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HPLC Method Development and Validation for Dissolution of Vancomycin Hydrochloride from the Capsules

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***CS Srinivas¹, SB Puranik²**

¹*Research Scholar, Bundelkhand University, Jhansi, India.*

²*Research Guide, Bangalore, Karnataka, India.*

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ABSTRACT

Aim of this research work was to develop and validate HPLC method for dissolution of vancomycin hydrochloride from the capsules. The method is found to be specific for the vancomycin capsules. A system suitability test was established and recorded. The method is found to be linear, precise and accurate between the ranges of 25-150%. The method is robust and precise. Hence, this method stands validated and can be used for routine and stability sample analysis.



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INTRODUCTION

Analytical methods validation is an important regulatory requirement in pharmaceutical analysis. High-Performance Liquid Chromatography (HPLC) is commonly used as an analytical technique in developing and validating assay methods for drug products and drug substances (1). Method validation provides documented evidence and a high degree of assurance that an analytical method employed for a specific test, is suitable for its intended use. Over recent years, regulatory authorities have become increasingly aware of the necessity of ensuring that the data submitted to them in applications for marketing approvals have been generated using validated analytical methodology. The International Conference on Harmonization (ICH) has introduced guidelines for analytical methods validation (2, 3). Both United States Food and Drug Administration (USFDA) as well as United States Pharmacopoeia (USP) refer to ICH guidelines (4-7).

Vancomycin is a glycopeptide antibiotic isolated from *Streptomyces orientalis* cultures by Laboratory Lilly, USA. It was first introduced in medical clinic in 1958. Vancomycin acts against almost all gram-positive organisms inhibiting the biosynthesis of the cell-wall mucopeptide and thereby causes cell lysis. Vancomycin is resistant to the proteolytic enzymes activity due to its complex molecular structure (8-10). Hence, vancomycin has been used as a primary anti-infectious agent against methicillin resistant *Staphylococcus aureus* (MRSA) and also to infections caused by *Clostridium difficile*. It is also the drug of choice for patients who are allergic to β -lactam antibiotics.

To the date a few quantification methods to determine the concentration of vancomycin in various pharmaceutical products as well as in biological fluids have been available (11-20). Among them, immune enzymatic techniques such as FPIA, EMIT, RIA (21) and chromatographic methods are the most relevant. The drawbacks available with immune enzymatic techniques have led to the development of various chromatographic techniques for the accurate and precise determination of vancomycin.

Among these methods, HPLC is the most sensitive and specific and has the ability to detect low levels with high precision and accuracy. The reverse phase liquid chromatography with gradient elution and UV detection method has been developed for the estimation of vancomycin released from the vancomycin capsules USP. The hard gelatin capsules contain vancomycin

hydrochloride equivalent to 125 and 250 mg of vancomycin along with poly ethylene glycol in it. The developed method was validated as per the ICH guidelines to ensure its specificity, linearity, accuracy and precision.

MATERIALS AND METHODS

Apparatus

Chromatographic separation and development work was performed on an Agilent gradient HPLC system (Agilent Technologies, Waldbronn, Germany). Waters symmetry® C18 (250 x 4.6 mm) I.D. column with 5 µm packing was used for separation. An autosampler equipped with a sample loop of 50 µL volume and a UV-VIS detector set at 230 nm was used for all injections. Analysis was performed at ambient temperature (25°C).

Phosphate buffer preparation

An aqueous buffer was prepared by mixing potassium phosphate dibasic and water. Then, the pH was adjusted to 3.2 using orthophosphoric acid. The prepared solution was filtered, degassed and used as dissolution media.

Mobile phase

Mobile phase was prepared by mixing phosphate buffer and acetonitrile in a ratio of 91:9 and degassed briefly. Optimization of mobile phase was also done based on observations for various parameters such as retention time, theoretical plates and resolution.

Standard Solution (Resolution Solution)

An accurately weighed quantity of vancomycin hydrochloride standard was added into a volumetric flask and the volume was made up to the required level using water.

Dissolution operation conditions

Dissolution was performed in the USP type I apparatus using water as medium. The washed bowls were filled with 900 mL of media and the temperature was maintained at $37 \pm 0.5^\circ\text{C}$. One capsule was placed in each basket and after the specified time (45 min), required volume of

specimen was collected and filtered through 0.45 µm membrane filter. The respective quantity of fresh medium was replaced in order to maintain the sink conditions.

