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Development and Validation of the Quantitative Analysis of Cefuroxime Sodium in Powder for Injection by Infrared Spectroscopy

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Abstract A simple, useful and reproducible infra red spectrophotometric method for quantitative determination of cefuroxime (CFU) in powder for injection was developed. This method involved the measurement of absorbance measurements of the band corresponding to aromatic ring centered by 1475-1600 cm^{-1}. The validation of analytical method was carried out to the study of the following analytical parameters: linearity, specificity, precision, accuracy and robustness. The linearity range was found to be 5.0 to 20.0 $\mu g.ml^{-1}$ (regression equation: $y = 0.5053x + 0.0114$, $r^2 = 0.9991$). The data show a good precision of the method, since the R.S.D. values are close to 2% and below 5%. The proposed method was successfully applied to the assay of cefuroxime in powder for injection.

Keywords Infrared Spectroscopy, Quantitative Determination, Quality Control

1. Introduction

Antibiotics are specific chemical substances produced by living organisms that are able to inhibit the action of other organisms. Molecules of cephalosporins are characteristically composed of three main parts, namely: β-lactam ring, free carboxyl acid group and a substituted amino acid side chain. The main representatives of this class are shown in Figure 1.

Replacement of R1 or R2 positions results in different compounds with different antimicrobial spectrum, power, bioavailability, half-life and toxicity profile. These differences are relatively small, however with rampant bacterial resistance, a study of this class of drugs requires ongoing research so that in the near future a new generation of pharmaceuticals being developed and introduced into clinical practice. The chemistry of cephalosporins has been widely explored because of their extensive medical applications[1].

Cefuroxime (CAS 56238-63-2) (Figure 2) is a cephalosporin of second generation with high antibacterial activity; it has enhanced in vitro activity against clinically important Gram-positives and Gram-negative microorganis ms[2].

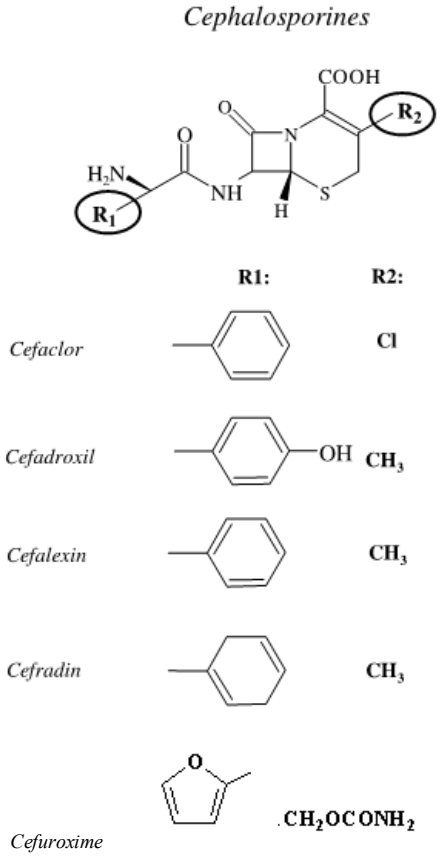

Figure 1. General structure of cephalosporin, showing the main substituents with their names

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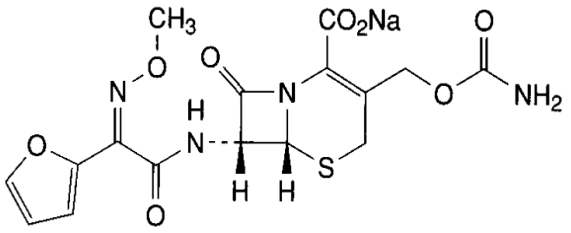

Figure 2. Chimical structure cefuroxime sodium (CAS 56238-63-2)

Infrared spectroscopy is an important technique used for the characterization of very complex mixtures. The portion of the infrared region most useful for analysis of organic compounds is having a wavelength of 2500-1600 nm[3]. Among the methods recommended by the British Pharmacopoeia identifying spectrometric in IR seems to emerge as the best method to identify approximately 60% of the substances[4].

