WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL RESEARCH

Research Article

Coden USA: WJPPCL

2026
Volume: 03
Issue: 03
Page: 01-24

METHOD DEVELOPMENT AND VALIDATION OF TEDIZOLID IN SOLID DOSAGE FORM BY RP-HPLC METHOD

Dr. K. Kaveri*[1], R. Jeevitha[2], B. Jana[2], L. Jayabharath[2], C. Jana[2], N. Jaisuriyan[2]

*[1]Principal, [2]B. Pharm Final Year Students.

Aadhibhagawan College of Pharmacy, Rantham, Thiruvannamalai, Tamil Nadu.

Received: 22 January 2026 **Revised: 10 February 2026** **Accepted: 02 March 2026**

Corresponding Author: Dr. K. Kaveri

Address: Principal, Aadhibhagawan College of Pharmacy, Rantham, Thiruvannamalai, Tamil Nadu.

Email Id: lgopipharma10@gmail.com

ABSTRACT

A simple, precise, accurate, and robust Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the quantitative determination of Tedizolid in pharmaceutical dosage forms. Method development trials were performed using different mobile phase compositions of ethanol and water on a C_{18} (250 × 4.6 mm, 5 µm) column. Among the tested conditions, the optimized chromatographic system consisted of DMSO: Buffer (90:10 v/v) in isocratic mode at a flow rate of 1.0 mL/min, with detection at 296 nm and an injection volume of 20 µL. The retention time of Tedizolid was found to be approximately 2.97 minutes.The method was validated as per ICH Q2 guidelines. System suitability parameters were within acceptable limits, with plate count around 3000 and tailing factor approximately 1.25. The method exhibited good linearity in the concentration range of 10–60 µg/mL with a correlation coefficient (r) of 0.9966. Precision studies showed %RSD less than 2% for system precision, method precision, and intermediate precision. Accuracy was confirmed by recovery studies at 50%, 100%, and 150% levels, yielding recoveries between 99.31% and 100.18%. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 22.85 µg/mL and 69.30 µg/mL, respectively. Solution stability studies indicated that the analyte remained stable for up to 16 hours.The assay of the marketed formulation (StariZo) showed 99.9% purity. The developed method was found to be specific, sensitive, reproducible, and suitable for routine quality control analysis of Tedizolid in bulk and pharmaceutical formulations.

KEYWORDS: Tedizolid, RP-HPLC, Method Development, Method Validation, ICH Q2 Guidelines, Linearity, Precision, Accuracy, LOD; LOQ, Pharmaceutical Analysis, Stability Studies.

1. INTRODUCTION

High-performance liquid chromatography (or High pressure liquid chromatography. HPLC) is a form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds. There are different modes of separation in HPLC. They are normal phase mode, reversed phase mode, reversed phase ion pair chromatography, affinity chromatography, Ion exchange chromatography and size exclusion chromatography (gel permeation or gel filtration chromatography).

Normal phase uses a polar stationary phase and a non-polar mobile phase. The polar analytes are retained for longer time and takes more time to elute because of its higher affinity towards stationary phase whereas the non-polar compounds travel faster and are eluted first because of its lower affinity towards stationary phase. Use of more polar solvents in the mobile phase will decrease the retention time of the analytes, whereas more hydrophobic solvents tend to increase retention times.

Reversed phase uses a non-polar hydrophobic packing with octyl or octa decyl functional group bonded to silica gel as stationary phase and an aqueous, moderately polar solvent as mobile phase. With the stationary phases, retention time is longer for molecules which are more non-polar, while polar molecules elute more readily. Reverse phase mode is the most popular mode for analytical and preparative separation of compounds of interest in, chemical biological, pharmaceutical, food and biomedical sciences. Most of the drugs in pharmaceuticals are polar in nature. The different columns used are octa decyl silane (ODS) or C18, C8, C4, etc., (In the order of increasing polarity of the stationary phase).

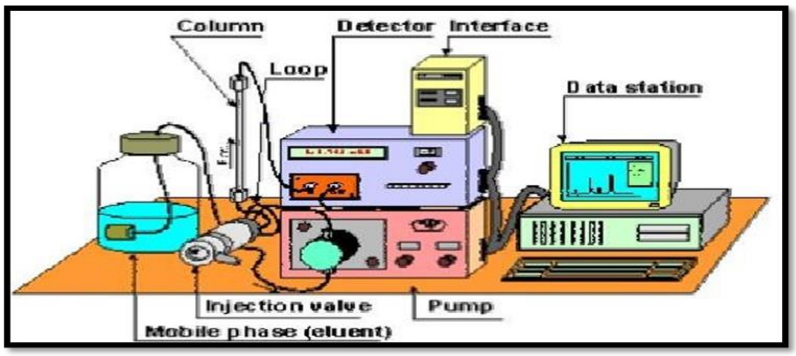


Fig. 1: Complete picture of HPLC System.

2. DRUG PROFILE

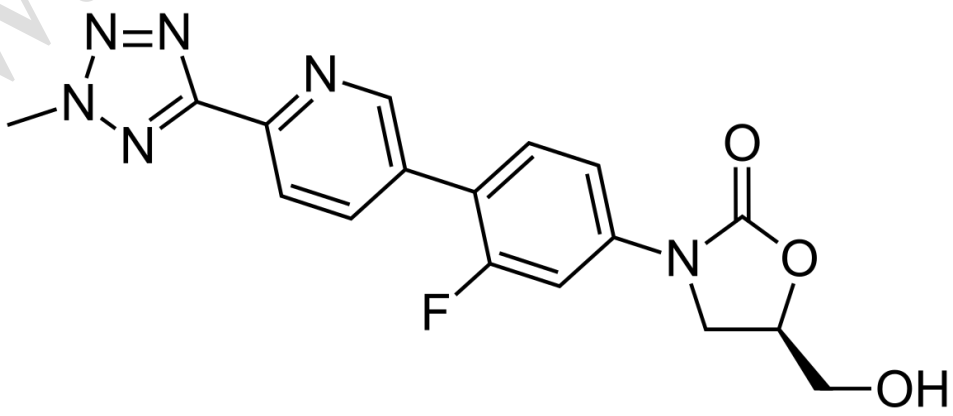
Drug Name: Tedizolid

- **IUPAC Name**: [(5R)-3-[3-fluor-4-[6-(2-methyltetrazol-5-yl) pyridin-3-yl]phenyl]-2-oxo-1,3-oxazolidin-5-yl]methyl dihydrogen phosphate
- **Molecular Formula:** C17H16FN6O6P
- **Molecular Weight:** 450.32 g/mol
- **Appearance:** White to off-white crytalline powder, odorless.
- **Melting Point:** 256.8℃
- **Solubility:** Soluble in DMSO and insoluble in water and ethanol.
- **Mono isotopic:** 450.08

Description

Tedizolid phosphate is a phosphate monoester formed by the formal condensation of equimolar amounts of phosphoric acid with the hydroxy group of tedizolid. It acts as a prodrug of tedizolid and is used to treat acute bacterial skin infections caused by certain susceptible bacteria, including Staphylococcus aureus (both methicillin-resistant [MRSA] and methicillin-susceptible strains), various Streptococcus species, and Enterococcus faecalis. It is classified into various chemical groups, such as carbamate esters, organofluorine compounds, oxazolidinones, pyridines, tetrazoles, and phosphate monoesters.

Drug interaction: Both linezolid and tedizolid are weak inhibitors of monoamine oxidase (MAO), which can theoretically result in interactions with serotonergic medications, such as selective serotonin reuptake inhibitors (SSRIs). However, tedizolid poses a lower risk of inducing serotonin syndrome compared to linezolid, making it a safer option for patients taking these drugs.



