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Saudi Journal of Medical and Pharmaceutical Sciences
Abbreviated Key Title: Saudi J Med Pharm Sci
ISSN 2413-4929 (Print) | ISSN 2413-4910 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article **Medicine**

Development and Validation of Stability- Indicating RP-HPLC Method for Determination of Impurities in Linezolid Oral Suspension Dosage Forms

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DOI: https://doi.org/10.36348/sjmps.2025.v11i12.015 | **Received:** 28.10.2025 | **Accepted:** 22.12.2025 | **Published:** 24.12.2025

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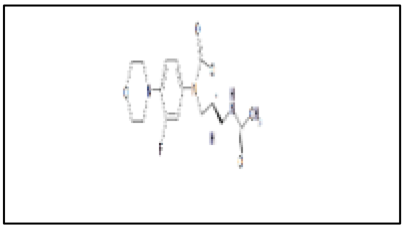
Abstract

A quality by design (QbD)-based on high resolution, stability-indicating high performance liquid chromatography (HPLC) method was developed for determining the impurities in Linezolid oral suspension dosage forms. Using this method six known were impurities qualified, and two degradants were quantified with excellent peak resolution. Using p^H 3.0 0.02M potassium phosphate buffer as buffer. For mobile phase-A consisted of 80% buffer, acetonitrile 10%, and 10% methanol in the ratio of 80:10:10(v/v/v), while mobile phase B consisted of methanol and buffer in the ratio of 60:40, (v/v) methanol and buffer. The column was maintained at a temperature of 30°C, with a flow rate of 1.0 mL/min. UV wavelength at 254 nm. The method exhibited high specificity and demonstrated linearity over the concentration range of 0.502–6.025 µg/mL, with a correlation coefficient (r^2) greater than 0.999. Accuracy exceeded 97%. The method was validated in accordance with the guidelines established by the International Council for Harmonisation (ICH), covering parameters such as specificity, linearity, precision, accuracy, and robustness. Forced degradation studies revealed that Linezolid oral suspension was sensitive to acid and oxidative stress conditions. The developed method is considered suitable for routine quality control applications, including impurity profiling and stability-indicating analysis of Linezolid oral suspension.

Keywords: HPLC, Method development, Validation, PDA.

1.INTRODUCTION

Linezolid is a synthetic antibiotic belonging to the oxazolidinone class, used as both an antibacterial and anti-infective agent. It is primarily indicated for the treatment of serious infections caused by Gram-positive bacteria that are resistant to multiple antibiotics. Chemically, it is known as N-[[(5S)-3-[3-fluoro-4-(4-morpholinyl) phenyl]-2-oxo-5-oxazolidinyl] methyl] acetamide (see Figure 1 for Linezolide and related impurities).



Linezolid

Citation: Nagajyothi Bhavanam, Govardhan Rao Thalluri, Ramakrishna Myneni, Nithin kumar Pallepati, Pranitha Sambu, Navaneeswar Reddy Kalluru (2025). Development and Validation of Stability- Indicating RP-HPLC Method for Determination of Impurities in Linezolid Oral Suspension Dosage Forms. *Saudi J Med Pharm Sci, 11*(12): 1236-1244.

