IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

METHOD DEVELOPMENT AND VALIDATION PROCESS FOR THE ESTIMATION OF LINEZOLID AND ITS FORMULATION USING THE REVERSE PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

Shubham N. Abhang[1*], Dr. Kranti Musmade[2], Dr. Sumit Joshi[3], Dr. Ganesh Y. Dama[4]

Student[1], Professor[2], H.O.D.[3], Principal[4]

[1-4]Department of Quality Assurance, Shradachandra Pawar College of Pharmacy, Dumbarwadi, Otur, Pune, Maharashtra, India-410504

Abstract:

The objective of this study was to develop a reliable and accurate RP-HPLC method for the determination of Linezolid content in drug formulations. The method development involved the optimization of various parameters, including the mobile phase composition, column selection, and detection wavelength. The Linezolid drug and its formulation were subjected to RP-HPLC analysis using the developed method. For the validation of the RP-HPLC method, parameters such as linearity, precision, accuracy, specificity, robustness, and system suitability were evaluated. The linearity of the method was determined by analyzing Linezolid standard solutions at different concentrations. Precision was assessed by repeatability and intermediate precision studies. Accuracy was determined by recovery studies conducted at various spiked levels.

The developed RP-HPLC method exhibited satisfactory results for all validation parameters. The linearity of the method was excellent over the specified concentration range. Specificity tests demonstrated that the method was selective for Linezolid, without interference from other components. The method showed robustness, as minor variations in the chromatographic conditions did not significantly affect the Linezolid peak. In conclusion, the developed RP-HPLC method proved to be reliable, accurate, and specific for the estimation of Linezolid drug and its formulation. The validation results indicated that the method is suitable for routine analysis of Linezolid samples, ensuring the quality control of Linezolid-based products and compliance with regulatory guidelines.

Keywords: Linezolid, bioavailability, Solubility, Qualitative analysis, RP-HPLC method.

Linezolid

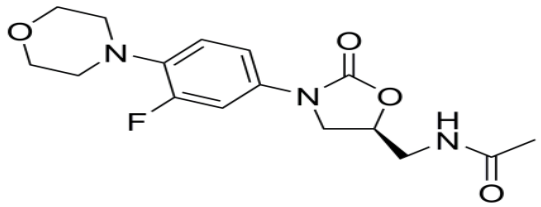


Figure No. 01: Structure of Linezolid

Linezolid is a synthetic antibiotic which is used for the treatment of infections caused by aerobic Gram-positive bacteria. Its effects are bacteriostatic against both enterococci and staphylococci and bactericidal against most isolates of streptococci. Linezolid exerts its antibacterial activity by inhibiting the initiation of bacterial protein synthesis - more specifically, it binds to the 23S ribosomal RNA of the 50S subunit and, in doing so, prevents the formation of the 70S initiation complex which is essential for bacterial reproduction.

Linezolid was initially approved in 2000 and was the first member of the oxazolidinone antibiotic class. A second member of this class, tedizolid, was approved by the FDA in 2014 and is considered generally more effective and tolerable than its predecessor.

Chemical name: N-[[(5S)-3-(3-fluoro-4-morpholin-4-ylphenyl)-2-oxo-1,3-oxazolidin-5-yl]methyl]acetamide

Molecular formula: $C_{16}H_{20}FN_3O_4$

Molecular weight: 337.35 g/mol

Melting Point: 181.5-182.5°C

Solubility: It is soluble in Water, Ethanol, Methanol, and Hexane.

Category: antibacterial

PKa: 1.8

Linezolid is indicated in adults and children for the treatment of infections caused by susceptible Gram-positive bacteria, including nosocomial pneumonia, community-acquired pneumonia, skin and skin structure infections, and vancomycin-resistant Enterococcus faecium infections. Examples of susceptible bacteria include Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, and Streptococcus agalactiae.

Linezolid is not indicated for the treatment of Gram-negative infections, nor has it been evaluated for use longer than 28 days.

Materials and Method

1. Procurement of drug sample

Table No. 1: Details of drug sample

Name of Drug	Quantity	Drug Supplier
Linezolid	5gm	

2. Reagents and chemicals

All the chemicals used are of HPLC and AR grade. Chemicals used are as follows

Table No. 2: Reagents and Chemicals List

Sr. No.	REAGENTS	GRADE	MANUFACTURES
1	Acetonitrile	HPLC	LOBA CHEMIE Pvt. Ltd
2	Water	HPLC	LOBA CHEMIE Pvt. Ltd
3	Orthophosphoric acid (OPA)	AR	PALLAV Chemical and Solvent Pvt. Ltd
4	Methanol	HPLC	LOBA CHEMIE Pvt. Ltd

Experimental Work

1. Identification of drug

Organoleptic properties of drug

The sample of Linezolid was checked for organoleptic properties such as colour and odour.

Melting point determination

Identification of Linezolid was done by checking its melting point and it was found in the range of 181.5-182.5°C Standard.

Solubility analysis

It is soluble in Water, Ethanol, Methanol, and Hexane.

Fourier Transform Infra-red Spectroscopy (FTIR)

The IR study of pure drug was carried out by using Fourier transform infrared spectrophotometer (BRUKER). Infrared absorption spectrum of Linezolid was recorded and interpreted over the wave number 400 to 600 cm-1 using Fourier Transform spectrophotometer (Bruker, ECO- ATR)

Selection of wave length

From the above stock solution further dilution were prepared and scanned between range of 200-400nm and spectra were obtain. The observed λmax for Linezolid was 258nm.

2. High Performance Liquid Chromatographic Method

Preparation of standard stock solutions

Accurately 10.0 mg weighed quantity of Linezolid was transferred to 100 mL volumetric flask. That was dissolved by adding 50 mL mobile phase and then the drug solution was diluted up to the mark with mobile phase to get the stock solution of 100 µg/mL of Linezolid.

The working standard solutions of drug were obtained by appropriate dilution of the respective stock solution with mobile phase.

0.1% OPA pH 4.0 preparation-

0.3 ml of ortho-phosphoric acid was mixed with 300 ml of HPLC grade water. Adjusted pH of 0.1% OPA to 4.0 using triethyl amine. Filtered through 0.45 µ filter paper. Sonicated for 5 minutes.

Mobile Phase Preparation

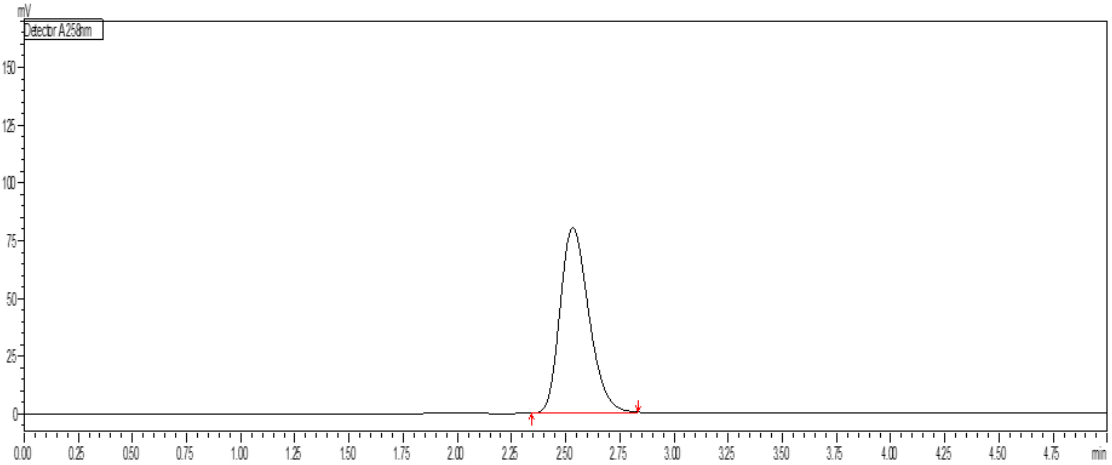
Mixed 700 ml of Acetonitrile, 300 ml of 0.1% OPA pH 4.0. Filtered through 0.45 µ filter paper. Sonicated for 5 minutes.

Trial and error method:

Table No.3: Trial and error method

Trial No.	Column Used	Mobile Phase	Flow Rate	Wavelength	Observation	Result
1.	X-Terra RP 18, 100 mm x 4.6 mm, 3.5µm	Acetonitrile : 0.1% OPA (70:30)	0.5ml/min	258 nm	No stable baseline and tailing peak. and theoretical plates found less	Method rejected
2.	Agilent C18, 250 mm x 4.6 mm, 5µm	Acetonitrile : Water (50:50)	0.6ml/min	258 nm	No stable baseline	Method rejected
3.	Agilent C18, 250 mm x 4.6 mm, 5µm	Acetonitrile Water pH 3.0 (70:30)	0.8ml/min	258nm	Some small peaks were observed.	Method rejected
4.	Agilent C18, 250 mm x 4.6 mm, 5µm	Acetonitrile Buffer pH 6.0 (60:40)	0.9ml/min	258nm	Peak shape was not proper	Method rejected

5.	Shimadzu C18, 250 mm x 4.6 mm, 5µm	ACN: 0.1% OPA pH 4.0 (70:30)	1.0ml/min	258nm	Peak shape, RT and baseline found satisfactory	Method accepted



Trial

Figure No. 2: Chromatogram of Trial 1

Table No. 4: Chromatographic Condition for Trial 1

Mobile phase	Acetonitrile : 0.1% OPA pH 3.0 (70 : 30)
Selection of column	100 mm x 4.6 mm, 5 µ , C18 Make- X-Terra
Flow rate	0.50 ml/min
Detection wavelength	258 nm
Injection Volume	10 µl
Conclusion	No stable baseline and tailing peak. and theoretical plates found less

Trial 2

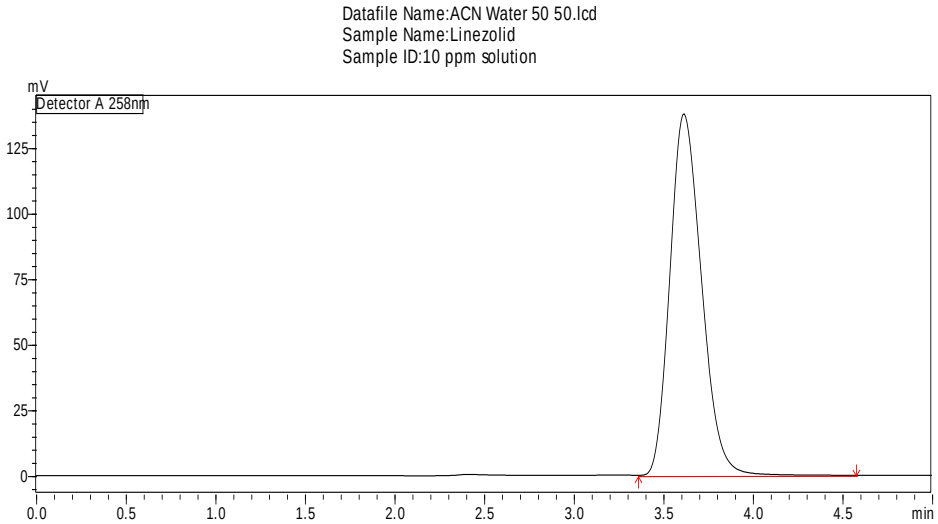


Figure No. 3: Chromatogram of Trial 2

Table No.5: Chromatographic Condition for Trial 2

Trial 3

Mobile phase	ACN : Water (50 : 50)
Selection of column	250 mm x 4.6 mm, 5 µ , C18 Make- Agilent
Flow rate	0.60 ml/min
Detection wavelength	258 nm
Injection Volume	10 µl
Conclusion	No stable baseline and theoretical plates were found less

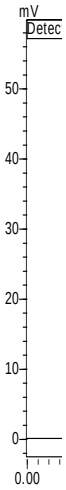


Figure No. 4: Chromatogram of Trial 3

Table No. 6: Chromatographic Condition for Trial 3

Mobile phase	ACN : Water pH 3.0 (70 : 30)
Selection of column	250 mm x 4.6 mm, 5 µ , C18 Make- Agilent
Flow rate	0.80 ml/min
Detection wavelength	258 nm
Injection Volume	10 µl
Conclusion	Some small peaks were observed.

