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Original Article

DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR THE ESTIMATION OF LINEZOLID IN LINEZOLID GEL

VIPUL K. SAPAVADIYA*[a], MIRAL V. SAPAVADIYA[b] PRAGNA K. SHELAT[a], ANITA N. LALWANI[a]

[a]Department of Pharmaceutics, K. B. Institute of Pharmaceutical Education and Research, Sector-23, GH-6 Road, Gandhinagar 382023, Gujarat, India, [b]Bajaj Healthcare Ltd., Formulation Division, at and post:, Manjusar, Vadodara 391775, Gujarat, India
Email: sapavadiyavipul@gmail.com

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ABSTRACT

Objective: Development and validation of new RP-HPLC method for the estimation of linezolid in linezolid gel.

Methods: Linezolid was chromatographed on a reverse phase symmetry C18 column (150 x 4.6 mm x 3.5 µm) in a mobile phase consisting of potassium dihydrogen phosphate buffer (pH 4.6 adjusted with 10% orthophosphoric acid) and methanol in the ratio of 55:45. The mobile phase was pumped at a flow rate of 1.2 ml/min with detection at 250 nm.

Results: The retention time for Linezolid was found about 2.94 min. The detector response was linear in the concentration of 20 µg/ml to 160µg/ml with correlation coefficient of 0.9997. The percentage recovery of Linezolid at target concentration was found to be 97.8%. The limit of detection and limit of quantification was found to be 10 µg/ml and 5 µg/ml respectively. All other validation parameter were within acceptance criteria.

Conclusion: The proposed method was found to be simple, fast, accurate, precise and reproducible and could be used for routine quality control analysis of Linezolid in Linezolid gel.

Keywords: Linezolid, RP-HPLC, Gel, Method Validation.

INTRODUCTION

Linezolid is a member of a new structural class of antibiotics, Oxazolidinones. The oxazolidinones have a good activity against Gram-positive bacteria. They act uniquely by inhibiting the formation of protein synthesis initiation in Gram-positive bacteria [1-3]. Linezolid is active after oral or intravenous administration. Linezolid is expected to increase the treatment options for severe infections due to Gram positive bacteria, particularly resistant infections (e. g. Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococci* (VRE)) [4-5]. Linezolid is a synthetic antibacterial agent of the oxazolidinone class. The chemical name for Linezolid is (*S*)-N-[3-[3-Fluoro-4-(4-morpholinyl) phenyl]-2-oxo-5-oxazolidinyl] methyl]-acetamide. The empirical formula is $C_{16}H_{20}FN_3O_4$. The chemical structure of the linezolid is presented in fig.1. Its molecular weight is 337.346 g/mol.

Literature survey reveals few analytical methods for the estimation of Linezolid from pharmaceutical dosage forms especially in gel dosage form, are available. This reported method was not economical in terms of mobile phase composition and run time [6-7]. The availability of an HPLC method with high sensitivity and specificity will be very useful for the determination of Linezolid in pharmaceutical formulations. Hence, a new method for determination of Linezolid in gel formulations with a short time of analysis (approximately 6 minutes) was developed and validated. The current method is concerned at rapid analysis of Linezolid in pharmaceutical dosage forms. It is fast and quick chromatographic method in terms of retention time and run time when compared with other reported methods described in the literature survey.

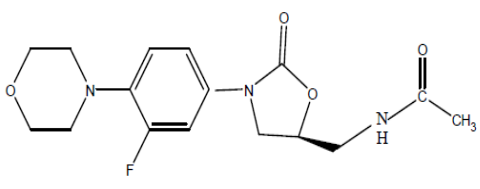

Fig. 1: Structure of Linezolid

MATERIALS AND METHODS

Chemicals and reagents

Pure Linezolid was received as a gift sample from Cadila Healthcare Limited, Vadodara, Gujarat, India. Methanol of HPLC grade was purchased from Merck (India) Ltd. Potassium dihydrogen phosphate and orthophosphoric acid of AR grade were purchased from Qualigen Fine Chemicals Ltd, Mumbai. Formulation of Linezolid Gel was prepared in-house.

