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UV-Spetrophotometric Method Development and Validation for Determination of Linezolid in Pharmaceutical Dosage Form.

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Research Article

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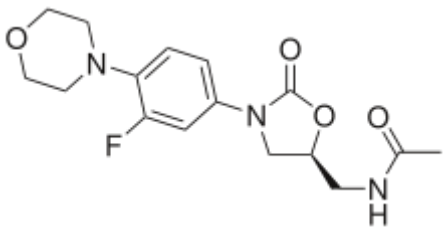
ABSTRACT

A simple, precise and economical second order derivative method has been developed for the estimation of Linezolid in bulk and pharmaceutical formulations. In this method Linezolid showed sharp peak at 252 nm when n= 1 and linearity was measured at 252 nm. It obeyed Beer's law in the concentration range of 1-6 mcg/ml. The LOD and LOQ were found to be 0.36 mcg/ml and 1.11 mcg/ml respectively. A recovery of Linezolid in tablet formulation was observed in the range of 98.30-101.09%. The proposed method is precise, accurate and reproducible and can be extended to the analysis of Linezolid in bulk and pharmaceutical formulations.

INTRODUCTION

Linezolid[1] is a synthetic antibiotic, the first of the oxazolidinone class, used for the treatment of infections caused by multi-resistant bacteria including streptococcus and methicillin-resistant Staphylococcus aureus (MRSA). The drug works by inhibiting the initiation of bacterial protein synthesis.Chemical name of Linezoild is N-{[(5S)-3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl]methyl}acetamide. It has a molecular formula of $C_{16}H_{20}FN_3O_4$ and a molecular weight of 337.346 g/mol.



Linezolid

Literature survey reveals that several analytical methods have been reported for the estimation of Linezolid by UV[2], LC-UV[3] , HPLC-UV[4,5], RP-HPLC[6,7,8,9] and HPTLC[10] methods. Apart from above one spectroscopic methods such as UV/Vis, difference spectrophotometric method, RP-HPLC etc., 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 and the statiscal analysis proved that method is reproducible and selective for the analysis of Linezolid in bulk drug and tablet formulations.

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EXPERIMENTAL

Instruments and reagents

A Elico-210 UV/VIS spectrophotometer was used with 1 cm matched quartz cell. All the chemicals used were of analytical grade. Hydrochloric Acid was procured from Loba Chem. Ltd., Mumbai. An analytically pure sample of Linezolid was obtained from Hetero Drugs Limited as a gift sample. Tablet of 600 mg were procured from local pharmacy.

Preparation of working standard drug solution

The standard Linezolid (100 mg) was weighed accurately and transferred to volumetric flask (100 ml). It was dissolved properly and diluted up to the mark with 0.05N Hcl to obtain final concentration of 1000 mcg/ml and the resulting solution was used as working standard solution.

Analysis of marketed formulations

For the estimation of Linezolid in tablets formulations by this method. 5 branded tablets were weighed and triturate to fine powder. Tablet powder equivalent to 10 mg of Linezolid was weighed and transfer into 100 ml volumetric flask than dissolved with 0.05N Hcl and further diluted with 0.05N Hcl. It was kept for ultrasonication for 30 min; this was filtered through Whatman filter paper No. 41 and then final dilution was made with 0.05N Hcl to get the final stock solution of 100 mcg/ml. From this stock solution, various dilutions of the sample solution were prepared and analysed.

Spectroscopic method

The spectra showed sharp peak at 252 nm when n=1 and linearity was measured at 252 nm (Fig 1). The absorbance difference at n=1 ($dA/d\lambda$) is calculated which was directly proportional to the concentration of the standard solution. The standard drug solution was diluted so as to get the final concentration in the range of 1-6 mcg/ml and scanned in the spectra. The calibration curve of $dA/d\lambda$ against concentration of the drug showed linearity. Similarly absorbance of sample solution was measured and amount of Linezolid was determined from standard calibration curve.

RESULTS AND DISCUSSION

As the drug Linezolid showed a broad spectrum, the spectroscopy method applied has the advantage that it locate the hidden peak in the normal spectrum, when the spectrum is not sharp and it also eliminate the interference caused by the excipients and the degradation products present, if any, in the formulation.

