Brazilian Journal of Analytical Chemistry
2023, Volume 10, Issue 40, pp 170-181
doi: 10.30744/brjac.2179-3425.TN-80-2022

BrJAC

TECHNICAL NOTE

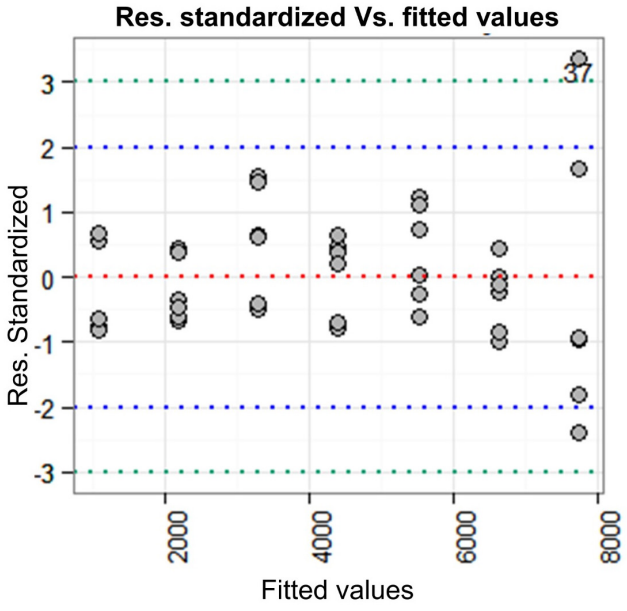
Development and Validation of a High Performance Liquid Chromatography Ultraviolet Detection Method for the Quantitative Determination of Vancomycin Hydrochloride

Priscilla Sete de Carvalho Onofre[1], Daniele Porto Barros[1], Gabriela Trindade de Souza e Silva[2], Fernando Luiz Affonso Fonseca[3], Paulo César Pires Rosa[2], Mavilde da Luz Gonçalves Pedreira[1], Maria Angélica Sorgini Peterlini[1]

[1]*Departamento de Enfermagem Pediátrica, Universidade Federal de São Paulo, Campus São Paulo, Rua Napoleão de Barros, 754, CEP 04024-002, São Paulo, SP, Brazil*

[2]*Faculdade de Ciências Farmacêuticas, Universidade Estadual de Campinas, Campinas, SP, Brazil*

[3]*Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, Campus Diadema, Diadema, SP, Brazil*



Vancomycin hydrochloride is a tricyclic glycopeptide that contains amino acids and sugars. This substance is indicated to treat serious infections caused by Gram-positive bacteria by intravenous infusion. The objective of this study was to develop and validate an analytical methodology by high performance liquid chromatography with ultraviolet detection (HPLC-UV) to determine vancomycin hydrochloride content by assessing the parameters of selectivity, linearity, working range, matrix effect, robustness, precision, and accuracy. The sample used was vancomycin hydrochloride in a vial and analyzes were carried out on HPLC-UV system with C18 reverse-phase column at 30 °C, pH=4 and diode-array detection (220 nm). The mobile phase was composed of acetonitrile and monobasic ammonium phosphate buffer (8:92 v/v), 1 mL min^{-1} flow rate, injection volume of 20 µL and 15 minute of run time. The method has been shown to be selective, free from mobile phase interference, diluent and other substances on vancomycin hydrochloride retention time; the method is linear in the range between 25 and 175 µg mL^{-1}; matrix effect showed parallelism between the lines, thus indicating the absence of interference of the matrix constituents in analysis of the

Cite: Onofre, P. S. C.; Barros, D. P.; Silva, G. T. S.; Fonseca, F. L. A.; Rosa, P. C. P.; Pedreira, M. L. G.; Peterlini, M. A. S. Development and Validation of a High Performance Liquid Chromatography Ultraviolet Detection Method for the Quantitative Determination of Vancomycin Hydrochloride. *Braz. J. Anal. Chem.* 2023, *10* (40), pp 170-181. http://dx.doi.org/10.30744/brjac.2179-3425.TN-80-2022

Submitted 28 August 2022, Resubmitted 07 February 2023, Accepted 11 February 2023, Available online 02 March 2023.

compound of interest; the method was robust with drug variations proportional to the deliberate changes caused by the change in the flow rate of the mobile phase and in the column temperature; the method showed accuracy at 25, 50, and 75 µg mL^{-1} concentrations, showing satisfactory recovery rate after addition of the standard. The analytical methodology described proved to be simple, fast, safe and was considered valid.