Trial 4

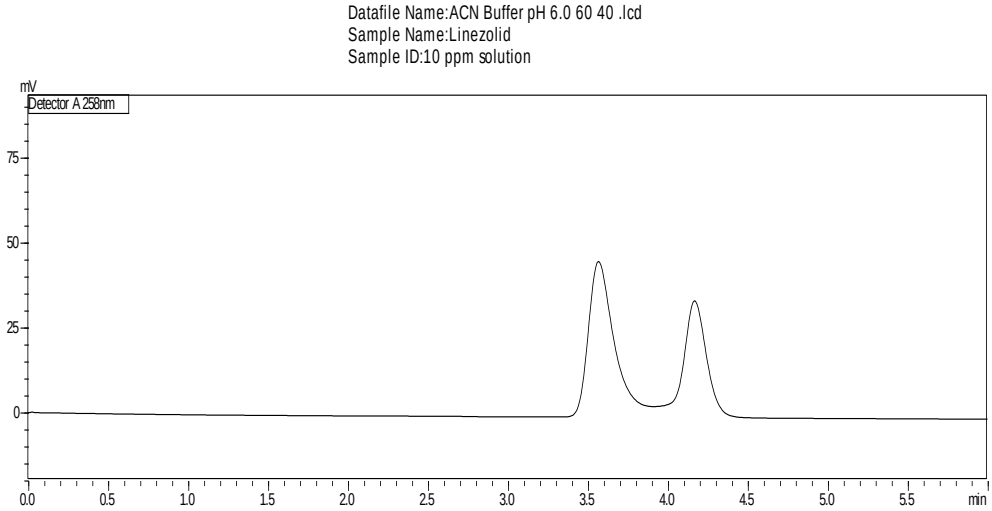


Figure No. 5: Chromatogram of Trial 4

Table No. 7: Chromatographic Condition for Trial 4

Mobile phase	ACN : Buffer pH 6.0 (60: 40)
Selection of column	150 x 4.6 mm, 5 µ , C18 Make- Agilent
Flow rate	0.90 ml/min
Detection wavelength	258 nm
Injection Volume	10 µl
Conclusion	Peak shape was not proper.

Trial 5

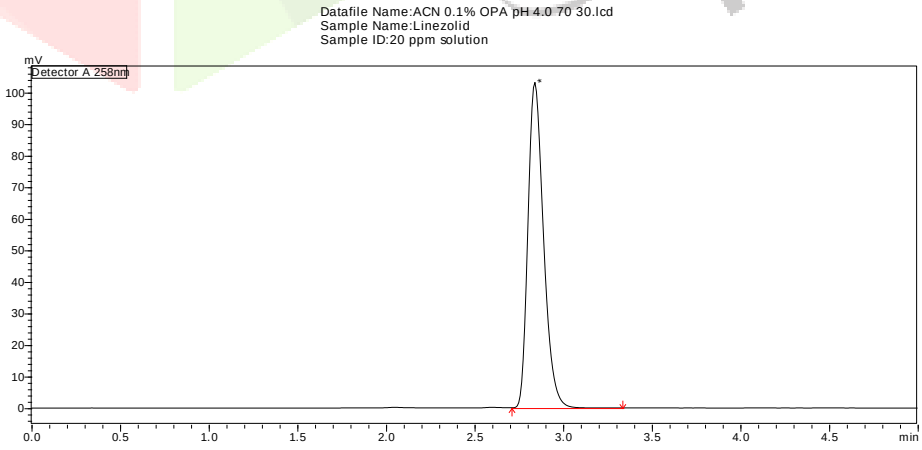


Figure No. 6: Chromatogram of Trial 5

Table No. 8: Chromatographic Condition for Trial 5

Mobile phase	ACN : 0.1% OPA pH 4.0 (70: 30)
Selection of column	250 x 4.6 mm, 5 µ , C18 Make- Shimadzu
Flow rate	1.0 ml/min
Detection wavelength	258 nm
Injection Volume	10 µl
Conclusion	Good Peak and Retention time observed (confirmed)

Optimized Chromatographic Conditions

Table No.9: Optimized Chromatographic Conditions

Mobile phase	ACN : 0.1% OPA pH 4.0 (70: 30)
Selection of column	250 x 4.6 mm, 5 µ , C18 Make- Shimadzu
Flow rate	1.0 ml/min
Detection wavelength	258 nm
Injection Volume	10 µl
Conclusion	Good Peak and Retention time observed (confirmed)

Validation of Developed RP-HPLC Method

1. Linearity

The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Test solutions of different concentration were injected separately and the chromatograms were recorded. A series of test preparations of Linezolid (5-40µg/ml) were prepared by taking 0.5, 1.0, 2.0, 2.5, 3.0, 4.0ml from the stock solution in six 100 ml volumetric flask and final volume make up to the mark with mobile phase.

2. Precision

Intraday and Interday Precision

Intraday precision study was carried out by preparing test solution of same concentration and analyzing it at two different times in a day. The same procedure was followed for two different days to determine interday precision. The result was reported as %RSD. The precision result showed a good reproducibility with percent relative standard deviation less than 2.

The % RSD obtained should be NMT 2.0.

3. LOD and LOQ:

LOD and LOQ determined by the following formula by taking the standard deviation of y- intercept and slope from the linearity curves.

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. Limits are prescribed as percentage or as parts per million. The limit of detection will not only depend on the procedure of analysis but also on type of instrument. A signal-to-noise ratio between 3:1 or 2:1 is generally considered acceptable for estimating the detection limit. It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

LOD= 3.3 (SD)/ S

Where, SD = Standard deviation,

S = Slope of the curve.

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and Accuracy. It is expressed as the conc. of analyte (e.g., percentage, parts per billion) in the sample. A typical signal-to-noise ratio is 10:1 or 20:1.

The S/N ratio should not less than 10.

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

LOQ= 10 (SD)/S

Where, SD = Standard deviation,

S = Slope of the curve.

4. Accuracy

Samples are prepared normally covering 50 % to 150 % of the nominal sample preparation concentration. These samples are analyzed and the recoveries of each are calculated. For this study,

- Prepare three preparation of each 50 %, 100 % and 150 % level and inject in to the chromatography.
- Make the injection lowest concentration to highest concentration.
- Calculate individual recovery, mean recovery and %RSD.

Acceptance Criteria:

- Individual and mean % recovery should be within 98.0 % to 102.0 %.

5. Repeatability

Repeatability precision study was carried out by preparing test solution of same concentration and analyzing it at five different times. The result was reported as %RSD. The precision result showed a good reproducibility with percent relative standard deviation less than 2.

6. Robustness

The robustness of an analytical procedure is an estimate of its capacity to last unchanged by slight but intentional change in the analytical method parameters. To assess HPLC method robustness some measurable factors were

intentionally changed. The study was carried out solution (20 µg/mL) by varying the flow rate (1.0, and 1.2 mL/min) and, Wavelength (256 and 258) respectively.

7. Ruggedness

The value for %RSD (Relative Standard Deviation) less than 2 for three successive injections of solution (20 µg/mL) by two different analysts of the sample solution from the same homogenous mixture at working concentrations, which indicate the method developed is rugged.

Results and Discussion

1 Identification of drug

1.1 Organoleptic properties of drug

Table No. 10: Organoleptic properties of drug

Sr. No.	Organoleptic Property	Linezolid
1	Colour	
2	Odour	Odourless

1.2 Melting point of drug

Table No. 11: Melting point of drug

Sr. No.	Name of drug	M.P. (˚C)
1	Linezolid	182˚C

1.3 Solubility Study:

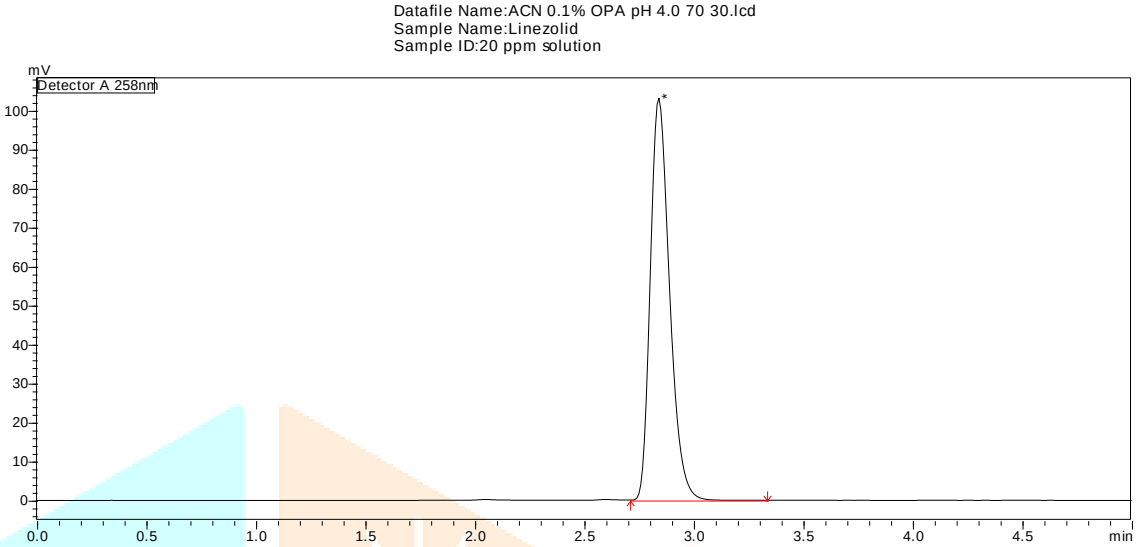
Solubility of Linezolid was observed by dissolving them in different solvents and the observed results are given in the table no.13

Table No. 12: Solubility Study of Linezolid

Sr. No.	Solvents	Solubility
1	Water	Soluble
2	Methanol	Soluble
3	Hexane	Soluble
4	Ethanol	Soluble

2 Preliminary HPLC method development

At the beginning of the experiment, trial run was performed as per section 7.2.5 of chapter7. The chromatogram and data obtained with first condition viz. ACN: 0.1% OPA pH 4.0 (70: 30) at 258nm and was as shown in Graph No. 02 and Table 14.