Preparation of linezolid gel

All the required ingredients of the formulation were weighed accurately. Polymer powder was dispersed in 50 ml of distilled water maintained at 95 °C. The dispersion was stirred at 95°C for 20 min using a magnetic stirrer to facilitate hydration of the polymer. After all other ingredients along with linezolid were added and further stirred for 10 min. Finally, required amount of distilled water added for preparation of gel with suitable consistency.

Instrumentation and chromatographic conditions

The chromatographic separation was carried out on HPLC system (Shimadzu Co, Tokyo, Japan) with UV-Visible dual absorbance detector (PDA). The analytical column Symmetry C18 column (150 x 4.6 mm x 3.5 µm) was used for separation. The mobile was pumped from the solvent reservoir into the column at a flow rate of 1.2 ml/min. The detection was monitored at 250 nm. The volume of injection loop was 10 µl. Prior to the injection of the drug solution, the column was equilibrated for at least 30 minutes with the mobile phase following through the system. The column was maintained at ambient temperature (40 °C).

Preparation of mobile phase

Potassium dihydrogen phosphate buffer (pH 4.6 adjusted with 10% orthophosphoric acid) and methanol were filtered separately through 0.45µ membrane filter. Filter solvents were mixed in the ratio of 55:45 (%v/v) and degassed before use.

Preparation of standard solution

Transfer an accurately weighed quantity of about 100mg of Linezolid in 100 ml volumetric flask. About 60 ml of the mobile

phase was added, sonicated to dissolve the drug completely and the volume was made up with the mobile phase. 5 ml of above solution was diluted to 50 ml with the mobile phase (100 µg/ml).

Preparation of sample solution

10g gels equivalent to 100mg of Linezolid were taken in a clean and dry 100 ml volumetric flask. 80 ml of the mobile phase was added, sonicated to dissolve for 5 to 10 minutes and make up the volume with mobile phase. 5 ml of the above solution was diluted to 50 ml with mobile phase and filtered through 0.45µ membrane filter (100 µg/ml).

Procedure

10 µl of filtered portion of sample and standard preparations were injected into chromatograms. The responses for the major peak were recorded and content of linezolid was calculated.

Selection of detection wavelength

The UV spectrum of diluted solutions for various concentrations of Linezolid in the mobile phase was recorded using UV visible spectrophotometer.

Validation parameters

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines [8].

1. Specificity

Standard solution, sample solution, blank solution and placebo solution were injected simultaneously into the system and chromatograms were recorded. The specificity of the method was determined by observing interference of any encounter ingredients in the formulation.

2. System suitability

Standard solution was injected six times into the system and chromatograms were recorded. % RSD (Relative standard deviation) of peak area, theoretical plates and tailing factor was calculated.

3. Precision

Method precision (Intra-day) of the proposed method was determined by injecting six replicate of sample solution. While intermediate precision (inter-day) was determined by injecting six replicate of sample solution on two different days. % RSD of peak area of the sample solution was calculated for intra-day and inter-day study.

4. Accuracy

Accuracy is the degree of agreement between a measured value and the accepted reference value. The accuracy of the method was determined by injecting triplicate samples at 3 different concentrations equivalent levels 50%, 100% and 150%. Predetermined amount of standard was added to this solution by spiking standard drug solution to the sample. The recovered amount of Linezolid, % RSD of recovery, % recovery of each concentration is calculated to determine the accuracy.

5. Limit of detection (LOD), Limit of Quantification (LOQ) and Linearity

LOD, LOQ and Linearity were determined by calibration curve method. 10 µl of each calibration standard solutions (2.5, 5, 10, 20, 40, 60, 80, 100, 120, 140 and 160 µg/ml) were injected into the HPLC system to get the chromatograms. Linearity curve was constructed by plotting the concentration of Linezolid on X-axis and average peak areas of standard Linezolid on Y-axis and regression equations was computed. LOD was considered at the lowest concentration till peak is detected and LOQ was considered at the lowest concentration of peak detection where % RSD of peak response was within range of not more than 2.0%.

