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Research Article

RP-HPLC Method Development & Validation For Estimation Of Linezolid In Bulk Drug And Solid Dosage Form

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ABSTRACT

The main aim of the present research work is to develop a sensitive, precise and accurate HPLC (High-Performance Liquid Chromatography) procedure for the selective estimation of Linezolid. An isocratic separation of Linezolid through column used was a Phenomenex ODS-3 with dimensions of 250 mm length and 4.6 mm inner diameter, packed with 5µm particle utilizing mobile phase composition of Methanol and 0.1% Ortho Phosphoric Acid in water, with a proportion of 70% Methanol and 30% water (v/v). The detection of the analyte was processed at the maximum wavelength of 258 nm and with 1 ml/min flow of the mobile phase. In the developed reversed-phase high-performance liquid chromatography (RP-HPLC) method, the analytes were separated using an isocratic program. The separation was performed on an HPLC system data acquisition and analysis was handled using Openlab EZ-Chrome Software. The results of the analysis were verified for linearity, accuracy, precision, robustness, limit of detection, and limit of quantification in the developed method. Five variable concentration levels of 2, 10, 20, 25 and 30µg/ml were used for the estimation of recovery and linearity. The %RSD was also under 2%, demonstrating the great degree of precision of the suggested approach.

INTRODUCTION

Linezolid is a synthetic antibiotic of oxazolidinone class used as antibacterial and anti-infective. It is used for the treatment of serious infections caused by Gram-positive bacteria that are resistant to several antibiotics.(2,3) It is chemically known as N-[[(5S) - 3- [3- fluoro- 4 - (4 morpholinyl) phenyl] – 2 – oxo – 5 -oxazolidinyl] methyl] acetamide. It is official in the Indian Pharmacopoeia.(3) Linezolid is a synthetic antibiotic used for the treatment of serious infections caused by Gram-positive bacteria that are resistant to several other antibiotics. Linezolid is active against most Gram-positive bacteria that

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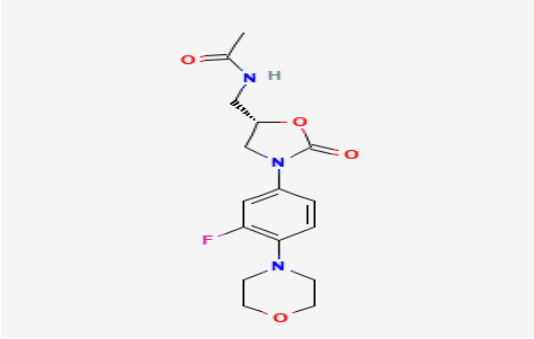
cause diseases including streptococci, vancomycin-resistant enterococci (VRE), and methicillin-resistant Staphylococcus aureus (MRSA). The main indication of linezolid is the treatment of severe infections caused by Gram-positive bacteria that are resistant to other antibiotics; it should not be used against bacteria that are sensitive to drugs with a narrower spectrum of activity, such as penicillin and cephalosporin. In both the popular press and the scientific literature, linezolid has been called a ‘‘reserve antibiotic’’. (1,2,4)



Structure of Linezolid

MATERIAL & METHOD:

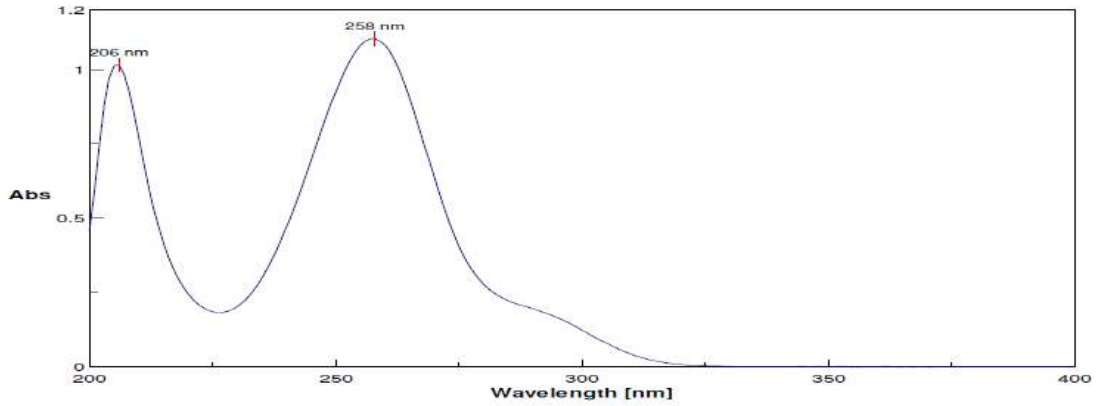
1. Methanol procured from Merck HPLC grade.
2. Acetonitrile procured from Merck HPLC grade.
3. Water procured from Siddhi Lab HPLC grade.
4. Ortho Phosphoric Acid procured from Qualigens HPLC grade

