Development and Validation of UV Spectrophotometric Method for the Estimation of Linezolid

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Abstract: *The objective of this study was to develop and validate a sensitive, simple, accurate, precise and cost-effective UV spectrophotometric method for the quantification of Linezolid (LNZ) in pharmaceutical formulations of lipid nanoparticles. Various analytical performance parameters, including linearity, range, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were determined in accordance with the International Conference on Harmonization (ICH) Q2 (R1) guidelines. The analysis was carried out using a mixture of 80% water and 20% methanol (v/v). The maximum absorption peak (λ_{max}) of LNZ was found to be 251 nm in the selected medium. Beer-Lambert's law was valid in the concentration range of 0.5–10 µg/ml, with a high correlation coefficient (R^2) of 0.9955. The study results affirm that the developed procedure is both accurate and precise, with reproducible outcomes. Additionally, it is characterized by its simplicity, affordability, and time efficiency. Consequently, this method can be effectively employed for the quantification of LNZ in prepared lipid nanoparticles.*

Keywords: Linezolid**,** UV spectrophotometric method, validation, *absorption* maxima

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1. 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.

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 [8, 9], 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, limit of detection (LOD) and limit of quantification (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.

2. Materials and Methods

2.1. Instruments

A Shimadzu UV–Visible spectrophotometer (UV -1800, Shimadzu Corporation, Kyoto, Japan) was used for all absorbance measurements with one cm matched quartz cells and Shimadzu electronic balance (AUX 220, Shimadzu Corporation, Kyoto, Japan) was used for weighing all samples.

2.2. Materials

Linezolid was obtained as a gift sample from Optrix Laboratories Private Ltd. (Telangana, India). Methanol was purchased from Qualigens Fine Chemicals, Mumbai (India), All chemicals and reagents used were of analytical grade. Double distilled water was used to prepare solutions wherever required and it was filtered before use through a 0.22 μm membrane filter.

2.3. Determination of absorption maxima

A standard stock solution of strength 10 µg/ml was prepared by dissolving 1 mg of LNZ in 20 ml of 80:20 v/v water and methanol in a 100 ml volumetric flask by manual shaking. The volume was adjusted with the same up to the mark to reach the final strength. The resulting solution was scanned in the UV range (200–400 nm). In the spectrum, LNZ showed maximum absorbance at 251 nm.

2.4. Validation Procedure

The method was validated according to ICH Guidelines in terms of linearity, range, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ) [12].

2.4.1. Linearity and range: As per 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. To create standard solutions in the range of 0.5 - 10 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.

2.4.2. Precision: The precision was assessed at two levels in accordance with 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 LNZ at three different concentration levels: 2, 5 and 8 µg/ml, each in triplicate. The % relative standard deviation (RSD) for absorbance was calculated to determine both intraday and interday variation.

2.4.3. 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 LNZ in the presence of excipients. The accuracy of the method was evaluated using the standard addition method. Pre-analysed samples of LNZ (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].

2.4.4. Limit of detection and limit of quantitation: The limit of detection (LOD) is the minimum analyte concentration that can be detected, though not necessarily precisely quantified. The limit of quantitation (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:

$$LOD = 3.3 \times \sigma/S \quad \text{.....(1)}$$

$$LOQ = 10 \times \sigma/S \quad \text{.....(2)}$$

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

3. Results and Discussion

3.1. Wavelength of maximum absorption

The wavelength of maximum absorption (λ_{max}) was determined to be 251 nm (Figure 1a) in the chosen medium. Furthermore, it was noted that there was no alteration in the λmax of the drug within this concentration range (0.5-10 µg/ml), as illustrated in Figure 1(b) by overlaying the drug's spectra.

