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Research Article **Analysis**

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A VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF LINEZOLID IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, precise, rapid and accurate Reverse phase HPLC method was developed for the estimation of Linezolid in tablet dosage form. A Thermohypersil C-18 column (250x4.6 mm, 5 µ) particle size with mobile phase consisting of mixture of Acetonitrile and water in the ratio of 60:40 (v/v) at pH 6.0 adjusted with ortho-phosphoric acid. The flow rate was 1.0 mL/min and the effluents were monitored at 254 nm. The retention time was 3.27 min. The detector response was linear in the concentration of 20-60 mcg/mL. The respective linear regression equation being y=98.543x+180.061.The limit of detection and limit of quantification was 0.5mcg/mL and 0.17mcg/mL respectively. The percentage assay of Linezolid was 99.94%. This method has been validated and shown to be specific, sensitive, precise, linear, accurate, rugged, robust and fast. Hence this method can be useful for the routine determination of Linezolid in bulk drug and in its pharmaceutical dosage form.

KEY WORDS : Linezolid, RP-HPLC and Tablets.

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INTRODUCTION

Linezolid is a member of a new structural class of antibiotics, Oxazolidinones. The oxazolidinones have a good activity against Gram-positive bacteria[1,2] . They act uniquely by inhibiting the formation of protein synthesis initiation in Gram-positive bacteria[3]. Cross-resistance with existing antibiotics has not been demonstrated to date. Linezolid is active after oral or intravenous administration. Linezolid is expected to increase the treatment options for severe infections due to Gram positive bacteria, particularly resistant infections (e.g. *Methicillin-resistant Staphylococcus aureus*, MRSA and Vancomycin-resistant Enterococci (VRE))[4,5]. Appropriate use of Linezolid is essential to minimize the risk of resistance development in Gram positive bacteria. The availability of both parenteral and oral formulations provides the opportunity to transfer appropriate patients to an oral formulation.

. Linezolid is a synthetic antibacterial agent of the oxazolidinone class. The chemical name for Linezolid is (S)-N-[[3-[3-Fluoro-4-(4-morpholinyl) phenyl]-2-oxo-5-oxazolidinyl] methyl]-acetamide. The empirical formula is $C_{16}H_{20}FN_3O_4$. Its molecular weight is 337.35. Literature survey reveals few analytical methods [6-12] for the estimation of Linezolid from pharmaceutical dosage forms and also in biological fluids. The availability of an HPLC method with high sensitivity and specificity will be very useful for the determination of Linezolid in pharmaceutical formulations. Due to the increasing importance of speed and reliability of analysis in pharmaceutical anaytical laboratories, a new method for determination of Linezolid in formulations with a short time of analysis (5 min) with retention time 3.27 minutes is described in this work. The current method is concerned at rapid analysis of Linezolid in pharmaceutical dosage forms. It is fast and quick chromatographic method in terms of retention time and run time when compared with other reported methods described in literature survey.

The chemical structure of Linezolid is represented as below.

Structure of Linezolid

EXPERIMENTAL

Materials and Reagents

Linezolid was obtained as a gift sample from M/s. Hetero Drugs, Hyderabad. Acetonitrile, and *ortho*-phosphoric acid and water used were of HPLC grade (Qualigens). Commercially available Linezolid tablets (Lizolid-600® tablets Glenmark (Integrace), India) were procured from local market.

Instrument

Quantitative HPLC was performed on liquid Chromatograph, Shimadzu LC 2010 dual λ detector equipped with automatic injector with injection volume 20 µL. The HPLC system was equipped with LC solution Software.

pH meter: The pH measurements were carried out with Elico, model LI 120, pH meter equipped with a combined glass-calomel

electrode calibrated using standard buffer solutions of pH 4.0, 7.0 and 9.2.

Materials/Reagents

Acetonitrile HPLC grade (Qualigens) and Water HPLC grade (Milli-Q), *orho*-Phosphoric acid (Rankem).Linezolid working and reference sample is obtained as gift sample from Hetero Labs, Hyderbad. Lizolid 600mg tablets were purchased from Glenmark.

