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Research Article

FTIR Spectrophotometry as a Green Tool for Quantitative Analysis of Drugs: Practical Application to Amoxicillin

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Amoxicillin is an antimicrobial agent that belongs to the penicillin compounds. Its bactericidal action causes a destruction of the cell wall of bacteria. It is widely used in clinical practice, and it belongs to the Brazilian National List of Essential Drugs (RENAME). In literature, there are some green analytical methods for the amoxicillin analysis; however, none of them is focused on its quantification in capsules. Therefore, the aim of this study was to validate an environmentally friendly analytical method for the analysis of this antimicrobial action in capsules, using spectrophotometry in the mid-infrared region. The analyses were performed in the spectral range of 1815–1736 cm^{-1}, and the samples were analyzed as potassium bromide pellets. The method was validated according to the ICH guidelines and Brazilian legislation. Linearity, selectivity, precision, accuracy, and robustness were evaluated and showed adequate results for method validation, in a concentration range of 0.5–1.5 mg/pellet. Thus, it is concluded that the validated spectrophotometric method is able to quantify amoxicillin in capsules. In addition, it is a fast, economical, and environmentally friendly method, since it does not use organic solvents, and it can be used for quality control of routine analysis of this drug.

1. Introduction

Amoxicillin is a broad-spectrum antimicrobial agent belonging to the class of penicillins and is widely used in clinical practice. Furthermore, it belongs to the Brazilian National List of Essential Drugs (RENAME). Amoxicillin is available in tablets, capsules, and powder for oral suspension. It is also available associated with potassium clavulanate, for the treatment of infections caused by β-lactamase-producing bacteria, and presented in the pharmaceutical dosage forms of tablets and oral suspension [1].

This penicillin is generally indicated for treating infections caused by microorganisms responsible for gonorrhea, pneumonia, bronchitis, otitis, or endocarditis. Furthermore, it is applied after infections of the urinary tract caused by *Haemophilus influenzae*. It can be used as an alternative treatment in cases of gastroenteritis and typhoid fever caused by *Shigella*. Amoxicillin is considered the drug of choice for the prophylaxis of bacterial endocarditis, and it can be combined with omeprazole and clarithromycin for combating *Helicobacter pylori* in the patients with peptic ulcer [2].

This drug has a rapid bactericidal action, acting on the cell wall of bacteria. It is very well-absorbed orally, and 60 to 70% are excreted renally. Generally, amoxicillin is administered three times daily as a 500 mg capsule, or even twice daily, and it is well tolerated by the organism. However, it may have some undesirable effects such as nausea, vomiting, and gastrointestinal irritation [3].

In literature, there are several published studies describing the development of the analytical methods for analysis of amoxicillin in different matrices. However, most of the present methods use highly potential toxic solvents for the operators and environment, for example,

high-performance liquid chromatography (HPLC) [4–8], ultraperformance liquid chromatography (UPLC) [9], or iodometry [10].

The quality of drugs has always been a concern of the World Health Organization (WHO). Since without the guarantee these products meet the required standards of quality, safety, and effectiveness, any health service is evidently affected [11]. Because of this, attention on the quality control of drugs is important. Pharmaceutical companies must ensure that their marketed products have adequate safety and efficacy for the population.

Therefore, the validation of the efficient analytical methods for use in the quality control of the marketed drugs is essential. The validation of an analytical methodology aims at verifying whether a tested method is suitable for a particular purpose, in other words, whether the method is able to analyze qualitatively and/or quantitatively a drug or related substances in pharmaceutical dosage forms [12–14].

The trend is that industries seek ways to reduce the impacts of their activities on the environment. Thus, they can adopt the position of reduction, prevention, or elimination of process waste. In this regard, there are some actions that can be taken, such as the replacement of the analytical methods that employs a high amount of organic solvents with others that do not use them, the replacement of a synthesis process by a greener one, or the exchange of raw materials or inputs by other less toxic ones [15].

In this context, the infrared spectrophotometry stands out. This method allows a quantification of substances without using organic solvents. This technique does not require any extraction step of the sample and therefore can also be used for substances with solubility problems.

Considering the importance of amoxicillin for clinical practice and the relevance of seeking green methods for the analysis of drugs, this work describes the development and validation of an innovative and environmental friendly analytical method, by spectrophotometry in the mid-infrared region, for quantification of amoxicillin in capsules.

