International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

A validated RP- HPLC method for estimation of linezolid in linezolid infusion.

Gaurav Gaur[1*],
Prof.vipin kukkar[1], Ramaniranjan Singh[1]

[1]Department Of Quality Assurance,Seth G.L.Bihani S.D. College Of Technical Education, Sriganganagar, Rajasthan, India

ABSTRACT
This paper presents a RP-HPLC method for the estimation of linezolid in linezolid infusions. The process was carried out on 150×4.6 mm µ, c18, bds, thermohypersil column using the mobile phase consisting of orthophosphoric buffer (pH3.4) and acetonitrile in ratio of 80:20 at flow rate of 1 ml/min; wavelength was fixed at 251 nm. Linezolid Retention time for linezolid was found to be 6.2 minute. The proposed method was found to be simple, precise, accurate and rapid for determination of linezolid in pharmaceutical infusion. The mobile phase is simple to prepare and Economical. Hence, it can be easily and conveniently adopted for routine analysis of linezolid in pharmaceutical infusion formulation.

Key words: Linezolid, RP-HPLC Estimation.

Correspondence to Author

Gaurav Gaur

Department Of Quality Assurance,Seth G.L.Bihani S.D. College Of Technical Education, Sriganganagar, Rajasthan, India

Email: gaurpharma@rediffmail.com

INTRODUCTION

Linezolid is from the oxazolidinone class of antibiotics and has shown good activity against Gram-positive micro-organisms and mycobacteria. It inhibits the formation of 70S subunits in bacterial ribosomes, which are essential components in the translation procedure. Because of this unique mechanism of action, linezolid can be used for the treatment of resistant micro-organisms, including meticillin-resistant Staphylococcus aureus, vancomycin- resistant enterococci and multidrug resistant Mycobacterium tuberculosis.

From literature survey it was found that various methods have been reported for the drug linezolid in various biological fluid & various oral formulation, but no HPLC methods were reported for pharmaceutical large volume parenteral dosage form so far .The present work
describes a simple, precise, and accurate reverse phase HPLC method for simultaneous estimation of linezolid in large volume parenteral.

EXPERIMENTAL

Linezolid working standard obtained from Venus

remedies limited, Acetonitrile HPLC grade, Merck India limited, Orthophosphoric acid, S.D. Himedia & Water for Injection is used throughout the experimental work.

INSTRUMENTATION

Quantitative HPLC was performed on an isocratic HPLC of waters 2996, using automatic injection loop and detector PDA W2996. The output – signal was monitored and integrated by empower 2 software.

Chromatographic condition:

The process was carried out on 150×4.6 mm µ, c18, bds, thermohypersil column using the mobile phase consisting of orthophosphoric buffer (pH3.4) and acetonitrile in ratio of 80:20 at flow rate of 1 ml/min, wavelength was fixed at 251 nm .The mobile phase was filtered through 0.2µ membrane filter and degassed.

Preparation of solution:

Weigh accurately 40 mg of Linezolid working standard & transfer to 100 ml volumetric flask ,add 50 ml of diluents and sonicate to dissolve make up volume with diluents up to mark. Pipette 5 ml of standard stock solution to 25 ml volumetric flask and make up volume with diluent up to mark.

For preparation of sample working solution, Pipette out 10 ml of sample (2mg/Ml Linezolid Infusion) in 50 ml volumetric flask, make up volume to the mark with diluents then transfer 5 ml of this solution in 25 ml volumetric flask make up volume to mark with mobile phase.

20µl of solution was injected into HPLC system to obtain chromatogram for standard drug solution and sample solution.

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded then 5 replicate of standard solution was injected and the chromatogram was recorded. The retention time of chromatogram was found to be 6.2.This procedure was repeated for the sample solution and assay value were calculated

METHOD VALIDATION

Linearity and range of method was determined on standard solution by analyzing 75% to 125 % of test concentration, and the calibration curve was plotted using AUC versus concentration of Standard solution. Accuracy of method was ascertained by recovery study by adding a known Amount of standard drug in placebo of formulation and analyzing method by proposed method. Precision was studied by analyzing six replicates of standard solution. Specificity was carried out by injecting placebo solution. Robustness of method was evaluated by performing the assay with variations in pH, flow rate and stability of analytical solution was determined. The chromatographic parameters were also validated by system suitability studies which were carried out on freshly prepared standard stock solution.