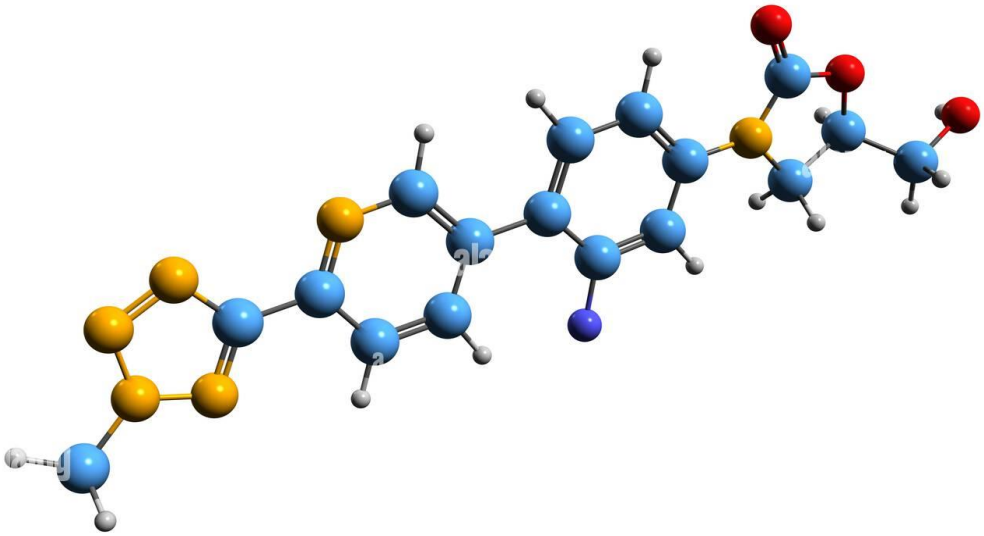


Fig. 2: Structure Of Tedizolid Phosphate.

Mechanism Of Action

Tedizolid exhibits bacteriostatic activity by inhibiting bacterial protein synthesis through its binding to the 23s ribosomal RNA of the 50s subunit. Its chemical structure is similar to that of linezolid.both are synthetic molecule containing an oxazolidinone ring (ring A) and a lateral chain at the C5 position, which enhance their activity against certain gram-positive bacteria and mycobacteria.

The primary chemical difference between the two compounds is that tedizolid contains a hydroxymethyl group in its side chain, which contributes to its activity against certain bacterial strains carrying the cfr gene. Additionally, tedizolid features a para-oriented D-ring structure, which increases its binding interactions with the peptidyl transferase center, thereby enhancing its potency compared to linezolid.

Pharmacodynamics

The antimicrobial spectrum of tedizolid (TDZ) includes clinically significant Gram-positive bacteria, such as methicillin-susceptible Staphylococcus aureus (MSSA), methicillin-resistant S. aureus (MRSA), methicillin-susceptible S. epidermidis, methicillin-resistant S. epidermidis, vancomycin-sensitive and vancomycin-resistant enterococci (VRE), penicillin-susceptible Streptococcus pneumoniae (PSSP), penicillin-resistant S. pneumoniae (PRSP), and other commonly reported cutaneous and respiratory pathogens. Most of these Gram-positive bacteria are susceptible to TDZ, with minimum inhibitory concentration (MIC) values of ≤ 0.5 mg/L.

However, compared to its activity against Gram-positive organisms, TDZ shows reduced potency against Gram-negative bacteria, such as Haemophilus influenzae (MIC: 16 mg/L) and Moraxella catarrhalis (MIC: 4 mg/L). Similar to linezolid (LNZ), TDZ binds to the 50S subunit of bacterial ribosomal RNA and inhibits protein synthesis.

Pharmacokinetics

Absorption: Tedizolid reaches peak plasma concentrations approximately three hours after oral administration and about one hour after intravenous administration. Its absolute oral bioavailability is around 91%, and food does not affect its absorption. When administered once daily, either orally or intravenously, tedizolid reaches steady-state concentrations within approximately three days. Following a single dose or at steady state, the maximum plasma concentration (Cmax) of tedizolid is 2.0 ± 0.7 mcg/mL and 2.2 ± 0.6 mcg/mL, respectively, for oral administration, and 2.3 ± 0.6 mcg/mL and 3.0 ± 0.7 mcg/mL, respectively, for intravenous administration. The time to reach maximum concentration (Tmax) has a median (range) of 2.5 hours (1.0–8.0) after oral administration and 3.5 hours (1.0–6.0) at steady state, while for intravenous administration, the median Tmax is 1.1 hours (0.9–1.5) after a single dose and 1.2 hours (0.9–1.5) at steady state. The area under the concentration-time curve (AUC) is 23.8 ± 6.8 mcg·hr/mL after a single oral dose and 25.6 ± 8.4 mcg·hr/mL at steady state, compared to 26.6 ± 5.2 mcg·hr/mL and 29.2 ± 6.2 mcg·hr/mL, respectively, for intravenous administration.

Volume of distribution: The volume of distribution for tedizolid after a single 200 mg intravenous dose ranges from 67 to 80 liters. In a study involving oral administration of 200 mg tedizolid at steady state, the volume of distribution was reported to be 108 ± 21 liters, whereas a single 600 mg oral dose resulted in an apparent volume of distribution of 113.3 ± 19.3 liters. Tedizolid has been shown to penetrate the interstitial space of both adipose and skeletal muscle tissues and is also present in the epithelial lining fluid and alveolar macrophages.

Protein binding: Approximately 70% to 90% of tedizolid binds to human plasma proteins.

Metabolism: Tedizolid is administered as a phosphate prodrug, which is converted into the active circulating form, tedizolid. Before excretion, the majority of tedizolid is metabolized in the liver into an inactive sulfate conjugate; however, this process is unlikely to involve cytochrome P450 enzymes.

Half-life: The half-life of tedizolid is approximately 12 hours.

Clearance: Tedizolid exhibits an apparent oral clearance of 6.9 ± 1.7 L/hr following a single dose and 8.4 ± 2.1 L/hr at steady state. Its systemic clearance is 6.4 ± 1.2 L/hr after a single dose and 5.9 ± 1.4 L/hr at steady state.

Side effects: Severe stomach pain, diarrhoea that is watery or bloody (even if it occurs months after your last dose)

Toxicity: Toxicity information for tedizolid is limited. In cases of overdose, patients may be at increased risk of experiencing severe adverse effects, including nausea, headache, dizziness, diarrhea, and vomiting. Symptomatic and supportive care is recommended.

Route of elimination: Following a single oral dose, approximately 82% of tedizolid is excreted in the feces and 18% in the urine. The majority is eliminated as the inactive sulfate conjugate, with only about 3% recovered unchanged. More than 85% of the elimination takes place within 96 hours.

3. MATERIALS AND METHODS

3.1 METHOD DEVELOPMENT

Diluent: Mix DMSO: Buffer in the ratio of 90:10

Mobile phase: Filtered and degassed mixture of DMSO: Buffer in the ratio of 90:10

Standard preparation: Weighed accurately about 0.100g of Tedizolid working standard into a 100ml volumetric flask, added 70ml of diluent, shake and sonicated to dissolve the content, made up the volume with diluent. Pipette out 5ml of resulting solution to 100ml volumetric flask made up with diluent. Filtered through 0.45 micron membrane filter. Collect the filtrate after discarding the few ml of the filtrate.

Assay preparation: Weigh 20 tablets, triturate to a fine powder. Weigh accurately about 0.100g powdered tablet (equivalent to 2.12g of Tedizolid) in to a 100ml volumetric flask. Added 70ml of diluent shake for 15 minutes and sonicated for 15 minutes, and made up the volume with diluent, pipette out 5ml of filtrate to 100ml with diluent. Filter the solution through 0.45 micron membrane filter. Collect the filtrate after discarding the first few ml of the filtrate.

Selection of chromatographic method: A RP C18:250x4.6mm 5µm column equilibrated with mobile phase DMSO: Potassium dihydrogen Orthophosphate (90:10%v/v, pH4) was used. Mobile phase flow rate was maintained at 1.0 ml / min. Detection wavelength 296 nm was selected by scanning standard drug over a wide range of wavelength 200 nm to 400 nm in U.V Spectroscopy. The sample was injected through 20 µl fixed loop, and the total run time was adjusted for 6 mins.

Table 1: Method Development For RP-HPLC Analysis Of Tedizolid.

