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Method development and validation of Vancomycin drug in bulk and dosage form by RP-HPLC technique

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Abstract

A simple, rapid, and selective RP-HPLC method was developed for the estimation of vancomycin hydrochloride concentration in injectable dosage forms. The method was developed using Nucleosil C18 – (250 mm × 4.6 mm x 10-µm) with a mobile phase composed of phosphate buffer (pH 2.2), acetonitrile, and water in the ratio of 50:50 (by volume), respectively. The mobile phase was pumped isostatically HPLC system at a flow rate of 1 mL/min and quantification of the analyte was based on measuring its peak areas at 235 nm. The retention time for vancomycin hydrochloride was about 10.15. The reliability of the proposed HPLC procedure was validated to linearity, ranges, precision, accuracy, specificity, and detection limit. The calibration curve was linear in the range of 50% to 150% of the working concentration. The range for the

analytical method was found to be 25 ppm to 75 ppm with a correlation coefficient of more than 0.9999. The proposed method proved to be selective and stability-indicating by the resolution of the analytes from the forced degradation (hydrolysis, oxidation, thermolysis, and photolysis) products. The validated HPLC method was successfully applied to the analysis of vancomycin hydrochloride in pharmaceutical dosage forms. The degradation products resulted from the storage of the drug under stress degradation conditions described by the International Conference on Harmonisation (ICH).

Keywords: vancomycin hydrochloride, RP-HPLC, bulk dosage form, injectables

Introduction

As a very common drug, discovered in 1956 by McCormick et al, Vancomycin is a notable antibiotic prescribed for combating gram-positive infections (1). Chemically it is a tricyclic glycopeptide, isolated from *Streptomyces orientalis* and Nocardia *lurida* (2). It was the first bactericidal antibiotic prescribed for penicillin-resistant staphylococci infections. With the wide availability of methicillin, the use of vancomycin was limited (3). However, it regained its popularity in the late 1960s as an agent for treating bacterial endocarditis as an alternative to penicillin. With the emergence of methicillin-resistant Staphylococcus *aureus* and multiply-resistant Staphylococcus *epidermidis* strains as clinical problems in the late 1970s, there has been renewed interest in vancomycin (4).

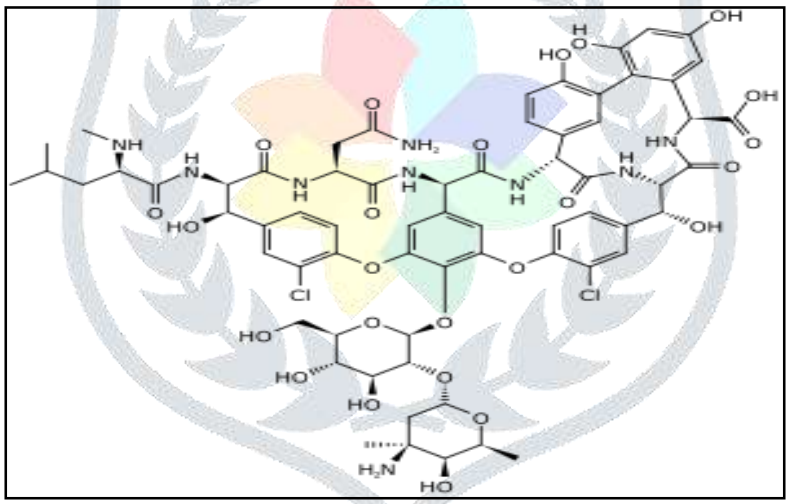

Figure 1: Structure of Vancomycin

The agent is bactericidal against most gram-positive cocci and rods but is ineffective against most gram-negative bacteria. It exerts its bactericidal effect by interfering with the phospholipid cycle of cell-well synthesis (5) and by altering plasma membrane function (6), thereby inhibiting ribonucleic acid synthesis (7). The bacterial cell wall contains peptidoglycan that encircles the whole bacteria (8).

