ISSN 1061-9348, Journal of Analytical Chemistry, 2013, Vol. 68, No. 1, pp. 33–38. © Pleiades Publishing, Ltd., 2013.

ARTICLES

Spectrophotometric Determination of Linezolid in Pharmaceuticals on the Basis of Coupled Redox-Complexation Reactions[1]

K. V. V. Satyanarayana and P. Nageswara Rao

Department of Chemistry, National Institute of Technology Warangal, Andhra Pradesh, 506004 India

Received September 30, 2010; in final form, June 27, 2011

Abstract—The present study describes three simple and sensitive spectrophotometric methods developed for the determination of linezolid (**LZD**) in pure and tablet forms. These methods are based on the oxidation of LZD by ferric chloride in the presence of 1,10-phenanthroline (method A), or 2,2'-bipyridyl (method B), or potassium ferricyanide (method C). The colored complexes were measured at 510, 522 and 758 nm for methods A, B and C, respectively. In all the methods, the absorbance is found to increase linearly with increasing LZD concentration. Beer's law is obeyed over the concentration ranges of 0.5–6.0, 0.5–9.0 and 1.0–9.0 μg/mL for methods A, B and C, respectively. The calculated molar absorptivity values are 5.8×10^4, 3.6×10^4 and 4.8×10^4 L/mol cm for methods A, B and C, respectively, and the corresponding Sandell`s sensitivities are 5.8×10^{-3}, 9.8×10^{-3} and 7×10^{-3} μg/cm^2, respectively. The developed methods are applied successfully to the determination of LZD in the pharmaceutical formulations and the results tallied well with label claims.

Keywords: linezolide, spectrophotometry, coupled redox-complexation, pharmaceutical formulations

DOI: 10.1134/S1061934813010127

Chemically, linezolid (LZD) is *N*-[(5*S*)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamidel (Fig. 1). It is the first drug of the class, oxazolidinone antibacterial, approved in US, UK and Canada for treatment of gram-positive infection.

Literature survey revealed that few analytical methods have been reported for the estimation of linezolid in biological fluids and pharmaceutical formulations, which include liquid chromatography [1–6], capillary electrophoresis [7] high performance thin layer chromatography [8] and spectrophotometry [9]. Critical literature survey revealed that no attempt has been made to determine LZD by Fe(III) in the presence of 1,10-phenanthroline, or 2,2'-bipyridyl, or ferricyanide. Hence, an attempt has been made in the present study to develop simple and sensitive spectrophotometric methods using the above reagents for the assay of LZD in pharmaceutical formulations. The methods are based on the reducing property of the cited drug, which is easily oxidized by iron(III). The formed iron(II) is made to interact with 1,10- phenanthroline, 2,2'-bipyridyl, or potassium ferricyanide to give colored complexes, whose absorbances are measured at 510, 522 and 758 nm for methods A, B and C, respectively. The developed methods have the advantages of speed, sensitivity, simplicity, and cost effectiveness. The results obtained are closely comparable to those of a reported method and the recovery tests are also found to be satisfactory.

EXPERIMENTAL

Apparatus. All the absorption spectra were recorded using UV-Vis-NIR spectrophotometer (Shimadzu 1601, Japan) equipped with 1 cm matched quartz cells by using a personal computer loaded with the UV-PC 3.9 software package. An electronic micro balance (Sartorius MC 5, Germany) was used for weighing the solid materials.

Materials and reagents. All solvents and reagents used were of analytical grade. Double-distilled water was used throughout the investigation. An aqueous solution of 0.05 M ferric chloride (S.D. Fine Chem., Mumbai, India) was prepared by dissolving 0.8111 g of the chemical in 100 mL of double distilled water and standardized by iodometric method. The stock solution was then diluted appropriately with double distilled water to get 0.005 M working concentration for

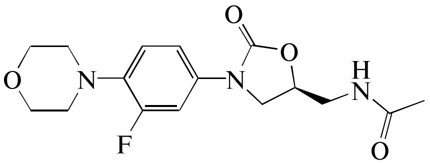

Fig. 1. Chemical structure of linezolid.

