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UV SPECTROSCOPIC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF VANCOMYCIN IN BULK AND ITS FORMULATION

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ABSTRACT

An UV-Spectroscopic validated method has been developedfor the determination of Vancomycin. 50% ethanol and 50% Distilled waterused as mobile phase composition. The method produced linear response over the wide concentration range of 25-150 Micro gram/Ml, with an average accuracy of 99.55%, as well as average intra- and inter-day variations of 1.0398 and 1.1676 %, respectively. The limits of detection and Quantitation of the method were 3.96 Micro grams / Ml and 12 Micro gram/Ml respectively.

INTRODUCTION

Vancomycin is have selective toxicity[1], that is they interact with a target present on the bacterial cell, the prokaryote, that is not present in the mammalian host, the eukaryote. For the ß-lactam drugs, this target is the cell wall. Mammalian cells lack cell walls, which for the bacteria are a critically important barrier protecting the organism from not only the vagaries of osmotic stresses, heat and viruses but also as a protection from anti-microbial[2] peptides, and host defense mechanisms. It is recommended intravenously as a first-line treatment for complicated skin infections, bloodstream infections, endocarditis, bone and joint infections, and meningitis caused by methicillin-resistant S. Aureus[3]. Chemicaly[4] 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 leucyl)amino] -20, 23, 26, 42, 44-pentaoxo-7,13-dioxa-21,24, 27, 41, 43 pentaazaoctacyclo [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-carboxylic acid, with molecular formula of

$C_{66}H_{75}Cl_2N_9O_{24}$ and molecular weight 1449.265 g/mol.The chemical stucture of drug was shown on fig:1. A survey of literature[6,7] revealed that few methods based on visible spectrophotometry for vancomycin hydrochloride have been reported. These reagent is easily obtainable, highly purified and are soluble in ethanol therefore the proposed methods have been satisfactorily applied for the determination of Vancomycin hydrochloride in pure and pharmaceutical preparations.The main objective of the present work was to develop a simple, accurate, precise and economic for determination of Vancomycin by UV in bulk and its tablet dosage form. The developed method to be validated in accordance to ICH Q2 (R1) guidelines [8,9].

MATERIALS AND METHODS

Chemicals and reagents:

The sample vancomycin (VANKING^R 500)I.P Injection was secured Neon Laboratories limited, Mumbai, India.solvent used as analytical grade ethanol and ,Sodium hydroxide,All the reagents used were of analytical grade and distilled water was used as diluent forfurther preparations of the drug.

Instrument specifications *:* The sample analysis was performed by using UV visible double beam specrophometershimadzu 1800. having deuterium lamp associated with spectra treats with UV probe software .The lamda max of vancomycin is adjusted to 280nm.

Methodology

Preparation of stock solution vancomycin pure drug 10 mg was weighed and transferred to a 10 ml volumetric flask and dissolved It was dissolved properly and diluted up to the mark with diluent to obtain final concentration of 1000 µg/ml. 10µg/ml solution was prepared from the stock solution was prepared using 50% ethanol .

Preparation of standard solution: Vancomycin pure 100 mg was weighed and transferred to a 100 ml volumetric flask and dissolved in ethanol. It was dissolved properly and diluted up to the mark with diluent to obtain final concentration of 1000 µg/ml. 100µg/ml solution was prepared from the stock solution was prepared using distilled water, which was used as working standard.

Preparation of working solutions*:* From the standard stock solution of vancomycin , appropriate aliquots were pipetted out in to 10 ml volumetric flasks and dilutions were made with distilled water to obtain working standard solutions of concentrations from 25-150µg/ml. Absorbance for these solutions were measured at 280 nm. The standard solution analytical concentration range was found to be 25-150 µg/ml.