S. No	Column used	Mobile phase	Isocratic/ Gradient	Inj. Vol.	Observation	Result
1.	C_{18};250x4.6mm, 5µm	DMSO: Buffer (50:50)	Isocratic	20µl	The theoretical plates are not within the limit	Method Rejected
2.	C_{18};250x4.6mm, 5µm	DMSO: Buffer (60:40)	Isocratic	20µl	The theoretical plates are not within the limit	Method Rejected
3.	C_{18};250x4.6mm, 5µm	DMSO: Buffer (70:30)	Isocratic	20µl	The tailing factor and theoretical plates are not within the limit	Method Rejected
4.	C_{18};250x4.6mm, 5µm	DMSO: Buffer (80:20)	Isocratic	20µl	The tailing factor and theoretical plates are not within the limit	Method Rejected
5.	Princeton SPHER C_{18};250x4.6mm, 5µm	DMSO: Buffer (90:10)	Isocratic	20µl	The tailing factor and theoretical plates are within the limit	Method Accepted

Table 2: Chromatographic System.

MOBILE PHASE	**Ethanol: Water (35:65)**
COLUMN	**C_{18};250 x 4.6 mm,5µm**
FLOW RATE	**1.0ml/min**
COLUMN TEMPERATURE	**Room Temperature**
WAVE LENGTH	**296nm**
INJECTION VOLUME	**20 micro liter**

3.2 ASSAY REPORT

Analysis:

Sample Name: Tedizolid Sample ID: Tedi005 File: 0023.RAW Date: 2025-10-06 PM 12:31:17

Chromatogram

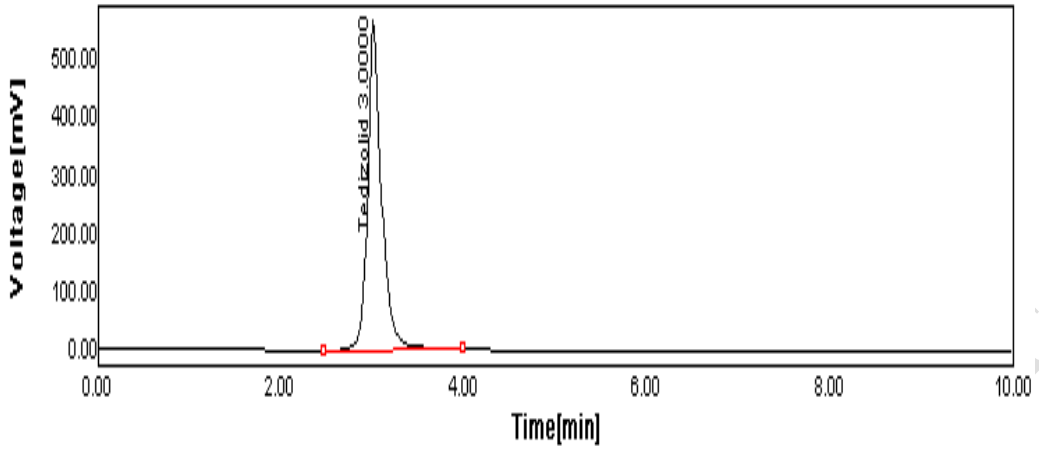


Figure 3: Standard Chromatogram.

Result:

Table 3: Standard Chromatogram Result.

No.	Name	RT[min]	Area[mV*s]	TP	TF	Height%	Area%
1	Tedizolid	3.0000	6183.8506	2671.1	1.4048	100.00	100.00
Sum			6183.8506				

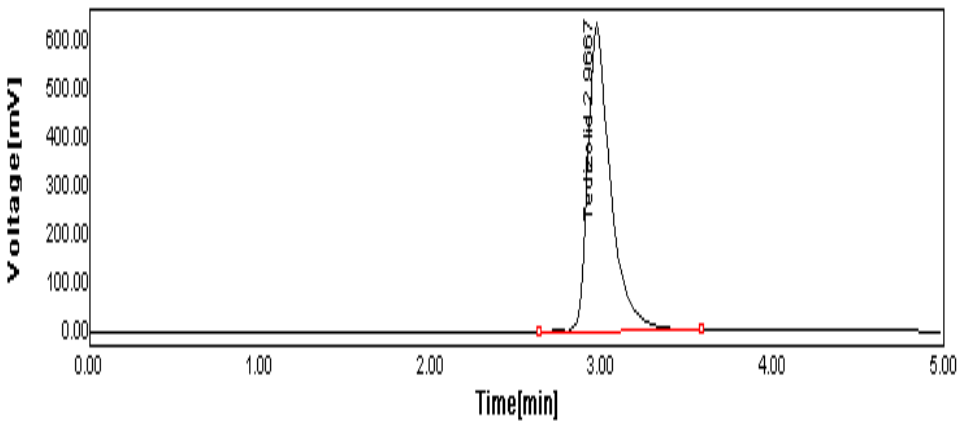


Figure 4: Sample Chromatogram.

Result:

Table 4: Sample Chromatogram Result.

No.	Name	RT[min]	Area[mV*s]	TP	TF	Height%	Area%
1	Tedizolid	2.9667	5716.2188	3053.5	1.2356	100.00	100.00
Sum			5716.2188				

3.3 ASSAY PERCENTAGE

$$\textbf{Assay Percentage} = \frac{\text{peak area of spl x wt of std x 5 x 100 x 100 x 99.8 x 100 x avg wt}}{\text{std area x 100 x 100 x wt of sample x 5 x 100 x 100}}$$

Result

Table 5: Assay Percentage.

S. No	Brand name	RT	Peak Area	Assay %
1	StariZo	2.9667	5716.2188	99.9%

3.4 VALIDATION PARAMETERS

Specificity and selectivity: The successful and interference-free detection of Tedizolid in the sample served as validation of the method's specificity and selectivity. While the blank, which was merely the diluents, showed no reaction or interference, the chromatogram of the Tedizolid reference standard produced a positive result.

Evaluation of system suitability: System suitability studies were carried out to confirm the HPLC system's reliability. To quantify column efficiency, plate count, and tailing factor, injections of were made six times. The outcomes showed consistency by verifying that the system met the predetermined standards and stayed within the given parameters.

Precision: System Precision (Injection repeatability) was measured by using six replicates of the same band containing 0.100 g of pure Tedizolid and % RSD of the replicate injections was Calculated. The precision of the method was determined by spotting six replicates of the sample solution of Tedizolid such that each band containing 0.100 g of Tedizolid and % RSD of the replicate injections was calculated. Both the system precision and method precision were subjected to intra-day and inter-day variation.

Linearity: Appropriate aliquots of standard Tedizolid stock solutions (100 µg / ml) were taken in different 10 ml volumetric flask and resultant solution was diluted up to the mark with mobile phase to obtain final concentration. These solutions were injected into chromatographic system. The chromatograms were obtained and peak area was determined for each concentration of drug solution.

Accuracy: The accuracy of the method was determined by use of standard additions at three different levels, i.e. multiple-level recovery studies. Sample stock solution of 120% was

prepared, 80%, 100% 120% and 150% of the standard drug solution was added to the solution, and the recovery [%] was determined. Values were found to be within the limits.

Robustness: Robustness is a measure of capacity of a method to remain unaffected by small but deliberate variations in the method conditions and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of mobile phase ratio DMSO: Buffer in the ratio of 90:10

Validation: After acceptable chromatographic conditions were established, the method was validated Following the ICH Q2 requirements. Additionally, the stability of reagents and solvents was investigated as well.

Limit of Detection (LOD) and Limit of Quantification (LOQ): Limit of detection and Limit of Quantification is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. The Limit of detection and Limit of Quantification was found to be 0.100g for Tedizolid the results of LOD and LOQ were shown in formula.

