RESEARCH PAPER

Development and Validation for Assessing the Related Substance of Linezolid by Rp-Hplc Method in Bulk and Injection Formulation

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Received: 16th Dec, 2025; Revised: 8th Feb 2026; Accepted: 12th Feb, 2026; Available Online: 28th Feb, 2026

ABSTRACT
A reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of related substances in Linezolid, an oxazolidinone class antibiotic, in both bulk and injection formulations. The method employed a Symmetry Shield C18 column (150 mm x 4.6 mm, 3.5 µm), with a mobile phase consisting of potassium dihydrogen phosphate buffer (pH 3.0 adjusted with ammonia and trifluoroacetic acid) and methanol, in a gradient elution mode. The analyte and impurities were detected at 254 nm. The method was validated as per ICH Q2(R1) guidelines and demonstrated excellent specificity, sensitivity, accuracy, linearity, and precision. Linearity was observed over a range of 0.06 to 11.18 ppm (r > 0.999). Recovery studies showed values between 105% and 116%. The developed method proved robust and stable for both standard and sample solutions, confirming its utility in routine quality control and stability studies of Linezolid formulations.
Keywords: Linezolid, RP-HPLC, Related Substances, Method Validation, Impurity Profiling, ICH Q2(R1), Bulk Drug, Injection Formulation.
How to cite this article: Raja D K, Gandhimathi R, Gandla K, Repudi L, Jaini PK, Chagarlamudi K. Development and Validation for Assessing the Related Substance of Linezolid by RP-HPLC Method in Bulk and Injection Formulation. Int J Drug Deliv Technol. 2026;16(18s): 994-998. DOI: 10.25258/ijddt.16.18s.112
Source of support: Nil.
Conflict of interest: None

INTRODUCTION
Linezolid is a synthetic antibiotic that belongs to the oxazolidinone class, with potent activity against Gram-positive bacteria, including multi-drug resistant strains like MRSA and VRE[1,2]. It is primarily used in the treatment of infections such as pneumonia, skin infections, and bacteremia. The accurate quantification of Linezolid-related impurities is crucial for ensuring drug safety and efficacy. Regulatory authorities, including the ICH, emphasize the detection and control of related substances to ensure pharmaceutical product quality[3]. The current study focuses on the development and comprehensive validation of an RP-HPLC method capable of identifying and quantifying related substances of Linezolid in bulk and injection formulations. The method was aimed to be robust, specific, linear, and precise for application in quality control laboratories.

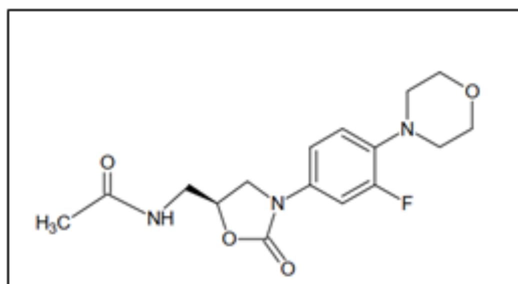

Figure 1. Linezolid Structure

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MATERIALS AND METHODS

2.1 Chemicals and Reagents: Linezolid reference standard and certified impurity standards (Impurities A–I, 5-HMF, N-oxide, etc.) were sourced from qualified vendors. Methanol, acetonitrile, trifluoroacetic acid, potassium dihydrogen phosphate, and ammonia solution of HPLC grade were used. Milli-Q water was used throughout the study.

2.2 Instrumentation: The analysis was carried out using a Waters Alliance HPLC system equipped with UV detection at 254 nm, and controlled by Empower software. The stationary phase used was Symmetry Shield C18 (150 mm × 4.6 mm, 3.5 µm).