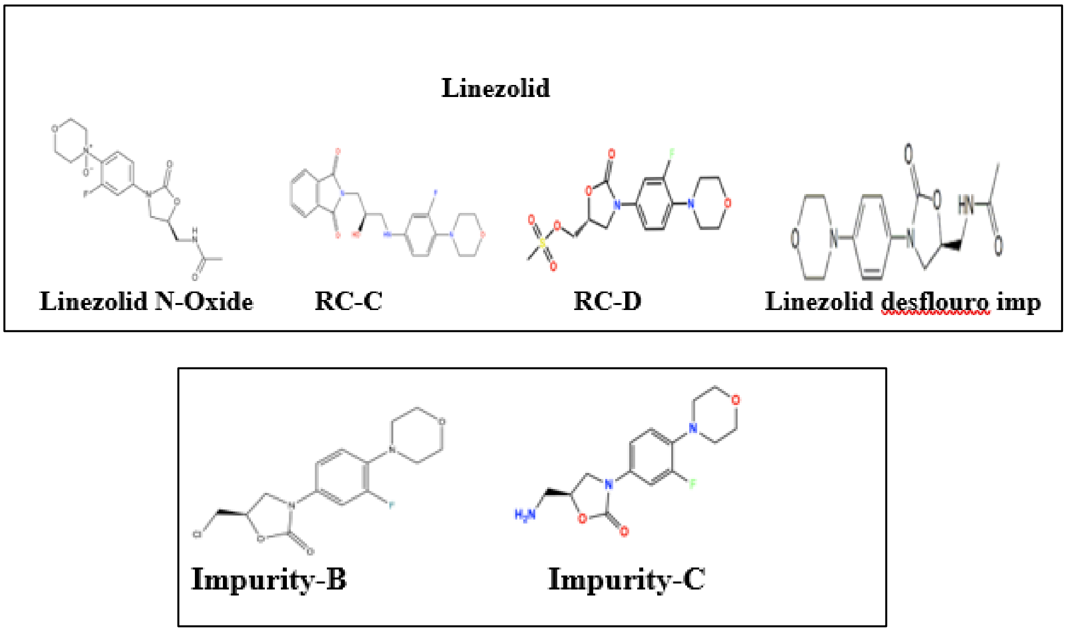


Figure 1: Chemical Structure of Linezolid and its impurities

It is freely soluble in water and alcohol, with a molecular formula of $C_{16}H_{20}FN_3O_4$. Linezolid is commonly prescribed for Gram-positive bacterial infections (Ahirrao *et al.,* 2021). A comprehensive review of the literature indicates that only a limited number of analytical methodologies have been reported for the determination of Linezolid and its related impurities. The documented methods for the quantification of Linezolid and associated degradation products are scarce and encompass a variety of analytical techniques. These include a micellar electrokinetic capillary chromatography (MEKC) method employe by UV detection (Michalska *et al.,* 2008), a liquid chromatography (LC) method (Raju *et al.,* 2012), and a high-performance liquid chromatography (HPLC) method (Reddy, K. et.,2002). A critical evaluation of the existing literature reveals that most of the high-performance liquid chromatography (HPLC) methods developed for the analysis of Linezolid, including those cited in official pharmacopoeias, employ Chiral pack, C18, or polymeric stationary phases. However, these methods frequently suffer from limitations such as poor peak symmetry and significant tailing, which compromise analytical performance. Considering these challenges, the present study was undertaken to develop a simple, robust, and reliable reversed-phase HPLC (RP-HPLC) method in accordance with the guidelines established by the International Council for Harmonisation (ICH), optimized for a C18 column and operable under reduced column temperature conditions. The study further aims to perform the comprehensive forced degradation studies as per ICH Q2(R2) guidelines and to validate a stability-indicating method for the quantification of Linezolid and its related impurities in oral suspension dosage forms. To the best of our knowledge, this investigation constitutes the first report of a validated stability-indicating RP-HPLC method specifically developed for Linezolid and its impurities in oral suspension formulations. Furthermore, forced degradation experiments conducted under various stress conditions confirmed the specificity of the method, with no interference observed from degradation products, excipients, or unidentified peaks.

2.MATERIALS AND METHODS

2.1 Chemical and reagents

The AR grade Potassium di hydrogen phosphate, Orthophosphoric acid were procured from VWR chemicals, USA. The HPLC grade of Acetonitrile and Methanol (J.T. beaker) with certified purity of 99.9% was purchased from Avantor performance materials, LLC, Radnor, PA, USA. High quality In-House purity water was used for the experiments (TOC <500ppb, pH about 7.0, Conductivity < 1.0 µs/cm, finally exposed to UV radiation and followed filtered through 0.2 µm filter). Linezolid and impurities were procured from Synzeal laboratories private limited, Ahmedabad, India.

2.2 Instrumentation and Software

Waters HPLC system Alliance e2695 separation module with auto injector, temperature controller for sample storage and column was used for current analysis. The signal output was observed through Empower 3 Software Build 3471 SPs Installed: Feature Release 3 DB ID: 2639633283. The LC column was Hypersil BDS C18, 250 x 4.6 mm, 5 µm, is manufactured by Thermo scientific. Analytical balance model AX205 (make: Mettler Toledo), sonicator (make: ENERTECH), Rotary shaker (make: REMI; model: RS – 24BL) were employed in this work.

2.3 Preparation of 0.02M potassium phosphate buffer solution

Weighed and transferred 2.72g of potassium di hydrogen phosphate into 1000ml of water and adjusted the pH 3.0 with diluted Orthophosphoric acid solution and mixed well.

Preparation of mobile phase-A

Mixed 800mL of buffer ,100mL of acetonitrile and 100mL of methanol in the ratio of 80:10:10 (v/v/v) and mixed well.

2.4 Preparation of mobile phase-B

Mixed 600ml of methanol and 400ml of buffer in the ratio of 60:40(v/v) and mixed well.

2.5 Preparation of diluent

Mixed 800 mL of buffer, 100ml of Acetonitrile and 100ml of methanol in the ratio of 80:10:10 (v/v/v) mixed well.

2.6 Preparation of standard stock solution

Accurately weighed and transferred approximately about 10 mg of Linezolid standard into a 250-mL volumetric flask. Added about 150 mL of diluent, sonicated to dissolved, diluted to volume with diluent, mixed well. The final concentration was approximately 40 µg/mL of Linezolid.

