International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 22s, 2025
https://theaspd.com/index.php

Sustainable and Economical in Vitro Analytical Method Development and Validation of Tedizolid Phosphate in Rabbit Plasma Using RP-HPLC Follows ICH M_{10} Guidelines

Dantinapalli VL Sagar[1], Meenakshi Sharma[2]
[1]Research Scholar, Department of Chemistry, School of Basic and applied Sciences, Raffles University, Neemrana-301705, 0009-0007-3371-2791
[2]Associate Professor, Department of Chemistry, School of Basic and applied Sciences, Raffles University, Neemrana-301705,0009-0001-8264-5149
Corresponding Author E-mail: dvl.sagar@gmail.com
Orchid id: 0009-0007-3371-2791

Abstract:
A simple, Accurate, precise method was developed for the simultaneous estimation of Tedizolid Phosphate in Rabbit plasma was developed and validated. By using Centrifugation technique, the sample preparation was prepared. Chromatogram run through Std Agilent C18 Column, 4 µm, and 4.6 mm X 250 mm, Mobile phase containing Acetonitrile: Buffer Ortho Phosphoric Acid taken in the ratio 60:40 v/v was pumped through column at a flow rate of 1.0 ml/min OPA.in this method was buffer. For the separation of Tedizolid Phosphate Internal Standard [IS] used is Ciprofloxacin. The Temperature was maintained at 30°C. Optimized wavelength selected was 252 nm. Retention time of Tedizolid Phosphate and Internal Standard were found to be 3.564 min and 2.982 min. The standard curve was linear (R2 >0.999) over the concentration range of 75 – 3000 ng/ml. All the analytical validation parameters were determined as per ICH guidelines the bioanalytical method developed approach was selective, robust, and reliable, as accuracy, precision, recovery, and other validation parameters were all within the recommendations' limitations. The peaks produced for the drug of interest and the internal standard were well separated from one another without any plasma interferences, and the peaks were symmetrical with an adequate tailing factor. The method has the potential to be very beneficial in therapeutic drug monitoring (TDM), bioequivalence research, pharmacokinetics studies, toxicology, and biomedical investigations.
Key Words: *Tedizolid Phosphate, Internal Standard, Ciprofloxacin, RP - HPLC, Bioanalysis, Rabbit Plasma.*

INTRODUCTION

The development and validation of bioanalytical methods for drug analysis in rabbit plasma represent a vital component of pharmacokinetic research and drug safety evaluations. Recent advancements in analytical technologies, particularly the integration of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), have markedly enhanced the precision and reliability of drug quantification in biological matrices. This sophisticated methodology facilitates the accurate measurement of drugs and their metabolites, thereby providing critical insights into their pharmacokinetic profiles in vivo. However, the complexity of sample preparation remains a significant challenge, as variations in extraction techniques can substantially affect analytical outcomes. As the field evolves, the need for continuous refinement and rigorous validation of bioanalytical methods becomes increasingly important, ensuring that the resulting pharmacokinetic data are both reliable and applicable in preclinical studies. This literature review seeks to consolidate existing knowledge on bioanalytical method development and validation, emphasizing the essential role these processes play in advancing drug safety and efficacy assessments.

Furthermore, the integration of bioanalytical methods with advanced computational techniques, such as pharmacokinetic modeling and simulation, presents an exciting frontier in drug development. By employing these modeling approaches, researchers can predict drug behavior in vivo more accurately, thereby enhancing the design of preclinical studies and optimizing dosing regimens. This synergy not only streamlines the drug development process but also aids in identifying potential adverse effects early on, ultimately leading to safer therapeutic options. As a result, the continuous evolution of bioanalytical methodologies, coupled with sophisticated modeling techniques, underscores the critical need for a holistic approach in drug safety and efficacy assessments, reinforcing the importance of rigorous validation processes throughout the research continuum

Tedizolid phosphate is an oxazolidinone class antibiotic that inhibits bacterial protein synthesis and is proven to be effective in the treatment of certain Gram-positive bacterial infections.

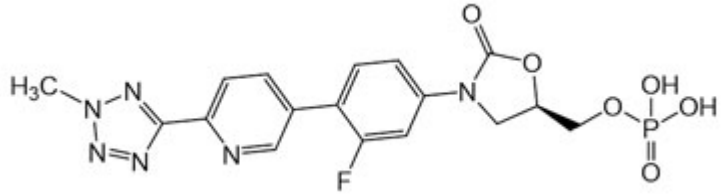

Figure 1: Chemical Structure of Tedizolid phosphate

CAS Number	:	856867-55-5
IUPAC Name	:	{[(5R)-3-{3-fluoro-4-[6-(2-methyl-2H-1,2,3,4-tetrazol-5-yl)pyridin-3-yl]phenyl}-2-oxo-1,3-oxazolidin-5-yl]methoxy}phosphonic acid
Molecular Weight (g/mol)	:	Average: 450.323
Molecular Formula	:	$C_{17}H_{16}FN_6O_6P$
Appearance	:	Powder
Physical State	:	Solid
Solubility	:	insoluble in Water (0.136 mg/mL in water)
Log p	:	4.89
pK Values (Predicted)	:	1.35 (Acidic), -1.6 (Basic).

Indication: Tedizolid is indicated for the treatment of acute bacterial infections of the skin and skin structure (ABSSSI). To prevent drug resistance, tedizolid should only be used for infections that are caused by susceptible bacteria.

Experimental Work:

Materials used In work:

Tedizolid Phosphate API and Internal Standard was obtained as a gift sample, From Akrivis Pharma pvt Ltd. K2 EDTA control plasma procured form Deccan Pathological labs, Hyderabad. Acetonitrile, Phosphate buffer , Methanol ,Sodium dihydrogen phosphate, Ortho-phosphoric acid of Analytical grade used in the Work

Methodology:

Preparation of solutions

All solutions performed sonication, were stored at room temperature, and were utilized within 24 hours after their production.

The next section outlines the methodology for preparing buffers and possible solutions.

Preparation of diluent (v/v):

Based up on the solubility of the drugs, diluent was selected, Water and Acetonitrile taken in the ratio of 50:50.

Preparation of stock solutions: -

Standard Preparation: Accurately Weighed and transferred 75mg of Tedizolid Phosphate working Standards into a 100ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 15 minutes and make up to the final volume with diluents, and filter the solution with Hplc nylon 0.5µm size filters (750 ppm/µg/ml of Tedizolid Phosphate)

Standard Working Solution: From the above Tedizolid Phosphate stock solution 0.010 ml, 0.020 ml, 0.030 ml, 0.080ml, 0.2 ml, 0.240 ml, 0.320 ml and 0.400 ml was pipette and transferred to 8 individual of 10 ml volumetric flask and make up the volume up to the mark with diluent to produce 75 µg/ml, 150 µg/ml, 225 µg/ml, 600 µg/ml, 1500 µg/ml, 1800 µg/ml, 2400 µg/ml and 3000 µg/ml.

Selection of an Internal standard:

Bioanalysis commonly favors the utilization of internal standards. In many instances, the utilization of labeled information systems may become impractical due to the exorbitant expenses involved and the challenges associated with procuring them from authorized channels. Hence, structural homologs of the Analyte are also employed as internal standards (IS). However, it is essential for the chosen information systems (IS) to exhibit the following properties:

1. The stability of the system is notably high.
2. There is no interference observed from the interstitial substance (IS) on the analyte.

3. The extraction efficiencies of the internal standard (IS) and the analyte are similar.
4. The analyte exhibits similar behavior during sample preparation and subsequent analysis, hence reducing analytical variability.
5. Please provide the concentration value that has been determined by calculations without any ambiguity.
6. The improvement of the overall reliability of the procedure.

Stock solution of internal standard (Ciprofloxacin)**:**

Standard Preparation: Accurately Weighed and transferred 75mg of Ciprofloxacin working Standards into a 100ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 15 minutes and make up to the final volume with diluents, and filter the solution with Hplc nylon 0.5µm size filters (750 ppm/µg/ml of Tedizolid Phosphate).