Vancomycin is not metabolized and is excreted primarily in the urine. Around 90% of the administered dose is excreted by glomerular filtration. It has a serum protein binding affinity of 50 to 60% and diffuses readily into body fluid compartments, including the cerebrospinal (9). In adults, a single intravenous dose of 1 g produces plasma concentrations of 15 to 30 µg/ml 1 hour after a 1- to 2-hour infusion (10). Its plasmatic half-life ranges from 4 to 11 hours, with an average of 6 hours in patients with normal renal function. The adverse

effects of vancomycin are hypotension and tachycardia, phlebitis, nephrotoxicity, ototoxicity, hypersensitivity reactions, chills, exanthema, and fever (11).

Vancomycin is mainly prescribed as a sterile powder for solution for injection in strengths 5g/ vial, 1g/vial, and 500mg/vial. Orally Vancomycin is available in capsule form in 250mg, and 500mg strengths. It is very important to establish an effective and robust method to assay the drug present in the sterile dosage forms in the Indian market. The literature suggests that vancomycin has been assayed by HPLC (12-13), micellar electrokinetic capillary chromatography (14), and spectrophotometry (15). High-performance liquid chromatography is a technique in analytical chemistry used to separate, identify and quantify each component in a mixture. Usman et al. developed a simple, rapid, and selective RP-HPLC method for the determination of vancomycin hydrochloride by using a mobile phase NH4H2PO4 (50 mM, pH 2.2)–acetonitrile (88:12, v/v) at a flow rate of 0.36 mL/min on a nuclear C18 column (125 mm × 4.6 mm, 5 µm) with UV detection at 205 nm (16).

In another method developed by Serri *et al*, the separation was achieved using a Capital C8 Optimal column (250 ◊ 4.6 mm i.d., 5 µm particle size) with a mobile phase composed of buffer citrate (pH 4), acetonitrile, and methanol in the ratio of 85:10:5 (by volume), respectively (17). Simultaneous estimation of the vancomycin and ceftriaxone in tablets was achieved on a Betasil C-1 column using a mobile phase consisting of a binary mixture of acetonitrile and triethylamine buffer adjusted to pH 3.5 ± 0.1 with orthophosphoric acid in a ratio of 20:80 (18). Hadwiger et al (2015), developed a method for injectable products using high-resolution liquid chromatography-mass spectroscopy for quality assessment of US markets (19). Similar methods for quality assessment of the Indian market for injectable products are low in number.

Therefore, the present study aims to develop and validate a simple reverse phase HPLC method for vancomycin in bulk dosage form and to verify the analytical method for assay of vancomycin HCl in Vancomycin Hydrochloride for Injection, USP 500mg/vial, 10mlby HPLC as per the ICH guidelines.

Materials and Methods

Chemicals and reagents

The laboratory (working) standards Vancomycin HCl working standard were received as gift samples from ----. FDC product of Vancomycin HCl was prepared with a label claim of 500mg/vial, 10ml. Solvents like Acetonitrile, Methanol were HPLC grade, and reagents Sodium Monohydrogen Phosphate Heptahydrate ($Na_2HPO_4•7H_2O$), Sodium dihydrogen phosphate ($NaH_2PO_4•H_2O$), hydrochloric acid, were analytical grade, and high purity. Milli-Q water for buffer was obtained from---.

HPLC method development

Chromatographic conditions and instruments

The performance of chromatographic analysis RP-HPLC (H.P.L.C- Waters- Alliance 510) instrument equipped with UV detector was used (UV- 484 Data Ace). The stationary phase was Nucleosil C18 - 250 mm × 4.6 mm x 10-µm column, HPLC column oven temperature 40°C, autosampler temperature 10°C, with the flow rate of 1 mL/min. The injection volume was maintained at 20 µL with a run time of 20 minutes, and

a wavelength of 235 nm was optimized. The isocratic mode was used for the mobile phase. Other instruments used in the validation like analytical balance (Mettler Toledo B204S), ultra sonicator, and pH meter were calibrated.