Graph No. 7: Chromatogram of preliminary trial

Table No. 13: Trial experiment graph

Name	Ret. Time	Area	Area%	Asymmetry	Theoretical Plates
Linezolid	2.840	631248	100.000	1.302	4292

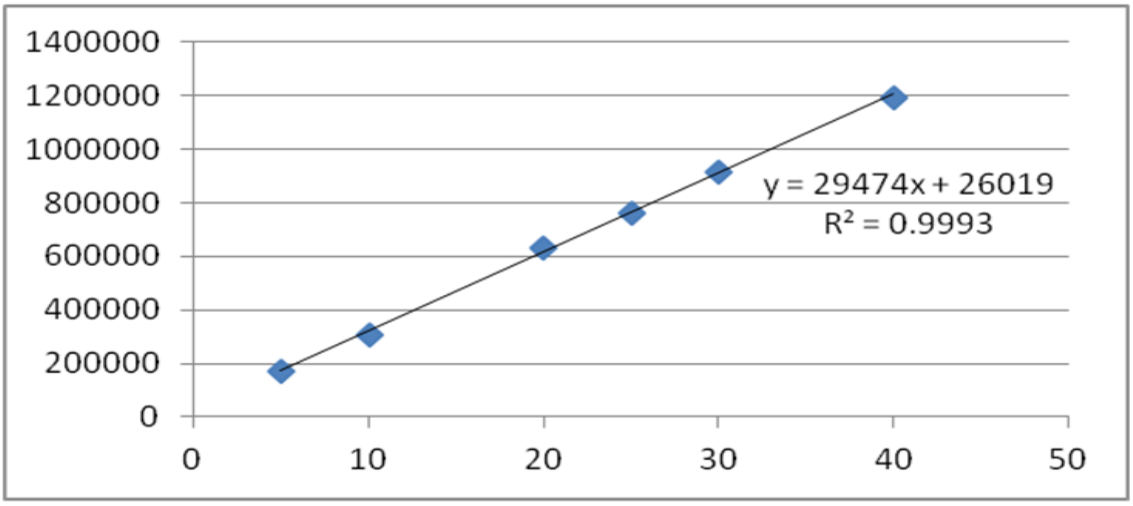
3. RP-HPLC method validation

3.1. Linearity and Range

Drug was found to be linear in the concentration range of 5-40 µg/ml. Results obtained are shown in Table no. 32 and calibration plot obtained was shown in Graph no. 25

Table No. 14: Data of calibration curve of Linezolid by HPLC method

Sr. No.	Linezolid Conc. (ppm)	Time	Peak Area	No. of Theoretical Plates	Asymmetry
1	5	2.843	173024	4338	1.305
2	10	2.842	309695	4325	1.303
3	20	2.841	631512	4296	1.305
4	25	2.842	760871	4329	1.305
5	30	2.842	915622	4315	1.305
6	40	2.845	1196963	4358	1.309



Graph No.8: Linearity graph of Linezolid

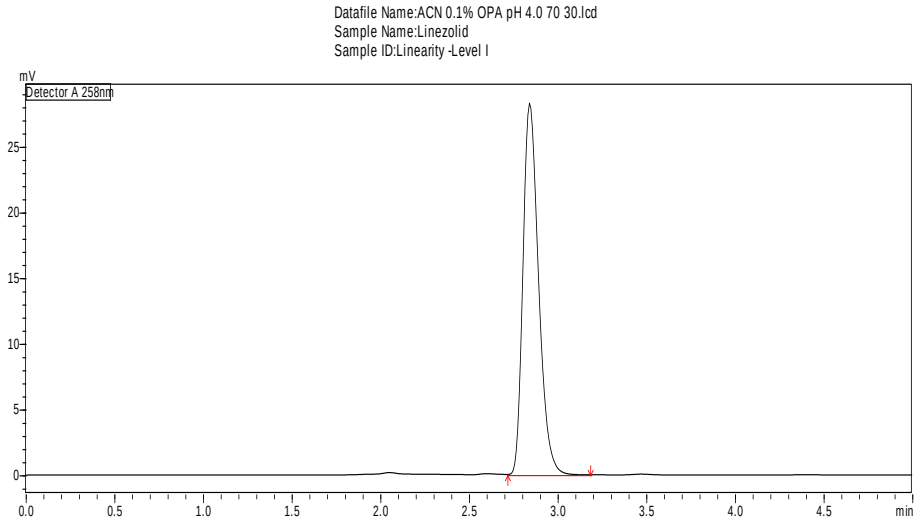
Data Interpretation: The method was found to be linear for Linezolid. The correlation coefficient of the plot was found to be 0.9993.

Optical characteristics

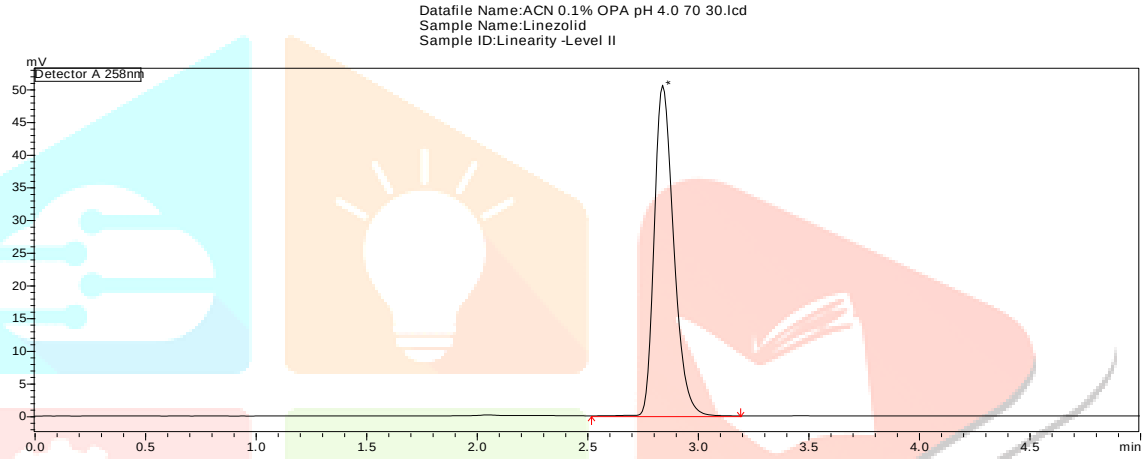
Optical characteristics and statistical data of linearity for Linezolid by HPLC method are summarized in Table no. 16.

Table No. 15: Optical characteristics for Linezolid

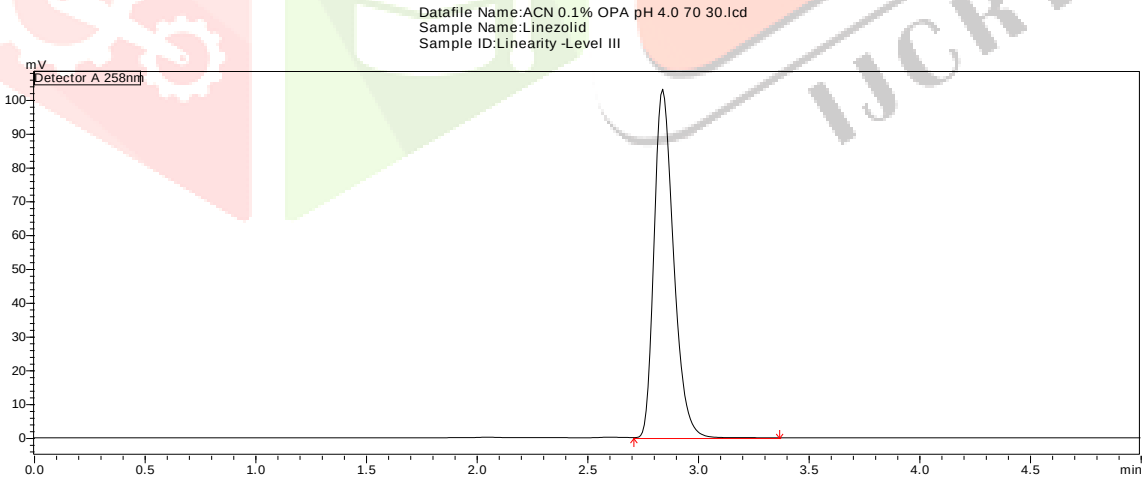
Sr. No.	Parameters	HPLC method
1	λ_{max}	258
2	Linearity	5-40
3	Regression equation[y]	y = 29474x + 26019
4	Slope[m]	29474
5	Intercept [c]	26019
6	Correlation coefficient [r^2]	0.9993
7	Limit of detection (LOD)	0.78
8	Limit of quantitation (LOQ)	2.34



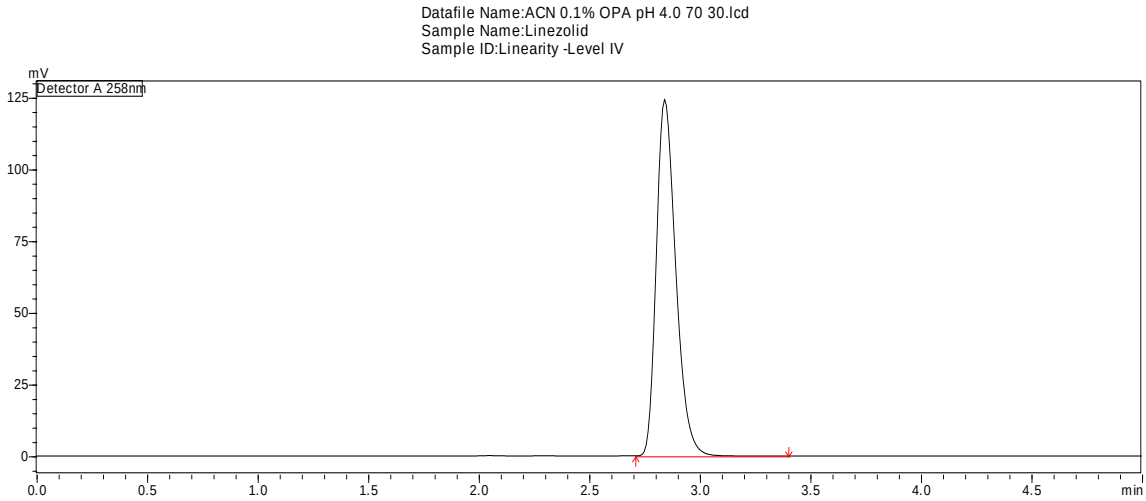
Graph No. 9 Chromatogram of Linearity 5µg/ml Linezolid



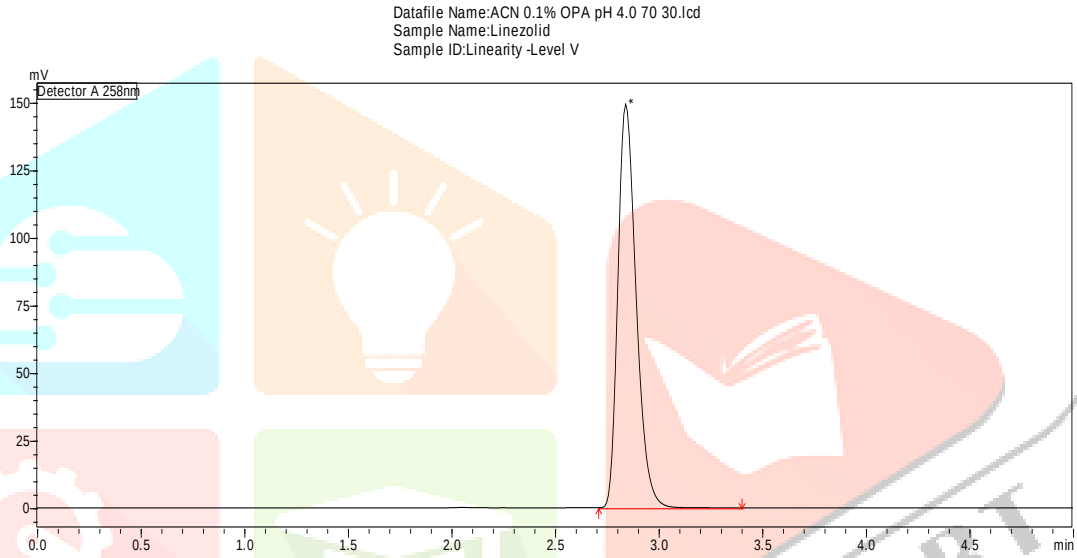
Graph No. 10: Chromatogram of Linearity 10µg/ml Linezolid