HPLC Conditions

The contents of the mobile phase were of acetonitrile and water 60:40 (v/v) adjusted pH 6.0 with *ortho*-phosphoric acid. They were filtered before use through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 mL/min. The run time was set at 5.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 254 nm.

Preparation of Standard Stock solution

A standard stock solution of the drug was prepared by dissolving 50.13 mg of Linezolid in 50 ml volumetric flask containing 30 ml of mobile phase, sonicated for about 15 min and then made up to 50 ml with mobile phase to get approximately 1000mcg/ml of standard stock solution.

Working Standard Solution: 1ml of the Standard stock solution was taken in 25 ml volumetric flask and thereafter made up to 25 ml with mobile phase to get a concentration of 40µg/ml.

Preparation of Sample solution: 20 tablets (Lizolid® 600 mg, Glenmark (Integrace)) were weighed, and then powdered. A sample of the powdered tablets, equivalent to 50.13 mg of the active ingredient, was mixed with 30 ml of mobile phase in 50 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication for complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by addition of mobile phase up to 50 ml to obtain a stock solution of 1mg/ml. The resultant solution was further diluted by taking 1 ml of the stock solution with 25ml of mobile phase to get the concentration of 40µg/mL.

Linearity: Aliquots of standard Linezolid stock solution were taken in different 10 mL volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Linezolid are in the range of 20-60 mcg/mL. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with UV detector at 254 nm and a Calibration graph was obtained by plotting peak area versus concentration of Linezolid (Figure-2).

Assay: 20 µL of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 3.273 minutes. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table-2.

Recovery Studies

Accuracy was determined by recovery studies of Linezolid, known amount of standard was added to the pre-analysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table-2. The study was done at three different concentration levels.

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

The limit of detection (LOD) and limit of quantification (LOQ) for Linezolid were found to be 0.5mcg/mL and 0.17mcg/mL respectively.

The signal to noise ratio is 3 for LOD and 10 for LOQ.

RESULTS AND DISCUSSION

For developing the method, a systematic study on the effect of various factors was carried out by varying one parameter at a time and keeping all other conditions constant, that is, OFAT (one factor at a time) mode of study. Method development consists of selecting the appropriate detection wave length and stationary and mobile phases.

Proper wavelength was needed to determine maximum detector response. From the spectrum, it is clear that Linezolid absorbs maximum light between 245 nm to 255 nm. The longer wavelength of 254 nm was selected since it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify Linezolid.

Preliminary development trials have been performed with octadecyl columns of different types and configurations from different manufacturers. Under these chromatographic conditions, the analysis of interest has exhibited poor peak efficiencies (N) and peak symmetries (Tailing factor); and a partial resolution (Rs) between drug and mobile phase components. Finally Thermohypersil C18 column (250 mm x 4.6 mm, 5µm) column was selected based on the peak shape and the baseline separation from the other interfering peaks in the formulation sample.

Different mobile phases were tested to optimize analytical performance. In order to get sharp peak and base line separation of the components, number of experiments by varying the composition of various solvents and its flow rate was carried. Finally, the contents of the mobile phase were acetonitrile and water in the ratio of 60:40 (v/v) at pH 6.0 adjusted with *ortho*-phosphoric acid has found to be best suitable for good resolution with acceptable system suitability parameters.

System Suitability: The system suitability tests were carried out on freshly prepared standard stock solution of Linezolid. The system was suitable for use if the tailing factors for Linezolid were $<$ 1.55. The Parameters studied to evaluate the suitability of the system are given in Table-3.

Specificity: The effect of wide range of excipients and other additives, usually present in the formulation, in the determination under optimum conditions were investigated. Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. The common excipients present in the formulation did not interfere with the elution or quantification of the method. The acceptance criteria for specificity, RSD should be less than 2%.

Linearity and Range: The plot of peak area of each sample against respective concentration of Linezolid was found to be linear in the range of 20–60 mcg/mL with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in Table-1. The respective linear regression equation being y=98.543x+180.061. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table-1.