Preparation of buffer solution

For the preparation of phosphate buffer, 20.214 g of $Na_2HPO_4 \cdot 7H_2O$ and 3.394 g of $NaH_2PO_4 \cdot H_2O$ to 800 volumes of water were taken, and pH 2.2 was adjusted with hydrochloric acid, and volume was made up to 1liter. The buffer and acetonitrile were mixed in the ratio of 80:20 and filtered through 0.2 µm Nylon membrane filter paper.

Preparation of diluent

The diluent used for the separation was acetonitrile and water in a ratio of 60:40. The diluents were filtered using 0.2 µm Nylon membrane filter paper and degassed.

Preparation of stock and standard solution

Preparation of Vancomycin HCl Standard Solution

About 50 mg of Vancomycin HCl working standard was weighed and transferred and 20 ml of diluent was added and sonicated to dissolve. Volume was further made up with the diluent. 1 ml of the solution was transferred to a 10ml volumetric flask, diluted and mixed. The solution was filtered through a 0.2µm nylon membrane filter.

Preparation of Test Solution

One ml of solution was diluted with 50ml of diluent and mixed manually, followed by sonication for 5min. 1 ml from the aforesaid solution was transferred into a 10ml volumetric flask. The volume was made by 10 ml diluent, and the solution was filtered.

Method Validation

System Suitability

For system suitability, Vancomycin HCl standard working solution was used. equal volumes of blank, five replicate injections of system suitability solution were separately injected. Followed by two injections of the test solution and the chromatogram was recorded. Any peak due to blank in the solution was disregarded. % RSD of five replicate injections of system suitability solution (Vancomycin HCl standard working solution) were calculated. Tailing factor and theoretical plates of the peak in the chromatogram obtained with the 5^{th} injection of system suitability solution (Vancomycin HCl working standard solution) were checked. For system suitability the theoretical plates should be not less than 2000, the tailing factor should be less than 2.0, and the % RSD should be not more than 2.0%.

Specificity

Selectivity was performed by injecting the diluent blank solution, excipient blend, system suitability solution, and test solution. The Vancomycin HCl peak should be well resolved from any other peak and each other. The diluent blank solution and excipient blend solution should not show any peak at the retention time of the Vancomycin HCl.

Linearity

For the linearity study, five standard solutions of Vancomycin HCl were prepared from the range starting from 50% to 150% of the theoretical concentration of assay preparation. The system suitability solution and the linearity solutions were injected as per the protocol. The linearity graph of concentration against peak response was plotted and the correlation coefficient was determined. The correlation coefficient should be greater than or equal to 0.999.

Precision:

Method Precision

Six test solutions of Vancomycin HCl in Vancomycin Hydrochloride for Injection, USP 500mg/vial, 10ml and were prepared as per the analytical method. The % RSD of % assay of six test solutions was calculated. % RSD of the results of six test solutions should not be more than 2.0%.

Intermediate Precision:

Six test solutions of Vancomycin Hydrochloride for Injection, USP 500mg/vial, and 10ml were prepared as per the analytical method on a different day. These test solutions were analyzed by a different analyst using different HPLC columns of the same make but having a different serial number and different HPLC systems. The % RSD of % assay results of twelve test solutions (six samples from method precision and six samples from intermediate precision) was calculated. % RSD of the results of twelve test solutions (six of method precision and six of intermediate precision) should not be more than 2.0%.

Robustness

Two test solutions of the same lot of Vancomycin HCl in Vancomycin Hydrochloride for Injection, USP 500mg/vial, 10ml as per analytical method were prepared. This solution along with diluent blank solution and system suitability solution was injected along with different chromatographic conditions like change in column lot, change in flow rate (± 0.2 ml/minute), and change in wavelength (± 2 nm).