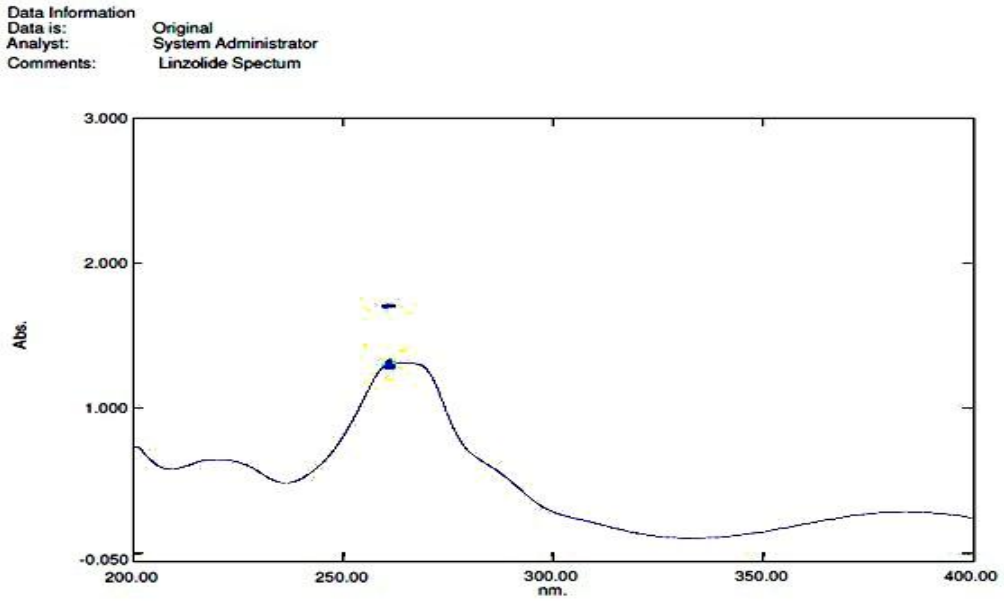

Figure1: Spectrum of Linezolid at 252nm

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The spectra showed sharp peak at 252 nm when n=1 and linearity was measured at 252 nm. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 1-6 mcg/ml with r^2= 0.997 and given in Table 1.

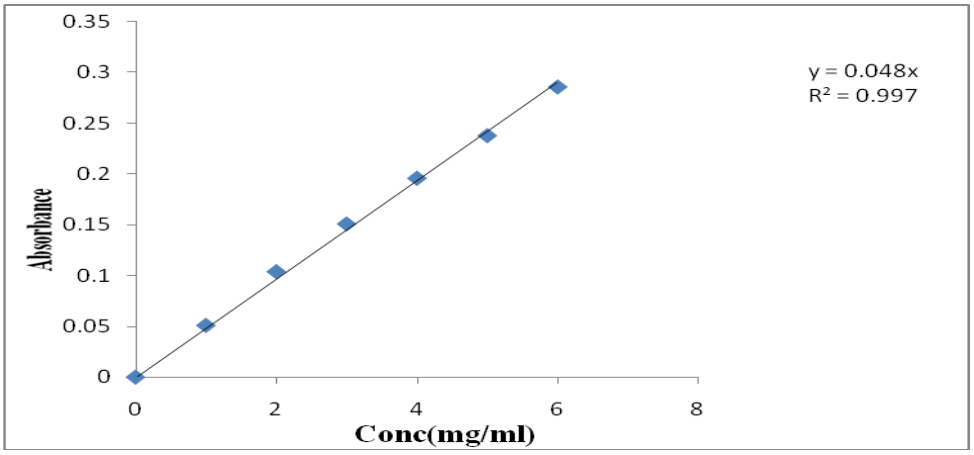

Fig 2: Calibration Curve For Linezolid at 252nm

Table 1: Results of calibration curve at 252 nm

SL.NO.	Conc. (mcg / ml)	Absorbance at 252 nm
1	0	0
2	1	0.051
3	2	0.104
4	3	0.151
5	4	0.196
6	5	0.238
7	6	0.286

Precision

From stock solution (1000mg/ml) take 1-6ml and make up the dilution to 10ml with 0.05N HCL solution. The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision results were expressed as standard deviation or relative standard deviation.

Table 2: Inter day & Intra day precision results for Linezolid

Conc mcg / ml	Inter-day Absorbance Mean ± SD**	Intra-day Absorbance Mean ± SD**
1	0.078 ± 0.01320	0.075 ± 0.00351
2	0.119 ± 0.00757	0.119 ± 0.00208
3	0.151 ± 0.00200	0.153 ± 0.00152
4	0.199 ± 0.00984	0.202 ± 0.01249
5	0.251 ± 0.02112	0.261 ± 0.01497
6	0.287 ± 0.00556	0.290 ± 0.007371

Acceptance criteria

% RSD of the six replicate injections should not more than 2.0%.

Recovery Studies

Recovery studies were carried out at three different levels i.e. 80%, 100% and 120% by adding the pure drug to the previously analysed tablet powder sample and shown in Table 3 and 3A. The percentage recovery value indicates non interference of the excipients used in formulation.

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Table 3: Recovery study Data

Brand name	Amount of sample (µg/ml)	Amount of drug added (µg/ml)	Amount Recovered** (µg/ml)	% Recovery± SD**
LIZOFORCE-600	20	15	15.20	101.09 ± 0.70
LIZOFORCE-600	20	20	19.66	98.30 ± 0.67
LIZOFORCE-600	20	25	25.06	99.61 ± 0.93

**Average of six determinations.

Table 3A: Analysis of tablet formulation

Tablet	Label claim	Amount found (mg)	%Recovery ± S.D**
LIZOFORCE-600	600	500.98	99.21 ± 0.028

**Average of six determinations.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Wavelength

The solution was prepared and observed in replicate for three times with (±2) wavelength i.e. 250nm and 254nm respectively

Table 4: Robustness summary

S. No	Condition	Modification	Mean Absorbance ± SD	% RSD (for Absorbance)
1	Wavelength (nm)	250	0.204 ± 0.001528	0.745
		254	0.152±0.002082	1.36

*Average of three determinations

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 5: Ruggedness studies of Linezolid by UV- visible spectroscopic method

S. No	Analyst-1		Analyst-2	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	4	0.182	4	0.181
2	4	0.179	4	0.179
3	4	0.181	4	0.180
	AVG	0.180	AVG	0.180
	SD	0.00152	SD	0.001
	%RSD	0.844	%RSD	0.556

Acceptance criteria

% RSD should not more than 2.0%.

CONCLUSION

A spectrophotometric method for quantifying Lizoforce-600 in formulation samples has been developed and validated. The proposed method is precise, accurate and reproducible and can be extended to the analysis of Linezolid in bulk and tablet formulations.

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