2.7 Preparation of standard solution (2µg/mL of Linezolid)

Pipetted out 5.0 mL of Linezolid Standard Stock Solution into a 100-mL volumetric flask, and diluted to volume with diluent, and mixed well. The final concentration was approximately 2µg/mL of Linezolid.

2.8 Chromatographic conditions

Chromatographic separation was achieved using 0.02 M monobasic potassium phosphate, dissolved in 1000 mL of water and adjusted to pH 3.0 with phosphoric acid, as the buffer solution. Mobile phase A consisted of a mixture of 800 mL of buffer, 100 mL of methanol, and 100 mL of acetonitrile in the ratio of 80:10:10 (v/v/v). Mobile phase B was composed of 600 mL of methanol and 400 mL of buffer in the ratio of 60:40 (v/v). The flow rate was set at 1.0 mL/min. The diluent was prepared using the same composition as mobile phase A (80:10:10), v/v/v). Chromatographic separation was performed on a Hypersil BDS C18 column (250 × 4.6 mm, 5 µm) particle size. The gradient programme was followed as MP-A 95%, MP-B 5% for 0min; MP-A 75%, MP-B 25% for 15min; MP-A 0%, MP-B 100% for 28min; MP-A 0%, MP-B 100%for 45min; MP-A 95%, MP-B 5% for 55min and column temperature 30℃, run time 55min. The injection volume was 10µL and the detection of components were made at 254 nm.

2.9 Preparation of Sample (1000 µg/mL of Linezolid)

Weighed accurately and transferred about 5g(5mL) of Linezolid oral suspension into a clean, dry 100-mL volumetric flask. Added 60 mL of diluent and sonicated for about 30 minutes with intermittent shaking. Then, dilute to volume with diluent and mixed well. Centrifuged the solution at 5000 RPM for 10 minutes and filtered it through a 0.45 µm glass fiber filter, discarding the initial 5 mL of filtrate. The final concentration was approximately 1000µg/mL of Linezolid.

3. RESULTS AND DISCUSSION

3.1 Method Development and Optimization

The objective of the present investigation was to establish a simple, robust, and stability-indicating high-performance liquid chromatographic method for the reliable separation of linezolid and its associated impurities with satisfactory resolution. Linezolid was known to contain several related impurities, including Linezolid N-oxide, RC-C, Linezolid Desfluoro impurity, RC-D, impurity C, and impurity B. However, according to the Drug Master File (DMF), Not all of these impurities were classified as degradation products; only Linezolid N-oxide and RC-C have been identified as degradants.

The separation of linezolid and its related impurities in pharmaceutical oral suspension dosage forms using liquid chromatography–mass spectrometry (LC–MS) techniques presents considerable challenges with respect to specificity, primarily due to the presence of placebo components comprising various inactive excipients. In this context, simple reversed-phase high-performance liquid chromatography (RP-HPLC) methods were often preferred in quality control laboratories due to their reproducibility, accuracy, and suitability for routine analysis. Considering a buffer solution at pH 3.0 was prepared using 0.01 M potassium phosphate, with pH adjustment carried out using orthophosphoric acid. A systematic approach to method development was employed, where in individual chromatographic parameters were varied sequentially one at a time while maintaining all other conditions constant, to optimize the analytical method. Since Linezolid exhibits good solubility in polar solvents, a reversed-phase high-performance liquid chromatography (RP-HPLC) technique was selected for its analysis. The selection of the stationary phase was based on critical chromatographic performance parameters, including system backpressure, peak shape, theoretical plate number, and the reproducibility of Linezolid retention time across successive injections. Based on a comprehensive assessment, the Enable Hypersil BDS C18 column (250 × 4.6 mm, 5 µm particle size) was identified as the most suitable column for achieving the desired separation. Optimization of the mobile phase composition was guided by the chemical structure of Linezolid and its structurally related impurities. Initial method development trials were conducted under isocratic conditions using a mobile phase consisting of 2.72 g of monobasic potassium phosphate and methanol in the ratio of 60:40 (v/v). These experiments were performed on HPLC system equipped with a photodiode array (PDA) detector and a Zodiac C18 column (150 × 4.6 mm, 5 µm particle size) at a flow rate of 1.0 mL/min. However, under these conditions, coelution of all analytes were observed at a single retention time, indicating inadequate resolution for impurity profiling.