Stability of Analytical Solution

System suitability solution and test solution of Vancomycin Hydrochloride for Injection, USP 500mg/vial, 10ml were prepared on 0^{th},12^{th}, 24^{th}, $36^{th,}$ and 48^{th} hour of experiment and stored these solutions at room temperature for every time interval up to 48 hrs and analyzed these solutions on 48 hrs with the freshly prepared test solution. The system suitability solution was prepared freshly at the time of analysis. The assay of Vancomycin Hydrochloride for Injection, USP 500mg/vial, 10ml in the sample was calculated. The analyte is considered stable if there is no significant change in % assay.

Results and Discussion

Method Development

Amongst the several trials for Vancomycin, the best result was obtained when buffer and Acetonitrile were used in the ratio of 80:20 at 235nm with a 1ml/min flow rate. The retention time was reported to be 10.156 minutes. The diluent used was a combination of acetonitrile and water in a ratio of 50:50. The chromatogram obtained for the optimized method is shown in Figure 2.

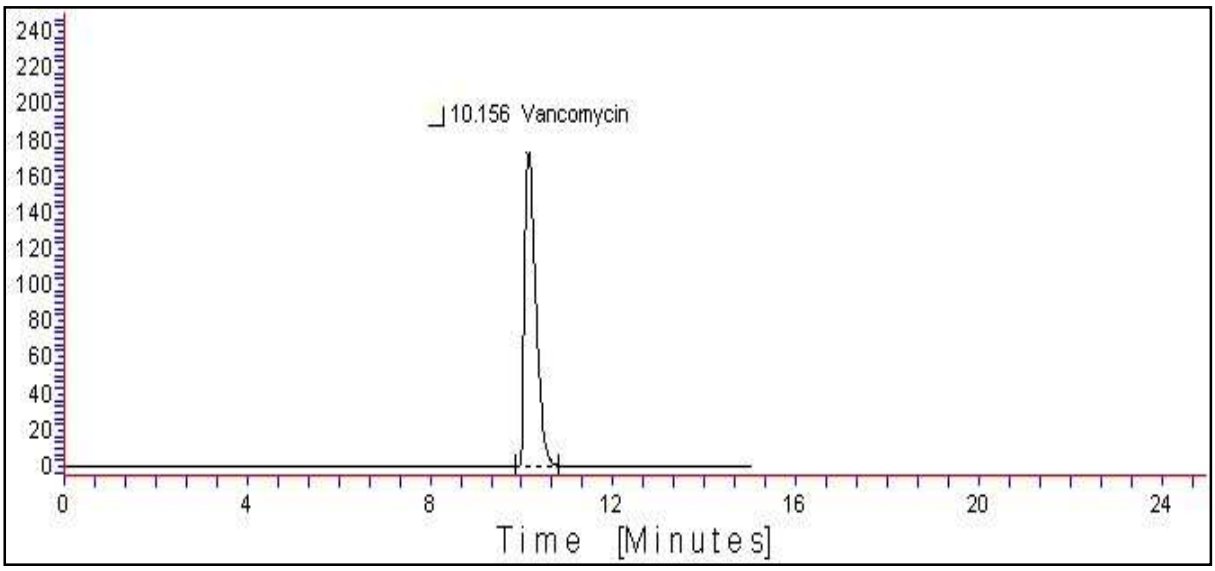

Figure 2: HPLC chromatogram of Vancomycin HCl

Method Validation

Specificity

Selectivity was performed by injecting the diluent blank solution, excipient blend, system suitability solution, and test solution. The Vancomycin HCl peak should be well resolved from any other peak and each other. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. All the injections were processed at the wavelength provided in the method. There was no interference observed from diluent blank solution, placebo with Vancomycin HCl peak. The method was found to be selective for vancomycin. Table 1 encapsulates the results for selectivity of the method for the analyte.

