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A NOVEL UV SPECTROSCOPIC METHOD DEVELOPMENT AND VALIDATION FOR DETECTION AND QUANTIFICATION OF VANCOMYCIN IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM

M.Susima*, Shaik Muneer, K.B. Chandra Sekhar, J. Komala Preethi, T. Latha, G. Koundilya, G.Ramesh
Department of Pharmaceutical Sciences, JNTUA- Oil Technological and Pharmaceutical Research Institute, Ananthapuramu-515001, A.P, India.

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ABSTRACT

The present study was aimed to develop a novel, simple, accurate, precise, robust and economic UV spectrophotometric method for the detection and quantification of vancomycin in bulk and its formulation. Quantification was carried at absorption maxima 280.50 nm using 0.01M Sodium hydroxide as diluent. The developed method was validated with accordance to ICH Q2 (R1) guidelines. Beer's law was obeyed in the concentration range of 40-240 µg/mL with correlation coefficient of 0.9991. The sensitivity was checked as the limit of detection and limit of quantification which were found to be 1.6 and 4.8 µg/mL respectively. Further studies of the accuracy and precision of the proposed method were performed using standard addition method; the mean quantitative recovery of such studies were found to be in the range 99.39 ± 0.41 to 101.24 ± 0.89 with RSD ≤ 2.0%. From the results it can be concluded that the developed method was specific, selective, precise and robust. This method could be successfully applied for analysis of vancomycin in its formulation.

Corresponding author
M.Susima
Department of Pharmaceutical Sciences,
JNTUA-OTPRI, Anantapur
A.P., India.
muneer.pharma@gmail.com

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INTRODUCTION

Vancomycin (VCN) is a glycopeptide antibiotic.1,2 Chemically it is (1S,2R,18R, 22S,25R, 28R,40S)-22-(2-amino-2-Oxoethyl)-48-[2-O-(3-amino-2,3,6-trideoxy-3-methyl-alpha-L-lyxo-hexopyranosyl)-beta-D-glucopyranosyloxy]-5,15-dichloro-2,18,32,35,37-pentahydroxy-19-[(N-methyl-D-leucyl)amino]-20,23,26,42,44-pentaoxo-7,13-dioxa-21,24,27,41,43-pentaazaocta cyclo[26.14.2.2(3,6).2(14,17).1(8,12).1(29,33).0(10,25).0(34,39)]pentaconta-3,5,8(48),9,11,14, 16,29(45),30,32,34,36,38,46,49-pentadecaene-40-carboxylicacid. Its chemical structure was shown in Figure 1 and it is used for full time treatment for the severe infection and susceptible strains due to methicillin resistant staphylococci (MRSA) virus(3-6), and *Streptococcus pneumonia*(7). It acts by inhibiting cell wall biosynthesis and also alters the bacterial cell membrane permeability and RNA synthesis.(8)

To the best of our knowledge, no reported analytically and official procedures are not available for determination of VCN in its formulation by using UV spectroscopic analysis. Therefore the main objective of this work is to develop a novel, accurate, precise and robust method for the detection and estimation of vancomycin in bulk as well as its formulation.

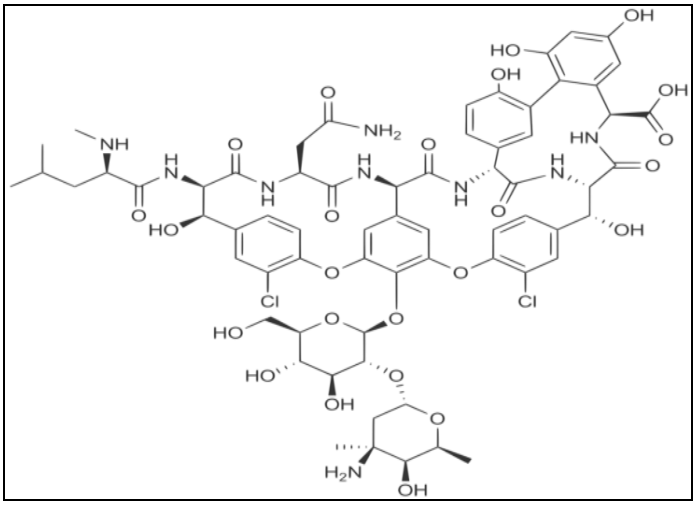

Figure 1: Structure of Vancomycin.

