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Development and Validation of Stability Indicating Rp-Hplc Method for Estimation of Linezolid in Bulk Drug and Pharmaceutical Dosage Form

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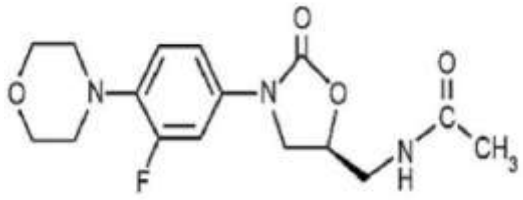
ABSTRACT: A simple, selective, linear, precise and accurate isocratic reverse phase- high performance liquid chromatography (RP-HPLC) method was developed and validated for linezolid in bulk and pharmaceutical dosage form. The drug compounds were subjected to a stress condition in order to conduct a force degradation investigation. Finally, the separation and quantification of drug from degradation products was carried out on a kromasil C18 column (250 mm 4.6 mm ID, 5 m), with a temperature of 40°C, a mobile phase of methanol: water (70:30) v/v, a flow rate of 1.0 ml/min, and detection at 258 nm. The retention time for linezolid was 2.9 minutes. In the concentration range of 10-150 ppm, linearity was observed. The linezolid recovery evaluation was found to be satisfactory, and the correlation coefficient was 0.9999, indicating that the methods' linearity was within specification limitations. The approach was validated in accordance with the International Conference on Harmonization (ICH) requirements. It is possible to use the proposed method.

KEYWORDS: Linezolid, HPLC Method, Degradation, Validation

I. INTRODUCTION:

DRUG: LINEZOLID
Structure:



$C_{16}H_{20}FN_3O_4$

Fig 1: chemical structure of linezolid

Chemical name	(S)-N-[[3-[3-Fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl] methyl] acetamide
Generic name	Linezolid
Brand name	Zyvox

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Class	Synthetic antibiotic, antimicrobial called oxazolidinones
Mol. Formula	$C_{16}H_{20}FN_3O_4$
Mol. Weight	337.35 g/mol
CAS Registry Number	165800-03-3
Antimicrobial spectrum	Mainly active against gram- positive organisms
pH value	4.3 to 5.3
pKa value	1.8
Polarity	Log P 0.232
solubility	Water-soluble (approximately 3mg/ml), it is also soluble in organic solvents such as methanol, ethanol, DMSO, dimethyl formamide.
bioavailability	~100% (oral)
Protein binding	Low (31%)
metabolism	Hepatic (50-70%, CYP not involved)
Dose	Oral or i.v. 600 mg every 12 hours for serious infections & 400 mg every 12 hours for uncomplicated infection [FDA Label]
Half life	4.2- 5.4 hours (shorter in children)
Excretion	Non renal, renal, and fec
Melting point	181.5 to 182.5 Ċ
Storage	Store at RT
U.V. spectrum	λ max 252- 259 nm

Linezolid was authorised by the US Food and Drug Administration (FDA) on April 18, 2000. Linezolid has been approved to treat infections caused by Vancomycin-resistant Enterococcus faecium, hospital-acquired pneumonia caused by Staphylococcus aureus, complicated skin and skin structure infections (SSSIs), uncomplicated SSSIs caused by methicillin-susceptible S.aureus or Streptococcus pyogenes, and community-acquired pneumonia caused by Streptococcus pneumoniae.

The antibiotic linezolid is the first member of the oxazolidinone class of antibiotics. The substance is a synthetic antibiotic that binds to rRNA and inhibits bacterial protein production. Most Gram-positive bacteria that cause disease, such as streptococci, vancomycin-resistant enterococci (VRE), and methicillin-resistant Staphylococcus aureus, are susceptible to linezolid (MRSA). It's also used to treat skin infections and pneumonia.

It can be used to treat a wide range of illnesses, including drug-resistant tuberculosis. It can be utilized in patients of all ages, as well as those who have liver illness or impaired renal function. In the treatment of skin and soft tissue infections, linezolid is more effective than glycopepetide antibiotics (vancomycin and teicoplanin) and beta-lactamase antibiotics (SSTIs). In the treatment of diabetic foot infections, linezolid looks to be both cheaper and more effective than vancomycin. It's also been utilized as a second-line treatment for capnocytophaga infections with outstanding success. Linezolid is an antibiotic oxazolidinone that is effective against most aerobic Gram-positive bacteria and mycobacteria. It seems to be bacteriostatic against staphylococci and enterococci, as well as bactericidal against most streptococci isolates.

Linezolid works by preventing the bacterial cell from producing proteins. By preventing the development of the 70S ribosomal initiation complex, it functions as a bacterial protein synthesis inhibitor. Bacterial ribosomes are made up of two subunits, 30S and 50S, each of which is made up of RNAs encased in proteins. These two units link together to start the process of protein synthesis and then separate after the process is finished. The ribosomal 30S subunit binds to mRNA to make formylmethionyl-tRNA, which subsequently forms an initiation complex with GTP

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and three initiation factors ([IFs] 1-3). After binding to the ribosomal 50S subunit to form the 70S ribosome, protein synthesis can commence.

II. EXPERIMENTAL WORK :

2.1 Selection of Analytical Wavelength

2.1.1. Selection of solvent

Methanol was selected as the solvent for dissolving Linezolid.