Keywords: validation studies, HPLC-UV, high performance liquid chromatography, vancomycin

INTRODUCTION

Vancomycin hydrochloride is a complex tricyclic glycopeptide drug that contains amino acids and sugars (Figure 1). This substance is indicated to treat serious infections caused by Gram-positive bacteria resistant to other lower potential antibiotics for both adults and child. This drug is mainly used to treat respiratory tract infections due to methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis,* as well as prophylaxis in major cardiac surgeries.[1-3]

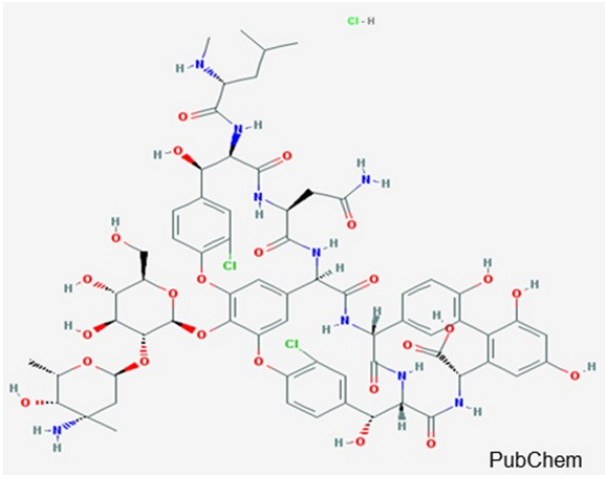


Figure 1. Chemical structure of vancomycin hydrochloride.

Analytical methods for the determination of vancomycin hydrochloride stability are described in several international compendiums using techniques such as high-performance liquid chromatography with ultraviolet detection (HPLC-UV), capillary electrophoresis, mass spectrometry, polarized immunofluorescence, radioimmunoassay, among others.[4-6]

The liquid chromatography is an appropriate method due to its relative low cost when compared with other techniques, and mainly due to the ease and speed in separating, identifying and quantifying the analyzed compounds. The HLPC has been used for both dosing this antibiotic in the product and in biological fluids.[4-6] Until now, this is the most widely used antimicrobial determination procedure, and it is even the official method for purity determination by the British Pharmacopeia and United States Pharmacopeia (USP).[7-9]

The choice for the appropriate analytical methodology is of fundamental importance for the quality control procedure of the active substance or pharmaceutical form. Thus, for the use of the analytical method or its adaptation, it is necessary to perform a validation study, to guarantee the efficiency in pharmacological analyses in routine uses, with reliable information about the sample.[10]

The validation of an analytical methodology produces evidence to confirm the proposed technique as reliable for what it applies, constituting a series of procedures, ensuring credibility to the measures obtained. The main purpose of this validation is to show that the analytical method is suitable for its purpose.[9-10]

The Brazilian Health Regulatory Agency (ANVISA), in the Collegiate Board Resolution (RDC) No. 166 of 2017,[11] defines the objective of validating active compounds as the demonstration that the method follows the analytical premises. The validation must ensure reliability in the results to which it is proposed, whether quantitative or qualitative determination of drugs.

The objective of this study was to develop and validate a fast and reliable analytical methodology by HPLC-UV, considering the parameters recommended by the RDC 166/2017 from ANVISA, to determine vancomycin hydrochloride content.

MATERIALS AND METHODS

Equipment and materials

The instruments and materials used in the experiment were: a high performance liquid chromatograph system with an ultraviolet detector (Agilent Technologies®-1260 Infinity Series HPLC Modular), an autosampler, a diode-array detector (DAD), a 4.6 x 250 mm C18 reverse-phase analytical column, particle size 5 µm (Waters®- Spherisorb ODS 2 Hypersil and Thermo Scientific®- ODS Hypersil), analytical balance (Shimadzu®- AUY220, Kyoto, Japan); 0.45 µm pore-coated polyvinylidene fluoride (PVDF) membrane (MF-Millipore®, type HAWP, Darmstadt, Germany); 13 mm diameter Nylonmembrane syringe filter with 0.45 µm pore size (Allcrom®-Membrane Solutions); Millipore filtration system for HA organic solvents, 0.45 µm; benchtop digital pH meter (Kasvi® K39-2014B, Curitiba, Brazil); magnetic stirrer (Warmnest® CJ-882A, Zhejiang, China); ultrasonic washer (Ultronique®—Eco-sonics, Indaiatuba, SP, Brazil); vacuum pump (Prismatec® 121-Type 2 VC, Itu, SP, Brazil) and type 1 ultrapure water purification system (Direct Q 3®-Merck Millipore®, Massachusetts, USA).