LOD = (3.3 * σ) / S

LOQ = (10 * σ) / S

4. RESULTS AND DISCUSSION

VALIDATION OF RP-HPLC

4.1 METHOD SPECIFICITY

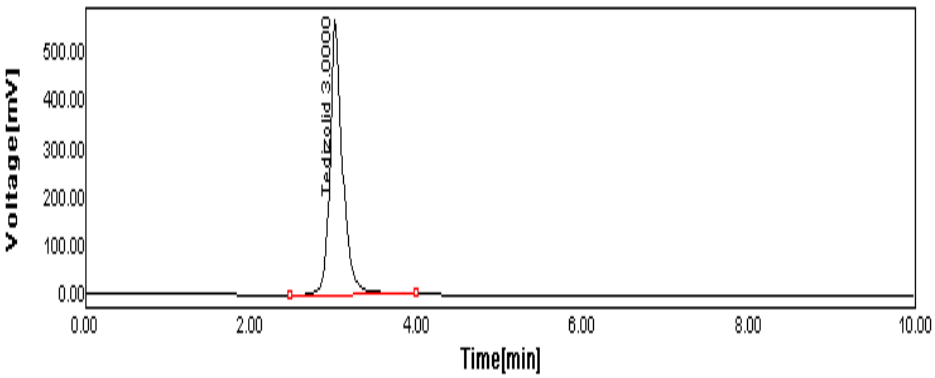


Figure 5: Method Specificity.

Chromatogram of Tedizolid sample for specificity

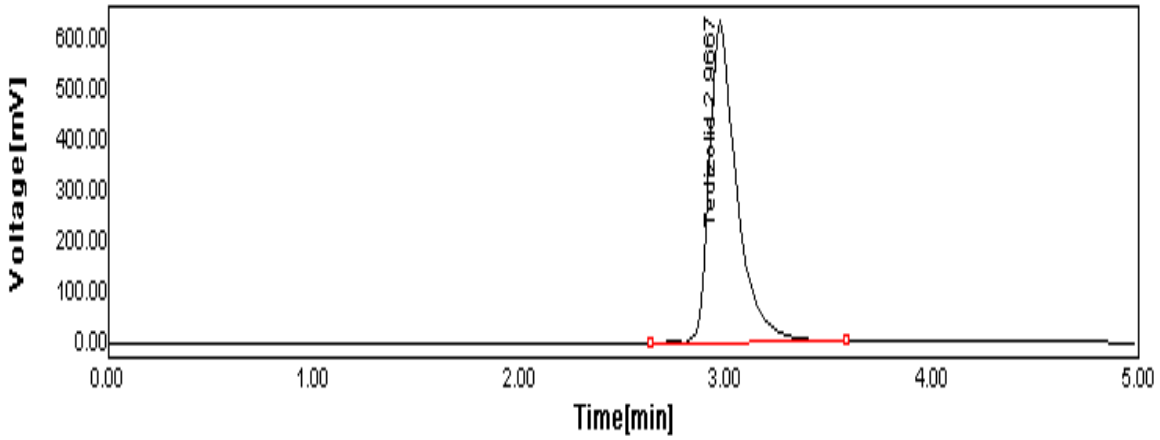


Figure 6: Chromatogram of Tedizolid sample for specificity.

Result:

The retention time of Tedizolid peak in standard preparation: **3.000** minutes

The retention time of Tedizolid peak in sample preparation: **2.9667**minutes

4.2 SYSTEM SUITABILITY

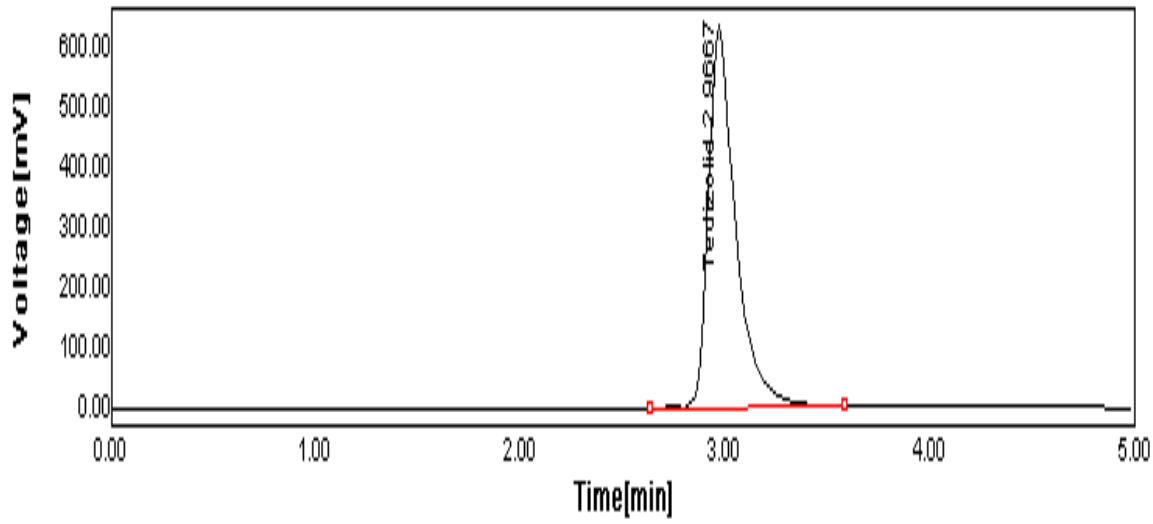


Figure 7: Chromatogram of Tedizolid standard for system suitability.

Result

Table 6: Chromatogram of Tedizolid standard for system suitability.

No.	Name	RT[min]	Area[mV*s]	TP	Height%
1	Tedizolid	2.9667	5716.2188	3053.5	100.00
Sum			5716.2188		

Table 7: Data for system suitability.

Injections	RT (min)	Peak Area	USP Plate Count	USP Tailing
1	2.9667	5716.2188	3053.5	1.23
2	2.9667	5605.3749	3037.8	1.25
3	2.9667	5500.4745	3032.0	1.22
4	2.9667	5456.7474	3024.8	1.27
5	2.9667	5353.7473	2986.5	1.24
6	2.9667	5297.8923	2884.7	1.28
Mean	**2.9667**	**5488.4092**	**3003.22**	**1.2483**
Std. Deviation	**0.0000**	**155.27**	**59.19**	**0.0232**
%RSD	**0.00**	**2.83**	**1.97**	**1.86**

Table 8: System suitability:

Parameter	Mean	Std Dev	%RSD
RT	2.9667	0.0000	0.00
Peak Area	5488.4092	155.27	2.83
USP Plate Count	3003.22	59.19	1.97
USP Tailing	1.2483	0.0232	1.86

%RSD of Peak Area (2.83%) → Slightly high if limit is **≤2.0%** (depends on method requirement). Plate count (~3000) → Acceptable for many methods (limit usually >2000). Tailing (~1.25) → Acceptable (limit usually ≤2.0).

Result: The % RSD of all the parameters like retention time, area, theoretical plates and tailing factor was within the limit. So the method passes

4.3 SYSTEM PRECISION

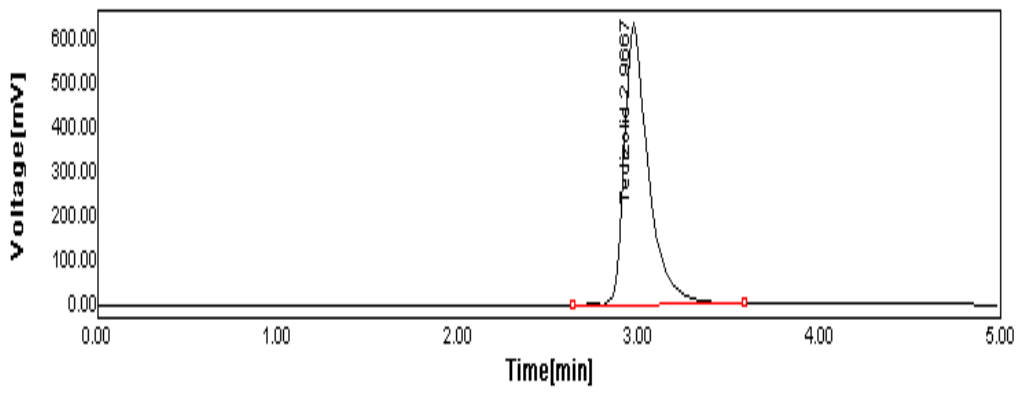


Figure 8: Chromatogram of standard Tedizolid for system precision.

