined following the International Conference on Harmonization (ICH) Q2 (R1) guidelines.

Results: The maximum absorption peak (λ_{max}) of Linezolid was determined to be 251 nm in the selected medium. Beer-Lambert's law was valid in the concentration range of 0.5–9 µg/ml, with a high correlation coefficient (R^2) of 0.9955. The proposed method exhibited a recovery ranging from 99.08 to 100.37% with % Relative Standard Deviation (RSD) value consistently below 2%.

Conclusion: The study findings confirm the accuracy, precision and reproducibility of the developed method. Additionally, it is characterized by its simplicity, affordability, and time efficiency. Thus, this method can be effectively employed for the quantification of Linezolid in lipid nanoparticles.

Keywords: Linezolid, UV spectrophotometric method, Validation, Absorption maxima, Analytical method

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INTRODUCTION

Linezolid, a synthetic antibiotic belonging to the oxazolidinone class, has emerged as a critical component in the treatment of drug-resistant bacterial infections. Its effectiveness in combating a range of gram-positive pathogens, including Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-Resistant *Enterococcus faecium* (VRE), has positioned it as an indispensable weapon in the global fight against antimicrobial resistance. The drug also plays a pivotal role in the treatment of bacterial pneumonia, VRE infections and skin and soft tissue infections, which are among the most common bacterial infections encountered in clinical practice [1]. It occurs as a white crystalline powder and is chemically known as (S)-N-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (fig. 1).

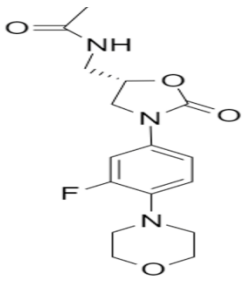

Fig. 1: Chemical structure of linezolid

Accurate quantification of Linezolid within lipid nanoparticles is a critical aspect of pharmaceutical development, quality control, and pharmacokinetic studies. Various analytical methods have been explored to quantify Linezolid, encompassing liquid chromatography [2, 3], ultraviolet spectroscopy [4], High-Performance Liquid Chromatography (HPLC) [5, 6], ultra-performance liquid chromatography (UPLC) [7], Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) [8, 9], Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) [10] and microbiological method [11]. However, these methods often involve complex sample preparation, costly instrumentation, and time-consuming procedures, which can limit their applicability in resource-constrained settings.

In this context, the development and validation of a simple, cost-effective, and reliable UV spectrophotometric method for the estimation of Linezolid presents a promising alternative. UV spectrophotometry is a well-established technique in pharmaceutical analysis, offering several advantages, including ease of use, minimal sample preparation, and reduced instrument costs. Such a method could address the need for efficient Linezolid quantification while maintaining accessibility for a wide range of healthcare facilities.

This research article discusses the development and validation of a UV spectrophotometric method for Linezolid estimation, which includes assessments of linearity, precision, accuracy, specificity, LOD and LOQ as per ICH Q2 (R1) guidelines [12, 13]. By offering a simpler and cost-effective analytical approach, this research contributes to the estimation of Linezolid in studies like entrapment efficiency, drug loading and *in vitro* drug release.

MATERIALS AND METHODS

Instrumentation

A Shimadzu UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) was utilized for all spectral measurements, employing one-centimetre-matched quartz cells. Additionally, the Shimadzu electronic balance (AUX 220, Shimadzu Corporation, Kyoto, Japan) was used for weighing all samples.

Materials

Linezolid was generously provided as a gift sample by Optrix Laboratories Private Ltd. (Telangana, India). Methanol was procured from Qualigens Fine Chemicals, Mumbai (India), All chemicals and reagents used were of analytical grade. For solution preparation, double distilled water was employed wherever necessary, and it was filtered through a 0.22 µm membrane filter before use.

Selection of absorption maxima for analysis of linezolid

A standard stock solution with a concentration of 10 µg/ml was prepared by dissolving 1 mg of Linezolid in 20 ml of 80:20 v/v water and methanol in a 100 ml volumetric flask through manual shaking. The volume was then adjusted with the same solvent up to the mark to reach the final concentration. The resulting solution was subjected to UV scanning in the range of 200–400 nm, revealing that Linezolid exhibited maximum absorbance at 251 nm.

Validation procedure

The method was validated according to ICH Guidelines in terms of linearity, range, accuracy, precision, LOD, LOQ, robustness and ruggedness [12, 13].

Linearity and range

According to ICH guideline Q2(R1), the linearity of an analytical procedure is defined as its ability, within a given range, to yield test results that are directly proportional to the concentration (amount) of analyte in the sample. The range of an analytical method is the interval between the upper and lower concentration of the analyte for which it has been demonstrated that the analytical procedure maintains a suitable level of precision, accuracy and linearity.