Reagents, drug, reference standard and solvents

The HPLC grade organic solvent acetonitrile (Carlo Erba®, Val-de-Reuil, France) was used to prepare the solutions; the following substances were also used: phosphoric acid 85.0% PA- ACS (Synth®, Diadema, SP, Brazil); monobasic ammonium phosphate (Sigma-Aldrich, Vetec®, Duque de Caxias, RJ, Brazil); Vancomycin hydrochloride equivalent to 500 mg in vial presentation as a lyophilized powder (Vancocina® CP - ABL Antibiotics from Brazil, Cosmópolis, SP, Brazil); distilled water (DA) in a 10 mL plastic ampoule (Isofarma Industrial Pharmaceutical Ltda, Eusébio, CE, Brazil); standard of vancomycin hydrochloride of USP considered a primary reference (USP, United States of America, 98.8% vancomycin content USP Catalog No. 1709007) and 0.9% Sodium Chloride (NaCl) (JP Indústria Farmacêutica SA, Ribeirão Preto, SP, Brazil).

Preparation of the mobile phase and standard solution

The mobile phase (MP) was prepared by combining monobasic ammonium phosphate (5 mg mL^{-1}) in deionized water and mixing this solution in a ratio of 92% to 8% of acetonitrile.

Subsequently, a 1 mg mL^{-1} solution of standard vancomycin hydrochloride solution in MP was prepared. The solution was homogenized and solubilized in an ultrasonic washer. Based on this solution, an aliquot for the preparation of 0.1 mg mL^{-1} solution was filtered and analyzed by chromatographic method.

Sample preparation

The drug vancomycin hydrochloride (500 mg) was reconstituted in 10 mL of DA (50 mg mL^{-1}) in the vial itself and then diluted in 0.9% NaCl solution at 5 mg mL^{-1}. Then this solution was diluted in a concentration of 0.1 mg mL^{-1}.

Chromatographic conditions

Chromatographic analyses were conducted in isocratic mode; with the column temperature set at 30 °C and DAD configured at 220 nm of wavelength, 1 mL min^{-1} flow rate, 20 µL injection volume and 15 min analytical run time. The quantification of vancomycin hydrochloride was performed by external standardization.

Parameter determination for method validation

The validation of the chromatographic method for the analysis of vancomycin hydrochloride concentrations was performed following the recommended guidelines for analytical methods regarding the selectivity, linearity, working range, matrix effect, robustness, precision and accuracy as required by the RDC 166/2017 of ANVISA.[11]

Selectivity

As for the assessment of the selectivity parameter, MP chromatograms, reference standard, vancomycin hydrochloride solution and sample diluent, consisting of 0.9% NaCl solution, were compared, as well as the purity signal.[11]

Matrix effect

In this study, five different concentrations were used in triplicate. Starting from the vancomycin hydrochloride solution prepared at 5,000 μg mL^{-1}, 0.2 mL aliquots were removed with micropipette and transferred to 10 mL volumetric flasks. Then the standard volumes were added to these flasks for the preparation of solutions in the range of 25–125 μL. The two resulting curves were compared and assessed by the *t*-test.

Linearity and linear range

The linearity was determined based on a mathematical relationship of the analyte average area for each aliquot and its respective theoretical concentration, thus obtaining an analytical curve by the linear equation y = ax + b, the coefficients a and b being estimated by linear regression. Therefore, three analytical curves were analyzed in the concentration range of 25–175 μg mL^{-1} (seven points) from a stock solution at 1 mg mL^{-1}, diluted with MP.