2.1.2. Preparation of standard stock solutions

To make the stock solution, weigh 10 mg Linezolid into a 20 mL volumetric flask, add 15 mL methanol, and sonicate to completely dissolve the standard, then dilute to the desired concentration with methanol (500 PPM). Methanol was used to dilute the 0.8 mL to 20 mL. (20 PPM)

2.1.3. Selection of analytical wavelength

Scanning was performed from 400nm to 200nm using Methanol as a blank and Linezolid standard solution (20 PPM). For each medication, the absorption maxima were calculated. The results showed that linezolid had the highest absorbance at 258 nm.

2.2 METHOD DEVELOPMENT BY RP-HPLC

2.2.1. Preparation of standard stock solution for Chromatographic development:

The Linezolid standard stock solution was created by dissolving 10 mg Linezolid in a 20 mL clean and dried volumetric flask, adding 15 mL methanol to completely dissolve it, and topping up the volume with methanol (500PPM).

Methanol was added to dilute the stock solution from 2 mL to 10 mL. (100 PPM).

It was made in the first HPLC experiment with methanol, and water from the second HPLC trial was utilized as diluents and injected in development trials.

Selection of analytical wavelength for HPLC method development: The wavelength of maximal absorption from the spectrophotometric study was chosen as the analytical wavelength for the examination, and it was 258 nm.

2.2.2. Optimization of HPLC Method

Reversed Phase Liquid Chromatography with Isocratic elution and UV detection.

Table: 1 Optimized Chromatographic Conditions for Linezolid

Parameter	Description
Mode	Isocratic
Column Name	Kromasil C18**,** 250 mm X 4.6mm ID, 5 µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	258 nm
Column Oven temp	40ºC
Mobile Phase	Methanol : Water (70:30%V/V)
Flow Rate	1.0 ml/min
Diluent	Water
Run time	10 Minutes

2.3 Preparation Of System Suitability Test (Linezolid Standard Solution):

Weighed about 25 mg of Linezolid and transferred it to a 50 mL volumetric flask, added 30-35 mL of methanol, sonicated to dissolve it, and filled the flask with methanol to the desired volume. Pipette 2 ml of standard stock solution into a 10 ml volumetric flask and top up with water to get the desired volume. Chromatograms were taken at 100 g/mL (working concentration).

System suitability is a pharmacopoeial criteria that determines if the chromatographic system is suitable for the analysis being performed. The studies were carried out by gathering data from five replicate injections of a standard medication solution and recording the outcomes.

Acceptance criteria

1. RSD should not be more than 2.0 % for five replicate injections of standard.
2. USP Tailing Factor/ Asymmetry Factor is not more than 2.0.
3. The column efficiency as determined for Plate Count should be more than 2000.

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2.4 Analysis of Marketed Test Sample:

Test sample that has been marketed the tablets with the name Lizomed 300 mg are chosen for examination and validation.

Average weight of test sample (Lizomed 300 mg**):**

Weighed the 20 tablets at a time and calculated average weight of tablet by following formula:

Average weight (mg) = Weight of 20 tablets (mg) / 20

Sample preparation of Marketed test sample:

20 tablets were weighed, then transferred to a mortar pestle and crushed to a fine powder. Butter paper was used to uniformly mix the components. Weighed the powder material equivalent to 100 mg of Linezolid and transferred it to a 100 mL volumetric flask that had been cleaned and dried. 70 mL methanol was added, and the mixture was sonicated for 15 minutes with occasional shaking. Allow 15 minutes for the solution to cool to ambient temperature before adding methanol to get the volume up to the desired level. 3-5 mL of the initial filtrate was discarded after filtering the solution using an appropriate 0.45 syringe filter. Further, 1 ml of filtered stock solution was diluted to 10 ml with water (100 mcg of Linezolid), the resultant solution was injected, and chromatograms and results were recorded.

Sample Prepared in duplicate. Summary of sample preparation as follows:

Sample	Sample (mg)	Diluted to (mL)	Volume taken	Diluted to (mL)
Sample 1	158.3	100	1	10
Sample 2	158.7	100	1	10

Formula for % Assay calculation:

$$\%\ Assay = \frac{LinezolidSplarea}{LinezolidStd\ avgarea} X \frac{LinezolidSTDwt\ (mg)}{50} X \frac{2}{10} X \frac{100}{Tabletsampleweight\ (mg)}$$

$$X \frac{10}{1} X \frac{Avgwtofsample\ (mg)}{LabelclaimofLinezolid\ (mg)} X\ 100$$

2.5 FORCE DEGRADATION OF PHYSICAL LAB MIXTURE

To establish if the analytical procedure for the assay was stability indicating or not, a physical lab mixture of tablet and placebos was subjected to various stress conditions to conduct forced degradation tests. Stress tests were carried out under acid/base hydrolysis, oxidation, and heat conditions, as prescribed in ICH Q1A. (R2). Thermal and sunlight were used to destroy medicinal compounds in the solid form. For each stressful scenario, several trials were conducted with increasing degrees of severity, resulting in a 5-20% reduction in performance.

Marketed tablet formulation (Lizomed 300 mg tablets) was available, but its excipients are unknown to us. If we perform force degradation on marketed test sample, there might be possibility of getting degradation product of excipients. When we will get degradation products, we cannot distinguish between the degradation products of Linezolid API and degradation products of excipients. Hence physically lab mixture of tablet prepared at lab level and used for force degradation purpose.

1. Tablet Preparation:

Considered for preparing physical tablet lab mixture is a marketed tablet formulation (Lizomed 300 mg tablets) with an average weight of 474 mg.