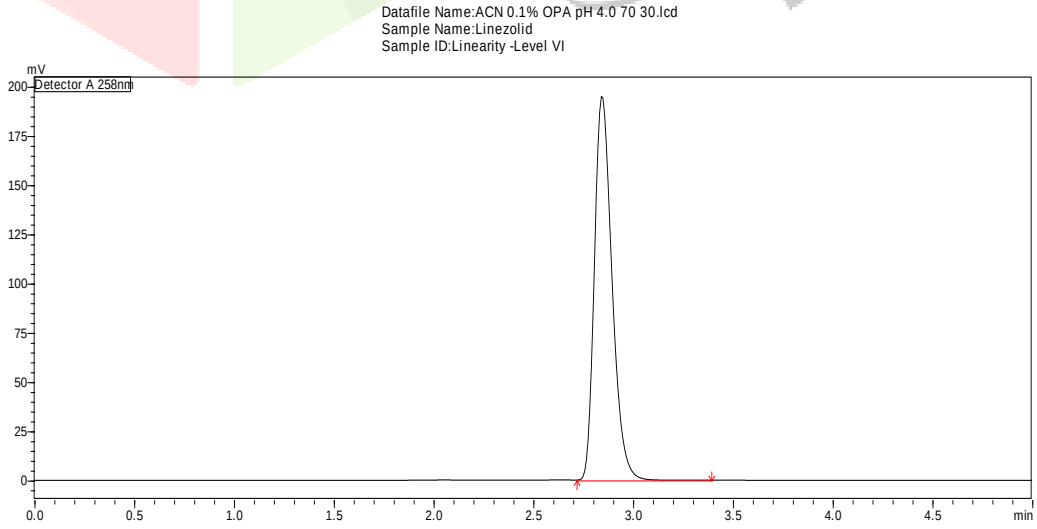
Graph No. 11: Chromatogram of Linearity 20µg/ml Linezolid



Graph No. 12: Chromatogram of Linearity 30µg/ml Linezolid



Graph No. 13: Chromatogram of Linearity 35µg/ml Linezolid



Graph No. 14: Chromatogram of Linearity 40µg/ml Linezolid

3.2. Accuracy

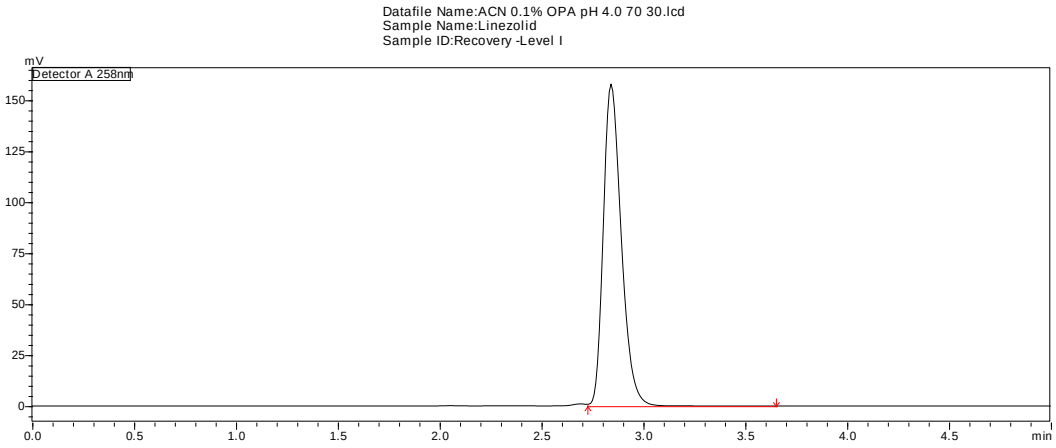
Accuracy was studied by standard addition method and % recovery found was within acceptable limit. Statistical validation is shown in Table No 17.

Table No. 16: Data for recovery study of Linezolid by HPLC method

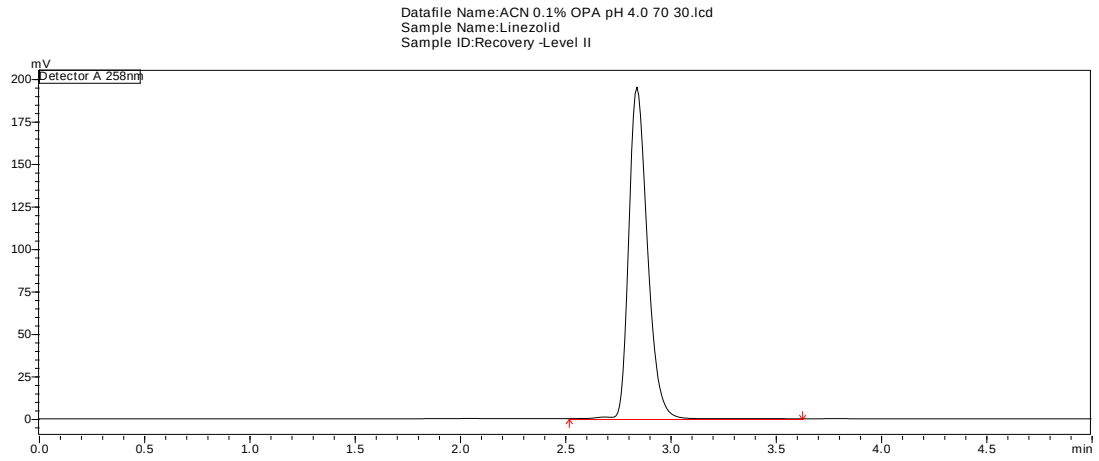
Level of addition	Standard added (ml)	Conc. (ml)	Total Conc. (µg/ml)	Area Obtained	Std Area	Drug recovered (µg/ml)	%Recovery
50%	1.0 ml	2.0 ml	30	911889	911891	30.91	102.8%
	1.0 ml	2.0 ml	30	911894		30.96	103.1%
	1.0 ml	2.0 ml	30	911892		30.94	103.0%
100%	2.0 ml	2.0 ml	40	1182639	1182635	40.14	100.4%
	2.0 ml	2.0 ml	40	1182632		40.10	100.2%
	2.0 ml	2.0 ml	40	1182636		40.12	100.3%
150%	3.0 ml	2.0 ml	50	1482467	1482460	50.28	100.5%
	3.0 ml	2.0 ml	50	1482462		50.25	100.2%
	3.0 ml	2.0 ml	50	1482469		50.29	100.6%

Table No 17: Statistical validation of Linezolid by HPLC method

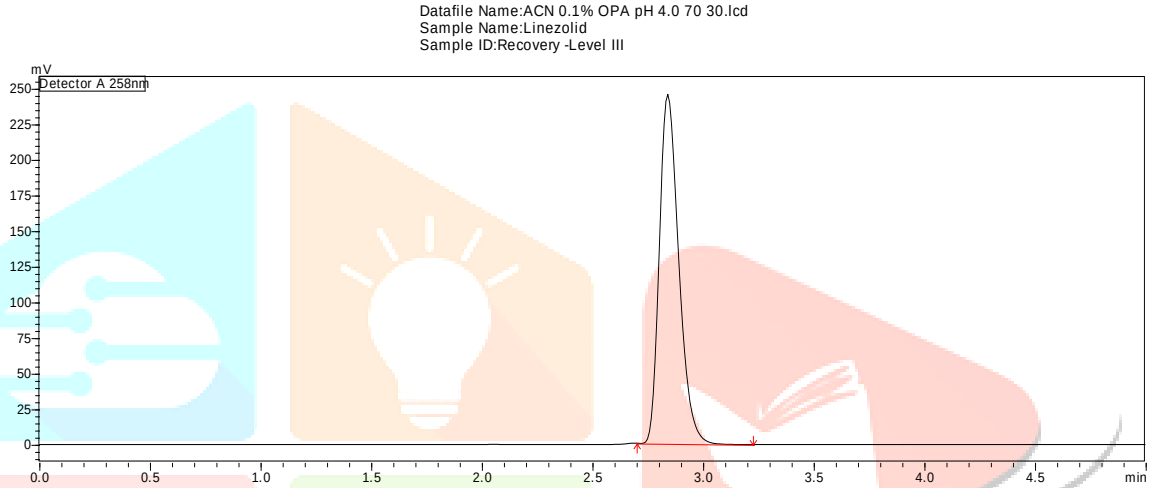
Levels	% Mean recovery	SD	%RSD
50%	103.2%	0.0311	0.0318
100%	100.3%	0.0188	0.0188
150%	100.4%	0.0124	0.0123



Graph No. 15: Chromatogram of % Recovery of Linezolid (Level 50)



Graph No. 16: Chromatogram of % Recovery of Linezolid (Level 100)



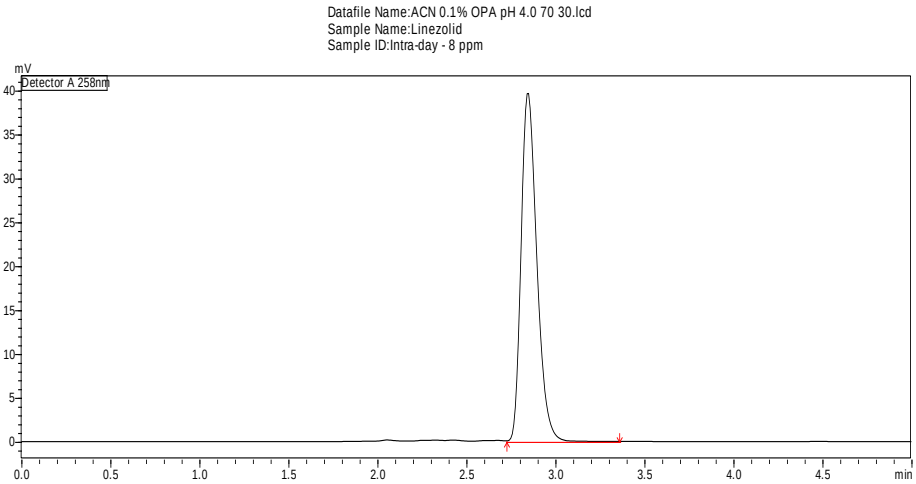
Graph No. 17: Chromatogram of % Recovery of Linezolid (Level 150)

3.3 Precision

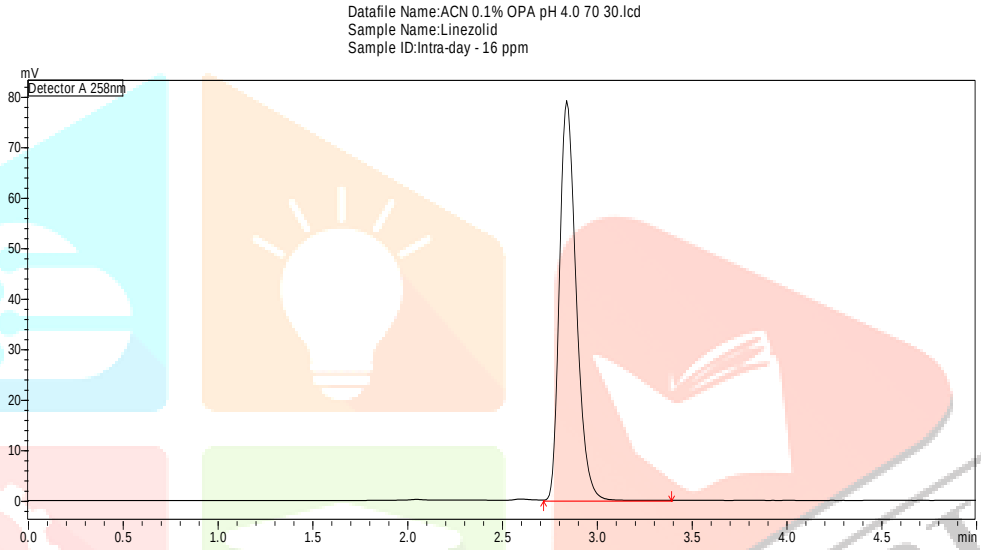
Intraday and interday precision assures the repeatability of test results. The % RSD found was below 2. Result of intraday and interday precision was shown in Table no. 19 and Table no. 38 respectively.

