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Development and Validation of UV- Spectrophotometric Method for estimation of Vancomycin Hydrochloride

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ABSTRACT

UV-spectrophotometry refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This method of analysis is gaining importance as it is rapid, simple, precise, less time consuming and highly accurate. The objective of the present investigation was to develop an accurate, rapid and robust method for determination of Vancomycin hydrochloride in pharmaceutical preparations by using UV spectrophotometric method. Vancomycin hydrochloride shows maximum absorbance at a wavelength of 281 nm, which is used for this study. The method provides a linear response from a quantitation range of 20 µg/ml to 100 µg/ml in phosphate buffer pH 6.8 with regression equation y = 0.0038x + 0.0045 and r^2 0.9994. Interday precision and accuracy was found to be below 0.2 and above 99.00% respectively for the developed method. Thus the developed method may be suitably applied for regular quality control of Vancomycin hydrochloride in bulk and pharmaceutical preparations.

Keywords: Vancomycin hydrochloride, spectroscopic method, validation.

INTRODUCTION

Vancomycin hydrochloride (VANCO) is a glycopeptide antibiotic which is active against Gram-positive organisms. It is a drug of choice in treatment of methicillin-resistant staphylococcal infections caused by *Staphylococcus epidermidis* and in treatment of colites caused by *Clostridium difficile*.[1]

Chemically it is (Sa)-(3*S*,6*R*,7*R*,22R,23*S*,26*S*,36*R*,38a*R*-[[2-*0*-Amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]oxy-3-(carbamoylmethyl)-10,19-dichloro 2,3,4,5,6,7,23,24,25,26,36,37,38,38a –tetradeca hydro-7,22, 28,30,32 - pentahydroxy - 6 - [(2*R*)-4-methyl-2-(methylamino)]valeramido] -2,5,24,38,39-pentaoxo-22*H*-8,11:18,21-dietheno-23,36-(iminomethano)-13,16:31,35-dimetheno-1*H*, 16*H*-[1,6,9] oxadiazacyclohexadecino [4,5-*m*] [10,2,16] benzoxadiazacyclo tetracosine-26-carboxylic acid, monohydrochloride (Fig-1).

VANCO is off white, odourless, free flowing powder having molecular weight 1485.71 and melting point 105°C. It is freely soluble in water, insoluble in ether and chloroform.[2, 3]

VANCO acts by inhibiting proper cell wall synthesis in gram-positive bacteria. As the mechanism of production of cell wall is different in gram negative organism VANCO is not active against most of the gram-negative bacteria. The large hydrophilic molecule is able to form hydrogen bond interactions with the terminal D-alanyl-D-alanine moieties of the NAM/NAG-peptides. This is a five-point interaction in normal circumstances. This binding of VANCO to the D-Ala-D-Ala prevents synthesis of cell wall of the long polymers of N-acetylglucosamine (NAG) and N-acetyl muramic acid (NAM) which is the backbone strands of the bacterial cell wall, and it prevents the backbone polymers that do manage to form from cross-linking with each other.[4, 5]

UV spectroscopic method for analysis is a versatile and expeditious method for analysis of different drug samples. The method developed in UV spectroscopy is less expensive compared to other methods like HPLC, GC, Mass spectroscopy etc., since it does not utilize costly solvents and exacting requirements for the analysis.[6] An extensive review of literature reveal that there is no suitable UV-spectrophotometric method for estimation of VANCO. Thus the objective of the present investigation was to develop an accurate, rapid and robust method for determination of VANCO in pharmaceutical preparations by using UV Spectrophotometric method.
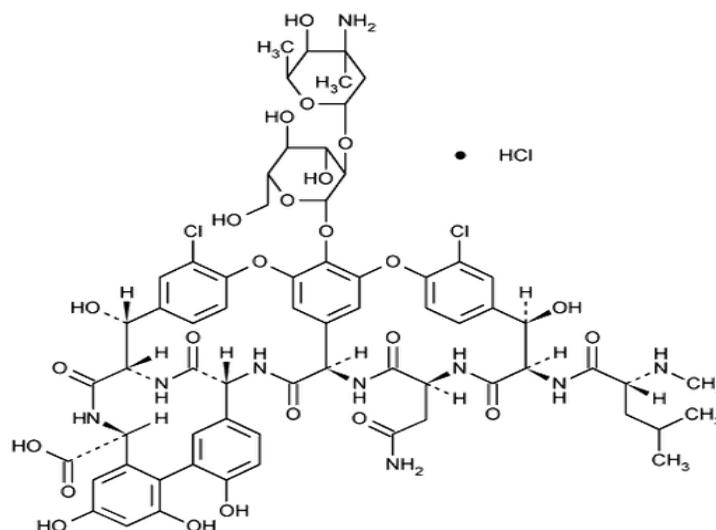
Figure 1: Chemical structure of Vancomycin hydrochloride.