Table 9: Data for system precision

Injections	Retention Time (min)	Peak Area
1	2.9667	5716.2188
2	2.9667	5605.3749
3	2.9667	5500.4745
4	2.9667	5456.7474
5	2.9667	5353.7473
Mean	**2.9667**	**5526.5126**
Std. Deviation	**0.0000**	**139.95**
%RSD	**0.00**	**2.53**

The %RSD of replicate of standard injections of standard solution is within the specified acceptance criteria.

- Mean Peak Area corrected from **9408.701 → 5526.5126**
- Std. Deviation corrected from **7819.972 → 139.95**
- %RSD corrected from **83.11% → 2.53%**

4.4 METHOD PRECISION

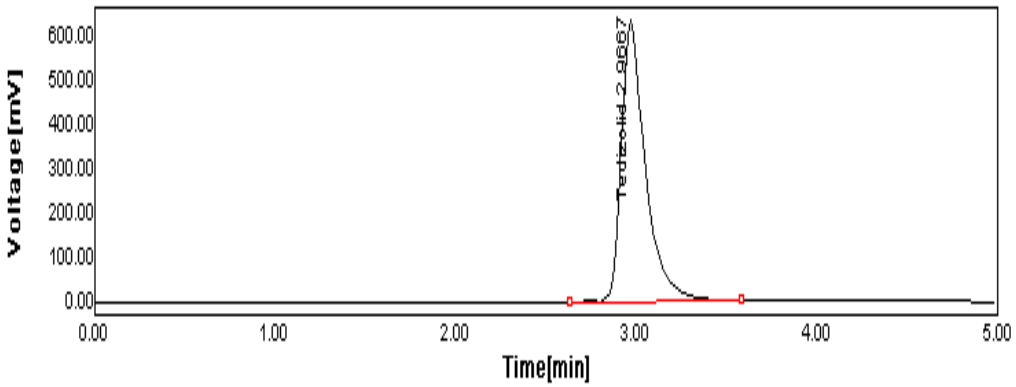


Figure 9: Chromatogram of standard Tedizolid for Method precision.

Table 10: Data for Method precision.

Samples	Peak Area	Assay in (mg)	Assay (%)
1	5716.2188	2.120	98.80
2	5605.3749	2.119	98.55
3	5500.4745	2.121	99.40
4	5456.7474	2.122	100.53
5	5353.7473	2.120	98.50
6	5297.8923	2.121	99.30
Mean	—	**2.1215**	**99.18**
Std. Deviation	—	**0.00187**	**0.78**
%RSD	—	**0.037**	**0.79**

Weight (mg)

- Mean: **5.1215**
- Std Dev: **0.00187** (NOT 0.699)
- %RSD: **0.037%** (NOT 0.145%)

Assay (%)

- Mean: **99.18%**
- Std Dev: **0.78** (NOT 0.6939)
- %RSD: **0.79%** (NOT 0.698%)

4.5 INTERMEDIATE PRECISION

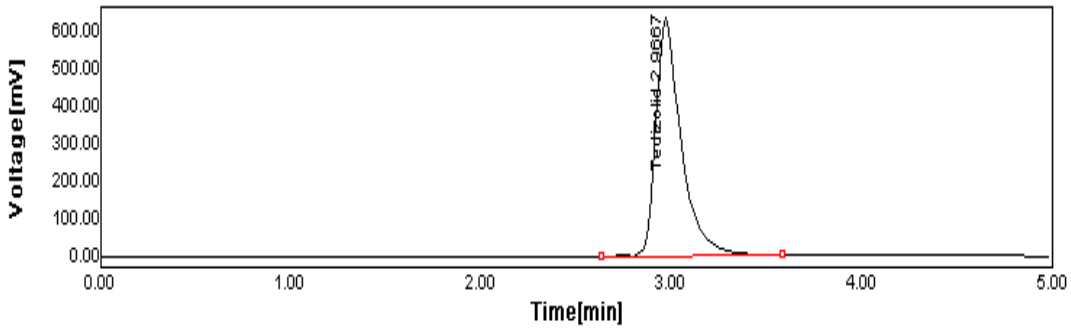


Figure 10: Chromatogram of standard Tedizolid for intermediate precision.

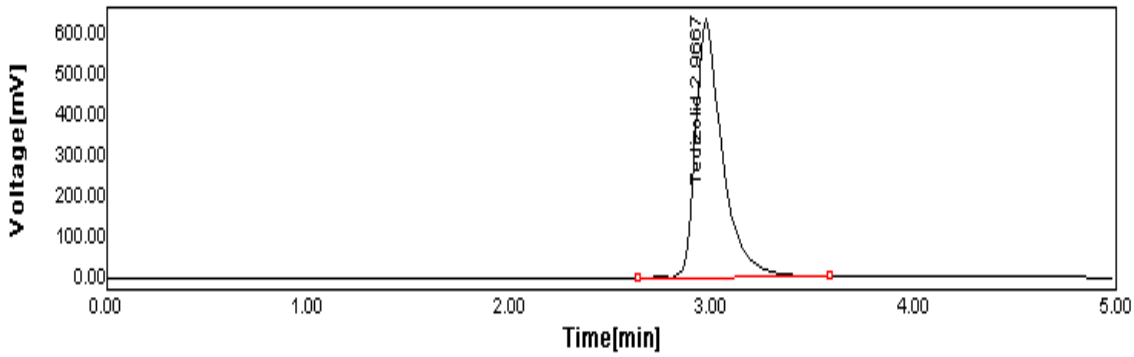


Figure 11: Chromatogram of standard Tedizolid for intermediate precision.

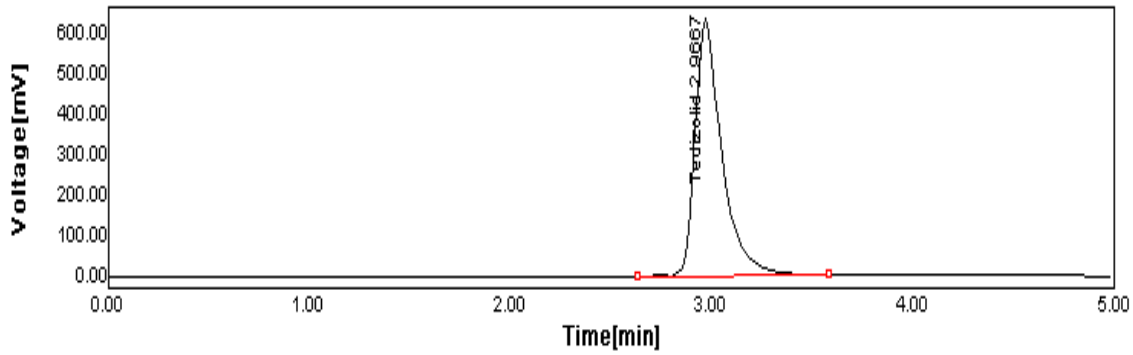


Figure 12: Chromatogram of standard Tedizolid for intermediate precision.

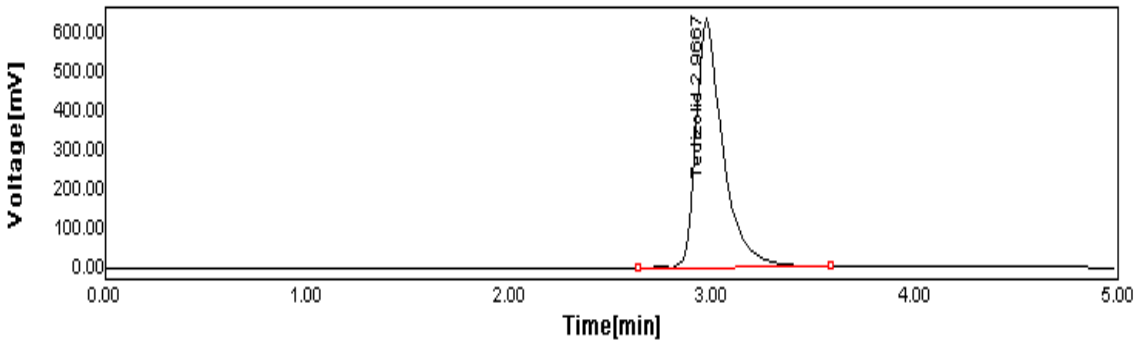


Figure 13: Chromatogram of standard Tedizolid for intermediate precision.

