IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
May 2022 Vol.:21, Issue:3

Drug Release of Vancomycin Hydrochloride Capsules by HPLC

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
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Submitted: 25 April 2022
Accepted: 30 April 2022
Published: 30 May 2022

Keywords: vancomycin capsules, HPLC, dissolution, related substances

ABSTRACT

Research work was to develop and validate the HPLC method for the 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 stable 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 the validated analytical methodology. The International Conference on Harmonization (ICH) has introduced guidelines for analytical methods validation (2, 3). Both the 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 a medical clinic in 1958. Vancomycin acts against almost all gram-positive organisms inhibiting the biosynthesis of the cell-wall mucopeptide and thereby causing cell lysis. Vancomycin is resistant to proteolytic enzyme 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 against infections caused by *clostridium difficile*. It is also the drug of choice for patients who are allergic to β-lactam antibiotics.

To 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 can 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 polyethylene glycol in it. The developed method was validated as per the ICH guidelines to ensure its specificity, linearity, accuracy, and precision.

METHODS

Apparatus

Chromatographic separation and development work was performed on an Agilent gradient HPLC system (Agilent Technologies, Waldbronn, Germany). Waters symmetry®C18 (250 x 4.6mm) 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

The mobile phase was prepared by mixing phosphate buffer and acetonitrile in a ratio of 91:9and degassed briefly. Optimization of the 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 a medium. The washed bowls were filled with 900 mL of media and the temperature was maintained at 37 ± 0.5°C. One capsule was placed in each basket and after the specified time (45 min), the required volume of

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

Procedure

After setting the chromatographic conditions, the instrument was stabilized by injecting a blank solution into it. All solutions were filtered through a 0.45µm 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,

$$\% \text{ Release} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{1} \times \frac{P}{100} \times \frac{100}{LC}$$

Where,

AT = Area response of vancomycin hydrochloride in the 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 the 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 the 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 analyzed. The peaks of blank and placebo should not interfere or the interference should be less than 0.5% of the 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 an identical sample. The precision of a method is usually expressed as the standard deviation or relative standard deviation (RSD) of a series of measurements.

a) System precision

The system precision was checked by injecting the standard vancomycin solution 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

To ensure the precision of the developed method, a homogenous solution of a single batch should be analyzed six times. The method should give consistent results for a single batch sample. The dissolution analysis of six vancomycin capsules of the 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 by changes in the instrument, analyst, and day. The intermediate precision study was performed on vancomycin capsules and the analysis was repeated by a different analyst on the different instruments using a different lot of columns 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 a 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 into the chromatograph and the average area response for vancomycin was recorded for 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 ±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% levels and these samples were analyzed 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 the analyte that has been demonstrated to be determined with suitable accuracy. The data for analyzing 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 indicates its reliability during normal usage. This has been evaluated by adjusting the parameters like column temperature by ±5°C, flow rate by ±0.2 mL/min, pH of the buffer by ±0.2 unit, and the organic phase ratio in the mobile phase by ±2.0%. The method should achieve all the system suitability parameters in the above-said variations to qualify for the robustness test.

RESULTS AND DISCUSSION

An 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.

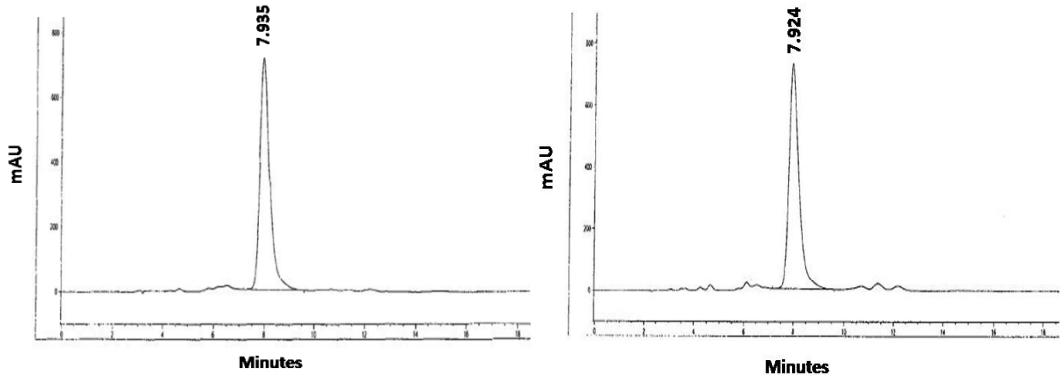

Figure 1. a) Standard Vancomycin b) Vancomycin released from capsules

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, and 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 in three different ways like system precision, method precision, and intermediate precision.