MATERIAL AND METHODS

Chemicals & Reagents

Analytically pure sample of vancomycin with purities greater than 99% was obtained as gift sample from Mylan Laboratories, Bangalore, Karnataka. The Vancomycin injection formulation (VANKING^{R} 500 Injection I.P. Neon Laboratories limited, Mumbai, India) for the study was purchased from local pharmacy. AR grade sodium hydroxide was purchased from Merck, Mumbai. Distilled water was prepared inhouse using MilliQ water system.

Instrumentation

A double beam UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1mm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. For scanning, the Wavelength range was selected from 400 to 200 nm with medium scanning speed. All experiments were performed at room temperature.

Preparation of standard stock solution

Stock solution was prepared by weighing about 10mg of VCN and transferred into 10ml volumetric flask (previously calibrated) and dissolve it in 5ml of solvent and sonicated for 5 min using ultrasonicator and further the volume was made upto the mark using 0.01M sodium hydroxide to get a concentration of 1mg/ml (solution A). From this various aliquots were prepared by an appropriate dilution of stock solution (solution A) with distilled water to cover wide range of the concentrations.

Selection of Analytical wavelength and calibration curve:

Standard solutions of VCN were scanned between 200-400nm in UV double beam spectrophotometer for wavelength selection and the solutions showed absorption maxima at 280.50 nm wavelength as shown in Figure 2. Hence 280.50 nm was selected as the analytical wavelength for the current study.

Stability of solution:

Prepared stock solution of VCN was stored at room temperature for 24 hours. After 24 hour, sample was rescanned and absorption maxima were noted using UV spectrometer. The absorption maxima of VCN showed at 280.50 nm and the %Relative standard deviation of absorbance was less than 1%.This shows that solution of VCN was stable in 0.01M Sodium hydroxide.

Analysis of formulation (assay of marketed formulation)

The analysis for formulation was carried by weighing an equivalent of 25 mg VCN drug into 25ml volumetric flask and the contents of the flask was thoroughly mixed and filtered by whatmann's filter paper if necessary. Further sonication was carried using ultrasonicator for 5 min to enhance solubility and finally the volume was made to upto the mark using 0.01M sodium hydroxide. From the above prepared solution various concentrations were prepared using distilled water.

Validation of developed method

Developed UV spectroscopic method was validated in terms of specificity/selectivity, precision, accuracy, linearity, LOD & LOQ, robustness according to ICH guidelines (9) discussed in following sections

Linearity and Range:

Calibration standards covering the range 40-240 µg/mL were prepared with the suitable dilution made from VCN stock solution, and analyzed in triplicate. The calibration curve was obtained by plotting the intensity of absorbance against of concentration. The slope and intercept of the calibration line were determined by linear regression using the least squares method.

Precision

Reproducibility of the method was checked by performing intra-day precision (six times a day) and inter-day precision (repeated triplicates for six consecutive days). Results are expressed in terms of standard deviation and percentage Relative standard Deviation (%RSD). The intraday and interday precision values in terms of %RSD was found to be 0.40 and 0.44 respectively.

Accuracy (% Recovery):

To check the accuracy of the developed method, recovery studies were carried out from pre-quantified sample at three different level of standard addition 50%, 100% and 150%. Percentage Recovery was the average of three determinations at each standard addition level. Percentage Recovery was found to be between 99.39%-101.24% which prove that the method were accurate. High percentage recovery values showed that the methods were free from interference of the excipients used in the formulation. The result was depicted in table 3.

LOD AND LOQ:

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. In this study LOD and LOQ were based on the standard deviation of response and the slope of corresponding curve following equation:

$$\mathbf{LOD = 3.3\ \sigma / S}$$

Where σ is standard deviation of Y-intercept and S is slope of calibration curve.

The limit of quantification (LOQ) is defined as the lowest concentration of calibration curve that can be measured with an acceptable accuracy, precision and variability. The value of LOQ was determined using following equation:

$$\mathbf{LOQ=10\ \sigma/S}$$

Robustness

Robustness was determined by performing the same proposed method on different wavelengths. The analysis showed %RSD less than 2 and indicates that the method developed is robust.