Formula for 1 tablets as follows:

Sr. No.	Ingredients	Role	Qty (mg)
1	Linezoid API	Active ingredient	300 mg
2	Placebo	NA	174 mg
Total			474 mg

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Total 10 gm of Physical lab mixture prepared.
Placebo prepared at lab level by using formula as follows:

Sr. No.	Ingredients	Role	Qty (mg)
1	Lactose	Filler	80
2	Starch	Binder	5
3	Magnesium stearate	Lubricant	5
4	Talc	Glidant	5
5	crospovidone	Disintegrants	5
Total			100 mg

Total 10 gm of placebo prepared.

1) Test sample as such:

Weighed the physical lab mixture equivalent to 100 mg of Linezolid (158 mg of physical lab mixture). Transfer to a clean, dry 100 mL volumetric flask, add 70 mL Methanol, and sonicate for 15 minutes with intermittent shaking. Methanol was used to get the volume up to the desired level. Filter the solution using a 0.45 PVDF syringe filter, discarding the first 3-5 mL. Dilute 1 mL of filtrate with 10 mL of water. (Linezolid 100 PPM)

2) Physical degradation:

A. Thermal degradation: In a petri dish, place an appropriate amount of physical lab mixture and place in a hot air oven at 105o C for 48 hours. After 48 hours, the sample was removed and placed in a desiccator to reach R.T.

B. Photolytic degradation: In a petri dish, place a sufficient amount of physical lab mixture and place in direct sunlight for 72 hours. After 72 hours, the sample was removed and placed in a desiccator to reach R.T.

3) Chemical degradation:

A. Acid degradation: Both the physical lab mixture of tablet and the placebo were forced to degrade in an acidic environment. For this experiment, 5 N HCl was made (42.5 ml of HCl diluted to 100 ml with water).

B. Base/Alkaline degradation: Under alkaline conditions, both the physical lab mixture of tablet and the placebo were forced to degrade. For the experiment, 5 N NaOH (20 gm NaOH dissolved in 100 ml water) was made.

C. Peroxide degradation: The physical lab mixture of tablet was subjected to forced deterioration. The investigation employed a 30 percent hydrogen peroxide solution and a 30 percent sodium sulfite solution.

2.6 VALIDATION OF RP-HPLC METHOD

The developed method for estimation of Linezolid was validated as per ICH guidelines for following
parameters.

1) FILTRATION STUDY:

The Linezolid Test sample was used in this study (Tablet solution). Filtration tests were conducted using both unfiltered and filtered test solutions. During the filtration process, 0.45 m PVDF and 0.45 m Nylon syringe filters were utilized, with 5 mL of the aliquot sample being discarded.

2) STABILITY OF ANALYTICAL SOLUTION :

For the standard and test sample solutions, a stability investigation was carried out. The stability test was carried out in a typical laboratory setting.
The solution was kept in a brightly lit laboratory for 12 and 24 hours before being examined.

3) SPECIFICITY:

The capacity to access the analyte definitively in the presence of components that are expected to be present is referred to as specificity.

To demonstrate the method's specificity, the following solution must be made and injected. (Purity of peak in standard and test sample solution checked)

I. Blank (Methanol as a diluent)
II. Placebo
III. Linezolid Standard solution
IV. Tablet test sample solution

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4) LINEARITY AND RANGE

Preparation of linearity solution

5 levels of Linearity was performed from 10% to 150% of working concentration

Linearity Linezolid stock solution:

Weighed 50 mg of Linezolid and dissolved in 50 mL with methanol. (1000 µg/mL)

Linearity levels prepared as follows:

Sr. No.	Level (%)	mL of stock solution	Diluted to with water (mL)	Linezolid Concentration (µg/mL)
1	10	0.2	20	10.00
2	50	1.0	20	50.00
3	100	2.0	20	100.00
4	125	2.5	20	125.00
5	150	3.5	20	150.00

Determination

Each level was injected three times and the mean area was computed. As shown in the results, the calibration curve was graphically shown as a function of analyte concentration in g/mL on the X-axis vs. mean area on the y-axis.

Acceptance criteria

Correlation Coefficient: NLT 0.98

Intercept: To be report

Slope: To be report

5) ACCURACY (% RECOVERY)

The accuracy will be tested between 50 and 150 percent of working concentration. Each accuracy level's solution was duplicated three times. Calculated percent RSD for each level and total recovery, as well as percent RSD for each level and overall recovery.

Accuracy levels details:

Refer Following table for each sample:

Level (%)	API (mg)	Placebo	Diluted to (mL)	Volume taken(mL)	Diluted to (mL)	Conc (µg/mL)
50	50.16	58.6	100	1.0	10	50.16
	50.21	58.4	100	1.0	10	50.21
	50.31	58.2	100	1.0	10	50.31
100	100.09	57.9	100	1.0	10	100.09
	100.12	59.1	100	1.0	10	100.12
	100.41	58.2	100	1.0	10	100.41
150	150.08	58.4	100	1.0	10	150.08
	151.00	58.9	100	1.0	10	151.00
	150.15	58.2	100	1.0	10	150.15

Acceptance criteria

1. % Recovery for each sample and Mean recovery and overall recovery should be in the range of 98-102%.
2. The Relative Standard Deviation should not be more than 2.0%.