Table No. 18: Data for intraday precision of Linezolid by HPLC method

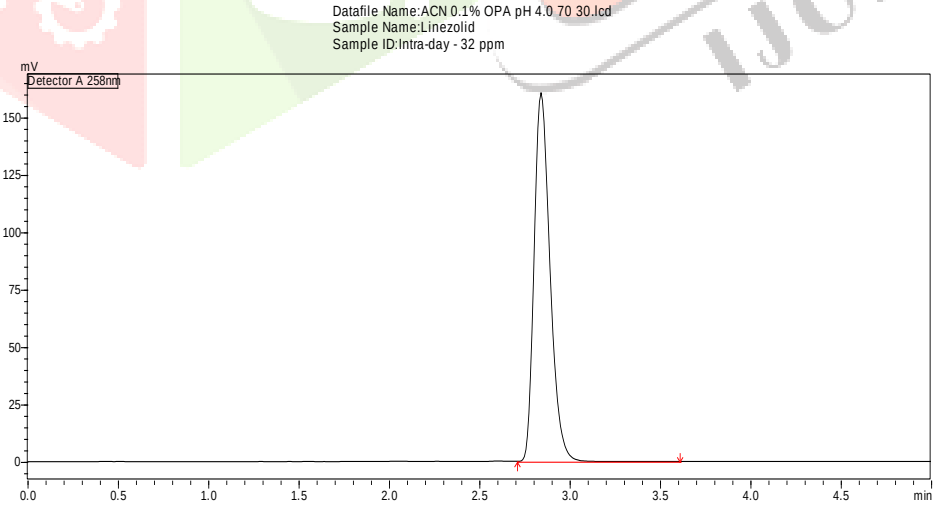
Sr.No.	Concentration	Area Set I	Area Set II	Area Set III	Area-Mean	% RSD
1	8 µg/ml	244020	244002	243970	243997	0.01%
2	16 µg/ml	484168	483909	484172	484083	0.03%
3	32 µg/ml	983112	983717	983544	982457	0.03%



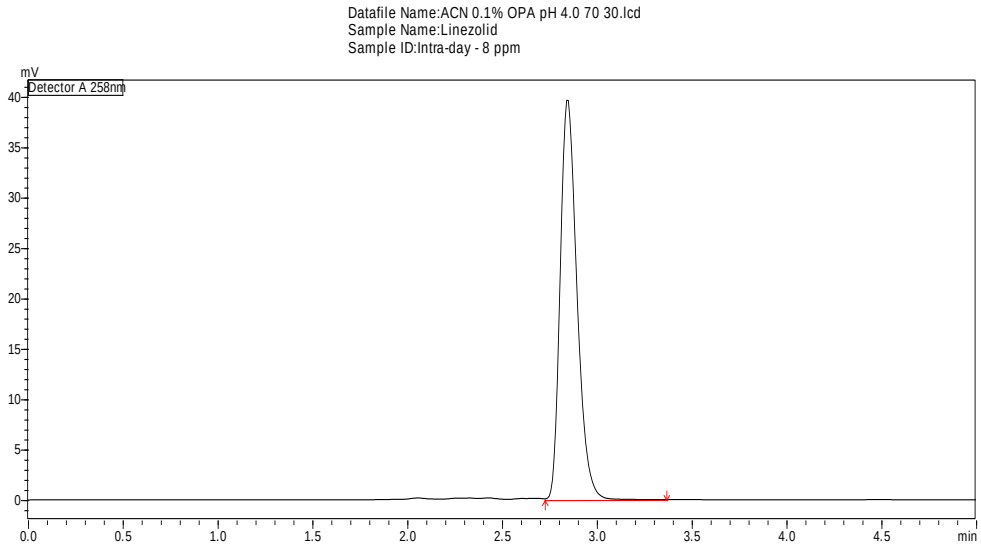
Graph No. 18: A Chromatogram of Intraday Precision Zero Hour of 8µg/ml Linezolid



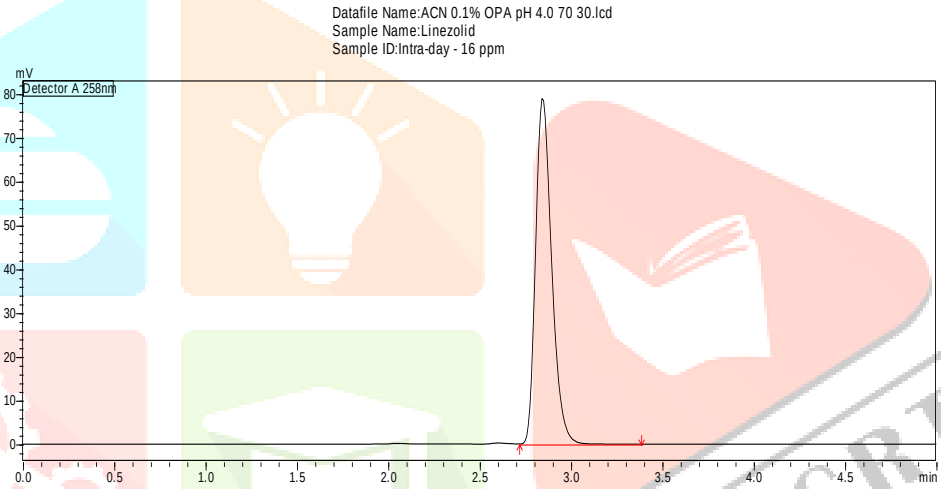
Graph No. 19: A Chromatogram of Intraday Precision Zero Hour of 16µg/ml Linezolid



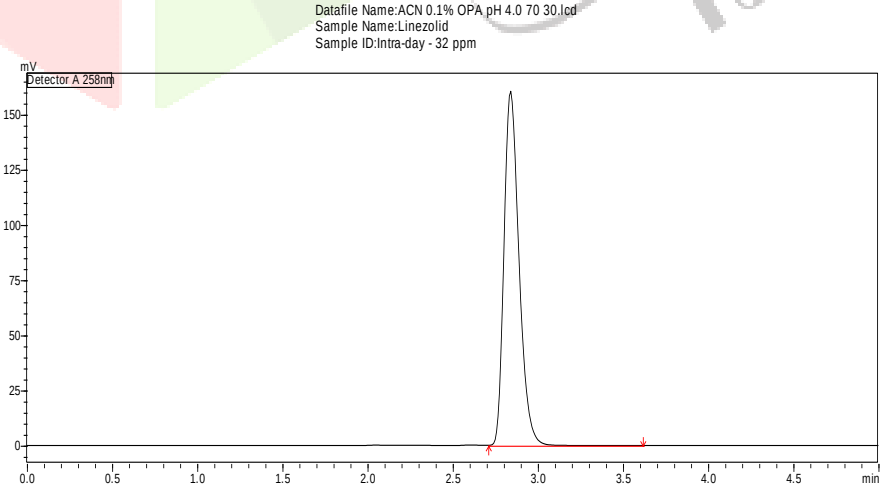
Graph No. 20: A Chromatogram of Intraday Precision Zero Hour of 32µg/ml Linezolid



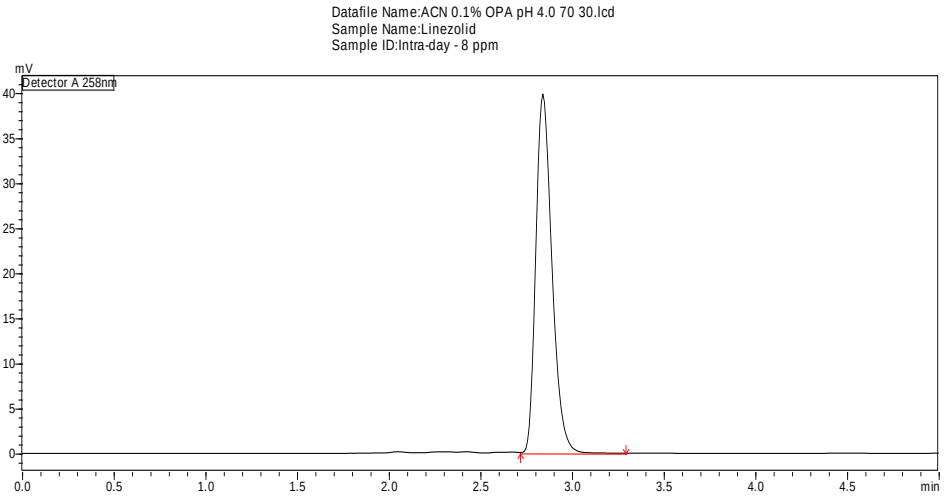
Graph No. 21: A Chromatogram of Intraday Precision Two Hour of 8µg/ml Linezolid



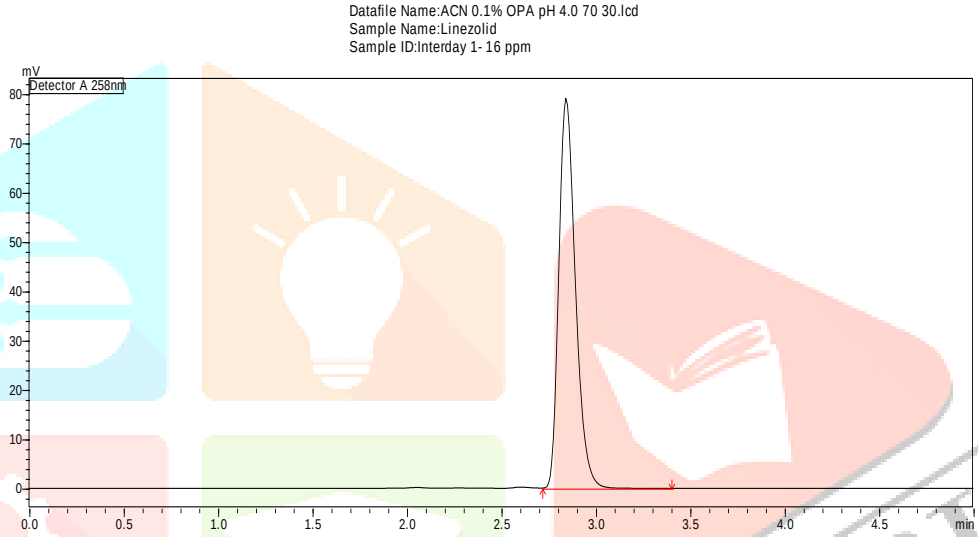
Graph No. 22: A Chromatogram of Intraday Precision Two Hour of 16µg/ml Linezolid



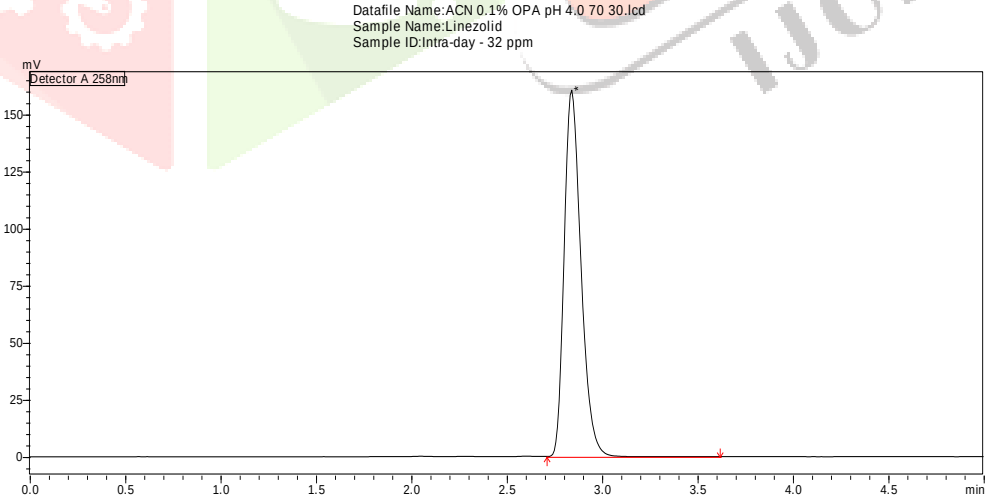
Graph No. 23: A Chromatogram of Intraday Precision Two Hour of 32µg/ml Linezolid



