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UV Spectrophotometric Method Development and Validation for Estimation of Vancomycin Hydrochloride in Bulk and IV Dosage Form

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ABSTRACT

Vancomycin Hydrochloride is an antibacterial prescription medicine approved by the U.S. FDA for the treatment of certain bacterial infections.[1] A UV-Spectroscopic validated method has been developed for the determination of vancomycin. The main objective of the present work was to develop an accurate, rapid, and economical method for the determination of vancomycin hydrochloride in pharmaceuticals by using uv spectrophotometric method. Vancomycin Hydrochloride shows maximum absorbance at a wavelength of 238.4nm, which is used for this study. The method provides a linear response from a quantitation range of 5-25µg/ml in ratio 20:80 methanol to water ratio with regression equation y=0.0208x + 0.018 and R^2= 0.9996. Interday precision and accuracy were 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 preparation.

INTRODUCTION:

Vancomycin is an amphoteric glycopeptide antibiotic, first isolated in 1956, that is active against a broad range of Gram-positive bacteria as well as some Gram-negative cocci. Vancomycin (VCM) is widely used to treat Methicillin-resistance Staphylococcus aureus (MRSA) infection [2]. It has a strong bactericidal activity, inhibiting the polymerization of peptidoglycans in the bacterial cell wall[3]. Because of toxicity, vancomycin was used primarily for alternative therapy before the recent emergence of methicillin-resistant and penicillin-resistant organisms. For patients allergic to both penicillin and cephalosporins, Vancomycin is an antibacterial medication in the glycopeptide class like penicillin [4]. vancomycin is FDA-approved for administration by either intravenous injection or oral route. Glycopeptide antibiotics such as vancomycin, ramoplanin, and teicoplanin are lifesaving drugs in certain clinical situations where first-line antibiotics (e.g., penicillin, cephalosporins) result in treatment failure [5,6].

IUPAC NAME:

(1S,2R,18R,19R,22S,25R,28R,40S)-48-[(2S,3R,4S,5S)-3-[(2S,4S,5S,6S)-4-amino-5-hydroxy- 4,6-dimethyloxan-2-yl]oxy-4,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-22-(2-amino-2- oxoethyl)-5,15-dichloro-2,18,32,35,37-pentahydroxy-19-[[(2R)-4-methyl-2-(methylamino)pentanoyl]amino]-20,23,26,42,44-pentaoxo-7,13-dioxa-21,24,27,41,43-pentazaoctacyclo[26.14.2.23,6.214,17.18,12.129,33.010,25.034,39]pentaconta-3,5,8(48),9,11,14,16,29(45),30,32,34(39),35,37,46,49-pentadecaene-40-carboxylicacid; hydrochloride.[7]

Its molecular formula is $C_{66}H_{76}Cl_3N_9O_{24}$ and the molecular weight is 1485.7[7]

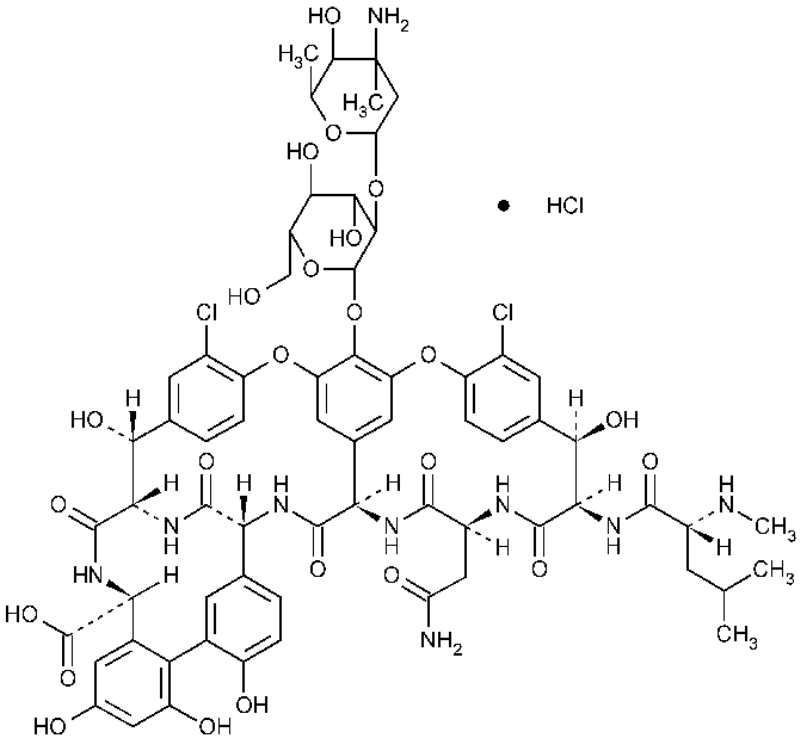

Figure no 1: Structure of vancomycin hydrochloride [8]