Procedure

After setting the chromatographic conditions, the instrument was stabilized by injecting blank solution into it. All solutions were filtered through 0.45 µm Nylon filter and the first 2 mL was discarded. The respective solutions were injected into the chromatographic system and the corresponding chromatograms were recorded. The area responses for all the peaks were measured. The percentage of drug release was calculated using the formula,

$$\% \text{ Release} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{I} \times \frac{P}{100} \times \frac{100}{LC}$$

Where,

AT = Area response of vancomycin hydrochloride in sample solution

AS = Average peak area response of the vancomycin in standard solution

WS = Weight of vancomycin hydrochloride standard in mg

DS = Dilution of standard solution

DT = Dilution of test/sample solution

P = Potency of vancomycin hydrochloride

LC = label claim of vancomycin in mg

The developed analytical method for vancomycin dissolution from the vancomycin hydrochloride capsules was observed for following parameters:

System suitability

To ensure that the analytical system is working fine and can give reliable results, the system suitability parameters are to be verified. The system suitability parameters were checked by injecting 5 different shots of standard solution into chromatograph and the chromatograms were recorded. The relative standard deviation of replicate injections should not be more than 2.0%.

Specificity

The specificity parameter of the developed method was verified by separately injecting the blank, placebo, standard and sample solutions into the chromatograph. The specificity of the method was also verified by examining the standard and sample solutions in dissolution media of pH 1.2, 4.5 and 6.8. The extent of interference from the blank and placebo peaks to the vancomycin peak was analysed. The peaks of blank and placebo should not interfere or the interference should be less than 0.5% of vancomycin peak.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of identical sample. The precision of a method is usually expressed as the standard deviation or relative standard deviation (RSD) of series of measurement.

a) System precision

The system precision was checked by injecting the standard vancomycin solution for 6 times into the system and the retention time and peak responses of the standard drug were recorded. The relative standard deviation for retention time and area response for vancomycin peak was calculated. The RSD between the six samples for retention time and area response should not be more than 1% and 2% respectively.

b) Method precision

In order to ensure the precision of the developed method, a homogenous solution of single batch should be analysed six times. The method should give consistent result for a single batch sample. The dissolution analysis of six vancomycin capsules of same batch was performed as per the procedure and the data obtained was compared with the standard solution to calculate the RSD. The RSD between the six units of vancomycin capsules should not be more than 6.0%.

c) Intermediate Precision

The intermediate precision should be carried out to ensure that the analytical results would remain unaffected with change in instrument, analyst and day. The intermediate precision study

was performed on vancomycin capsules and the analysis was repeated by different analyst on different instrument using different lot of column on a different day. The percentage of drug release was calculated and RSD was determined between sample and standard. The RSD of the percentage of vancomycin release in six different determinations should not be more than 6.0%.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by a well defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. A linearity stock solution was prepared by dissolving specified quantity of standard vancomycin in dissolution media and from this the desired linearity solutions were prepared as per Table 1.

Levels	Volume of Linearity stock solution Added (In ml)	Total volume (In ml)	Concentration (In ppm)
1	0.7	50	70.0616
2	1.0	50	100.0880
3	1.5	50	150.1320
4	2.3	50	230.2024
5	2.6	50	260.2288
6	2.9	50	290.2552
7	3.2	50	320.2816
8	3.5	50	350.3080
9	4.3	50	430.3784

The above solutions were injected in to the chromatograph and the average area response for vancomycin was recorded at each level. The slope, intercept, coefficient of correlation and regression coefficient (R square) was calculated and test the intercept for statistical equivalence to zero. The correlation coefficient and regression coefficient should not be less than 0.995 for vancomycin and the percentage of intercept should be within $\pm 5.0\%$ of the response at 100% level.

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its range. The

accuracy analysis was performed on the vancomycin capsules at three different levels and in triplicates at each level. A known quantity of vancomycin hydrochloride was spiked at 25%, 50%, 100% and 150% level and these samples were analysed as per the procedure. From this the percentage of recovery was calculated and the individual and mean percentage of recovery should be between 90.0% and 110.0%.

