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DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING METHOD BY ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF VANCOMYCIN DRUG IN VANCOMYCIN INJECTION

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ABSTRACT

The Analytical Method Development and Validation of Vancomycin drug in Vancomycin Injection is particularly designed to intended purpose of Pharmaceutical Industries. A simple, precise, rapid and accurate reverse phase UPLC method was developed for the estimation of Vancomycin drug in Vancomycin Injection. Waters xterra RP18, 150mm x 3.9mm, 5µm with mobile phase consisting of monobasic ammonium phosphate buffer 0.05M and acetonitrile were mixed in the ratio 90:10 (v/v) respectively and degassed for about 10 minutes. Isocratic mode of chromatography technique was used. The flow rate was 0.7 mL/min and the eluents were monitored at 230 nm. The retention time of the Vancomycin peak is about 3.5 minutes. The detector response was linear in the concentration of 5.03 to 15.08 mcg/mL, Y-intercept is -49657 and slop is 754605.4. The percentage assay of Vancomycin was 100%. The method was validated by determining its accuracy, precision and linearity with inline of International Council on Harmonization guidelines, ICH Q2R1.

KEYWORDS: Vancomycin Injection, Analytical Method by UPLC, Reverse Phase Chromatography, Stability Indicating, Isocratic mode of Chromatography Technique and Industrial Intended Purpose.

INTRODUCTION

Vancomycin is a Glycopeptide Antibacterial. The chemical classification of vancomycin is Glycopeptides. Vancomycin is a branched tricyclic glycosylated peptide with bactericidal activity against most organisms and bacteriostatic effect on enterococci. At a site different from that of penicillins and cephalosporins, vancomycin binds tightly to the D-alanyl-D-alanine portion of cell wall precursors, thereby interfering with bacterial cell wall synthesis. This leads to activation of bacterial autolysins that destroy the cell wall by lysis. Vancomycin may also alter the permeability of bacterial cytoplasmic membranes and may selectively inhibit RNA synthesis.

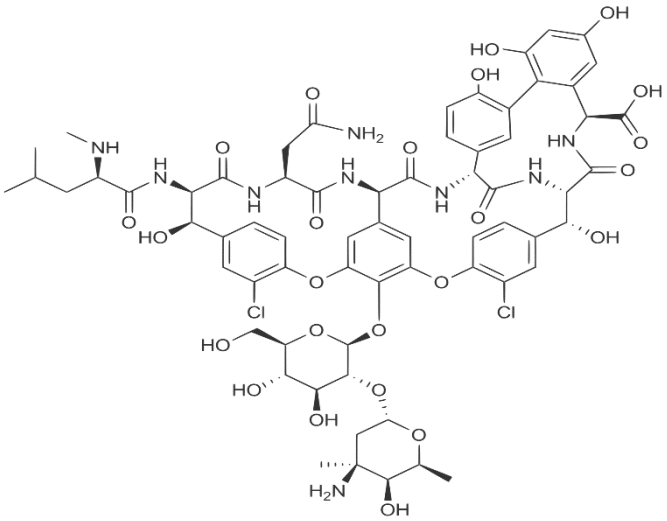


Fig. 1: Structure of Vancomycin.

The molecular formula is $C_{66}H_{75}Cl_2N_9O_{24}$

Vancomycin is an antibiotic used to treat a number of bacterial infections. It is used as the treatments of complicated skin infections, bloodstream infections, endocarditis, bone and joint infections and meningitis caused by methicillin-resistant Staphylococcus aureus. Vancomycin Injection is available in market as 5 mg/mL concentration.

In the literature, several analytical techniques like HPLC, GC, UPLC, ICP-MS, ICP-OES methods including Voltammetric and UV spectrophotometric methods have been referred for determination of Vancomycin drug in Vancomycin Injection. The main purpose of the present study was to establish relatively simple, sensitive and validated liquid chromatographic method for the determination of Vancomycin in Vancomycin Injection. The method was validated by determining its accuracy, precision and linearity as per ICH guidelines.

Experiment

Materials and Methods: Procured commercially available Vancogen 500mg/mL from Alkem Laboratories as a sample, Waters xterra RP18, 150mm x 3.9mm, 5µm HPLC column, Acetonitrile, Methanol, monobasic ammonium phosphate and HPLC grade water (Qualigens) were procured from market. Vancomycin Working standard was procured from the Centaur Laboratories Pvt. Ltd as a gift material.