A survey literature [9,10] revealed that few methods based on UV-visible spectrophotometry for vancomycin hydrochloride have been reported. The reagents used in this are easily obtainable and highly purified. The proposed method has 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 determination of vancomycin by UV in bulk and pharmaceutical dosage form. The developed method is to be validated following ICH Q2 (R1) guidelines. [11,12]

MATERIALS AND METHOD

Vancomycin was obtained as a gift sample from Hetero Labs, limited, Telangana. IV infusion of Cipla was purchased from the local market. IV contains 250 mg of vancomycin hydrochloride. All chemicals and reagents were of analytical grade.

Instruments-

A Double beam UV-spectrophotometer (systemic -2201 spectrophotometer) was used for the detection of absorbance, sonicator (Microclean 1103) and weighing balance (SHIMADZU AY220) were used for experimental purposes.

Chemicals and Reagents-

Methanol, Distilled water, and Whatman filter paper were used.

Experimental Work-

Method Development

Solubility of vancomycin was performed in solvent methanol and distilled water in a ratio of 20:80 and the UV-spectra of the drug in this solution was recorded. The absorbance value of the drug was higher at absorption maxima with methanol and distilled water in a ratio of 20:80 was selected as a solvent for further investigation as it is more economical.

Preparation of Diluent –

20 ml of methanol was added to a 100 ml volumetric flask and diluted up to the mark with distilled water.

Preparation of standard stock solution –

Weigh accurately 10 mg of drug was transferred to a 10 ml volumetric flask and the volume was made up to the mark with diluent. (Concentration1000 µg/ml) further, pipette out 2 ml from the above solution and dilute up to the mark with diluent (50 µg/ml).

Determination of absorption maxima-

The stock solution of 10 µg/ml was prepared and was scanned in the range of 200-800 nm for the analysis of absorption maxima of vancomycin hydrochloride. The obtained result gives the maximum wavelength at 238.4 nm.

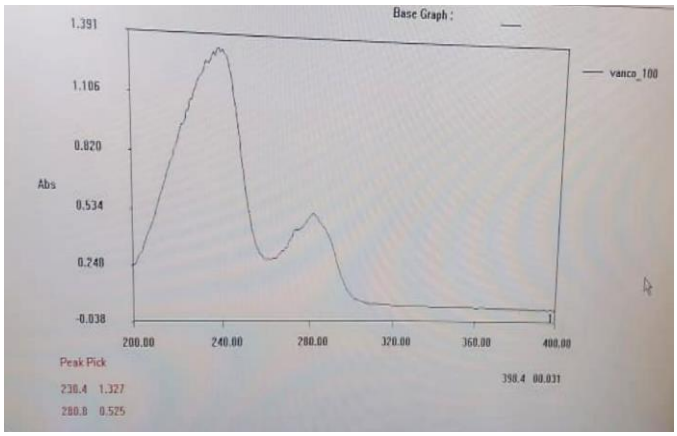

Figure no 2 UV -Visible Spectra of vancomycin hydrochloride

Procedure for determination of calibration curve –

The above stock solution (5,10,15,20,25 µg/ml) solution was prepared by diluting aliquots of (1,2,3,4,5 ml) in diluent and volume was made up to the mark using diluent.

Assay of vancomycin Hydrochloride –

From IV infusion powder equivalent of 15 mg was weighed accurately and transferred into a 10 ml volumetric flask and diluted with diluent up to the mark. Further, pipette out 1 ml from the above solution and diluted with diluent up to the mark. Further, pipette out 1 ml from the above solution and diluted with diluent up to the mark. (15 µg/ml).

RESULT AND DISCUSSION

METHOD VALIDATION –

The developed method was validated as per ICH guidelines. The parameter ICH assessed were specificity, linearity, range, accuracy, precision, LOD, LOQ, and Robustness.

Linearity and range –

The method is linear over the range of 5-25 µg/mlwithR^2 a value of 0.9996. (Refer to Table No 01) The analytical parameter range is the difference between upper and lower concentration limits and the range for this method was found to be 5-25 µg/ml.

