Macedonian pharmaceutical bulletin, 68 (Suppl 1) 99 - 100 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.045
Short communication

A validated isocratic RP-HPLC method for determination of linezolid in pharmaceutical dosage forms

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Introduction

Linezolid is an oral and parenteral antibiotic that belongs to a new group of synthetic antibiotics known as fluorinated oxazolidinones. It is indicated for Gram–positive infections and has been approved for vancomycin-resistant enterococcal infections, including bacterial pneumonia, skin and skin tissue infections, and infections related to susceptible organisms complicated by bacteremia (Hashemian et al., 2018).

Linezolid has been assayed in dosage forms by spectrophotometry, liquid chromatography, high–performance thin–layer chromatography and micellar electrokinetic chromatography (Mohapatra et al., 2011). However, there is yet no monograph on linezolid in the current European Pharmacopoeia. Therefore, we aimed to develop simple, fast and reliable RP-HPLC method for determination of linezolid in dosage forms in the presence of its degradation products. The method performance was further fully validated according to requirements in the ICH Q2(R1) Guideline (ICH, 2019).

Materials and methods

The method was developed using Shimadzu Nexera–I LC-2040C 3D Plus Ultra-High-Performance Liquid Chromatography system equipped with quadruple pump, automatic sampler and PDA detector. All data was processed with the LabSolutions 5.106 Version software.

Chromatographic separation was performed on a reversed-phase column Agilent ZORBAX SB C18 (250 x 4.6 mm I.D., particle size 5 µm), in an isocratic mode. The mobile phase consisted of a mixture of methanol and water acidified with o-phosphoric acid, pH 2.6, 50:50 (*V/V*). The flow rate was kept at 1.0 mL/min. Wavelength was selected by scanning a standard solution of linezolid over 200–400 nm using Model Lambda 12 (Perkin Elmer) UV-visible spectrophotometer and the wavelength of 254 nm was chosen for detection of linezolid. The injection volume was 20 µL. All separations were performed at a temperature of 30°C ± 2°C.

Linezolid USP reference standard and Linezolid related compound C were used in the study. Methanol and o-phosphoric acid were purchased from Merck (Darmstadt, Germany). Double-distilled water was used to prepare the solutions. Samples of Linezolid 2 mg/mL solution for infusion were obtained commercially.

Standard and sample preparation

The working concentration of the standard and the sample solution was 0.12 mg/mL. The standard solution was prepared by dissolving the linezolid reference standard with mobile phase. To prepare the sample solution, 3 mL volume of Linezolid 2 mg/mL solution for infusion was transferred into a volumetric flask and diluted with the mobile phase up to 50 mL. Before the injection in the HPLC system, both the standard and sample solutions were filtered through a 0.45-µm nylon syringe filter.

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Results and discussion

The selection of chromatographic conditions and mobile phase composition were based on system suitability parameters (resolution, tailing factor), run time and ease of preparation.

The method specificity was demonstrated with the resolution (Rs = 4.01) between the peaks obtained from the linezolid active substance and its closest eluted impurity, linezolid related compound C ((S)-5-(Aminomethyl)-3-(3-fluoro-4-morpholinophenyl) oxazolidin-2-one

The linearity of the method was evaluated by linear regression analysis and calculated by the least-square regression method. The calibration curve was constructed with 8 concentration levels ranging from 27.11 – 325.32 µg/mL. We obtained the following equation: y = 58922x – 28075, and concluded that the method is linear in the given range, with a correlation coefficient of 0.9999 (R^2 = 0.9999).

The precision of the methods was determined by repeatability (intraday precision) and intermediate precision (interday precision) and was expressed as relative standard deviation (RSD, %) of a series of measurements. The repeatability was evaluated by assaying 6 replicates of sample solution at the working concentration (0.12 mg/mL) on the same day. The intermediate precision was studied by comparing the results obtained on three different days. Based on the results for RSD (0.06% and 0.11%, for repeatability and intermediate precision, respectively), we concluded that the proposed method has acceptable repeatability and intermediate precision (RSD < 2%).

Accuracy is the degree of agreement between a measured value and the accepted reference value. Accuracy of the method was determined by calculating recovery of linezolid by standard addition method. Known amount of linezolid (54.2 – 277.8 µg/mL) was added to pre quantified sample solution and the amount of linezolid was determined. The average recovery was 100.77% (99.31 % – 101.43%) which confirmed the method accuracy.

Robustness is the ability to provide accurate and precise results under a variety of conditions. In order to measure the extent of method robustness, the most critical parameters (column temperature and flow rate) were deliberately changed while keeping the other conditions constant. We calculated the RSD of the tailing factor and retention time of the linezolid in the chromatograms after deliberately changing the working temperature (30°C - 2°C) and flow rate (1.0 mL/min ± 0.1 mL/min). The results of robustness study (RSD ranging from 0.12 % - 0.69%) indicated that the proposed method is robust (RSD ≤ 2%).

System suitability testing is an integral part of liquid chromatographic method validation performed to check and ensure on-going performance of a chromatographic system. It was estimated by 6 repeated injections of working standard solution at 100% of test concentration (0.12 mg/mL linezolid) and evaluated through the folowing parameters: capacity factor, tailing factor and theoretical plate. The results obtained for capacity factor (5.5), tailing factor (0.967) and theoretical plate (7040) confirmed that the proposed HPLC system is suitable for determination of linezolid in the phramaceutical dosage forms.

Conclusion

The proposed RP–HPLC method allows simple, accurate and precise determination of linezolid in pharmaceutical dosage forms, in the presence of its degradation products and related compounds. The advantages of the method include short run time, simple sample and mobile phase preparation, isocratic mode of elution, and excellent peak symmetry. Therefore, the developed method can be applied for the routine analysis for determination of linezolid in pharmaceutical dosage forms in quality control laboratories.

References

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