6) PRECISION

I. Repeatability:

Preparation of sample solution (6 Samples prepared):

20 tablets were weighed, then transferred to a mortar pestle and crushed to a fine powder. Butter paper was used to uniformly mix the components. Weighed the powder material equivalent to 100 mg of Linezolid and transferred it to a 100 mL volumetric flask that had been cleaned

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and dried. 70 mL methanol was added, and the mixture was sonicated for 15 minutes with occasional shaking. Allow 15 minutes for the solution to cool to ambient temperature before adding methanol to get the volume up to the desired level. 3-5 mL of the initial filtrate was discarded after filtering the solution using an appropriate 0.45 PVDF syringe filter. Further, 1 ml of filtered stock solution was diluted to 10 ml with water (100 mcg of Linezolid), the resultant solution was injected, and chromatograms and results were recorded.
Six samples prepared.

Precision (Repeatability) Sample details are as follows:

Sample	Powder wt. (mg)	Diluted to (mL)	Volume taken(mL)	Diluted to (mL)
Sample 1	158.16	100	1.0	10
Sample 2	158.91	100	1.0	10
Sample 3	159.21	100	1.0	10
Sample 4	158.06	100	1.0	10
Sample 5	158.41	100	1.0	10
Sample 6	158.22	100	1.0	10

Acceptance criteria:
% Assay: 90-110% for each sample and mean assay value
% RSD for % assay value of 6 samples: NMT 2%

I. Intermediate precision:
Six Samples prepared

Intermediate Precision Sample details are as follows:

Sample	Powder wt. (mg)	Diluted to (mL)	Volume taken(mL)	Diluted to (mL)
Sample 1	158.41	100	1.0	10
Sample 2	158.06	100	1.0	10
Sample 3	158.33	100	1.0	10
Sample 4	159.04	100	1.0	10
Sample 5	158.11	100	1.0	10
Sample 6	158.71	100	1.0	10

7) ROBUSTNESS
Blank and Standard solution were injected under different chromatographic conditions as shown below.
a) Changes in flow rate by ±10%. (±0.1ml/min)
b) Change in column oven temperature. (± 2ºC)
c) Change in wavelength (± 3 nm)

III. RESULT AND DISCUSSION

1. Selection of analytical wavelength
Linezolid STD solution: (20 PPM)
Linezolid showed maximum absorbance at 258 nm.

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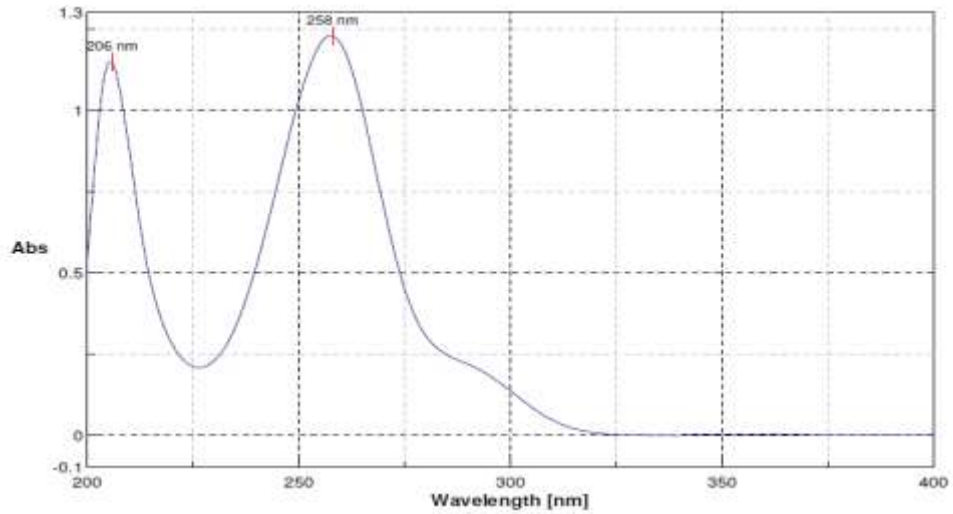

Fig.1: UV spectrum of linezolid

2. Optimization of HPLC method

The linezolid eluted well, and there was excellent chromatography. (Final dilution made in water)

It was concluded that the chromatographic conditions employed in this trial produced a better peak, good retention time, and tailing factor, hence they were used for method validation.

