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Research Article

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New Derivative Spectrophotometric Methods for the Determination of Linezolid - An Antibacterial Drug

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ABSTRACT

Linezolid is an antibacterial used for the treatment of serious infections caused by Gram-positive bacteria that are resistant to several other antibiotics. Three simple, rapid and sensitive zero, first and second derivative spectrophotometric methods are developed for the determination of Linezolid in pharmaceutical dosage forms. The absorption maxima was found to be at 250 nm in Method A (D_0) and shows linearity over the concentration range of 0.1-75 µg ml^{-1} with regression equation 0.0516x + 0.0013 (r^2 = 0.999). In Method B (D_1) the amplitude was chosen (240 -262 nm) and in Method C (D_2) the minima (251 nm) was chosen for spectral calculations. Linezolid follows Beer-Lambert's law over the concentration range of 0.1-60 µg ml^{-1} (r^2= 0.999) in first order (D_1) and 0.1-75 µg ml^{-1} (r^2= 0.999) in second order derivative spectroscopy respectively. The proposed methods can be successfully applied for the determination of Linezolid in pharmaceutical formulations and validated according to ICH guidelines.

Keywords: Linezolid, derivative spectroscopy, validation, ICH.

INTRODUCTION

Linezolide [1] (LNZ) chemically, (s)-N-[[3-[3-fluoro-4(4-morpholinyl) phenyl]-2-oxo-5-azolidinyl] methyl] acetamide (Fig 1) with molecular formula $C_{16}H_{20}FN_3O_4$ and molecular weight 337.346g/mol was the first oxazolidinone to be developed and approved for clinical use. Linezolid is a synthetic antibiotic used for the treatment of serious infections caused by Gram-positive bacteria that are resistant to several other antibiotics [2-3]. Linezolid is active against most Gram-positive bacteria that cause diseases including streptococci, vancomycin-resistant enterococci (VRE), and methicillin-resistant Staphylococcus aureus (RSA) [4-7]. The main indication of linezolid is the treatment of severe infections caused by Gram-positive bacteria that are resistant to other antibiotics; it should not be used against bacteria that are sensitive to drugs with a narrower spectrum of activity, such as penicillins and cephalosporins.

A literature review revealed that the HPLC method has been the technique of choice for the separation and determination of linezolid in biological fluids. Thus far, several HPLC methods have been described to analyze linezolid in various body fluids including LC=MS=MS, microbore LC=ESI-MS=MS, HPLC with electrospray tandem mass spectrometry, LC methods using ultraviolet (UV) detection [8-14], and fluorescence detection [15]. For the assays in the pharmaceutical dosage forms, the methods reported in literature are HPLC [16-19], capillary electrophoresis [20] and HPTLC [21].

In this study simple, rapid, precise, and accurate spectrophotometric methods have been developed for the determination of Linezolid in pharmaceutical dosage form and validated as per the ICH guideline [22-23].

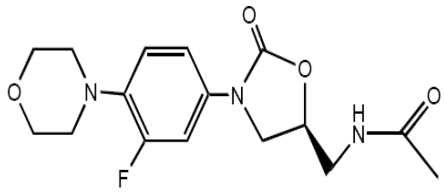

Fig 1: Chemical Structure of Linezolid

EXPERIMENTAL SECTION

Instrumentation

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Denver, Germany).

Chemicals and reagents

Analytical grade Formic acid (Merck) was purchased. Linezolid (amorphous form) was obtained as gift sample from Glenmark pharmaceuticals (India) was used as such without further purification.

Preparation of Stock and sample Solution

The standard solution of Linezolid was prepared by dissolving accurately about 25 mg of the Linezolid with Methanol in a 25 ml volumetric flask. The stock solution was further diluted with 0.05% formic acid to obtain required (0.1-75 µg ml^{-1}) sample solutions.

Procedure:

Zero-Order Derivative spectrometry (Method A)

The drug solution was scanned (200-400 nm) against reagent blank and the absorption spectrum (Fig 2) was recorded. The absorption maximum (λmax) was observed at 250 nm and the absorbance of all the sample solutions (0.1-75 µg ml^{-1}) was recorded at that λ_{max}. A graph was plotted by taking the concentration of the solutions on the x-axis and the corresponding absorbance values on the y-axis.