Final concentration: From the above solution, take 1ml of solution and spiking blank plasma with working stock dilutions of Analyte to produce 10µg/ml ISD concentration.

Preparation of calibration curve (CC) standards and quality control (QC) samples

Quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 75 µg/ml (Standard-1/LLOQ), 150 µg/ml (Standard-2), 225 µg/ml (Standard-3/LQC), 600 µg/ml (Standard-4), 1500 µg/ml (Standard-5/MQC), 1800 µg/ml (Standard-6), 2400 µg/ml (Standard-7/HQC) and 3000 µg/ml (Standard-8/ULOQ).

Table1 CC spiking solutions of Tedizolid Phosphate:

Spiking solution	**stock solution in PPM**	**pipeteout in ml**	**make up in ml**	**spiking in ml**	**make upon ml**	**final conc in ng/ml**
Standard-1	750	0.010	10	0.25	2.5	75
Standard-2	750	0.020	10	0.25	2.5	150
Standard-3	750	0.030	10	0.25	2.5	225
Standard-4	750	0.080	10	0.25	2.5	600
Standard-5	750	0.200	10	0.25	2.5	1500
Standard-6	750	0.240	10	0.25	2.5	1800
Standard-7	750	0.320	10	0.25	2.5	2400
Standard-8	750	0.400	10	0.25	2.5	3000

Table 2 Preparation of QC spiking solutions:

Spiking solution	**pipeteout in ML**	**make up in ML**	**spiking in ML**	**make upon ML**	**final conc in ng/ml**
LLOQ	0.010	10	0.25	2.5	75
LQC	0.030	10	0.25	2.5	225
MQC	0.200	10	0.25	2.5	1500
HQC	0.320	10	0.25	2.5	2400
ULOQ	0.400	10	0.25	2.5	3000

The solutions containing CCs and QCs were stored in a deepfreeze at a temperature of -20°C. A volume of 0.25 mL of spiked samples was tightly closed and stored in multiple pre-labeled vials at a temperature of -20°C.

- CC standards.
- QC samples.
- Standard blank (with spiking IS and analyte).

- Standard zero sample (spiking of IS working solution to blank plasma during sample processing).
- These Samples were subsequently used for conducting various validation experiments and analyzing animal study samples.

Extraction procedure for Bio-Sample analysis.

The protein precipitation method was employed to extract Tedizolid Phosphate from rabbit plasma, utilizing Ciprofloxacin as an internal standard (IS), in the subsequent procedure.

In this experiment, a total of 750µl of plasma was combined with 50µl of internal standard and an additional 20µl of Tedizolid Phosphate. The mixture was subjected to a 15-second cyclomixing process. Following this, 1 ml of acetonitrile was added to the mixture, and the resulting solution was subjected to vertexing for a duration of 2 minutes. Subsequently, the solution was centrifuged at a speed of 3200 rpm for a period of 5 minutes, allowing for the collection of the supernatant sample. To ensure the removal of any impurities, the sample was then filtered using a polyvinylidene fluoride or polyvinylidene difluoride 0.45µ filter. Finally, 10 µL of the filtered sample was injected into the high-performance liquid chromatography (HPLC) system for further analysis.

Data analysis

The Analyst software version empower 2 was used to data acquisition and analysis, and additionally, a validated excel sheet was used to compute the statistics like mean, SD and %CV for analytical values generated during method validation.

Validation Methodology in bioanalytical method: -

System Suitability Parameter

System Suitability test are performed that the test mixture is essential to check the specifications of a liquid chromatographic system. The System suitability testing limits are acceptance criteria that must be prior to sample analysis**.**

Methodology: The experiment involves the administration of six quality control samples of MQC (specifically, 40 ng/ml) from a single vial at the beginning of the study.

Acceptance criteria: The criteria acceptance accordingly as the % CV of the retention time (RT) should be ≤ 2.00 %., The % CV of the area ratio should be ≤ 5.00 %.

Auto Sampler Carryover

Carry-over is an alteration of a measured concentration due to residual analyte from a preceding sample that remains in the analytical instrument, during validation carry-over should be assessed by analyzing blank samples after the calibration standard at the ULOQ.

Methodology: The high-performance liquid chromatography (HPLC) technology was evaluated in order to investigate the potential occurrence of carry-over. The carryover was evaluated by injecting the following samples in a sequential manner.

- Blank refers to a solution that is used as a mobile phase and contains water as the solvent.
- Standard QC (ULOQ).
- Blank
- Standard QC (ULOQ)
- lower standard (AQ LLOQ)

Acceptance criteria: - The carryover area response in subsequent injections of RS or STD Bulk after aqueous or extracted ULOQ should be ≤ 20.00 % of the equivalent aqueous or extracted LLOQ standard area.

Specificity and Screening of Biological matrix

Specificity is the ability of a bioanalytical method to detect and differentiate the analyte from other substances, including its related substances (e.g., substances that are structurally similar to the analyte, metabolites, isomer, impurities, and degradation products formed during sample preparation or concomitant medications that are expected to be used in the treatment of patients with the intended indication).

Methodology: Specificity is determined by the injecting six samples of standard solution and the LLOQC sample solution and

Acceptance criteria: - check the % Interference Response of interfering peaks in STD Blk at the retention time of analyte should be ≤20.00 % of that in LLOQ and At least 80 % of the matrix lots (Biological Sample) with intended anticoagulant should be within the acceptance criteria.

Sensitivity

Sensitivity is often interpreted as related to the detection/determination ability, LLOQ based on precision and accuracy (bias) data, this is probably the most practical approach and defines the LLOQ as the lowest concentration of a sample that can still be quantified with acceptable Limit.

Methodology: - the sensitivity is performed by injecting six injections of lower concentration of sample (LLOQ).

Acceptance criteria: -the acceptance criteria of sensitivity of LLOQ are At least 67 % (4 out of 6) of samples should be within 80.00-120.00 %.

Matrix Factor evaluation

A matrix effect is defined as an alteration of the analyte response due to interfering and often unidentified component(s) in the sample matrix. During method validation it is necessary to evaluate the matrix effect between different independent sources/lots.

Methodology: - The matrix effect should be evaluated by analyzing at least 3 replicates of **low and high QCs (LQC and HQC),** each prepared using matrix from at least 6 different sources/lots.

Acceptance criteria: - The accuracy should be within ±15% of the nominal concentration and the precision (per cent coefficient of variation (%CV)) should not be greater than 15% in all individual matrix sources/lots.

Linearity (Calibration Curve and Range)

the relationship between the nominal analyte concentration and the response of the analytical platform to the analyte, Calibration standards, prepared by spiking matrix with a known quantity of analyte, span the calibration range and comprise the calibration curve. Calibration standards should be prepared in the same biological matrix as the study samples.

Methodology: The calibration range is obtained by injecting 6 concentrations of calibration standards not including blank and zero samples and establishing the concentration-response relationship by the sample regression model method

Acceptance criteria: The % accuracy for all CC standards except of LLOQ (STD 1) standard should be within 85.00-115.00 %. The % accuracy for LLOQ standard should be within 80.00-120.00 %.

Rugged Linearity

Linearity ruggedness is a measure for the susceptibility of a method to small changes that might occur during routine analysis,

Methodology: The calibration range is obtained by injecting 6 concentrations of calibration standards not including blank and zero samples and establishing the concentration-response relationship by the sample regression model method and

Acceptance criteria: The % accuracy for all CC standards except of LLOQ (STD 1) standard should be within 85.00-115.00 %. The % accuracy for LLOQ standard should be within 80.00-120.00 %.

Precision and Accuracy (Intra-day)

Accuracy and precision should be determined by analysing the QCs within each run (within-run) and in different runs (between-run). Accuracy and precision should be evaluated using the same runs and data.

Methodology: -

The test is performed injecting the QC samples were injected 6 replicates at each qc concentration level in each analytical run.

Acceptance criteria: - The overall accuracy at each concentration level should be within ±15% of the nominal concentration, except at the LLOQ, where it should be within ±20%. The precision (%CV) of the concentrations determined at each level should not exceed 15%, except at the LLOQ, where it should not exceed 20%.