---

[1] The article is published in the original.

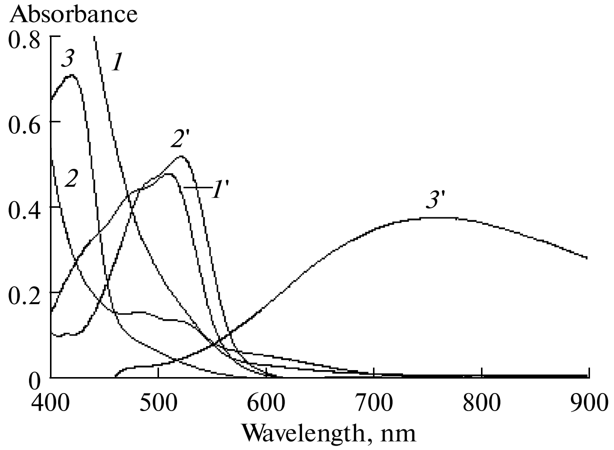

Fig. 2. Absorption spectra of : *1*—Fe(III)–1,10-phenanthroline, *2*—Fe(III)–2,2'-bipyridyl, *3*—Fe(III)–$K_3[Fe(CN)_6]$ against water before and *1', 2', 3'*—after reduction with 3, 5, 3 μg/mL of linezolid, respectively, against reagent blank.

methods A, B and C. The solution was prepared afresh just before the experiment. 1,10-phenanthroline (0.02 M) was prepared by dissolving 0.3604 g of 1,10-phenanthroline (S.D. Fine Chem., Mumbai, India) in 100 mL of double distilled water.

Standard LZD was procured from Symed laboratories, Hyderabad, India. A standard solution containing 500 μg/mL of LZD was prepared by dissolving accurately 0.0250 g of pure drug in 50 mL of methanol in a calibrated flask. The solution was further diluted with double distilled water to get working concentration of 50 μg/mL LZD.

Method A. Different aliquots of stock reference solution (50 μg/mL) in the range of 0.1–1.2 mL were transferred into a series of 10 mL standard flasks. To each flask, 2.0 mL of 0.005 M ferric chloride and 3.0 mL of 1,10-phenanthroline (0.02 M) were added. The flasks were kept in a hot water bath at a temperature of 75 ± 1°C for 20 min and then immediately cooled to room temperature (27 ± 1°C) using cold water. The solutions were made up to volume with double distilled water. The absorbance of each solution was measured at 510 nm against the reagent blank.

Method B. Varying aliquots of stock reference solution (50 μg/mL) in the range of 0.1–1.8 mL were transferred into a series of 10 mL standard flasks. To each flask, 1.0 mL of 0.005 M ferric chloride and 2.5 mL of 2,2'-bipyridyl (0.02 M) were added and the flasks were kept in a water bath (75 ± 1°C) for 20 min, then immediately cooled to room temperature (27 ± 1°C) using cold water. The solutions were made up to volume with double distilled water. The absorbance of each solution was measured at 522 nm against the proper blank.

Method C. Different aliquots of stock reference solution (50 μg/mL) in the range of 0.2–1.8 mL were transferred into a series of 10 mL standard flasks. To each flask 3.5 mL of ferric chloride (0.005 M) and 3.0 mL of potassium ferricyanide (0.002 M) were added. The contents were mixed well and kept aside for 30 min at (27 ± 1°C). Finally, 0.5 mL of 1 M HCl was added to each flask. The contents of the flasks were diluted to mark with double distilled water and mixed thoroughly. The absorbances of the resulting solutions were measured at 758 nm for LZD against a reagent blank prepared similarly.

In all the three methods, the calibration graph was constructed by plotting the absorbance versus concentration of the LZD in μg/mL. The concentration of the unknown was read from the calibration graph or computed from the regression equation.