2.3 Chromatographic Conditions: - Mobile Phase A: Potassium dihydrogen phosphate buffer with pH adjusted to 3.0 using ammonia and TFA - Mobile Phase B: Methanol - Gradient mode: Optimized for best separation - Flow Rate: 1.0 mL/min - Injection Volume: 10 µL - Column Temperature: 45°C - Sample Cooler Temperature: 25°C

2.4 Preparation of Solutions: Standard, sample, and impurity solutions were prepared using validated protocols. Diluent was a mixture of Mobile Phase A and methanol (1:1). Sensitivity and linearity solutions were prepared over a wide concentration range.

RESULTS AND DISCUSSION

3.1 System Suitability: System suitability testing was conducted before analysis to confirm system performance. Linezolid exhibited a sharp, symmetrical peak with a tailing factor between 1.0–1.05, indicating good peak symmetry. The number of theoretical plates exceeded 145,000, confirming the high efficiency of the column and optimized mobile phase. These results suggest that the system is capable of delivering consistent and reliable chromatographic performance for Linezolid and its impurities.

3.2 Specificity: The method was shown to be specific for Linezolid and its impurities, with no interference from blank, placebo, or excipients at the retention time (~42.4 min) of the active compound. Known impurities were clearly separated from the Linezolid peak, confirming the method's selectivity. Chromatograms of spiked samples indicated resolution factors >2.0 between all critical pairs. The specificity of the method is critical for ensuring accurate quantification of impurities in real samples.