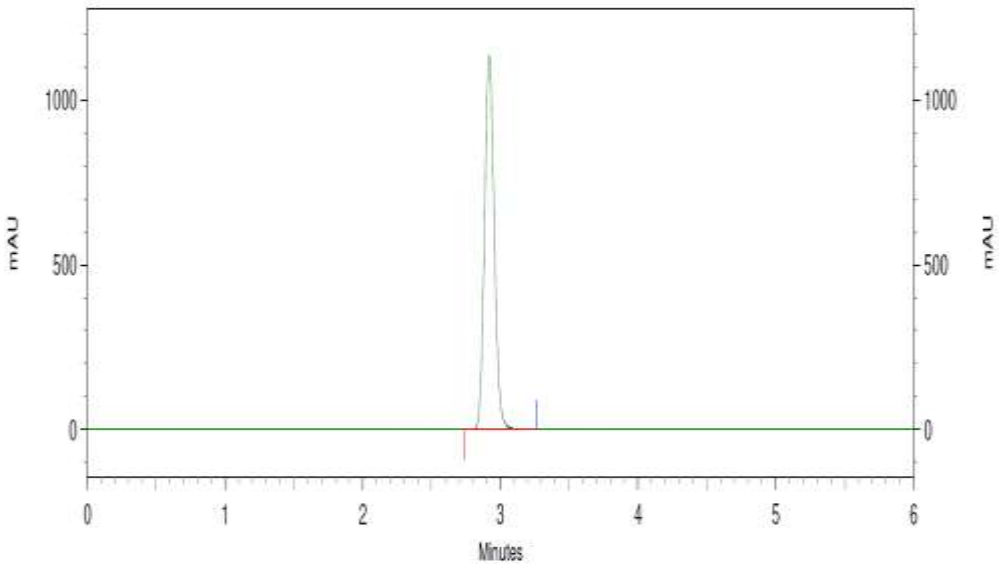

Fig.2: Chromatogram of optimized method

3. Results for System Suitability Test of Linezolid

Sr No.	Standard solution	Area	Asymmetry	Theoretical plates
1	Standard_1	113751481	1.37	5041
2	Standard_2	113585719	1.37	5079
3	Standard_3	113652497	1.36	5091
4	Standard_4	112751437	1.37	5064

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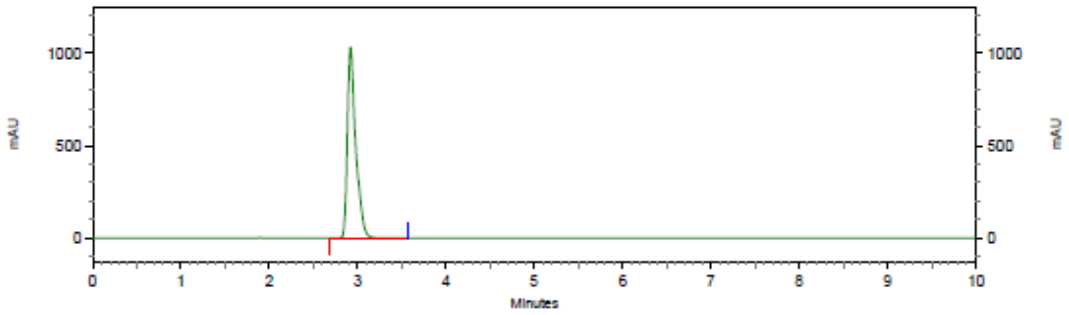
5	Standard_5	113287341	1.36	5037
Mean		**113405695**	**1.37**	**5062**
STD Dev		**404644.60501**		
% RSD		**0.36**		

Data interpretation: The data given above shows that the procedure conforms with system suitability parameters. As a result, the chromatographic method can be determined to be adequate for the required analysis.

Sample Name: SST STANDARD_1



VWD: Signal A, 258 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Linezolid	2.93	113751481	1.37	5041
Totals		113751481		

Fig.3: Chromatogram of Standard solution 1 of system suitability solution.

4. Analysis of Marketed Test samples (Assay)
a) Lizomed 300 mg Tablet:
Weight of 20 tablets = 9.4800 gm

Average weight of tablet = 9.4800 /20 = 0.474 gm = 474.0 mg

Assay results of Lizomed 300 Tablet

Sample	Area	% Assay	Mean Assay
Sample 1	110652718	97.54	97.36
Sample 2	110514097	97.18	

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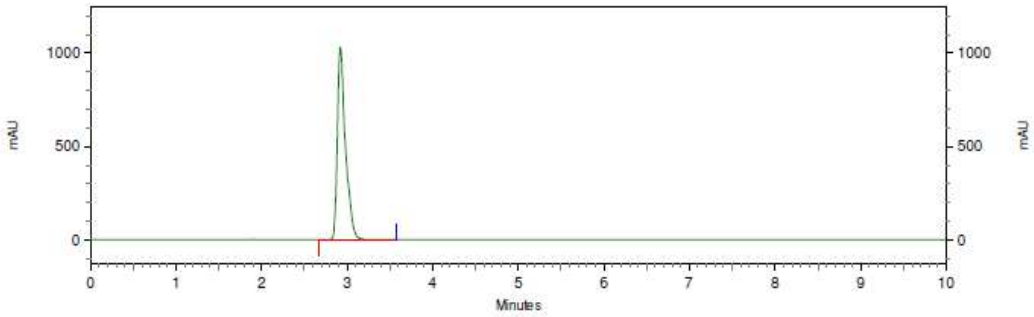
Sample Name: ROUTINE SAMPLE_1



VWD: Signal A, 258 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Linezolid	2.92	110652718	1.37	5121
Totals		110652718		

Fig. 4: Typical chromatogram 0f Lizomed 300 Tablet sample.

Acceptance criteria:
1) % Assay found should be in the range of 90-110%.

Data interpretation:

From the above results, it can be concluded that the assay result is within the limit for selected marketed test sample and sample can be used for validation.