Procedure for pharmaceutical formulations. Ten tablets containing linezolid were weighed and ground into a fine powder. An amount of the powder equivalent to 0.0250 g of LZD was weighed into a 50-mL volumetric flask. 30 mL methanol were added to it and the contents were shaken thoroughly for about 15 min. The solution was filtered using Whatmann no. 41 filter paper into a 50-mL volumetric flask and then made up to mark with methanol. The filtrate was diluted with double distilled water to get a concentration of 50 μg/mL of LZD for methods A, B and C. A suitable aliquot was then subjected to analysis.

RESULTS AND DISCUSSION

1,10-Phenanthroline and related compounds are used for the spectrophotometric analysis of different metals and other substances either directly through the formation of metal chelates and mixed ligand complexes, or indirectly for the determination of reductants and oxidants. It is known that 1,10-phenanthroline and 2,2'-bipyridyl form highly stable, intensely red colored, water-soluble chelates with iron(II). The complex formation stabilizes the iron(II) oxidation state, as the redox potentials of these complexes are higher than that of the iron-aqua complex. Trace amounts of the reductants are conveniently measured through spectrophotometric determination of ferrous iron using 1,10-phenanthroline (phen) or 2,2'-bipyridyl (bipy).

To a solution consisting of Fe(III) and 1,10-phenanthroline or 2,2'-bipyridyl, when LZD is added, Fe(III) is reduced to Fe(II) by LZD. Fe(II) so formed readily combines with 1,10-phenanthroline or 2,2'-bipyridyl to form a red-colored complex, $[Fe(phen)_3]^{2+}$, with an absorption maximum at 510 nm or $[Fe(bipy)_3]^{2+}$ exhibiting an absorption maximum at 522 nm. Fig. 2 shows the absorption spectra of these colored complexes.

Optimum reaction conditions. In order to optimize the proposed spectrophotometric methods, the effect of experimental variables was studied. Studies were performed by altering each variable in turn while keeping the other variables constant.

Effect of pH. The effect of pH for the quantitative determination of LZD in methods A and B was studied in the pH range of 3.0–6.0. It was observed that the absorbance of the complex decreased in the presence of the buffer media in the studied pH range in comparison to the absorbance without addition of any buffer. It may be noted that pH of the solution without buffer is 5.0, where the highest absorbance was obtained. Therefore, further studies were performed without addition of any buffer.

Effect of reagent concentration. The effects of reaction variables in methods A and B such as concentration of Fe(III) and 1,10-phenanthroline or 2,2'-bipyridyl have been investigated by keeping the concentration of LZD constant at 5.0 μg/mL. These results indicate that maximum absorbance was obtained when 2.0 mL of 0.005 M of ferric chloride were added for method A and when 1.0 mL of 0.005 M of ferric chloride was added in the case of method B. Larger volumes of iron(III) chloride had effect on the sensitivity of the reaction. Similar observations were noted when the varied volumes of 0.02 M 1,10-phenanthroline or 2,2'-bipyridyl solution were added to fixed amounts of LZD (5.0 μg/mL) and diluted to 10 mL after the formation of the colored complex. The results of this study reveal that 3.0 mL of 0.02 M reagent solutions is optimum for Fe^{2+}-phen (method A). However, in the case of Fe^{2+}-bipy (method B) 2.5 mL of 0.02 M reagent is adequate.

Effects of temperature and heating time. The effects of temperature and heating time on the formation of the colored complexes were also studied. The reaction of LZD with both reagents proceeded very slowly at room temperature and therefore, higher temperature was employed to accelerate the reaction. Maximum absorbance was obtained after heating the reaction mixture on a water bath at 75°C for about 20 min in case of both the Fe^{2+}-phen and Fe^{2+}-bipy complexes. Further heating caused no appreciable change in the absorbance. The color of the complex formed remained stable at room temperature for more than 12 h.