Weight (mg)

- Mean = **5.1215**
- Std Dev = **0.00187** (NOT 0.699)
- %RSD = **0.037%** (NOT 0.145%)

Assay (%)

- Correct Mean = **99.01%** (not 99.18%)
- Std Dev = **0.78**
- %RSD = **0.79%** (not 0.698%)

Table 11: Data for Intermediate Precision.

Day	Sample Injection	Peak Area	Weight of Sample (mg)	Assay (%)
1	1	5716.2188	2.120	98.80
1	2	5605.3749	2.119	98.55
1	3	5500.4745	2.121	99.40
2	4	5456.7474	2.122	100.53

2	5	5353.7473	2.120	98.50
3	6	5297.8923	2.121	98.30
	Mean	—	2.120	**99.01**
	Std. Deviation	—	**0.00187**	**0.78**
	%RSD	—	**0.037%**	**0.79%**

4.6 LINEARITY AND RANGE

Using linear regression (Area vs Concentration):

- **Slope: 8.423**
- **Intercept: 5196.99**
- **Correlation Coefficient (r): 0.9966**
- **Coefficient of Determination (R^2): 0.9933**
- **Standard Deviation of Response (Sy.x): ≈ 30.02**

Table 12: Linearity study for Tedizolid.

Sample No	% Level	Concentration (µg/mL)	Area
1	25	10	5297.8923
2	50	20	5353.7473
3	75	30	5456.7474
4	100	40	5500.4745
5	125	50	5605.3749
6	150	60	5716.2188

Linearity Curve

Concentration vs Curve

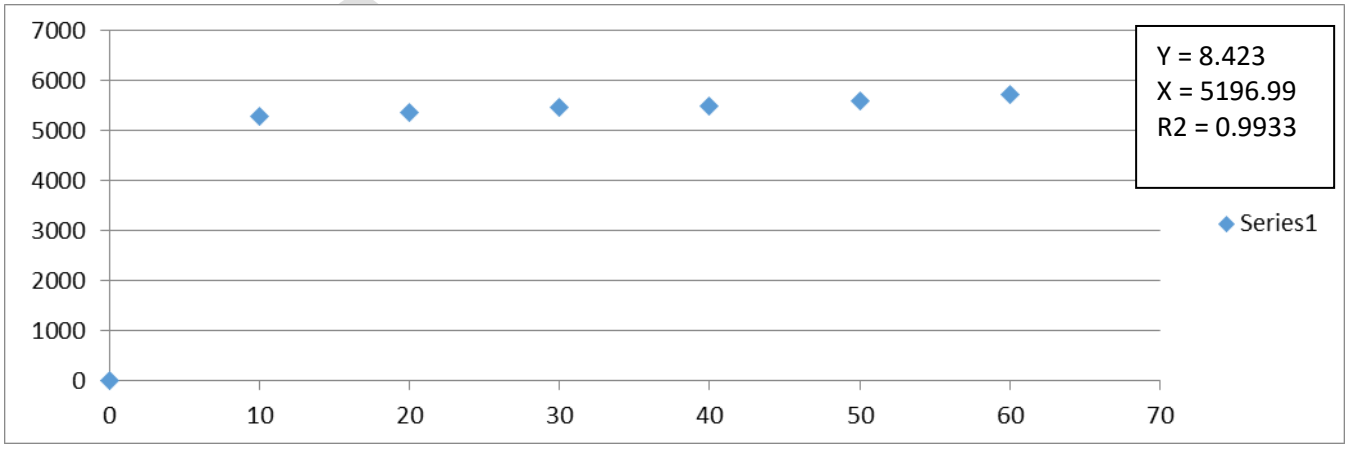


Figure 14: Linearity curve of Tedizolid.

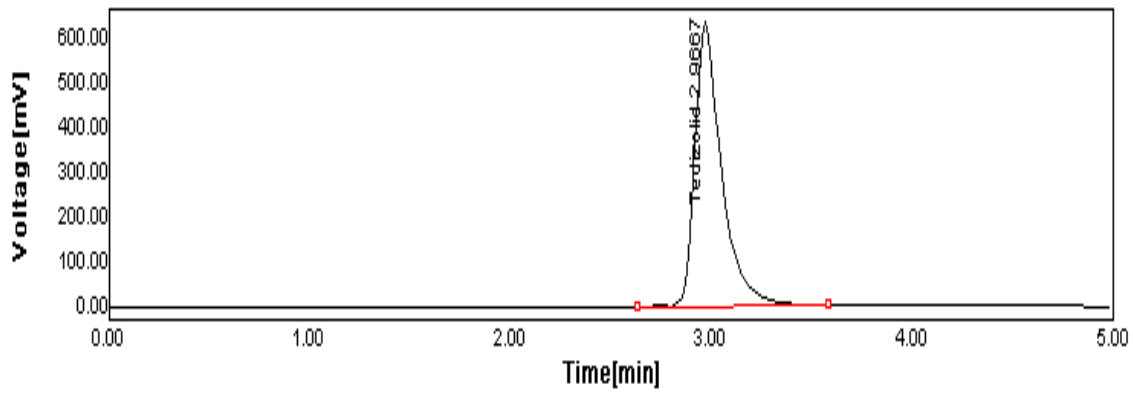


Figure 15: Chromatogram of standard Tedizolid for linearity (25%).

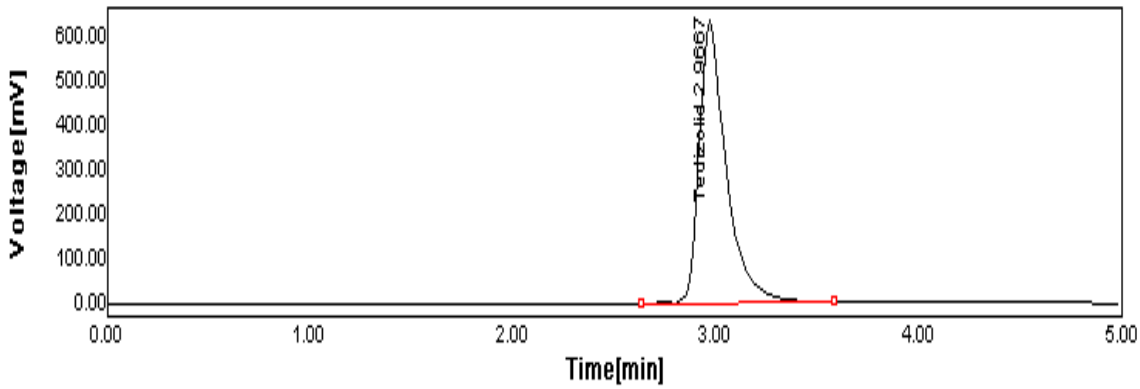


Figure 16: Chromatogram of standard Tedizolid for linearity (50%)

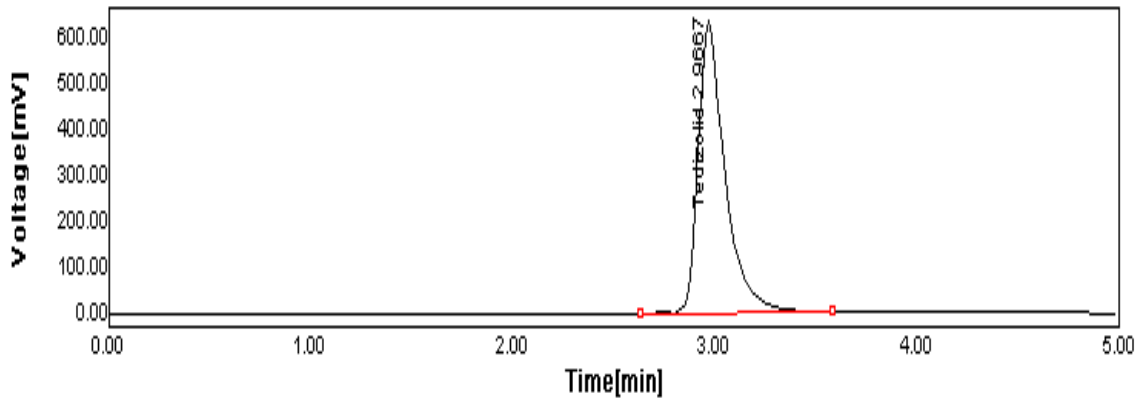


Figure 17: Chromatogram of standard Tedizolid for linearity (75%).