6. Robustness

Robustness is the ability to provide accurate and precise results under a variety of conditions. In order to measure the extent of method robustness, the most critical parameters were interchanged while keeping the other parameters unchanged and in parallel, the chromatographic profile was observed and recorded. The studied parameters were changed in flow rate by±0.2, change in detection wavelength by±2 nm and change in the composition of the mobile phase (±5% of methanol).

RESULTS AND DISCUSSION

Method development

Selection of detection wavelength

The maximum absorbance at different concentration in the mobile phase was observed at 250 nm wavelength (fig. 2). Hence, this wavelength was used for detection of Linezolid.

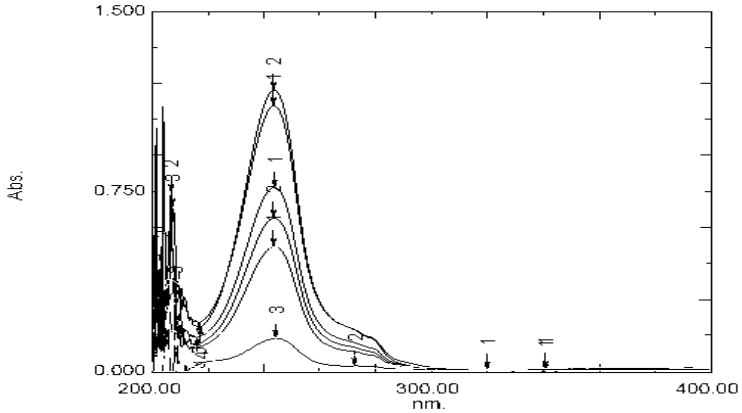

Fig. 2: UV Spectrum of Linezolid at different concentration

A Reverse phase HPLC method was developing the system suitability parameters like tailing factor (T), the number of theoretical plates (N), the runtime and the cost effectiveness. The optimized method developed resulting in the election of Linezolid at 2.94 min. Fig. 3 represent chromatograms of the standard solution (100µg/ml). The total run time is 6 minutes. System suitability tests are an integral part of method development and used to ensure adequate performance of the chromatography system. Retention time (Rt), the number of theoretical plates (N) and peak tailing factor was evaluated for six replicate injections of the standard at the working concentration. The results are given in table 1.

In order to test the applicability of the developed method to a linezolid gel formulation, Linezolid gel formulation was

chromatographed at working concentration (100µg/ml). System suitability parameters were within the acceptable limits, ideal for the chromatographies sample. Integration of the separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample with % RSD within the limit of not more 2.0%. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram (Fig.3) of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug linezolid by the proposed HPLC method.

Method validation

Specificity

The specificity of the HPLC method is illustrated in Fig.-3 where complete separation of Linezolid was noticed in the presence of gel excipients. In addition, there was no any interference at the retention time in the chromatogram of the placebo solution. In peak purity analysis with PDA, purity angle was less than purity threshold observed for the analyte which shows that the peaks of analyte were pure and excipients in the formulation does not interfere with the analyte. Hence, Specificity of linezolid is established.