Method C. ***Absorption spectra.*** The formation of the Prussian blue complex was employed in the qualitative detection of Fe(II). The complex was formed due to the reaction between Fe(II) and hexacyanoferrate(III). The chromogenic reagent blank [Fe(III) mixed with hexacyanoferrate(III) in acidic medium] did not show strong absorption in the visible region of 600–900 nm. The investigated LZD is oxidized by iron(III) chloride in acidic medium to produce iron(II). The iron(II) ion reacts with potassium hexacyanoferrate(III) to produce bluish green iron(II) ferricyanide complex with an absorbance maximum at 758 nm as shown in Fig. 2. Therefore, the formation of the Prussian blue complex is the basis for development of a sensitive spectrophotometric method for the determination of LZD. The optimum conditions were established by varying parameters, such as the concentration of iron(III), ferricyanide and acid, reaction time and the order in which the reagents were added.

Optimum concentration of iron(III) and ferricyanide. One milliliter of 50.0 μg/mL of LZD and 2.0 mL of 0.002 M ferricyanide were mixed thoroughly and different volumes (1.0 to 4.0 mL) of 0.005 M Fe(III) were added to this solution. These solutions were made up to 10 mL in the calibrated flasks. The absorption spectra of these solutions were recorded. The highest absorbance was obtained with 3.5 mL of ferric chloride. The influence of the amount of potassium ferricyanide on the absorbance of the complex was studied by adding different volumes of 0.002 M reagent to the fixed amounts of LZD (5.0 μg/mL) and iron(III) chloride (3.5 mL; 0.005 M). The highest absorbance was obtained with 3.0 mL of 0.002 M ferricyanide reagent solution.

Effect of nature of acid and its concentration. The reaction product, Prussian blue, was found to flocculate within 20–30 min of color development. To delay the flocculation, acid was added after full color developmentand before dilution. Hydrochloric acid was found to give more stable color and reproducible results compared to sulphuric acid. A 0.5 mL of 1 M hydrochloric acid in a total volume of 10 mL was found to be optimum.

Influence of reaction temperature and standing time. The influences of the reaction temperature and the standing time on the absorbance have been investigated. It was found that the absorbance reached its maximum at room temperature. In order to make determination of LZD both sensitive and simple, room temperature was chosen as the optimum reaction condition. The absorbance was measured at various standing time intervals when the reaction was maintained at room temperature. It was observed that the absorbance was maximum when the standing time was 30 min. Therefore, 30 min and room temperature were selected for further experiments. The developed color remained stable at room temperature for more than 12 h.

Effect of order in which reagents were added. After fixing all other parameters, a few experiments were performed in order to ascertain the influence of the order in which reagents were added. The maximum absorbance and highest stability were obtained when the order of addition was: LZD drug, iron(III) solution, ferricyanide solution and hydrochloric acid. The same order of addition was followed throughout the investigation.

VALIDATION STUDY OF THE PROPOSED METHODS

Linearity and Sensitivity

Under optimum experimental conditions, a linear relation was obtained between absorbance and concentration of LZD in the range 0.5–6.0 μg/mL in method A, 0.5–9.0 μg/mL in method B and 1.0–9.0 μg/mL in method C. The regression analysis of the plot using the method of least squares was made in order to evaluate the intercept (a), slope (b), correlation coefficient (r^2) and standard deviations of slope and intercept. In all