RESULTS AND DISCUSSION

Vancomycin has the zero order absorbance overlay spectra maxima at 280.50 nm. The spectra of standard and overlay spectra were shown in Figure 2 and 3. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 40-240 µg/ml with correlation coefficient (r^2) was found to be higher than 0.999. Recovery studies were carried out at three different levels i.e. 50 %, 100 %, and 150 % by adding the pure drug to the previously analysed sample. Percentage recovery for vancomycin was determined by all the methods and they were found to be under acceptance criteria which are 99.20% to 101.69 % according to ICH guidelines. The percentage recovery value indicates noninterference from excipients used in formulation. The precision was carried out as described in method. The values obtained in the repeatability (precision) shows that there is no significant difference in the precision values; hence the developed method can be used to analyze the vancomycin in its bulk and pharmaceutical dosage form formulation. The mean assay of the precision value is 99.1323%. The LOD determined as the amount drug was found to be 1.53698 µg/mL and the LOQ was determined as the lowest concentration was found to be 4.6575 µg/mL in formulation. The summary of Optical characteristics with other parameters were shown in table 5.

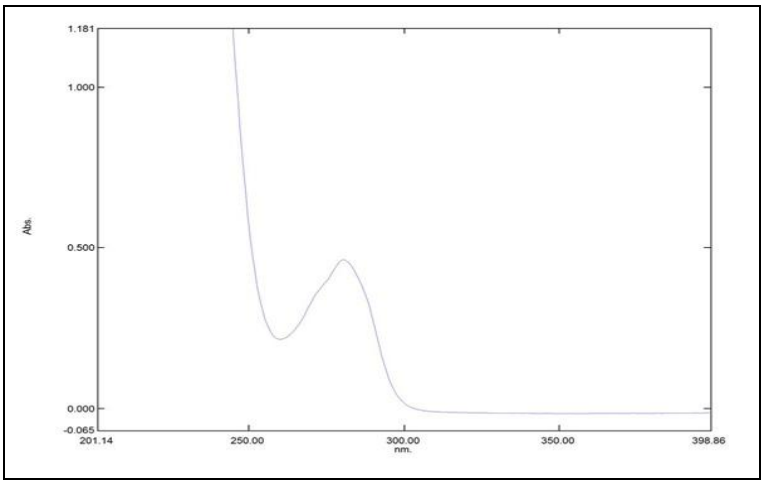

Figure 2. Standard spectrum of VCN showing maximum absorption at 280.5nm.

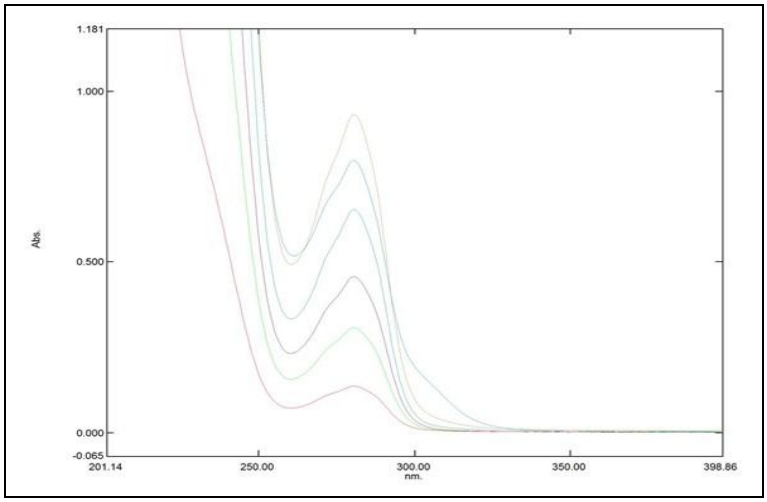

Figure 3. Overlay spectrum of VCN.

Linearity:

Calibration curve data were constructed in the range of the expected concentration of 40µg/ml to 240µg/ml. Beers laws was obeyed over this concentration range. The regression equation was found to be Y=0.004x-0.0084. The correlation coefficient(r^2) of the standard curve was found to be 0.9991. The stock solutions and working standards were made in 0.01M NaOH. Calibration curve is presented in the Figure 4.