5 .FORCE DEGRADATION OF PHYSICAL LAB MIXTURE
Result summary of Force degradation of Physical lab mixture:

Sample Name	Treatment	Exposure condition	% Assay	% Degradation
Physical lab mixture	Sample as such	NA	97.75	Nil
	Thermal	105°C for 48 Hours	97.17	Nil
	Photolytic	Direct sunlight for 72 hours	96.76	Nil
	Acid	10 mL of 5 N HCl for 12 Hour at R.T.	92.05	5.70
	Base	10 mL of 5 N NaOH for 12 Hour at R.T.	82.25	15.50
		10 mL of 5 N NaOH for 6 Hour at R.T.	88.61	9.14
	Peroxide	10 mL of 30% H2O2 for 12 Hour at R.T.	98.36	Nil
		10 mL of 30% H2O2 for 24 Hour at R.T.	98.12	Nil

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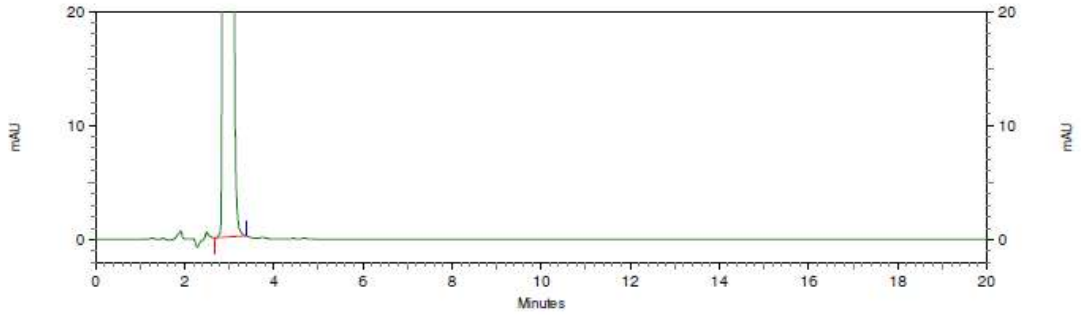
1) Control sample chromatogram (Sample as such)

Sample Name: SAMPLE AS SUCH



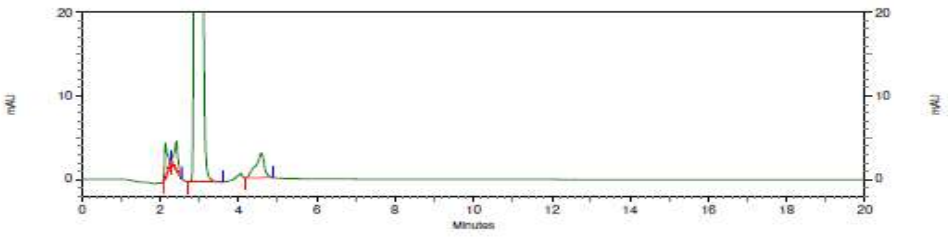
VWD: Signal A, 258 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Linezolid	2.92	110751867	1.37	5345
Totals		110751867		

Fig. 5: Typical chromatogram of sample as such

2) Acid Degradation

Sample Name: ACID SAMPLE 12 HOURS



VWD: Signal A, 258 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)	Resolution (USP)
Degradation product 3	2.14	315172	1.71	5257	0.00
Degradation product 4	2.41	398165	0.89	3487	1.97
Linezolid	2.94	104686341	1.40	4926	3.16
Degradation product 5	4.59	749762	0.79	2632	6.29
Totals		106149440			

Fig.6: Chromatogram of sample exposed at Acid condition for 12 hour

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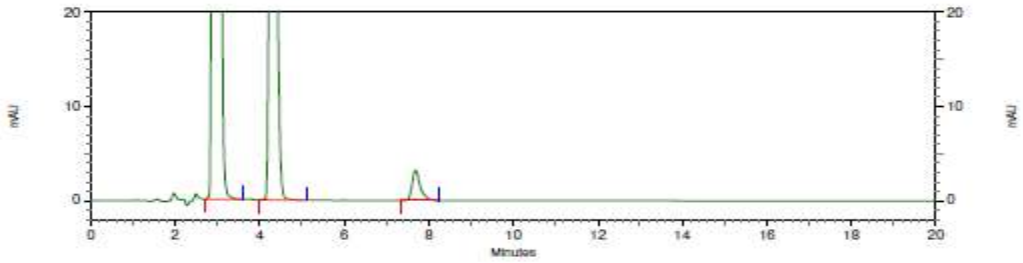
3) Base degradation

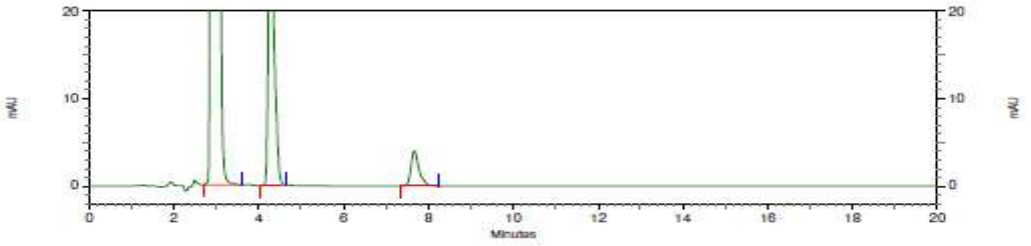
Sample Name: SAMPLE BASE 12 HOURS



VWD: Signal A, 258 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)	Resolution (USP)
Linezolid	2.93	93482417	1.39	5096	0.00
Degradation product 1	4.29	16298055	1.51	7666	7.63
Degradation product 2	7.69	717208	1.42	8339	12.78
Totals		110497680			

Fig.7: Chromatogram of sample exposed at Basic condition for 12 hour.

Sample Name: SAMPLE BASE 6 HOURS



VWD: Signal A, 258 nm Results Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)	Resolution (USP)
Linezolid	2.93	101028147	1.38	4940	0.00
Degradation product 1	4.28	6420103	1.46	7869	7.68
Degradation product 2	7.67	881198	1.40	8663	12.96
Totals		108329448			

Fig.8: Chromatogram of sample exposed at Basic condition for 6 hour.