Table 1. Analytical and regression parameters of proposed methods

Parameter	Method A	Method B	Method C
λ_{max}, nm	510	522	758
Beers law limit, μg/mL	0.5–6.0	0.5–9.0	1.0–9.0
Molar absorptivity, L/mol cm	5.8×10^4	3.5×10^4	4.8×10^4
Sandell's sensitivity, μg/cm^2	0.0058	0.0098	0.007
Regression equation ($Y = a + bC$)			
Slope (b)	0.1715	0.1055	0.1427
Intercept (a)	-8×10^{-4}	6.0×10^{-3}	-5.0310^{-2}
Correlation coefficient, r^2	0.999	0.9992	0.9994
Standard deviation of slope, S_b	2.17×10^{-3}	1.006×10^{-3}	1.347×10^{-3}
Standard deviation of intercept, S_a	7.995×10^{-3}	5.373×10^{-3}	7.581×10^{-3}
Confidence interval of slope, ($\pm tS_b$)*	0.0048	0.0023	0.0031
Confidence interval of intercept, ($\pm tS_a$)*	0.017	0.0121	0.0175
Detection limit LOD, μg/mL	0.15	0.17	0.17
Quantification limit LOQ, μg/mL	0.46	0.52	0.53

* The values were calculated at 95% confidence level.

Table 2. Evaluation of precision and accuracy

Proposed method	LZD taken, μg/mL	Intraday precision ($n = 6$)*			Interday precision ($n = 6$)*		
		LZD found, μg/mL	% RSD	% RE	LZD found, μg/mL	% RSD	% RE
Method A	1	1.011	1.58	1.1	0.994	2.1	–0.6
	3	3.043	1.91	1.43	3.003	1.87	0.1
	5	4.991	1.12	–0.18	5.027	2.16	0.54
Method B	2	1.986	1.01	–0.7	2.005	1.94	0.25
	5	5.048	1.82	0.96	5.017	1.51	0.34
	8	7.858	0.86	–1.78	7.994	1.2	–0.08
Method C	2	2.030	1.82	1.5	1.996	1.54	–0.2
	5	5.017	1.12	0.34	5.01	1.7	0.2
	8	7.980	0.83	–0.25	8.06	2.07	0.75

* Mean value of six determinations; RE—relative error; RSD—relative standard deviation.

cases, Beer's law plots are found to be linear with good correlation coefficients as shown in Table 1. The moderately high sensitivity of the method was indicated by the fairly high value of molar absorptivity and low values of Sandell`s sensitivity. The limits of detection (**LOD**) and limits of quantitation (**LOQ**) [10] were determined using the formula: LOD or LOQ = $k\mathrm{SD}a/b$, where k (constant) = 3.3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope.

Precision and Accuracy

The precision and accuracy of the proposed methods were determined at three different concentrations of LZD. At each concentration, six replicate determinations were made on the same day (for intraday precision). Such determinations were also carried out on five consecutive days (for inter day precision). The results are shown in Table 2.

Accuracy and Recovery

The accuracy and reliability of the methods were ascertained through recovery experiments. Pure LZD was added at two different levels to pre-analyzed formulations, and the total content was found by the proposed methods. The recoveries of the pure drug added to the tablet powder are shown in Table 3 confirming that the proposed methods are not liable to interfer-

Table 3. Results of recovery experiments by standard addition method

			Proposed methods (%)Recovery* ± SD		
Proposed methods	Formulation taken, μg/mL	Pure drug added, μg/mL	Lizolid-600	Lizoforce-600	Linox-600
Method A	3	1.5	100.07 ± 1.63	102.75 ± 2.31	99.7 ± 0.67
	3	3	102.11 ± 2.07	101.84 ± 1.25	98.12 ± 1.51
Method B	3	1.5	101.1 ± 1.55	102.1 ± 0.83	100.33 ± 1.25
	3	3	100.84 ± 2.63	102.32 ± 1.47	99.7 ± 1.15
Method C	4	2	100.98 ± 1.65	101.23 ± 0.9	98.83 ± 1.92
	4	4	100.83 ± 1.55	101.57 ± 2.31	100.1 ± 1.28

* Mean value of three determinations; SD—standard deviation.