Area results were assessed visually at first, followed by the least squares method, preceded by the Cochran test, followed by residue analysis.[11]

Robustness

This test was elaborated to assess the ability of the chromatographic method to resist small changes in analytical parameters, thus the analytical column temperature of 28 °C and 32 °C and the MP flow of 0.8 mL min^{-1} and 1.2 mL min^{-1} were modified. In order to assess the impact of these changes, the following parameters were evaluated: peak purity of the compound of interest, retention factor (K) and resolution (RS).[9,11] Therefore, Equations 1 and 2 were used.

$$K = t\,c/t\,s \qquad \text{Equation 1}$$

where:
tc = time required by the sample components
ts = time required by the eluent

$$Rs = \frac{2(tr1 - tr2)}{w1 + w2} \qquad \text{Equation 2}$$

where:
tr1 = retention time of the sample
tr2 = retention time of the peak prior to the analyte of interest
w1 = peak width of the analyte of interest
w2 = peak width prior to the analyte of interest

Precision

In the intraday accuracy study, nine replicates of individually prepared a 100 µg mL^{-1} concentration were used. The samples were assessed under the same operating conditions, same analyst and same instrumentation, in a single analytical run.

In order to identify possible variations of the data collection site due to analyses performed on different days or by different analysts, the intermediate precision or reproducibility analysis was performed. In this test, different samples were assessed on different non-consecutive days.

Accuracy

The accuracy (recovery) test was performed by comparing the results achieved in the analysis of vancomycin hydrochloride solution samples with the results obtained by analyzing samples containing known standard concentrations.

The quantities of 25, 50 and 75 µg were added to the standard sample solution. These quantities are considered low, medium and high concentration measurements, respectively, and they were analyzed in three replicates of each level. The recovery factor (% R) was calculated by subtracting the concentration determined in the added sample (C_b) from the concentration determined in the non-added sample (C_a), then the result was divided by the added concentration (C_c), and finally the value was multiplied by 100, as shown in Equation 3.[11]

$$\%R = \frac{(Cb - Ca)x\ 100}{Cc} \qquad \text{Equation 3}$$

where:
C_a= concentration determined in the non-added sample
C_b= concentration determined in the added sample
C_c= added concentration

RESULTS AND DISCUSSION

This is an experimental study to develop and validate the analytical methodology for quantification of vancomycin hydrochloride.

The analytical strategy of validation and the analysis steps were conducted according to ANVISA RDC 166/2017[11] and the analytical performance parameters determined by experiments were: selectivity, linearity, working range, matrix effect, robustness, accuracy, and precision appropriate to the analysis.

The performance of the analysis tests provided satisfactory results in relation to the validation criteria of the proposed analytical methods.

The study was conducted at the Laboratory of Nursing Experiments (Laboratório de Experimentos de Enfermagem – LEEnf), Paulista Nursing School, Federal University of São Paulo, SP, Brazil.

In the selectivity or specificity test, no substances eluting near or at the same retention time of the test compound were detected. The compound peak was shown as high purity. Figure 2 shows the chromatograms analyzed.