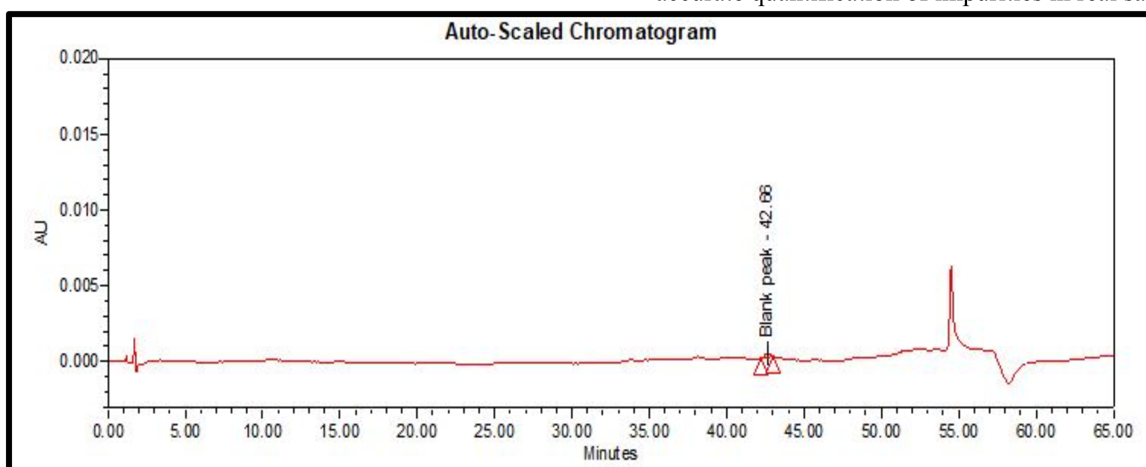

Figure 2. *Chromatogram of blank*

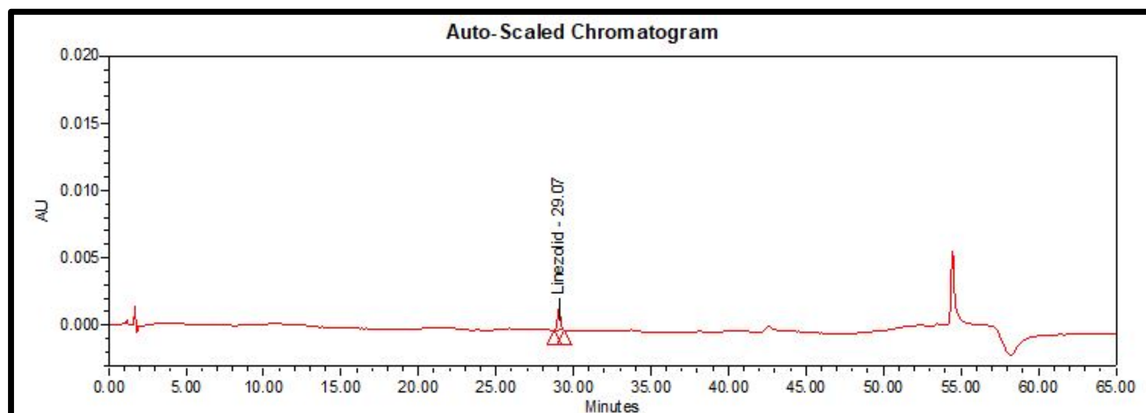

Figure 3. Chromatogram of sensitivity solution

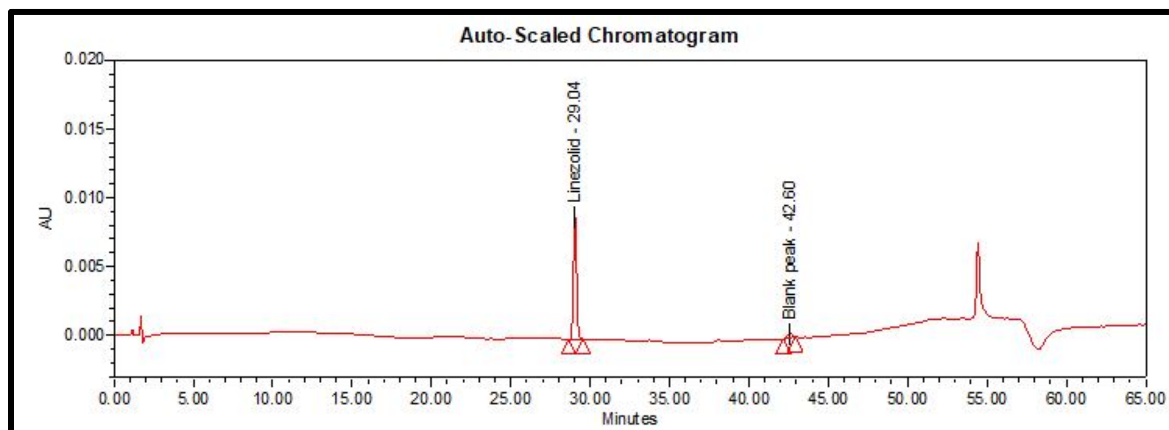

Figure 4. Chromatogram of standard solution

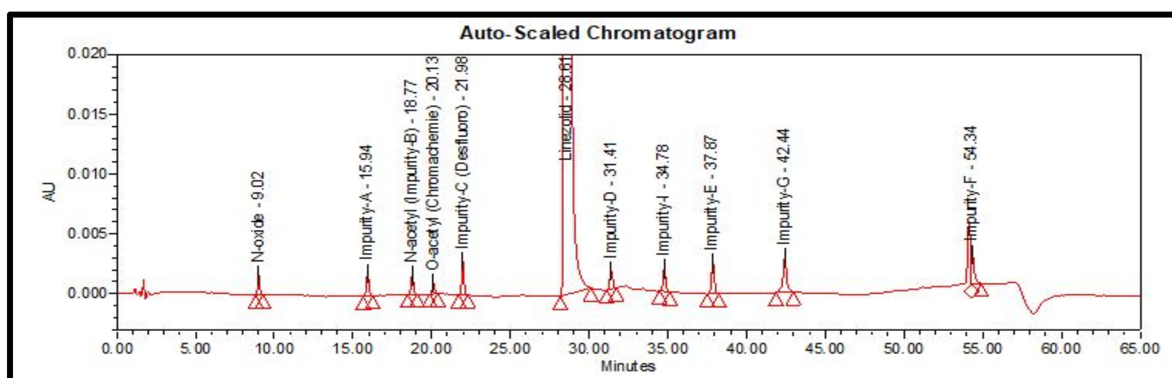

Figure 5. Chromatogram of mixed impurity sample showing Linezolid and separated impurity peaks

3.3 Linearity: Linearity was evaluated over a wide concentration range for Linezolid and its impurities. Calibration curves were constructed by plotting peak area versus concentration. Correlation coefficients (r^2) were all >0.999, confirming linear responses for all analytes across tested ranges [4,5].