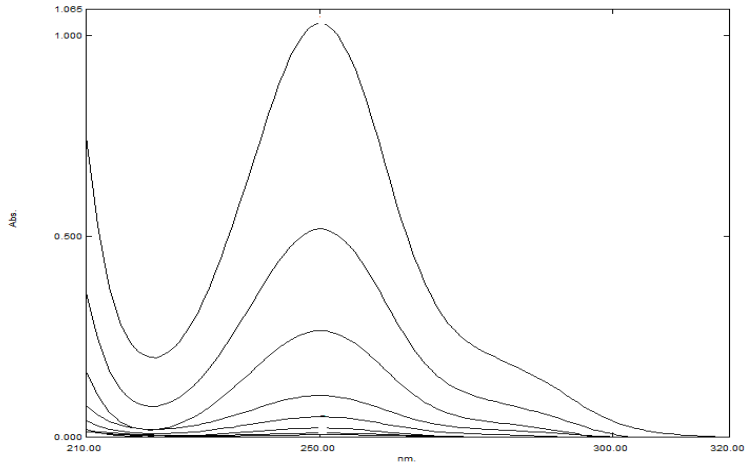

Fig 2: **Overlay Absorption Spectrum of Linezolid (D_0)**

First-Order Derivative spectrometry (Method B)

The drug solution was scanned (200-400 nm) against reagent blank and the absorption spectrum was recorded. This spectrum was derivatised to get first order derivative spectra (Fig 3) and the zero crossing point was found to be at 250 nm. The minima and maxima were observed at 262 and 240 nm in the spectrum and therefore the amplitude was chosen to record the derivative absorbance of the sample solutions (0.1-60 µg ml^{-1}). A graph was plotted by taking the concentration of the solutions on the x-axis and the corresponding amplitude values on the y-axis.

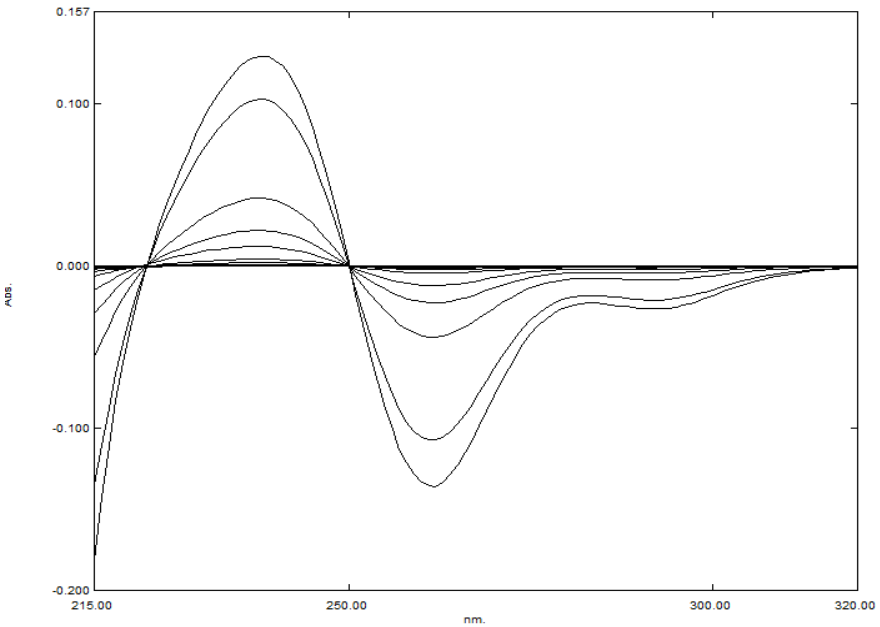

Fig 3: First order derivative overlay spectrum (D_1) of Linezolid

Second-Order Derivative spectrometry (Method C)

The Linezolid drug solution was scanned (200-400 nm) against reagent blank and the absorption spectrum was recorded and was derivatised to get second order derivative spectra (Fig 4). This spectrum shows minima at 251 nm and was chosen for the analytical determinations.