Method Validation

Linearity and range: Linearity is defined as the ability to obtain test results, which were directly proportional to the concentration of an analyte in the sample within a given range. Linearity data for the spectrophotometric method was obtained at an absorption maximum of 280 nm by using five concentrations in the range of 25–150µg/ml. A calibration curve was obtained by plotting absorbance against concentration by considering five observations .Linearity data for the UV method was obtained by using five concentrations within the range of 25–150 µg/ml. A calibration curve was obtained plotting peak area against concentration by considering five observations. The regression of vancomycin concentration over its absorbance was found to be y= 0.005x- 0.0605 and R^2 as 0.999(where y is the absorbance and x is the concentration of Vancomycin). The linearity data was shown table no 1.

Precision*:* The degree of closeness of agreement between a series of measurements obtained from multiple samplings of the same homogeneous sample under the prescribed condition was determined.

Table 1: Results of calibration curve at 280 nm for Vanomycin

S.No	Concentration (µg/ml)	Absorbance
1	25	0.148
2	50	0.285
3	75	0.418
4	100	0.580
5	125	0.715
6	150	0.845

Table 2: Accuracy results for vancomycin

S. No	Spike Level	Absorbance	µg/ml Added	µg/ml Found	% Recovery
1	50 %	0.29	1.487	1.477	99.32
2	100 %	0.589	9.915	10.00	100.99
3	150 %	0.862	13.3854	13.171	98.43

Table 3: Summary of validation parameters obtained for proposed UV

S No.	**Parameters**	**Results**
1.	Absorption Maxima (nm)	280
2.	Beer's-Lambert's range (µg/ml)	25-150
3.	Regression equation (y)*	Y = 0.005x + 0.004
4.	Slope (b)	0.005x
5.	Intercept (a)	0.004
6.	Correlation coefficient (r^2)	0.999
7.	Intraday precision (% RSD)**	1.0398
8.	Interday precision (% RSD)**	1.1676
9.	Accuracy (% mean recovery)	99.55
10.	Limit of detection (µg / ml)	3.96(µg / ml)
11.	Limit of quantification (µg / ml)	12(µg / ml)
12.	Assay of tablets (%Purity)	99.15%

*Y = bx + a where x is the concentration of vancomycin in (µg / ml) and Y is the absorbance of the respective λ max. **Average of Six determinations