In our study, the standard solutions were prepared in the range of 0.5-9 µg/ml. The dilutions of the stock solution were prepared by diluting the required aliquot with the solvent system. The absorbance of each solution was measured at 251 nm using the same solvent system as the blank. A calibration curve was constructed by plotting concentration on the x-axis and absorbance on the y-axis and linearity was determined using a regression equation. This experiment was repeated 3 times.

The range is determined by verifying that the analytical procedure consistently maintains a satisfactory level of linearity, accuracy and precision when tested on samples containing the analyte amounts within or at the extremes of the specified range of the analytical procedure [12, 13].

Precision

The precision was assessed at two levels following the ICH, Q2 (R1) recommendations i.e. repeatability and intermediate precision [12].

The repeatability of the drug sample was evaluated through intraday variation involving the analysis of three concentrations with three replicates each, performed three times a day, totalling a minimum of nine determinations spanning the specified procedure's range. On the other hand, intermediate precision was determined by assessing interday variation over three different days for the quantification of Linezolid at three different concentration levels: 2, 5 and 8 µg/ml, each in triplicate. The % RSD for absorbance was calculated to determine both intraday and interday variation [13].

Accuracy

Accuracy is defined as the closeness of the test results obtained using the analytical method to the true value [14]. The method was further validated to assess its sensitivity in estimating Linezolid in the presence of excipients. The accuracy of the method was evaluated using the standard addition method. Pre-analyzed samples of Linezolid (4 µg/ml) were spiked with an additional 50%, 100% and 150 %, of the standard drug and the mixtures were analyzed using the proposed method. The experiment was conducted in triplicate. The % recovery and % relative standard deviation were calculated at each concentration level for each sample [12, 13].

Limit of detection and limit of quantitation

The LOD is the minimum analyte concentration that can be detected, though not necessarily precisely quantified. The LOQ represents the lowest analyte concentration that can be accurately and precisely quantified under the defined operational conditions of the method. The calculations for the LOD and LOQ of the drug were performed following equations according to ICH guidelines using the following equations:

$$\text{Limit of detection (LOD)} = 3.3 \times \frac{\sigma}{S}$$

$$\text{Limit of quantification (LOQ)} = 10 \times \frac{\sigma}{S}$$

Where σ = the standard deviation of the response; S = the slope of the regression line [13].

Robustness

The robustness of the UV analytical method was determined by analysing the 5 µg/ml Linezolid

solutions at different temperatures i.e. 25±10 °C and wavelengths (λmax) i.e. 251±2 nm [15].

Ruggedness

The ruggedness of the proposed method was assessed for a concentration of 5 µg/ml of Linezolid by analysing aliquots from a homogenous slot. Two analysts performed the analysis under identical operational and environmental conditions [16, 17].

RESULTS AND DISCUSSION

Wavelength of maximum absorption

This serves as a key parameter for subsequent analyses and method optimization. The wavelength of maximum absorption (λmax) was identified at 251 nm (fig. 2) in the selected medium. Furthermore, it was noted that there was no alteration in the λmax of the drug within this concentration range (0.5-9 µg/ml), as illustrated in (fig. 3) by overlaying the drug's spectra. Sapavadiya *et al.* reported λmax at 250 nm for the detection of Linezolid by RP-HPLC in the mobile phase of Potassium dihydrogen phosphate buffer (pH 4.6) and methanol in the ratio of 55:45 (%v/v) [18].

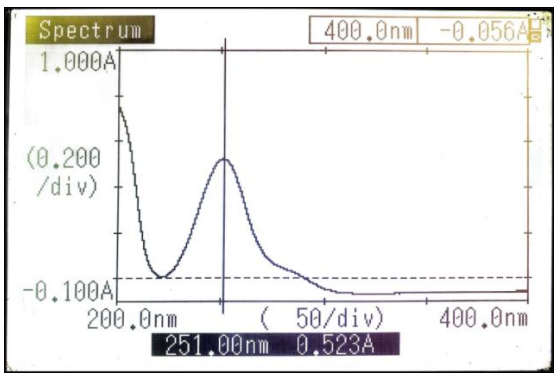

Fig. 2: UV Absorption spectra of Linezolid at 251 nm

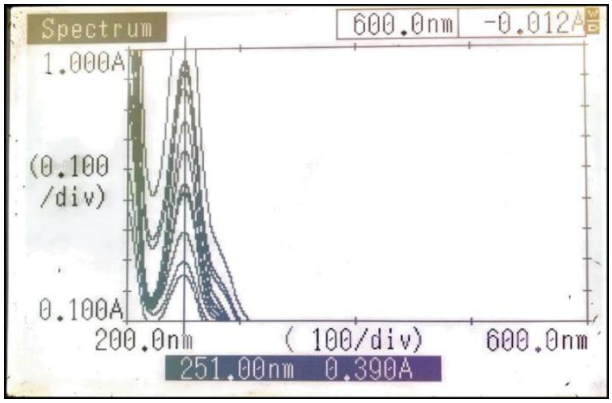

Fig. 3: Overlay UV spectra of linezolid in 80:20 v/v water and methanol showing maximum absorbance at 251 nm