Instrumentation: UPLC, Waters ACQUITY UPLC H-Class system with equipped Diode Array Detector and automatic injector with injection volume 5µl. The UPLC data was analysed with Empower-3 Software, SARTOURIUS Analytical balance with model of MSA225P-100-DA and FISHER SCIENTIFIC pH METER with model of XL15.

UPLC Conditions: The contents of mobile phase were mixture of 0.05M monobasic ammonium phosphate in Water and Acetonitrile in the ratio of 90:10 (v/v) respectively and degassed for about 10 minutes. The run time was set about 8 minutes and the column temperature was 45ºC. Prior to injection of Blank solution and sample solution, the column was equilibrated for at least 60 minutes with the mobile phase flowing through the system. The eluents were monitored at 230 nm.

Preparation of Diluent: Mixed HPLC grade water and methanol in the ratio of 90:10 (v/v).

Preparation of standard solutions (10 µg/mL): A standard stock solution of the Vancomycin drug was prepared by 10.0 mg of Vancomycin working standard was weighed and transferred into a 100 mL volumetric flask and diluted to volume with the diluent and mixed well. 10.0 mL of the above standard stock solution was pipetted into 100 mL volumetric flask, diluted to volume with diluent and mixed well.

Preparation of Sample solution (10 mcg/mL): Accurately 1.0 mL of the sample solution (Vancogen 500mg/mL) was taken and diluted with diluent up to 250 mL and mixed well. 1.0 mL of this solution was pipetted into 200 mL volumetric flask, diluted to volume with diluent and mixed well.

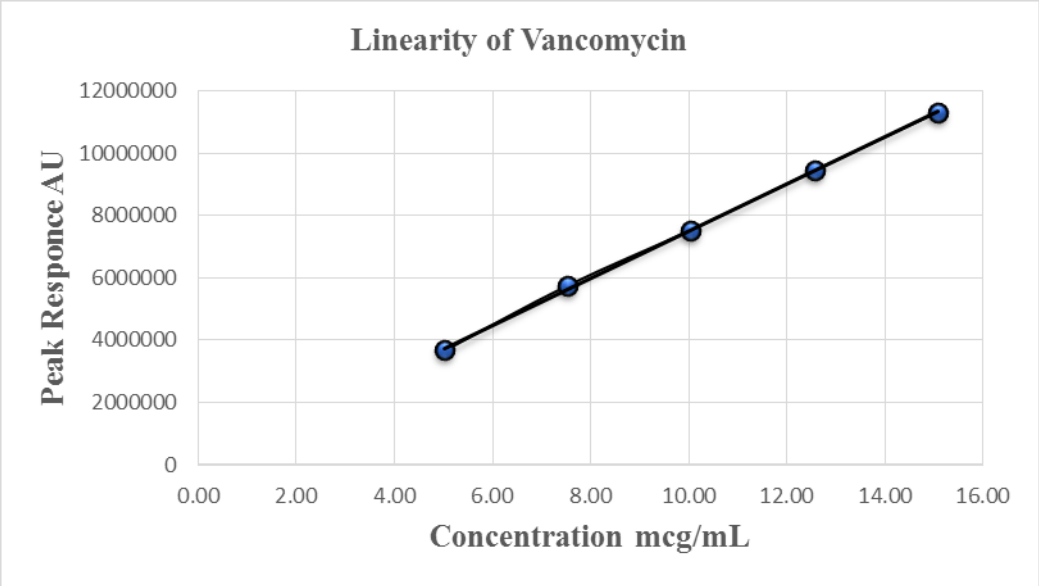
Linearity: Linearity of Detector response was established by plotting a graph of concentration (in mcg/mL) versus peak area and determining correlation coefficient, y-intercept and slope. Vancomycin linearity solutions were prepared in the concentration range from about 50% (5 mcg/mL) to about 150% (15 mcg/mL) level of the target concentration

(10 mcg/mL) and injected into the UPLC system. The detector response was found to be linear and the results were found to be within limit, refer table 1 and 2.

Table 1: Linearity data.

% of Linearity Level	Concentration (mcg/mL)	Peak Area
50	5.03	3664051
75	7.54	5746088
100	10.05	7528106
125	12.56	9431133
150	15.08	11301259
Correlation coefficient, NLT 0.997	0.999	
Y-intercept	-49657	
Slope	754605.4	

Table 2: Linear Regression of Calibration curve.