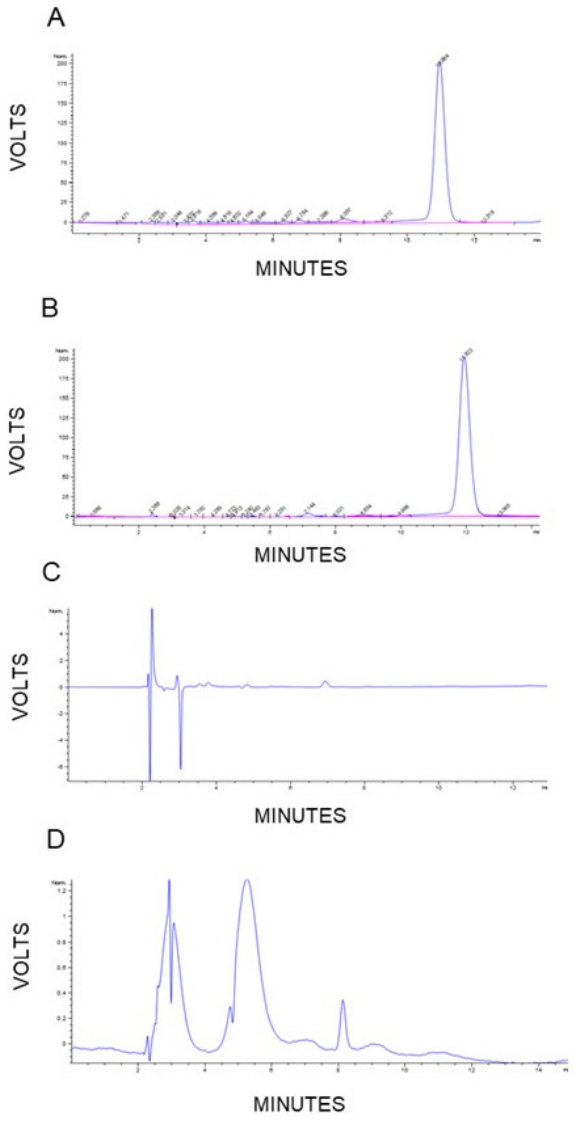


Figure 2. A. Chromatographic separation of vancomycin hydrochloride **sample**; **B.** Chromatographic separation of the vancomycin hydrochloride reference **standard**; **C.** Chromatographic analysis of diluent (0.9% sodium chloride solution) **D.** Mobile Phase chromatographic analysis report. Analyses performed in the isocratic mode method, C18 column at 30 °C, 220 nm wave, with Mobile Phase at a ratio of 92% buffer-solution and 8% acetonitrile, at a flow rate of 1 mL min^{-1} and 20 µL of injection volume.

The chromatograms in Figure 2 present that at the retention time of the vancomycin hydrochloride solution, no interfering substances were observed to elute at or near the standard time (Figure 2 C and D).

The investigation of selectivity conducted in this study was able to confirm that MP and diluent related to vancomycin hydrochloride standard do not have structurally similar components that could be present during the analyses at the same chromatographic peak. The presence of this peak was only verified in the drug solution.[11]

In the chromatograms analyzed, the vancomycin hydrochloride peak was unique in its retention time, with a gaussian distribution peak, and no other degradation or impurity peak was observed in the peak vicinity, therefore the method can be considered selective for the tested conditions.

Thus, seven duplicates of the vancomycin hydrochloride standard concentrations were analyzed for each of the three curves performed for the linearity assay. Table I shows the analytical curves of the method with the respective coefficients of determination (R^2) using the DAD ultraviolet detector in the concentration range 25–175 µg mL^{-1} (n=7 points).

Table I. Analytical curves (y=ax+b) for vancomycin hydrochloride determination by HPLC-UV in the linear range of 80% to 120%

Replicates	Analytical curves	R^2
Replicate 1	y = 45.544x – 147.66	0.9993
Replicate 2	y = 43.927x + 43.169	0.9997
Replicate 3	y = 43.983x – 12.339	0.9995
Resulting curve	y = 44.436x – 34.75	0.9999

Note: y = peak area of interest; x = concentration in μg mL^{-1}; R^2 = coefficient of determination.

Figure 3 shows the overlap of the curves obtained by the linearity study as well as the residual graph.

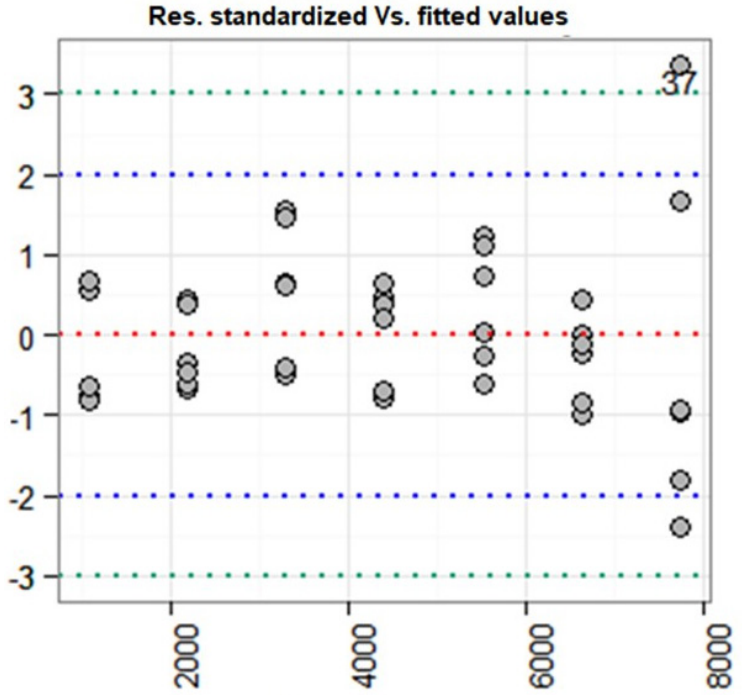


Figure 3. Graph of the residual *versus* adjusted values.