Rugged Precision and Accuracy (Inter-Day)

Accuracy and precision should be evaluated using the same runs and data.

Methodology: -The test is performed injecting the QC samples were injected 6 replicates at each qc concentration level in each analytical run

Acceptance criteria: the overall accuracy at each concentration level should be within ±15% of the nominal concentration, except at the LLOQ, where it should be within ±20%. The precision (%CV) of the concentrations determined at each level should not exceed 15%, except at the LLOQ, where it should not exceed 20%.

Recovery:

Recovery was determined by measuring the peak areas obtained from prepared plasma samples with those extracted blank plasma spiked with standards containing the same area with known amount of Drug.

Methodology: -The recoveries for Tedizolid Phosphate at LQC, MQC and HQC levels the results demonstrated that the bioanalytical method had good extraction efficiency by injecting the six samples of LQC, MQC and HQC with the main drug and check the interference with un-extracted and extracted

Acceptance criteria:

The % CV of recovery at each QC level should be ≤ 15.00 %. The overall mean recovery % CV for all QC levels should be ≤ 20.00 %.

Recovery of Internal Standard

The measuring the peak areas obtained from prepared plasma samples with those extracted blank plasma spiked with Internal Standards containing the same area with known amount of Drug.

Methodology: -The recoveries for IS at 6 replicates the results demonstrated that the bioanalytical method had good extraction efficiency by injecting the six samples and check the interference with unextracted and extracted.

Acceptance criteria: The % CV of recovery at each QC level should be ≤ 15.00 %. The overall mean recovery % CV for all QC levels should be ≤ 20.00 %.

Reinjection Reproducibility

Reproducibility of the method is assessed by replicate measurements of the QCs and is usually included in the assessment of precision and accuracy. However, if samples could be reinjected (e.g., in the case of instrument interruptions or other reasons such as equipment failure), reinjection reproducibility should be evaluated and included in the Validation Report or provided in the Bioanalytical Report of the study where it was conducted.

Methodology: -The reproducibility was performed by injecting the qc samples in 6 replicates and check the acceptance limits.

Acceptance criteria: The % mean accuracy for LQC, MQC and HQC samples should be within 85.00-115.00 % and for the LLOQ QC sample it should be within 80.00-120.00 %.

Stabilities

Stability evaluations should be carried out to ensure that every step taken during sample preparation, processing and analysis as well as the storage conditions used do not affect the concentration of the analyte.

Methodology: -The stability is assessed by long term stock solution stability and Matrix samples stability at -28±5 °C for 37 days & -80±5 ^{0}C, stability testing is performed by injecting the QC samples of high and low concentrations(HQC and LQC) with taken biological matrix

Acceptance criteria: The mean concentration at each QC level should be within ±15% of the nominal.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

Based on drug solubility and P^{ka} Value following conditions has been used to develop the method estimation of Tedizolid Phosphate as per current ICH guidelines.

Optimization of the chromatographic conditions

For developing the method for the assay of Tedizolid Phosphate, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all the other conditions constant. The following studies were conducted for this purpose. A high purity advance C18 column was chosen as the stationary phase for this study. The mobile phase and the flow rate in order to get sharp peaks and base line separation of the components, the author has carried out a number of experiments by varying the commonly used solvents, their compositions and flow rate. To effect ideal separation of the drug under isocratic conditions, mixtures of commonly used solvents like water, methanol and acetonitrile with or without buffers in different combinations were tested as mobile phases on Agilent C18 stationary phase. A binary mixture of acetonitrile and 0.1% OPA buffer in a ratio of 60:40 v/v was proved the most suitable of all the combinations since the chromatographic peaks obtained were well defined, resolved, and free from tailing. A mobile phase flow rate of 0.1 mL/min found to be suitable.

Optimized method:

Chromatographic conditions

Mobile phase	Acetonitrile: OPA (60:40 v/v)
Flow rate	1.0 ml/min
Column	Agilent C18 (4.6 x 250mm, 5µm)
Wave length	252 nm

Column temperature	30°C
Injection volume	20 µL
Run time	10.0 min
Buffer	Ortho - Phosphoric Acid

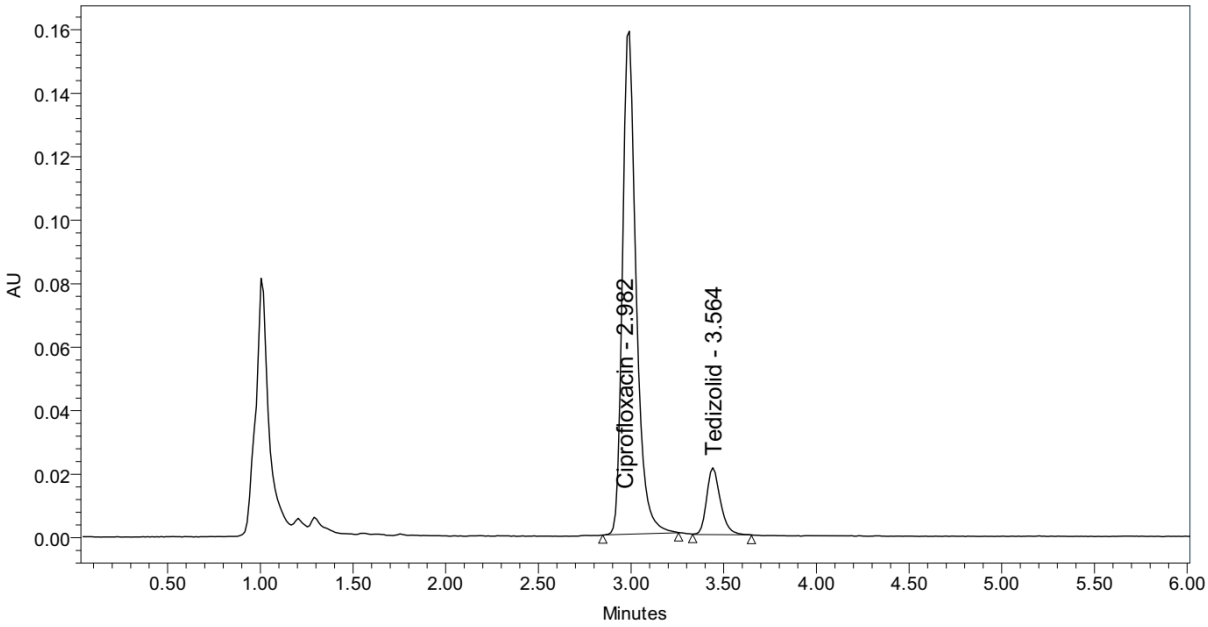

Figure 2 : Chromatogram of Optimized

Table 3: Observation of Optimized Chromatogram

	Peak Name	RT	Area	s/n	USP Plate Count	USP Tailing	USP Resolution
1	Ciprofloxacin	2.982	22012	1823.0	4810.1	1.3	
2	Tedizolid	3.564	551232	2388.3	5412.0	1.3	3.2

Observation: Tedizolid Phosphate and Internal Standard were eluted at 2.982 min, 3.564 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated. Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits

METHOD VALIDATION

1) System suitability of Tedizolid Phosphate

This system suitability method intended to guarantee that the HPLC system is working in such a way that correct and reproducible data may be submitted to regulatory agencies with confidence. This procedure includes signal stability, carryover, and instrument response tests.

Table 4: System Suitability of Tedizolid Phosphate

System Suitability						
Analyte	Tedizolid Phosphate	ISTD	Ciprofloxacin			
Sample Name		Analyte Area	Analyte RT (min)	ISTD Area	ISTD RT (min)	Area Ratio
AQ MQC		119044	3.58	1383770	2.97	0.0860
AQ MQC		119058	3.58	1383994	2.97	0.0860

AQ MQC	119036	3.58	1383999	2.97	0.0860
AQ MQC	119025	3.58	1382622	2.97	0.0861
AQ MQC	119087	3.58	1383873	2.97	0.0861
AQ MQC	119033	3.58	1383986	2.97	0.0860
MEAN		**3.584**		**2.968**	**0.08603**
SD		**0.0000**		**0.0000**	**0.000030**
%CV		**0.00**		**0.00**	**0.04**

Discussion: plate count, tailing factor, resolution of Tedizolid Phosphate was According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits. The % CV of the retention time (RT) should be ≤ 2.00 %.