The infrared spectra are caused by different modes of vibration and rotation of a molecule. At wavelengths below 25 nm radiation has enough energy to cause changes in the levels of vibrational energy of the molecule, and these changes are accompanied by changes in the levels of rotational energy, so that energy is large enough to cause vibrational transitions in ground state of the molecule along with the rotations associated. This means that the infrared absorption curves are much more specific than the ultraviolet and visible region, though some peaks may, however lose sharpness[5].

Infrared spectrum of a sample may be obtained by passing a beam of infrared light through the sample. Examination of the transmitted light reveals how much energy was absorbed at each wavelength. This can be done with a monochromatic beam, which changes in wavelength over time, or by using a Fourier transform instrument to measure all wavelengths at once. From this, a transmittance or absorbance spectrum can be produced, showing at which infrared wavelengths the sample absorbs. Analysis of these absorption characteristics reveals details about the molecular structure of the sample[6].

Infrared spectroscopy is widely used in both research and industry as a simple and reliable technique for measurement and quality control. It is of especial use in forensic analysis in both criminal and civil cases and has been highly successfully for applications in both organic and inorganic chemistry[7].

Several analytical procedures are available in the literature for the analysis of antimicrobial[8-19], high performance liquid chromatography[19-25], capillary electrophoresis[26], fluorimetry[27-30], polarography[31-35], titrimetry[36] and bioassay[37-39]. Although infrared spectroscopy is officially accepted for the identification of various compounds, the literature shows few publications that use this technique for quantitative analysis[40-44].

Therefore, the aim of this study was to develop and validate a new and novel method of infrared spectroscopy for the quantitative determination of cefuroxime powder for solution for injection.

2. Experimental

2.1. Chemicals

Cefuroxime (CFU) reference substance (purity 97.40%) was kindly supplied by Glaxosmithkline and cefuroxime sodium powder for injection Cellofarm Farmacêutica (São Paulo, Brazil). Cefuroxime sodium powder for injection (Zencef®) were claimed to contain 750 mg (as anhydrous base) of active drug.

Potassium bromide (Merck, Darmstadt, Germany) used to the preparation of translucent pellets was of analytical grade and was previously dried at 120ºC for 2 h.

2.2. Instrumentation and Analytical Conditions

2.2.1. Equipment

A conventional SHIMADZU IR Spectrometer Model FTIR 8300 (Tokyo, JP) with spectral digitalization was used for the analysis and obtaining data and respective absorption regions (wavelength region of 500-4000 cm^{-1} at 2 cm^{-1} intervals). After obtaining the IR spectrum and with the assistance of the IR Solution software, quantitative analysis was carried out in the spectral region between 1800 and 1700 cm-1, related to a carbonyl band of the cefuroxime molecule, and this band had its height analyzed in terms of absorbance.

2.2.2. Obtaining of Analytical Curve

Equivalent amounts of 5.0, 7.0, 10.0, 15.0 and 20.0 mg of cefuroxime reference substance (previously diluted with potassium bromide 1:10, w/ w) were taken and diluted with sufficient amounts of potassium bromide to obtain 250 mg pellets. The powders were mixed and ground until obtaining a homogeneous mixture. Thus, this mixture was compressed in a mechanical die press for 15 minutes to obtain translucent pellets, through which the beam of the spectrometer can pass.

2.2.3. Determination of Cefuroxime in the Pharmaceutical Dosage Form

Preparation of cefuroxime RS pellets

Amounts of powder equivalent to 1.5 mg of cefuroxime (15.0 mg of the 1:10 dilution in potassium bromide) were taken and homogenized with 235 mg of potassium bromide, making the total pellet weight of 250 mg. The determinations were performed in triplicate.