2. Experiment

2.1. Equipment. For spectrophotometric analysis, a spectrophotometer FTIR Shimadzu (Kyoto, Japan), IR Prestige-21 model, was used. This equipment was connected to a computer to use the "IR Solution" software for analysis of the spectra. The construction of calibration curves was performed using Microsoft Excel (2013).

The other equipment used is as follows: H51 analytical balance (Mettler Toledo™, Barueri, Brazil) and oven ECB 1.2 Digital (Odontobrás™, Ribeirão Preto, Brazil).

2.2. Chemicals and Reagents. The pharmaceutical company União Química (São Paulo, Brazil) kindly donated amoxicillin reference standard (AMX RS) (declared a purity of 98.9%) and amoxicillin (AMX) in the pharmaceutical dosage form of the capsule (Uni Amox™, 500 mg/capsule, União Química, São Paulo, Brazil). Adjuvants present in this pharmaceutical dosage form are sodium lauryl sulfate, croscarmellose sodium, and magnesium stearate. These substances were purchased from Sigma-Aldrich (São Paulo, Brazil). Potassium bromide (KBr) analytical grade was also used (Synth™, Diadema, Brazil).

2.3. Qualitative Analysis. To perform the method, the spectrophotometer described in Equipment was used. For the preparation of AMX RS pellets, an equivalent to 2.0 mg of AMX RS was accurately weighted and then homogenized with 148.0 mg of KBr, previously powdered, and dried at 105°C in an oven to constant weight, in order to obtain a 150 mg pellet (at 2 mg/pellet). This mixture was compressed by a mechanical press for 10 minutes to obtain a translucent pellet. The spectral region included in the analysis was from 4000 to 400 cm^{-1} (the mid-infrared region). The analysis was held in transmittance, and the spectrum was obtained with the aid of "IR Solution" software (Shimadzu, Kyoto, Japan). The same procedure was performed with amoxicillin in capsules. Finally, a comparison of the spectra obtained from AMX RS and AMX capsule was performed to verify the similarity between them.

In addition, three independent pellets were prepared, containing each adjuvant present in the pharmaceutical dosage form (croscarmellose sodium, sodium lauryl sulfate, and magnesium stearate), in a concentration of 1.0 mg/pellet. For this, 1.0 mg of each component was homogenized with 149.0 mg of KBr. Each mixture was compressed by a mechanical press for 10 minutes to obtain translucent pellets. The spectral region included in the analysis was from 4000 to 400 cm^{-1} (the mid-infrared region). This procedure was performed to define the best spectral region to be used in the quantitative analysis (a region without interference of adjuvants).

2.4. Quantitative Analysis

2.4.1. Obtaining the Calibration Curve. To perform the method, the spectrophotometer described in "Equipment" section was used. Samples were analyzed as KBr pellets containing the drug. After obtaining the spectrum in the mid-infrared range (4000–400 cm^{-1}), the spectral region from 1815.0 to 1736.0 cm^{-1} was selected, which corresponds to a characteristic band of the amoxicillin molecule (carbonyl), and its height was quantitatively analyzed in absorbance.

During preliminary tests, five concentrations of amoxicillin were selected for the analytical curve, as follows: 0.5, 0.75, 1.0, 1.25, and 1.50 mg/pellet.

For the preparation the pellets, the amounts equivalent to 0.50, 0.75, 1.00, 1.25, and 1.50 mg of AMX RS (previously diluted in potassium bromide of 1:10, w/w) were taken and diluted with sufficient amounts of KBr to obtain 150 mg pellets. The powders were mixed and ground to obtain a homogeneous mixture. Thereafter, this mixture was compressed with a mechanical press for 10 minutes to obtain translucent pellets.