To address this, the mobile phase was modified to consist of 2.72 g of monobasic potassium phosphate (0.02 M) in 1000 mL of water, with the pH adjusted to 3.0 using orthophosphoric acid. This buffer was then mixed with methanol and acetonitrile in a 60:20:20 (v/v/v) ratio. And change the column to hypersil BDS 250×4.6 5µm. Although the main peak was eluted, several impurity peaks were not adequately resolved. To further address the resolution issue, a gradient elution program was implemented for improved separation of all impurities. Initially, mobile phase A consisted of 100% buffer, and mobile phase B comprised 100% methanol. However, the main peak separated from all peaks, but all impurity peaks were not eluted. 10% methanol was added to mobile phase A, but not all impurities were eluted. To enhance elution, 10% acetonitrile was introduced into mobile phase A, which allowed all impurities to elute; however, impurities B and C exhibited late elution. To achieve earlier elution of impurities B and C, 30% buffer was added to mobile phase B. While this adjustment led to earlier elution of B and C, overall resolution among all impurities remained inadequate. Finally, increasing the buffer content in mobile phase B to 40% resulted in satisfactory resolution of all impurity peaks.

Based on these experiments, the final optimized conditions were established. The Hypersil BDS C18 column (250 × 4.6 mm, 5 µm) was selected as the stationary phase. The elution method was gradient, with the column temperature maintained at 30°C and detection monitored at 254 nm. The injection volume was 10µL, with a flow rate of 1.0 mL/min. The typical retention time of Linezolid was approximately 24.5 minutes (see Table 1&Table 2).

Table 1: Optimized Method development conditions:

Mobile phase	**Column**	**Flow rate(ml/mint) elution mode column temp injection volume**	**Extraction solvent/diluent**	**Observation**	**Result**
0.02M potassium phosphate buffer and methanol in the ratio of (60:40) (v/v)	Zodiac C18 column (150 × 4.6 mm, 5 µm particle size)	1.0ml/mint isocratic 30°C,10µL	Water and methanol in the ration of 50:50 (v/v)	all analytes was observed at a single retention time, indicating inadequate resolution for impurity profiling	Rejected
0.02M potassium phosphate buffer pH 3.0 and methanol and acetonitrile in the ratio of (60:20:20)(v/v/v)	Hypersil BDS C18 column (250 × 4.6 mm, 5 µm particle size)	1.0ml/mint Isocratic 30°C,10µL	Water and methanol in the ration of 50:50 (v/v)	the main peak was eluted, several impurity peaks were not adequately resolved	Rejected
0.02M potassium phosphate buffer (pH 3.0), adjusted with orthophosphoric acid. Mobile phase A consisted of 100% buffer, mobile phase B consisted of 100% methanol	Hypersil BDS C18 column (250 × 4.6 mm, 5 µm particle size))	1.0ml/mint gradient 30°C,10µL	Water and methanol in the ration of 50:50 (v/v)	the main peak was eluted, several impurity peaks were not adequately resolved	Rejected
0.02M potassium phosphate buffer (pH 3.0), adjusted with orthophosphoric acid. Mobile phase A consisted of 90% buffer and 10% methanol,	Hypersil BDS C18 column (250 × 4.6 mm, 5 µm	1.0ml/mint gradient 30°C,10µL	Water and methanol in the ratio of (50:50) (v/v)	All peaks were eluted, but not separated	Rejected

mobile phase B consisted of 100% methanol	particle size)				
0.02M potassium phosphate buffer (pH 3.0), adjusted with orthophosphoric acid. Mobile phase A consisted of 80% buffer and 10% methanol and 10% Acetonitrile in the ratio of(80:10:10), mobile phase B consisted of 100% methanol	Hypersil BDS C18 column (250 × 4.6 mm, 5 µm particle size)	1.0ml/mint gradient 30°C,10µL	Mixed buffer and methanol and acetonitrile in the ratio of (80:10:10)(v/v/v),diluent changed due to interference	All peaks were separated from the Linezolid peak, but impurity B& C exhibited late elution.	Rejected
0.02M potassium phosphate buffer (pH 3.0), adjusted with orthophosphoric acid. Mobile phase A consisted of 80% buffer and 10% methanol and 10% Acetonitrile in the ratio of(80:10:10), mobile phase B consisted of 60% methanol and 40% buffer in the ratio (60:40)(v/v).	Hypersil BDS C18 column (250 × 4.6 mm, 5 µm particle size)	1.0ml/mint gradient 30°C,10µL	Mixed buffer and methanol and acetonitrile in the ratio of (80:10:10)(v/v/v),diluent changed due to interference in blank	All peaks were separated from the Linezolid peak, and impurity B& C exhibited earlier elution and all peaks resolution were satisfied	Approved

Table 2: Specificity results – Retention times and Relative retention times of specified impurities

Impurity Name/Peak Name	RT from Spike Sample Minutes)	RRT from Spike Sample
Linezolid N-Oxide	5.74	0.23
Related compound-C/Linezolid amine/Impurity-A	11.49	0.46
Linezolid desflouro impurity	16.93	0.68
Linezolid	24.59	1.00
Related compound-D	27.30	1.11
Impurity-C	38.25	1.55
Impurity-B	40.40	1.64

3.2 Method Validation

The method was validated based on International Conference on Harmonization (ICH) Q2(R2) Guidelines. (Hareesh Divadari *et al.,* 2024; Naveen Maddukuri *et al.,*2025). Validation parameters included linearity, precision, accuracy, robustness, specificity and forced degradation (Teja Kami Reddy *et al.,* 2024; Lankalapalli PK *et al.,*2025).