Quality Control Samples

1) Auto sampler carryover of Tedizolid Phosphate

The carryover was tracked back to the injection valve and eradicated by converting from a partial loop injection to a full loop injection, which allowed more effective cleansing of the sample flow channel. The HPLC system's susceptibility to carryover was shown to be dependent on the detection method's absolute sensitivity and the mass of Analyte injected at the assay's lower limit of quantitation (LLOQ).

Table 5 Auto sampler carryover of Tedizolid Phosphate

Auto sampler Carryover				
Analyte	Tedizolid Phosphate		**ISTD**	Ciprofloxacin
Sample ID	Peak Area		% Carryover	
	Drug	ISTD	Drug	ISTD
Unextracted samples				
RS	0	0	N/A	N/A
AQ ULOQ	236370	236382	**0.00**	**0.00**
RS	0	0		
AQ LLOQ	5953	5941	N/A	N/A
Extracted samples				
STD Blk	0	0	N/A	N/A
ULOQ	236362	236359	**0.00**	**0.00**
STD Blk	0	0		
LLOQ	5948	5940	N/A	N/A

Discussion: - The area obtained is less than 20 % of extracted LLOQ standard area to un-extracted area by injected of replicate manner.

Specificity and Screening of Biological Matrix

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present

Table 6 : Specificity and Screening of Biological Matrix of Tedizolid Phosphate

Specificity and Screening of Biological Matrix						
Analyte	Tedizolid Phosphate			**ISTD**	Ciprofloxacin	
S.No.	**Sample ID**	**Response**		**% Interference**		**Pass/Fail**
		Drug	**ISTD**	**Drug**	**ISTD**	
1	STD Blk1	0	0	0.00	0.00	Pass

2	LLOQ1	5943	1383770			
3	STD Blk2	0	0	0.00	0.00	Pass
4	LLOQ2	5948	1383776			
5	STD Blk3	0	0	0.00	0.00	Pass
6	LLOQ3	5937	1383780			
7	STD Blk4	0	0	0.00	0.00	Pass
8	LLOQ4	5639	1383769			
9	STD Blk5	0	0	0.00	0.00	Pass
10	LLOQ5	5950	1383775			
11	STD Blk6	0	0	0.00	0.00	Pass
12	LLOQ6	5948	1383770			

Observation: We did not find and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

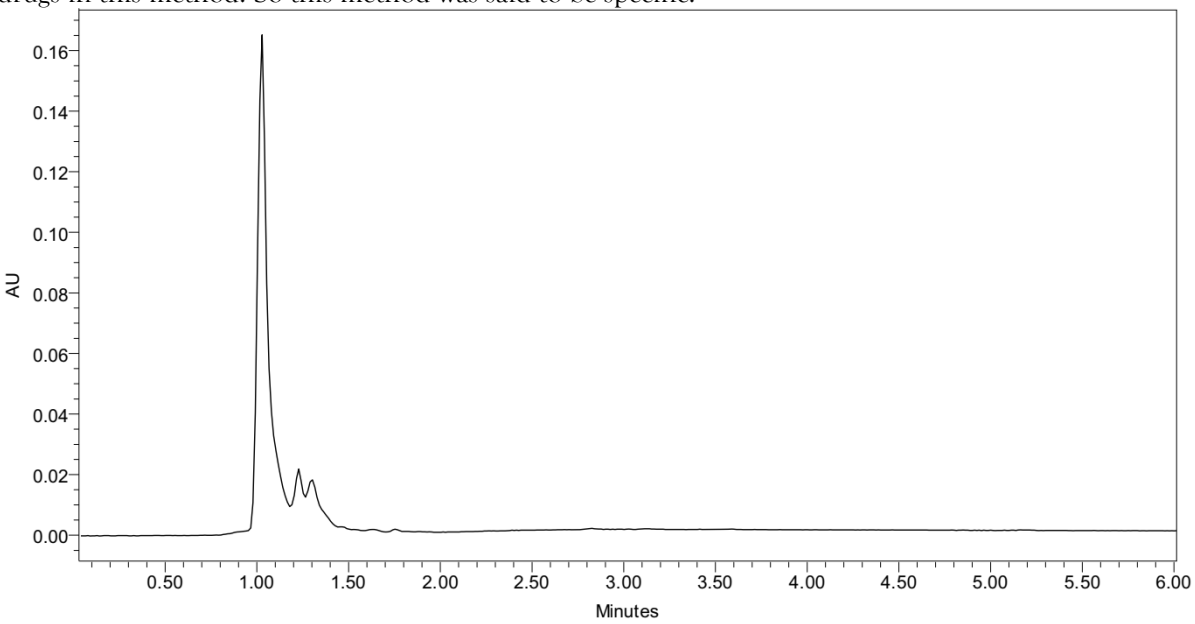

Figure 3 Representative Chromatogram of a Blank Plasma Sample

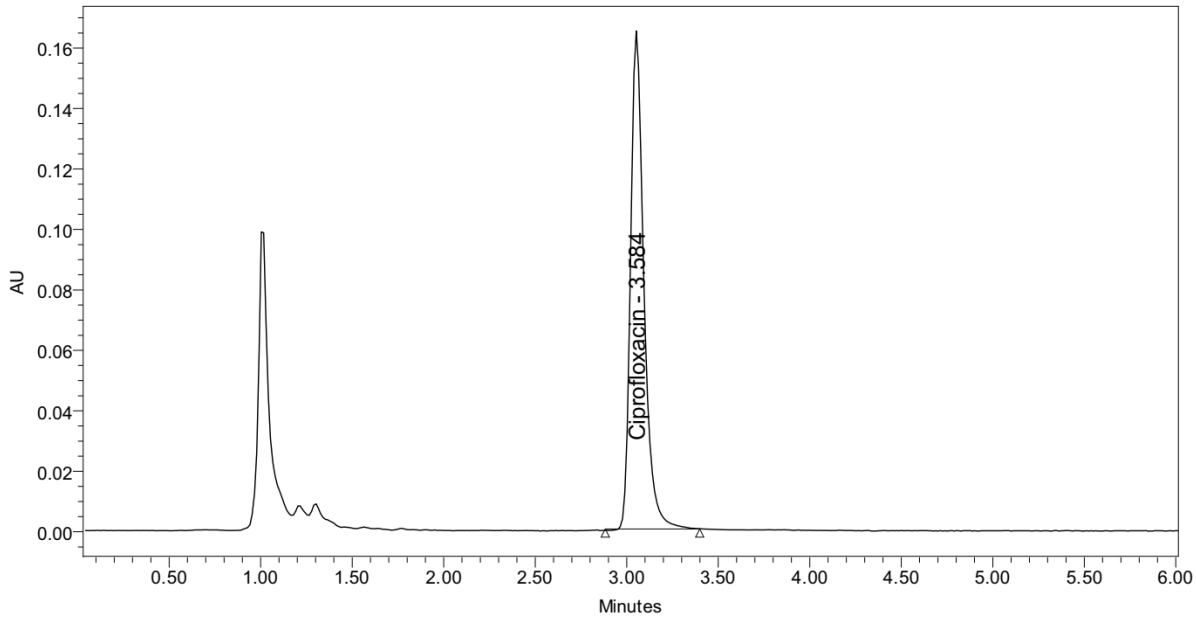

Figure 4 Representative Chromatogram of Blank Plasma with Internal Standard Sample

Discussion – The response areas obtained of analyte and internal standard are less than 20% and 5 % of LLoq Area. We did not find and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific

2) Sensitivity

A sensitivity is defined as "the lowest analyte concentration that can be measured with acceptable accuracy and precision i.e., LLoQ