2.4.2. Determination of Amoxicillin in the Pharmaceutical Dosage Form. Amoxicillin content in capsules was calculated by Equation (1), and its percentage content was calculated by Equation (2):

$$C_s = A_s \times \frac{C_{RS}}{A_{RS}}, \quad (1)$$

$$C_s\% = C_s \times \frac{100}{C_t}. \quad (2)$$

where C_s is the concentration of AMX in the sample (mg/pellet), A_s is the absorbance of the AMX sample pellet, C_{RS} is the concentration of AMX RS solution (mg/pellet), A_{RS} is the absorbance of the AMX RS pellet, $C_s\%$ is the percentage concentration of AMX in the sample, and C_t is the theoretical concentration of AMX in the sample (mg/pellet)

2.4.3. Method Validation. The method was validated by analyzing the following parameters: linearity, precision, accuracy, robustness, and selectivity, as recommended by ICH guidelines and Brazilian legislation [12, 13]. Limits of detection and quantification are not required for the validation of this class of the analytical method (Class I), according to ICH guidelines and Brazilian legislation [12, 13].

(1) Linearity. To check the linearity of the method, five concentrations of AMX RS (0.5 to 1.5 mg/pellet) were used and evaluated on three different days. Linearity was confirmed by linear regression of least squares and statistical analysis by ANOVA.

(2) Precision. Precision was evaluated in two different ways: by intermediate precision (interday and between analysts) and repeatability (intraday). Interday precision was performed by analysis of six pellets of AMX RS (at 1.0 mg/pellet) on three different days, at the same experimental conditions. Similarity between the absorbances obtained on different days was evaluated by analysis of variance (ANOVA). Between-analyst's precision was carried out by the analysis of six pellets of AMX RS (at 1.0 mg/pellet) performed by two different analysts. In this case, the absorbances were compared by *F*-test and *t*-test. Regarding intraday precision, it was evaluated by the analysis of six consecutive pellets of AMX RS (at 1.0 mg/pellet) in the same day and at the same working conditions. Thereafter, the percentage relative standard deviation value (RSD) between the absorbances was calculated [13].

(3) Accuracy. Accuracy was performed by the analysis of AMX RS recovery, in triplicate, at three levels (R1, R2, and R3), from 80 to 120% of the working concentration of the method (1.0 mg/pellet). The pellets for the recovery assay are prepared in accordance with the description in Table 1.

The recovery percentage was calculated by the equation determined by the Association of Official Analytical Chemists (AOAC) [16].

(4) Robustness. Robustness aims at showing the reliability of the method after small variations in the analytical parameters. The following parameters were varied: temperature of the workroom (21°C, with the air conditioning on, and 26 C, with the air conditioning off), compression time of the pellets (2 minutes above and below the working compression time), and KBr brand (Shimadzu). For this, six pellets of AMX RS (at 1.0 mg/pellet) were analyzed under each previously described condition. Similarity of the results was evaluated by the *F*-test and *t*-test, comparing the normal working conditions.

(5) Selectivity. Selectivity was analyzed in order to verify the ability of the method to quantify the drug in the presence of the adjuvants present in the pharmaceutical dosage form of the capsule [12, 13]. The used method has been previously described in Qualitative analysis.

3. Results

3.1. Qualitative Analysis. Figure 1 shows an overlay of the absorption spectra of the AMX RS and sample. Figure 2 displays the absorption spectra of each adjuvant present in the capsules. The spectral range chosen for quantitative analysis (from 1815 to 1736 cm^{-1}) is also identified in both figures.

3.2. Quantitative Analysis

3.2.1. Linearity. Three calibration curves, generated on three different days, were analyzed by a graphical representation. The average values of absorbance were plotted against their respective concentrations. A final analytical curve was obtained by averaging the three analytical curves, resulting in a correlation coefficient (r) of 0.9971, as shown in Figure 3.

ANOVA calculated for the data of the analytical curve showed an $F_{calculated}$ of 521.45 and $F_{critical}$ of 0.00445.

3.2.2. Precision. Precision was evaluated according to the repeatability (intraday precision) and intermediate precision (interday precision) and between analysts. Intraday precision shows a RSD of 3.80% among the absorbance values. Table 2 shows the absorbances obtained for the evaluation of interday precision and between analysts.

The absorbances obtained for interday precision (Table 2) were compared by ANOVA. $F_{calculated}$ and $F_{critical}$ were 1.94 and 3.68, respectively. To calculate the precision between analyst, *F*-test and *t*-test were used (Table 3). As the result, $t_{calculated}$ and $t_{critical}$ were 1.94 and 2.23, respectively, showing no significance.

3.2.3. Accuracy. Accuracy was evaluated by the recovery test and conducted at three different concentration levels, and the results are shown in Table 3.