Robustness: The robustness of the method was assessed by deliberate alteration of the experimental conditions. The mobile phase flow rate was changed by 0.2 units from 1.0 mL/ min to 0.8 and 1.2 mL/ min, the amount of acetonitrile in the mobile phase was varied by ±10%, and the effect of detection wavelength was studied at 252 and 256 nm. During these tests all other conditions were held constant at the optimum values.

From the typical chromatogram of Linezolid as shown in Figure-1, it was found that the retention time was 3.273 min. A mixture of acetonitrile and water 60:40 (v/v)

adjusted pH 6.0 with *ortho*-phosphoric acid as the mobile phase at a flow rate of 1.0 ml/min was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extractions were involved. A good linear relationship (r^2=0.9999) was observed between the concentration range of 20-60 mcg/mL. Low values of standard deviation are indicative of the high precision of the method. The assay of Linezolid tablets was found to be 99.94%. From the recovery studies it was found that about 99.97% of Linezolid was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible.

Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage forms of Linezolid within a short analysis time.

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Table-1
Linear Regression Data for Calibration curves

Drug	Linezolid
Concentration range (mcg/mL)	20-60
Slope (m)	98.543
Intercept (b)	180.061
Correlation coefficient	0.9999
% RSD	0.05

Table-2
Results of HPLC Assay and Recovery studies

Sample	Amount claim (mg/tablet)	% found by the proposed method	% Recovery*
1.	600	99.43	99.97
2.	600	99.55	98.365
3.	600	99.46	98.247

Average of three different concentration levels

Table-3
Validation Summary

Validation Parameter	Results
System Suitability	
Theoretical Plates (N)	5863
Tailing factor	1.11
Retention time in minutes	3.27
% Area	99.96
LOD (mcg/mL)	0.5
LOQ (mcg/mL)	1.7

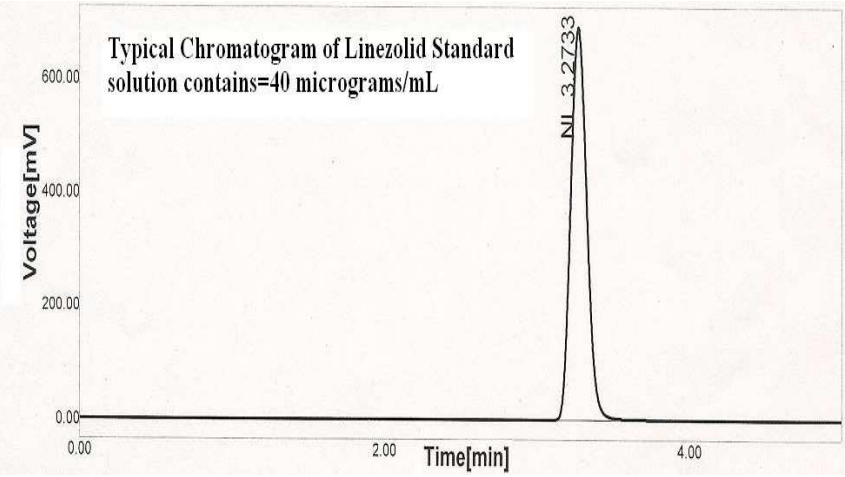

Figure-1
Typical Chromatogram of Linezolid by HPLC

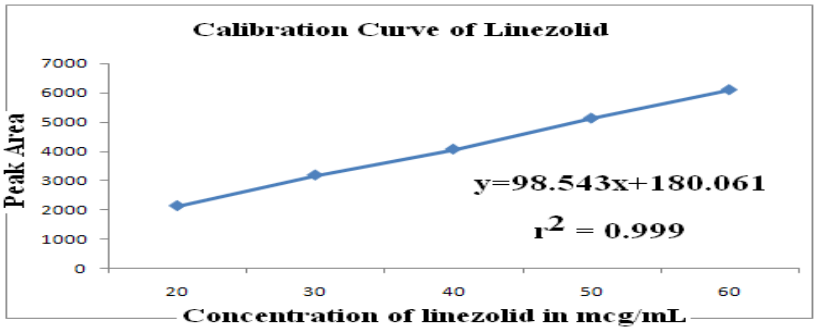

Figure-2
Calibration curve of the Linezolid by RP-HPLC.