Preparation of cefuroxime Sample Pellets

The contents of twenty vials of cefuroxime in powder for injection solution were mixed. From this mixture, amounts of 1.5 mg of cefuroxime (15.0 mg of the 1:10 dilution in potassium bromide) were taken and homogenized with 235 mg of potassium bromide, making the total pellet weight of 250 mg. The determinations were performed in triplicate.

Readings were made at wavelength region of 500-4000

cm-1 and absorbances were monitored at 1475-1600 cm-1 region, that corresponding to the aromatic ring absorption region of the molecule.

2.2.4. Calculation of Cefuroxime Content in the Sample

The cefuroxime concentration in the sample was calculated by Equation 2 and its percentage content was calculated by Equation 1.

$$Cs = As \times \frac{Crs}{Ars} \quad (1)$$

$$Cs\% = Cs \times \frac{100}{Ct} \quad (2)$$

Where:
Cs = Sample concentration
Cs% = Percentage sample concentration
Crs = Concentration of reference substance (mg)
As = Sample absorbance
Ars = Reference substance absorbance
Ct = Theoretical concentration of cefuroxime in the sample

2.3. Method Validation

The method was validated by determining the following parameters: linearity, precision, accuracy, robustness, and detection and quantification limits, according to the literature recommendation[45].

2.3.1. Linearity

To asses linearity of the method, doses of reference substance were evaluated on 3 different days. Regression lines were calculated by the least-squares method. Statistical evaluation was made by ANOVA. For the infrared spectrometric method, linearity was verified by analysis of 5 points at concentration range 5.0-20.0 mg.

2.3.2. Precision

Table 1. Preparation of pellets for the recovery assay of the method of IR spectroscopy for cefuroxime (CFU)

	CFU sample[1] (mg)	CFU RS[1] (mg)	KBr_2 (mg)	Final concentration (mg/pellet)
Sample	1.0	-	249.0	1.0
R1	1.0	0.1	248.9	1.1
R2	1.0	0.2	249.8	1.2
R3	1.0	0.4	249.6	1.4
Standard	-	1.0	249.0	1.0

[1]diluted 1:10 (w/w) in KBr.
[2]sufficient amount for the preparation of pellets with a total weight of 250 mg

The precision of the method was evaluated in two requisites: repeatability and intermediate precision. Repeatability (intra-assay) was studied by the performance of seven determinations of the sample in a concentration of 10.0 mg/pellet, all in the same day and identical working conditions. Intermediate precision (inter-assay) was assessed by performing the assay for a second analyst and also in three different days under the same experimental conditions. At the end of test, the relative standard deviation percentage (R.S.D.) values of the determinations were analysed[45].

2.3.3. Accuracy

Accuracy was attained via the recovery assay, in which known quantity of c RS was added to known quantity of the sample[45]. The recovery was performed in the 3 levels, R1, R2 and R3, and the pellets were prepared according to the Table 1, in triplicate.

The recovery percentage was calculated by the equation determined by the Association of Official Analytical Chemists (AOAC)[46].

2.3.4. Robustness

The robustness of the method was evaluated with the purpose of showing the reliability of the analysis concerning small variations in its working parameters. In other words, it shows that the validity of the method is maintained even with small variations in its working conditions. The following parameters were individually varied: total pellet weight, brand of potassium bromide and time compression. The obtained responses were evaluated according to the R.S.D. among the dosages.

2.3.5. Detection and Quantification Limits

The detection (LOD) and quantification (LOQ) limits were calculated based on the intercept standard deviation and the curve slope, as described in the literature[45]. Three different curves were performed for the obtainment of the necessary data for the calculation. The values were calculated by the equations 3 and 4.

$$LOD = 3.3\left(\frac{SD}{a}\right) \quad (3)$$

$$LOQ = 10\left(\frac{SD}{a}\right) \quad (4)$$

Where:
a = inclination of the analytical curve
SD = intercept standard deviation

3. Results and Discussion

Quality Control is a very important step in the process of drug manufacturing, as it ensures its safety and efficacy. Thus, research on quality control of pharmaceutical products to identify the content of active and the study of physical and chemical characteristics of the drug are essential to ensure the quality of the final product.