3.2.1 System suitability

System suitability parameters (tailing factor, number of theoretical plates) were assessed by injecting a blank diluent followed by Linezolid solution (2µg/ml). See Table 3 for system suitability results.

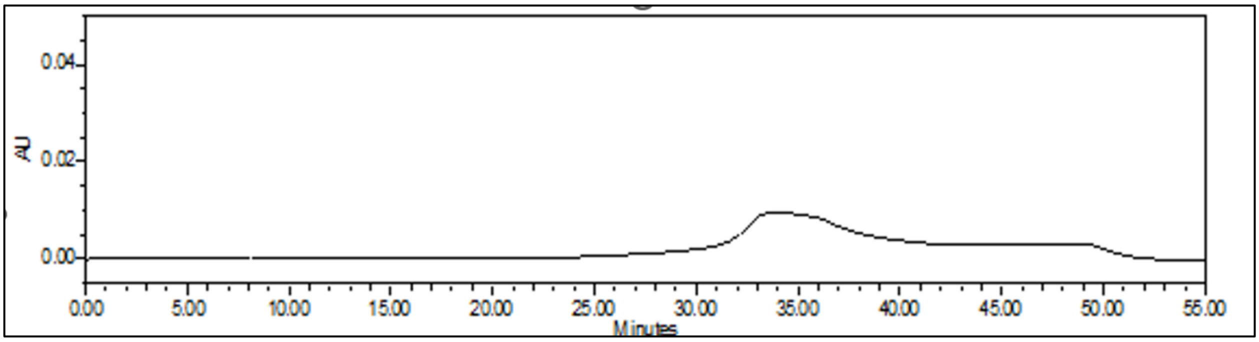
Table 3: System suitability results

Name of the parameter	Observed Value	Acceptance criteria
USP tailing factor for Linezolide peak from standard solution	1.0	NMT 2.0
USP Plate count for Linezolid peak from standard solution	125837	NLT 2500
% RSD of Linezolid peak areas for six replicate injections of standard solution	0.74	NMT 5.0%

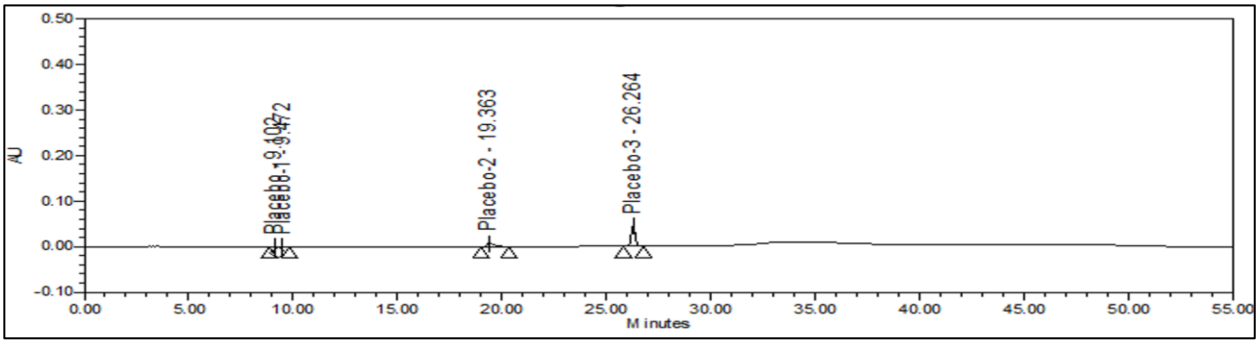
3.2 .2 Specificity

3.2.2.1 Interference test

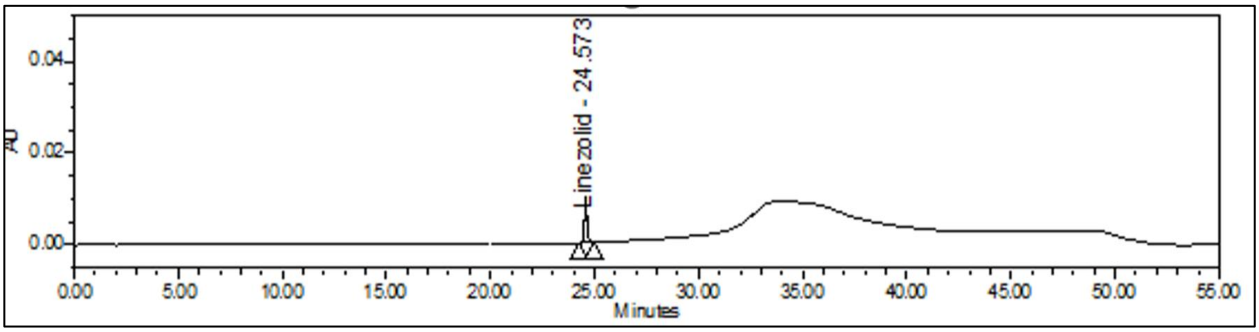
Prepared blank, placebo ,impurities (Linezolid N-Oxide, RC-C,RC-D, Desfluoro impurity, Impurity-C, Impurity-B) as per the optimized test procedure and verified the interference of excipients, and diluent peaks at retention time of active peaks (Prasanna Kumar Lankalapalli *et al.,* 2024). The results showed no interference at active peaks (see figures .2,3,4,5)