Table - 2: System suitability - Selectivity

Sr. No.	Area of Vancomycin HCl
1	2958.29
2	2968.93
3	2969.21
4	2957.03
5	2996.48
Mean	**2969.99**
Standard Deviation (±)	**15.88**
(%) Relative Standard Deviation	**0.53**

Linearity

For the linearity study, five standard solutions of Vancomycin HCl were prepared from the range starting from 50% to 150% of the theoretical concentration of assay preparation. The system suitability solution and the linearity solutions were injected as per the protocol. The linearity graph of concentration against peak response was plotted and the correlation coefficient was determined. The correlation coefficient should be greater than or equal to 0.999. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table-3 for system suitability- linearity results).

Table 3: System suitability - Linearity of standard

Sr. No.	Area of Vancomycin HCl
1	3127.946
2	3110.681
3	3144.525
4	3123.237
5	3158.738
Mean	**3133.03**
Standard Deviation (±)	**18.80**
(%) Relative Standard Deviation	**0.60**

The average peak area of Vancomycin HCl peak at each concentration level was determined and the linearity graph was plotted against the sample concentration in percentage. The results of the linearity study are as given in Table 4.

Table 4: Results of linearity of standard

Linearity Level	Sample Concentration (in %)	Sample Concentration(in ppm)	Peak Area	Correlation Coefficient
Level – 1	50	25.0	1516.38	**0.999**
Level – 2	75	37.5	2362.94	
Level – 3	100	50.0	3224.00	
Level – 4	125	62.5	4043.59	
Level – 5	150	75.0	4977.45	

The linearity plot of peak area of Vancomycin HCl Vs. standard concentration in percentage is presented in figure-2.

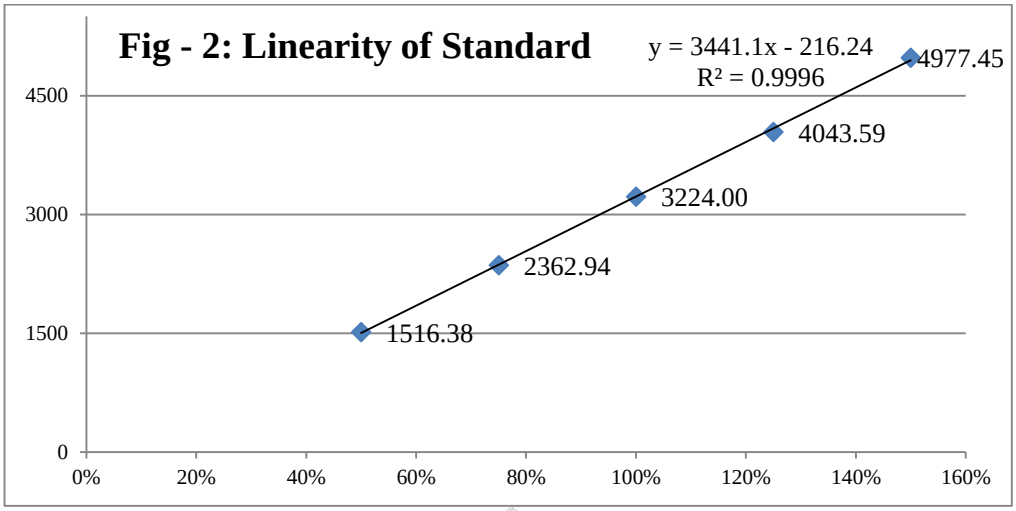

Figure 2: Linearity graph of Vancomycin HCl standard

A linearity graph of the average area at each level against the concentration (%) was plotted and was found to be a straight-line graph. The correlation coefficient was found to be more than 0.999. Hence it was concluded that the method is found to be linear in the range of 50% to 150% of the working concentration. The range for the analytical method was found to be 25 ppm to 75 ppm.

Precision:

Method Precision:

% RSD of the results of six test solutions should not be more than 2.0%. The system suitability criterion was found to meet the pre-established acceptance criteria as per the analytical method. The results of the assay obtained from six test solutions preparations are presented in Table – 5. The % RSD of the six assay results is found less than 2.0% and meets the pre-established acceptance criteria. Hence, it is concluded that the method is precise (Figure 3).

