



Received on 13 April, 2011; received in revised form 21 May, 2011; accepted 26 June, 2011

ESTIMATION OF TEICOPLANIN IN PARENTERALS BY RP-HPLC

Ravi Pratap Pulla*¹, B. S. Sastry², Y. Rajendra Prasad³ and N. Appala Raju⁴

Department of Pharmaceutical Chemistry, SSJ College of Pharmacy¹, V.N.Pally, Gandipet, Hyderabad-500 075, Andhra Pradesh, India

Department of Pharmaceutical Chemistry, Principal & Professor, Medak Institute of Technology-Pharmacy², Kothapet (V), Near Narsapur, Shivampet, Medak District- 502 334, Andhra Pradesh, India

Department of Pharmaceutical Chemistry, Professor, Andhra University- College of Pharmaceutical Sciences³, Peda Waltair, Visakhapatnam- 530 003, Andhra Pradesh, India

Department of Pharmaceutical Chemistry, Associate Professor, Sultan-UI-Uloom College of Pharmacy⁴, Mount Pleasant, Road No# 3, Banjara Hills, Hyderabad-500 034, Andhra Pradesh, India

ABSTRACT

Keywords:

Teicoplanin,
RP-HPLC,
Estimation,
Parenterals

Correspondence to Author:

Ravi Pratap Pulla

Department of Pharmaceutical Chemistry,
SSJ College of Pharmacy, V.N.Pally,
Gandipet, Hyderabad-500 075, Andhra
Pradesh, India

A simple, precise, rapid and accurate Reverse Phase HPLC method was developed for the estimation of Teicoplanin in Parenteral dosage form. An Xterra RP C18, 250x4.6 mm, column with 5 μ m particle size and the mobile phase consisting of 0.02M Potassium Dihydrogen Orthophosphate + 0.02M Dipotassium Hydrogen Orthophosphate + 0.5% Triethyl Amine in water pH: 3.2 adjusted with Orthophosphoric Acid: Acetonitrile (40:50). Acetonitrile in the isocratic mode was used. The flow rate was 0.5 ml/min and the effluents were monitored at 210 nm. The retention time was 4.178 min. The detector response was linear in the concentration of 24.1-289.2 mcg/mL for Teicoplanin, the respective linear regression equation being $Y (-242937.9235) = 71026.949x + 174544.8363$ for Teicoplanin. The Limit of Detection (LOD) and The Limit of Quantification (LOQ) were 1.205 mcg/mL and 3.615 mcg/mL respectively for Teicoplanin. The percentage assay of Teicoplanin was 99.64 %. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Teicoplanin in bulk drug and in its pharmaceutical dosage forms.

INTRODUCTION: Teicoplanin, chemically is Ristomycin A 34- O- [2-(acetylamino)- 2- deoxy-.beta.- D- glucopyranosyl]- 22, 31- dichloro- 7- demethyl- 64- O- demethyl- 19- deoxy- 56- O-[2- deoxy- 2- [(8- methyl- 1-oxononyl)amino]-.beta.- D- glucopyranosyl]- 42- O- .alpha.- D- mannopyranosyl (**figure 1**). The Empirical formula is $C_{88}H_{97}Cl_2N_9O_{33}$ and the Molecular weight is 1879.7 g/mol. Teicoplanin is an antibiotic used in the prophylaxis and treatment of serious infections caused by Gram-positive bacteria, including methicillin-

resistant *Staphylococcus aureus* and *Enterococcus faecalis*. It is a glycopeptide antibiotic extracted from *Actinoplanes teichomyceticus*, with a similar spectrum of activity¹ to vancomycin. Its mechanism of action is to inhibit bacterial cell wall synthesis². It works by stopping the bacteria making their cell walls and by disrupting their cell membranes³. Together, the cell wall and membrane form a barrier between the bacterial cell contents and the external environment. By disrupting this barrier, Teicoplanin kills the bacteria

that are causing the infection⁴⁻⁹. Its strength is considered to be due to the length of the hydrocarbon chain. Literature survey reveals a few chromatographic methods to determine the Teicoplanin in Parenteral dosage form and in biological fluids. So far, no assay methods by liquid chromatography were reported for the estimation of Teicoplanin in pharmaceutical

dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of Teicoplanin in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reverse-phase HPLC method for the estimation of Teicoplanin in bulk drug samples and in pharmaceutical dosage form.