System precision

The results of the system precision analysis were presented in 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 Hydrochloride	
Injection no.	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 shown 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

	% Released	
Set No.	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 these experiments, the results were consistent and the percentage of RSD is within 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 a 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

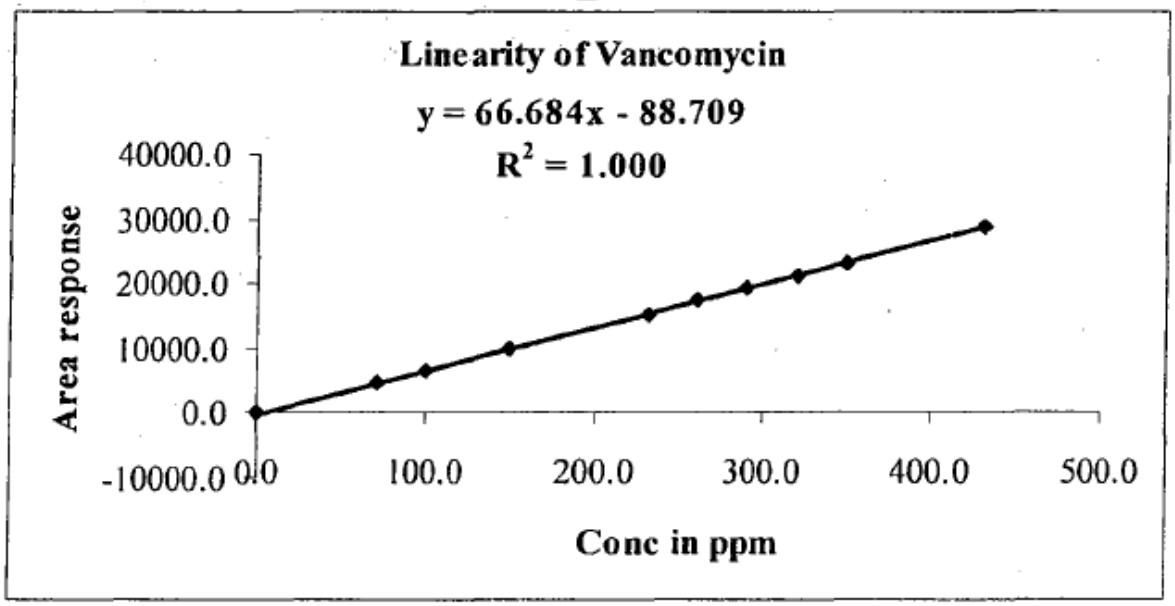

Figure 2. The graph displaying the linearity between different concentrations of vancomycin and its area response

Accuracy

Accuracy is usually determined in 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 the newly developed method with the results of the 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 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%.

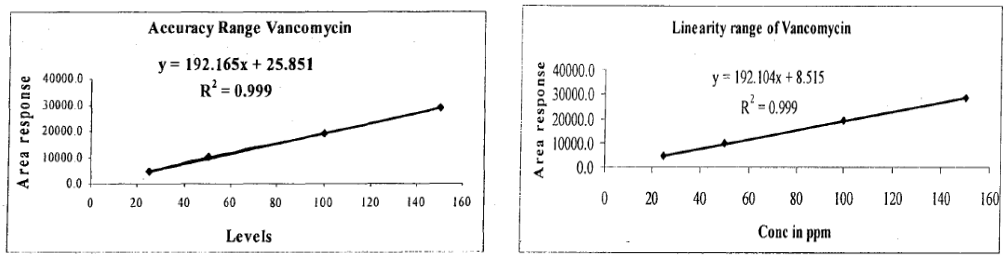

Figure 3. The graph displaying the accuracy and linearity range of the HPLC method for vancomycin estimation

Robustness

Certain known variations have been made to the HPLC conditions to evaluate the robustness of the same. The parameters altered and their consequences in terms of percentage of RSD have been given in table 6. The robustness acceptance criteria were 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 were 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 the 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 stable sample analysis.

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