Graph No. 24: A Chromatogram of Intraday Precision Four Hour of 8µg/ml Linezolid



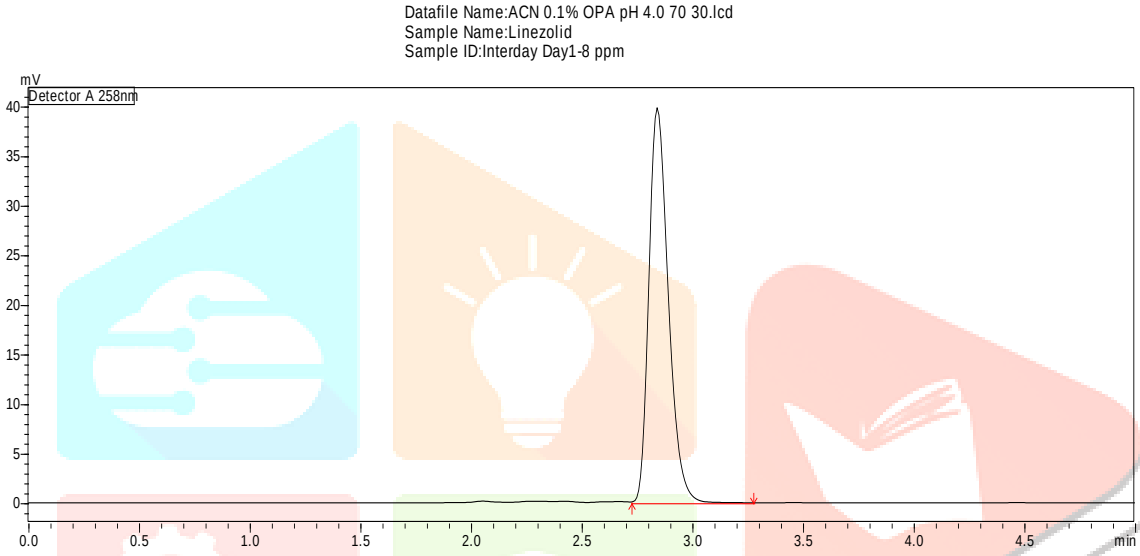
Graph No. 25: A Chromatogram of Intraday Precision Four Hour of 16µg/ml Linezolid



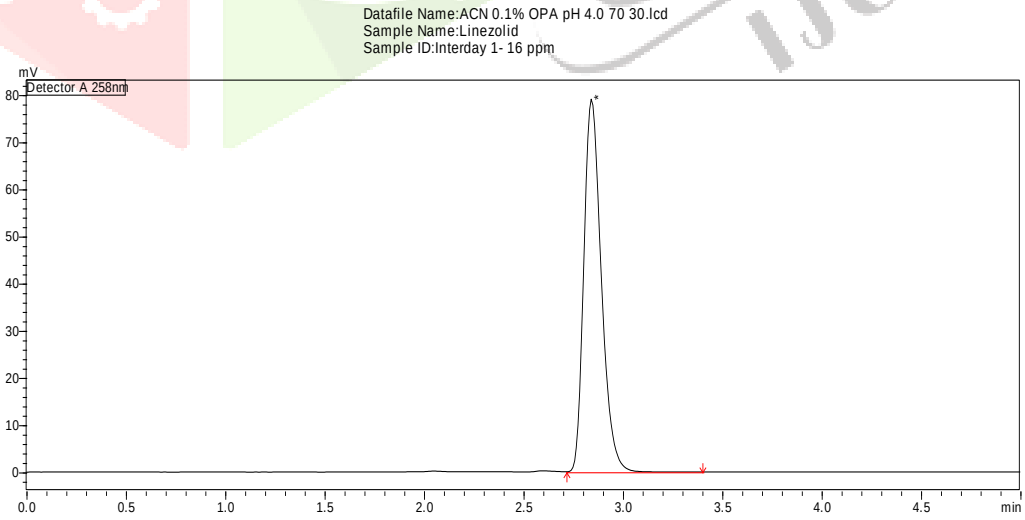
Graph No. 26: A Chromatogram of Intraday Precision Four Hour of 32µg/ml Linezolid

Table No. 19: Data for interday precision of Linezolid by HPLC method

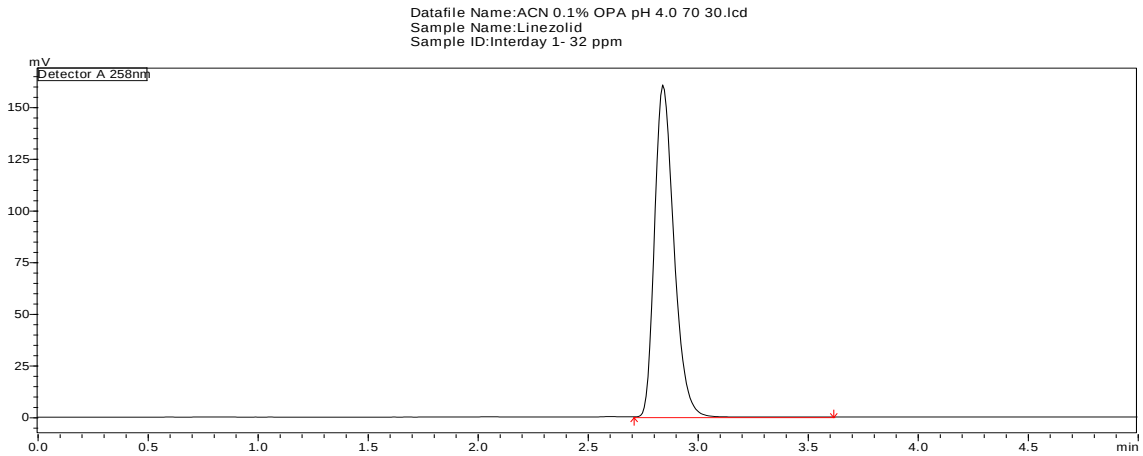
Sr.No.	Concentration	Area Day 1	Area Day 2	Area Day 3	Mean Area	% RSD
1	8 µg/ml	243508	241970	243753	243077	0.40%
2	16 µg/ml	484172	483719	484434	484108	0.07%
3	32 µg/ml	983855	983770	982719	983448	0.06%



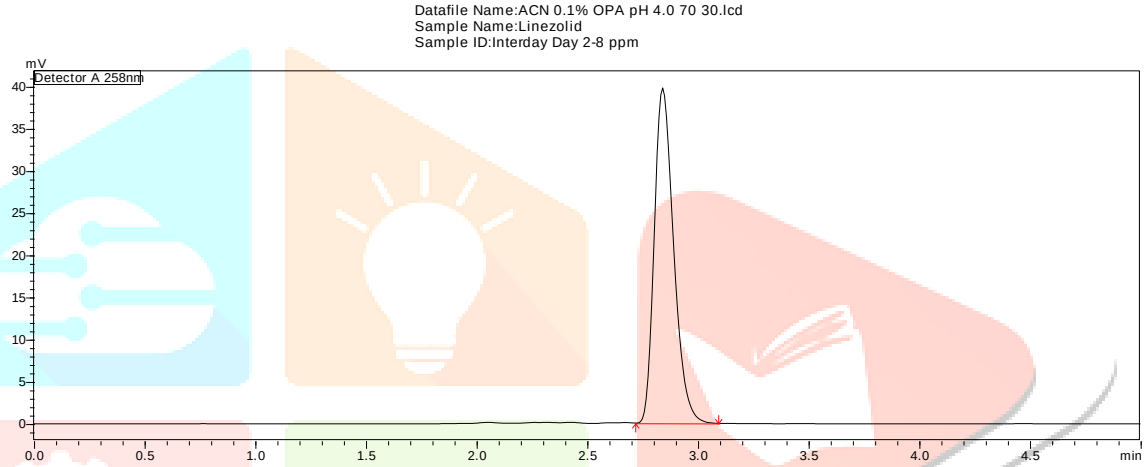
Graph No. 27: A Chromatogram of Interday Precision Day 1 of 8µg/ml Linezolid



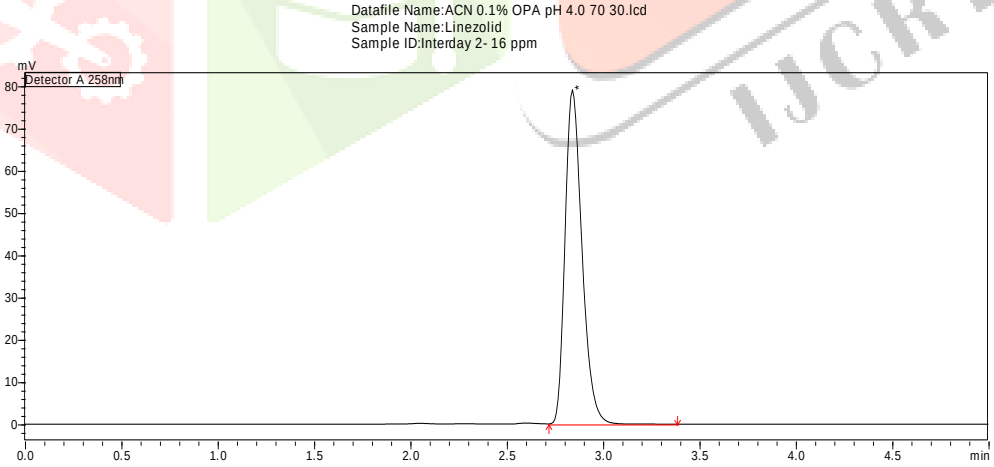
Graph No. 28: A Chromatogram of Interday Precision Day 1 of 16µg/ml Linezolid



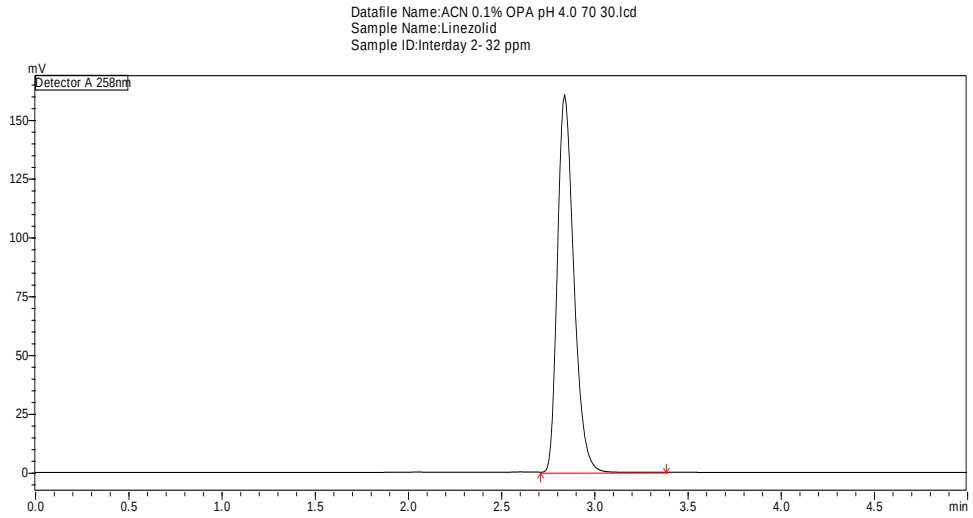
Graph No. 29: A Chromatogram of Interday Precision Day 1 of 32µg/ml Linezolid