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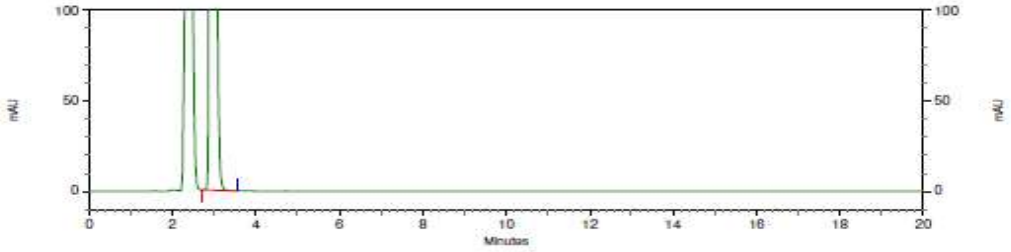
3) Peroxide degradation

Sample Name: SAMPE PEROXIDE 12 HOURS

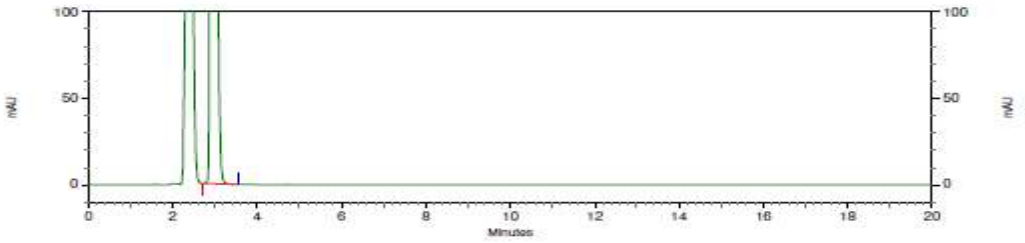


VWD: Signal A, 258 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Linezolid	2.94	111724586	1.39	5019
Totals		111724586		

Fig.9: Chromatogram of sample exposed at Peroxide condition for 12 hours.

Sample Name: SAMPE PEROXIDE 24 HOURS



VWD: Signal A, 258 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Linezolid	2.93	110886541	1.38	5137
Totals		110886541		

Fig.10: Chromatogram of sample exposed at Peroxide condition for 24 hour.

6. VALIDATION OF RP-HPLC METHOD

1. Results of Filter Study:

Sample description	Area	% Absolute difference
Unfiltered	110746084	NA
0.45 µ PVDF filter	110651794	0.09
0.45 µ Nylon filter	110579718	0.15

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2. Results of Solution Stability.

Sample solution			Standard solution		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**% Absolute difference**
Initial	110684517	NA	**Initial**	113521067	NA
12 Hours	110149718	0.48	**12 Hours**	113011971	0.45
24 Hours	109141872	1.39	**24 Hours**	111841937	1.48

Acceptance criteria: % Absolute difference of Stability solution: NMT 2.0 w.r.t. Initial solution.

Data interpretation: Standard and Test solution was found stable up to 24 Hrs. Hence both solutions can be used up to 24 Hrs.

3. Results of Specificity.

Description	**Observation**
Blank	No interference at R.T. of Linezolid due to blank
Placebo	No interference at R.T. of Linezolid due to placebo
Standard solution	Peak purity was 0.997
Test Solution	Peak purity was 0.985

Standard and Test sample solution: Peak purity: NLT 0.95
Peak purity for Standard as well as test solution was well within limits. Hence developed chromatographic method passed the criteria for specificity.

Level	**Conc (µg/mL)**	**Area**	**Mean**	**% RSD**
10%	10.00	11374380	11383029	0.281
		11356241		
		11418466		
50%	50.00	57206636	57290563	0.130
		57349847		
		57315205		
100%	100.00	113824897	113568220	0.210
		113526718		
		113353045		
125%	125.00	141770960	141749550	0.082
		141624068		
		141853621		
150%	150.00	169765043	169449247	0.180
		169156308		
		169426389		