Precision studies: The precision of test method was evaluated by preparing six homogeneous sample preparations under prescribed conditions from the same sample lot and analysed as per the developed procedure within the short interval time. 5 µL of sample solutions were injected into the UPLC system, which was pre-saturated by the mobile phase and analytical column. Before injecting the sample solutions in the sample set or sample sequence, system suitability parameters were verified, which are related to UPLC system and the particular analytical method integral limitations. All the system suitability parameters were within the acceptance criteria and blank injection was represented to its good base line and chromatography. The retention time was found as 3.5 minutes for Vancomycin active moiety. The amount of drug present per each sample preparation was calculated by comparing the

peak area of the sample solution with that of the standard solution. The data are presented in table 3. Typical chromatograms of Blank solution and Vancomycin sample solution as shown in fig 2 & 3.

Table 3: Results of Precision studies.

Sample Preparations	% Assay of Vancomycin	
	Analyst-1	Analyst-2
Preparation – 1	99.9	100.4
Preparation – 2	100.1	100.3
Preparation – 3	99.8	99.9
Preparation – 4	100.1	100.4
Preparation – 5	99.6	100.5
Preparation – 6	100.3	100.8
Individual Average	99.9	100.4
Individual (% RSD), NMT 2	0.3	0.3
Overall Average	100.2	
Overall % RSD, NMT 2	0.3	

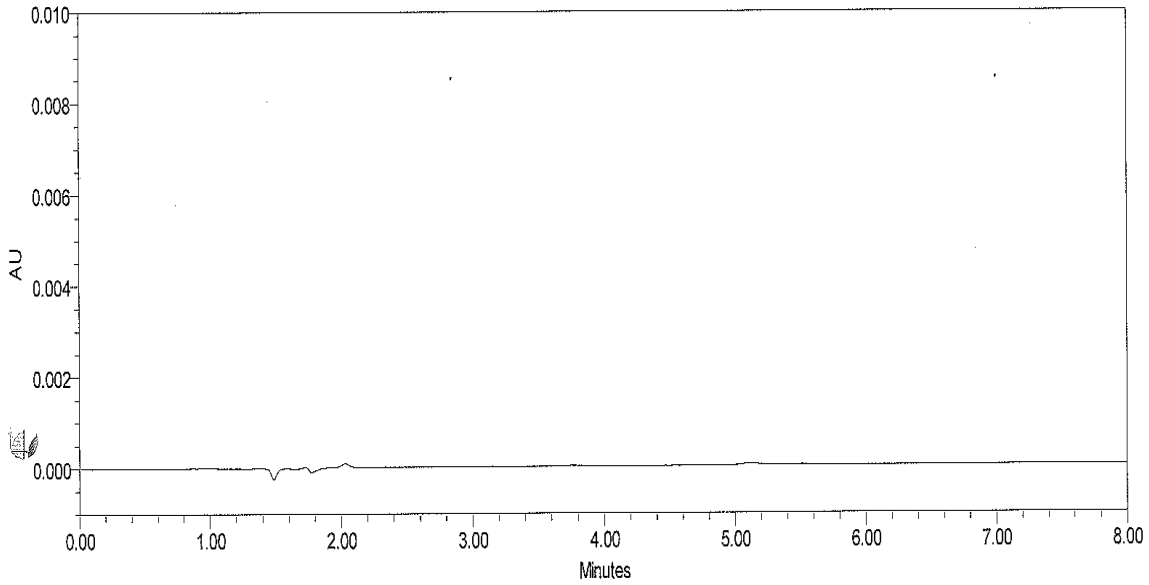


Fig. 2: Typical Chromatogram of Blank solution by UPLC.

