SSRG International Journal of Applied Chemistry
ISSN: 2393 – 9133 / https://doi.org/10.14445/23939133/IJAC-V9I1P103

Volume 9 Issue 1, 18-24, Jan-Apr 2022

Original Article

Development and Validation of RP-LC Method for Linezolid in Pharmaceutical Formulations

Syed Mastan Ali[1], Ponnuri Bharath[2], V. Siva Ramakrishna[3], D. Ramachandran[4]

[1,2,3,4]*Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, A.P, India*

Received Date: 10 February 2022
Revised Date: 25 March 2022
Accepted Date: 27 March 2022

Abstract *- A new, simple, rapid, selective, precise and accurate isocratic reverse-phase high-performance liquid Chromatography assay method has been developed to estimate Linezolid in tablet formulations. The separation was achieved using column Inert sustain C18 (250×4.6 mm, 5µ) in the mobile phase consisting of methanol and pH 3.0 phosphate buffer in the ratio of (45:55, v/v). The flow rate was 1.0 mL/min-1, and the separated Linezolid was detected using a UV detector at 251 nm. Column temperature 45°C and sample temperature ambient and injection volume 20 µL. The retention time of Linezolid was noted to be 4.95 min, respectively, indicative of a rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.*

Keywords *- Liquid Chromatography, Linezolid, Validation.*

I. INTRODUCTION

The oxazolidinones represent the first truly new class of antibacterial agents to reach the marketplace in several decades [1]. Linezolid, (s)-N-[[3-[3-fluoro-4(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl] acetamide (Figure 1) was the first oxazolidinone to be developed and approved for clinical use. Linezolid is a synthetic antibiotic used to treat serious infections caused by Gram-positive bacteria that are resistant to several other antibiotics [2-3]. Linezolid is active against

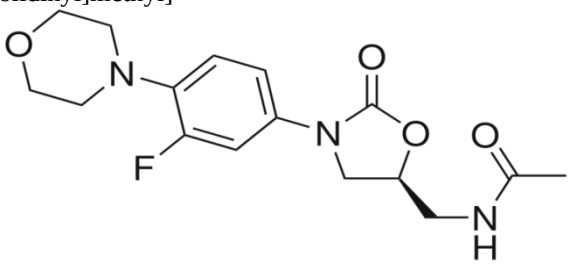

Fig. 1.1 Chemical structure of Linezolid

Most Gram-positive bacteria cause diseases, including streptococci, vancomycin-resistant enterococci, and methicillin-resistant Staphylococcus aureus [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. In both the popular press and the scientific literature, Linezolid has been called a reserve antibiotic, which should be used sparingly to remain effective as a drug of last resort against potentially intractable infections [8].

A literature review revealed that the HPLC method had 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, LC methods using ultraviolet (UV) detection [9–15] and fluorescence detection [16]. For the assays in the pharmaceutical dosage forms, the methods reported in the literature are HPLC,[17–20] capillary electrophoresis, [21] TLC followed by densitometric and first derivate spectrophotometry [22], and HPTLC by densitometric analysis [23]. However, any generally recommended or rapid stability-indicating analytical method for determining Linezolid in pharmaceutical dosage form has not yet been described in any pharmacopoeia or literature. As a result, in this study, a novel rapid, selective, linear, precise, and sensitive stability-indicating RP-HPLC method with the UV detection to estimate Linezolid in pharmaceutical dosage form was developed and validated as per the ICH guideline.

II. EXPERIMENTAL

A. Chemicals and Reagents

Analytical-grade Ammonium dihydrogen phosphate, and orthophosphoric acid, were from Merck Chemicals Mumbai, India. Acetonitrile, methanol and water, both HPLC-grades, were from Merck Chemicals. Mumbai, India.

B. Instrumentation

Waters 2489 U.V-Visible detector/2695 Separation Module, Empower 2 software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model), Centrifuge Eppendorf 5810 were used in the present assay.