INSTRUMENTS:

HPLC system, specifically a Jasco HPLC Binary Gradient System with model number 1260 Infinity by Agilent. The pump is identified as DEAX02386, and the detector as DEACX16446. The column utilized is a Phenomenex ODS-3, measuring 250 mm X 4.6 mm with a particle size of 5 µm. The software employed for operation is Openlab EZ Chrome. The analytical balance utilized is an Azcet High Precision Balance, model CY 224C, with a maximum capacity of 220 grams and a minimum readability of 0.001 grams. The pH meter employed is a digital pH meter manufactured by LabIndia. For sonication purposes, a Bio-technic Ultra Sonicator with a capacity of 13.5 litres is used. Additionally, two types of filters are employed: a Nylon membrane with a pore size of 0.45 µm and a PVDF membrane also with a pore size of 0.45 µm.

Selection of analytical wavelength

Methanol as a blank and Linezolid standard solution (20 ppm) was scanned from 400 nm to 200 nm. Absorption maxima was determined for drug. Linezolid showed maximum absorbance at 258 nm shown in results.



UV spectrum of Linezolid

METHOD DEVELOPMENT BY RP – HPLC

Preparation of standard stock solution:

Linezolid standard stock solution was prepared by transferring 20 mg Linezolid into a 20 mL clean and dried volumetric flask added about 15 mL of

Methanol to dissolve it completely and made volume up to the mark with methanol. (1000 ppm). Further diluted 2 ml of stock solution to 20 mL with Methanol. (100 ppm).

Chromatographic Conditions:

Detector: U.V. Detector Column: Phenomenex ODS-3 Column Dimension: (250 mm X 4.6 mm i.d.) 5µm Column Oven temperature: 35°C, Injection Volume: 20 µl Wavelength: 258 nm, Mobile phase: Methanol: 0.1% OPA in Water (70:30) Flow Rate: 1.0 ml/min.

Sample preparation of Marketed test sample:

Weighed 20 tablets and determine the average weight. Then transferred in mortar pestle and crushed to fine powder. Mixed the contents with butter paper uniformly. Weighed the powder material equivalent to 50 mg of Linezolid and transferred to clean and dried 50 mL of volumetric flask. Added 35 mL of Methanol, sonicated for 10 minutes with intermittent shaking. After 10 minutes allow to cool he solution to room temperature and made volume up to the mark with Methanol. Filtered the solution through suitable 0.45 µ Nylon syringe filter discarding first 3-5 mL of filtrate. Further diluted 0.5 ml of filtered stock solution to 25 ml with methanol (20 ppm). Injected the resultant solution and chromatograms were recorded and results are recorded.

System suitability

System suitability is a Pharmacopoeial requirement and is used to verify, whether the chromatographic system is adequate for analysis to be done. The tests were performed by collecting data from five replicate injection of standard drug solution and the results are recorded.

Linearity & Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. 5 levels of Linearity was performed from 10%, 50%, 100%, 125%, 150% of working concentration.

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

Detection limit: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation limit:

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. As per ICH Q2R1 guidelines LOD and LOQ was determined by using the approach Based on the Calibration Curve in which residual standard deviation of a regression line was calculated and determined the LOD and LOQ by using following formula:

$$LOD = 3.3\ \sigma / S$$

$$LOQ = 10\ \sigma / S$$

Accuracy (% Recovery):

The accuracy of the analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value of the value found, Accuracy will be conducted in the range from 50 %, 100%, 150 % of working concentration. Solution of each accuracy level was prepared in triplicate. Calculated % Recovery for each sample, Mean % recovery for each level and overall recovery and also calculated % RSD for each level and % RSD for overall recovery.