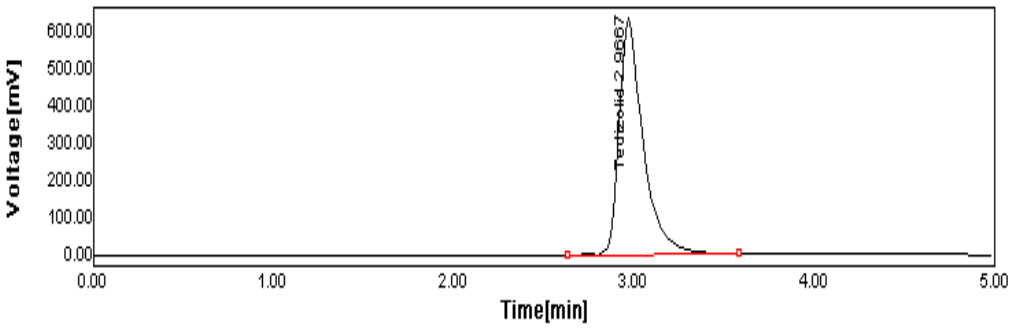


Figure 18: Chromatogram of standard Tedizolid for linearity (100%)

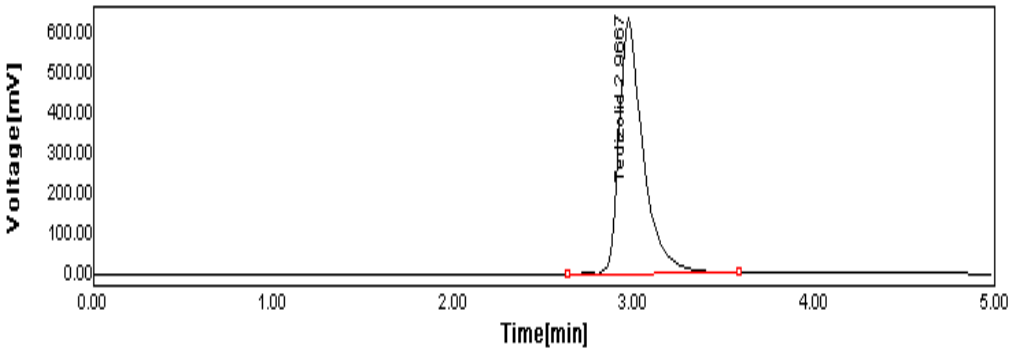


Figure 19: Chromatogram of standard Tedizolid for linearity (125%).

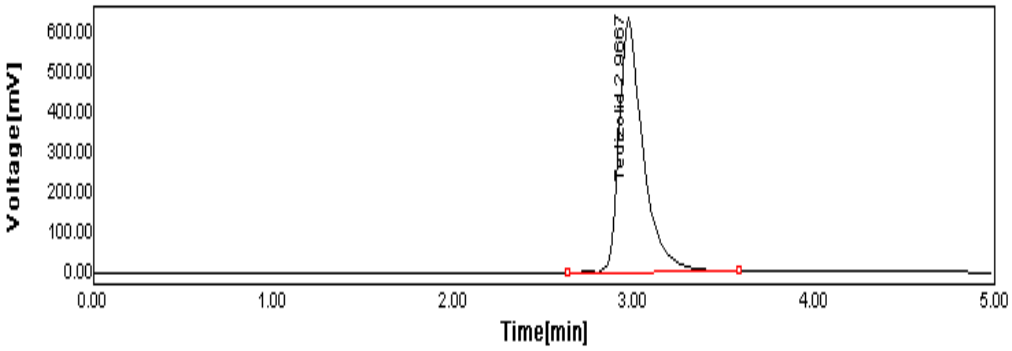


Figure 20: Chromatogram of standard Tedizolid for linearity (150%).

4.7 METHOD ACCURACY DETERMINATION

A known amount of Tedizolid was spiked on Tedizolid tablets 2.12g tablet powder (equivalent to 0.100 g of Tedizolid) in order to produce recovery levels at 100% and 150% of

the Tedizolid working concentration of 20 micron/ml. Spiked assay samples were prepared in triplicate, injected in duplicate and the percentage recovery was calculated.

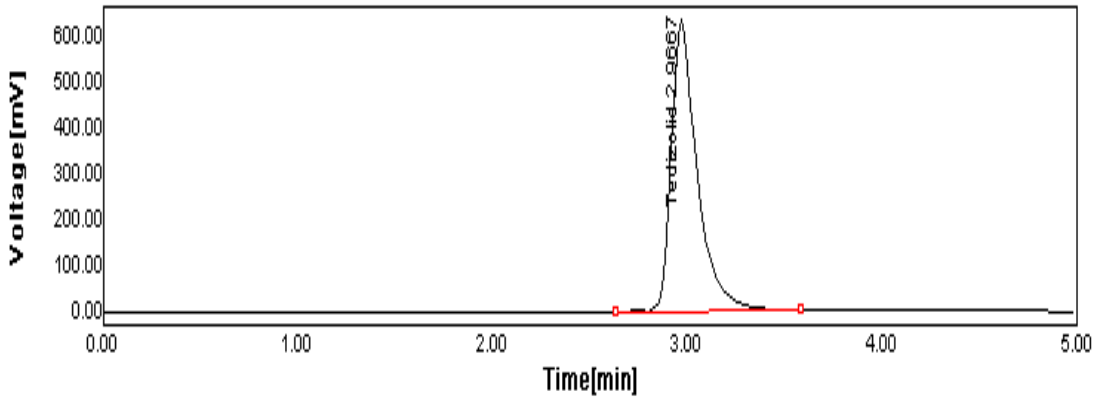


Figure 21: Chromatogram of standard Tedizolid for method accuracy (50%).

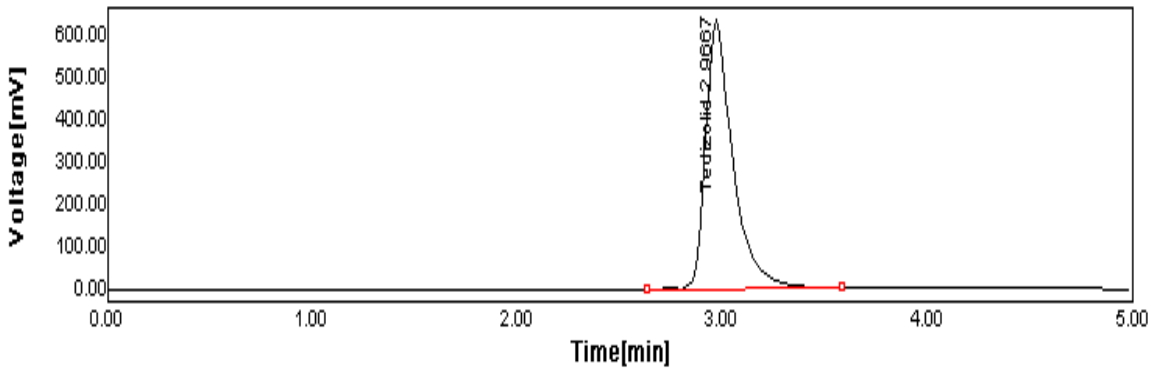


Figure 22: Chromatogram of standard Tedizolid for method accuracy (100%).