C. Preparation phosphate Buffer pH 2.5

Weighed accurately, 0.23 g of Potassium dihydrogen phosphate was dissolved in 1000.0 mL of milli-Q water and adjusted the pH to 3.02 with orthophosphoric acid. The solution was filtered through 0.45µ filter paper and degassed.

D. Mobile Phase Preparation

Mixed accurately 450 volumes of buffer and 550 acetonitrile in the ratio of 45:55 v/v degassed and sonicated.

E. Diluent Preparation

Mixed accurately 550 volumes of buffer and 450 volumes of acetonitrile in the ratio of 550:450 v/v degassed and sonicated

F. Standard Preparation

Weighed accurately 30.10 mg of the Linezolid working standard into a 50 mL volumetric flask, added 30 mL of diluent sonicate to dissolve, and diluted to volume with diluent and mixed well. Taken 5 mL of this solution transferred to 50 mL volumetric flask diluted to volume with diluent.

G. Sample Preparation

Weighed 10 tablets, taken average weight and crushed into fine powder. Accurately weighed and transferred equivalent to 100 mg of Linezolid sample powder into 100 mL volumetric flask, added 70 mL of diluent, sonicate for 20 minutes with intermediate shaking to dissolved, then diluted to the volume with diluent, mixed well and filtered the solution through 0.45 µm PVDF membrane filter. Diluted 3 mL of this solution into a 50 mL volumetric flask diluted to volume with diluent and mixed well.

H. Chromatographic Conditions

Chromatographic analysis was performed on the Inert sustain C18 (250x4.6 mm, 5µ) column. The mobile phase consisted of pH 3.0 phosphate buffer and acetonitrile in the ratio of 45:55%v/v. The flow rate was 1.0 mL/min, the Column temperature was 45°C, and sample temperature was ambient, the injection volume was 20 µL, and detection was performed at 251 nm using a photodiode array detector (PDA).

III. METHOD DEVELOPMENT

Spectroscopic analysis of compound Linezolid showed maximum UV absorbance (λmax) at 251 nm. To develop a suitable and robust LC method for the determination of Linezolid, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Agilent Zorbax SB C18 with the following different mobile phase compositions like that buffer and acetonitrile in the ratio of 80:20 v/v 65:35 v/v & 55:45. It was observed that when Linezolid was injected, Peak Tailing was not satisfactory.

The Inert sustain C18 (250 x 4.6 mm, 5µ) column was used for the next trial, and the mobile phase composition was changed slightly. The mobile phase composition was buffer and acetonitrile in the ratio of 45:55 v/v. Respectively as eluent at flow rate 1.0 mL/min. UV detection was performed at 251nm. The retention time of Linezolid is 4.95 minutes, and the peak shape was good.

The method's chromatogram of the Linezolid standard using the proposed method is shown in (Fig. 1.2.) system suitability results are presented in Table 1.1.

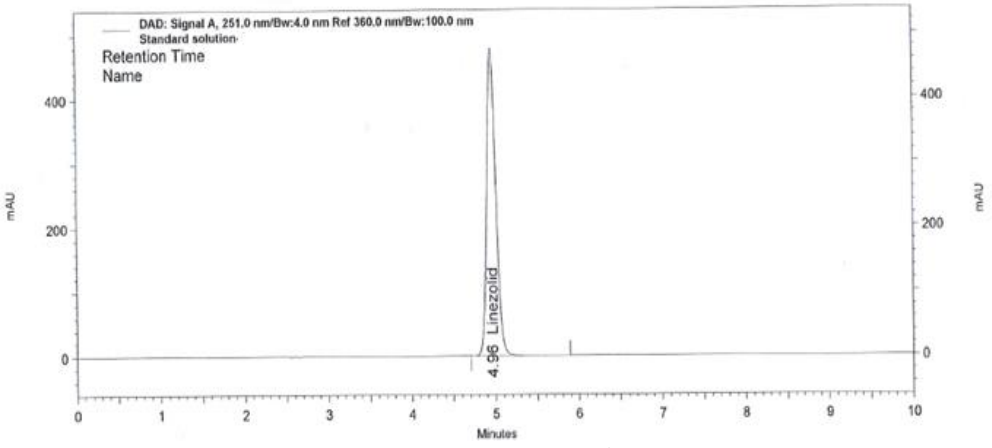

Fig. 1.2 Typical chromatogram of standard

IV. METHOD VALIDATION

The developed RP-LC method was extensively validated for assay of Linezolid using the following parameters.