In order to reduce environmental impacts of their activities on the environment, industries must seek alternatives to reduce, prevent or eliminate chemical residues in their routine processes. Thus, the replacement of analytical methods that employ organic solvents for others that do not employ them is an action may be taken for this purpose[47]. In that context, the infrared spectroscopy is a good option because it is a method that does not use organic solvents and also allows the quantification of compounds.

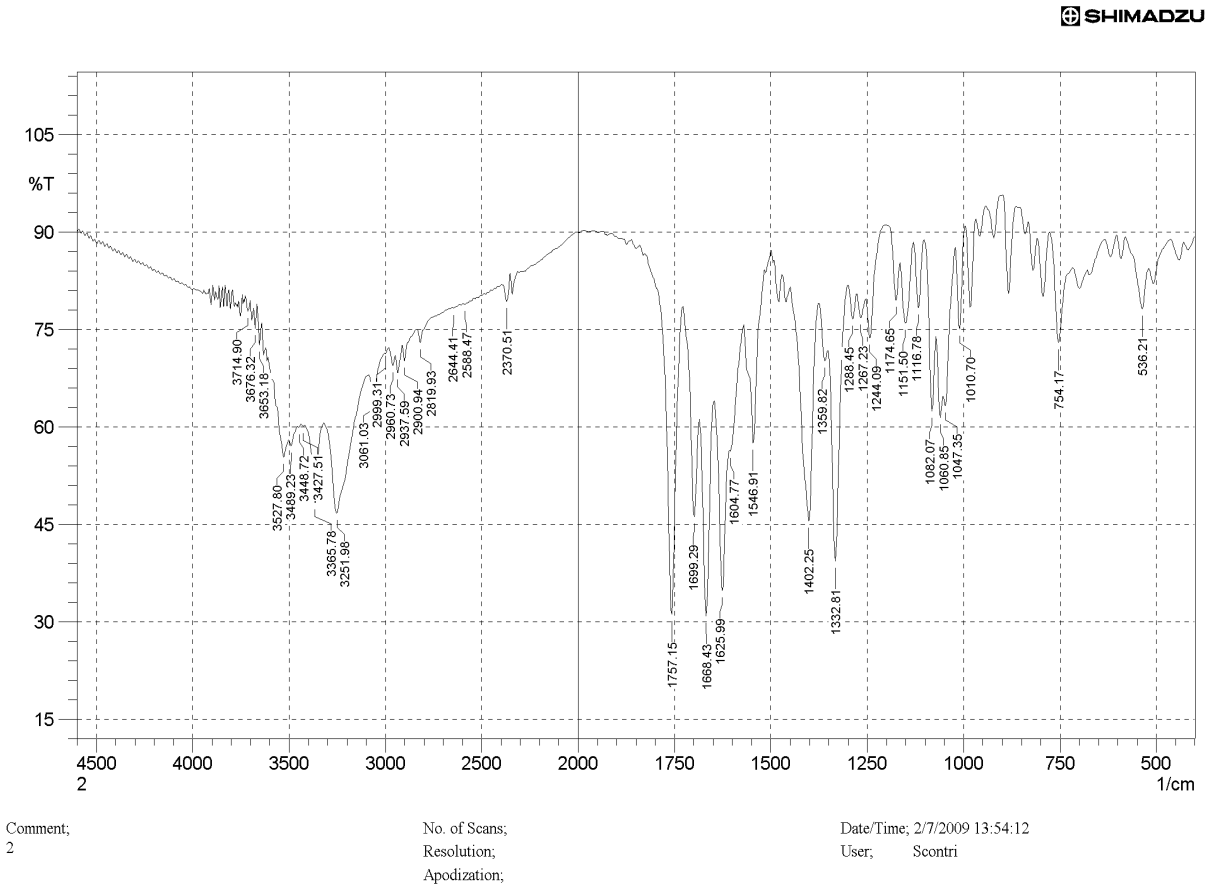

Figure 3. Infrared spectrum of cefuroxime RS (concentration of 1 mg/pellet)