A calibration curve was constructed within the concentration range of 0.5-9 µg/ml by plotting concentration on the X-axis and absorbance on the Y-axis. The data for the calibration curve is presented in (table 1), while the curve is depicted in (fig. 4).

Table 1: Calibration curve data for linezolid

Concentration (µg/ml)	Mean absorbance at 251 nm	% RSD	Regressed absorbance	Equation of line
0.5	0.067±0.0020	1.493	0.0989	y = 0.1034x+0.0472
1	0.182±0.0057	1.583	0.1506	Correlation
2	0.255±0.0035	1.379	0.254	coefficient
3	0.389±0.0025	0.647	0.3574	R^2 = 0.9955
4	0.441±0.0032	0.728	0.4608	Slope
5	0.554±0.0036	0.651	0.5642	m = 0.1034
6	0.657±0.0042	0.634	0.6676	Intercept
7	0.765±0.0025	0.329	0.771	c = 0.0472
8	0.883±0.0042	0.471	0.8744	
9	0.984±0.0035	0.357	0.9778	

*The data is expressed as mean±SD, n=3.

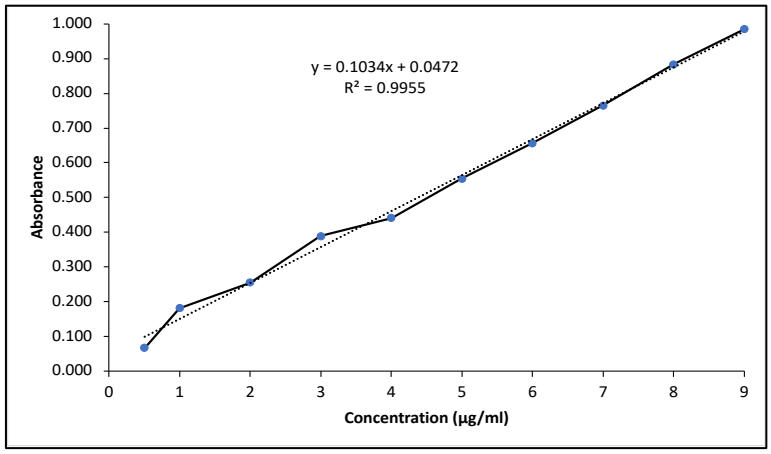

Fig. 4: Calibration curve of linezolid

Method validation

Linearity and range

Linearity refers to the ability of the analytical method to produce test results that are directly proportional to the concentration of the analyte in the sample across a specified range. The linearity and range of the UV method were assessed by constructing a calibration curve using standard solutions of the analyte at concentrations ranging from 0.5 to 9 µg/ml. The absorbance was determined in triplicate, and the mean absorbance range (n=3) was found to be 0.067-0.984, with RSD values below 2 %, as shown in table 1. The calibration curve exhibited a linear relationship within the concentration range of 0.5-9 µg/ml, with a correlation coefficient (R^2) of 0.9955. This value closely aligns with those determined by Naik and Pai (2013) and Nagaraju *et al.*, (2014) i.e. 0.991 and 0.997, respectively [19, 4]. The linear regression equation was determined to be y = 0.1034x+0.0472 [13].

Precision

The precision of the UV method was evaluated to ascertain both its repeatability and intermediate precision. The assessment involved

analysing Linezolid at three different concentration levels 2, 5 and 8 µg/ml of Linezolid in triplicate. The results of repeatability (intraday precision) and intermediate (interday) precision were expressed in terms of % RSD. The study of intraday and interday precision for the developed method confirmed adequate sample stability and method reliability, as all %RSD values were below 2%, as depicted in table 2. The results indicate a high level of consistency and reliability in the UV method for Linezolid analysis. The low % RSD values obtained for both intraday and interday precision demonstrate minimal variability in measurements. This suggests that the method is capable of producing reliable and reproducible results over time, thereby enhancing confidence in the analytical findings.