Table7 Sensitivity of Tedizolid Phosphate

Sensitivity			
Analyte	Tedizolid Phosphate	**ISTD**	Ciprofloxacin
S No.			**LLOQ**
			Nominal Concentration (ng/mL)
			75.000
			Nominal Concentration Range (ng/mL)
			(60.000-90.000)
			Calculated Concentration (ng/mL)
1			75.290
2			75.240
3			75.170
4			74.650
5			74.350
6			75.020
n			6
Mean			74.9533
SD			0.37495
% CV			**0.50**
% Mean Accuracy			**99.94**

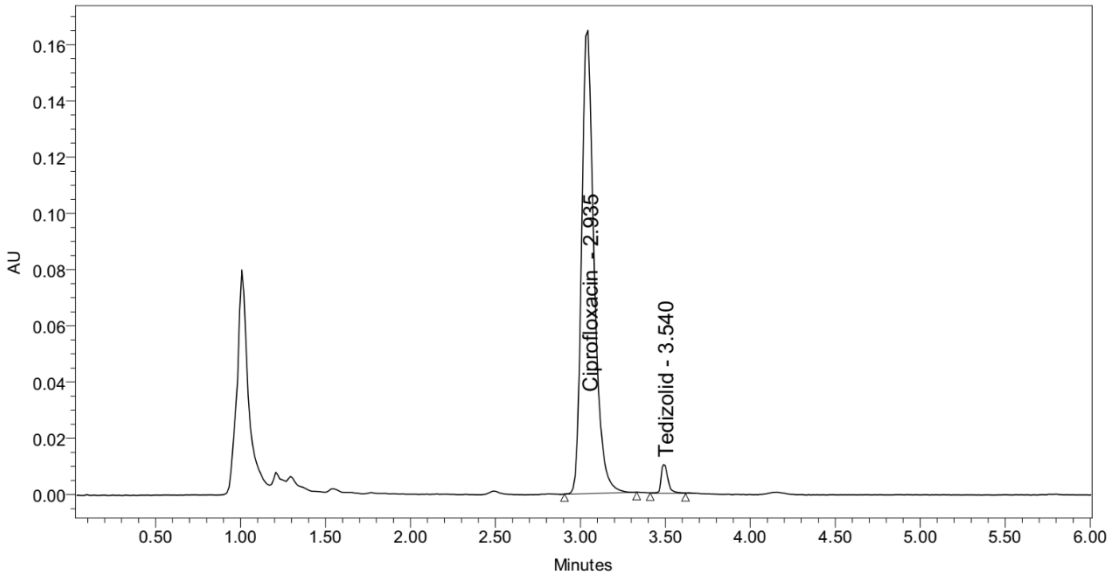

Figure 5 : LLOQ Chromotogram

Discussion: The LLOQ concentration was found between 80-120 % and % Coefficient of variation found to be 0.50% and mean of 6 injections was found to be 99.94% % within the acceptance limits. As the limit of Sensitivity % CV was less than "20%" the system Sensitivity was passed in this method.

Matrix factor evaluation

Table 8 : Matrix factor evaluation (absence of matrix factor)

Matrix Effect

Analyte	Tedizolid Phosphate	**ISTD**	Ciprofloxacin
S. No.	**Plasma Lot No.**	**HQC**	**LQC**
		Nominal Concentration (ng/mL)	
		2400.000	225.000
		Nominal Concentration Range (ng/mL)	
		(2,040.000-2,760.000)	(191.250-258.750)
		Calculated Concentration (ng/mL)	
1	LOT1	2406.63	226.48
		2402.75	227.02
		2407.26	226.78
2	LOT2	2408.25	226.74
		2407.84	227.65
		2408.63	226.48
3	LOT3	2406.25	227.08
		2405.15	225.45
		2406.38	225.65
4	LOT4	2405.42	225.85
		2407.20	225.65
		2408.21	226.05
5	LOT5	2406.24	226.35
		2407.29	226.74
		2408.52	225.06
6	LOT6	2406.74	225.54
		2407.56	226.51
		2408.95	226.25
n		18	18
Mean		**2406.9596**	**226.2961**
SD		**1.50860**	**0.66987**
% CV		**0.06**	**0.30**
% Mean Accuracy		**100.29**	**100.58**
No. of QC Failed		**0**	**0**

Discussion- The Evaluation of Matrix by injecting the QC samples of high and low concentrations in 6 lots the %Mean obtained was 100.29% and 100.58% of HQC and LOQ and % CV obtained are 0.59% and 1.67% of HQC and LOQ. As the limit of CV was less than "20%" the system Matrix was passed in this method.

Linearity:

Table 9 Linearity of Tedizolid Phosphate

Linearity								
Analyte	Tedizolid Phosphate						ISTD	Ciprofloxacin
	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
	Nominal Concentration (ng/mL)							
	75.000	150.000	225.000	600.000	1500.000	1800.000	2400.000	3000.000
	Nominal Concentration Range (ng/mL)							
	(60.000-90.000)	(127.500-172.500)	(191.250-258.750)	(510.000-690.000)	(1,275.000-1,725.000)	(1,530.000-2,070.000)	(2,040.000-2,760.000)	(2,550.000-3,450.000)
	Back Calculated Concentration (ng/mL)							
	74.954	149.846	226.854	602.860	1499.840	1803.470	2402.965	2998.580
	75.025	150.080	225.987	603.750	1498.850	1804.954	2403.025	3001.587
	74.956	150.024	225.620	603.850	1500.240	1804.680	2402.954	3002.846
n	3	3	3	3	3	3	3	3
Mean	74.9783	149.9833	226.1537	603.4867	1499.6433	1804.3680	2402.9813	3001.0043

SD	0.04043	0.12219	0.63366	0.54501	0.71557	0.78967	0.03821	2.19187
%CV	0.05	0.08	0.28	0.09	0.05	0.04	0.00	0.07
% Mean Accuracy	99.97	99.99	100.51	100.58	99.98	100.24	100.12	100.03

Final Conc in ng/ml	ISD(area)	Drug(area)	Area response ratio
0	0	0	0
75	1383771	5944	0.0043
150	1381492	11945	0.0086
225	1382984	17877	0.0129
600	1383611	47890	0.0346
1500	1382987	119017	0.0861
1800	1382232	145919	0.1056
2400	1383360	195674	0.1414
3000	1383580	236376	0.1708

Discussion: regression co-efficient value were

Parameter	Tedizolid Phosphate
Conc range (µg/mL)	75 – 3000 ng/ml
Co-relation	0.9999

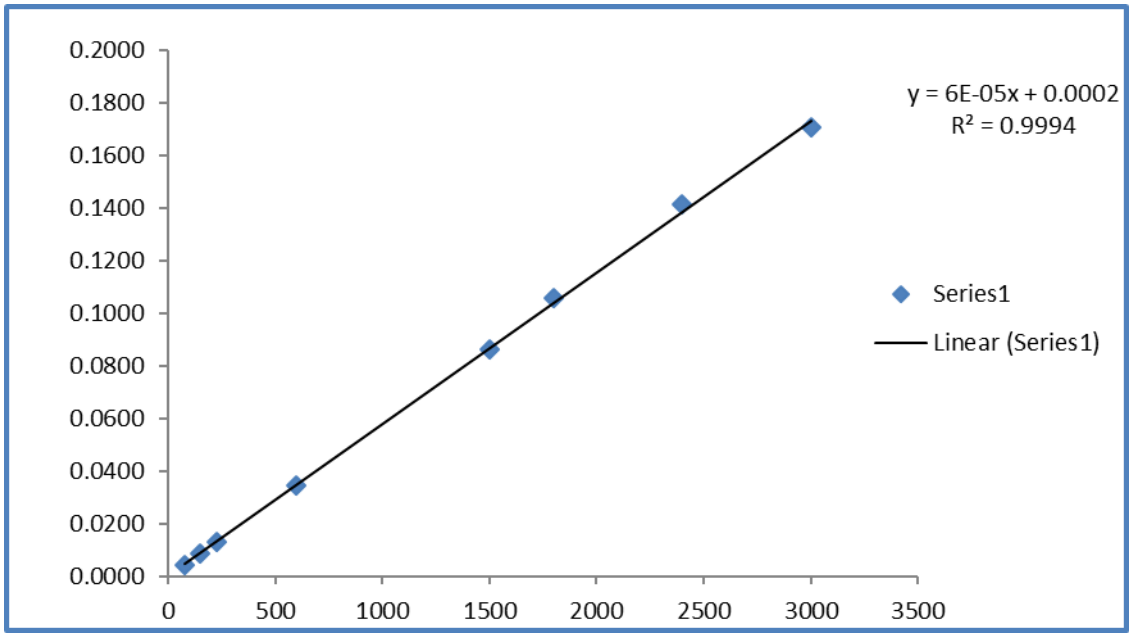

Figure 6 Representative Calibration Curve for Regression Analysis

Discussion :- Calibration was found to be linear over the concentration range of 75 to 3000 ng /ml. The coefficient correlation (r^2) value was found consistently greater than 0.999 in all the cases. This indicating linearity of results and an excellent correlation between peak area ratios for each concentration of analytes.