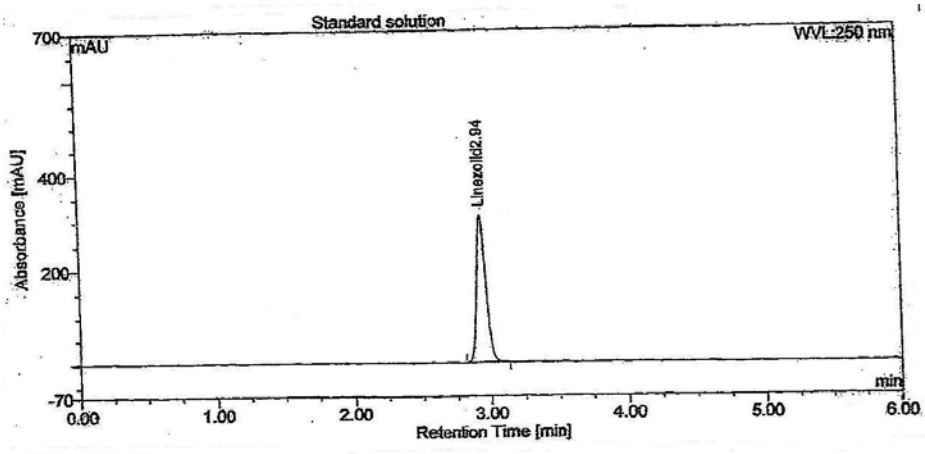

Fig. 3: Typical Chromatograms of Linezolid

System suitability

The System is considered suitable if tailing factor should not be more than (NMT) 2.0, theoretical plate count should not less than (NLT) 2000 and % RSD for peak area of six replicate injections of Linezolid standard should NMT 2.0. Results of system suitability are presented in table 1 which shows % RSD, theoretical plate count and tailing factor were within acceptance criteria. The retention time of the drug in the mobile phase was found approximately about 2.94 minutes which proved method is fast as compare to other reported methods. Based above result, it can be concluded that reproducibility of the proposed method was established.

Precision

The intra-day and inter-day precision results were shown in Table-2. % RSD of peak area for six replicates of sample preparation for intra-day and inter-day was 1.25 and 1.43 respectively which was within the acceptable criteria of not more than 2.0 which proves repeatability of methods. Hence method precision and intermediate precision of the method were established.

Table 1: System suitability parameters

S. No.	Injection no.	Peak area	Theoretical plate count (NLT 2000)	Tailing factor (NMT 2.0)	Retention time
1	Standard-1	7845.36	6825	1.36	2.94
2	Standard-2	7812.66	6878	1.45	2.92
3	Standard-3	7795.30	6955	1.22	2.93
4	Standard-4	7825.14	6790	1.09	2.93
5	Standard-5	7625.36	7014	1.19	2.94
6	Standard-6	7925.66	6744	1.47	2.94
Mean Peak Area		7804.91			
% RSD (NMT 2%)		1.27			

Table 2: Intra-Day and Inter-Day Precision of Linezolid

S. No.	Intra-Day precision*		Inter-Day precision*	
	Injection no.	Peak area	Injection no.	Peak area
1	Sample-1	7569.63	Sample-1	7489.75
2	Sample-2	7656.22	Sample-2	7599.35
3	Sample-3	7769.88	Sample-3	7685.66
4	Sample-4	7489.23	Sample-4	7545.22
5	Sample-5	7654.12	Sample-5	7769.32
6	Sample-6	7589.56	Sample-6	7510.29
Mean		7621.44	Mean	7599.932
% RSD (NMT 2%)		1.25	% RSD (NMT 2%)	1.43

*Concentration of Linezolid 100 µg/ml

Accuracy

Recovery results of linezolid are presented in table-3. The % mean recovery amount of Linezolid was within the range of 90% to 110% and % RSD was within the limit of not more than 2.0%. Hence, accuracy of the proposed method is established.

LOD, LOQ and Linearity

The results of LOD, LOQ and linearity are presented in Table-4. Results show that a phenomenal correlation exists between peak area and concentration of drug within the range of 20-160µg/ml with Correlation coefficient of 0.9997. Linearity curve is shown in fig. 4. % RSD till detection of peak response using 10µg/ml was found within range of not more than 2.0% and peak was detected at the lowest concentration of 5µg/ml. Hence, linearity of the proposed method was found in the range of 20 µg/ml to 160 µg/ml. LOD and LOQ of linezolid were found 10 µg/ml and 5 µg/ml respectively.

Robustness

The results of robustness study are shown in Table-5. Theoretical plate, tailing factor and % RSD was within acceptance criteria for different change condition of flow rate, detection wavelength and mobile phase composition. Results indicated that the small change in the conditions did not significantly affect the determination of Linezolid. Hence, robustness of the proposed method was established.