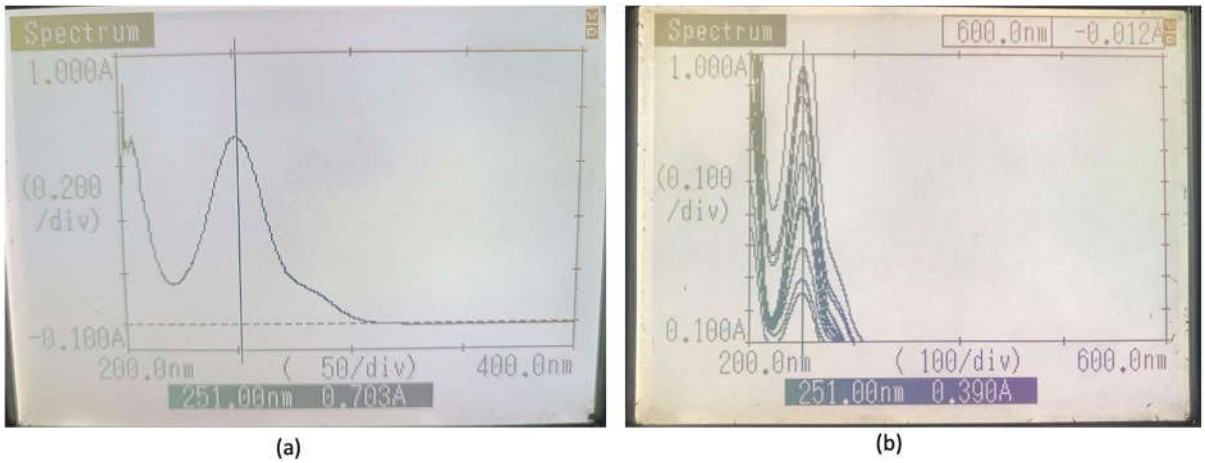

Figure 1(a) Absorption spectra of LNZ (b) Overlay UV spectra of LNZ in 80:20 v/v water and methanol showing maximum absorbance at 247.5nm

A calibration curve was constructed within the concentration range of 0.5-10 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 Figure 3.

Table 1. Calibration curve data for LNZ

Concentration (µg/ml)	Mean Absorbance at 251 nm ± SD (n = 3)	% RSD	Regressed Absorbance	Equation of Line

0.5	0.067±0.002	1.492537313	0.0989	y = 0.1034x + 0.0472 Correlation coefficient $R^2 = 0.9955$ Slope m = 0.1034 Intercept c = 0.0472
1	0.182±0.0057	1.583227429	0.1506	
2	0.255±0.0035	1.379012271	0.254	
3	0.389±0.0025	0.647498665	0.3574	
4	0.441±0.0032	0.728372414	0.4608	
5	0.554±0.0036	0.65082153	0.5642	
6	0.657±0.0042	0.634009949	0.6676	
7	0.765±0.0025	0.329112225	0.771	
8	0.883±0.0042	0.471320604	0.8744	
9	0.984±0.0035	0.357019782	0.9778	