Precision

Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous test under the prescribed conditions. Precision is of two types, Repeatability

and Intermediate precision. It is performed on tablet test sample.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

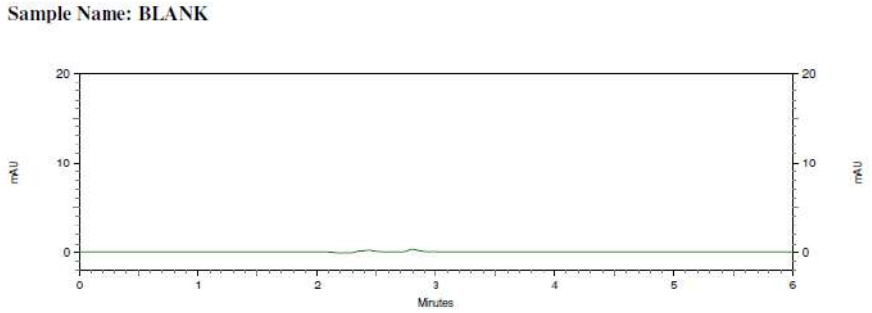
Blank and Standard solution were injected under different chromatographic conditions as shown below.

a. Changes in flow rate by ±10%. (± 0.1ml/min)
b. Change in column oven temperature. (± 2ºC)
c. Change in wavelength (± 3 nm)

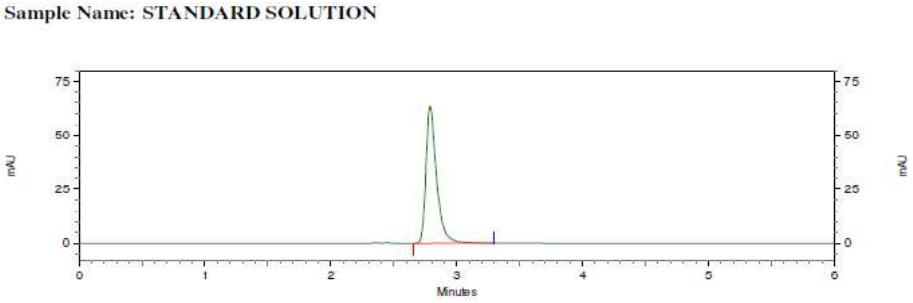
RESULT & DISCUSSION:

Optimized Chromatographic Conditions

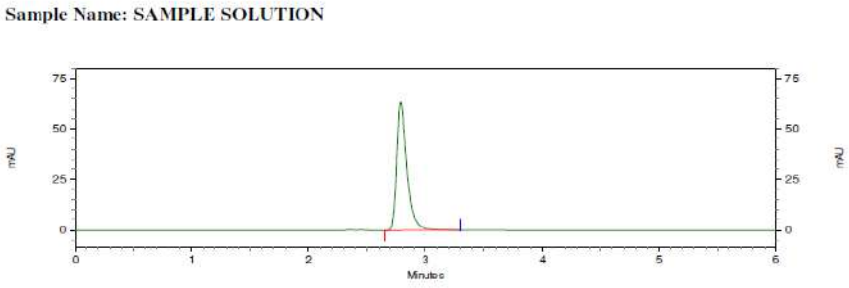
Parameter	Description
Mode	Isocratic
Column Name	Phenomenex ODS-3, 250 mm X 4.6 mm, 5 µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	258 nm
Column Oven temp	35ºC
Mobile Phase	Methanol : 0.1% OPA in Water (70 : 30 % v/v)
Flow Rate	1.0 ml/min
Diluent	Methanol
Run time	6 Minutes



Typical chromatogram of Blank solution.



Typical chromatogram of Standard solution.



Typical chromatogram of Test sample solution.

System Suitability Test of Linezolid:

RESULTS

Sr No.	Standard solution	Area	Asymmetry	Theoretical plates
1	Standard_1	6469513	1.35	7538
2	Standard_2	6429601	1.35	7543
3	Standard_3	6452910	1.35	7531
4	Standard_4	6430091	1.34	7554
5	Standard_5	6448073	1.35	7549
Mean		**6446038**	**1.35**	**7543**
STD Dev		**16784.84**		
% RSD		**0.26**		

Specificity:

Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present. Blank, standard solution prepared and injected to check peak purity.

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 0.988
Test Solution	Peak purity was 0.983

Precision

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Precision was performed on Test sample.

Repeatability:

The preparation of sample solutions involved creating six separate samples to ensure repeatability and consistency in the experimental procedure.

Intermediate precision:

It is performed by doing analysis on another day to check reproducibility of results. Samples prepared in same manner as that of Repeatability parameter (6 Samples prepared.