Linearity corresponds to the ability of the method to provide results directly proportional to the concentration of the analyzed substance. This linearity is obtained when the signal measured by each concentration of the analyte of interest is correlated, and the linear relationship is verified based on the mathematical treatment of the linear regression.[9,11] Thus, Cochran's test was carried out to assess the variance of experimental errors undergoing visual analysis of the residual graph (Figure 3). The calculated value for this test was smaller than the tabulated ones, then the variance of experimental errors (y values) are equal; the dataset therefore is homoscedastic. This observation coincides with the visual analysis of the graph, the variance seems constant, consequently, the dataset can be assessed by linear regression analysis.

Accordingly, in the mathematical model for linear regression analysis of the interval calibration curve proposed for the study, the R^2 was calculated (and it was shown within the acceptable value) proving

that the method is linear, as presented in Table I. Data corroborate ANVISA Resolution 166/2017,[11] which states that R^2 must be equal to or higher than 0.99; so, the method can be considered linear.

The matrix effect was measured by comparing the angular coefficients of the calibration curves and the fortified sample curve at five points of standard analysis (between 25–125 µg mL^{-1}) within the linear working range established by the linearity curve.

In Figure 4, parallelism between the lines can be observed, thus indicating the absence of interference of the matrix constituents in the analysis of the compound of interest.[11]

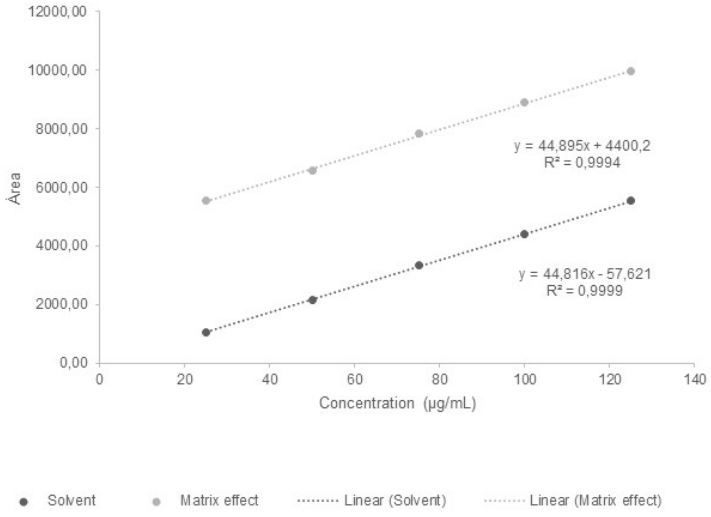


Figure 4. Curve obtained by the matrix effect in parallel to the linearity curve.

The robustness test was assessed by performing experiments with different chromatographic conditions by changing the MP flow rate and the column temperature. Those changes follow the recommendation of ANVISA RDC 166/2017.[11] Table II shows the results of the analyzed parameters.

Table II. Robustness of the vancomycin hydrochloride method according to variations in mobile phase flow and column temperature in relation to the original analysis conditions

Analysis parameter	Proposed method	MP flow rate 0.8 mL min^{-1}	MP flow rate 1.2 mL min^{-1}	Temperature 28 °C	Temperature 32 °C
Retention factor (K)	3.6	3.9	4.0	4.3	3.9
Previous Peak Resolution (RS)	2.3	3.0	2.1	2.6	2.3
Posterior Peak Resolution (RS)	4.0	4.7	4.0	3.5	2.8
Retention time (RT)	8.72	11.65	7.95	10.42	9.4
Purity	999.999	999.97	999.976	999.973	999.969

Note: MP = Mobile Phase.

The method is considered robust when the results of small deliberate changes caused by the analytical parameters of the method during the robustness test indicated that the sample varies proportionally with the programmed conditions and does not affect the performance of the analytical methodology.[9,11-12] In this

analysis, in the case of vancomycin hydrochloride, the K factor must present better resolution than the other peaks, with a value higher than 2. For the system under study was found based on Equation 1 the lowest K value equal to 3.9 (Table II).

The degree of separation quantification RS is considered robust when the value is higher than 2[9] corroborating the study under analysis, which presented RS of 2.1, calculated based on Equation 2, with a maximum retention time of 11.6 minutes. Thus, the mobile phase selected for validation even with the small modifications in the chromatographic conditions showed good resolution and within 15 minutes of running (Table II).