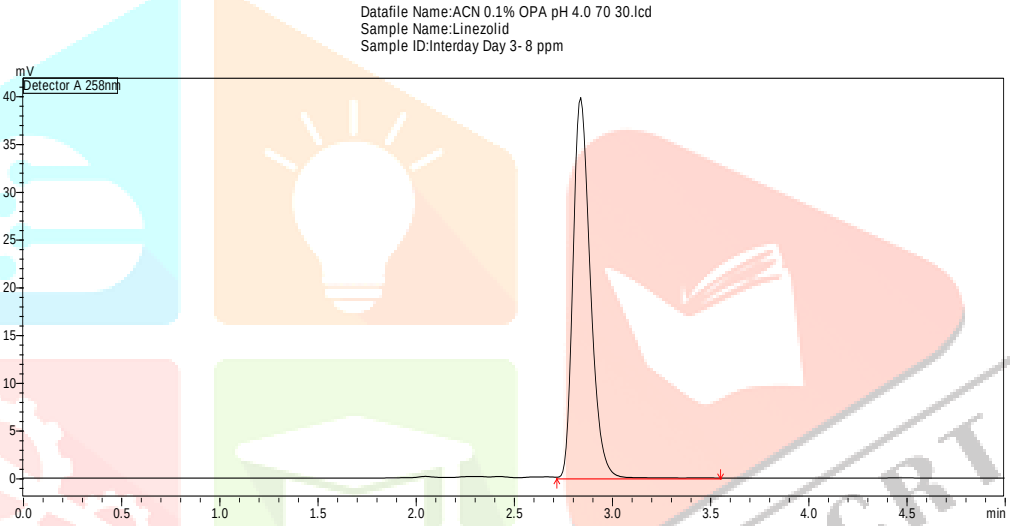
Graph No. 30: A Chromatogram of Interday Precision Day 2 of 8µg/ml Linezolid



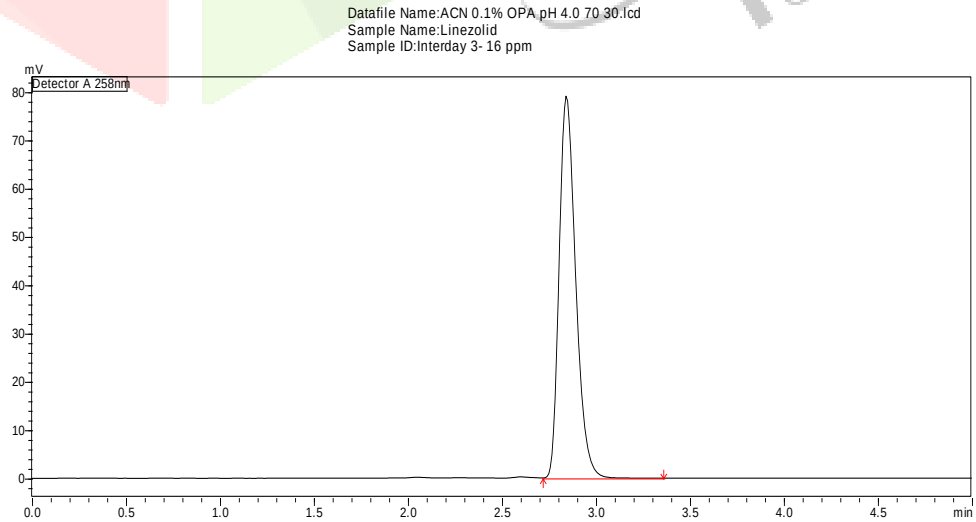
Graph No. 31: A Chromatogram of Interday Precision Day 2 of 16µg/ml Linezolid



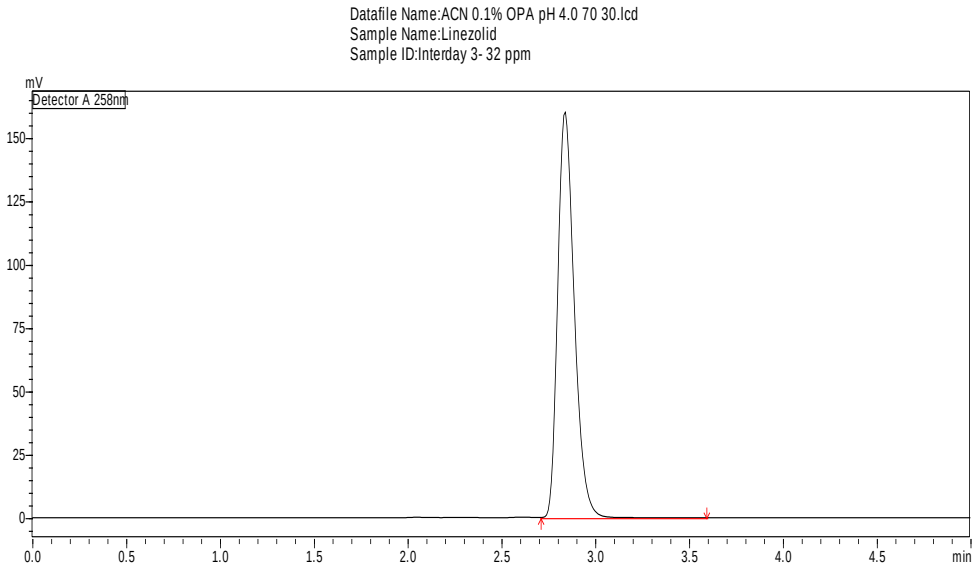
Graph No. 32: A Chromatogram of Interday Precision Day 2 of 32µg/ml Linezolid



Graph No. 33: A Chromatogram of Interday Precision Day 3 of 8µg/ml Linezolid



Graph No. 34: A Chromatogram of Interday Precision Day 3 of 16µg/ml Linezolid



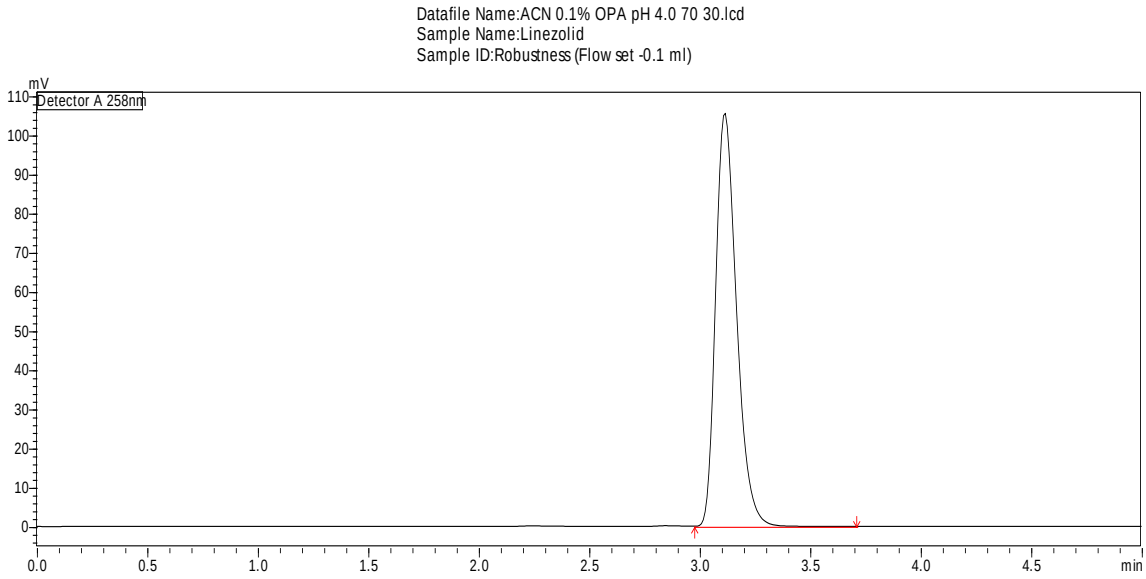
Graph No. 35: A Chromatogram of Interday Precision Day 3 of 32µg/ml Linezolid

3.4 Robustness

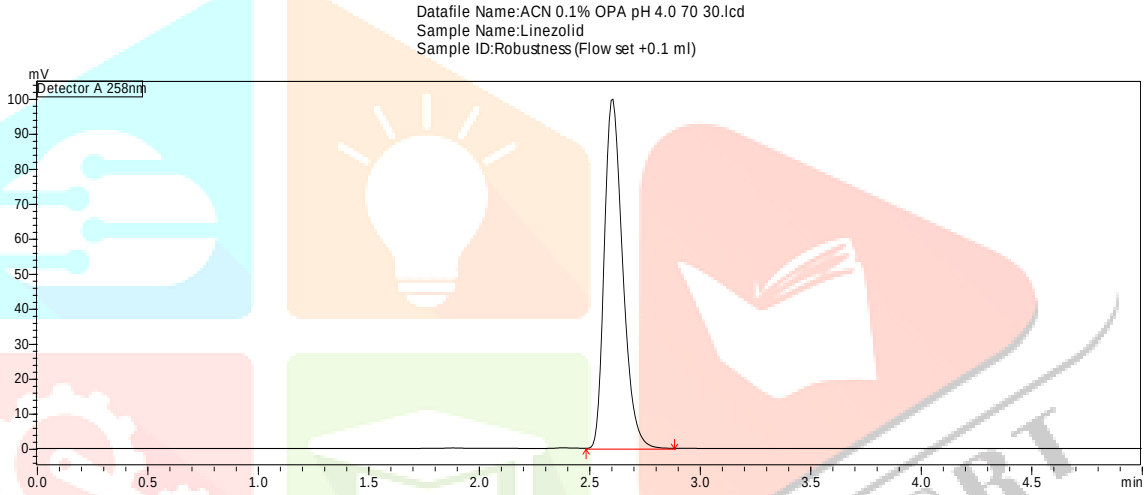
Robustness was studied by different deliberate variations in the chromatographic conditions. Results are shown in Table no 21.