Table 5: Results of method precision

Test Solution	% Assay of Vancomycin HCl
1	99.82
2	100.16
3	100.73
4	100.17
5	100.52
6	100.47
Mean	**100.31**
Standard Deviation (±)	**0.33**
(%) Relative Standard Deviation	**0.32**

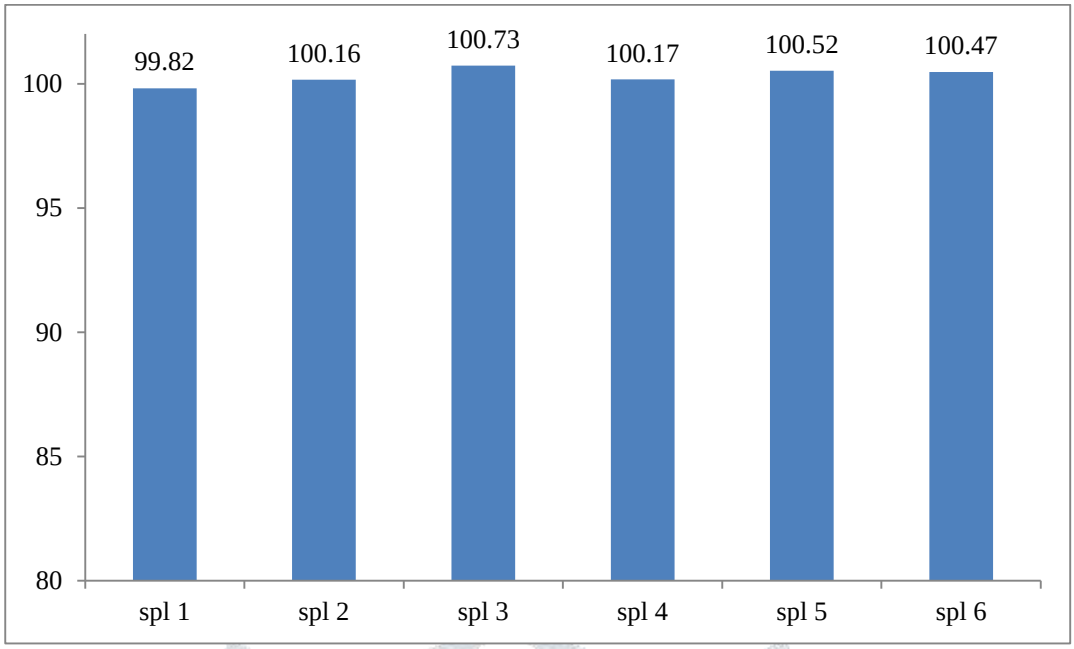

Figure 3: Comparative results of method precision

Intermediate Precision:

% RSD of the results of twelve test solutions (six of method precision and six of intermediate precision) should not be more than 2.0%. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table -6 for system suitability results). The results of assay obtained from six test solutions are presented in Table - 8. % RSD of assay results from method precision and intermediate precision (12 results) are presented in Table -8.

Table - 6: System suitability - Intermediate precision

Sr. No.	Area of Vancomycin HCl
1	2862.66
2	2866.40
3	2883.40
4	2861.91
5	2832.86
Mean	**2861.45**
Standard Deviation (±)	**18.20**
(%) Relative Standard Deviation	**0.64**

Table - 7: Results of intermediate precision

Test Solution	% Assay of Vancomycin HCl
1	99.77
2	102.28
3	98.86
4	99.61
5	99.67
6	99.65
Mean	**99.97**
Standard Deviation (±)	**1.18**
(%) Relative Standard Deviation	**1.18**