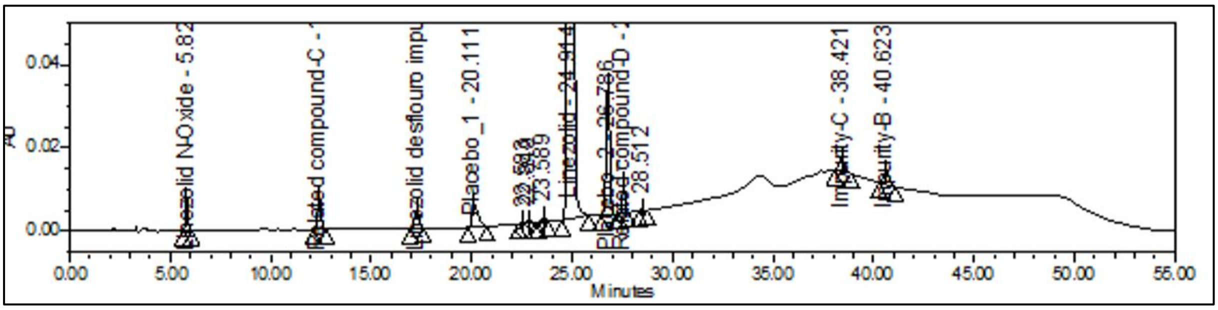
Figure#2: Typical Chromatogram of Blank



Figure#3: Typical Chromatogram of Placebo



Figure#4: Typical Chromatogram of Standard solution



Figure#5: Typical Chromatogram of Spiked sample

3.2.2.2 Degradation studies

Degradation studies were conducted to determine the specificity and stability-indicating properties of the proposed method. The stress conditions applied included acidic, alkaline, oxidative, and aqueous environments

To prove the stability indicating power of optimized analytical method performed the forced degradation in various conditions like acid, base, water, peroxide, conditions as per the current ICH Q2(R2) guidelines.

For Acid and Base samples weighed and transferred approximately 5g(about 5mL) of Linezolid oral suspension into 100 ml volumetric flask, added 5 mL of 2N HCl or 2N NaOH, mixed, kept on water bath at 60°C for about 4hr, neutralized with 5 mL of 2N

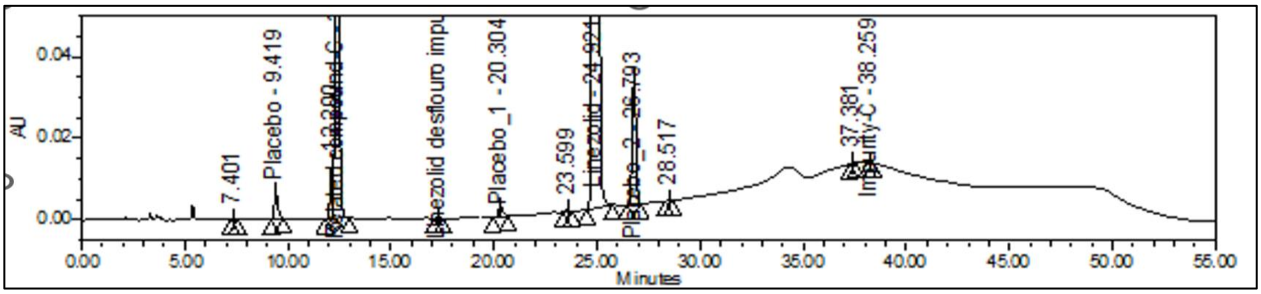
NaOH or 2N HCl, then added about 40 ml of diluent, kept on sonicator for 30 minutes with intermittent shaking. Then, dilute to volume with diluent and mixed well. Centrifuged the solution at 5000 RPM for 10 minutes and filtered it through a 0.45 µm glass fiber filter, discarding the initial 5 mL of filtrate. For peroxide sample weighed and transferred approximately 5g(about 5mL) of Linezolid oral suspension into 100 ml volumetric flask, added 5 mL of 10%peroxide solution, mixed, kept on water bath at 60°C for 4hr, then added about 40 ml of diluent, kept on sonicator for 30 minutes, diluted to volume with diluent.