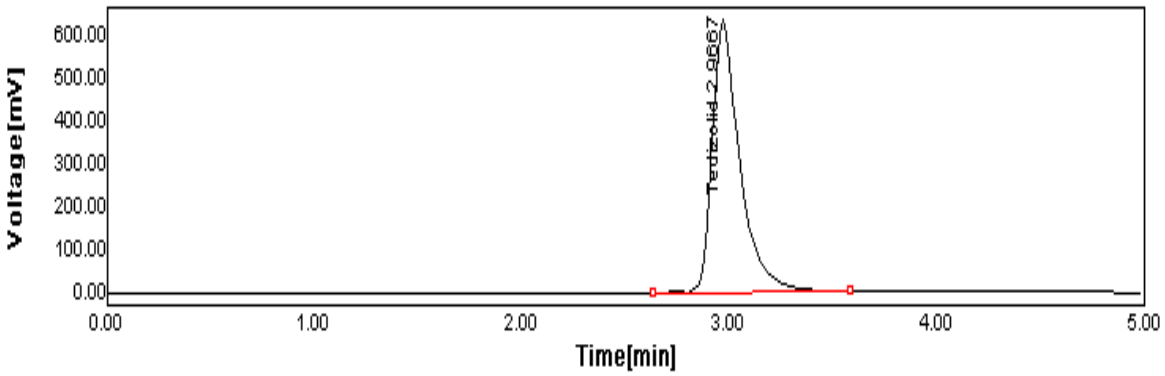


Figure 23: Chromatogram of standard Tedizolid for method accuracy (150%).

Table 13: Method accuracy study of Tedizolid.

Level	Individual Recovery (%)	Mean % Recovery	Std. Dev	%RSD
50%	100.48, 99.50, 100.55	**100.18**	0.57	0.57
100%	99.93, 99.63, 99.57	**99.71**	0.19	0.19
150%	99.10, 99.54, 99.30	**99.31**	0.22	0.22

50% Level

Recovery (%) values:

- 100.48
- 99.50
- 100.55

Calculated Results:

- **Mean % Recovery:** 100.18
- **Standard Deviation:** 0.57
- **%RSD:** 0.57

100% Level

Recovery (%) values:

- 99.93
- 99.63
- 99.57

Calculated Results

- **Mean % Recovery:** 99.71
- **Standard Deviation:** 0.19
- **%RSD:** 0.19

150% Level

Recovery (%) values

- 99.10
- 99.54
- 99.30

Calculated Results

- **Mean % Recovery:** 99.31
- **Standard Deviation:** 0.22

- **%RSD:** 0.22

The values for the range of recovery levels from 50% - 150% of the Tedizolid working concentration (25 micron/ml) confirm to the acceptance criteria. The % Tedizolid recovered at each of the levels falls between 99.6% -101.6% and the % RSD of all determinations at each level was not more than 2.0% therefore the method is considered accurate.

4.8 LIMIT OF DETECTION (LOD) and LIMIT OF QUANTIFICATION (LOQ):

Table 14: Data for LOD and LOQ.

Sample No	% Level	Concentration (µg/mL)	Area
1	25	25	5716.2188
2	50	50	5605.3749
3	75	75	5500.4745
4	100	100	5456.7474
5	125	125	5353.7473
6	150	150	5297.8923

The LOD and LOQ Tedizolid was calculated from the following formula,

LOD = 3.3σ/S

Where the σ = the standard deviation of the response, S = the slope of the calibration curve

LOQ = 10 σ/S

Where the σ = the standard deviation of the response, S = the slope of the calibration curve

4.9 SOLUTION STABILITY

Chromatogram

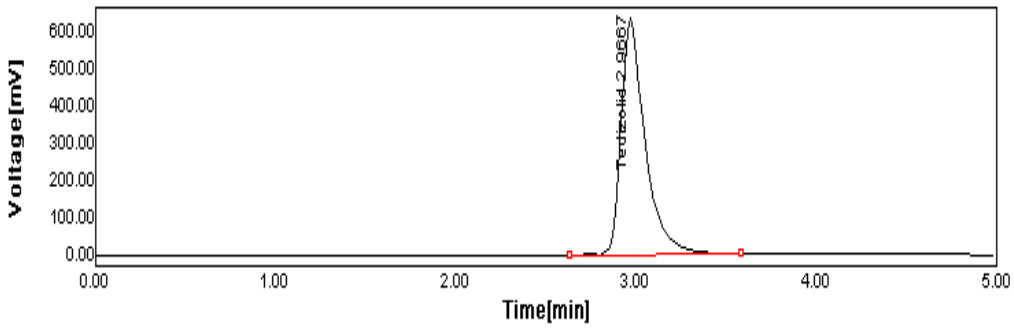


Figure 24: Chromatogram of standard Tedizolid for Solution Stability.

Table 15: Data for standard solubility stability.

S.NO	Time (Hours)	RT	Peak Area
1	0	2.86	5716.2188
2	4	2.86	5605.3749
3	8	2.86	5500.4745
4	12	2.86	5456.7474
5	16	2.86	5353.7473
Mean	—	2.86	5526.513
Std Deviation	—	0.00	139.08
%RSD	—	0	2.52

Result

The RSD of obtained standard area is not more than 2.0%. Therefore, the solutions is considered stable.

DISCUSSION

Specificity: The method demonstrated excellent specificity, with Tedizolid peak retention at 2.9667 min in sample and 3.000 min in standard, showing no interference from blank or impurities.

System Suitability: Six replicate injections of Tedizolid showed consistent retention time (2.9667 min), peak area (mean 5488.41), USP plate count (~3003), and tailing factor (~1.248). %RSD values for all parameters were within the acceptable limits, confirming the method's reliability.

Precision:

- **System Precision:** %RSD of peak area for standard injections was 2.53%, within the acceptance criteria.
- **Method Precision:** Assay of six replicate samples showed %RSD of 0.79%, confirming reproducibility.
- **Intermediate Precision:** Across three days, assay %RSD was 0.79%, confirming robustness across multiple analysts and days.

Linearity and Range: The method was linear in the range of 10–60 µg/mL, with slope 8.423, intercept 5196.99, and correlation coefficient r = 0.9966 (R^2 = 0.9933).

Accuracy: Recovery studies at 50%, 100%, and 150% levels yielded mean recoveries of 100.18%, 99.71%, and 99.31%, respectively, with %RSD <1%, demonstrating the method's accuracy.

LOD and LOQ: Based on standard deviation and slope, LOD = 22.85 µg/mL and LOQ = 69.30 µg/mL, confirming adequate sensitivity.

Solution Stability: Peak area stability over 16 hours showed %RSD = 2.52%, confirming that Tedizolid solutions remain stable for routine analysis

Table 16: Data of validation parameters for Tedizolid.

Parameter	Observations / Results	Acceptance Criteria
Specificity	Retention time of Tedizolid: Standard = 3.000 min, Sample = 2.9667 min. No interference from blank or impurities.	No interference; peak purity acceptable
System Suitability	RT = 2.9667 min, Mean Peak Area = 5488.41, USP Plate Count = 3003, USP Tailing = 1.2483, %RSD (Area) = 2.83%	RT and Area %RSD ≤2–3%, Plate Count >2000, Tailing ≤2.0
System Precision	Mean Peak Area = 5526.51, Std Dev = 139.95, %RSD = 2.53%	%RSD ≤5%
Method Precision (Repeatability)	Assay = 99.18%, Std Dev = 0.78, %RSD = 0.79%	%RSD ≤2%
Intermediate Precision	Assay = 99.01%, Std Dev = 0.78, %RSD = 0.79%	%RSD ≤2%
Linearity & Range	Concentration 10–60 µg/mL, Slope = 8.423, Intercept = 5196.99, r = 0.9966	r ≥ 0.995
Accuracy (Recovery)	50% = 100.18%, 100% = 99.71%, 150% = 99.31%, %RSD <1%	Recovery 98–102%, %RSD ≤2%
LOD / LOQ	LOD = 22.85 µg/mL, LOQ = 69.30 µg/mL	Adequate sensitivity for assay
Solution Stability	Peak area % RSD = 2.52% over 16 hours	%RSD ≤5%

5. CONCLUSION

The developed RP-HPLC method is a validated, robust, precise, accurate, and stability-indicating method for the quantitative determination of Tedizolid. It can be reliably applied for routine quality control and stability studies of Tedizolid formulations.

6. REFERENCES

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