CONCLUSION

It can be seen from the results presented that the proposed procedure has good precision and accuracy. Results of the analysis of pharmaceutical formulations revealed that proposed methods are suitable for their analysis with virtually no interference of the usual additives present in the pharmaceutical formulations. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage forms of Linezolid within a short analysis time.

REFERENCES

1. Aoki, H., Ke, L., Poppe, S. M., Poel, T. J., Weaver, E. A., Gadwood, R. C., Oxazolidinone antibiotics target the P site on *Escherichia coli* ribosomes. Antimicrobial Agents and Chemotherapy, 46: 1080–5, (2005)
2. Munoz Bellido, J. L., Gutierrez Zufiaurre, M. N., Sanchez Hernandez, F. J., Yague Guirao, G., Segovia Hernandez, M. & Garcia-Rodriguez, J. A. In vitro activity of Linezolid, synercid and telithromcin against genetically defined high level fluoroquinolone-resistant methicillin-resistant *Staphylococcus aureus.* International Journal of Antimicrobial Agents, 20: 61–4, (2002)
3. Ballow, C. H., Jones, R. N., Biedenbach, D. J. & North American ZAPS Research Group. A multicenter evaluation of Linezolid antimicrobial activity in North America. Diagnostic Microbiology and Infectious Diseases, 43: 75–83, (2002)
4. Stefani, S., Mezzatesta, M.L., Tempera, G., Debbra, E., Schito, A. M., Nicoletti, G, Comparative activity of Linezolid against staphylococci and enterococci isolated in Italy. Clinical Microbiology and Infection,; 8: 368–72, (2002)
5. Slatter, J. G., Stalker, D. J., Feenstra, K. L., Welshman, I. R., Bruss, J. B., Sams, J. P., Pharmacokinetics, metabolism, and excretion of Linezolid following an oral dose of [^{14}C]Linezolid to healthy human subjects. Drug Metabolism and Disposition, 29: 1136–45, (2001)
6. C. M. Tobin, J. Sunderland, L. O. White and A. P. MacGowan et al, A simple, isocratic high-performance liquid chromatography assay for Linezolid in human serum, J. Antimicrob. Chemother., 48 (5): 605-608, (2001)
7. C. M. Tobin, J. Sunderland, L. O. White and A. P. MacGowan et al, A high performance liquid chromatography (HPLC) assay for Linezolid in continuous ambulatory peritoneal dialysis fluid (CAPDF). Journal of Antimicrobial Chemotherapy, 51(4): 1041-1042, (2003)
8. Narayana ChL, Suresh T, Rao SM, Dubey PK, Babu JM, A validated chiral HPLC method for the enantiomeric separation of Linezolid on amylose based stationary phase, J Pharm Biomed Anal., 32(1), 21-8, (2003)
9. Loreis I Bebawy, A Spectrophotometric and HPLC Determination of Linezolid in Presence of Its Alkaline-Induced Degradation Products and in Pharmaceutical Tablets, Analytical Letters, 36(6):1147-1161, (2003)
10. M. Mathrusri Annapurna, K. Satish Kumar and M.V.V. Sai Gangadhara Reddy, New Derivative Spectrophotometric Methods for the Determination of Linezolid - An Antibacterial Drug, Journal of Chemical and Pharmaceutical Research, 4(1): 714-718, 2012
11. Patel SA, Patel PU, Patel NJ, Patel MM, Bangoriya UV. Determination of Linezolid in pharmaceutical dosage forms by liquid

chromatography and ultraviolet spectroscopy. . J AOAC Int.;90(5):1272-7, (2007)

12. Sharmistha Mohapatra, M. Mathrusri Annapurna, B. V. V. Ravi Kumar, Mohammed Anwar, Musarrat Husain Warsi & Sohail Akhter, Validated stability indicating RP-HPLC method for the estimation of Linezolid in a pharmaceutical dosage form, Journal of Liquid Chromatography & Related Technologies , 34(18):2185-2195, (2011)