3.2.4. Robustness. Robustness was evaluated by small variations in working parameters, such as the temperature of the

Table 1: Preparation of pellets for the recovery assay of the method of FT-IR spectrophotometry for amoxicillin.

	AMX sample (mg) (diluted 1 : 10 w/w in KBr)	AMX RS (mg) (diluted 1 : 10 w/w in KBr)	Amount of KBr (mg)[1]	Final theoretical concentration (mg/pellet)
Sample	5.0	—	145.0	0.50
R1	5.0	3.0	142.0	0.80
R2	5.0	5.0	140.0	1.00
R3	5.0	7.0	138.0	1.20
Reference standard	—	5.0	145.0	0.50

[1]Sufficient amount for the preparation of pellets with a total weight of 150 mg. AMX: amoxicillin; AMX RS: amoxicillin reference standard.

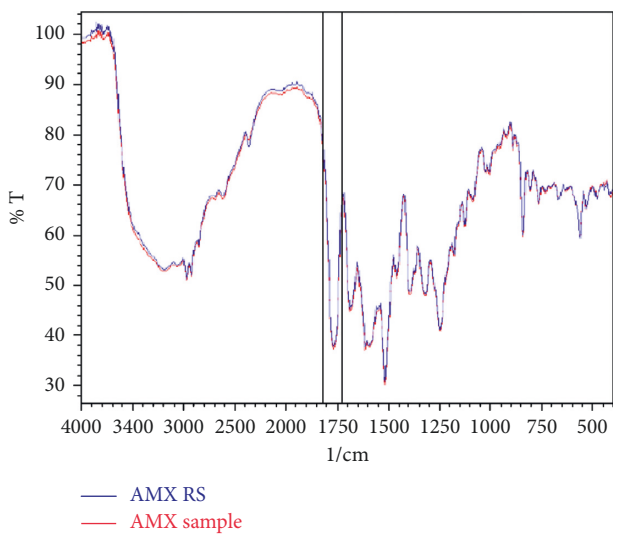

Figure 1: Overlap of the absorption spectra in the mid-infrared region of AMX RS and in the pharmaceutical dosage form of capsules. AMX RS: amoxicillin reference standard; AMX: amoxicillin. Spectral range chosen for quantitative analysis (from 1815 to 1736 cm^{-1}) is highlighted.

workroom, pellet compression time, and KBr brand. The obtained experimental values were evaluated by F-test and t-test, by comparing the absorbance values from the normal and varied conditions. The obtained absorbances are presented in Table 4.

For the temperature of the workroom, the values of $t_{calculated}$ and $t_{critical}$ were 4.96 and 2.36, respectively. This result is statistically significant.

Regarding the pellet compression time, the absorbances obtained for the normal working condition (10 minutes) were compared, individually, with each varied condition (8 and 12 minutes). The comparison between 8 and 10 minutes showed $t_{calculated}$ and $t_{critical}$ of 2.04 and 2.26, respectively. This result is statistically significant. In relation to the higher pellet compression times comparison (10 and 12 minutes), the $t_{calculated}$ was 1.97 and $t_{critical}$ 2.31, showing no significance.

Concerning the KBr brand, the absorbances obtained from two different brands were compared (Synth and Shimadzu). In this case, the values of $t_{calculated}$ and $t_{critical}$ were 1.21 and 2.23, respectively, showing no significance.

3.2.5. Selectivity. Results obtained from selectivity analysis have been previously described in Qualitative analysis. Figure 1 presents an overlay of the absorption spectra of the AMX RS and sample. Figure 2 presents the absorption spectra of each adjuvant present in the analyzed pharmaceutical dosage form. The spectral range chosen for quantitative analysis (from 1815 to 1736 cm^{-1}) is also identified in both figures.

3.3. Determination of Amoxicillin in the Pharmaceutical Dosage Form. Three quantifications of amoxicillin in the capsules were performed, according to Equations (1) and (2). Table 5 shows the results of these assays.

4. Discussion

Environmental preservation is an important issue nowadays and is increasingly a concern of chemical and pharmaceutical industries. In order to reduce the environmental damage caused by toxic waste generation, companies look for alternatives to reduce, prevent, and even eliminate the generation of chemical waste from their processes. One option is to replace methods that use organic solvents by others that do not use the environmentally friendly methods [15, 17].