RESULTS AND DISCUSSION

The typical chromatogram obtained from the formulation is presented in Figure 1. The Retention time for linezolid was found to be 6.2 minutes.USP plate count and USP tailing was to be 10236 and 1.08 respectively. Linearity and range of method was determined on standard solution by analyzing 75% to 125 % of test concentration. Correlation coefficient was found to be 0.9997. Accuracy of the method was ascertained by recovery study. The concentration of standard spiked to the sample was 75% - 125% of the assay level. Recovery data from the study is reported in Table 1 .The method was found to be accurate with percent recoveries between 99.5% and 100.67%. There was good repeatability of proposed method with percentage RSD 0.51. The results of specificity studies indicated no interference from excipients and mobile phase; the peak response was due to linezolid only. The solution was was found to be stable at room temperature for 24 hours.

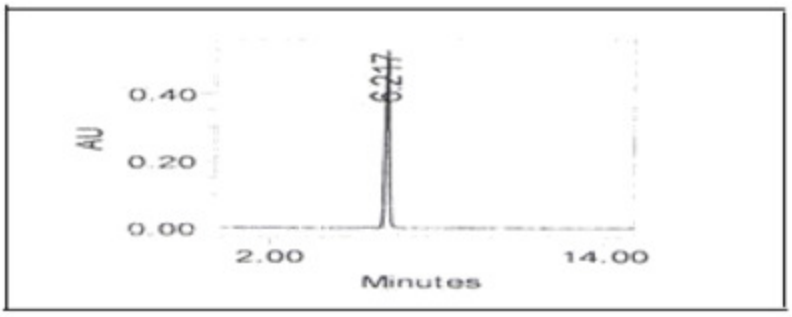

Figure 1. Typical chromatogram of the sample solution

Table 1 recovery study

Sample ID	Statistical analysis Amount recovered*
S1- 75%	Mean*=100.67% ± 0.152
S2-100%	Mean*=99.5% ± 0.14
S3-125%	Mean*=100.69% ± 0.27

**Each value is average of three determinations ± SD*

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of linezolid in pharmaceutical infusion formulation. The mobile phase is simple to prepare and Economical. The sample recoveries in all formulations were in good agreement with their Respective label claims. Hence, it can be easily and conveniently adopted for routine analysis of linezolid in pharmaceutical infusion formulation.

ACKNOWLEDGEMENTS

The authors are grateful to P.Ramaniranjan, Qualitry control manager, Venus remedies limited, panchkula, for providing facilities for research work.

REFERENCES

1. 1. LM., Li, J., Nation, R.L. and Rayner, C.R. 2006, 'High-performance liquid chromatographic method for simple and rapid determination of linezolid in human plasma' biomedical chromatography, vol.20, Issue 8, Pp. 782-786
2. 2. Buerger,C, Joukhadar,C, Muller,M. And Kloft,C.2003 , 'Development of a liquid chromatography method for the determination of linezolid and its application to in vitro and human micro dialysis samples', journal of chromatography, vol .796,issue 1, Pp. 155-164
3. 3. Validation Of Analytical Procedures: Text And Methodology Q2 (R1)' ICH Harmonized Tripartite Guideline, 2005, pp 1-7
4. 4. Snyder, L.R., Kirkland, J.J., and Glajch, J.L. (2003) Practical HPLC Method Development, 2nd ed., Wiley-Interscience
5. 5. Tobin, C. M., Sunderland, J. , Lovering, A. and MacGowan, A. P. 2003, 'A high performance liquid chromatography (HPLC) assay for linezolid in continuous ambulatory peritoneal dialysis fluid (CAPDF)', Journal of antimicrobial chemotherpy , vol.51 ,issue -4, Pp. 1041 -1042
6. 6. Tobin, C. M., Sunderland, J. , White, L. O. and MacGowan A. P. 2001, 'A simple, isocratic high-performance liquid chromatography assay for linezolid in human serum',Journal of Antimicrobial Chemotherapy, Volume48, Issue5, Pp. 605-608.

7. 7. Toutain, J, Boselli, E, Djabarouti, S, Allaouchiche, B, Xuereb, F, Bernadou, JM, Ba B, Saux and MC,Breilh, D 2004, 'Determination of linezolid in plasma and bronchoalveolar detection using a fully automated ,extraction method' journal of chromatography B pp. 145-50.
8. 8. Traunmüller, F, Mauric, O, Popovic, M. and Joukhadar, C., 'Spectrophotometric and HPLC Determination of Linezolid in Presence of Its Alkaline-Induced Degradation Products and in Pharmaceutical Tablets' Analytical letters,vol.36,issue 6,page no.1147-1161

*****