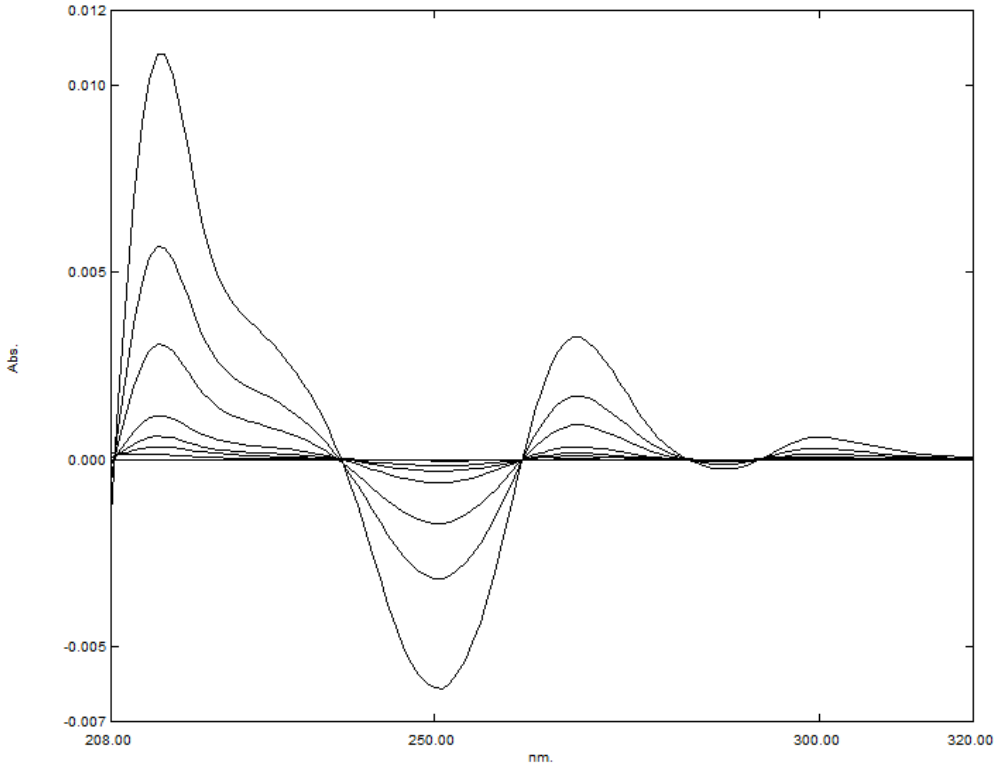

Fig 4: Second order derivative overlay spectrum (D_2) of Linezolid

A series of solutions (1-75 µg ml^{-1}) were prepared, scanned against reagent blank and their minima values were recorded. A graph was plotted by taking the concentration on the x-axis and the corresponding minima values on y-axis.

Assay procedure for the commercial formulations (Tablets)
Linezolid (600 mg of Linezolid per tablet) is available in the local market with brand names Zyvox (Glenmark) and Lizoforce (Microcryson) and were purchased for the study.

20 tablets were collected from two different brands and LNZ equivalent to 25 mg was weighed extracted with methanol separately, sonicated and make up to volume with methanol in three different 25 ml volumetric flasks (1 mg/ml) and filtered. The dilutions were made from this stock with 0.05% formic acid as per the requirement.

A series of solutions (0.1–75.0 µg/ml for Method A and C; 0.1–60.0 µg/ml for Method B) were prepared, scanned and the corresponding values were recorded and calibration curves were drawn. A straight line was obtained and the results obtained were shown in Table 1.

Table: 1 Assay of commercial formulations

Brand	Labelled Amount (mg)	*Amount obtained (mg)			% Recovery			% RSD*		
		Method			Method			Method		
		A	B	C	A	B	C	A	B	C
I	600	599.91	599.89	599.83	99.99	99.98	99.97	0.86	0.65	0.76
II	600	599.73	599.86	599.78	99.96	99.95	99.96	0.98	0.87	0.93

**Each value is average of three determinations*

Precision and Accuracy
The precision study was done as per the ICH guidelines by recording the response of six replicates in Method A and B (20µg/ml) and Method C (20µg/ml) and the % RSD was calculated.

Accuracy was evaluated as per the ICH guidelines by the percent recovery studies by the addition of 80%, 100%, and 120% of pure sample solution to the pre-analysed formulation solution. For the present study 20 µg/ml of LNZ solution extracted from the formulation was taken and 80%, 100%, and 120% of pure sample solution (i.e. 16, 20 and 24 µg/ml) were added and the % RSD was calculated.

RESULTS AND DISCUSSION

Beer's law was obeyed in the concentration range of 0.1–75.0 µg/ml for both the methods A and C and 0.1–60.0 µg/ml for method B. The linear regression equations were found to be y = 0.0516x + 0.0013, y = 0.0043x-0.0002 and y = 0.0003x-0.00001 for method A, B and C with correlation coefficient 0.999.

The % RSD values in precision studies were found to be 0.4354, 0.5465 and 0.7658 for method A, B and C which are less than 1% indicating that the method is more precise. The % RSD values in accuracy studies were found to be 0.8192, 0.9143 and 0.8769 for method A, B and C which are less than 1% indicating that the method is more accurate.

CONCLUSION

The present methods can be employed for the estimation of Linezolid in pharmaceutical formulations successfully. The percentage of purity was found to be 99.95-99.99.

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