A. Specificity Blank and Placebo Interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo were injected into the chromatograph in the defined chromatographic conditions, and the blank and placebo chromatograms were recorded. The chromatogram of the blank solution (Fig. 1.3) showed no peak at the retention time of the Linezolid peak. This indicates that the diluent solution used in sample preparation does not interfere with Linezolid estimation in Linezolid tablets. Similarly, the chromatogram of the placebo solution (Fig. 1.4) showed no peaks at the retention time.

Linezolid peak. This indicates that the placebo used in sample preparation does not interfere with Linezolid estimation in Linezolid tablets.

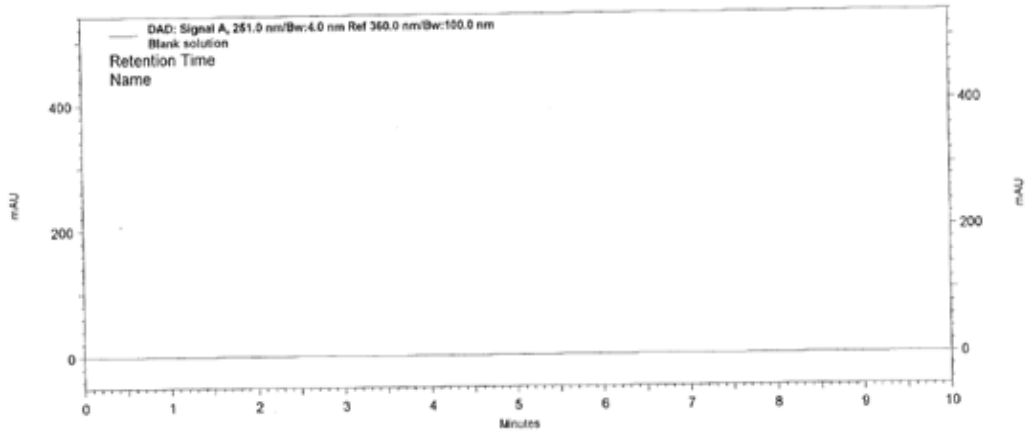

Fig. 1.3 Typical chromatogram of blank

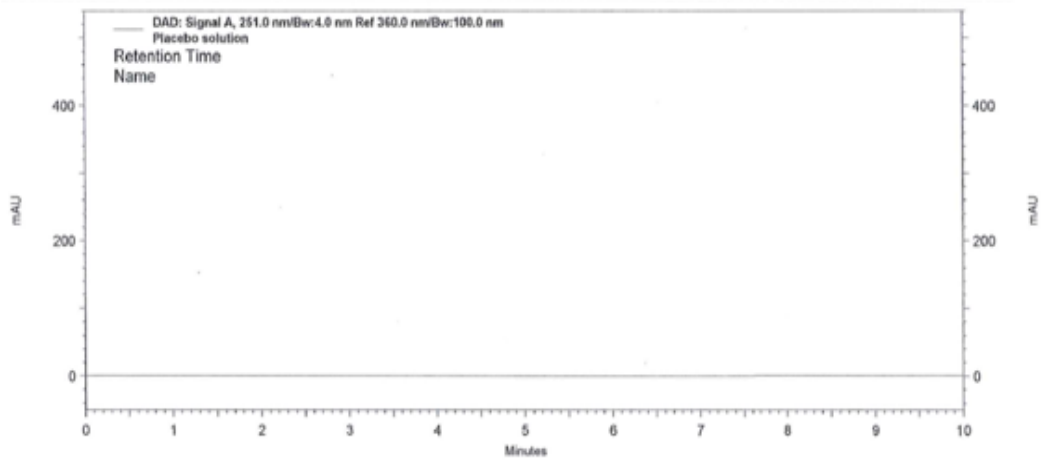

Fig. 1.4 Typical chromatogram of placebo

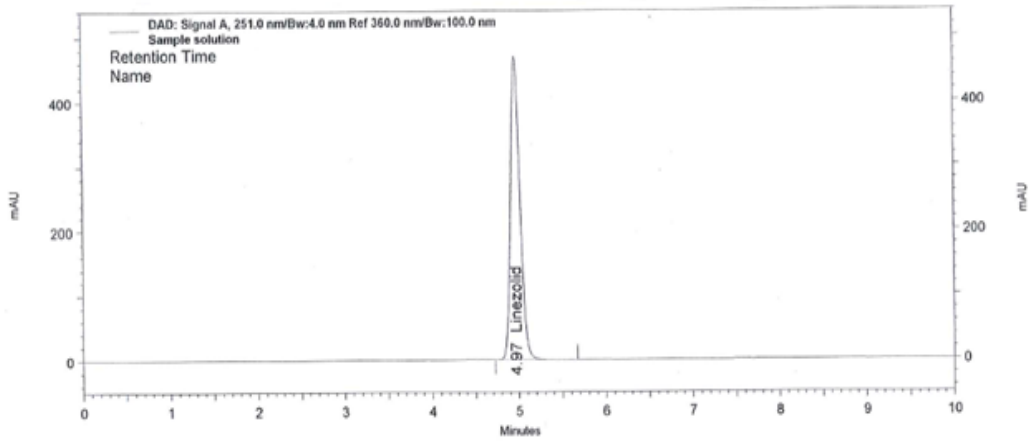

Fig. 1.5 Typical chromatogram of sample

Table 1.1. System suitability parameters for Linezolid by the proposed method

Name of the Compound	Retention Time	Theoretical plates	Tailing factor
Linezolid	4.96	10195	1.1

B. System Precision

The standard solution was prepared as per the test method, injected into the HPLC system six times and evaluated the % RSD for the area responses. The data are shown in Table 1.2.

Table 1.2. System precision data for Linezolid