Table 3: Recovery data of Linezolid

S. No.	Recovery level	Amount taken (mg)	Amount added (mg)	Total amount (mg)	Amount recover (mg)	% recovery	Mean % recovery	% RSD (NMT 2.0%)
1	50 %	50	25	75	73.65	98.20	95.7	1.81
2					70.25	93.67		
3					71.35	95.13		
4	100 %	100	25	125	120.45	96.36	97.8	1.62
5					122.66	98.13		
6					123.52	98.82		
7	150 %	150	25	175	168.25	96.14	96.7	1.03
8					169.35	96.77		
9					170.25	97.29		

Table 4: LOD, LOQ and Linearity of Linezolid

S. No.	Concentration(µg/ml)	Mean peak area	% RSD*
1	160.0	12710.55	1.35
2	140.0	10980.61	1.05
3	120.0	9490.25	0.72
4	100.0	7845.62	0.45
5	80.0	6225.45	0.44
6	60.0	4852.65	0.89
7	40.0	3224.56	1.46
8	20.0	1520.14	1.22
9	10.0	825.56	1.65
10	5.0	390.25	10.25
11	2.5	Not Detected	--
Correlation coefficient (r)		0.9997	
Slope		78.96	
Y intercept		0.12	
LOD (µg/ml)		10	
LOQ (µg/ml)		5	

* % RSD should not be more than 2.0%

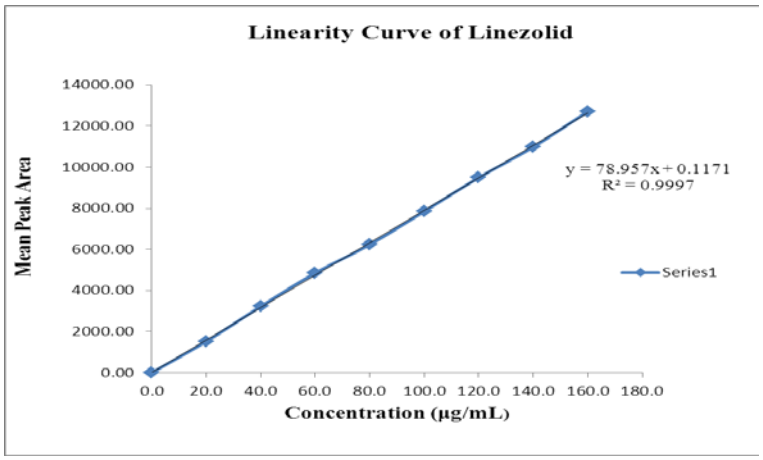

Fig. 4: Calibration curve of Linezolid

Table 5: Robustness of Linezolid

S. No.	Change parameter	Optimized parameter	Used condition	Theoretical plate	Tailing factor	% RSD
1	Flow rate	1.2 ml	1.0 ml/min	6480	1.39	1.88
2	(±0.2 ml/min)		1.4 ml/min	4560	1.34	0.92
3	Detection wavelength (±2 nm)	250 nm	299 nm	5589	1.08	1.11
4			303 nm	6253	1.22	1.24
5	Mobile phase composition (±5%)	Buffer: methanol (55:45)	Buffer: methanol (57.8:42.2)	6014	0.95	1.35
6			Buffer: methanol (52.2:47.8)	5244	1.17	1.68

CONCLUSION

In this present study, an attempt has been made to develop RP-HPLC method for the determination of Linezolid in gel dosage form. The results of the validation process showed that the proposed method is authenticated and found within predetermined limits, and fitness for purpose. It can be seen that the proposed procedure have good precision and accuracy. Results of the analysis of pharmaceutical formulations revealed that proposed methods are suitable for their analysis with virtually no interference of the usual additives present in the pharmaceutical formulations. Hence, this method can easily and conveniently adopt for routine quality control analysis of Linezolid in linezolid gel formulation.

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CONFLICT OF INTERESTS

Declared None

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