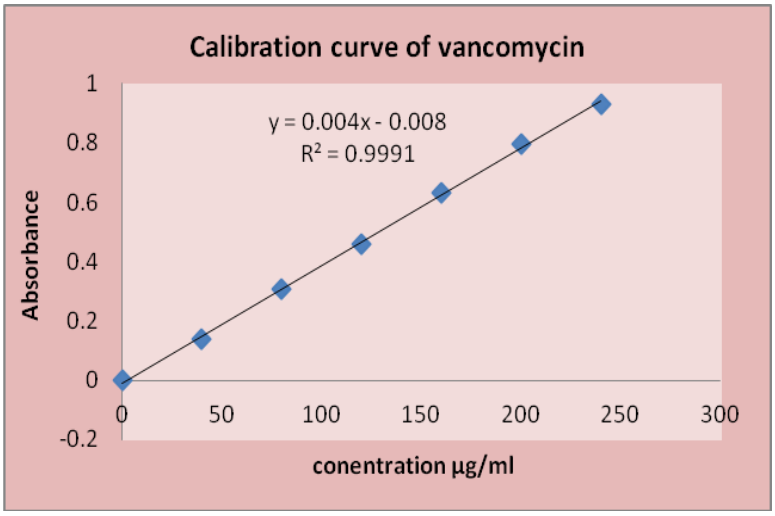

Figure 4. Calibration curve of VCN.

Accuracy:

The mean % recovery was found to be 100.42%. Hence accuracy of the method passed.(limits **:** mean % recovery must be in between 98-102%. The result was shown in table 1.

Table 1: Recovery study of Vancomycin.

Concentration level (%)	Absorbance	Amount added	Amount found	% recovery	% mean recovery
50	0.231	29.7397	30.06508	101.094	
	0.229	29.7397	29.80477	100.219	101.24
	0.234	29.7397	30.45553	102.407	
100	0.459	118.9588	119.4794	100.438	
	0.461	118.9588	120	100.875	100.656
	0.46	118.9588	119.7397	100.656	
150	0.678	267.6573	264.7289	98.9059	
	0.681	267.6573	265.9002	99.3435	99.3922
	0.685	267.6573	267.462	99.927	

Precision:

The intra-day and inter-day variation in the absorbance of drug solution was calculated in terms of coefficient of variation (C.V.). The results are furnished in table 3 .The % RSD of method precision was found to be less than 2%. Hence method passed the test and results shown in table 2.

Table 2: Precision study of Vancomycin.

S. No	Precision Study			
	Intra Day	Assay	Interday	Assay
1	0.46	99.78	0.457	99.13
2	0.457	99.13	0.463	100.43
3	0.459	99.56	0.461	100
4	0.463	100.43	0.458	99.34
5	0.461	100	0.460	99.78
6	0.459	99.56	0.461	100
Mean	0.459833	99.74333	0.46	99.78
Std Deviation	0.001863	0.404585	0.002	0.43501
% RSD	0.405232	0.405626	0.434783	0.435969

LOD & LOQ:

The limit of detection (LOD) and limit of quantification (LOQ) were calculated and was found to be 1.53698 and 4.6575 µg/mL respectively. The results are shown in table 3.

Table 3: Results showing LOD and LOQ.

S. No	Validation Parameter	Slope	Standard Deviation	Value (µg/mL)
1	Limit of Detection (LOD)	0.004	0.001863	1.536975
2	Limit of Quantification (LOQ)			4.6575

Robustness:

Robustness was analyzed at three different wavelengths by ±2nm (278.5,280.5 and 282.5nm) was checked and %RSD was calculated. The results are shown in Table 4.

Table 4: Result of Robustness.

S. No	Robust parameter	Wavelength (nm)	Absorbance ± Std Dev (n=3)	% RSD
1		278.50	0.454 ± 0.000249	0.549
2	Wavelength (±2nm)	280.50	0.460 ± 0.00082	0.177
3		282.50	0.458 ± 0.00125	0.272

Table 5: Summary of Optical characteristics and Other Parameters.

S No.	PARAMETERS	RESULTS
1	Absorption Maxima (nm)	280.50
2	Beer's-Lambert's range (µg/ml)	40-240µg/ml
3	Regression equation (y)*	Y = 0.004 X -0.0084
4	Slope (b)	0.004
5	Intercept (a)	0.0084
6	Correlation coefficient (r^2)	0.9991
7	Intraday precision (% RSD)**	0.405232
8	Interday precision (% RSD)**	0.442427
9	Accuracy (% mean recovery)	100.42
10	Limit of detection (µg / ml)	1.53698
11	Limit of quantification (µg / ml)	4.6575
12	Assay (%Purity)	99.1323

CONCLUSION

The results and the statistical parameters demonstrate that the proposed UV spectrophotometric method was fully validated and found to be simple, sensitive, accurate, precise, reproducible, and robust and relatively inexpensive. These levels of accuracy and precision obtained indicate suitability of the developed method for the quality control analysis of the vancomycin in its formulation. So, the developed method can be recommended for the routine of Vancomycin in pharmaceutical preparations.

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