Instrumentation

A double beam UV spectrophotometer (UV-1800, Shimandzu, Japan) connected with a computer was used for the study. This instrument has resolution of 1 nm spectral bandwidth over entire wavelength range (1,100 nm to 190 nm). The UV spectrophotometer has wavelength accuracy of ±0.1 nm and reproducibility of ±0.1 nm. One centimeter matched quartz cell was used to record the absorption of test and reference solutions. Obtained data was analyzed using UVProbe software (Version 2.34).

MATERIALS AND METHODS

Vancomycin hydrochloride was a gift sample from Aurobindo Pharma, Hyderabad, India. Sodium hydroxide and potassium dihydrogen phosphate were procured from SD Fine Chemicals, Mumbai, India. All other chemicals and solvents used in this study were of analytical grade and were used without further purification.

Spectrum measurement

A standard solution of VANCO (40μg/ml) in phosphate buffer pH 6.8 was prepared and scanned between 200 to 400 nm using UV-visible spectrophotometer.

Preparation of standard solution and calibration curve

Stock solution was prepared by dissolving 100 mg of VANCO in 100 ml of phosphate buffer pH 6.8 to obtain concentration of 1000 μg/ml. From the stock solution 20, 40, 60, 80 and 100 μg/ml dilution were prepared by taking 2, 4, 6, 8, 10 ml of stock solution respectively in 100 ml volumetric flask and adjusting the volume with phosphate buffer pH 6.8. The absorbance of each sample was measured at λmax, against blank phosphate buffer pH 6.8 on UV-visible spectrophotometer. The above procedure was repeated for three times and average of three reading of absorbance was calculated. Taking these readings a calibration curve was plotted by taking concentration of VANCO in X-axis and corresponding absorbance in Y axis.

Analytical method validation

The method was validated for linearity, accuracy and precision.

Linearity

For an analytical method, linearity is its ability to obtain test results, within a given range, that are directly proportional to the concentration of analyte in the sample.[7] Linearity can be documented as the linear regression curve of the measured response with corresponding increasing concentration of analyte. Linearity of the analytical method was examined to confirm that Beer's law operates over the range of interest. To check the linearity of the UV method of VANCO, the standard solutions were prepared at 20, 40, 60, 80 and 100 μg/ml. concentrations and absorbance were taken at 281 nm in UV spectrophotometer. The method can be considered linear for estimation of VANCO if it is linear over 20 to 100 μg/ml range. Least square regression method was used for determination of regression coefficient, r and the equation $y = ax + b$ for the best fitting line.

Accuracy

Accuracy or trueness of analytical method is given by the extent by which the value obtained deviates from the true value.[8] The "true" value is the result which would be observed in absence of error. Accuracy of the assay is defined as the percentage of the agreement between the measured value and the true value.[9]

Precision

Precision refers to the extent of variability of a group of measurement observed under similar experimental conditions.[10] Precision provides an indication of random errors which is generally subdivided into two cases: repeatability and reproducibility. Precision is commonly expressed in terms of imprecision. It is computed as either standard deviation, variance or Coefficient of variation (CV) of the test results. Large standard deviation is indication of less precision. Often, the maximum and minimum precision estimates are of interest. Measuring of precision quantitatively is critically depended on the stipulated conditions, which in turn depend on factors affecting the variability of the results from a measurement method. Laboratory, time elapsed between measurements, operator, calibration of equipment etc. are some of the factors which affects variability of the results.[11]

To evaluate the precision of the UV method of VANCO, standard solutions containing known amounts of pure drug (20, 60 and 100 μg/ml) were prepared and analyzed in three replicates. Intraday precision was determined by measuring absorbance of samples on the same day while absorbance values were determined for three consecutive days for interday precision.