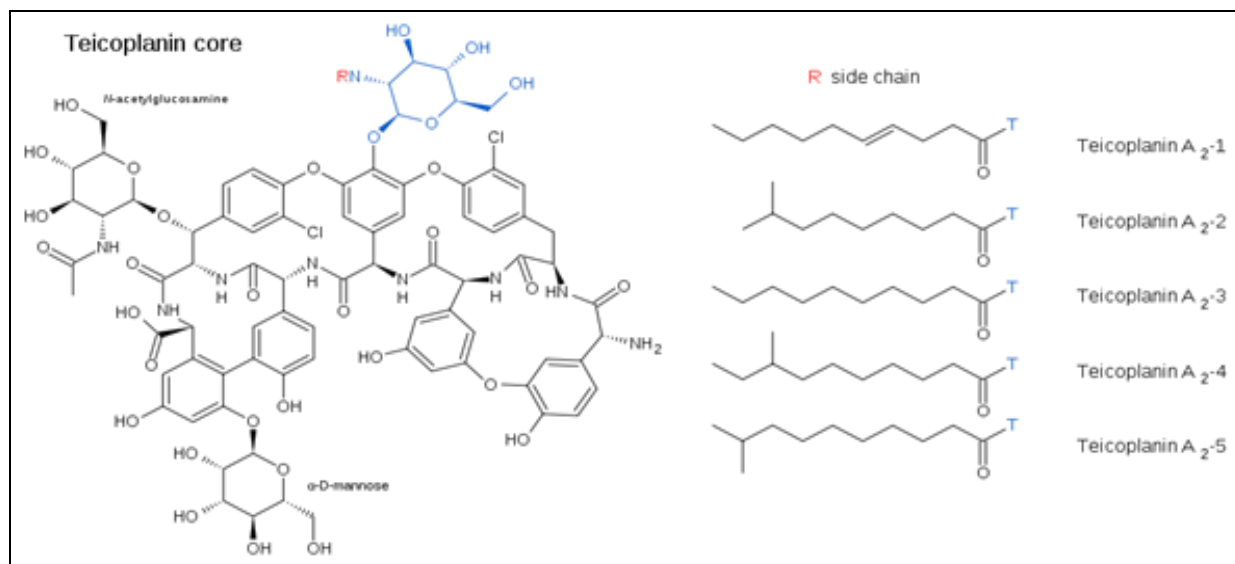


FIG. 1: TEICOPLANIN

EXPERIMENTAL:

Materials and Methods: Teicoplanin was obtained as a gift sample from Cipla Limited, Mumbai. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available Vial Ticocin[®] 200 mg, Cipla Limited was procured from local market.

Instrument: Quantitative HPLC was performed on Liquid Chromatograph, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 10 μ l, and 2693 pump. An Xterra, RP-C18 column (250x4.6 mm i.d; particle size 5 μ m) was used. The HPLC system was equipped with Empower Software.

HPLC Conditions: The contents of the mobile phase were 0.02M Potassium Dihydrogen Orthophosphate + 0.02M Dipotassium Hydrogen Orthophosphate + 0.5% Triethyl Amine in water pH: 3.2 adjusted with Orthophosphoric Acid: Acetonitrile (40:50). Acetonitrile in the isocratic mode has been used. They were filtered before use, through a 0.45 μ m membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 0.5

ml/min. The run time was set at 30.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 210 nm.

Preparation of Standard Stock solution: A standard stock solution of the drug was prepared by dissolving 241 mg of Teicoplanin in 100 ml volumetric flask containing 30 ml of diluents 0.02M Potassium Dihydrogen Orthophosphate + 0.02M Dipotassium Hydrogen Orthophosphate + 0.5% Triethyl Amine in water pH: 3.2 adjusted with Orthophosphoric Acid: Acetonitrile (40:50), sonicated for about 15 min and then made up to 100 ml with Water: Acetonitrile to get standard stock solution of 2.41 mg/mL of Teicoplanin.

Working Standard solution: 5 ml of the above stock solution was taken in 50 ml volumetric flask and made up to 50 ml with diluent, Water: Acetonitrile to get a concentration of 241 μ g/ml of Teicoplanin.

Preparation of Sample solution: Twenty Vials (Ticocin® 200 mg, Cipla Limited, Mumbai) were taken and the contents were emptied and transferred into volumetric flask containing 20 ml of the diluent and diluted upto 100 ml to get the stock solution 2.41 µg/ml. Take this 5 ml of this stock solution and further diluted upto 50 ml to get the working sample solution of 241 µg/ml.

Linearity: Aliquot of standard Teicoplanin stock solution was taken in a different 10 ml volumetric flask and diluted up to the mark with the mobile phase such that the final concentration of Teicoplanin was in the range of 24.1-289.2 µg/ml. Each of the drug solution (10 µL) was injected three times into the column, and the peak area and retention time were recorded. Evaluation was performed with PDA detector at 210 nm and a calibration graph was obtained by plotting peak area versus concentration of Teicoplanin (**Figure 2**).