For water degradation, the sample weighed and transferred approximately 5g (about 5mL) of Linezolid oral suspension into 100 ml volumetric flask, added 5 mL of water, at 60°C for 4hr on water bath. kept on sonicator for 30 minutes, diluted to volume with diluent. Above all samples Centrifuged the solution at 5000 RPM for 10 minutes and filtered it through a 0.45 µm glass fiber filter, discarding the initial 5 mL of filtrate. During the acid, Base, water, peroxide Linezolid showed major degradation in acid and oxidative conditions. All chromatograms showed no interference at the retention time of Linezolid and its impurities. The peak purities were monitored by using Empower 3 software, the purity angle is less than that of purity threshold in all stress conditions. All the conditions results were shown in

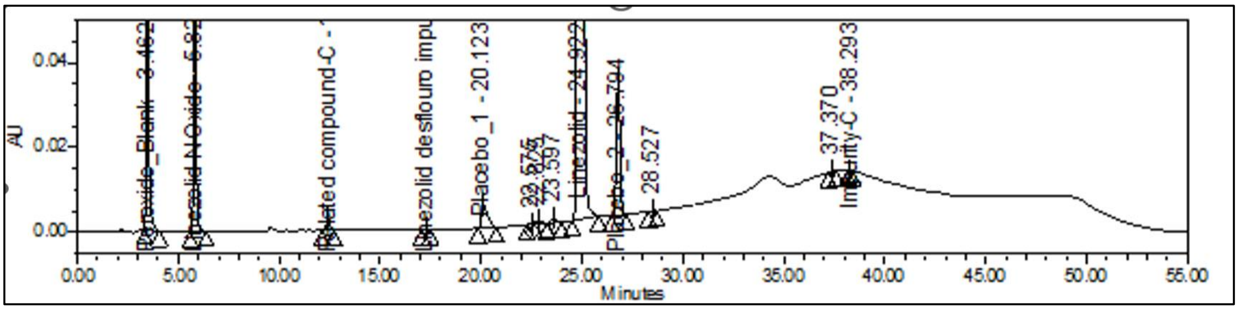
Table 4(see figures 6,7)

Table 4: Forced degradation as per ICH for Linezolid

Sample name & Condition	**Known impurities**						**Total impurities**	**% Assay**	**Total impurities+%Assay**	**Mass balance**	**Purity Angle**	**Purity Threshold**
	N-oxide	**RC-C**	**Desflouro**	**RC-D**	**Imp-C**	**Imp-B**						
Not Stressed	ND	0.04	0.010	ND	ND	ND	0.17	100.2	100.2	100.4	0.196	0.244
5mL_2N HCL_60°C_4 Hours	ND	2.59	0.01	ND	ND	ND	2.97	97.1	100.1	99.7	0.154	0.242
5mL_2N NaoH_60°C_4 Hours	ND	0.04	0.01	ND	0.01	ND	0.18	99.5	99.7	99.3	0.204	0.247
5mL_Water60°C_4Hours	3.20	0.08	0.01	ND	0.00	ND	3.42	97.7	101.1	100.7	0.202	0.247
5mL_10%Peroxide_60°C_4 Hours	ND	0.04	0.01	ND	0.00	ND	0.16	100.4	100.6	100.2	0.189	0.241



Figure#6: Typical Chromatogram of Acid sample



Figure#7: Typical Chromatogram of Peroxide sample

3.2.3 Linearity

To prove the linearity of the optimized method, a sequence of concentrations was prepared for Linezolid (0.5119 µg/mL to 4.0957 µg/mL), N-Oxide (0.5021 µg/mL to 6.0529µg/mL), Related Compound-C (0.5079µg/mL to 4.0634 µg/mL) over a concentration range of 0.05% to 200% relative to the 100% specification, using appropriate volumes of stock solutions. A calibration curve was created by plotting peak response against concentration. The component showed a correlation coefficient greater than 0. 999.The results were shown in **Table 5.**

3.2.4 Selectivity

The limit of detection (LOD) and Limit of quantification (LOQ) values for Linezolid and impurities were established by signal to noise ratio(S/N) method. The method demonstrated a high sensitivity for detecting impurities, with the Limit of Quantitation (LOQ) and Limit of Detection (LOD) observed at 0.05% and 0.02%.

3.2.5 Precision

Precision measures the degree of agreement between individual test results when applying a procedure or method to a homogeneous sample (Myneni RK *et al.,* 2025). It is typically expressed as variance or standard deviation (SD). Under normal conditions, precision reflects the degree of repeatability or reproducibility.

Six individual samples were taken from a homogeneous mixture and spiked at the 100% level to assess reproducibility. The precision of the analysis was evaluated based on the relative standard deviation (RSD) values of the six impurity-spiked sample results.