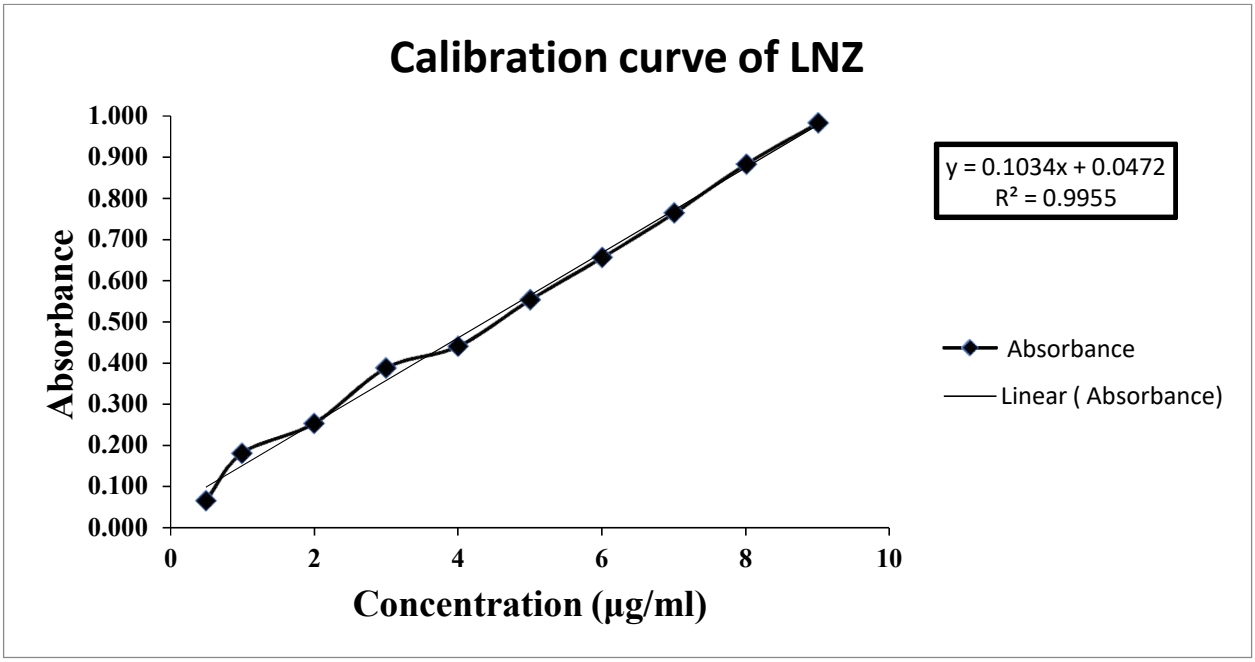

Figure 2. Calibration curve of LNZ

3.2. Method validation

3.2.1. Linearity and Range: The absorbance of the prepared dilutions (0.5-10 µg/ml) was determined in triplicate and the mean absorbance range (n=3) was found to be 0.103-0.846 with RSD values below 2 % as shown in Table 1. The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the samples in the concentration range of 0.5 -10 µg/ml was linear with a correlation coefficient (R^2) of 0.9955. The linear regression equation was found to be Y = 0.1034X + 0.0472.

3.2.2. Precision: The precision was assessed by analyzing LNZ at three different concentration levels 2, 5 and 8 µg/ml of LNZ in triplicate. The results of repeatability **(**intraday precision) and intermediate (interday) precision were expressed in terms of % RSD. The intraday and interday precision study of the developed method confirmed adequate sample stability and method reliability where all RSD were below 2% as shown in Table 2.

Table 2. Precision of the proposed method

Concentration (µg /ml)	Intraday precision		Concentration (µg /ml)	Day	Interday Precision	
	Mean absorbance ± SD	% RSD			Mean absorbance ± SD	% 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

3.2.3. Accuracy: The standard addition method involved the addition of drug at concentrations of 2 µg/ml (50%), 4 µg/ml (100%), and 6 µg/ml (150%) in a sample solution of 4 µg/ml. The proposed method displayed a recovery of 99.08-100.37% (% RSD < 2)when a standard drug solution was added to the previously analyzed test solution. The values for percentage recoveries and relative standard deviations (% RSDs) are presented in Table 3.

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 ± SD (n = 3)		
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

3.2.4. Limit of detection (LOD) and limit of quantitation (LOQ): LOD and LOQ of this method were determined by the standard deviation method. The values of LOD and LOQ were found to be 0.417 and 1.263 µg/ml respectively.

Table 4 summarizes all the UV spectrophotometric parameters of LNZ.

Table 4. UV spectrophotometric parameters of Linezolid

S. No.	Parameters	Results
1.	Absorption maxima (nm)	251
2.	Linearity range (µg/ml)	0.5-10
3.	Regression equation	y = 0.1034x + 0.0472
4.	Slope	0.1034
5.	Intercept	0.0472
6.	Correlation coefficient (R^2)	0.9955
7.	Recovery (%)	99.08-100.37
8.	LOD (µg/ml)	0.417
9.	LOQ (µg/ml)	1.263

Conclusion

The outcomes and statistical values indicate that the suggested UV spectrophotometric technique is straightforward, quick, precise, and specific. Consequently, it is suitable for the quantitative analysis of Linezolid in lipid nanoparticle formulations without any interference from common excipients and related compounds, making it suitable for routine testing.

Conflict of Interest

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

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