Range

The range of an analytical method is the interval between the upper and lower levels of analyte that has been demonstrated to be determined with a suitable accuracy. The data for analysing the range capability of the developed method has been derived from the linearity and accuracy studies. The calculated RSD on 12 determinations should not be more than 6.0%

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. This has been evaluated by adjusting the parameters like column temperature by $\pm 5^{\circ}\text{C}$, flow rate by ± 0.2 mL/min, pH of buffer by ± 0.2 unit and the organic phase ratio in mobile phase by $\pm 2.0\%$. The method should achieve all the system suitability parameters in the above said variations in order to qualify the robustness test.

RESULTS AND DISCUSSION

A HPLC method for studying the dissolution of vancomycin hydrochloride from the capsules has been developed and validated for its suitability, specificity, precision, linearity, accuracy and robustness.

System suitability

The chromatographic method was optimized for effective separation and the quality of the same was monitored through system suitability parameters. The relative standard deviation for the analytical peak area of two replicate injections should be less than 2.0%. From the data obtained it was found that the RSD value is well within the limits and hence the method passes the system

suitability testing. The chromatogram obtained from the standard and sample vancomycin solutions using the developed method has been presented in Figure 1.

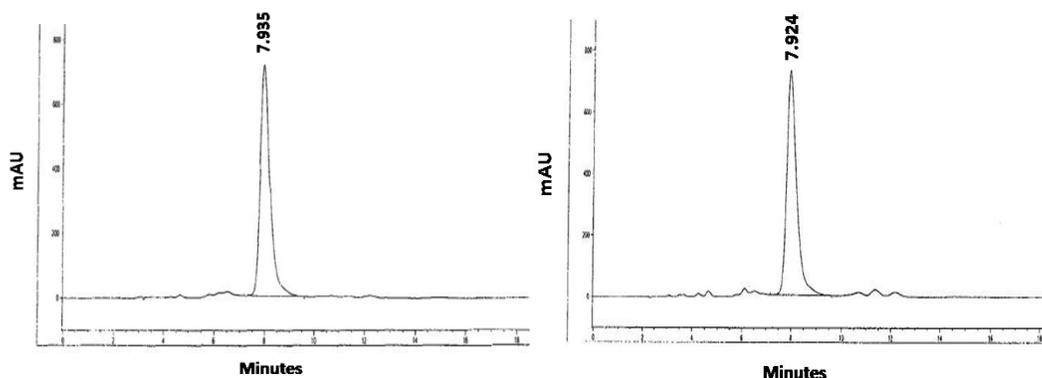


Figure 1. a) Standard Vancomycin b) Vancomycin released from capsules

Validation

Method validation was performed as per the ICH guidelines for specificity, linearity, precision, accuracy, range and robustness.

Specificity

The specificity analysis was carried out by injecting different samples like dissolution media, placebo, standard and sample drug solutions into the chromatograph. The retention time of standard and sample vancomycin was determined as 7.935 and 7.924. Further from the studies, it was found that the dissolution media and placebo were not interfering with the desired drug peak and hence the developed HPLC method of determination of vancomycin released from the capsule is specific to this drug.

Precision

The precision studies were carried out to authenticate that the method is producing consistent results for the tested samples. This has been performed by three different ways like system precision, method precision and intermediate precision.

System precision

The results of system precision analysis were presented in the Table 2. From the data, it was confirmed that the retention time and area response for the six different samples were consistent and the percentage of relative standard deviation is well within the limits. Hence, the developed method meets the system precision requirement of validation.

Table 2. The retention time and area response data of six injections of standard vancomycin

Injection no.	Vancomycin Hydrochloride	
	Area response	Retention Time (min)
1	20239.10000	7.415
2	20232.90000	7.406
3	20227.20000	7.402
4	20222.20000	7.399
5	20218.30000	7.401
6	20215.40000	7.404
Mean	20225.85000	7.40450
Stdev	9.01793	0.00568
RSD	0.0%	0.1%

Method precision

The method precision results were provided in Table 3. The percentage release of vancomycin from the six units of vancomycin capsule was presented and the data has showed that the results were consistent and the percentage of RSD is within the limits.

Table 3. Percentage of drug release from six units of vancomycin capsules

Set No.	% Released	
	Vancomycin Hydrochloride	
	250mg	125mg
1	103.4	108.4
2	102.8	108.0
3	102.7	108.4
4	103.7	108.7
5	103.7	108.8
6	103.3	109.2
Mean	103.3	108.6
Stdev	0.43643	0.40925
RSD	0.4%	0.4%

Intermediate precision

The intermediate precision study was performed using two different HPLC instruments, two different analysts on different days and also by changing the columns. In all this experiments, the results were consistent and the percentage of RSD is in acceptable limits. This has proved that the developed method for estimating vancomycin release from the capsules is rugged.

