A New and Ecological Method to Quantify Vancomycin in Pharmaceutical Product by Infrared Spectrometry

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Abstract
Vancomycin, an antimicrobial, does not present quantitative method by infrared spectrometry in the literature for the evaluation of a pharmaceutical product. This technique is considered a clean alternative because in the main, there is no solvent involved and the generation of waste is reduced. So, the aim of this study was to develop and validate a new, ecological, low cost and fast method by infrared spectrometry using KBr and band between 1450–1375 cm–1. It was linear in the range of 1.0–2.0 mg/150 mg, with a correlation coefficient of 0.9994. Selective when the spectra of vancomycin reference and sample were compared. Precise by repeatability (2.29%) and intermediate precision (3.12%). Accurate with average recovery of 99.37% and robust when strength and compression time of the pellets and KBr brand were varied. Considering all the methods found in literature, there is not one using infrared spectrometry for quantitative purpose, so the method developed and validated could be considered an innovation and clean alternative. This is due to the fact that it is fast, easy to handle, low cost, and non-toxic as well as generating minimal waste. The method can be applied in the routine analysis of vancomycin dosage form and is an important option for the current and sustainable pharmaceutical analysis.

Keywords: Vancomycin; infrared spectrometric method; green analytical chemistry; sustainable alternative; pharmaceutical analysis.

1. Introduction
Vancomycin is the first glycopeptide antibiotic discovered. It has a molecular formula C66H75Cl2N9O24 and molecular mass 1449.27 g mol–1. Its mechanism of action comprises the disruption of cell wall of Gram-positive bacteria, being the only antibiotic used nowadays for the treatment of infections caused by the methicillin-resistant Staphylococcus aureus (MRSA).1-3 The main nucleus in glycopeptide antibiotics is an heptapeptide, which has a different substituent depending on the antibiotic.4-5 Figure 1 shows the structure and the substituents of vancomycin.

Inhibition of the bacterial cell wall occurs when this drug binds to the final residues of D-Ala-D-Ala of peptidoglycan by van der Waals forces and five hydrogen bonds, presenting strong binding, preventing the bind of peptidoglycan.5-7

In the context of the importance of vancomycin in the drug scenario, the development of analytical methods for its evaluation becomes extremely fundamental. Some analytical methods for this drug both physico-chemical8-14 and microbiological were found in literature.15-18 The spectrophotometry is a kind of very useful analytical method, which provides precise results and can be applied to quantify drugs. Only seven studies were found using this technique in literature for vancomycin and all of them in the UV region.19-25 However, the spectrometry in the infrared region is also an alternative, mainly because it can also be applied in the quantitative analysis of drugs, and has already been done to other drugs.26-34 No study relat-
ing this technique to vancomycin was found in literature, so the aim of this work was to develop and validate a spectrometric method in the infrared region for the quantification of vancomycin dosage form.

2. Experimental

2.1. Materials and Reagents

Vancomycin reference (declared content 96.30%) and lyophilized powder for injection (sample) 500 mg (labeled content) were used in spectrometric analysis in the infrared region (IR) and they were kindly donated by the ABL Antibióticos do Brasil (Cosmópolis, São Paulo, Brazil). Potassium bromide (KBr, Neon\textsuperscript{TM}, Suzano, São Paulo, Brazil), previously maintained in an oven for 24 h before the analysis was used as diluent for pellets preparation, each pellet contained 150 mg. A pool containing vancomycin reference or sample was prepared in the ratio of 1:10 and the pellets were obtained by weighing the appropriate amount from this pool.

2.2. Equipment

An analytical balance DV215CD (Discovery, Ohaus\textsuperscript{®}, São Paulo, Brasil) was used. An agate mortar and pestle were used to prepare the pellets, which were transferred to a compression system. A spectrophotometer IR-Prestige-21 (Shimadzu\textsuperscript{®}, Japan) was also used and the readings were performed using the software IR Solution\textsuperscript{®}.

2.3. Method Development

2.3.1. Pellets Preparation

Pellets of vancomycin reference and sample were prepared using KBr as a diluent. An amount of 20 mg of vancomycin reference and 180 mg of KBr were weighed and homogenized using the mortar and pestle. From this mixture an appropriate amount (16 mg) was taken and added to 134 mg of KBr in order to obtain 1.60 mg/150 mg tablets. The same procedure was done to the vancomycin sample, considering the average weight from twenty vials of vancomycin (504.92 mg). An amount of 20.19 mg vancomycin sample and 179.81 mg of KBr were weighed and homogenized. Then an amount of 16 mg was weighed and added to 134 mg of KBr, obtaining a final concentration of 1.60 mg/150 mg. This powder was transferred to the compression system for 7 min at 90 kN. After this period, pellets were placed on the spectrophotometer and the readings were performed at 1450–1375 cm\textsuperscript{-1}.

2.4. Method Validation

2.4.1. Validation Parameters

The validation procedure was performed according to the International Conference on Harmonization\textsuperscript{35} specifications for linearity, selectivity, limits of detection and quantification, precision, robustness, and accuracy.

Linearity: An analytical curve was obtained in the range of 1.00 mg/150 mg to 2.00 mg/150 mg. For this, 99 mg of the vancomycin sample and 891 mg of KBr was weighed and transferred to the mortar and mixed. From this pool, an appropriate amount was weighed in order to obtain pellets of 1.00, 1.20, 1.40, 1.60, 1.80, and 2.00 mg/150 mg. The linearity assay was performed on three different days and in triplicate. The data obtained were evaluated by regression analysis. The equation of the line was determined by linear regression analysis using the method of the least squares. Analysis of Variance (ANOVA) was also performed using the absorbance values obtained for each concentration.

Selectivity: Selectivity of the method was determined by the absorbance obtained for the vancomycin reference and sample, as well as the evaluation of the overlap of their spectra.

Limits of detection and quantification: The limits of detection (LOD) and quantification (LOQ) were obtained from the three calibration curves, using the Equations 1 and 2, respectively:

\[
\text{LOD} = 3 \times \frac{SD}{a} \quad (1)
\]

\[
\text{LOQ} = 10 \times \frac{SD}{a} \quad (2)
\]

SD: standard deviation

\(a\): average slope

Precision: Precision was evaluated by repeatability and intermediate precision. Repeatability assay was performed using six replicates of the same concentration (1.60 mg/150 mg) on the same day, with the same analyst, under the same conditions of analysis. Intermediate precision assay was performed by another analyst on a different day, under the same conditions of analysis. The preci-
sion was evaluated by RSD (%) values. The method was considered precise when RSD (%) values were lower than 2.00%.

Robustness: The robustness assay of the method was performed using 1.60 mg/150 mg tablets and small variations in three fundamental parameters: time of compression (normal: 7 min, variation: 5 and 9 min), strength of compression (normal: 90 kN, variation: 88 and 92 kN) and KBr brand (normal: Neon®, variation: Dinâmica®). Each condition was analyzed in triplicate, on the same day and with the same analyst. The results were analyzed by F-test and t-test compared to normal conditions. The method was considered robust when $t_{\text{calculated}}$ was smaller than $t_{\text{critical}}$.

Accuracy: The accuracy of the method was demonstrated by the recovery test, in triplicate and at three levels, 80, 100 and 120%, considering 1.60 mg/150 mg (100%). A standard pool was prepared with the vancomycin reference (30 mg + KBr: 270 mg). The sample pool was also prepared under the same conditions (sample: 30.29 mg + KBr: 272.60 mg). From the reference pool an amount of 10 mg was weighed and added to 140 mg of KBr, obtaining a pellet of 1.00 mg/150 mg. The same was done to the sample pool. The 3 levels, 80, 100 and 120%, were prepared using the same amount of the sample pool (10 mg) and a different amount of the reference pool, 28, 60 and 92 mg (equivalent to 2.80, 6.00 and 9.20 mg), respectively. Average recoveries, expressed in terms of percentage recovered from the standard and RSD (%), were determined. Method was considered accurate when recovery levels were 98 to 102% and RSD (%) values were lower than 2.00%.

