Research Article

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DETERMINATION OF VANCOMYCIN BY USING RP-HPLC METHOD IN PHARMACEUTICAL PREPARATIONS

K.Nirmala and R. Ramesh Raju*

Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India

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***Corresponding author**

E-mail: rrraju1@gmail.com

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ABSTRACT

A novel, simple and economic reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the estimation of vancomycin in tablet dosage forms with high precision and accuracy. Sample separation was achieved on C18 column (250 X 4.6mm i.d., 5µm) in isocratic mode using methanol, Acetonitrile (ACN) and 0.1% Ortho phosphoric acid (OPA) in the ratio (25:72:3) (v/v/v) as mobile phase, pumped in to the column at flow rate of 1 ml/min and the detection of eluent from the column was carried out using variable wavelength detector at 229 nm. The total run time was 7 min and the column was maintained at ambient temperature. The retention time of Vancomycin was 2.822 min. The standard curves were linear over the concentration range of 2-12 µg/ml and the LOD and LOQ values for Vancomycin were 0.1 and 0.03 respectively. The recovery was found to be 99 percent and the % RSD of intraday and inter day precision was found less than 2%, respectively. The percentage amount of two different marketed tablet formulation of Vancomycin was found to be 99.5%.The method was validated as per ICH (International council of Hormonisation) guidelines. Validation studies demonstrated that the proposed RP-HPLC method is simple, specific, rapid, reliable and reproducible. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the routine quality control analysis of Vancomycin in tablet dosage forms.

Keywords: Vancomycin, RP-HPLC, ICH guidelines, Validation and C18column.

INTRODUCTION

Vancomycin was first isolated in 1953 by Edmund Kornfeld from a soil sample collected from the interior jungles of Borneo[1]. *Amycolatopsis orientalis* is main constituent of vancomycin and it plays the key role for treatment[2]. The original indication for vancomycin was for the treatment of pencillin resistant *Staphylococcus aureus*[2,3]. Vancomycin is a glycopeptides antibiotic being isolated from both *Steptomyces orientalis* and *Nocardia lurida*. It was introduced in 1956 because of its strong bactericidal activity against many gram-positive bacteria, particularly *Staphylococus aureus*. Because of its toxicity, Vancomycin was relegated to the role of alternate therapy when antibiotics such as methicillin became available[4,5,6].

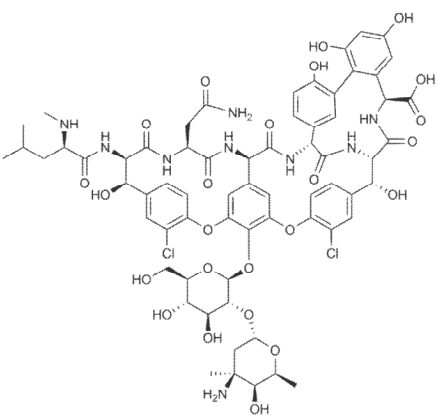


Figure 1: Structure of Vancomycin

Molecular formula of vancomycin is $C_{66}H_{75}Cl_2N_9O_{24}$ and molecular weight is 1449.3 $g.mol^{-1}$. Structure of vancomycin is given in Figure 1. Vancomyicin is used for full time treatment for the severe infection and susceptible strains due to methicillin resistant staphylococci (MRSA) virus, the increasing number of methicillin-resistant isolates of *S.aureus*, *Staphylococcus epidermidis*[7-10] and *Streptococcus pneumoniae*[11]. Similar to problems of treating patients allergic to beta-lactam antibiotics, led to the rehabilitation of vancomycin[7,9,12]. In addition, it seems that much of the toxicity was due to impure preparations[9]. It is indicated for penicillin allergic patients, especially those who cannot receive or have failed to respond to other drugs including penicillins or cephalosporins. Vancomycin is not active *In-vitro* against Gram negative bacilli, mycobacteria and fungi, bactericidal action results inhibition of cell wall biosynthesis and also alters the bacterial cell membrane permeability and RNA synthesis. There is no cross resistance between vancomycin and other antibiotics. Many microbiological assay procedures lasting from 24 to 48 h have been developed for monitoring vancomycin[11,13,14]. Radioimmunoassay (RIA) and fluorescent polarization immunoassay (FPIA) have been developed[15,16] and two chromatographic procedures have also been described[17,18,19].The present report describes a simple, accurate, and very sensitive high-pressure liquid chromatographic assay (HPLC) and it was used commonly in hospitals.

MATERIAL AND METHODS

Drugs and Chemicals

Working standard of vancomycin was obtained from well reputed research laboratories**.** HPLC grade water, methanol, 0.1% Ortho Phosphoric Acid (OPA) was purchased from E. Merck (Mumbai, India).

Instrumentation

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6 mm, Electronic balance-DENVER (SI234), a manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used to analysis of the HPLC PEAK and UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance of drug.

Determination of wavelength of maximum absorbance

The standard solutions of Vancomycin were scanned in the range of 200-400nm against mobile phase as a blank.Vancomycin showed maximum absorbance at 229 nm.So the wavelength selected for the determination of Vancomycin was 229nm.

Chromatographic equipment and conditions

The development and validation of the assay was performed on α Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, manual injector Rheodyne valve with 20µL fixed loop, PEAK LC software was used.

The mobile phase consisting of Methanol, acetonitrile (HPLC grade) and 0.1% OPA were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 25:72:3 (v/v/v) into the column at a flow rate of 1ml/min. The detection was monitored at 229 nm and the run time was 7 min. The volume of injection loop was 20 µl. Prior to injection of the drug solution the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature

Standard and sample solutions

Standard solution was prepared by adding 10 mg amount of Vancomycin. Drug was used accurately weighed and dissolved in 10 ml mobile phase in volumetric flask to get 1000ppm and it is standard solution. From the standard solution on serial dilutions 100ppm concentration was prepared. 20 tablets of Vancomycin were grinded to a fine, unifom size powder. From this powder approximately 25ml mobile phase were added then the solution was sonicated for 30min.The flask was filled to volume with mobile phase and mixed well and filtered. After filtration an amount of the solution was diluted with mobile phase to concentration up to 6ppm.