Table 1. Linearity data

Analyte	Linearity Range (ppm)	Correlation Coefficient (r^2)
Linezolid	0.06 – 11.18	0.9999
Impurity A	0.06 – 11.00	0.9998
Impurity B	0.07 – 10.50	0.9999
N-oxide	0.05 – 10.00	0.9997
O-acetyl	0.06 – 9.50	0.9996
5-HMF	0.06 – 10.00	0.9999

3.4 Precision: System precision was evaluated by injecting six replicates of the standard solution. Method precision was assessed using six individual preparations of the same sample. %RSD values were below 2% for Linezolid and all impurities, indicating excellent precision of the method.

Table .2. Precision Study

Impurity	% RSD (n=6)
Linezolid	0.80
N-oxide	1.92
Impurity A	1.68
Impurity B	2.14
O-acetyl	2.95

3.5 Accuracy: Accuracy was established by recovery studies at four concentration levels: LOQ, 50%, 100%, and 150% of target analyte concentration. The recovery of each impurity was within the acceptable range of 98–120% [6]. This indicates that the method is accurate for quantifying impurities in Linezolid injection.

Table.3 Accuracy study

Impurity	Level	% Recovery	% RSD
Descarbonyl	100%	110.3%	2.10
N-Acetyl	100%	112.8%	2.57
N-Desacetyl N-Acetyl	100%	116.2%	3.18

3.6 LOD and LOQ: The LOD and LOQ were established based on signal-to-noise ratios of 3:1 and 10:1, respectively. LOD was found to be 0.015%, and LOQ 0.050%. These values were experimentally verified and are suitable for trace-level detection of impurities in formulation samples [7].

3.7 Solution Stability: Stability testing revealed that standard solutions were stable for up to 8 days, and sample solutions for 66 hours at room temperature (25 ± 3°C). No significant degradation or change in peak area was observed during the stability period [8].

3.8 Robustness: Robustness was tested by making deliberate changes to flow rate (±0.2 mL/min), column temperature (±5°C), and detection wavelength (±2 nm). The method remained unaffected by these small changes, demonstrating its robustness for routine analysis [9].

CONCLUSION

A simple, specific, robust, and validated RP-HPLC method was successfully developed for the estimation of related substances in Linezolid bulk and injection formulations. The method fulfilled all ICH Q2(R1) criteria including accuracy, linearity, precision, specificity, LOD, LOQ, stability, and robustness. The developed method is suitable for routine impurity profiling, stability monitoring, and regulatory compliance in pharmaceutical quality control.

ACKNOWLEDGMENTS

The author is highly obliged to Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu, India and Chaitanya Deemed to be University, Hyderabad, Telangana for providing the required facilities and their constant encouragement and support for us throughout the entire and also thankful to Lee Health Pharm.Pvt.Ltd.Hyderabad, India. For gifted standards samples of Linezolid and its formulations

CONFLICT OF INTERESTS

No conflict of interest was declared.

AUTHOR CONTRIBUTIONS

All of the authors made major contributions to this work, took part in its review and editing, and gave their final approval for publishing which can be used to confirm their research profiles. The writers' ORCID identifies, are listed below:

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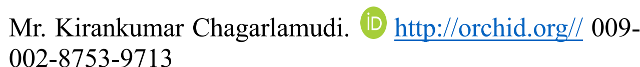
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