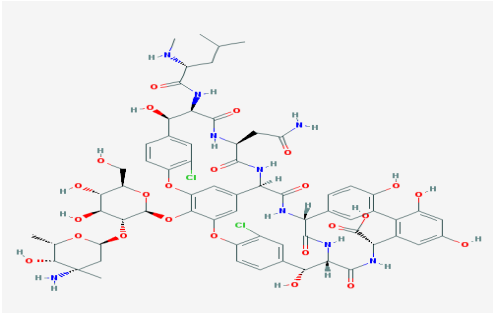

Figure 1:Chemical structure of vancomycin

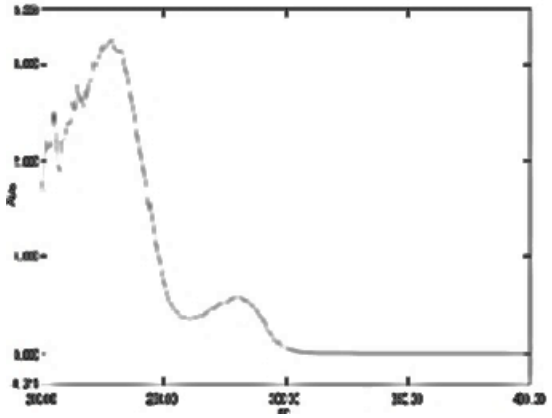

Figure 2: UV spectra of Vancomycin

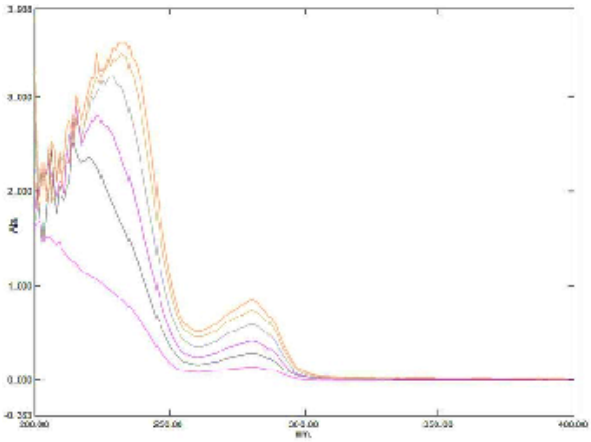

Figure 3: Overlay UV spectra of Vancomycin

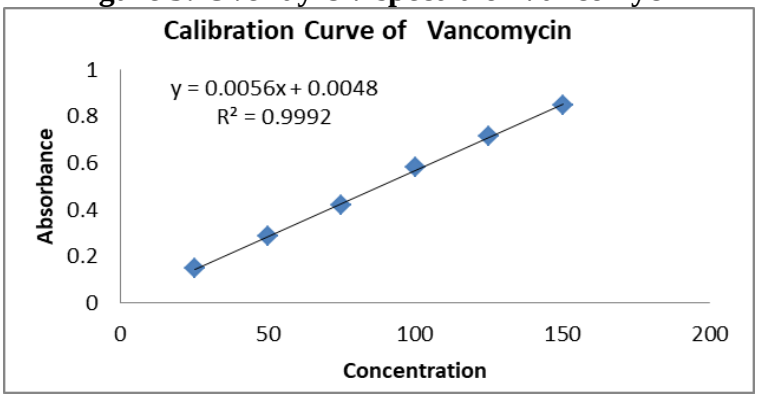

Figure 4: Linearity curve for vancomycin

The intra-day precision was performed by analyzing six replicate standard solutions on the same day, and inter-day precision was performed by analyzing a series of standard solutions for 3 consecutive days using the proposed U V method .The precision value of inter and intra-day were found to be 0.844 and 1.17231 respectively which was found to be within limits i.e. < 2.

Detection and quantification limits: Limit of detection (LOD) represents the lowest amount of analyte in the sample which can be detected. Limit of quantification (LOQ) represents the lowest amount of analyte, which can be quantitatively determined. The above parameters are calculated based on the standard deviation of the response and the slope. The standard deviation was calculated based upon the calibration

curve. LOD = 3.3σ/SLOQ = 10σ/S .The LOD and LOQ values for vancomycin were 3.96 µg/ml µg/ml and 12.0 µg/ml µg/ml respectively.

Robustness: Robustness is defined as the measure of its capacity to remain unaffected by small but deliberate variation in method parametersof the conditions on the determination of vancomycin. The different variations are change of wave length by ± 2 nm from developed spectroscopic conditions. The concentration of the solution analyzed was 100µg/ml., and it provides an indication of its reliability during normal range.bt using UV. The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%. From the observation it was found that the % RSD was within limit at all variable condition.

RESULTS AND DISCUSSION

Linearity: Regression value must be not more than 0.999. Llinearity of Vancomycin within 25-150µg/ml with regression value of 0.9997. the linearity data was shown in table 1 and fig 3 and 4.

Accuracy: Accuracy was performed by three different spiked concentrations like 50%, 100% and 150%. The percentage mean recovery was found to be 99.58%. The results were shown in table 2. All the validation parameters are present in within the limit. The summary of validation parameters were explained in table 3.

CONCLUSION

A novel, precise, economical, accessible, reliable and reproducible method for estimation of vancomycin in bulk and its tablet dosage form using UV method were developed and were validated as per ICH guidelines. The wide range of linearity establishes a further scope of promoting the proposed methods for estimation of vancomycin. The RSD values for all the validation parameters were found to be less than 1, indicating that the proposed UV method were trusts worthy. This methods have ample scope and application in industry for estimation of vancomycin.

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