No. of injections	Peak area response
1	4555654
2	4545420
3	4564722
4	4529045
5	4578069
6	4577550
Average	4558410.00
SD	19137.90
% RSD	0.42

C. Method Precision

The precision of the test method was evaluated by doing an assay for six samples of Linezolid tablets as per the test method. The content in mg and % label claim for linezolid for each test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The data are shown in Table 1.3.

Table 1.3. Method precision data for Linezolid

No.of Preparation	% assay
Preparation 1	99.1
Preparation 2	99.9
Preparation 3	100.3
Preparation 4	100.7
Preparation 5	101.8
Preparation 6	100.1
Average	100.3
SD	0.9
%RSD	0.9

D. Linearity

The linearity of an analytical method is its ability to obtain test results that have a definite mathematical relation to the concentration of the analyte. The linearity of response for Linezolid was determined in the range of 50% to 150 % (30, 45, 60, 75, and 90 µg/mL for Linezolid). The calibration curve of the analytical method was assessed by plotting concentration versus peak area and represented graphically. The correlation coefficient [r2] was found to be 1.000. Therefore the HPLC method was found to be a standard linear curve and was calculated and given in Figure 1.6 to demonstrate the linearity of the proposed method. From the data obtained, which is given in Table 1.4, the method was linear within the proposed range.