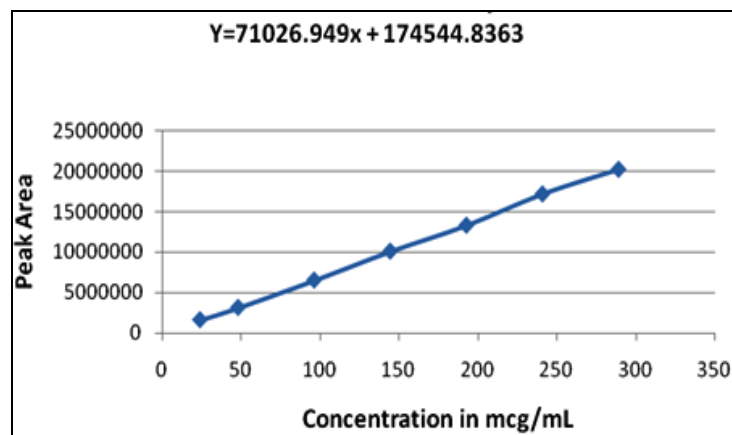


FIG. 3: CALIBRATION CURVE OF TEICOPLANIN BY RP-HPLC

The plot of peak areas of sample against respective concentration of Teicoplanin was found to be linear in the range of 24.1-289.2 µg/ml with correlation coefficient of 0.9995. Linear regression least square fit data obtained from the measurements are given in **Table 1**. The respective linear regression equation being $Y (-242937.9235) = 71026.949x + 174544.8363$ for Teicoplanin. The regression characteristics, such as slope, intercept, and %RSD was calculated for this method and given in **Table 1**.

TABLE 1: LINEAR REGRESSION DATA OF CALIBRATION CURVE

Parameter	Teicoplanin
Concentration range (µg/ml)	24.1-289.2
Slope (m)	71026.949
Intercept (b)	-242937.9235
Correlation coefficient	0.9995
% RSD	0.42

Assay: 10 µl of sample solution (Ticocin® Vial 200 mg, Cipla Limited) was injected into the injector of liquid chromatograph. The retention time was found to be 4.178 min for Teicoplanin. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in **Table 2**.

Recovery Studies: Accuracy was determined by recovery studies of Teicoplanin. A known amount of standard was added to the preanalyzed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in **Table 2**. The study was done at three different concentration levels.

TABLE 2: ASSAY & RECOVERY OF TEICOPLANIN IN PARENTERAL DOSAGE FORM

Tablet Formulation	Amount Claim (mg/Vial)	Amount Obtained (mg)* by proposed method	** % Recovery by the Proposed method
1	200	196.71	91.00
2	200	197.27	95.30
3	200	199.54	111.30

* Average of three determinations; ** after spiking the sample

RESULTS AND DISCUSSION: The system suitability tests were carried out on freshly prepared standard stock solutions of Teicoplanin. Parameters that were studied to evaluate the suitability of the system are given in **Table 3**.

TABLE 3: VALIDATION SUMMARY: SYSTEM SUITABILITY

Parameter	Teicoplanin
Theoretical Plates (N)	-----
Tailing factor	1.81
Retention time (Minutes)	4.178
Resolution	-----
% Peak Area	99.93
LOD (µg/ml)	1.205
LOQ (µg/ml)	3.615

Limit of Detection (LOD) and Limit of Quantification (LOQ): The Limit of Detection (LOD) and The Limit of Quantification (LOQ) were 1.205 mcg and 3.615 mcg respectively for Teicoplanin. From the typical chromatogram of Teicoplanin as shown in **Figure 3**, it was found that the retention time was found to be 4.178 min. A mixture of 0.02M Potassium Dihydrogen

Orthophosphate + 0.02M Dipotassium Hydrogen Orthophosphate + 0.5% Triethyl Amine in water pH: 3.2 adjusted with Orthophosphoric Acid: Acetonitrile (40:50) in the isocratic mode, was found to be the most suitable as mobile phase to obtain the peaks well defined and free from tailing.