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

	Sample	Test Sample (mg)	Area	% Assay
Repeatability	Sample 1	85.3	6430274	99.46
	Sample 2	85.2	6318641	97.85
	Sample 3	84.9	6324520	98.29
	Sample 4	85.2	6420317	99.43
	Sample 5	85.3	6350387	98.23
	Sample 6	85.1	6307921	97.80

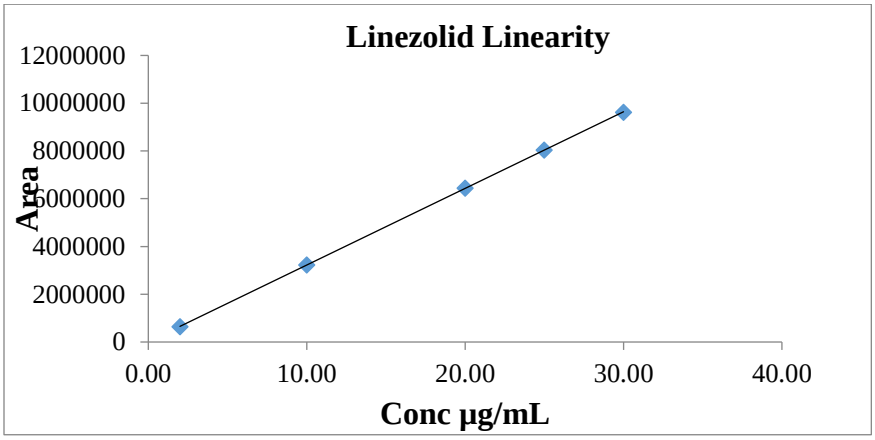
	Mean			**98.51**
	Std Dev			**0.7504**
	% RSD			**0.762**
Intermediate precision (Inter-Day)	Sample 1	85.1	6329146	98.13
	Sample 2	85.5	6492543	100.19
	Sample 3	84.8	6309719	98.17
	Sample 4	85.6	6320341	97.42
	Sample 5	85.2	6412378	99.30
	Sample 6	85.4	6299784	97.33
	Mean			**98.42**
	Std Dev			**1.1173**
	% RSD			**1.135**
RepeatabilityPlus Inter-day	**Mean**			**98.467**
	Std Dev			**0.9085**
	% RSD			**0.923**

Linearity and Range:

Linearity of an analytical method is its ability to obtain test results that are proportional to the concentration of analyte in samples within a given range.

Results of HPLC Linearity Data for Linezolid:

Level	**Conc (µg/mL)**	**Area**	**Mean**	**% RSD**
10%	2.00	641684	641717	0.211
		643090		
		640378		
50%	10.00	3238364	3235029	0.430
		3219740		
		3246984		
100%	20.00	6440348	6442582	0.278
		6461503		
		6425894		
125%	25.00	8023715	8044162	0.229
		8049317		
		8059453		
150%	30.00	9629460	9626683	0.226
		9603648		
		9646941		



LOD = 0.138 µg/mL

Quantitation limit (LOQ):

LOQ = 10 σ / S

LOQ = 10 x 3.3 x 13377.652 / 320964.4678

LOQ = 0.417 µg/mL

Accuracy (Recovery):

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method is determined by applying the method to analyzed samples to which known amounts of analyte have been added. Accuracy will be conducted in the range from 50 % to 150 % of working concentration. Solution of each accuracy level was prepared in triplicate.

Result and statistical data of Accuracy of Linezolid

Level (%)	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	3217451	9.98	10.12	98.62	99.54	1.1343
	3248609	10.08	10.00	100.80		
	3208671	9.96	10.04	99.20		
100	6435861	19.97	20.04	99.65	99.20	0.4486
	6403176	19.87	20.12	98.76		
	6419703	19.92	20.08	99.20		
150	9653072	29.95	30.04	99.70	99.62	0.4516
	9686134	30.05	30.04	100.03		
	9609792	29.82	30.08	99.14		

Filtration Study:

Filtration study of an analytical procedure checks the interference of extraneous components from filter, deposition on filter bed and compatibility of filter with sample. Performed on tablet test sample.

Sample description	Area	% Absolute difference
Unfiltered	6462018	NA
0.45 µ PVDF filter	6430710	0.48
0.45 µ Nylon filter	6440354	0.34

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Following changes made under Robustness:

- Change in Wavelength
- Change in flow rate
- Change in column oven temperature

Result of Robustness study:

Analysis of Marketed Test samples (Assay)

Megazolid 600 mg Tablet:

Weight of 20 tablets = 20.4120 gm

Average weight of tablet = 20.4120 / 20 = 1.0206 gm

Assay results of Glezolide 600 mg

Sample	Area	% Assay	Mean Assay
Sample 1	6432914	99.39	**98.90%**
Sample 2	6346901	98.40	