Linearity

The linearity data for the developed method was presented in Table 4. From the statistical treatment of the data of vancomycin hydrochloride, it was clear that the response of vancomycin is linear between 50-150% levels. The correlation coefficient and regression coefficient are greater than 0.995. The linear regression equation was $y = 66.684x - 88.709$, where slope = 66.684 and intercept = -88.709. In addition, the analysis of residuals showed that the values were randomly scattered around zero and hence it has good fit to the linear model. To evaluate whether the y-intercept is significantly different from zero, the p-value was determined. The p value for vancomycin was found to be 0.08 and hence it is statistically equivalent to zero. Thus, the method has qualified the test for linearity

Table 4. Linearity data of vancomycin hydrochloride

Sr. No.	Vancomycin in ppm	Area response
1	0.000	0.00000
2	70.0616	4521.75220
3	100.0880	6575.47217
4	150.1320	9957.66260
5	230.2024	15207.95000
6	260.2288	17282.65000
7	290.2552	19254.35000
8	320.2816	21176.55000
9	350.3080	23237.50000
10	430.3784	28734.00000
	Slope	66.684
	Intercept	-88.709
	Intercept %	-0.5%
	Correlation. Coefficient.	1.000
	Regression Coefficient	1.000

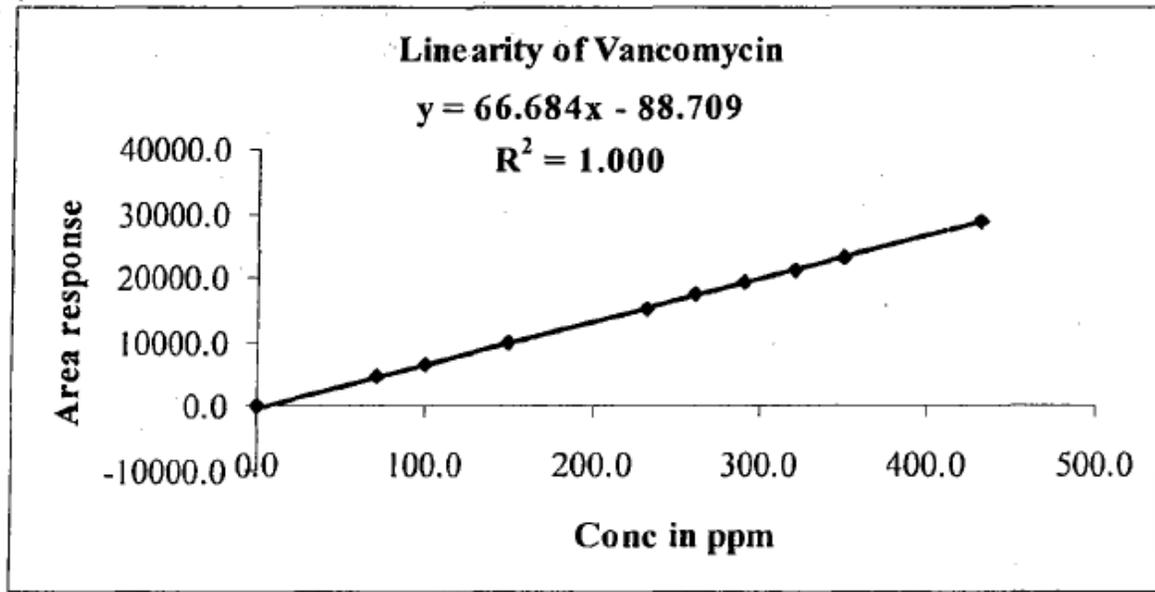


Figure 2. The graph displaying the linearity between different concentrations of vancomycin and its area response

Accuracy

Accuracy is usually determined by either of four ways (Shabir et al., 2004). First, it can be assessed by analyzing a sample of known concentration and comparing the measured value to the true value. The second one is to compare the results of newly developed method with the results of existing well-characterized procedure that is known to be accurate. The third approach is based on the recovery of known amounts of analyte. This is performed by spiking analyte in placebo samples. Then, the percentage of recovery should be calculated. The fourth approach is similar to the third one and it is used when it is not possible to prepare a blank sample matrix without the presence of the analyte.