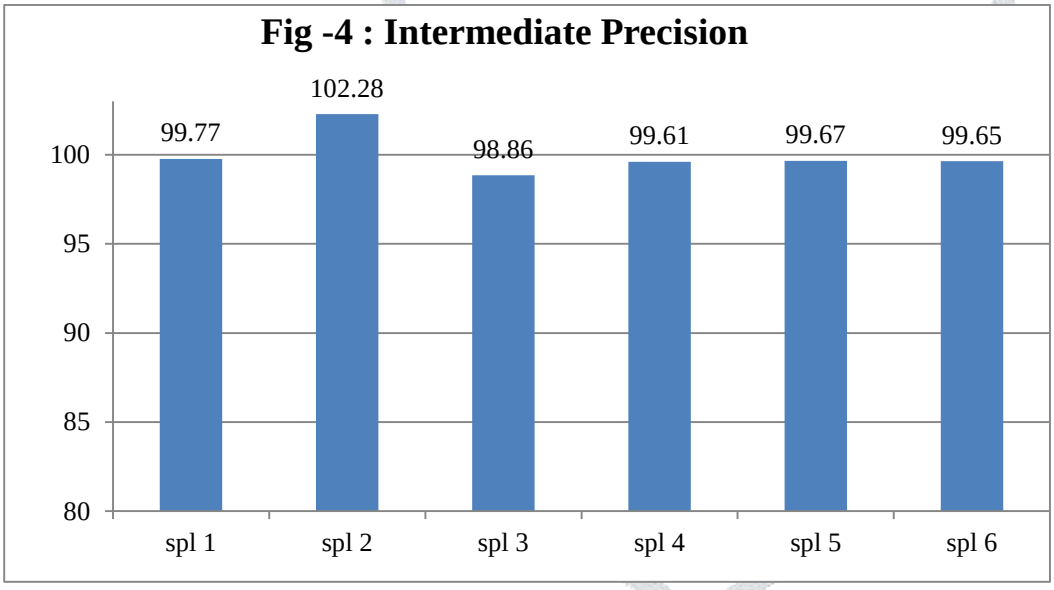

Figure 4: Comparative results of intermediate precision

Table 8: Results of twelve test solutions of Vancomycin HCl (six of method precision & six of intermediate precision)

Analysis performed during method precision study By Analyst 1 on system 1 and column 1 on day 1	
Same column	**% Assay of Vancomycin HCl**
1	99.82
2	100.16
3	100.73
4	100.17

5	100.52
6	100.47
Analysis performed during the intermediate precision study By Analyst 2 on system 2 and column 2 on day 2	
Column sr. no.	**015322030142 01**
Test Solution	**% Assay of Vancomycin HCl**
7	99.77
8	102.28
9	98.86
10	99.61
11	99.67
12	99.65
Mean of twelve samples	**100.14**
Standard Deviation (±)	**0.84**
(%) Relative Standard Deviation	**0.84**

The analysis was carried out on six test solutions of the same lot of the drug product by two different analysts using two different types of equipment within the same laboratory using two different columns of the same make but having different serial numbers on two different days. The % RSD of the twelve assay results (six of method precision and six from intermediate precision) is found to be less than 2.0 %. Thus, the method is found to be rugged and precise.

Robustness:

The analysis of the same lot of Vancomycin Hydrochloride for Injection, USP 500mg/vial, 10ml was carried out under different conditions of column lot, flow rate & wavelength. The system suitability was found to meet the pre-established criteria at all the conditions and the % RSD between results obtained with the changed condition and average result of method precision is not more than 2.0 %. Results for different chromatographic column conditions are enlisted in Tables 9-10 respectively.

Table - 9: System suitability - Robustness with change in Column

Sr. No.	**Area of Vancomycin HCl**	
	Same column	**Different column**
1	2977.06	2866.2
2	2957.68	2886.85
Mean	**2967.37**	**2876.53**
Standard Deviation (±)	**13.70**	**14.60**
(%) Relative Standard Deviation	**0.46**	**0.51**

The assay results obtained with the change in column are as given in Table 10.