Table 1.4. Linearity studies for Linezolid by the proposed method

S.No.	Linearity concentration	Concentration (µg/mL)	Area response
1	50%	30.21	2281187
2	75%	45.43	3478123
3	100%	60.65	4562145
4	125%	75.08	5712741
5	150%	90.62	6843789
Correlation coefficient:			1.0000
Slope (m):			75488.36
Intercept (y):			16250.83

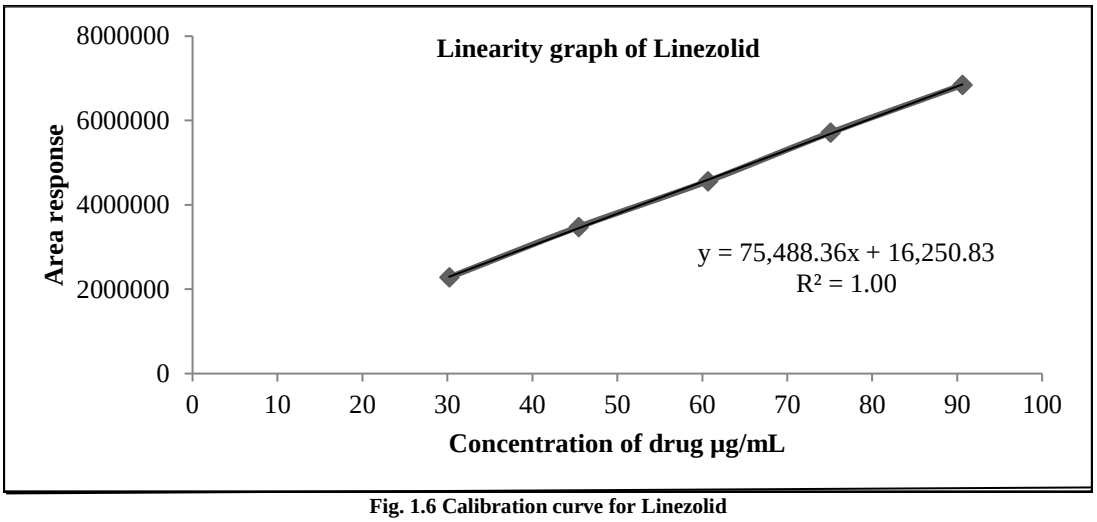

Fig. 1.6 Calibration curve for Linezolid

E. Accuracy

The method's accuracy was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on a composite blend collected from 20 tablets of Linezolid, analyzed as per the proposed method. The mean percentage recovery for 50%, 100%, and 150% levels was 99.2, 99.5 and 99.3. They are within acceptable limits. Therefore, the HPLC method for the determination of assay of Linezolid in the formulation was accurate. The data obtained, which is given in **Table 1.5,** the method was found to be accurate.

Table 1.5. Recovery studies for Linezolid by the proposed method

Level	% Recovery	Mean % Recovery
50%	100.3	99.2
	98.3	
	98.9	
100%	99.4	99.5
	100.2	
	98.9	
150%	99.5	99.3
	99.6	
	98.9	

F. Solution Stability of Analytical Solutions

Standard and sample solutions were kept for about 48 hrs at room temperature in transparent bottles in an autosampler and refrigerator 2-8°C. The stability of standard and sample solutions was determined by comparing "old" standard solutions with freshly prepared standard solutions.

Table 1.6. Results for solution stability of standard

Time Interval (hrs)	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
12	0.96	0.98
24	0.95	0.97
48	0.95	0.97

Table 1.7. Results for solution stability of sample at room temperature

Time Interval (hrs)	%Assay	% of Assay difference
Initial	100.3	NA
12	99.9	0.4
24	99.7	0.6
48	99.1	1.2

Table 1.8. Results for solution stability of sample in the refrigerator

Time Interval (hrs)	%Assay	% of Assay difference
Initial	100.3	NA
12	100.0	0.3
24	99.9	0.4
48	99.6	0.7

G. Filter Validation

Performed the filter validation for the sample solution. One portion of the solution was centrifuged, and the other was filtered through 0.45 µm PVDF and 0.45 µm Nylon filters.

Table 1.9. Results for Filter validation of Linezolid

S.No.	Filter details	% Assay	The difference when compared to Centrifuged
1	Centrifuged Sample	100.3	NA
2	0.45 µm PVDF Filtered Sample	101.1	0.8
3	0.45 µm Nylon Filtered Sample	100.9	0.6

a) Observations

- 0.45 µm PVDF filtered sample when compared with the centrifuged sample was 0.8.
- 0.45 µm Nylon filtered sample, when compared with the centrifuged sample, was 0.6.

b) Conclusion

The above results concluded that both 0.45 µm PVDF and 0.45 µm Nylon filters were compatible.