The purity of the peak was stable in relation to the purity presented in the proposed method (Table II). According to the robustness test premise, the small variations caused do not affect the analytical procedure.

In this study, in the precision test to verify the single-day repeatability, nine replicates at 100% of the test concentration individually prepared were used. The samples were assessed under the same operating conditions, single analyst and same instrumentation, in a single analytical run and with three injections each.[11]

The theoretical concentration was estimated based on the analytical curve equation and was expressed in μg mL^{-1}. The results obtained with this test are presented in Table III.

Table III. Intra-run accuracy test (intermediate test)

Replicates (sample)	Average sample areas (intermediate test)	Standard Deviation	CV (%)	Estimated theoretical concentration (μg mL^{-1})
1	4574.80	3.12	0.07	103.73
2	4574.80	3.12	0.07	103.73
3	4725.27	10.89	0.23	107.12
4	4451.54	34.18	0.77	100.96
5	4417.67	12.70	0.29	100.20
6	4442.59	40.34	0.91	100.76
7	4451.54	34.18	0.77	100.96
8	4417.67	12.70	0.29	100.20
9	4442.59	40.34	0.91	100.76
Overall average concentration				4499.8
Overall standard deviation				104.2
Relative standard deviation (%)				2.3

Note: CV = Coefficient of variation.

As to the intra-run accuracy, a satisfactory result expressed by CV 0.91% was obtained, remaining below the acceptable limit value of 5%. The relative standard deviation (RSD) value found in the experiment was below 5% (Table III), indicating the accuracy of the method, thus corroborating the limits expressed in compliance with the analytical validation rules of the ANVISA.[11]

Table IV. Inter-run accuracy test

Replicates (sample)	Average sample areas
1	4602.40
2	4504.50
3	4503.10
4	4498.00
5	4495.10
6	4496.20
7	4495.00
8	4501.50
9	4497.00
Overall average concentration	4510.31
Overall standard deviation	34.71
Relative Standard Deviation (%)	0.77

The intermediate precision was assessed by analyzing vancomycin hydrochloride solutions at a concentration of 100 μg mL^{-1}, on different and non-consecutive days, obtaining RSD of 0.8%. These results show that the method provides results with acceptable accuracy on different days of analysis and involving two distinct analysts (Table IV).

In the accuracy test for 25 μg mL^{-1}, 50 μg mL^{-1}, and 75 μg mL^{-1} concentrations, it was presented for each theoretical concentration: mean replica areas, relative standard deviation, and recovery (Table V).

Table V. Data obtained for accuracy test evaluation

Theoretical standard of vancomycin concentration added (μg mL^{-1})	Experimental vancomycin concentration (μg mL^{-1})	Mean of the area	Relative Standard Deviation	Recovery (%)
125	122.90	5535	0.107	99.9
150	145.13	6585	0.062	99.6
175	172.55	7829	0.284	101.3

The accuracy of the method was determined by studying the original matrix recovery by adding a known amount of the reference standard used for the study of vancomycin hydrochloride.

According to the ANVISA Resolution RDC 166/2017,[11] the standard deviation in the accuracy test should not exceed 15%, a premise met at the three concentrations studied, therefore, the accuracy of the method being validated was ensured.

The high percentages obtained in the recovery (near 100%) indicate the accuracy of the method. Regarding the RSD, the acceptable or true value is 5%, indicating that the assay is within the established recommendations (Table V).[11]

CONCLUSIONS

The method developed and validated according to ANVISA RDC 166/2017[11] parameters for the determination of vancomycin hydrochloride by HPLC-UV was simple, fast and efficient, and it can be safely applied to the analysis of solution containing vancomycin hydrochloride.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The researchers thank the members of the SEGTEC Group (Brazil) for their support during data collection, the “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES) for providing the PhD scholarship to Priscilla Sete de Carvalho Onofre, and the “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq), for granting research support [No. 462183/2014-9].