Table - 10: Results for change in column

Flow rate →	**Same column**	**Different column**
Sample	**% Assay**	
Test solution	99.82	100.48
Average assay result from method precision	100.31	100.31
Mean	**100.61**	**100.40**
Standard Deviation (±)	**0.35**	**0.12**
(%) Relative Standard Deviation	**0.34**	**0.12**

Change in Flow Rate (± 0.2 mL/minute):

The normal Experimental Condition was a flow rate of 1.0ml/minute. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table – 11 for system suitability results).

Table - 11: System suitability - Robustness with change in flow rate

Sr. No.	**Area of Vancomycin HCl**	
	0.8 mL/minute	**1.2mL/minute**
1	3599.05	3011.65
2	3654.9	3021.6
Mean	**3626.97**	**3016.63**
Standard Deviation (±)	**39.49**	**7.04**
(%) Relative Standard Deviation	**1.09**	**0.23**

The assay results obtained with different flow rate conditions are as given in Table 12.

Table - 12: Results for change in flow rate

Flow rate →	**0.8 mL/minute**	**1.2mL/minute**
Sample	**% Assay**	
Test solution	101.55	99.94
Average assay result from method precision	100.31	100.31
Mean	**100.93**	**100.13**
Standard Deviation (±)	**0.88**	**0.26**
(%) Relative Standard Deviation	**0.87**	**0.26**

Change in Wavelength (± 2 nm)

The wavelength used for experimentation was 235nm. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table - 13 for system suitability results).

Table - 13: System suitability - Robustness with change in wavelength

Sr. No.	**Area of Vancomycin HCl**	
	233 nm	**237 nm**
1	3154.723	3071.84
2	3140.473	3054.43
Mean	**3147.60**	**3063.14**
Standard Deviation (±)	**10.08**	**12.31**
(%) Relative Standard Deviation	**0.32**	**0.40**

The assay results obtained with different wavelength conditions are given in Table - 14.

Table - 14: Results for change in wavelength

Wavelength	**228 nm**	**232 nm**
Sample	**% Assay**	
Test solution	101.65	100.49
Average assay result from method precision	100.31	100.31
Mean	**100.98**	**100.40**
Standard Deviation (±)	**0.95**	**0.13**
(%) Relative Standard Deviation	**0.94**	**0.13**

The analytical method meets the pre-established acceptance criteria for robustness study as per protocol. Thus, the method is robust.

Stability of Analytical Solution:

System suitability solution and test solution of Vancomycin Hydrochloride for Injection, USP 500mg/vial, 10ml were prepared on 0^{th},12^{th}, 24^{th}, $36^{th,}$ and 48^{th} hour of experiment and stored these solutions at room temperature for every time interval up to 48 hrs and analyzed these solutions on 48 hrs with the freshly prepared test solution. The analyte was considered stable if there is no significant change in % assay. The system suitability was found to meet the pre-established criteria and the % RSD between assay results obtained for freshly prepared test solution and the stored test solutions is less than 2.0%. There was no significant change in assay level observed up to 48Hrs for test solution at room temperature. Thus, it can be concluded that the solution is stable up to 48Hrs at room temperature. The assay results obtained during the solution stability experiment are given in Table- 16.

Table - 16: Results for solution stability

% Assay results calculated against the freshly prepared system suitability standard	
Sample	**% Assay of Vancomycin HCl**
0^{th} hr	101.90
12^{th} hr	101.50
24 hr	100.51
36 hr	98.37
48 hr	101.81
Mean	**100.82**
Standard Deviation (±)	**1.48**
(%) Relative Standard Deviation	**1.46**

Conclusion:

The analytical method of assay of Vancomycin HCl in Vancomycin hydrochloride for Injection, USP 500mg/vial, 10ml by HPLC was found to be suitable, selective, specific, precise, linear, accurate, and robust. The analytical solution is found to be stable up to 48 Hrs at room temperature. Hence, it is concluded that the analytical method is validated and can be used for routine analysis and stability study.

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