Table No.01 Result of linearity

Sr no	Concentration (µg/ml)	Absorbance
1	5	0.124
2	10	0.232
3	15	0.332
4	20	0.433
5	25	0.545

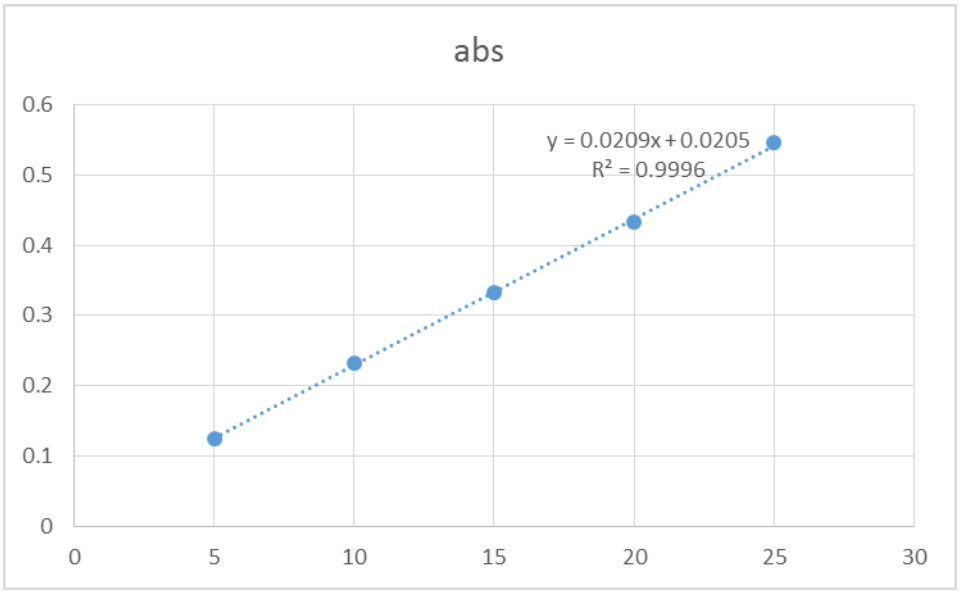

Fig 3: Calibration curve for Vancomycin Hydrochloride

Assay-

The absorbance of 15 µg/ml of hydrochloride was determined and percent purity was calculated. (Refer Table no 2).

Table no 2: ASSAY RESULT OF VANCOMYCIN

Formulation	Concentration	Amount obtained	% Recovery
Vanlid intravenous infusion IP 250	15 µg/ml	15.05 µg/ml	100.3

Precision

The precision of an analytical procedure is usually expressed as variance, standard deviation, or the coefficient of variation of a series of measurements. Intraday and Interday precision was performed by using a concentration of 15 µg/ml. The % RSD was found within the limit which is NMT 2%. Hence the parameter was valid.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value

found. The accuracy of an analytical method is expressed in terms of % recovery, accuracy, reported in Table No 3.

Table No 3: **RESULT OF ACCURACY**

Name of drug	Recovery level in %	Spiked amount (µg/ml)	Amount found(µg/ml)	% Recovery
VANCOMYCIN HYDROCHLORIDE	80	12	11.98	99.7
	100	15	14.98	99.86
	120	18	17.98	99.88

Precision

The precision of an analytical procedure is usually expressed as variance, standard deviation, or the coefficient of variation of a series of measurements. Intraday and Interday precision was performed by using a concentration of 15 µg/ml. The % RSD was found within the limit which is NMT 2%. Hence the parameter was valid. (Refer to Table NO 4 AND 5)

Table No 4: RESULT OF PRECISION INTRADAY

Sr no	Concentration	Absorbance
1	15µg/ml	o.176
2		0.173
3		0.176
4		0.174
5		0.171
6		0.175
7	Standard deviation	0.0019
8	% RSD	1.1143

Table No 5: RESULT OF PRECISION INTERDAY

Sr no	Concentration	Absorbance
1	15µg/ml	0.170
2		0.171
3		0.176
4		0.172
5		0.170
6		0.174
7	SD	0.0024
9	% RSD	1.3948

Limit of detection (LOD) and limit of quantitation (LOQ)

The sensitivity of the developed method was determined in terms of LOD and LOQ and it was calculated using the standard deviation method.

Table No 6: LOD and LOQ

LOD	1.390523503
LOQ	4.213707584

Robustness

The evaluation of robustness should be considered during the development phase. Robustness is usually demonstrated by making small deliberate changes to one of the operating parameters of the method, analyzing samples and comparing the results to those obtained using the prescribed method.

Wavelength change:

The effect of a small change in wavelength (λ_{max}±1 nm) 0n test result was studied.

Table No 7: Robustness

Wavelength (nm)	238.4	237.4	239.4
Absorbance	1.327	1.299	1.3

CONCLUSION

An analytical UV Spectrophotometric method developed and validated thoroughly for the estimation of vancomycin in API and IV Infusion dosage form. The above method was found to be easy, reproducible, simple, accurate, precise.

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