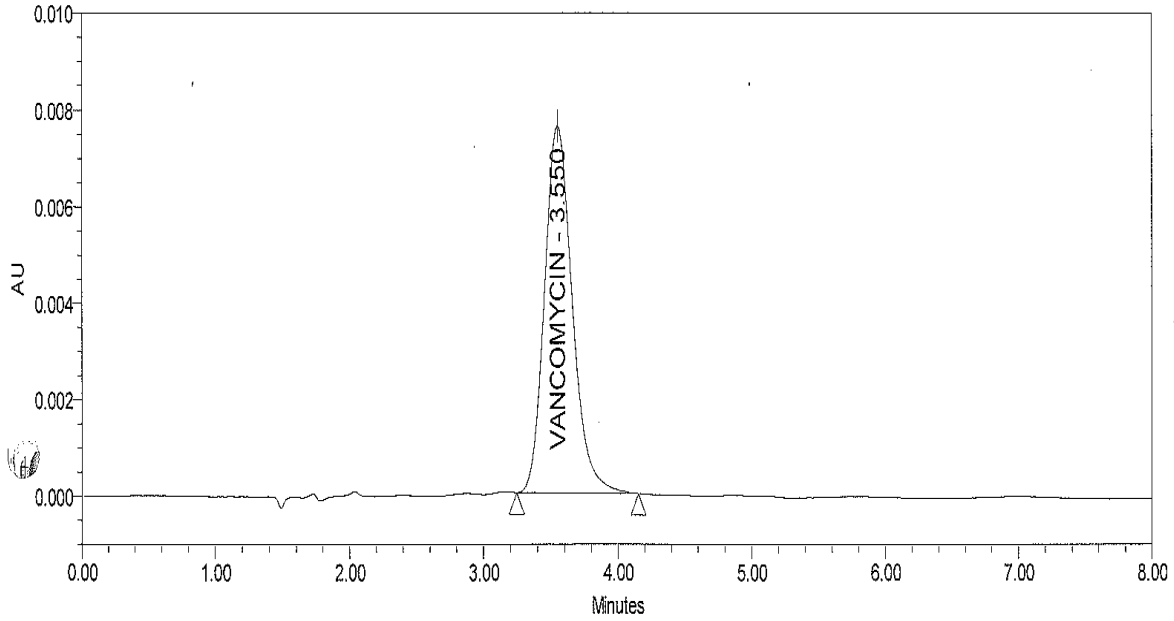


Fig. 3: Typical Chromatogram of Vancomycin sample solution by UPLC.

Recovery Studies

Accuracy is the closeness of agreement between the conventional true value and the values found. The value accepted as a conventional true value or the accepted reference value. several methods of determining accuracy are available, at this analytical method development of Vancomycin was verified by spiking method. Accuracy is often considered quantitatively expressed as measurement uncertainty. There are different approaches for measurement uncertainty estimation, but in practice the approach based on validation data is often the most convenient.

The Vancomycin drug was prepared as recovery solutions with respective concentrations of method range and injected into the UPLC system. Results of recovery study are shown in table 4. The study was done from about 50% (5 mcg/mL) to about 150% (15 mcg/mL) level of the test concentration (10 mcg/mL) at three different sample preparations for each level.

Table 4: Results of Recovery studies.

Recovery Level	Preparations	% RECOVERY			
		Injection-1	Injection-2	Mean	Overall Mean
50% Level	Preparation -1	100.3	100.6	100.2	100.3
	Preparation -2	100.6	100.5	100.4	
	Preparation -3	100.1	100.2	100.1	
100% Level	Preparation -1	100.2	100.3	100.8	100.4
	Preparation -2	100.7	100.5	100.2	
	Preparation -3	100.5	100.3	100.1	
150% Level	Preparation -1	100.3	100.2	100.1	100.3
	Preparation -2	100.4	100.5	100.6	
	Preparation -3	100.5	100.1	100.4	

System Suitability: The system suitability tests were carried out on freshly prepared standard solution of Vancomycin. The parameters studied to evaluate the suitability of the system are given in table 5.

Table 5: Validation Summary.

System suitability parameters	Observed Values	
	Analyst- 1	Analyst-2
USP Tailing factor for Vancomycin peak in standard solution (NMT 2.0)	1.1	1.1
Relative Standard Deviation for peak areas of Vancomycin from five replicate injections of standard solution (NMT 2.0%)	0.1	0.3
USP Plate count for Vancomycin peak in standard solution (NLT 2000)	16653	11235

RESULTS AND DISCUSSION

The results of the study showed that the proposed RP-UPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Vancomycin drug in Vancomycin Injection. In this present developed of UPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r2=0.999) was observed between the concentration range of 5.03 mcg/mL to 15.08 mcg/mL. Low values of standard deviation are indicative of the high precision of the method. The assay of Vancomycin Injection was found as 100%. From the recovery studies it was found that the Vancomycin was recovered which indicates high accuracy of the method. The absence of additional unknown peaks in the chromatogram indicates non-interference of the common excipients used in formulation.

CONCLUSION

Evaluated the critical process paraments of UPLC Analytical method development of Vancomycin drug in Vancomycin Injection and optimised. Analytical method validation was performed with suitable GMP and GLP guidelines. This analytical method is accurate, precise, linear and robust. The developed and validated method is exactly suitable to regular quality control testing at pharmaceutical industries.

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