Precision and accuracy (intra-day runs of Tedizolid Phosphate)

Table 10: precision data for intra-day runs of Tedizolid Phosphate

Precision and Accuracy				
Analyte	Tedizolid phosphate		Ciprofloxacin	
	HQC	MQC1	LQC	LLOQ QC
	Nominal Concentration (ng/mL)			
	2400.000	1500.000	225.000	75.000

	Nominal Concentration Range (ng/mL)			
	(2,040.000-2,760.000)	**(1,275.000-1,725.000)**	(191.250-258.750)	(60.000-90.000)
	Back Calculated Concentration (ng/mL)			
	2402.510	1508.954	226.385	74.955
	2401.658	1499.845	226.745	75.756
	2400.245	1500.745	226.984	75.025
	2409.548	1500.325	227.325	75.956
	2397.658	1498.568	226.954	75.085
	2398.056	1498.546	227.685	75.962
n	6	6	6	6
Mean	2401.6125	1501.1638	227.0131	75.4564
SD	4.33443	3.92164	0.45163	0.48399
%CV	0.18	0.26	0.20	0.64
% Mean Accuracy	100.07	100.08	100.89	100.61
	2407.654	1499.845	227.840	75.026
	2406.954	1500.652	226.990	74.965
	2408.035	1500.569	226.856	75.622
	2407.645	1500.995	226.954	75.211
	2399.674	1499.584	227.845	75.625
	2406.954	1508.632	226.385	75.764
n	6	6	6	6
Mean	2406.1527	1501.7128	227.1450	75.3688
SD	3.20255	3.43059	0.58235	0.34409
%CV	0.13	0.23	0.26	0.46
% Mean Accuracy	100.26	100.11	100.95	100.49
	2407.654	1504.258	226.845	75.652
	2406.358	1500.250	226.846	75.056
	2408.475	1499.654	226.521	75.964
	2406.541	1500.657	226.984	75.056
	2408.541	1500.652	227.035	75.763
	2406.451	1499.658	226.159	75.977
n	6	6	6	6
Mean	2407.3367	1500.8549	226.7317	75.5782
SD	1.02198	1.72638	0.33284	0.42253
%CV	0.04	0.12	0.15	0.56
% Mean Accuracy	100.31	100.06	100.77	100.77
Between Batch Precision and Accuracy				
n	**18**	**18**	**18**	**18**
Mean	**2405.0339**	**1501.2439**	**226.9632**	**75.4678**
SD	**3.91079**	**2.99910**	**0.47305**	**0.40502**
%CV	**0.16**	**0.20**	**0.21**	**0.54**
% Mean Accuracy	**100.21**	**100.08**	**100.87**	**100.62**

Rugged Precision and Accuracy (inter-day runs of Tedizolid Phosphate)

Table 11: precision data for inter-day runs of Tedizolid Phosphate

Ruggedness Precision and Accuracy				
Analyte	Tedizolid phosphate		**ISTD**	Ciprofloxacin
	HQC	**MQC1**	**LQC**	**LLOQ QC**
	Nominal Concentration (ng/mL)			
	2400.000	**1500.000**	**225.000**	**75.000**
	Nominal Concentration Range (ng/mL)			

	(2,040.000-2,760.000)	(1,275.000-1,725.000)	(191.250-258.750)	(60.000-90.000)
	Calculated Concentration (ng/mL)			
Different Column	2398.562	1499.685	226.541	75.745
	2397.658	1500.520	226.845	75.993
	2408.023	1498.485	226.956	75.952
	2402.659	1499.562	226.745	75.048
	2406.452	1503.548	226.953	74.963
	2398.065	1500.658	227.056	75.046
n	**6**	**6**	**6**	**6**
Mean	**2401.9032**	**1500.4097**	**226.8493**	**75.4576**
SD	**4.53053**	**1.72459**	**0.18483**	**0.48904**
% CV	**0.19**	**0.11**	**0.08**	**0.65**
% Mean Accuracy	**100.08**	**100.03**	**100.82**	**100.61**
Different Analyst	2405.056	1504.658	227.956	74.956
	2402.654	1497.965	226.845	74.962
	2406.984	1499.786	226.845	75.066
	2403.054	1498.956	227.956	74.968
	2406.398	1500.856	226.456	75.542
	2404.785	1498.625	226.942	75.079
n	**6**	**6**	**6**	**6**
Mean	**2404.8218**	**1500.1410**	**227.1667**	**75.0953**
SD	**1.73437**	**2.42809**	**0.63381**	**0.22544**
% CV	**0.07**	**0.16**	**0.28**	**0.30**
% Mean Accuracy	**100.20**	**100.01**	**100.96**	**100.13**

Discussion: The intraday and inter day accuracy and precision was assessed by analysing six replicates at five different QC levels like LLOQ, LQC, MQC and HQC. Accuracy and precision method performance was evaluated by determined by six replicate analyses for Tedizolid Phosphate at four concentration levels, i.e., 75µg/ml(LLOQ), 225µg/ml (LQC), 1500µg/ml (MQC) and 2400µg/ml HQC.
The intra-day and inter day accuracy of plasma samples were assessed and excellent mean % accuracy was obtained with range varied from 100.62-100.87%, and 100.08% - 100.21% for intraday and 100.13% - 100.96% and 100.01% - 100.20% for inter day respectively. The precision (%CV) of the analytes and plasma samples were calculated and found to be 0.21%- 0.54% and 0.16% - 0.20% for intraday and 0.28% -0.30% and 0.07% - 0.16% for inter day respectively.

Recovery of Tedizolid Phosphate-

Table 12: Recovery of Tedizolid Phosphate

Recovery – Analyte						
Analyte	Tedizolid Phosphate					**ISTD**
S No.	**HQC**		**MQC1**		**LQC**	
	Unextracted Response	Extracted Response	Unextracted Response	Extracted Response	Unextracted Response	Extracted Response
1	195638	195362	119020	119010	17892	17871
2	198578	196876	119123	119001	17883	17870
3	197564	195369	119266	119126	17890	17881
4	197459	195788	119045	119023	17888	17876
5	194973	193856	119176	119026	17874	17870
6	198890	197548	119269	119158	17896	17889
n	6	6	6	6	6	6
Mean	197184	195800	119150	119057	17887	17876
SD	1571.77	1293.84	106.72	66.97	7.76	7.63

% CV	**0.80**	**0.66**	**0.09**	**0.06**	**0.04**	**0.04**
% Mean Recovery	**99.30**		**99.92**		**99.94**	
Overall % Mean Recovery	**99.720**					
Overall SD	**0.3651**					
Overall % CV	**0.37**					

Recovery - Internal standard

Table 13: Recovery of Ciprofloxacin (IS)

Recovery - Internal standard		
Analyte	**ISTD**	Ciprofloxacin
S.No.	Un extracted Area Ratio	Extracted Area Ratio
1	1383621	1377070
2	1368849	1309094
3	1381550	1375360
4	1322395	1301622
5	1396072	1379873
6	1304540	1376166
n	6	6
Mean	1359504.5	1353197.5
SD	37122.29	37162.77
% CV	2.73	2.75
% Mean Recovery	**99.54**	