V. RESULTS & DISCUSSION

An RP-HPLC method for estimation of Linezolid was developed and validated as per ICH guidelines. A simple, accurate and reproducible reverse phase HPLC method was developed to estimate Linezolid in Linezolid tablet formulations. The optimized method consists of a mobile phase consisting of methanol and pH 3.0 phosphate buffer in the ratio of (45:55, v/v) with Inert sustain C18 (250×4.6 mm, 5µ) column. The retention time of Linezolid was found to be 4.95 minutes. The developed method was validated as per ICH Q2A (R1) guideline. The proposed HPLC method was linear over 30.21-90.62 µg/mL, and the correlation coefficient was found to be 1.000. The relative standard deviation for method precision was found to be 0.9%.

The accuracy studies were shown as % recovery for Linezolid at 50%, 100% and 150% levels. The limit of % recovered is shown in the range of 98 and 102%, and the results obtained were found to be within limits. Hence the method was found to be accurate.

The solution stability of the standard and samples are stable up to 48 hrs on benchtop and refrigerator (2-8°C). Performed the filter validation for sample solution both 0.45 µm PVDF and 0.45 µm Nylon filters were compatible.

VI. CONCLUSION

We have developed a fast, simple and reliable analytical method for the determination of Linezolid in pharmaceutical preparation using RP-HPLC, as there is no interference between blank and placebo at the retention time of Linezolid. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements.

The method is simple, reproducible, and with good accuracy and linearity. It allows the analysis of Linezolid in its different pharmaceutical dosage forms reliably.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Chemistry, Acharya Nagarjuna University, Guntur. Andhra Pradesh, India, for providing facilities to carry out this research work.

CONFLICT OF INTERESTS

The authors claim that there is no conflict of interest.

REFERENCES

[1] Zurenko G. E, Gibson J. K, Shinabarger D. L, Aristoff P. A, Ford C.W, Tarpley W. G, Novel Oxazolidinone–Quinolone Hybrid Antimicrobials. Curr. Opin. C. Pharmacol. 1 (2001) 470–476.

[2] Brickner S. J, Oxazolidinone Antibacterial Agents. Curr. Pharm. Des. 2 (1996) 175–194.

[3] Xu G. Y, Zhou Y, Xu M. C, A Convenient Synthesis of Antibacterial Linezolid from (S)-Glyceraldehyde Acetonide. Chin. Chem. Lett. 17 (2006) 302–304.

[4] Silva P. S. L, Neto H. M, Sejas L. M, Development and Validation of an Agar Diffusion Assay Stability-Indicating to Determination of the Potency of Linezolid in Tablets in the Presence of Photodegradation Products. Braz. J. Infect. Dis. 11 (2007) 297–299.

[5] Reis A. O, Cordeiro J. C. R, Machado A. M. O, Sader H. S, Determination of Linezolid and its Achiral Impurities Using Sweeping Preconcentration by Micellar Capillary Electrophoresis. Braz. J. Infect. Dis. 5 (2001) 243–251.

[6] Abb J, Bacterial Infection Following Refractive Surgery. Diagn. Microbiol. Infect. Dis. 43 (2002) 319–321.

[7] Patel R, Rouse M. S, Piper K. E, Steckelberg J. M, Diagn. Linezolid Therapy of Vancomycin-Resistant Enterococcus faecium Experimental Endocarditis. Microbiol. Infect. Dis. 34 (1999) 119–122.

[8] Wilson A. P, Cepeda J. A, Hayman S, Whitehouse T, Singer M, Bellingan G, In vitro Susceptibility of Gram-Positive Pathogens to Linezolid and Teicoplanin and Effect on Outcome in Critically Ill Patients. J. Antimicrob. Chemother. 58 (2006) 470–473.