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4) Linearity and Range

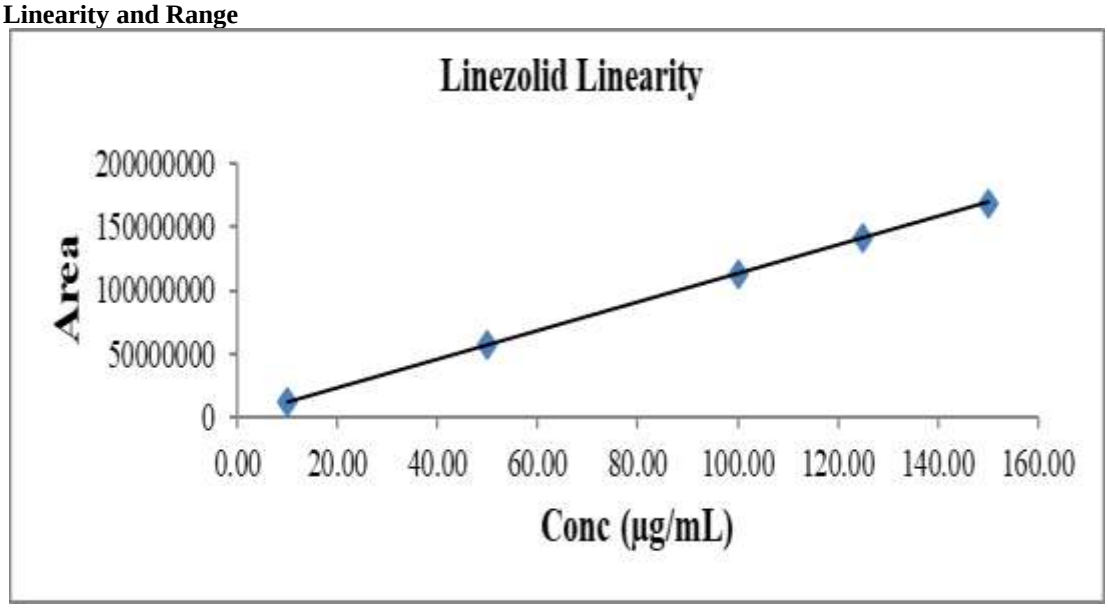

Fig. 11: Calibration curve of Linezolid

Data of linearity of Linezolid:

Sr no.	Parameter	Result value	Acceptance criteria
1	Beer's linearity range	10-150 µg/mL	NA
2	Correlation coefficient (R^2)	0.99999	NLT 0.98
3	Intercept	462364.1634	To be report
4	Slope	1129031.697	To be report
5	% RSD for area at each level	NA	NMT 2.0

The respective linear equation for Linezolid was
Y = M X + C
Y = 1129031.697 x + 462364.1634
Where, x = concentration of Analyte in µg/mL
y = is area of peak.
M = Slope
C= Intercept

5) Limit of Detection (LOD) and Limit of Quantitation (LOQ):

σ = 346383.749 (Residual standard deviation of a regression line)

S = 1129031.697 (Slope)

Detection limit (LOD):
LOD = 3.3 σ / S
LOD = 3.3 x 346383.749 / 1129031.697
LOD = 1.012 µg/mL

Quantitation limit (LOQ):
LOQ = 10 σ / S
LOQ = 10 x 346383.749 / 1129031.697
LOQ = 3.068 µg/mL

6) Accuracy

Result and Statistical Data of Accuracy of Linezolid

Level (50 %)	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	56648172	50.03	50.16	99.74	**100.38**	**0.6279**
	57413608	50.71	50.21	101.00		
	57194281	50.51	50.31	100.40		
100	114364187	101.01	100.09	100.92	**99.81**	**1.0791**
	113051973	99.85	100.12	99.73		

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	112284631	99.17	100.41	98.77		
150	167541863	147.97	150.08	98.59	**99.85**	**1.1980**
	170958412	150.99	151.00	99.99		
	171650148	151.60	150.15	100.97		

Overall Recovery: 100.01% and % **RSD for Overall Recovery:** 0.907

7) Precision

Result of Intra- day and Inter- Day Precision for Linezolid test sample assay:

	Sample	**Test Sample (mg)**	**Area**	**% Assay**
Repeatability	Sample 1	158.16	111541398	98.41
	Sample 2	158.91	110514193	97.05
	Sample 3	159.21	112541361	98.64
	Sample 4	158.06	113514061	100.22
	Sample 5	158.41	110325914	97.19
	Sample 6	158.22	110652814	97.59
	Mean			**98.18**
	STD DEV			**1.1858**
	% RSD			**1.208**
Intermediate precision (Inter-Day)	Sample 1	158.41	113107184	99.64
	Sample 2	158.06	110107184	97.21
	Sample 3	158.33	112651013	99.29
	Sample 4	159.04	110210391	96.70
	Sample 5	158.11	110652418	97.66
	Sample 6	158.71	110136814	96.84
	Mean			**97.89**
	STD DEV			**1.2694**
	% RSD			**1.297**
Repeatability Plus Inter-day	**Mean**			**98.037**
	STD DEV			**1.1811**
	% RSD			**1.205**

8) Result of Robustness Study:

Change in Parameter	**R.T.**	**Standard area**	**Asymmetry**	**Theoretical plates**
Wavelength by +3 NM (261 NM)	2.92	103609903	1.37	5025
Wavelength by -3 NM (255 NM)	2.92	116504704	1.37	5047
Flow rate by +10% (1.1mL/min)	2.62	103666592	1.40	4755
Flow rate by -10% (0.9mL/min)	3.25	127066293	1.36	5211
Column oven temp by +2ºC (42 ºC)	2.91	114442814	1.35	5234
Column oven temp by -2ºC (38 ºC)	2.93	111352417	1.40	4812

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Data interpretation: From the above results, it was concluded that the system suitability test result was found well within the limits and analytical method was robust.

Table: summary of validation parameters of HPLC method

Parameters	Linezolid
Linearity (µg/ml)	10-150 µg/ml
Linearity Intercept	462364.1634
Linearity equation	Y= 1129031.697x + 462364.1634
Correlation coefficient (R^2)	0.99999
Acid degradation %	5.70%
Base degradation %	15.50%
Precision (%RSD)	1.20
Accuracy	100.01%

IV. CONCLUSION:

The HPLC method developed for assessing stability was found to be straightforward, accurate, sensitive, precise, specific, and fast. On a regular basis, this method can be used to test linezolid in bulk and pharmaceutical forms such as tablets. This method was also used to check the product's quality and stability after being exposed to various storage conditions and stress. Some of the advantages from the above results-

a. Robust analytical method.
b. Method can be applied for routine analysis of drug.
c. Quantification done by using less amount of organic solvents.
d. Degradation study is also performed for better knowing of drug.

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