REFERENCES

(1) Costenaro, P.; Minotti, C.; Cuppini, E.; Barbieri, E.; Giaquinto, C.; Donà, D. Optimizing Antibiotic Treatment Strategies for Neonates and Children: Does Implementing Extended or Prolonged Infusion Provide any Advantage? *Antibiotics* **2020**, *9* (6), 329. https://doi.org/10.3390/antibiotics9060329

(2) Rybak, M. J.; Le, J.; Lodise, T. P.; Levine, D. P.; Bradley, J. S.; Liu, C.; Mueller, B. A.; Pai, M. P.; Wong-Beringer, A.; Rotschafer, J. C.; Rodvold, K. A.; Maples, H. D.; Lomaestro, B. M. Therapeutic monitoring of vancomycin for serious methicillin-resistant Staphylococcus aureus infections: A revised consensus guideline and review by the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the Society of Infectious Diseases Pharmacists. *Am. J. Health-Syst. Pharm*. **2020**, *77* (11), 835–864. https://doi.org/10.1093/ajhp/zxaa036

(3) McNeil, J. C.; Kaplan, S. L.; Vallejo, J. G. The Influence of the Route of Antibiotic Administration, Methicillin Susceptibility, Vancomycin Duration and Serum Trough Concentration on Outcomes of Pediatric Staphylococcus aureus Bacteremic Osteoarticular Infection. *The Pediatric Infectious Disease Journal* **2017**, *36* (6), 572–577. https://doi.org/10.1097/INF.0000000000001503

(4) Barco, S.; Mesini, A.; Barbagallo, L.; Maffia, A.; Tripodi, G.; Pea, F.; Saffioti, C.; Castagnola, E.; Cangemi, G. A liquid chromatography-tandem mass spectrometry platform for the routine therapeutic drug monitoring of 14 antibiotics: Application to critically ill pediatric patients. *J. Pharm. Biomed. Anal*. **2020**, *186*, 113273. https://doi.org/10.1016/j.jpba.2020.113273

(5) Lima, T. M.; Seba, K. S.; Gonçalves, J.; Cardoso, F.; Estrela, R. A Rapid and Simple HPLC Method for Therapeutic Monitoring of Vancomycin. *J. Chromatogr. Sci*. **2018**, *56* (2), 115–121. https://doi.org/10.1093/chromsci/bmx089

(6) Masse, M.; Genay, S.; Martin, M. A.; Carta, N.; Lannoy, D.; Barthélémy, C.; Décaudin, B.; Odou, P. Evaluation of the stability of vancomycin solutions at concentrations used in clinical services. *Eur. J. Hosp. Pharm*. **2020**, *27* (e1), e87–e92. https://doi.org/10.1136/ejhpharm-2019-002076

(7) Parker, S. L.; Guerra, V. Y. C.; Ordóñez, M. J. L.; Roger, C.; Lipman, J.; Roberts, J. A.; Wallis, S. C. An LC–MS/MS method to determine vancomycin in plasma (total and unbound), urine and renal replacement therapy effluent. *Bioanalysis* **2017**, *9*, 911-24. https://doi.org/10.4155/bio-2017-0019

(8) Zhang, M.; Moore, G. A.; Young, W. Determination of vancomycinin human plasma, bone and fat by liquid chromatography/tandem mass spectrometry. *J. Anal. Bioanal. Tech*. **2014**, *5*, 1-9. https://doi.org/10.4172/2155-9872.1000196

(9) United States Pharmacopeia Convention, 2019. The national formulary. USP 32nd Rev., NF 27th Ed. Rockville (MD). Available at: https://www.uspnf.com/official-text/proposal-statuscommentary/usp-32-nf-27 (accessed 2022-06-05).
(10) Ribani, M.; Bottoli, C. B. G.; Collins, C. H.; Jardim, I. C. S. F.; Melo, L. F. C. Validação em métodos cromatográficos e eletroforéticos. *Quim. Nova* **2004**, *27* (5), 771-80. https://doi.org/10.1590/S0100-40422004000500017
(11) Ministério da Saúde, Agência Nacional de Vigilância Sanitária. Resolução RDC No 166. Dispõe sobre a validação de métodos analíticos e dá outras providências. *Diário Oficial da União*, 25 de julho 2017. Available at: https://www.in.gov.br/materia/-/asset_publisher/Kujrw0TZC2Mb/content/id/19194581/do1-2017-07-25-resolucao-rdc-n-166-de-24-de-julho-de-2017-19194412 (accessed 2022-04-20).
(12) International Conference on Harmonisation (ICH). ICH Expert Working Group. Harmonised tripartite guideline. Validation of analytical procedures: text and methodology Q2(R1). In: International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. 2005 Nov. Geneva. https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf