UNIVERSIDADE FEDERAL DE SANTA CATARINA
CENTRO DE CIÊNCIAS DA SAÚDE
DEPARTAMENTO DE PATOLOGIA
CURSO DE FARMÁCIA

Eliza Bianchini de Paula

VALIDAÇÃO E IMPLANTAÇÃO DE UM MÉTODO PARA MONITORAMENTO TERAPÊUTICO DE VANCOMICINA EM UM HOSPITAL UNIVERSITÁRIO

Florianópolis - SC
2023

Eliza Bianchini de Paula

VALIDAÇÃO E IMPLANTAÇÃO DE UM MÉTODO PARA MONITORAMENTO TERAPÊUTICO DE VANCOMICINA EM UM HOSPITAL UNIVERSITÁRIO

Trabalho de Conclusão de Curso submetido ao curso de Farmácia do Centro Ciências da Saúde Campus Reitor João David Ferreira Lima da Universidade Federal de Santa Catarina como requisito parcial para a obtenção do título de Bacharel em Farmácia.

Orientador(a): Prof.(a) Dr.(a) Claudia Regina dos Santos

Florianópolis - SC

2023

Paula, Eliza Bianchini de
VALIDAÇÃO E IMPLANTAÇÃO DE UM MÉTODO PARA MONITORAMENTO TERAPÊUTICO DE VANCOMICINA EM UM HOSPITAL UNIVERSITÁRIO / Eliza Bianchini de Paula ; orientadora, Claudia Regina dos Santos, 2023.
23 p.

Trabalho de Conclusão de Curso (graduação) - Universidade Federal de Santa Catarina, Centro de Ciências da Saúde, Graduação em Farmácia, Florianópolis, 2023.

Inclui referências.

1. Farmácia. 2. Validação de Método. 3. Monitoramento Terapêutico. 4. Vancomicina. I. Santos, Claudia Regina dos. II. Universidade Federal de Santa Catarina. Graduação em Farmácia. III. Título.

Eliza Bianchini de Paula

VALIDAÇÃO E IMPLANTAÇÃO DE UM MÉTODO PARA MONITORAMENTO TERAPÊUTICO DE VANCOMICINA EM UM HOSPITAL UNIVERSITÁRIO

Este Trabalho de Conclusão de Curso foi julgado adequado para obtenção do título de Farmacêutico generalista e aprovado em sua forma final pelo Curso Farmácia

Florianópolis, 23 de junho de 2023.

_____

Coordenação do Curso

Banca examinadora

_____

Prof.a Claudia Regina dos Santos, Dra.

Orientador(a)

_____

Prof. - Filipe Carvalho Matheus, Dr.

Universidade Federal de Santa Catarina

_____

Prof.a Camila Marchioni, Dra.

Universidade Federal de Santa Catarina

Florianópolis, 2023

AGRADECIMENTOS

Agradeço a Deus, fonte da minha paz e da minha alegria, quem me trouxe até aqui. Agradeço a minha família, meu pai e minha mãe pelo apoio e pelo sustento nesses últimos anos, sustento espiritual, emocional e também financeiro. Agradeço a todos os meus amigos e todos aqueles que lá de Blumenau lembraram, oraram por mim ou mandaram uma mensagem de apoio, e também os amigos que fiz por aqui que me mantiveram no foco, me acolheram e me socorreram em alguns momentos. Agradeço a Lauren, amiga especial, que nos momentos de desabafo, nos momentos de angústia ou indignação estava ali para conversarmos. Agradeço também a MUNIL e ao meu PG que tornaram as minhas semanas mais leves, mais tranquilas me fizeram lembrar do meu propósito e da minha missão. Assim também agradeço todos aqueles que trabalharam comigo ao longo desse processo e tornaram esse trabalho possível, minha orientadora profª Claudia e meus colegas do LPTOX que me ajudaram muito Janer, Liana e profª Camila. Tenho a honra de ter a todos vocês como parte da minha história, obrigada!

O meu corpo e o meu coração
poderão fraquejar,
mas Deus é a força do meu coração
e a minha herança para sempre.
Salmos 73:26

SUMÁRIO

Este Trabalho de Conclusão de curso é apresentado na forma de artigo científico que será submetido para publicação na revista **Brazilian Journal of Pharmaceutical Sciences (BJPS)**, cujas instruções aos autores podem ser encontradas na página <https://s3-us-west-2.amazonaws.com/clarivate-scholarone-prod-us-west-2-s1m-public/wwwRoot/prod4/societyimages/bjps-scielo/Guideline-guia%20autores2023.pdf> e no anexo I.

ISSN (online): 2175-9790
Qualis Capes para Farmácia: B2

VALIDATION AND IMPLEMENTATION OF A METHOD FOR VANCOMYCIN THERAPEUTIC MONITORING IN A UNIVERSITY HOSPITAL

Eliza Bianchini de Paula[1], Claudia Regina dos Santos[2]

[1]College of pharmacy, Federal University of Santa Catarina, Florianopolis, Santa Catarina, Brazil
[2]Patology department, Federal University of Santa Catarina, Florianopolis, Santa Catarina, Brazil

Vancomycin is a hospital antibiotic used in Gram+ bacteria infection. The recommended plasma concentration is 15 - 20 µg/mL, values above can cause nephrotoxicity and ototoxicity. This, added to the intra-individual pharmacokinetic variations, makes it an excellent candidate for therapeutic monitoring, which, through the measurement and interpretation of plasma levels, ensures effective and safe individualized doses. So, this study is a method validation for the determination of vancokinemia by high performance liquid chromatography with a UV detector and the application in a Santa Catarina - Brazil hospital. The method obtained linearity between 5 – 100 µg/mL with a Lower Limit of Quantitation of 5 µg/mL and Limit of Detection of 1.45 µg/mL, presented adequate repeatability, intermediate precision, intraday/interday accuracy according to legislation and no matrix effect. Samples were stable for 24 hours at room temperature, 48 hours post-processing, up to 8 months stored and up to 2 freeze/thaw cycles. The method was applied to four patients, with 12 determinations performed, 66% indicated the need for a change in dose. In conclusion, the method was successfully validated and applied in the hospital, with challenges to be resolved in the continued use of the method.

Keywords: Vancokinemia. Method validation. Dose adjustment.

INTRODUCTION

Vancomycin is a glycopeptide potent antibiotic, for hospital use, used in the treatment of gram-positive bacteria infection, mainly methicillin-resistant Staphylococcus aureus. Bacterial strains are considered sensitive to vancomycin when the Minimum Inhibitory Concentration (MIC) is above 4 µg/mL, knowing that it is desirable that the minimum serum concentration is 4 times above the MIC, that is close to 16 µg/mL (Bertoluci, 2007), the plasma concentration recommendation at the time of lowest plasma concentration (valley) is 15 to 20 µg/mL (Mulubwa et al., 2020).

However, plasma values above 20 µg/mL can cause adverse effects such as nephrotoxicity to the patient (Filippone, Kraft and Farber, 2017). Several studies present oxidative stress as the mechanism of vancomycin nephrotoxicity in proximal tubules. Still according to Filippone, Kraft and Farber (2017), nephrotoxicity is defined when there is an increase of 50 µg/dL or 50% in the patient's serum creatinine compared to the baseline dose in two or more consecutive measurements after a few days of treatment. According to Elyasi, Khalili, Dashti-Khavidaki and Mohammadpour (2012), renal

toxicity by vancomycin was observed in 10-20% of patients after conventional therapy and 30-40% in high doses of vancomycin. According to the same study, doses above 4 g/day or therapy for more than 7 days and concomitant use with other nephrotoxic agents are also risk factors for the development of nephrotoxicity. Added to this, concentrations above 30 µg/mL can lead to ototoxicity. This adverse effect is rare but irreversible, possible risk factors for this adverse effect are age over 53 years, exposure for more than two weeks and concomitant use with other ototoxic drugs (Uda et al., 2019).

In addition to these risks, there are patient intra-individual pharmacokinetic variations that may interfere with the absorption, distribution, biotransformation and excretion of the drug. After administration, the molecule is 50% bound to plasma proteins, however, according to Cusumano et al. (2020) elderly and very sick patients may have reduced protein binding. In addition, the elimination half-life varies from 3 to 6 h with normal renal function and is excreted unchanged in the urine (Filippone; Kraft; Farber, 2017), predominantly by glomerular filtration, so it is known that in patients with anuria, the half-life can reach 7.5 days. As for the volume of distribution (VD) of the drug, it is 0.4 to 1 L/kg (Goti et al., 2018), and in elderly, obese or critically ill patients, VD values may be increased (Cusumano et al., 2020). According to Oliveira et al (2020), patients with renal insufficiencies are the major challenge because physiological changes significantly affect antimicrobial pharmacokinetics, reaching inadequate drug concentrations in plasma.

Thus, there is a narrow therapeutic window for treatment to avoid therapeutic failure, reduce bacterial resistance and avoid nephrotoxicity and ototoxicity. These factors, added to the pharmacokinetic intra-individual variability of the drug, make therapeutic monitoring (TM) an important tool to individualize the dose and ensure effective treatment.

For proper monitoring, 3 phases must be well suited, pre-analytical, analytical and post-analytical. Sample collection is an important factor in the pre-analytical phase as it must be carried out at the correct time (CFF, 2020). According to Heckler and Hahn (2020), vancomycin TM can be performed with a collection in the valley and in the stationary phase, which is when the concentration is at equilibrium in the plasma. This can be done by collecting the sample 30 minutes before the fourth dose, when the patient has normal renal function. In the analytical phase, it is essential that laboratories follow the specific and updated legislation in Brazil established by ANVISA (CFF, 2020). A validated method that ensures the reliability of the data must be used, in addition to internal quality parameters. It is also important for the pharmacist to know the possible interferences of the method, which may give a wrong result, such as bilirubinemia, hemolyzed samples, hyperlipidemias and interaction with other drugs. The post-analytical phase, in turn, is the interpretation of the results that must be correlated with the patient's clinical data, it is at this moment that the dose-response information added to the knowledge of pharmacokinetics can be used to revise the dose, when necessary, in order to fulfill the goal of the TM: to maximize the chance of success of the pharmacotherapy (CFF, 2020).

This study presents the development and validation of a method to determine vancokinemia and application to patients at a hospital in the state of Santa Catarina – Brazil.

MATERIALS AND METHOD

Reagents and Standards

Vancomycin hydrochloride (Sigma-Aldrich®) was used to prepare the standards. In addition, a pesticide called Imidacloprid (Sigma-Aldrich®) was used as an internal standard (I.S.) at a concentration of 52 µg/mL. For mobile phase preparation and sample preparation, Methyl Alcohol (Methanol) and Acetonitrile, both HPLC grade from Sigma-Aldrich® were used. For pH 4 buffer solution was used Sodium Acetate and Acetic Acid HPLC grade (Biograde®). Before analysis, the mobile phase solutions were filtered through a nylon filter membrane 47 mm x 0.45 µm.

Samples

Human plasma was obtained from the Toxicological Researches Laboratory of the University Hospital Polydoro Ernani de São Thiago, where the tests were performed and used as a sample for carrying out the tests and analytical curves. For the selectivity tests, serum from the Biochemistry and Immunology sector of the same hospital were used.

Sample preparation

A simple method of protein precipitation with acetonitrile was used. The points were pipetted and added with the I.S and vortexed for 10 seconds. Then, 1 mL of acetonitrile was added, vortexed for 30 seconds and centrifuged for 15 minutes at 3500 rpm. 1mL of the supernatant was separated for complete drying in a gaseous nitrogen sample concentrator at 50 ºC. Then, it was resuspended with 500 µL of a solution in a concentration equal to the initial proportion of the mobile phase of the method, that is, 10% of the 7:3 solution of methanol and acetonitrile and 90% of the pH 4 acetic buffer

Preparation of standard solutions

The vancomycin standard solution was prepared by weighing 0.125 g of Vancomycin Hydrochloride in analytical balance and transferring it to a 25 mL volumetric flask, completing with ultrapure water at 10% methanol arriving at a solution of 5 mg/mL, this solution was used to prepare 5 more solutions in the following concentrations (mg/mL) 4, 2.5, 1.5, 0.75 and 0.25 all in ultrapure water with 10% methanol. These were called mother-solutions and from them, at the time of analysis, the plasma curve was prepared directly in the test tube by pipetting 10 µL of each of these solutions and adding 490 µL of white plasma, thus reaching the concentration of the points on the curve 5, 15 , 30, 50, 80 and 100 µg/mL.

The I.S was prepared by weighing 0.026 g of Imidacloprid and transferring it to a 10 mL volumetric flask and completing it with 100% Methanol, due to its solubility characteristics, arriving at a solution with a concentration of 2.6 mg/mL, by adding 10 µl of this solution to the curve points and samples (500 µL) arrive the concentration of 52 µL/mL.

Instrumentation and chromatographic conditions

A High Performance Liquid Chromatography system with ultraviolet detector (HPLC-UV) (Shimadzu®) was used, with a C18 reverse column (4.6 mm x 15 cm x 5 µm) and UV detector in the wavelength range from 215 to 254 nm .

The mobile phase consisted of a biphasic system: acetic buffer of pH 4.0 (A): (Methanol: Acetonitrile) in the proportion of 70:30 (v/v) (B), with a gradient starting with 10% of phase B and 90% of phase A, following the phase proportion variations according to table I:

TABLE I: Gradient used in the method for determination of vancokinemia

METHOD GRADIENT	
Time (min)	Solvent B (%)
0	10
4	60
9	30
12	10
22	10

Under these conditions, the Retention Time (RT) of vancomycin obtained was 11.4 minutes and the internal standard RT was 14.1 minutes.

Validation

The method was validated according to Anvisa's RDC 116/2017 (Brazil). The tests performed were: lower limit of quantitation (LQ) and detection (LD), linearity, repeatability, intermediate precision, intraday and interday accuracy, residual effect, selectivity, matrix effect and short-term stability, long-term stability, post-processing and freezing and thawing cycles, and in the long-term stability test the samples were stored for up to 8 months.

For the linearity test, a calibration curve was prepared in quintuplicate, with this same curve the intraday precision and accuracy tests were analyzed. For the selectivity test, serum from 6 different sources were analyzed, one of which was lipemic and the other hemolyzed. For the matrix effect, a standard curve was made at the same concentrations as the points on the calibration curve to compare the statistically significant difference between the angular coefficients of the curve performed in plasma and standard, using the Student's T test in Microsoft excel®. For the stability test, a calibration curve was prepared on the day the points were prepared and compared with the short-term test, post-processing, after 1, 2 and 3 cycles of freezing and thawing and the long-term test where were analyzed at 15 days, 1.5 months, 3.5 months, 4.5 months, 6 were analyzed months, 7 months and 8 months.

Application of the method

For the sample collection, an orientation protocol was elaborate in order to implement the collection in the therapy valley, respecting the balance time of the drug in the body, being 3 to 5 plasma half-lives. Knowing that the half-life of vancomycin is from 3 to 6 hours, the necessary steady state after the first administration is from 18 to 30 hours considering 6 hours as the half-life.

In addition to the vancokinemia determinations of these patients, clinical information collected, such as the day of initiation of therapy, vancomycin administration dose and patient information such as gender and clinical conditions in order to relate to the results found. After the vancomycin quantification analysis, data analysis were performed jointly in order to identify the number of determinations within the reference interval of 15 – 20 µg/mL and the number of determinations that had an indication of dose adjustment based on the result found.

This study was submitted and approved by the Ethics Committee for Research with Human Beings at UFSC and followed the approved procedures

RESULTS AND DISCUSSION

Analytical method

The method validation used to quantify the patients' vancokinemia was carried out in accordance with RDC Resolution No. 166 of 2017, which establishes criteria for the validation of analytical methods (RDC 166, 2017, Brazil). Vancomycin presented RT of 11.4 minutes and IS RT of 14.1 minutes, as shown in figure 1A. Linearity was verified between 5 µg/mL and 100 µg/mL as seen in figure 1B. In addition, figure 1C shows the peak response at different concentrations of all peaks on the analytical curve, and the IS that remained at the same concentration and the same response at all points.

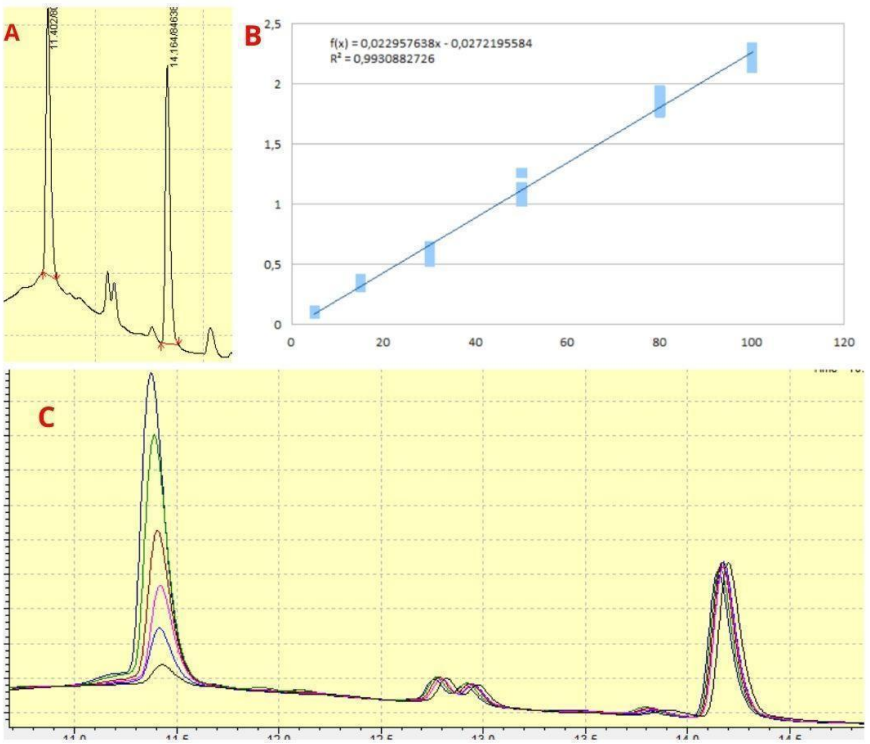

FIGURE 1: RT of vancomycin and IS (A) Linearity of the method (B) Comparison of the chromatogram of the different points of the curve (C)

According to RDC 166/2017 (Brazil) the method linearity must be demonstrated through its ability to obtain analytical responses directly proportional to the concentration of an analyte in a sample, it must be performed with at least 5 points and in triplicate. The result obtained in the graph was performed with a curve in quintuplicate using 6 points, and a correlation coefficient r = 0.996 was observed, thus determining the linearity of the method. The limit of quantitation (LQ) was established at 5 µg/mL and the limit of detection (LD) was obtained according to the formula described in RDC N°. 166 of 2017 (Brazil), obtaining a result of a LD of 1.45 µg/mL.

Precision and Accuracy

Repeatability, intermediate precision and intraday and interday accuracy were evaluated according to RDC 166/2017 (Brazil).

TABLE II: Method validation results

Concentration (µg/mL)	Repeatability (CV%)	Intermediate precision (CV%)	Intraday Accuracy (Average Error %)	Intermediate Accuracy (Average Error %)	Matrix effect (p<0,05)
5	9.59	1377	15.2	14.16	0.2113
15	7.45	13.49	5.73	10.71	
30	8.5	7.93	8.36	6.11	
50	7.99	6.27	5.43	12.62	
80	3.29	7.27	2.93	14.08	
100	3.17	6.9	2.94	5.45	

The results obtained in Table II were obtained using the preparations described above. A CV and error equal to or less than 15% were used as acceptance criteria, except for the LQ where CV and error equal to or less than 20% were acceptable, as described in RDC 899 of 2003 (Brazil). It was concluded that they were considered adequate for the studies of repeatability, intermediate precision, intraday accuracy and interday accuracy.

Matrix Effect

To matrix effect, after Student's t test evaluation a value of 0.2113 was obtained, concluding that there was no significant difference considering the $p<0.05$ criterion (Table II). Despite the favorable results and the determination of absence of matrix effect, it was decided to continue using the matrix itself to perform the curves, seeking more reliable and real results.

Selectivity

For selectivity analysis, six different samples were analyzed: a hemolyzed one, a lipemic one and four normal ones and the areas found was 4.16, 17.91, 6.07, 5.00, 8.23 and 35.01% of LQ, respectively. These areas found had a RT of 11.3 minutes, a few seconds before the vancomycin retention time of 11.4 minutes. According to RDC 27/2012 (Brazil), the interfering elements' responses peak cannot exceed 20% of the LQ area. All peaks remained below the legal limit except one of the samples considered normal that obtained an area of 35.01%, therefore the selectivity of the method presents restrictions. Possible interfering drugs can be investigated in this method.

Stability

The stability tests were carried out according to RDC 899/2003 (Brazil), which for the analysis of drugs in biological liquids, having evaluated:

- Short term stability;
- Stability after freeze-thaw cycles;
- Long-term stability;
- Post-processing stability;

For all stability tests, a curve was prepared on day 1 in blank plasma that was fortified with vancomycin at concentrations of 15 µg/mL, low quantitative control (LQC), 50 µg/mL, medium quantitative control (MQC) and 80 µg/mL, high quantitative control (HQC) in triplicate, this curve was used as day 0, as shown in Figure 2. The Brazilian Resolution states that “samples will be considered stable when no deviation greater than 15% is observed in the value obtained from newly prepared samples, with the exception of LQ, for which deviation of up to 20% is accepted.” (RDC 899, 2003, Brazil)

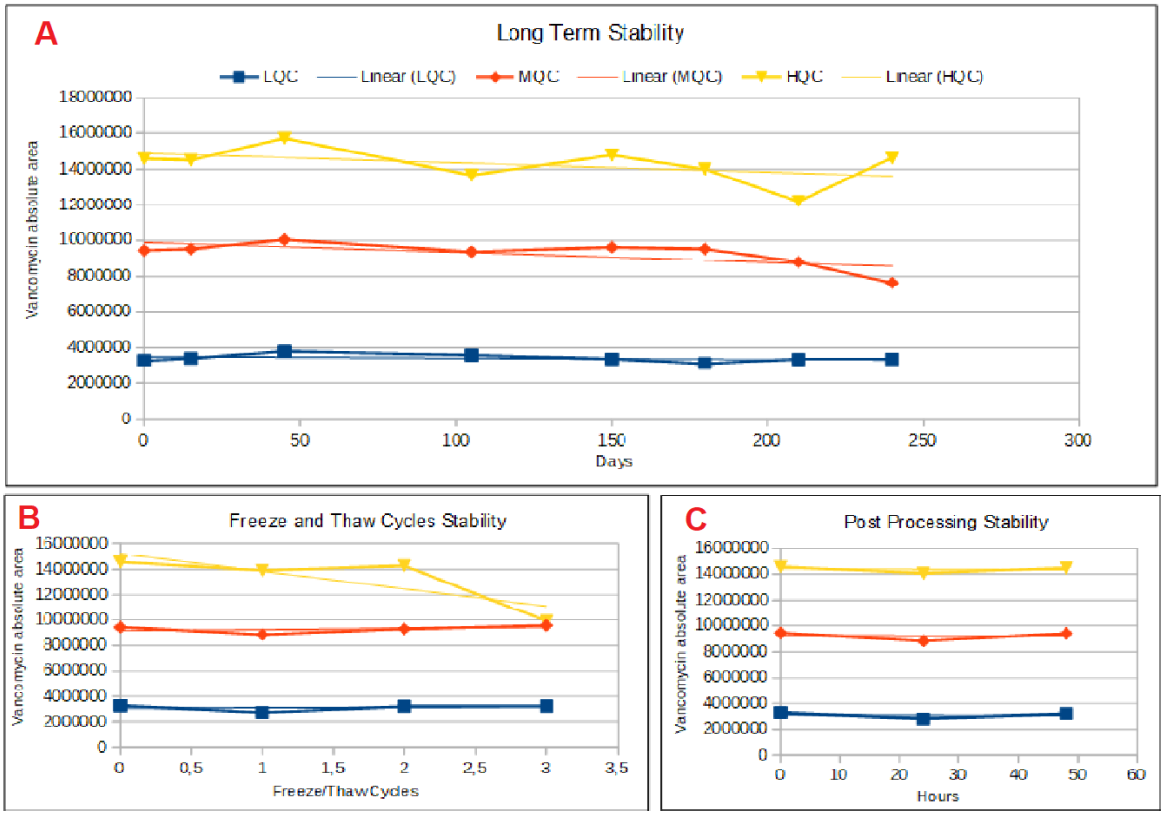

FIGURE 2: Results of long term stability tests (A); results of freeze and thaw cycles stability tests (B); and results of post processing stability tests (C)

*LQC = Low quantitative control, MQC= Medium quantitative control, HQC = High quantitative control

Short Term Stability

The samples were submitted for 24 hours at room temperature, approximately 23°C, and compared to day 0. For curve approval purposes, a curve was obtained with the samples submitted to room temperature and the CV (%) was observed. which must be less than 15% and the concentration which must have a difference of less than 15% from the expected concentration. As a result, the short-term stability test was approved.

Stability after freezing and thawing cycles

To analyze the stability after cycles of freezing and thawing, samples in the concentrations of LQC, MQC and HQC were submitted to 24h at freezing temperature, and then these samples were analyzed, this process was repeated three times, thus obtaining a graph as shown in figure 2B. Note that LQC and MQC remained stable until the third cycle, but HQC had a drop in response in the third cycle. Thus, a maximum of two cycles were defined for the analysis of these samples (Figure 2B).

Long-term stability

For the long-term stability test, samples of LQC, MQC and HQC concentrations were used, which were frozen separately and remained frozen and were analyzed in the following elapsed times after freezing: 15 days, 1.5, 3.5 , 4.5, 6, 7 and 8 months. As approval criteria, CV (%) and concentration obtained less than 15% compared to the expected concentration were considered. In Figure 2A, the samples remained stable over the 8 months analyzed.

Post-process stability

A post-processing stability test was also carried out, as described in RDC 899/2003 (Brazil), maintained for 24 h and 48 h at room temperature to then be analyzed in the equipment. The same approval criteria of the other stability tests were used, in this case a of the LQC replicates failed in accuracy, when processed 24 hours after processing and also the same replicate showed problems when analyzed in 48 hours at room temperature. The other replicates obtained approval, and the mean of the absolute area of vancomycin compared to the curve of the day 0 remained stable as seen in figure 2C, so it was considered approved in post-processing stability.

Residual effect

Residual effect analysis was performed using a standard at the High Limit of quantitation (HLQ) used in the curve (100 µg/mL) and blank standards subsequently analyzed. An area in RT similar to that of vancomycin was observed in all whites, which generated an average of 19.61% of the LQ (limit of quantitation) and peaks in the IS retention time, which generated an average of 2.26%. The legislation recommends a maximum of 20% of the LQ and 5% for the IS (RDC 27/2012, Brazil). A result was then obtained that could indicate the presence of a residual effect, however, when comparing the blank analyzed before starting the test and the blanks analyzed after analyzing the samples in HLQ, no difference was noticed, all ranged from 18% to 22% regardless of the processed HLQ and therefore this was considered a selectivity failure and not a residual effect.

Application at the Hospital

For application data were collected from four patients who were using vancomycin admitted to the Intensive Care Unit (ICU), who were applied to the vancokinemia quantification method validated according to previous results. A code system was used to preserve the identity of each patient as prescribed in the Informed Consent Form that each patient or accompanying family member signed, so patients will be called A1, A2, A3 or A4. The results are organized in table III in order to facilitate understanding.

TABLE III: Results found in patients admitted to the ICU

	A1	**A2**	**A3**	**A4**
Sex	Male	Male	Male	Male
Pathology	Esophageal tear and bacterial pneumonia	Acute endocarditis and septicemia	Sepsis, cirrhosis of the liver and malignant neoplasm of the colon	Third degree burn
Initial creatinine (mg/dL)	0.91	10.5	1.1	6.2
Initial Dose (mg/day)	2500	333	2000	1000
Final dose (mg/day)	3000	375	3000	750
Vancokinemia 1 (µg/mL)	5.5**	17.3	29.7***	25.5*
Vancokinemia 2 (µg/mL)	5.7**	-	17.1	15.0***
Vancokinemia 3 (µg/mL)	14.8	-	56.5***	-

Vancokinemia 4 (µg/mL)	30.3*	-	-	-
Vancokinemia 5 (µg/mL)	11.4	-	-	-
Vancokinemia 6 (µg/mL)	27.9*	-	-	-

*Collection performed at the appropriate time with dose reduction or withdrawal of vancomycin.

** Collection performed at the appropriate time with dose increase.

*** Collection carried out at inappropriate times

Among the collections carried out, the patient A1 was the one who had the most vancokinemia determinations, in all 6 collections were performed. He entered the ICU due to a laceration in the esophagus that ended up causing bacterial pneumonia and started using vancomycin at a dose of 2,500 mg/day. Four of the collections performed on this patient influenced a dose change, two increases after vancokinemia 1 and 2 (Table III) of 50% and 20% of the dose, respectively, to reach the therapeutic range that was obtained in the 3rd determination of vancokinemia. However, later, there was a dose decrease due to a vancokinemia of 30.3 µg/mL accompanied by an increase in the patient's creatinine that reached 1.12 mg/dL, this dose decrease proved to be efficient since the next determination (vancokinemia 5) obtained a result of 11.42 µg/mL, below the therapeutic range, but the dose was not readjusted. After ten days, another collection that resulted in a vancokinemia of 27.9 µg/mL, accompanied a creatinine increase (2.98 mg/dL) greater than 50% of the patient's baseline creatinine, which according to Filippone, Kraft and Farber (2017) characterizes renal injury therefore it led to the discontinuation of vancomycin.

The patient A4 also had dose reduced after vancokinemia. He was admitted to the ICU with third-degree burns and started using vancomycin at a dose of 1,000 mg/day. The first collection was performed obtaining a result of 25.5 µg/mL (Table III) which led to a dose reduction of 25%, another collection was performed in this patient. However, it was observed that the collection was out of balance and the result was disregarded. This patient did not perform any further dose changes or collections.

The patient A3 entered the ICU due to sepsis with an abdominal focus, in addition, the patient had a malignant neoplasm of the colon and liver cirrhosis, started vancomycin at a dose of 2,000 mg/day, with an increase to 3,000 mg/day before any measurement of vancokinemia. In this patient, were performed three collections, however the first collection and the last one were considered inappropriate due to the time not being in accordance with the therapy valley (table III) the only one performed at a time appropriate to the protocol led to a result of 17.1 µg/mL and no change in dose was made. It was observed that among 12 collections of the total, at least 3 were performed at a time not in accordance with the protocol, as per table III, making these results invalid for dose adjustment. It is understood that these failures were due to lack of communication within the ICU team, which was

still adapting to the new method. In order to prevent these errors from occurring again, a form was created to be completed by the ICU pharmacy and the nursing team with an actual time of collection and ensure that the valley has been collected before starting vancomycin administration.

Furthermore, another patient analyzed was A2, admitted to the hospital's ICU due to septicemia and acute endocarditis, started using vancomycin at a dose of 1,000 mg every 72 hours (333 mg/day). He had changes of 12% in dose previously the first collection of vancokinemia, which resulted in 17.3 µg/mL and therefore no further adjustment, was performed in this patient until the day the patient was discharged.

In summary, excluding inadequate results, 33% of the results were within the therapeutic range of 15 µg/mL to 20 µg/mL. 33% of the results were below the therapeutic range, and 33% were above the therapeutic range. Among the results below the therapeutic range, 66% resulted in an increase in the dose of vancomycin, only one of the results, for patient A1 of 11.42 µg/mL the choice was to maintain the dose. As for the results above the therapeutic range, 100% of the results led to a decrease in the dose used in the patient.

In conclusion, the analytical method was validated and proved to be reproducible, with good accuracy and analytical precision. With selectivity limitations, which can be verified in relation to the possible interferences of the method. As for implementation, difficulties were observed in sample collection at the correct time, due to lack of preparation and understanding of the importance of this test in the team involved. Measures such as training and a collection protocol made available in order to obtain the correct collection. In addition, this work contributed to facilitating the safe change of dose for patients using vancomycin. However, in a continuation beyond the valley, it is also important to analyze the peak, defined in the literature as the moment of highest plasma concentration of the drug, and through pharmacokinetic calculations, to individualize the dose of each patient using vancomycin.

REFERENCES

Agência Nacional de Vigilância Sanitária (Brasil). Resolução nº 166, de 24 de julho de 2017. Validação de métodos analíticos. Diário Oficial da União, 25 jul. 2017. Seção 1, p. 87.

Agência Nacional de Vigilância Sanitária (Brasil). Resolução nº 27, de 17 de maio de 2012. Requisitos mínimos para a validação de métodos bioanalíticos empregados em estudos com fins de registro e pós-registro de medicamentos. Diário Oficial da União, 10 de maio de 2012.

Agência Nacional de Vigilância Sanitária (Brasil). Resolução nº 899, de 29 de maio de 2003. Guia para validação de métodos analíticos e bioanalíticos métodos analíticos. Diário Oficial da União, 6 de março de 2003.

Bertoluci DF de F. Uso de vancomicina nas infecções por Staphylococcus aureus e epidermides em pacientes queimados: monitoramento das concentrações plasmáticas após infusão intermitente [dissertation]. São Paulo: Universidade de São Paulo, Faculdade de Ciências Farmacêuticas; 2007 [cited 2022 Jun 12]. Available from:

https://www.teses.usp.br/teses/disponiveis/9/9139/tde-13092007-100702/publico/DanieleFFBertoluci.pdf

CONSELHO FEDERAL DE FARMÁCIA: Monitorização Terapêutica de Medicamentos. Brasília: 2020. 88 p. [cited 2022 Jun 12] Available from: https://www.cff.org.br/userfiles/MONITORIZACAO%20-%20VERSAO%20PARA%20CONSULTA%20PUBLICA.pdf.

Cusumano JA, Klinker KP, Huttner A, Luther MK, Roberts JA, LaPlante KL. Towards precision medicine: Therapeutic drug monitoring–guided dosing of vancomycin and β-lactam antibiotics to maximize effectiveness and minimize toxicity. American Journal of Health-System Pharmacy [Internet]. 2020 Jun 15;77(14):1104–12. Available from: https://academic.oup.com/ajhp/article/77/14/1104/5857350?login=false

Elyasi S, Khalili H, Dashti-Khavidaki S, Mohammadpour A. Vancomycin-induced nephrotoxicity: mechanism, incidence, risk factors and special populations. A literature review. European Journal of Clinical Pharmacology [cited 2022 jun 28]. 2012 Mar 13;68(9):1243–55. Available from: https://link.springer.com/article/10.1007%2Fs00228-012-1259-9

Filippone E, Kraft W, Farber J. The Nephrotoxicity of Vancomycin. Clinical Pharmacology & Therapeutics [cited 2021 nov 21]. 2017 Jun 5;102(3):459–69. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5579760/

Goti V, Chaturvedula A, Fossler MJ, Mok S, Jacob JT. Hospitalized Patients With and Without Hemodialysis Have Markedly Different Vancomycin Pharmacokinetics: A Population Pharmacokinetic Model-Based Analysis. Therapeutic Drug Monitoring. [cited 2022 jul 24] 2018 Apr;40(2):212–21. Available from: https://journals.lww.com/drug-monitoring/Fulltext/2018/04000/Hospitalized_Patients_With_and_Without.7.aspx.

Heckler AM, Hahn SR. Implementation of a vancomycin therapeutic monitoring protocol in adults. Revista de Epidemiologia e Controle de Infecção. [cited 2022 mai 22] 2020 Jul 7;10(3). Available from: https://online.unisc.br/seer/index.php/epidemiologia/article/view/14582

Mulubwa M, Griesel HA, Mugabo P, Dippenaar R, van Wyk L. Assessment of Vancomycin Pharmacokinetics and Dose Regimen Optimisation in Preterm Neonates. Drugs in R&D. [cited 2021 nov 21] 2020 Apr 8;20(2):105–13. Available from: https://pubmed.ncbi.nlm.nih.gov/32266599/

Uda K, Suwa J, Ito K, Hataya H, Horikoshi Y. Ototoxicity and Nephrotoxicity With Elevated Serum Concentrations Following Vancomycin Overdose: A Retrospective Case Series. The Journal of Pediatric Pharmacology and Therapeutics. [cited 2021 nov 21] 2019 Sep 1;24(5):450–5. Available from: https://pubmed.ncbi.nlm.nih.gov/31598110/

Ye ZK, Li C, Zhai SD. Guidelines for Therapeutic Drug Monitoring of Vancomycin: A Systematic Review. Conly J, editor. PLoS ONE. [cited 2022 mai 22] 2014 Jun 16;9(6):e99044. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0099044.

ANEXO I: Instructions to authors (BJPS)

Brazilian Journal of Pharmaceutical Sciences

Brazilian Journal of Pharmaceutical Sciences

SCOPE AND POLICY

The Brazilian Journal of Pharmaceutical Sciences (BJPS) is a peer-reviewed electronic journal published continually by the School of Pharmaceutical Sciences of the University of São Paulo. The purpose of the Brazilian Journal of Pharmaceutical Sciences is to publish manuscripts that significantly contribute to knowledge in all areas of Pharmaceutical Sciences, including:

1. Medicinal Chemistry & Pharmacognosy
2. Pharmaceutical Technology, Drug development, Pharmaceutics & Biopharmaceutics, Drug Delivery
3. Clinical Pharmacy and Pharmacokinetics, Pharmaceutical Care, Pharmaceutical Services
4. Clinical-Bioanalytical Chemistry and Pharmaceutical Analysis
5. Food Science and Nutrition, including Food Analysis and Technology, Nutrigenomics, Immunonutrition, Functional foods and supplements, Food bioactive compounds, the interaction between drugs and foods
6. Vaccines and Biologicals
7. Cosmetology
8. Toxicology

Journal's policy, which are used as guidelines for the review of manuscripts:

1. Out of scope

The paper must report on recent advances in pharmaceutical technology, biopharmaceutics, pharmaceutical biotechnology, medication use, and pharmacist services of major importance.

Immediate rejection criteria:

a. Description of analytical methods with no relation to pharmaceutics, biopharmaceutics, or pharmaceutical biotechnology.

b. Mere description of compound synthesis, processes, validation, etc. without any pharmaceutical application.

c. Inappropriate promotion of a trademark or a product.

d. Regulatory issues with no relation to pharmaceutics, biopharmaceutics, or pharmaceutical biotechnology.

2. Too preliminary

A paper must be based on a thorough and extensive study, using established or well-described methods and including proper controls. Research must be hypothesis-driven and conclusions must be supported by the data presented.

Immediate rejection criteria:

a. No proof of concept in either an in vivo or an in vitro evaluation.

b. No clear indication of the use of the product or formulation.
c. No clear description of the materials & methods used in the pharmaceutical field.
d. Use of inadequate or insufficient methods.
e. Inappropriate statistical analysis.
f. Lack of proper controls.
g. Lack of coherent discussion of the results.

3. Lack of novelty:

The study described in the manuscript must represent a novel approach.

Immediate rejection criteria

a. Repetition of previously published data, either partially or entirely.
b. Simple variation of parameters of a formulation, processes, synthesis, etc.
c. Modification of well-known delivery systems with no novelty and/or benefit.
d. Plagiarism (intentional and unacknowledged copying of other's work) and self-plagiarism (re-use of parts of an author's previously published work without proper attribution).

Before entering the review process, all manuscripts will go through a similarity check using the plagiarism detection software iThenticate. If the editors agree that there is a high percentage of similarity to other texts, the manuscript will be rejected immediately.

The following papers **will not be accepted** for publication:

a. Studies on human subjects not approved by an accredited Ethics Committee or without written informed consent from the subject or legal guardian.
b. Studies on animals not approved by an accredited Ethics and Animal Care Committee.
c. Manuscripts describing plant extract activity that do not Identify quali and quantitative chemical markers of the extract.

PREPARATION OF THE MANUSCRIPT

a. Manuscripts must be submitted in English.
b. Submission of a manuscript to BJPS implies that the data have not been published previously and will not be submitted for publication elsewhere while the manuscript is under review.
c. Co-authors should be individuals who have contributed substantially to the content of the paper.

Manuscripts in accordance to the "Preparing your manuscript section" will be submitted for peer review to at least two independent, anonymous referees indicated by the Associated Editors. Based on peer review, the Associate Editors will suggest manuscript acceptance or not to the Editor-in-Chief, who is responsible for the final decision.

In the case revision is suggested, the authors are asked to resubmit the manuscript incorporating the suggestions and recommendations of the referees within 15 calendar days. If the revised version is not received within the time specified from the date of the notice, the manuscript process will be canceled. All revisions must be accompanied by a letter detailing the changes made to the original document and answering all the reviewer comments, on a point-by-point basis. All alterations must be identified in the revised manuscript.

Manuscripts must have their copyright enclosed as a file to the BJPS. The manuscript will not be sent to reviewers if the signed copyright was not included in the submission package. This document must be hand-signed by all authors, no exception. Later, if the manuscript was accepted, an English certificate will be requested for the last version of the manuscript.

The dates of receipt and acceptance will be published for each article. Authors are expected to return reviewed manuscripts to the Journal within 15 calendar days and to return galley proofs of accepted manuscripts within 72 hours. The total number of "late" days will be added to the submission date at the time of publication.

Authors are required to suggest 4 potential reviewers with information on institutional and e-mail addresses. At least two of these potential reviewers must be from countries other than the corresponding author. The Editors reserve the right to nominate these or other reviewers for manuscript evaluation.

Manuscripts that do not agree to the Instructions will be refused prior to peer review.

Disclosure instructions

Authors must disclose the use of generative AI and AI-assisted technologies in the writing process by adding a statement at the end of their manuscript in the core manuscript file, before the References list. The statement should be placed in a new section entitled 'Declaration of Generative AI and Aiassisted technologies in the writing process'.

This declaration does not apply to the use of basic tools for checking grammar, spelling, references etc. If there is nothing to disclose, there is no need to add a statement.

Statement model:

During the preparation of this work the author(s) used [NAME TOOL / SERVICE] in order to [REASON]. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

MANUSCRIPT CATEGORIES

The authors should state in the cover letter that the manuscript is intended to be Full-length Original Paper, Short Communication, Review Article, Mini-review article, Concepts and Comments and Book Reviews.

The Journal will also publish Thematic or Congress Abstracts Supplements under invitation by the Editors or previous approval by the Editorial Board.

BJPS will publish the following type of articles:

Full-length Original Paper

Each manuscript should clearly state its objective or hypothesis; the experimental design and methods used; the essential features of any interventions; the main outcome measures; the main results of the study; and a discussion placing the results in the context of published literature.

The manuscript should contain:

a. abstract of no more than 200 words

b. no more than 6 keywords

c. a running title to be used as a page heading, which should not exceed 60 letters and spaces

d. manuscript's main body is divided into separate sections (Introduction, Material and Methods, Results and Discussion).

e. no more than 40 references (without exceptions)

f. Supplementary data can be submitted as a *Supplementary information* session.

Short Communication

Short communication is **a report on a single subject**, which should be concise but definitive. The scope of this section is intended to be wide and encompass methodology and experimental data on subjects of interest to the readers of the Journal.

The manuscript should contain:

a. abstract of no more than 200 words

b. no more than 6 keywords

c. a running title to be used as a page heading, which should not exceed 60 letters and spaces

d. manuscript's main body is divided into separate sections (Introduction, Material and Methods, Results and Discussion), without a separate section for conclusions

e. no more than 20 references (without exceptions)

f. no more than three illustrations (figures and/or tables)

Review Article

A review article should provide a synthetic and critical analysis of a relevant area and should not be merely a chronological description of the literature. A review article by investigators who have made substantial contributions to a specific area of Pharmaceutical Sciences will be published by invitation of the Editors. However, an outline of a review article may be submitted to the Editors without prior consultation. If it is judged appropriate for the Journal, the author(s) will be invited to prepare the articlefor peer review.

The manuscript should contain:

a. abstract of no more than 250 words

b. no more than 6 keywords

c. a running title to be used as a page heading, which should not exceed 60 letters and spaces manuscript main body divided into sections with appropriate

titles and subtitles

d. no more than 90 references (without exceptions)

Mini-review Article

A mini-review is focused on a restricted part of a subject normally covered in a review article. The structure of the mini-review follows the same rules as the review.

Concepts and Comments

The Concepts and Comments section provides a platform for readers to present ideas, theories and views.

The manuscript should contain:

a. abstract of no more than 250 words

b. no more than 6 keywords

c. a running title to be used as a page heading, which should not exceed 60 letters and spaces

d. manuscript's main body is divided into sections with appropriatetitles and subtitles

e. no more than 40 references (without exceptions)

Book Reviews

Written by experts nominated by the Editors or written by theAuthors.

PREPARING YOUR MANUSCRIPT

Cover Letter

It is important that you include a cover letter with your manuscript. Take the time to consider why this manuscript is suitable for publication in the *Brazilian Journal of Pharmaceutical Sciences*. Why will your paper inspire other members of your field, and how will it drive research forward. Please explain these points in your cover letter.

The cover letter should also contain the following information:

a. Title of article.

b. Name(s), filiation and ORCID number of all author(s).

c. Information of Corresponding Author: name and e-mail (full address and telephone number are optional informations).

Authorship requirements

Only people who directly contributed to the intellectual content of the paper should be listed as authors. Manuscripts must be submitted electronically only. Confirmation of submission will be sent by email to all authors, for their agreement.

Authors should meet all of the following criteria, thereby taking public responsibility for the content of the paper:

a. Conceived, planned, and carried out the experiments presented in the manuscript or interpreted the data, or both.

b. Wrote the paper, or reviewed successive versions.

c. Approved the final version.

d. Holding positions of administrative leadership, contributing to patients, and collecting and assembling data, however important to the research, are not by themselves criteria for authorship. Any person who has made a substantial, direct contribution to the work but cannot be considered an author should be cited in the Acknowledgment section, with permission and include a description of his/her specific contribution to the research.

Text format

a. The text of a manuscript can only be accepted as a Microsoft Word file created with MS Word as a "doc", "docx" or "RTF" document.

b. Manuscripts should be sent in 30-36 lines, 1,5 spaced,

c. Each page should contain the page number in the upper right-hand corner starting with the title page as page 1.

d. Report all measurements in Système International, SI (http://physics.nist.gov/cuu/Units) and standard units where applicable

e. Names of plants, animals and chemicals should be mentioned according to International Rules available.

f. Names of drugs can follow the International rules (DCI) or current Brazilian rules (DCB)

g. Trademarks may be mentioned only once in the text (between parenthesis and initial in capital letter)

h. Do not use abbreviations in the title and limit their use in the abstract and text.

i. The length of the manuscript and the number of tables and figures must be kept to a minimum.

j. Ensure that all references are cited in the text.

k. Generic names must be used for all drugs. Instruments may be referred to by proprietary name; the name and country of the manufacturer should be given in parenthesis.

ORGANIZATION OF THE MANUSCRIPT

Most articles published in BJPS will be organized into the following sections:

Title

Running Title Authors (full names)

Corresponding author information (Abbreviated name, institutional address, phone, e-mail, ORCIDlink)

Abstract, Keywords **INTRODUCTION MATERIAL AND METHODS**

First Subtitle (if there is any) *Second subtitle* (if there is any)**RESULTS**

DISCUSSION ACKNOWLEDGMENTSREFERENCES

Tables with a descriptive title and footnote legends

Figures with a descriptive legend and uniformity in format.

Continuous page numbers are required for all pages including figures. There are no specific length restrictions for the overall manuscript or individual sections. However, we request authors to present and discuss their findings concisely. We recognize that some articles will not be best presented in our research article format. If you have a manuscript that would benefit from a different format, please contact the editors for further discussion.

TITLE PAGE

Title

The title should be as short and informative as possible, should not contain non-standard acronyms or abbreviations, and should not exceed two printed lines. The title should be centered and written in bold as the example below:

FREEZE-DRYING OF AMPICILLIN SOLID LIPID NANOPARTICLES USING MANNITOL AS CRYOPROTECTANT

Running title

This short title, to be used as a page heading, should not exceed 60 letters and spaces.

Authors and Affiliations

Full name (matched with superscript numbers identifying affiliation) must be written in bold and centered. Institution(s) (Department, Faculty, University, City, State, Country) of each author (in English must be centered and written in italic.

Example:

Hongmei Xia[1*] , Yongfeng Cheng[2] , Yinxiang Xu[3] , Zhiqing Cheng[1]

[1]College of Pharmacy, Anhui University of Chinese Medicine, Hefei, People's Republic of China, [2]School of Life Science, University of Science and Technology of China, Hefei, People's Republic of China, [3]Zhaoke (Hefei) Pharmaceutical Co. Ltd., Hefei, People's Republic of China

Corresponding author

One of the authors should be designated as the corresponding author. It is the corresponding author´s responsibility to ensure that the author list is accurate and complete. If the article has been submitted on behalf of a consortium, all consortium members and affiliations should be listed in the Acknowledgments section. Provide the name, email address, and ORCID number of the author to whom correspondence should be sent identified with an asterisk.

Abstract

Since abstracts are published separately by Information Services, they should contain sufficient hard data to be appreciated by the reader. The abstract should not exceed 200 words and should be prepared in a single paragraph without topics and no margins. The abstract should briefly and clearly present the objective, experimental approach, new results as quantitative data if possible, and conclusions. It should mention the techniques used without going into methodological detail and mention the most important

results. Abbreviations should be kept to a minimum and should be defined in both the Abstract and text. Please do not include any reference citations in the abstract. If the use of a reference is unavoidable, the full citation should be given within the abstract.

Keywords:

A list of keywords or indexing terms (no more than 6) should be included avoiding generic terms.

Keywords must be separated by dots with only the first letter of the first word in upper case.

Example:

Apoptosis pharmacokinetics. Toxicology.

INTRODUCTION

The Introduction should put the focus of the manuscript into a broader context and reflects the present state-of-art of the subject. This should state briefly and clearly the objectives of the investigation with reference to previous works. The introduction should justify the hypothesis of the study. An extensive review of the literature should be avoided and when possible replaced by recent reviews of the subject.

MATERIAL AND METHODS

These should be described in sufficient detail that the work can be reproduced. Well-established procedures and techniques require only a citation of the original source, except when they are substantially modified. Reports of experimental studies on humans and animals must certify (including the number of protocols) that the research received prior approval by the appropriate institutional review Ethics Committee.

RESULTS

Results must be presented clearly and concisely and in a logical order. This section should provide the results of all of the experiments required to support the conclusions of the paper. When possible, use figures or tables to present data rather than text. Large datasets, including raw data, should be submitted as supplementary files; these are published online and linked to the article.

DISCUSSION

Discussion should interpret the results and assess their significance in relation to existing knowledge. Speculation not warranted by actual data should be avoided. The Discussion should spell out the major conclusions and interpretations of the work including some explanation of the significance of these conclusions.

ACKNOWLEDGMENTS

When appropriate, briefly acknowledge technical assistance, advice, and contributions from colleagues. People who contributed to the work but do not fit the criteria for authors should be listed in the Acknowledgments section, along with their contributions. Donations of animals, cells, or reagents should also be acknowledged. You must also ensure that anyone named in the Acknowledgments agrees to being so named. Financial support for the research and fellowships should be acknowledged in this section (agency and grant number).

Figures

Figures must be submitted in high-resolution version (300 dpi). They must be submitted separately from the text, in the file upload section of the submission platform.

Preparing figure files for submission

The use of figures is mandatory for original articles since it increases the clarity of data. The use of color figures in articles is free of charge. The following guidelines must be observed when preparing figures. Failure to do so is likely to delay the acceptance and publication of the article.

a. Each figure of a manuscript should be submitted as a single file.

b. Figures should be numbered in the order they are first mentioned in the text, and uploaded in this

order.

c. Figure titles and legends should be provided in the main manuscript as a List of Figures, not in the graphic file.

d. The aim of the figure legend should be to describe the key messages of the figure, but the figure should also be discussed in the text.

e. An enlarged version of the figure and its full legend will often be viewed in a separate window online, and it should be possible for a reader to understand the figure without moving back and forth between this window and the relevant parts of the text.

f. The legend itself should be succinct, while still explaining all symbols and abbreviations. Avoid lengthy descriptions of methods. Statistical information should be given as well as the statistical tests used.

g. Arrows or letters should be used in the figure and explained in the legend to identify important structures.

h. Figures with multiple panels should use capital letters A, B, C, etc. to identify the panels.

i. Each figure should be closely cropped to minimize the amount of white space surrounding the illustration. Cropping figures improves accuracy when placing the figure in combination with other elements when the accepted manuscript is prepared for publication.

j. Individual figure files should not exceed 5 MB. If a suitable format is chosen, this file size is adequate for extremely high-quality figures.