2.5. Content Analysis

The preparation of the pellets was carried out according to section 2.3.1. Absorbance was measured at 1450–1375 cm$^{-1}$. Pellet was submitted to the compression system for 7 min under 90 kN. The values were compared using the Equation 3.

$$Cs = \frac{As \times Cr}{Ar}$$

$Cs$: concentration of sample pellet;
$As$: absorbance of sample pellet;
$Cr$: concentration of reference pellet;
$Ar$: absorbance of reference pellet.

Content measurement was considered adequate when content was 90–115%.

2.6. Comparison of Methods

The results of the vancomycin final product content obtained using the proposed method were compared with the results obtained by a microbiological method using turbidimetry.

3. Results and Discussion

3.1. Method Development and Validation

An appropriate amount of standard vancomycin was weighed and added to KBr in order to obtain pellets of

![Figure 2](image1.png)

Figure 2. Overlapping spectra of vancomycin standard and sample.

| Parameters               | 1450–1375 cm$^{-1}$ |
|--------------------------|---------------------|
| Linearity range (mg/150 mg) | 1.0–2.0             |
| Slope                    | 0.38                |
| Intercept                | 0.00                |
| Correlation coefficient (r)| 0.99                |
| Regression               | 371.33 (4.75)       |
| Lack of fit              | (3.26)              |
The data was validated by ANOVA (Table 1), which showed significant linear regression (F_{calculated} = 371.33 > F_{critical} = 4.75, p = 0.05), and no significant lack of fit (F_{calculated} = 3.12 < F_{critical} = 3.26, p = 0.05). In this way, the method can be considered linear.

The linearity was proven by the regression analysis using the least squares method, obtaining a correlation coefficient (r) of 0.99. It was also proven by the Analysis of Variance (ANOVA), which showed significant linear regression and no significant lack of fit.

The selectivity of the method was analyzed by comparing the spectra of vancomycin reference (black) and sample (red) (Figure 2).

The selectivity assay of the method was performed by the overlapping spectra of vancomycin reference and sample, which identified the drug.

The LOD and LOQ obtained were, respectively, 0.01 and 0.03 mg/150 mg. The limits are low, which show the sensitivity of the method.

Precision was proven by repeatability (RSD 2.29%) and by intermediate precision (RSD 3.12%). Both showed adequate results of RSD (%) and the intermediate precision was also analyzed by F-test and t-test, showing values of F_{calculated} < F_{tabulated} (3.74 < 5.05) and t_{calculated} < t_{tabulated} (2.21 < 2.23), respectively. So, the method can be considered precise. The results for repeatability and intermediate precision are shown in Table 2.

The robustness of the method was performed by small variations in three parameters. The method can be considered robust for these varied parameters when values of test t_{calculated} were smaller than the t_{critical}. The results are shown in Table 3.

The robustness of the method is an important parameter to be evaluated, which assures the results obtained. Variations performed in strength of compression,

| Day | Average content (%) | Final content (%) | RSD (%) |
|----|---------------------|------------------|--------|
| 1  | 101.66              | 102.35           | 1.09   |
| 2  | 101.75              |                  |        |
| 3  | 103.63              |                  |        |

The results for recovery are shown in Table 4.

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time of compression and KBr brand showed that the method remains robust and the quality of the results are reliable. This parameter is also useful in the development of other conditions for other drugs and samples, serving as a start for the conditions to be tested.