It is in this context, the infrared spectrophotometry stands out because it is a method that allows to quantify substances without organic solvents. It is suitable for drugs with solubility problems, since they can be analyzed in the solid form [18]. Infrared spectrophotometry is based on the fact that the chemical bonds of the molecules have natural vibrational frequencies. Each molecule only absorbs selected frequencies of radiation in the infrared region, which are equivalent to its natural vibrational frequencies. This absorption increases the amplitude of vibrational motion of the chemical bonds. Thus, the frequency of vibration may be associated with a particular type of the band [19, 20].

In addition to the absence of organic solvents, the spectrophotometric method in the infrared region has other advantages, such as being a rapid technique that does not require a pretreatment of the sample and assisting in detecting impurities. Another advantage is the low cost of materials for the manufacture of pellets. On the other hand, this method requires high control of humidity and temperature of the working environment.

The development of the analytical methods by spectrophotometry in the mid-infrared region has been successfully used in the quantification of other drugs [18, 21–36].

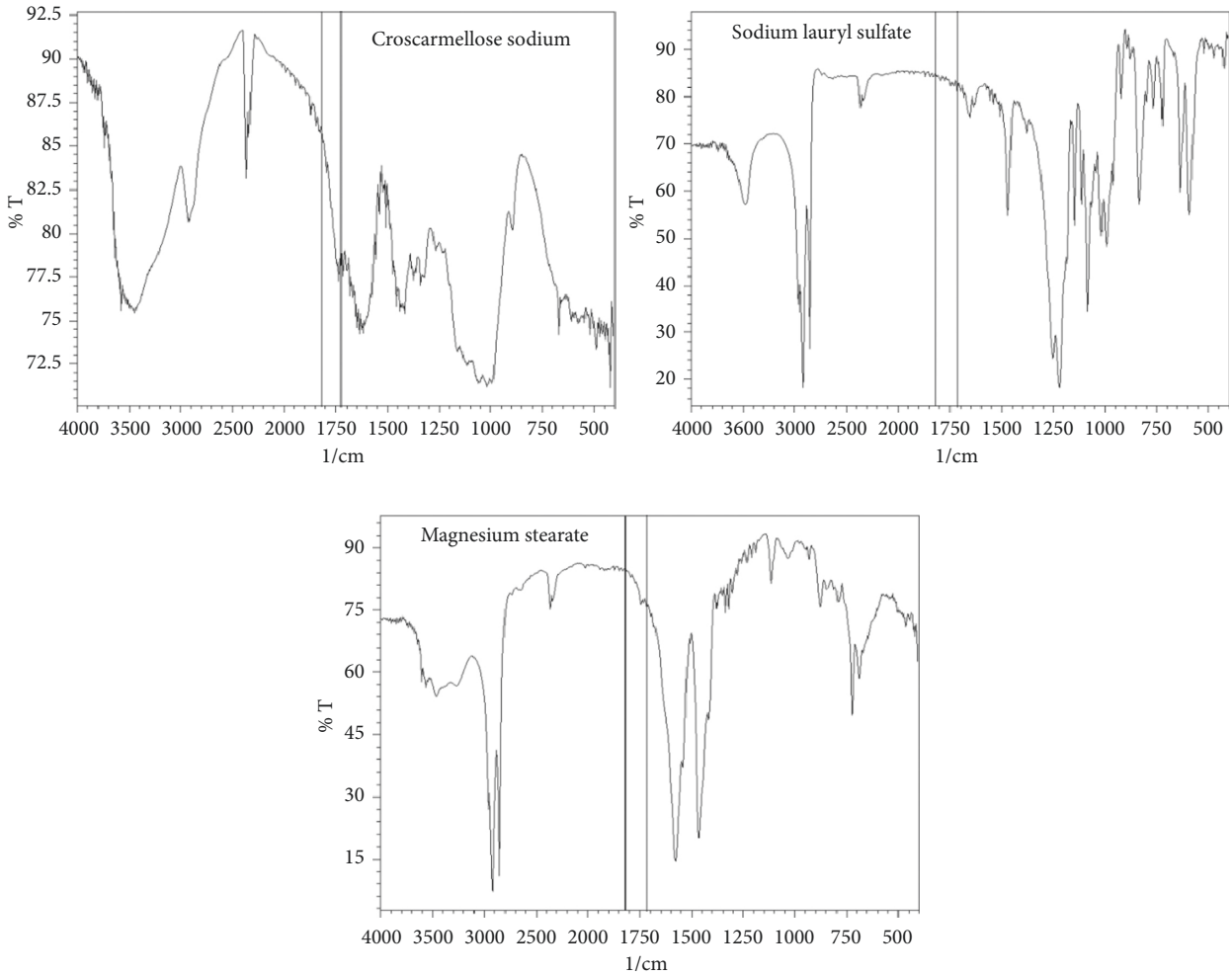

Figure 2: Absorption spectra in the mid-infrared region of the adjuvants present in the pharmaceutical dosage form of the capsule. Spectral range chosen for quantitative analysis (from 1815 to 1736 cm^{-1}) is highlighted.

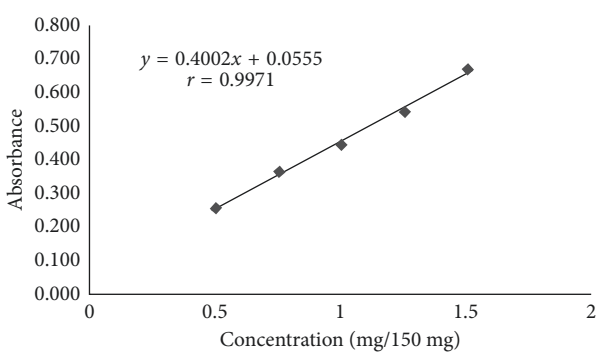

Figure 3: Graphical representation of the amoxicillin analytical curve by FT-IR spectrophotometry.

To develop the proposed method, the first step was a qualitative test. For this, an overlap of the spectra obtained with amoxicillin reference standard and amoxicillin in capsules was carried out (Figure 1). It was noticeable that both spectra have the same absorption bands, confirming the absence of adjuvants interference, impurities, or degradation products. The similarity between the spectra is a strong indicative of the identity of amoxicillin in the pharmaceutical dosage form.

The second step for the development of the quantitative method was to select a spectral range in the spectrum of amoxicillin in the mid-infrared region, to be quantitatively analyzed. For this, a comparison between the spectrum obtained with amoxicillin reference standard and each adjuvant present in the composition of amoxicillin in capsules was performed (Figure 2). It was observed that, between 1815 and 1736 cm^{-1}, which represents the amoxicillin carbonyl band, there was no adjuvants interference. Thus, this was the spectral range selected for the quantitative analysis.

After the selection of the spectral range for the quantitative analysis, the analytical curve was constructed. With this purpose, many concentrations of AMX RS were tested, and five of them were selected for the method, as follows: 0.5, 0.75, 1.0, 1.25, and 1.50 mg/pellet. Thereafter, the method was validated according to the ICH guidelines and Brazilian legislation [12, 13].

Three calibration curves, generated on three different days, were analyzed by a graphical representation. The average values of absorbance were plotted against their

Table 2: Absorbances obtained for the evaluation of interday and between analysts precision to validate the spectrophotometric method in the mid-infrared region for the analysis of amoxicillin capsules.

Interday			Between analysts	
Day 1	Day 2	Day 3	Analyst 1	Analyst 2
0.466	0.447	0.424	0.466	0.447
0.472	0.417	0.456	0.472	0.417
0.458	0.413	0.445	0.458	0.413
0.437	0.445	0.436	0.437	0.445
0.436	0.447	0.417	0.436	0.447
0.432	0.417	0.457	0.432	0.417

Table 3: Accuracy of the spectrophotometric method in the mid-infrared region for analysis of amoxicillin capsules.

	Theoretical concentration (mg/pellet)	Real concentration (mg/pellet)	Recovery (%)	RSD %	Average recovery (%)
R1	0.800	0.798	99.800	1.45	
R2	1.000	1.010	101.040	0.66	100.420
R3	1.200	1.205	100.420	0.80	

RSD: relative standard deviation.

Table 4: Absorbances obtained during the robustness evaluation of the spectrophotometric method in the mid-infrared region for the analysis of amoxicillin capsules.

Temperature of workroom		Pellet compression time			KBr brand	
21°C*	26°C	8 min	10 min*	12 min	Synth*	Shimadzu
0.472	0.495	0.415	0.428	0.435	0.430	0.437
0.447	0.494	0.420	0.424	0.434	0.433	0.433
0.460	0.504	0.418	0.427	0.432	0.435	0.431
0.458	0.508	0.417	0.417	0.413	0.434	0.432
0.447	0.491	0.422	0.421	0.434	0.439	0.430
0.491	0.503	0.413	0.417	0.433	0.432	0.426

*Normal working condition.

Table 5: Determination of amoxicillin in the capsules.

Assay	AMX content (mg/capsule)	AMX content (%)	Average content (%)	RSD (%)
1	501.535	100.307		
2	501.150	100.230	100.340	0.122
3	502.350	100.470		

AMX: amoxicillin; RSD: relative standard deviation.

respective concentrations. A final analytical curve was obtained by averaging the three analytical curves, resulting in a correlation coefficient (r) of 0.9971, as shown in Figure 3. The r value is situated in accordance with the required r values determined by the validation guidelines in the area of acceptance. Statistical analysis (ANOVA) of the results showed adequate regression of the calibration curve, as the $F_{\text{calculated}}$ (521.45) was higher than F_{critical} (0.00445).

Precision was evaluated according to the repeatability (intraday precision) and intermediate precision (interday) and between analysts. The intraday precision showed adequate response, as the calculated RSD between the absorbances obtained from six samples, analyzed at the same day and same experimental conditions, was 3.80%.

For interday and between analysts precision (Table 2), the absorbances obtained in three different days and by a second analyst were compared by ANOVA and F-test and t-test, respectively. Regarding the interday precision, the $F_{\text{calculated}}$ was lower than F_{critical}, and the precision between analysts $t_{\text{calculated}}$ was lower than t_{critical}. These data show the appropriate precision (interday and between analysists) of the method, since the absorbances obtained were statistically equivalent.

Accuracy of the method was also proved, since the recovery assay provided an average value of 100.42% (Table 3). This value is in accordance with the range recommended by the Horwitz Trumpet [37].

Robustness was evaluated by small variations in the following analytical conditions: temperature of the workroom, pellet compression time, and KBr brand. The obtained experimental values were evaluated by the F-test and t-test, by comparing the absorbance values from the normal and

varied conditions (Table 4). For the temperature of workroom, $t_{calculated}$ was higher than $t_{critical}$. It means the method is not robust for this aspect. In this way, the temperature of workroom must be strictly controlled for performing the method. When the air conditioning is turned off, the humidity of the environment increases, and this can be the main cause of the lack of robustness for this parameter. Regarding the other varied parameters (pellet compression time and KBr brand), all of them showed to be robust, since $t_{calculated}$ were lower than $t_{critical}$.

Selectivity of the method was carried out in order to verify whether the adjuvants present in the pharmaceutical dosage form were able to interfere with the analysis of amoxicillin. Figures 1 and 2 show that there are no interferences in the spectral range selected for quantitative analysis, so the selectivity was evidenced. It is important to highlight that, in Figure 2, each adjuvant was prepared in a concentration higher than that they are in the capsules, in order to better analyze the absorption bands. For this reason, the absorption bands presented in the excipient spectra are more prominent than that in Figure 1.

Three quantifications of amoxicillin in the capsules were performed, and the results are presented in Table 5. The average amoxicillin content was 100.34%, which is in accordance with the range recommended by the Brazilian Pharmacopoeia (90 to 120%) [20].

In this way, the method met all the requirements of the ICH guidelines [13] and Brazilian legislation [12] for validation of the analytical methods and can be used in quality control laboratories for the analysis of this drug. In addition, it presents low cost maintenance and is an environmentally friendly method.

5. Conclusion

The method has fulfilled all the requirements of the ICH guidelines and Brazilian legislation for the validation of the analytical methods. Spectrophotometry in the mid-infrared region for the quantification of amoxicillin in capsules showed adequate linearity, accuracy, precision, selectivity, and robustness for the pellet's compression time and KBr brand. On the other hand, the temperature of the workroom must be strictly controlled for performing the method. Therefore, this method can be used as an innovative alternative in quality control laboratories, since it is fast, clean (for the analysts and environment), presents low cost maintenance, and does not generate toxic chemical waste.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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