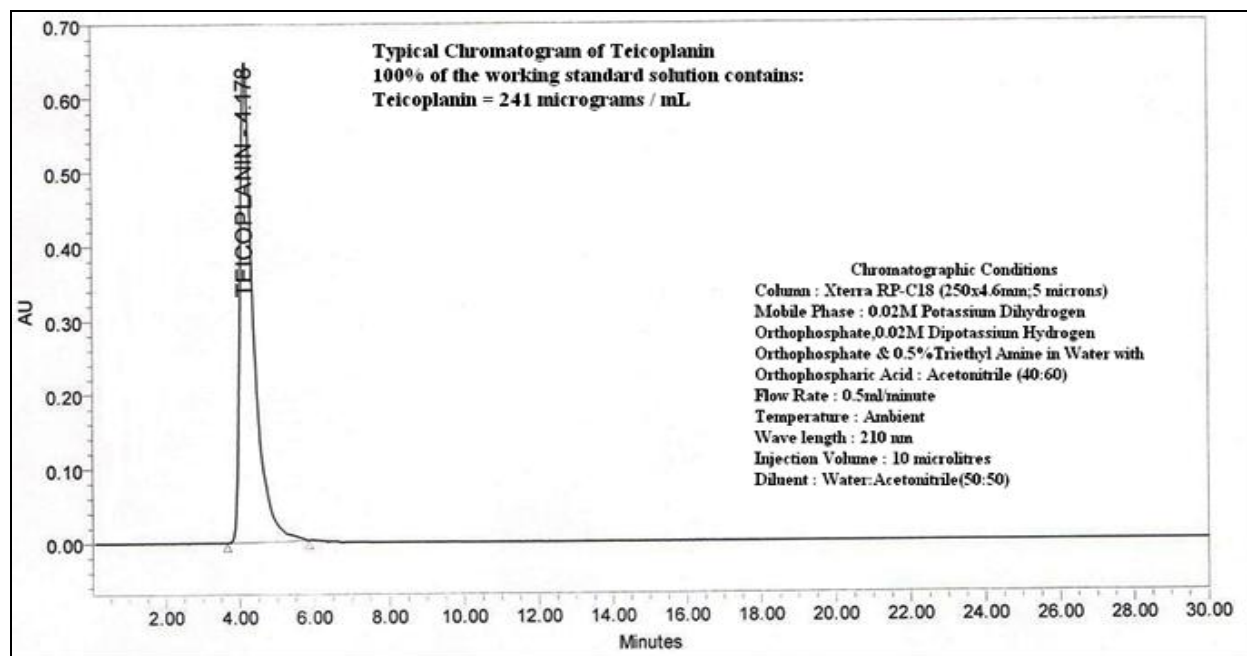


FIG. 3: TYPICAL CHROMATOGRAM OF TEICOPLANIN BY RP-HPLC

In the present developed HPLC method, the standard and sample preparation required less time and no tedious extractions were involved. A good linear relationship ($r=0.9995$) was observed between the concentration range of 24.1-289.2 $\mu\text{g/ml}$ for Teicoplanin. Low values of standard deviation are indicative of the high precision of the method. The assay of Teicoplanin vials was found to be 99.64 %. From the recovery studies it was found that about 99.2% of Teicoplanin was recovered which indicates high accuracy of the method.

The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and parenteral dosage forms of Teicoplanin within a short analysis time.

ACKNOWLEDGEMENTS: The authors are grateful to M/s Cipla Limited, Mumbai for the supply of Teicoplanin as a gift sample.

REFERENCES:

- de Lalla F, Nicolin R, Rinaldi E, Scarpellini P, Rigoli R, Manfrin V, Tramarin A (1992). "Prospective study of oral teicoplanin versus oral vancomycin for therapy of pseudomembranous colitis and Clostridium difficile-associated diarrhea." *Antimicrob Agents Chemother* 36 (10): 2192-6.
- Gilpin M, Milner P (1997). Resisting changes -- Over the past 40 years the glycopeptide antibiotics have played a crucial role in treating bacterial infections. But how long can it continue?. Royal Society of Chemistry. - includes picture of Teicoplanin's structure.
- Bernareggi A, Borghi A, Borgonovi M, Cavenaghi L, Ferrari P, Vékey K, Zanol M, Zerilli L (1992). "Teicoplanin metabolism in human". *Antimicrob Agents Chemother* 36 (8): 1744-9.
- Jadeja, L., et al. 1983. *Antimicrob. Agents Chemother.* 24: 145-146.
- Verbist, L., et al. 1984. *Antimicrob. Agents Chemother.* 26: 881-886
- Babul, N. and Pasko, M. 1988. *Drug Intell Clin Pharm.* 22: 218-226
- Campoli-Richards, D.M., et al. 1990. *Drugs.* 40: 449-486.
- Teichomycins, new antibiotics from Actinoplanes teichomyceticus nov. sp. Parenti F. et al., *J. Antibiotics* 1978, 31, 276.
- Teicoplanin. Goldstein B. P. et al. In: *Glycopeptide antibiotics* edited by Nagarajan R. Dekker New York. 1994, 273.