Table No. 20: Data for Robustness study of Linezolid by HPLC method

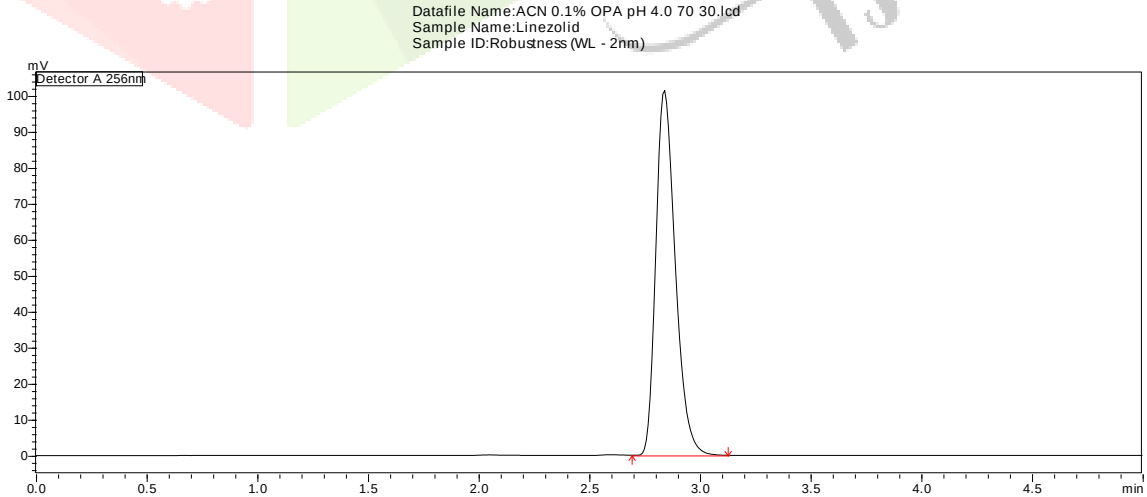
Sr. No.	Parameter	Condition	Area	Mean	SD	%RSD
1	Change in Flow rate (ml/min)	1.0	619347	619267.3	78.526	0.01268
2		1.0	619265			
3		1.0	619190			
1		1.2	616297	616288	97.81104	0.015871
2		1.2	616186			
3		1.2	616381			
1	Change in Wavelength (nm)	256	620253	620454.3	255.6762	0.041208
2		256	620368			
3		256	620742			
1		258	621753	621750.3	107.0249	0.017213
2		258	621856			
3		258	621642			



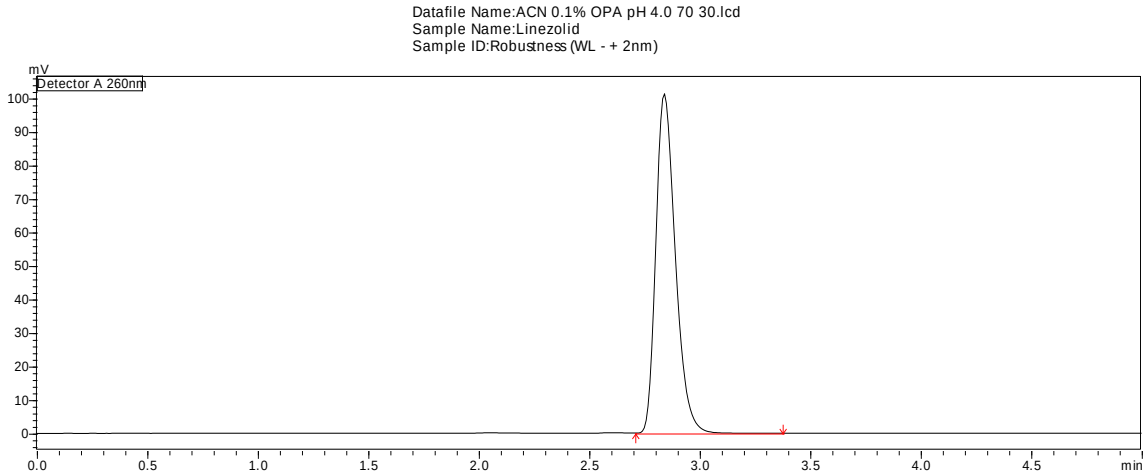
Graph No. 36: Chromatogram of 20µg/ml Linezolid at flow rate -0.1 ml/min



Graph No. 37: Chromatogram of 20µg/ml Linezolid at flow rate +0.1 ml/min



Graph No. 38: Chromatogram of 50µg/ml Linezolid at Wavelength 256nm



Graph No. 39: Chromatogram of 50µg/ml Linezolid at Wavelength 260nm

3.5 Limit of detection and limit of Quantitation

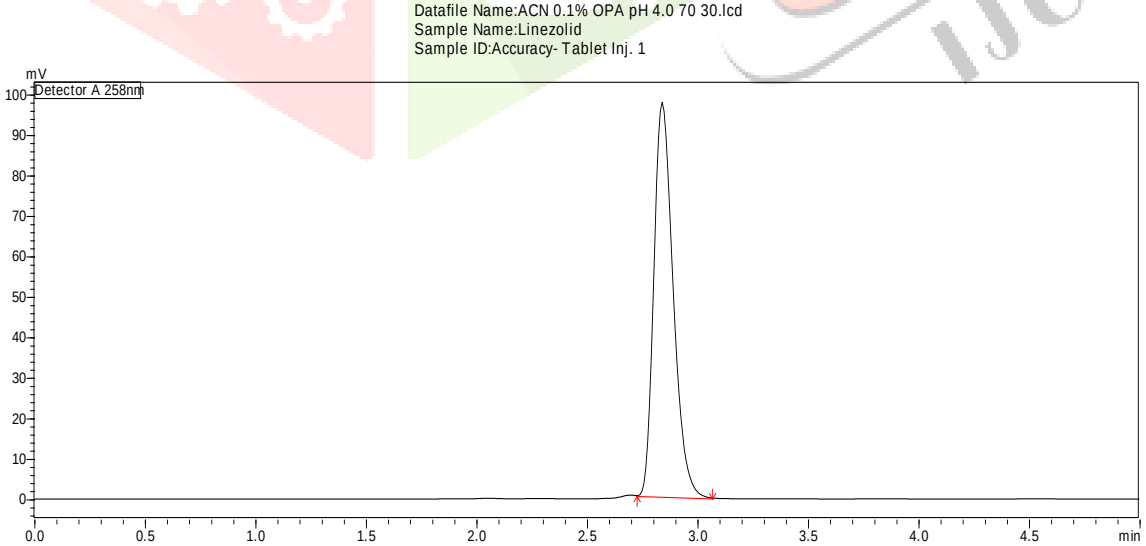
The results of LOD and LOQ are presented in Table No. 22

Table No. 21: Results of LOD and LOQ values of Linezolid

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Linezolid	0.78	2.34

3.6 Analysis of Marketed Formulation

The assay of marketed formulation was performed as per the procedure provided in section 7.6., chapter 7. Linezolid that can be quantified using proposed method was shown in Table 22.



Graph No. 40: Chromatogram of Linezolid in marketed formulation

Table No. 22: Result of assay of Linezolid in marketed formulation.

Marketed Formulation	Label Claim	Observed amount (mg)	% Assay
LIZOLID	600 mg Linezolid	600.31	100.05

Conclusion

The RP-HPLC method developed for estimation of Linezolid was validated as per ICH Q2 (R1) guidelines using various parameters. In this project, as per our objective RP-HPLC method was developed and validated on analytical column Agilent (250× 4.6mm, 5µm), with mobile phase ACN: 0.1% OPA pH 4.0 (70: 30). The flow rate was kept at 1.0 ml /min and UV detection was carried out at 258 nm. The retention time for Linezolid was found to be 2.840 min. RP-HPLC method has been developed for estimation of Linezolid in tablet dosage form. The proposed method was validated and it was found to be simple, sensitive, precise, and robust and it can be used for the routine analysis of Linezolid in bulk. All result are in acceptable limits such as %RSD is less than 2%, Tailing Factor less than 2, theoretical plates more than 2000.

References

1. Luxminarayan L, Sharma N, Viswas A, Khinchi MP. A review on chromatography techniques. Asian Journal of Pharmaceutical Research and Development. 2017 Mar 1:1-08.
2. Van der Wal S, Snyder LR. Photometric detection at 185 nm for high-performance liquid chromatography with either isocratic or gradient elution: Assay of mixtures of polyethylene glycol oligomers. Journal of Chromatography A. 1983 Jan 21; 255:463-74.
3. Vibha G et al. Development and validation of HPLC method – a review. International Research Journal of Pharmacy 2012; 2(4): 22-23.
4. Willard HH, Merritt LL, Dean JJA, Frank AS. Instrumental Method of Analysis.7th ed. New Delhi: CBS Publishers and Distributors; 1986.p.1-4.
5. Garry D Christian. Analytical chemistry. 4th ed. London: University of Wellington; 2001.p.1-4.
6. Robert D et al. Introduction of chemical analysis. 1st ed. New Delhi: IVY Publishing house; 2001.p.145.
7. A.H.Beckett and J.B stenlake Practical Pharmaceutical Chemistry Part-2. 4th edition. EBS Publishers and Distributors; 2000.p. 275-286.
8. Fong GW, Lam SK. HPLC in the Pharmaceutical Industry. New York: Marcel Dekker Inc; 1991.p.47.
9. Sethi P D. High performance liquid Chromatography. 1st ed. New Delhi: CBS publishers and distributors; 1996.p.17-19.
10. Kaur H. Spectroscopy. 3rd ed. Meerut: Pragati Prakashan Educational publishers;2007.p.1-5,237-314.
11. Lough WJ, Wainer I W. High Performance Liquid Chromatography; Fundamental Principles and Practice. Glasgow (UK): Blackie Academic & Professional; 1995.p. 23-8.
12. Turowski M et al. Selectivity of stationary phases in reversed-phase liquid chromatography based on the dispersion interactions. J. Chromatography. A.2001; A (911): 177-190.
13. Joachim Ermer, Miller. Method Validation in Pharmaceutical Analysis. Weinheim: Wiley-vch Verlag GmbH & Co; 2005.p.132.
14. Vikram Kumar et al. An Overview on HPLC Method Development, Optimization and Validation process for drug analysis. Phar. Chem. Jour. 2015; 2(2): 30-40.
15. Vibha Gupta et al. Development and validation of HPLC method – a review. Int. Res J Pharm. App Sci. 2012 August; 2(4): 17-25.
16. Murugan S.A. Review on method development and validation by using HPLC. IJNTPS 2013 October; 3(3): 78-81.
17. Boak LM, Li J, Nation RL, Rayner CR. High-performance liquid chromatographic method for simple and rapid determination of linezolid in human plasma. Biomedical Chromatography. 2006 Aug;20(8):782-6.
18. Peng GW, Stryd RP, Murata S, Igarashi M, Chiba K, Aoyama H, Aoyama M, Zenki T, Ozawa N. Determination of linezolid in plasma by reversed-phase high-performance liquid chromatography. Journal of pharmaceutical and biomedical analysis. 1999 Jun 1;20(1-2):65-73.

19. Slatter JG, Stalker DJ, Feenstra KL, Welshman IR, Bruss JB, Sams JP, Johnson MG, Sanders PE, Hauer MJ, Fagerness PE, Stryd RP. Pharmacokinetics, metabolism, and excretion of linezolid following an oral dose of [14C] linezolid to healthy human subjects. Drug Metabolism and Disposition. 2001 Aug 1;29(8):1136-45.
20. Fortuna S, De Pascale G, Ragazzoni E, Antonelli M, Navarra P. Validation of a new HPLC-UV method for determination of the antibiotic linezolid in human plasma and in bronchoalveolar lavage. Biomedical Chromatography. 2013 Nov;27(11):1489-96.
21. Swoboda, S., Ober, M., Anagnostakos, K., Geiss, H.K., Weigand, M.A. and Hoppe-Tichy, T., 2007. A simple isocratic HPLC assay to determine linezolid concentrations in different biomatrices for in vivo and in vitro studies.
22. Mohapatra S, Annapurna MM, Ravi Kumar BV, Anwar M, Warsi MH, Akhter S. Validated stability indicating RP-HPLC method for the estimation of linezolid in a pharmaceutical dosage form. Journal of liquid chromatography & related technologies. 2011 Nov 1;34(18):2185-95.
23. Warsi MH, Mohapatra S, Asfer M, Yusuf M, Ali A, Rahman MA, Ali A, Qadir A, Jain GK. Development and antibacterial investigation of Linezolid loaded SPIONs and HPLC method development for quantitative analysis of Linezolid. Journal of AOAC International. 2023 Jun 12:qsad071.
24. Ferrone V, Carlucci M, Cotellese R, Raimondi P, Cichella A, Di Marco L, Genovese S, Carlucci G. Development of a dried blood spot HPLC-PDA method for the analysis of linezolid and ciprofloxacin in hospital-acquired pneumonia patients. Drug testing and analysis. 2017 Oct;9(10):1611-9.
25. Mohammed SA, Eissa MS, Ahmed HM. Simple protein precipitation extraction technique followed by validated chromatographic method for linezolid analysis in real human plasma samples to study its pharmacokinetics. Journal of Chromatography B. 2017 Feb 1;1043:235-40.