RESULTS AND DISCUSSION

The standard solution (40μg/ml) of VANCO in phosphate buffer pH 6.8 gives a characteristics spectrum when scanned between 200 to 400 nm in UV-visible spectrophotometer. The absorption maxima (λ_{max}) was obtained at 281 nm.
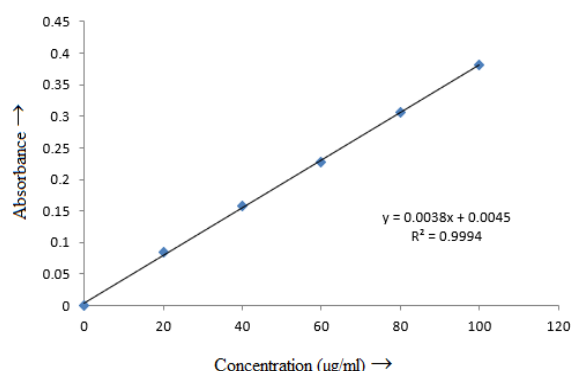

Figure 2: Calibration curve of Vancomycin hydrochloride in phosphate buffer pH 6.8.

The value of absorbance with respective concentration of VANCO solution were plotted to obtain the standard curve in phosphate buffer pH 6.8. Figure 2 shows the calibration curve of VANCO in phosphate buffer pH 6.8. A linear relationship was found between absorbance and the concentration of drug at the concentration range of 20 to 100 μg/ml, with the regression equation y = 0.0038x + 0.0045 and r^2 0.9994.

Precision and Accuracy:

Result of intraday and interday precision and accuracy for the UV spectroscopic assay method for VANCO in phosphate buffer pH 6.8 are shown in table 1 and 2 respectively. Precision is expressed as relative standard deviation (RSD). Formula used to calculate RSD and accuracy is as follows

$$RSD = \frac{\text{Standard deviation}}{\text{Mean concentration}} \times 100$$

$$Accuracy = \frac{\text{Mean observed concentration}}{\text{Actual concentration}} \times 100$$

Table 1: Intraday Precision and Accuracy

Concentration (μg/ml)		Precision (%)	Accuracy
Actual	Observed		
20	20.01±0.030	0.150	100.050
60	60.04±0.061	0.102	100.072
100	100.12±0.036	0.036	100.120

Table 2: Interday Precision and Accuracy

Concentration (μg/ml)		Precision (%)	Accuracy
Actual	Observed		
20	19.92± 0.055	0.276	99.583
60	59.48±0.065	0.110	99.133
100	99.01±0.021	0.021	99.013

Obtained results shows no significant difference between the actual amounts of drug added and observed concentration, which indicates the accuracy of the developed method.

CONCLUSION

The UV-spectrophotometric method developed is simple, with adequate accuracy and precision. Further this method is rapid and more economical than the other reported methods in literature. Hence the developed method can be applied for quality control and routine analysis of Vancomycin hydrochloride in bulk and in pharmaceutical preparations.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

REFERENCES

1. Barna JC, Williams DH. The structure and mode of action of glycopeptide antibiotics of the vancomycin group. Am Rev microbial, 1984; 38:339-357
2. United States Pharmacopeia and National Formulary (USP 34-NF 29). Vol 3. Rockville, MD: United States Pharmacopeia Convention; 2011. P. 4564-4566.
3. Indian Pharmacopoeia. Vol. 2, Government of India, ministry of Health and Family Welfare, Controller of publications. New Delhi; 2014. P. 1655-1657.
4. Kirby WMM. Vancomycin therapy of severe staphylococcal infections. J Antimicrobial Chemotherapy, 1984; 14 (Suppl D):73-78.
5. Pauly DJ, Musa DM et al. Risk of nephrotoxicity with combination vancomycin-aminoglycoside antibiotic therapy. Pharmaco-therapy 1990; 10:378-382.
6. Dubey SK, Kumar S, Mudakavi RJ, Deshpande S, Jain AK. Development and validation of UV-Spectrophotometric method for determination of Enalapril maleate. International Journal of Advances in Pharmaceutical Sciences., 2010; 1:375-380.
7. Brown EP, Weiner JA, Lin S, et al. Optimization and qualification of an Fc Array assay for assessments of antibodies against HIV-1/SIV. Journal of Immunological Methods. 2018; 455:24-33.
8. Srivastava MM: High Performance Thin Layer Chromatography (HPTLC). Springer, First Edition 2011.
9. Merodio M, Campanero MA, Mirshahi T, Mirshahi M, Irache JM. Development of a sensitive method for the determination of ganciclovir by reversed-phase high-performance liquid chromatography. Journal of Chromatography A. 2000; 870:159-167.
10. Bolton S, Swarbrick J: Basic definition and concept In: Pharmaceutical statistics: practical and clinical and application. Marcel Dekker, New York, Third Edition 1990.
11. Pryseley A, Mintiens K, Knapen K et al. Estimating precision, repeatability, and reproducibility from Gaussian and non-Gaussian data: A mixed models approach. Journal of Applied Statistics, 2010; 37(10):1729-1747.