The accuracy of the developed method was determined by the standard recovery test. Recovery values are shown in Table 4, as well as the RSD (%). The method can be considered accurate, considering the average recovery in three days of analysis, in accordance to 98–102% specified by AOAC\textsuperscript{36} and RSD (%) values below 2%.

### 3.2. Content Analysis

The content of vancomycin in the final product was analyzed and is shown in Table 5. Content analysis is not a parameter required for validation but it is also important to perform because it assures the content of the drug in the marketed sample. The analysis showed that the content of vancomycin is within the limits stipulated by Japanese Pharmacopoeia (90–115%).\textsuperscript{25}

### 3.3. Comparison of Methods

The result of the comparison between the proposed method (physico-chemical) and the turbidimetric method (microbiological) was made by the vancomycin content values in the final product and is shown in Table 6. An antimicrobial must always be analyzed using 2 methods, 1 physico-chemical and 1 microbiological, as many times physico-chemical methods are unable to assess the real potency of the antimicrobial.

In this case, the proposed method was directly compared with the microbiological method and was statistically equivalent, which allows its reliable use in the evaluation of the vancomycin final product.

Spectrometric method in infrared region is an important technique that can be used to quantify drugs, mainly because of its ecological characteristics and simplicity to perform. It also protects the environment, due to the less generation of waste, and the analyst, who does not need to get in touch with solvents.

### 4. Conclusions

In the present work a spectrometric method in infrared region was developed for the evaluation of vancomycin in lyophilized powder. This method is by itself greener when compared to a chromatographic method or a spectrophotometric method in the UV region, because there is no use of solvents, which generates less waste, for example. It is sensitive and proven to be precise to quantify the drug, being a great alternative to the routine quality control process of vancomycin in chemical and pharmaceutical laboratories. The method is also linear in the range of 1.00 to 2.00 mg/150 mg, selective, precise, robust, and accurate. It is worth remembering that each drug has specific characteristics and specific analysis rigidities. Many times, the method of analysis of one drug is not necessarily useful for another, so this work is important, which shows the ideal and eco-friendly conditions for the analysis of vancomycin in final product by spectrometry in infrared region. It generates less waste, does not use solvents, is fast and very simple to perform the test. Furthermore, it is advantageous because the sample can be analyzed in solid state, does not require the use of diluents or solvents, does not expose analysts to vapors that can be toxic and it can be used in production line control.

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### Declaration of interest

The authors have no financial or other potential conflicts of interest.
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Povzetek
Za določanje protimikrobne učinkovine vankomicina v farmacevtskih proizvodih v literaturi ni najti kvantitativne metode z infrardečo spektrometrijo. Ta tehnika se smatra za čistejšo alternativo, saj v splošnem ne uporablja nobenih topil in je tudi količina odpadkov zmanjšana. Cilj te raziskave je bil razviti in validirati novo ekološko, poceni in hitro metodo z infrardečo spectrometrijo z uporabo KBr in traka 1450–1375 cm\(^{-1}\). Metoda je bila linearna v območju 1,0–2,0 mg/150 mg s korelacijskim koeficientom 0,9994. Selektivna ob primerjavi spektra referenčnega vankomicina in vzorca. Natančna s ponovljivostjo 2,29\% in vmesno ponovljivostjo 3,12\%. Točna s povprečnim izkoristkom 99,37\% ter robustna, če smo spreminjali moč in čas kompresije peletov ter znamko KBr. Ob primerjavi z metodami v literaturi ni nobene, ki bi uporabljala infrardečo spektrometrijo za kvantitativne namene, zato predstavljeno validirano metodo lahko smatramo za inovacijo in čistejšo alternativo. Razlog je, da je hitra, enostavna za uporabo, poceni, nestrippena ter proizvaja minimalno odpadkov. Metodo se lahko uporabi za rutinsko analizo vankomicina v farmacevtskem prašku in predstavlja pomembno možnost za sodobno in trajnostno farmacevtsko analitiko.