[9] Ba B. B, Binso B. B, Quentin C, Saux M. C, Determination of Linezolid in Growth Media by High-Performance Liquid Chromatography with On-Line Extraction. J. Chromatogr. B. 854 (2007) 104–108.

[10] Buerger C, Joukhadar C, Muller M. M, Kloft C, Penetration of Linezolid into Soft Tissues of Healthy Volunteers after Single and Multiple Doses. J. Chromatogr. B. 796 (2003) 155–164.

[11] Tobin C. M, Sunderland J, White L. O, MacGowan A. P, Ventriculitis Due to a Hetero Strain of Vancomycin Intermediate Staphylococcus aureus (hVISA) Successful Treatment with Linezolid in Combination with Intraventricular Vancomycin. J. Antimicrob. Chemother. 48 (2001) 605–608.

[12] Borner K, Borner E, Lode E. H, Inter. Penetration of Linezolid into Soft Tissues of Healthy Volunteers after Single and Multiple Doses. J. Antimicrob. Agents. 18 (2001) 253–264.

[13] Stefanie S, Michael O, Konstantinos A, Heinrich K. G, Markus A. W, Torsten H. T, A Simple Isocratic HPLC Assay to Determine Linezolid Concentrations in Different Biomatrices for In vivo and In vitro Studies. J. Chem. Lab Med. 45 (2007) 1019–1022.

[14] Boak L. M, Jian L, Nation R. L, Rayne C. R, High-Performance Liquid Chromatographic Method for Simple and Rapid Determination of Linezolid in Human Plasma. J. Biomed. Chrom. 20 (2005) 782–786.

[15] Tobin C. M, Sunderland J, Lovering A. M, MacGowan A. P, A High-Performance Liquid Chromatography (HPLC) Assay for Linezolid in Continuous Ambulatory Peritoneal Dialysis Fluid (CAPDF). J. Antimicrob. Chemother. 51 (2003) 1041–1042.

[16] Cavazos-Rocha N, Vera-Cabrera L, Welsh-Lozano O, Waksman-De-Torres N, Salazar-Cavazos M. L, Simultaneous Determination and Validation of Antimicrobials in Plasma and Tissue of Actinomycetoma by High-Performance Liquid Chromatography with Diode Array and Fluorescence Detection. J. Pharm. Biomed. Anal. 43 (2007) 1775–1781.

[17] Bebaway L. I, Spectrophotometric and HPLC Determination of Linezolid in the presence of Its Alkaline-Induced Degradation Products and Pharmaceutical Tablets. Anal. Lett. 36 (2003) 1141–1161.

[18] Lopes C. C. G. O, Salgado H. R. N, Development and Validation of an Agar Diffusion Assay Stability-Indicating to Determination of the Potency of Linezolid in Tablets in the Presence of Photodegradation Products. Chromatographia. 69 (2009) S129–S135.

[19] Reddy K. V. S. R, Rao M. S, Reddy O. G, Suresh T, Moses Babu J, Dubey P. K, Vyas K, Isolation and Characterization of process-related Impurities in Linezolid. J. Pharm. Biomed. Anal. 30 (2002) 635–642.

[20] Michalska K, Pajchel G, Tyski S, Determination of Linezolid and Its Achiral Impurities Using Sweeping Preconcentration by Micellar Capillary Electrophoresis. J. Pharm. Biomed. Anal. 48 (2008) 321–330.

[21] Bebaway L. I, Spectrophotometric and HPLC Determination of Linezolid in the presence of its Alkaline-Induced Degradation Products and Pharmaceutical Tablets. Anal. Lett. Talanta. 60 (2003) 945–954.

[22] Agrawal H, Mahadik K. R, Paradkar A. R, Kaul N, Stability Indicating HPTLC Determination of Linezolid as Bulk Drug and in Pharmaceutical Dosage Form. J. Med. Econ. 29 (2003) 1119–1126.

[23] ICH, Stability Testing of New Drug Substances and Products (Q1AR2), ICH Harmonised Tripartite Guideline, ICH: Geneva. (2003).