CONCLUSION

- A successful attempt to determine linezolid in bulk and dosage form using high performance liquid chromatography was made in the current study activity. The development of an appropriate, suitable, and easy-to-use RP-HPLC method was the focus of the current work.
- Several methods have been published for determining Linezolid in bulk drugs or in pharmaceutical dose forms, according to a literature review. Thus, a novel, sensitive, and appropriate reversed-phase high performance liquid chromatography method was created and validated in the current study for the measurement of linezolid in bulk drug and pharmaceutical dosage form.
- In the developed reversed-phase high-performance liquid chromatography (RP-HPLC) method, the analytes were separated using an isocratic program. The mobile phase consisted of a mixture of Methanol and 0.1% Ortho Phosphoric Acid in water, with a proportion of 70% Methanol and 30% water (v/v). The separation was performed on an HPLC system equipped with a UV-visible detector, and data acquisition and analysis were handled using Openlab EZ-Chrome Software. The chromatographic column used was a Phenomenex ODS 3 with dimensions of 250 mm length and 4.6 mm inner diameter, packed with 5µm particle size. The flow rate of the mobile phase was set at 1.0 ml/min, and the detection of the analytes occurred at a wavelength of 258 nm.

REFERENCES:

1. Makwana, B., Patel, V. G. (2014). RP-HPLC Method Development and Validation for the Analysis of Pharmaceutical Drug - Linezolid. International Journal of Science and Research, 3(2), February.
2. Sushma, S., Pushpa Latha, E. (2015). Method Development And Validation Of Spectrophotometric Method For The Estimation Of Linezolid In Pure And Tablet Dosage Form. June 3(2), 82-88.
3. Mohapatra, S., Annapurna, M. M., Ravi Kumar, B. V. V., Anwar, M., Warsi, M. H., Akhter, S. (2011). Validated Stability Indicating RP-HPLC Method For The Estimation Of Linezolid In A Pharmaceutical Dosage Form. Journal of Liquid Chromatography & Related Technologies, 34(18), 2185-2195. DOI: 10.1080/10826076.2011.585548
4. Ali, S. M., Bharath, P., Ramakrishna, V. S., Ramachandran, D. (2022). Development and Validation of RP-LC Method for Linezolid in Pharmaceutical Formulations. SSRG International Journal of Applied Chemistry,

9(1), 18-24. DOI: 10.14445/23939133/IJACV9I1P103.

5. Abdel Kawy, M., Weshahy, S. A., Shokry, D. S. (2012). Validated Stability Indicating Assay of Linezolid by Spectrophotometric and High-Performance Liquid Chromatographic Methods. Australian Journal of Basic and Applied Sciences, 6(3), 767-778.
6. Gurmeet Chhabra, S., Jain, S., Jain, D. K. (2015). Development and Validation of Stability-Indicating Method for Assay of Linezolid Immediate Release Tablet by Reversed Phase HPLC. Int. J. Pharm. Pharm. Res, 4, 47-55.
7. Dighe, Y., Laware, R. B., Dighe, S. B. (2020). Analytical Method Development and Validation of Linezolid. Research Journal of Science and Technology, 12, DOI: 10.5958/2349-2988.2020.00002.9.
8. Agrawal, H., Mahadik, K. R., Paradkar, A. R., Kaul, N. (2003). Stability Indicating HPTLC Determination of Linezolid as Bulk Drug and in Pharmaceutical Dosage Form. Drug Dev Ind Pharm, 29(10), 1119-1126. DOI: 10.1081/ddc-120025869.
9. Patel, S. A., Patel, P. U., Patel, N. J., Patel, M. M., Bangoriya, U. V. (2007). High-Performance Thin Layer Chromatographic Method for Estimation of Linezolid in Tablets. Indian Journal of Pharmaceutical Sciences, 69(4), 571-574.
10. Prasanti, J. K., Sundar, B. S. (2012). LC Method Development and Analysis of Linezolid in Human Plasma of Feeding Mothers. Journal of Pharmacy Research, 5(5), 2413-2417.
11. Akhtar, J., Baghel, M., & Patel, D. (2013). Development of analytical method for simultaneous estimation of Cefixime and Linezolid in tablet dosage form. Research Journal of Pharmacy and Technology, 6(5), 592-594, January.
12. Kumar, L., Yadav, Y. S., Rathnanand, M. (2017). Simultaneous Determination of Linezolid and Levamisole Hydrochloride in a Fixed Dose Combination. Indian Journal of Pharmaceutical Education and Research, 51(4), Oct-Dec.

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