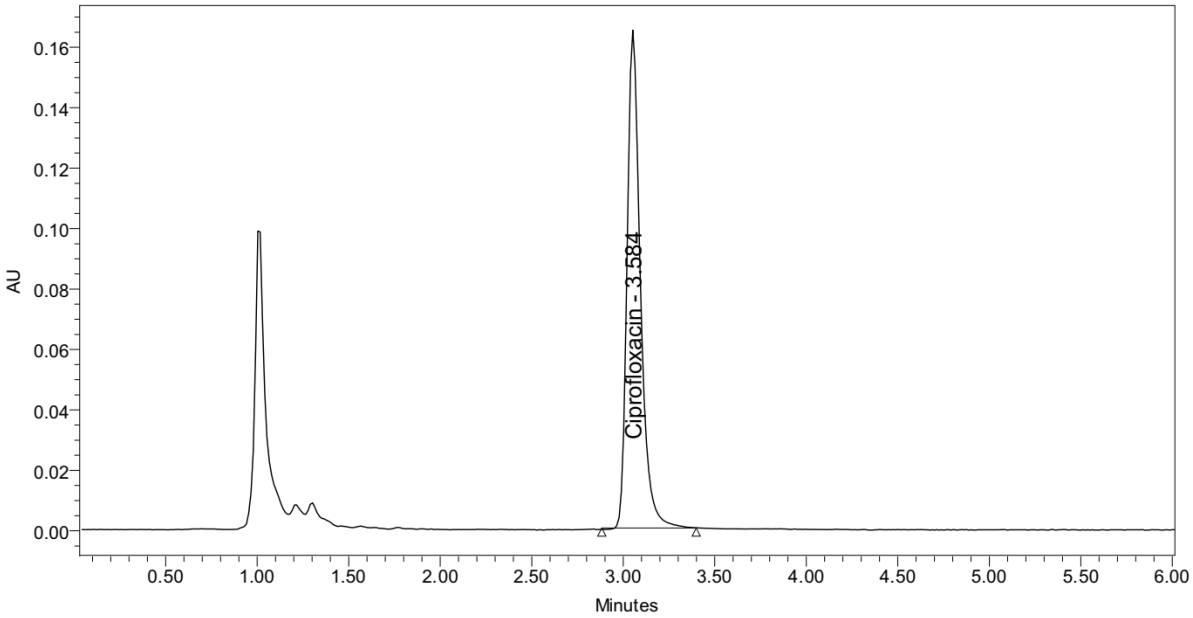

Figure 7 Recovery Chromatogram of Internal Standard

Discussion: Recovery was determined by measuring the peak areas obtained from prepared plasma samples with those extracted blank plasma spiked with standards containing the same area with known amount of Tedizolid Phosphate and . The overall % mean recovery for was found to be 99.54% at LQC, MQC and HQC levels and % CV ranged from 2.73 – 2.75% for IS, The results demonstrated that the bioanalytical method had good extraction efficiency. The results demonstrated that the bioanalytical method had good extraction efficiency.

Rugged Linearity:

Table 14 : Rugged Linearity of Tedizolid Phosphate

Ruggedness Linearity							
Analyte	Tedizolid Phosphate				**ISTD**	Ciprofloxacin	
STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
Nominal Concentration (ng/mL)							
75.000	150.000	225.000	600.000	1500.000	1800.000	2400.000	3000.000
Nominal Concentration Range (ng/mL)							
(60.000-90.000)	(127.500-172.500)	(191.250-258.750)	(510.000-690.000)	(1,275.000-1,725.000)	(1,530.000-2,070.000)	(2,040.000-2,760.000)	(2,550.000-3,450.000)
Calculated Concentration (ng/mL)							
Different Column							
75.955	153.568	226.845	603.578	1499.658	1806.628	2402.451.	3001.469
Different Analyst							
75.746	150.054	227.658	604.025	1499.860	1807.045	2402.656	3008.746

Discussion: Linearity ruggedness is a measure for the susceptibility of a method to small changes that might occur during routine analysis, The calibration range is obtained by injecting 8 concentrations (75 ng/ml - 3000ng/ml) of calibration standards not including blank and zero samples and establishing, The calibration curves were appeared linear and the coefficient of correlation was found to be 0.999 for Tedizolid Phosphate.

Reinjection Reproducibility

Table 15 : Reinjection Reproducibility of Tedizolid Phosphate

Reinjection Reproducibility				
Analyte	Tedizolid Phosphate		**ISTD**	Ciprofloxacin
	HQC	**MQC1**	**LQC**	**LLOQ QC**
	Nominal Concentration (ng/mL)			
	2400.000	1500.000	225.000	75.000
	Nominal Concentration Range (ng/mL)			
	(2,040.000-2,760.000)	(1,275.000-1,725.000)	(191.250-258.750)	(60.000-90.000)
	Calculated Concentration (ng/mL)			
	2406.58	1499.86	226.84	74.956
	2399.65	1500.25	226.85	75.841
	2398.51	1499.63	227.06	75.056
	2407.62	1498.21	226.95	75.074
	2406.64	1499.25	226.88	75.159
	2404.76	1503.26	226.75	75.055
n	6	6	6	6
Mean	2403.9610	1500.0776	226.8882	75.1903
SD	3.90779	1.70583	0.10737	0.32534
% CV	**0.16**	**0.11**	**0.05**	**0.43**
% Mean Accuracy	**100.17**	**100.01**	**100.84**	**100.25**

Discussion:- The % mean accuracy for LQC, MQC and HQC samples was found to be 100.84%, 100.01%, 100.17% and % Cv was found to be 0.05, 0.11, 0.16 and LLOQ was found 100.25 and % Cv was found to be 0.43. The results demonstrated that the bioanalytical method had good extraction efficiency.

Stabilities

Long-term stock solution stability

Table 16 stability of Tedizolid Phosphate (zero days)

DAY ZERO ASSESSMENT BATCH		
Analyte	**ISTD**	Ciprofloxacin
S No.	**HQC**	**LQC**
	Nominal Concentration (ng/mL)	
	2400.000	225.000
	Nominal Concentration Range (ng/mL)	
	(2,040.000-2,760.000)	(191.250-258.750)
	Calculated Concentration (ng/mL)	
1	2408.524	226.854
2	2408.695	226.954
3	2406.642	226.325
4	2405.650	227.096
5	2405.632	226.854
6	2497.560	227.963
n	0	6
Mean	2422.1170	227.0077
SD	36.98375	0.53600
% CV	**1.53**	**0.24**
% Mean Accuracy	**100.92**	**100.89**

Discussion- In bench-top stability, six replicates of LQC & HQC samples (225 and 2400 ng/ml) were analyzed for 9 hours at room temperature on the laboratory bench. The % mean stability was calculated and found to 100.89% for LQC and 100.92% for HQC respectively.

Matrix samples stability at -28±5 °C for 37 days

Table 17 : Matrix samples stability at -28±5 °C for 37 days

Long Term Analyte Stability in Matrix				
Analyte Name	Tedizolid Phosphate	**Temperature**	-28	±5 ^{0}C
S No.	**HQC**		**LQC**	
	Nominal Concentration (ng/mL)			
	2400.000	2400.000	225.000	225.000
	Nominal Concentration Range (ng/mL)			
	(2,040.000-2,760.000)	(2,040.000-2,760.000)	(191.250-258.750)	(191.250-258.750)
	Calculated Concentration (ng/mL)			
	Comparison Samples	Stability Samples	Comparison Samples	Stability Samples
1	2408.65	2404.56	226.854	226.958
2	2407.65	2406.69	226.745	226.845
3	2408.65	2405.66	227.124	226.956
4	2405.26	2408.97	227.066	227.038
5	2407.97	2405.62	227.163	226.845

6	2407.63	2404.37	226.845	224.054
n	6	6	6	6
Mean	2407.6358	2405.9776	226.9662	226.4493
SD	1.24863	1.68750	0.17309	1.17582
% CV	**0.05**	**0.07**	**0.08**	**0.52**
%Mean Accuracy	**100.32**	**100.25**	**100.87**	**100.64**
% Mean Stability	**99.93**		**99.77**	