To assess ruggedness (intermediate precision), the analysis was repeated on different days. The precision of the analysis was determined for each individual impurity at the 100% concentration level. The intra-day and inter-day precision results were shown in Table 5.

3.2.6 Accuracy

The accuracy of the method was demonstrated with freshly prepared Three levels (LOQ,100% &200%). Prepared each sample in triplicate preparation range from 200% level and concentrations of N-Oxide 6.0182µg/mL and RC-C 4.0019µg/mL and six sample preparation ranges from LOQ and 100% level and concentrations of N-Oxide 0.4965µg/mL and 3.0091µg/mL and RC-C 0.4952µg/mL and 2.0009µg/mL of impurities. The recovery was calculated in terms of the amount estimated to the amount spiked. All the method validation parameters results were shown in **Table 5**.

Table 5: Method validation summary for Linezolid

Validation parameters	Linezolid	Linezolid N-Oxide	Related compound-C
RRT w.r.to Linezolid	1.000	0.23	0.46
Linearity range(µg/ml)	0.5119-4.0957	0.5021-6.0259	0.5079-4.0634
Coefficient(r^2)	0.999963	0.999991	0.999981
Slope	40955.649218	20088.170270	42522.087490
(%) Y-intercept	91.809599	270.310661	193.834901
Bias	0.1	0.4	0.2
Residual sum of squares	565471.481723	74749.262731	313156.206619
RRF	1.00	0.49	1.04
LOQ concentration(ppm)	0.517	0.496	0.495
LOD concentration(ppm)	0.207	0.198	0.198
ACC-LOQ mean, %RSD(n=6)	103.1, 1.2	101.6,1.6	100.8,1.4
ACC-100% mean %RSD(n=6)	101.2 ,0.4	102.5,1.8	101.3,1.8
ACC-200% mean %RSD(n=3)	100.1 ,0.2	101.2,2.1	102.1,2.2
Method precision %RSD(n=6)	1.0	1.3	1.1
Intermediate precision % RSD (n=12)	0.8	1.5	1.4

3.2.7 Stability of mobile phase & sample solutions

The stability of Linezolid and Impurity compounds in the precision spiked sample solution was evaluated at room temperature at every 24Hrs frequency intervals up to 72Hrs with the fresh preparation of mobile phase every time. The stability of the mobile phase was also determined by analyzing a freshly prepared precision-spiked sample solution at 24-hour intervals. It was found that the sample, standard, and mobile phase solutions remained stable for up to 48 hours at room temperature.

3.2.8 Robustness

The robustness of the proposed RP-HPLC method was carried out by altering the experimental conditions such as analytical column, column temperature, flow rate, p^H variation (± 0.2) and mobile phase composition. The method was established by introducing small changes in experimental conditions like ± 1. All the conditions results were shown in **Table 6.**

Table 6: Robustness as per ICH for Linezolid

Robustness Conditions	**RRT**		**USP plate count**	**USP Tailing**	**%RSD**
	Linezolid N-Oxide	**RC-C**			
As per Methodology	0.23	0.46	125837	1.1	0.5
Decreased flow rate: 0.9mL/min	0.24	0.49	120787	1.2	0.8
Increased flow rate: 1.1mL/min	0.24	0.46	12654	1.1	0.6
Decreased Column oven temperature: 25°C	0.25	0.45	12089	1.0	0.5
Increased Column oven temperature: 35°C	0.22	0.48	12987	1.0	0.8
Decreased pH variation: pH 2.8±0.05	0.24	0.47	12546	1.3	0.9
Increased pH variation: pH 3.2±0.05.	0.23	0.46	12345	1.0	1.2
Organic composition variation in Mobile Phase-A decreased Acetonitrile composition: Buffer: Acetonitrile: Methanol (800:90:100)	0.25	0.49	12456	1.5	1.0
Organic composition variation in Mobile Phase-A Increased Acetonitrile composition: Buffer: Acetonitrile: Methanol (800:110:100)	0.24	0.45	12786	1.3	1.2
Organic composition variation in Mobile Phase-B Decreased Methanol composition: Buffer: Methanol (400:580)	0.23	0.47	12098	1.2	0.9
Organic composition variation in Mobile Phase-B Increased Methanol composition: Buffer: Methanol (400:620)	0.24	0.48	12565	1.3	1.3

4. CONCLUSION

A simple, economical, rapid and RP-HPLC stability indicating method has been effectively optimized for quantification of impurities. The optimized method was further validated for specificity, linearity, precision and accuracy, Robustness parameters. Further, stress studies were executed under various ICH stress conditions and proved stability indicating nature. Explicitly, the method was developed and validated and in quality control lab for stability analysis.

Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data sharing is not applicable to this article as no new data was created

Acknowledgements: The authors would like to thank Aurore life science Pvt. Ltd. (India) for providing a gift sample of the standard Linezolid.

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