Please note that it is the responsibility of the author(s) to obtain permission from the copyright holder to reproduce figures (or tables) that have previously been published elsewhere. In order for all figures to be open-access, authors must have permission from the rights-holder if they wish to include images that have been published elsewhere in non-open-access journals. Permission should be indicated in the figure legend, and the original source included in the reference list.

Supported file type

The following file format can be accepted: TIFF (suitable for images) or JPEG with 300 dpi, and Word file for the manuscript.

Tables

a. Tables must be submitted in Word (.doc) or Excel (.xls), not as an image.

b. Tables must be numbered consecutively with Roman numerals in the text.

c. Tables must have a concise and descriptive title.

d. All explanatory information should be given in a footnote below the table. Footnotes should be used to explain abbreviations and provide statistical information, including statistical tests used.

e. All abbreviations must be defined in this footnote, even if they are explained in the text.

f. Tables must be understandable without referring to the text.

g. Tables occupying more than one printed page should be avoided, if possible.

h. Vertical and diagonal lines should not be used in tables; instead, indentation and vertical or horizontal space should be used to group data.

Citations

References should be prepared and listed according to Vancouver's standard reference style. Entries should be arranged in alphabetical order by the author at the end of the paper. All authors' names should be given. The accuracy and completeness of reference data is the responsibility of the authors.

Only published references should be included in the reference list. Meeting abstracts, conference talks, or papers that have been submitted but not yet accepted should not be cited. Limited citation of unpublished work should be included in the body of the text only. All personal communications should be supported by a letter from the relevant authors.

References should be cited in the text by the authors' names, with only the first letter in capital letter followed by the year of publication. For more than three authors, the first has to be cited followed by the expression *et al.* (in italic). Small letters close to the year must differentiate references of the same authors and year of publication

Examples:

(Zhang, 2017)

(Ima, Souza, 2015)

(Fujisawa, Atsumi, Kadoma, 1989)(Aviral *et al*., 2009)

(Liu *et al.*, 2011a)(Liu *et al.*, 2011b)

References

Published Papers: Write all author's names up to 6 authors and followed by et al. (in case there are more than 6), Title (Only the first letter in upper case). Journal abbreviation without dots. Year;Volume(issue number):first page-last page.

Abe T, Fukushima N, Brune K, Boehm C, Sato N, Matsubayashi H, et al. Genome-Wide allelotypes of familial pancreatic adenocarcinomas and familial and sporadic intraductal papillary mucinous neoplasms. Clin Cancer Res. 2007;13(20):6019-25.

Ali A, Iqbal F, Taj A, Iqbal Z, Amin MJ, Iqbal QZ. Prevalence of microvascular complications in newly diagnosed patients with Type 2 diabetes. Pak J Med Sci. 2013,29(4): 899-902.

Calvo A, Gimenez MJ. Ex Vivo Serum Activity (Killing Rates) After Gemifloxacin 320 mg Versus Trovafloxacin 200 mg Single Doses Against Ciprofloxacin-Susceptible and -Resistant Streptococcus pneumonia. Int J Antimicr Ag. 2007;20:144-6.

Article accepted for publication but not yet published: First 6 authors followed by et al. Title. Journal (abbreviation in normal font), Year of expected publication (in press) at the end of the citation.

Janiszewski M, Lopes LR, Carmo AO, Pedro MA, Brandes RP, Santos CXC, et al. Regulation of NAD(P)H oxidase by associated protein disulfide isomerase in vascular smooth muscle cells. J Biol Chem. 2005 (in press).

Internet Communication: Ensure that URLs are active and available. Provide DOI, if available.

Brasil. Ministério da Saúde, Secretaria de Vigilância em Saúde. Leishmaniose visceral grave: normas e condutas [Internet]. Brasília (DF): Ministério da Saúde, 2006. [citado 2008 Jan 7]. 60 p. (Série A. Normas e Manuais Técnicos). Disponível em: http://dtr2001.saude.gov.br/editora/produtos/livros/ pdf/06_0072_M.pdf

CAPES Statistics. [cited 2006 Mar 16]. Available from: http:// www.capes.gov.br/capes/portal.

Developmental toxicology. [cited 2015 Apr 10]. Available from: http://www.devtox.org/nomenclature/organ.php.

Whole Book: Authors, Book title, Edition, City, Publisher, Year.

Hewitt W. Microbiological assay for pharmaceutical analysis: a rational approach. Boca Raton: CRC Press; 2003.

Jenkins PF. Making sense of the chest x-ray: a hands-on guide. New York: Oxford University Press; 2005. 194 p.

Laws:

Agência Nacional de Vigilância Sanitária (Brasil). Resolução nº. 259, de 20 de setembro de 2002. Regulamento Técnico para Rotulagem de Alimentos Embalados. Diário Oficial da União 23 set 2002; Seção 1.

Milech A, et al., Oliveira JEP, Vencio S, organizadores. Diretrizes da Sociedade Brasileira de Diabetes. São Paulo: A.C. Farmacêutica;2016.

Book Chapter: Authors, Chapter Title, Editors, Book title, Edition, City, Publisher, Year, Pages of citation.

Conference or Symposium Proceedings: Cite papers only from published proceedings.

Hejzlar RM, Diogo PA. The use of water quality modelling for optimizing operation of a drinking water reservoir. In: Proceedings of the International Conference Fluid Mechanics and Hydrology. 1999 Jun 23-26; Prague. Prague: Institute of Hydrodynamics AS CR; 1999. p 475-482.

Report

Rojko JL, Hardy WD Jr. Feline leukemia virus and other retroviruses. In: Sherding RG, editor. The cat: diseases and clinical management. New York: Churchill Livingstone; 1989. p. 229-332.

World Health Organization. WHO. Department of Mental Health and Substance Abuse. Mental health atlas 2005. Geneva: World Health Organization; 2005. 409 p.

Proceedings of the 10th annual meeting of the Canadian Society for Pharmaceutical Sciences. *J Pharm Pharm Sci*. 2007 Dec 3;10(4):1s-186s.

Audiovisual Material

Physician's Desk Reference (PDR). Release 2003.1AX. [CD- ROM]. Montvale: Thomson PDR; 2003.

Computer Program

Dean AG, Dean JA, Coulombier D, Brendel KA, Smith DC, Burton AH, et al. *Epi info, version 6.04: a word processing database and statistics program for public health on IBM- compatible microcomputers.* [Computer program]. Atlanta: Centers of Disease Control and Prevention;1998. World Health Organization. WHO. Working to overcome the global impact of neglected tropical diseases, First WHO report on neglected tropical diseases. Geneva, Switzerland: WHO Press; 2010.

Patent

Larsen CE, Trip R, Johnson CR. Methods for procedures related to the electrophysiology of the heart. Patent No.5.529.067. Novoste Corporation; 1995.

Thesis and Dissertations

Joselevitch C. Visão no ultravioleta em Carassius auratus (Ostariophysi, Cypriformes, Cyprinidae):

estudo eletrofisiológico do sistema cone - células horizontais. [Master's dissertation]. São Paulo: Instituto de Psicologia, USP; 1999.

Marcolongo R. Dissolução de medicamentos: fundamentos, aplicações, aspectos regulatórios e perspectivas na área farmacêutica. [dissertação]. São Paulo: Universidade de São Paulo, Faculdade de Ciências Farmacêuticas; 2003.

Unpublished results and Personal communication: Reference should appear in the text with the individual name(s) and initials and not in the reference list.

(Santos CS, da-Silva GB, Martins LT, unpublished results).

It is assumed that the author has obtained permission from the source when personal communication is cited.

HOW TO SUBMIT THE MANUSCRIPT

Submission should be sent electronically through the ScholarOne system (https://mc04.manuscriptcentral.com/bjps-scielo).

If you need further assistance, please contact the Journal staff (bjps@usp.br).