The analysis was carried out by adding known quantities of vancomycin to the placebo and the amount of drug recovered after analysis was presented in the Table 5. From the results, it was clear that the percentage of recovery was within the limits of 90-110% and this proved that the method is accurate.

Table 5. Accuracy/recovery of Vancomycin from samples with known concentrations

Sr. No	Level (about)	Area Response	mg added	mg recovered	% Recovery	Mean % Recovery	% RSD
1	25%	4522.29102	64.38	60.74	94.3	94.4	0.2
2		4527.98145	64.28	60.82	94.6		
3		4523.60620	64.42	60.76	94.3		
4	50%	10007.40000	134.58	134.41	99.9	99.8	0.4
5		10028.35000	134.44	134.69	100.2		
6		9971.78496	134.70	133.93	99.4		
7	100%	19305.50000	269.55	259.30	96.2	96.1	0.2
8		19231.25000	269.39	258.30	95.9		
9		19270.35000	269.09	258.83	96.2		
10	150%	28775.00000	403.63	386.49	95.8	95.8	0.1
11		28717.95000	403.29	385.72	95.6		
12		28790.10000	403.38	386.69	95.9		
Across all levels						96.5	2.2

Range

The range of determination of the developed procedure was derived from linearity and accuracy studies. This analysis revealed that the present method provides an acceptable degree of linearity, accuracy, and precision when applied to samples containing different amounts of analyte within the specified range, of the test method. The accuracy and linearity range of the HPLC method for estimation of vancomycin released from the capsules was given in Figure 3. From the results, it was concluded that the method is linear, precise and accurate between 50-150% levels of target concentration and the range of the method is 25 – 150%.

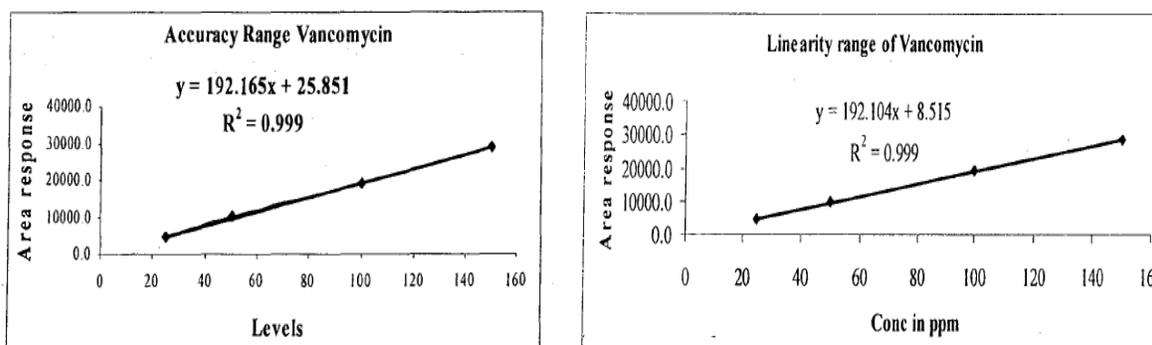


Figure 3. The graph displaying the accuracy and linearity range of HPLC method for vancomycin estimation

Robustness

Certain known variations have been made to the HPLC conditions in order to evaluate the robustness of the same. The parameters altered and its consequences in terms of percentage of RSD have been given in Table 6. The robustness acceptance criteria was similar to the conditions fixed in the system suitability studies and the result has revealed that the developed method of vancomycin estimation is robust.

Table 6. Parameters altered to ensure the robustness of the method

	The Relative standard deviation of replicate injections of standard solution should be NMT 2.0 %.
Initial	0.0%
Decrease in Flow rate	0.2%
Increase in Flow rate	0.1%
Decrease in Temperature	0.1%
Increase in Temperature	0.1%
Decrease in pH	1.0%
Increase in pH	0.1%
Decrease in Organic phase	0.1%
Increase in Organic phase	0.1%

CONCLUSION

The proposed HPLC method for dissolution of vancomycin hydrochloride from the capsules has been developed and validated. The method is found to be specific for the vancomycin capsules. A system suitability test was established and recorded. The method is found to be linear, precise and accurate between the ranges of 25-150%. The method is robust and precise. Hence, this method stands validated and can be used for routine and stability sample analysis.

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