Table 2: Precision of the proposed method

Concentration (µg/ml)	Intraday precision		Concentration (µg/ml)	Day	Interday precision	
	Mean absorbance*	% RSD			Mean absorbance*	% RSD
2	0.254±0.0026	1.042	2	1	0.256±0.0021	0.814
				2	0.253±0.0021	0.824
				3	0.256±0.0015	0.597
5	0.552±0.0020	0.377	5	1	0.553±0.0015	0.276
				2	0.556±0.0020	0.360
				3	0.554±0.0032	0.581
8	0.883±0.0015	0.173	8	1	0.884±0.0021	0.235
				2	0.884±0.0051	0.173
				3	0.887±0.0020	0.225

*The data is expressed as mean±SD, n=3.

Accuracy

The standard addition method comprised introducing the drug at concentrations of 2 µg/ml (50%), 4 µg/ml (100%), and 6 µg/ml (150%) into a sample solution containing 4 µg/ml. The proposed method demonstrated a recovery ranging from 99.08 to 100.37%, with % RSD value consistently below 2%. This was observed when a standard drug solution was added to the previously analysed test solution. The corresponding values for percentage recoveries and % RSDs are detailed in table 3. The ability to accurately recover known concentrations of the drug from the sample solution reinforces confidence in the method's accuracy and suitability [20].

Table 3: Accuracy as recovery of the proposed method

% of standard spiked to the sample	Sample concentration (µg/ml)	Amount (µg)		% drug recovered	% RSD
		Total including spiked sample	Spiked sample determined*		
50	4	6	5.95±0.07	99.22	1.12
100	4	8	7.93±0.09	99.08	1.09
150	4	10	10.04±0.05	100.37	0.50

*The data is expressed as mean±SD, n=3.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ of this method were determined by the standard deviation of the response and the slope of the calibration curve. The values of LOD and LOQ were found to be 0.417 µg/ml and 1.263 µg/ml, respectively. These values indicate the sensitivity of the method and the lowest concentration of Linezolid that can be reliably detected and quantified with acceptable precision and accuracy [16]. Notably, similar findings for LOD and LOQ were reported by Nagaraju *et al.* [4].

Robustness

The robustness of the UV method was assessed by intentionally varying wavelength and environmental temperature conditions and evaluating their effect on the analytical results. The absorbance of 5 µg/ml sample solution was measured at 251±2 nm wavelengths. Results (table 4) showed that % RSD remained within acceptable limits, indicating the method's robustness against wavelength variations. Similarly, % RSD values were found to be within acceptable limits, demonstrating the method's resilience to temperature fluctuations.

Table 4: Robustness studies of the method

Condition	Parameter	Absorbance	Mean	SD	%RSD
Change in wavelength	249 nm	0.551	0.553	0.0015	0.276
	251 nm	0.553			
	253 nm	0.554			
Change in temperature	15 °C	0.551	0.554	0.0025	0.455
	25 °C	0.554			
	35 °C	0.556			

n=3.

The results of the robustness evaluation suggest that the UV method is robust and reliable, showing minimal impact on the analytical results under variations in the tested parameters. This underscores the method's suitability for routine analysis in practical applications [15]. Similarly, robustness was also established for the method developed for the quantitative estimation of Nefopam hydrochloride [21].

Ruggedness

The method's ruggedness was evaluated by analysing it with two analysts. The absorbance was measured for the same concentration solution of 5 µg/ml six times. The results as shown in table 5, fell within the acceptable range, with a % RSD of less than 2% [16, 17]. The result suggests that the UV method demonstrates good ruggedness, indicating its reliability when implemented by different analysts [21].

Table 5: Ruggedness studies by two analysts

	Analyst I	Analyst II
Concentration	0.554	0.553
5 µg/ml	0.553	0.554
	0.554	0.551
	0.552	0.554
	0.554	0.552
	0.554	0.553
Mean Absorbance	0.554	0.553
SD	0.00084	0.00107
% RSD	0.151	0.193

n=6.

CONCLUSION

In this study, we have successfully developed and rigorously validated a UV spectrophotometric method for the quantitative analysis of Linezolid in pharmaceutical formulations. The method offers practical advantages such as simplicity, accuracy, cost-effectiveness, and rapid analysis time. Consequently, it will prove to be well-suited for the quantitative analysis of Linezolid in lipid nanoparticle formulations. Importantly, it demonstrates the ability to operate without interference from common excipients and related compounds, rendering it suitable for routine testing. The successful validation according to international guidelines ensures its suitability for regulatory compliance and routine pharmaceutical analysis applications.

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Nil

AUTHORS CONTRIBUTIONS

Iti Chauhan: Conceptualization, Investigation, Data Analysis, Writing-original Draft

Lubhan Singh: Supervision, Data Analysis, Writing-reviewing and editing

CONFLICT OF INTERESTS

The author declares no conflict of interest, financial or otherwise.

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