Table 4. Results of determination of linezolid in tablet forms by the proposed methods

Pharmaceutical preparations*	Amount per tablet (mg)	% Found** ± SD			
		Reference method	Method A	Method B	Method C
Lizolid-600[a]	600	100.47 ± 0.7	99.68 ± 0.95 $t = 1.64$ $F = 1.84$	100.6 ± 1.33 $t = 0.21$ $F = 3.61$	99.98 ± 0.77 $t = 1.15$ $F = 1.21$
Lizoforce-600[b]	600	101.75 ± 0.96	101.7 ± 1.83 $t = 0.06$ $F = 3.63$	100.98 ± 1.19 $t = 1.31$ $F = 1.86$	101.81 ± 2.02 $t = 0.066$ $F = 4.43$
Linox-600[c]	600	100.36 ± 1.21	100.51 ± 1.75 $t = 0.173$ $F = 2.1$	99.89 ± 2.07 $t = 0.478$ $F = 2.92$	99.8 ± 1.31 $t = 0.77$ $F = 1.17$

Notes: * Marketed by: [a] Glenmark, Mumbai, India; [b] Mankind, Delhi, India; [c] Unichem, Mumbai, India.

** Mean value of six determinations. The theoretical values of t (2.228) and F (5.05) at confidence limit at 95% confidence level ($p = 0.05$).

ence by tablet fillers, excipients and additives usually found in the pharmaceutical preparations.

Analysis of the Pharmaceutical Formulations

The proposed methods were applied to the determination of LZD in commercially available tablets. The applicability of the proposed methods for the assay of this drug in tablets was examined and the results are shown in Table 4. The performance of the methods was assessed using the t-test for accuracy and F-test for precision in comparison with the reference method [6]. The results showed that the t- and F-values are less than the theoretical value for 95% confidence level, suggesting that the proposed methods are comparable to the reference method with respect to accuracy and precision. The results are given in Table 4.

* * *

The proposed methods are quite simple and do not require any pretreatment of the drug and tedious extraction procedure. Moreover, the methods are free from interferences by common additives and excipients. The methods are good with respect to sensitivity, precision, accuracy and stability of the colored species for ≥12 h. These newly developed methods can be extended for routine analysis of linezolid in pharmaceutical formulations.

ACKNOWLEDGMENTS

The authors are very thankful to Director, National Institute of Technology, Warangal for providing financial assistance and research facilities.

REFERENCES

1. Klaus, B., Ellen, B., and Hartmut, L., *Int. J. Antimicrob. Agents*, 2001, vol. 18, p. 253.
2. Narayana, C.L., Suresh, T., Rao, S.M., Dubey, P.K., and Babu, J.M., *J. Pharm. Biomed. Anal.,* 2003, vol. 32, p. 21.
3. Buerger, C., Joukhadar, C., Muller, M., and Kloft, C., *J. Chromatogr. B,* 2003, vol. 796, p. 155.
4. Peng, G.W., Stryd, R.P., Murata, S., Igarashi, M., Chiba, K., and Aoyama H., *J. Pharm. Biomed. Anal.,* 1999, vol. 20, p. 65.
5. Cristiani, C.G.O.L. and Salgado, H.R.N., *Chromatographia*, 2009, vol. 69, p. S129.
6. Satish, A.P., Paresh, U.P., Natubhai, J.P., Madhabhai, M.P., and Andurvisha, V.B., *J. AOAC Int.*, 2007, vol. 90, p. 1272.
7. Manley, H.J., McClaran, M.L., Bedenbaugh, A., and Peloquin, C.A., *Perit. Dial. Int.,* 2002, vol. 22, p. 419.
8. Agrawal, H., Mahadik, K.P., Paradkar, A.R., and Kaul, N., *Drug Develop. Ind. Pharm.,* 2003, vol. 29, p. 1119.
9. Dharuman, J., Ravichandran, V., Krishna Pillai, M., Sulaiman, E., Lavanya, S., and Jayalakshmi, S., *Indian J. Pharm. Sci.,* 2004, vol. 66, p. 235.
10. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceutical for Human Use, ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated November 6, 1996, incorporated in November 2005. London.