Matrix samples stability at -80±5 °C for 37days

Table 18 : Matrix samples stability at -80±5 °C for 37 days

Long Term Analyte Stability in Matrix				
Analyte Name	Tedizolid Phosphate	**Temperature**	-80	±5 ^{0}C
S No.	**HQC**		**LQC**	
	Nominal Concentration (ng/mL)			
	2400.000	2400.000	225.000	225.000
	Nominal Concentration Range (ng/mL)			
	(2,040.000-2,760.000)	(2,040.000-2,760.000)	(191.250-258.750)	(191.250-258.750)
	Calculated Concentration (ng/mL)			
	Comparison Samples	Stability Samples	Comparison Samples	Stability Samples
1	2408.26	2407.06	226.756	226.755
2	2406.26	2406.58	226.065	226.856
3	2405.28	2406.55	225.965	227.956
4	2404.37	2400.33	226.857	226.845
5	2405.80	2404.04	226.765	227.856
6	2403.00	2402.26	227.966	226.963
n	6	6	6	6
Mean	2405.4938	2404.4670	226.7290	227.2051
SD	1.78040	2.74718	0.71731	0.54784
% CV	**0.07**	**0.11**	**0.32**	**0.24**
%Mean Accuracy	**100.23**	**100.19**	**100.77**	**100.98**
% Mean Stability	**99.96**		**100.21**	

Discussion:-Long-term stock solution stability for the Tedizolid Phosphate was determined at a concentration of LQC-HQC level after a storage period of 37 days at -28 °C & -80 °C in refrigerator. The % mean stability of the Tedizolid Phosphate was found to be 99.93% 99.77% at 28 ± 5°C and 99.96%, 100.21% at 80 ± 5°C respectively. Long term stock solution stability for the was determined at a concentration of LQC-HQC level after a storage period of 37 days at -28°C& -80°C in refrigerator.

Summary and Conclusion

A simple, Accurate, precise method was developed for the estimation of Tedizolid Phosphate in Rabbit plasma by using Rp-hplc was developed and validated. A simple, Accurate, precise method was developed for the simultaneous estimation of Tedizolid Phosphate in human plasma was developed and validated. By using Centrifugation technique, the sample preparation was prepared. Chromatogram run through Std Agilent C18 Column, 5 µm, and 4.6 mm X 250 mm, Mobile phase containing Acetonitrile: Buffer Ortho phosphoric acid taken in the ratio 60:40 v/v was pumped through column at a flow rate of 1.0 ml/min.OPA.in this method was buffer. For the separation of Tedizolid Phosphate Internal Standard [IS] used is Ciprofloxacin. The Temperature was maintained at 30°C. Optimized wavelength selected was 252 nm. Retention time of Tedizolid Phosphate and Internal Standard were found to be 3.564 min and 2.982 min. The standard curve was linear (R2 >0.999) over the concentration range of 75 – 3000 ng/ml. All the analytical validation parameters were determined as per ICH guidelines the bioanalytical method developed approach was selective, robust, and reliable, as accuracy, precision, recovery, and other validation parameters were all within the recommendations' limitations. The peaks produced for the drug

of interest and the internal standard were well separated from one another without any plasma interferences, and the peaks were symmetrical with an adequate tailing factor. The method has the potential to be very beneficial in therapeutic drug monitoring (TDM), bioequivalence research, pharmacokinetics studies, toxicology, and biomedical investigations.

REFERENCES

1. B.k Sharma, Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23rd Edition Goel publication , Meerut, (2007)
2. Lindholm.J, Development and Validation of HPLC Method for Analytical and Preparative purpose. Acta Universitatis Upsaliensis, pg . 13-14, (2004).
3. Rashmin, An introduction to analytical Method Development for Pharmaceutical formulations. Indoglobal Journal of Pharmaceutical Sciences , Vol.2 , Issue 2, Pg 191-196 (2012).
4. Malvia R, Bansal V , Pal O.P and Sharma P.K. A Review of High Performance Liquid Chromatography. Journal of Global Pharma technology (2010)
5. Douglas A Skoog, F. James Holler, Timothy A. Niemen, Principles of Instrumental Analysis Pg 725-760.
6. Dr.S. Ravi Shankar, Text book of Pharmaceutical analysis, Fourth edition, Pg 13.1-13.2
7. David G.Watson. Pharmaceutical Analysis, A text book for Pharmacy students and Pharmaceutical Chemists. Harcourt Publishers Limited; 2nd Ed., Pg 221-232.
8. Remingtonn's The Sciences and Practise of Pharmacy, 20th Edition (2000)
9. Connors Ka. A Textbook of Pharmaceutical Analysis, Wiley intersciences Inc; Delhi, 3rd Ed, Pg 373-421, (1994)
10. Gurdeep R.Chatwal , Sham K .Anand, Instrumental Methods of Chemical Analysis , Pg 2.566-2.638 (2007)
11. David G. Watson Pharmaceutical Analysis, A text book for pharmacy students and Pharmaceutical Chemists. Harcourt Publishers Limited; 2nd Ed.,Pg- 267-311
12. Nasal.A, Siluk.D, and Kaliszan.R. Chromatographic Retention Parameters in Medicinal Chemistry and Pharmacology, Pubmed, Vol.10, Issue 5 Pg no-381-426, March (2003)
13. Ashok Kumar, Lalith Kishore, navpreet Kaur , Anroop Nair. Method Development and Validation for Pharmaceutical Analysis. International Pharmaceutica Sciencia, Vol 2, Issue 3, Jul-Sep (2012)
14. Kaushal.C, Srivatsava.B, A Process of Method Development: A Chromatographic Approach. J Chem Pharm Res, Vol.2, Issue 2, 519-545, (2010)
15. Vibha Gupta, Ajay Deep Kumar Jain, N.S.Gill, Kapil, Development and Validation of HPLC method. International Research Journal of Pharmaeutical and Applied Sciences, Vol 2, Issue 4, Jul-Aug (2012)
16. Hokanson GC. A life cycle approach to the validation of analytical methods during Pharmaceutical Product Development. Part 1: The Initial Validation Process. Pharm Tech (1994) 92-100
17. Green JM. A Practicle guide to analytical method validation, Anal Chem (1996) 305A-309A
18. ICH, Validation of analytical procedures: Text and Methodology. International Conference on Harmonization, IFPMA , Geneva , (1996)
19. Ewelina rutkowska, Karolina paj k and Krzysztof J"ewiak* Lipophilicity – Methods of determination and its role in medicinal chemistry Acta Poloniae Pharmaceutica n Drug Research, Vol. 70 No.1 pp. 3n18, (2013).
20. IUPAC. Compendium of Chemical Terminology, 2nd edn. (The Gold Book). PAC69, 1137 (1997). Glossary of terms used in computational drug design (IUPAC Recommendations.
21. K. D. Tripathi, Essentials of Medical Pharmacology, 6th Edition, Jaypee brother's medical publishers (P) LTD, p-254-255.
22. Indian Pharmacopoeia, Indian Pharmacopoeial Commission, Controller of Publication, Government of India, Ministry of health and Family Welfare, Ghaziabad, India, 2 (2010) 1657-1658.
23. British Pharmacopoeia, The British Pharmacopoeial Commission, the stationary office, UK, London, 1408-1409 2 (2011).
24. http://www.drugbank.ca/drugs/DB00331.
25. Benoit Viollet, Bruno Guigas, Nieves Sanz Garcia, Jocelyne Leclerc, Marc Foretz, and Fabrizio Andreelli, cellular and molecular mechanisms of Samidorphan: An overview, Clincal Science (London), 122(6): 253–270,(2012).
26. https://go.drugbank.com/drugs/DB09042
27. Eman M. Moaaz et al. (2021), Stability-Indicating Determination of Tedizolid Phosphate in the Presence of its Active Form and Possible Degradants, Journal of Chromatographic Science 60(7).
28. Ajit Anerao et al. (2017), Enantioseparation of Tedizolid phosphate by RP-HPLC, using β-Cyclodextrin as a Chiral Mobile Phase Additive, J App Pharm Sci; 7(10): 30-36.