RESULT AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation, the quality of the chromatography was monitored by applying the following system suitability tests, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were, tailing factor ≤2.0 and theoretical plates >2000. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table 1 and standard chromatogram was given in Figure 2.

Validation

Method validation was performed according to ICH (International council of Hormonisation) guidelines for specificity, range of linearity, accuracy, precision and robustness.

Table 1: Column parameters and values

Test	Result
Elution	Isocratic
A.P.I Conc	6 ppm
Mobile Phase	MeOH ::ACN:0.1% OPA (25:72:3)
pH	5.4
Column	C18
Wave Length	229nm
Flow Rate	1ml/min
Runtime	7 min
Retention Time	2.822
Area	193908
Theoretical Plates	6829
Tailing Factor	1.08
Pump Pressure	21.2 psi

Table 2: Peak area and RSD determination of system

Conc 6 ppm		Precision 1	Precision 2	
Day -1	Injection	Area-1	Area-2	
	1	193766	196035	R.S.D1 = 0.611
	2	195120	195565	
Day-2	3	196133	195151	R.S.D 2 = 0.25
	4	196495	195120	
	5	196556	195949	
	6	196983	196303	

Table 3: Range of Linearity

Conc. in ppm	Area	
2	63795	Intercept =3147
4	127610	
6	193909	Slope = 30979
8	252381	
10	318772	C.C = 0.9994
12	366713	

Table 4: Limit of Detection and Limit of Quantification

Test-4	L.O.Q	0.03 ppm
Test-5	L.O.D	0.1 ppm

Table 5: Robustness Study

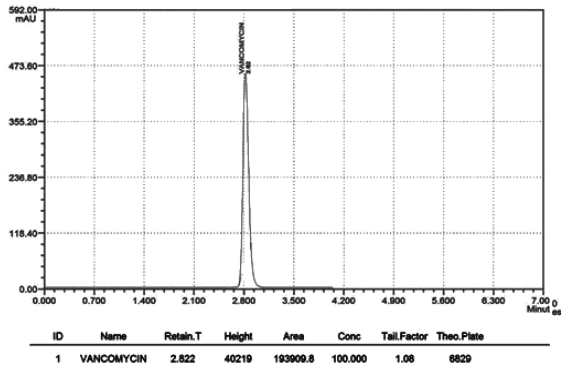
Parameter	Modification	Peak Area	% of change
M.Phase	MeOH ::ACN:0.1% OPA(30:67:3)	195492	0.816
pH	5.7	196278	1.22
Wavelength	234	196647	1.41

Table 6: Recovery Study

Recovery	Conc. of sample	Recovery	% of recovery
50%	4 ppm	3.975	99.38
100%	8 ppm	7.91	98.87
150 %	12 ppm	11.95	99.58

Table 7: Drug Estimation

Brand	Strength	Sample concentration	Amount estimated	% of drug estimated
Vancocin HCl Capsules	250mg	6.0ppm	5.97grms	99.50



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	VANCOMYCIN	2.822	40219	193909.8	100.000	1.08	6829
	Sum:		40219	193909.8	100.0000		

Figure 2: Standard Chromatogram

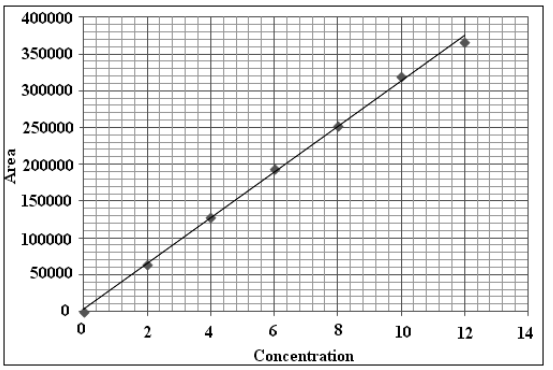


Figure 3: Linearity of Vancomycin

Accuracy

The accuracy of the HPLC method was assessed by adding known amount of drug solution to a drug solution of known concentration and subjecting the samples to the proposed HPLC method.

Precision

To study precision, six replicate standard solutions of Vancomycin (6ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table 2.

$$\% \text{ RSD} = \frac{\text{STD dev of X } 100\%}{\text{Mean}}$$

Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 2, 4, 6, 8, 10, 12, µg/ml for Vancomycin. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was (y=mx+c) where m=slope and c=intercept; y = 3147+ 30979x (r= 0.9994). Linearity values can show in Table 3.

Limit of Detection and Limit of Quantification

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.1 ppm dilution Peak was not clearly observed, based on which 1.5ppm is considered as Limit of Detection and Limit of Quantification is 0.03 ppm and the values are given in Table 4.

Robustness: Conc.:6 ppm

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored such as retention time, area, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describes the Robustness and given in the Table 5.

Recovery

Recovery test was performed at 3 different concentrations i.e., 4ppm, 8ppm and 12ppm. The accuracy was expressed in terms of recovery and calculated by multiplying the ratio of measured drug concentration compare to the expected drug concentration, so as to give the percentage of recovery. Results are given in Table 6.

Formulation

(Table 7)

CONCLUSION

The proposed method for the assay of Vancomycin in tablets or capsules is very simple and rapid. It should be emphasized that it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it and to evaluate the stability of pharmaceutical formulations.

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