Infrared spectroscopy is based on the fact that when molecules absorb energy, undergo a transition to a state of higher energy or excited state, and only vibrational energy transitions occur in the mid-infrared region. The vibrations induced by infrared radiation include strains and tensions of inter-atomic bonds and changes of bonds angles[48]. Considering that the molecule absorbs only selected frequencies in the infrared radiation, it will only absorb those that correspond to its natural vibrational frequency, which causes an increase in the amplitude of vibrational motion of its chemical bonds. Thus, the vibration frequency can be associated with a particular bond type[49].

Besides the fact that it does not use organic solvents, the infrared spectroscopy technique has other advantages. This is a rapid technique which requires minimum or no pre-treatment of the sample provides accuracy comparable to other methods and also helps in the detection of counterfeiting and impurities. Through this technique, the sample can be scanned, on average, up to 64 times in any physical state and taking less than a minute with high resolution and precision[50]. Furthermore, it is a suitable technique for drugs with solubility problems, since they can be prepared in the pellet form[43].

The infrared spectrum of cefuroxime RS is shown in Figure 3.

Results obtained through the infrared spectroscopic method for cefuroxime powder for injection are displayed in Table 2, which shows mean, s.e.m. and R.S.D. values. Quantities of the drug found were in accordance with values claimed by the manufacturer (100.25%), indicating the applicability of the proposed method to pharmaceutical analysis. This method showed good precision, with R.S.D. value found to be less than 2% (1.00%).

Table 2. Experimental values obtained for the determination of cefuroxime by infrared spectroscopic proposed method

Sample	Found, mg	Teor, %	Mean ± S.E.M.	R.S.D. %
I	1.5202	101.35		
II	1.4857	99.05		
III	1.5147	100.98	100.25 ± 0.44	1.00
IV	1.4803	98.69		
V	1.5084	100.56		
VI	1.5130	100.87		

R.S.D. = relative standard deviation
S.E.M. = standard error of the mean
each value is the mean of three determinations

3.1. Method Validation

3.1.1. Linearity

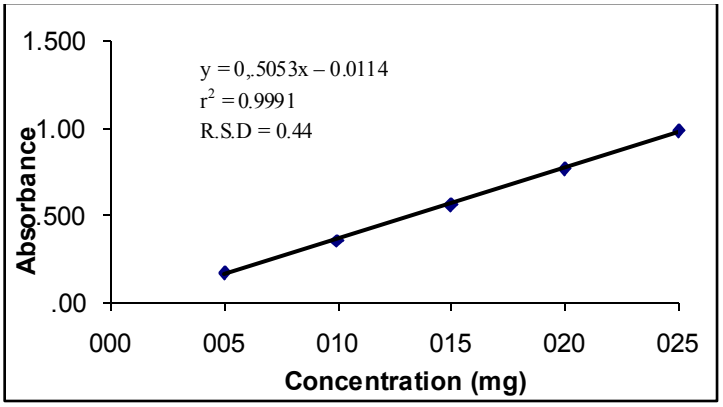

Figure 4. Graphical representation of cefuroxime analytical curve by IR spectroscopy

Table 3. Determination of the analytical method precision for cefuroxime by IR spectroscopy

Inter-days					Between analysts			
Sample	**Day**	**Content1 (g/vial)**	**Content1 (%)**	**R.S.D.2 (%)**	**Analyst**	**Content1 (g/vial)**	**Content1 (%)**	**R.S.D.2 (%)**
1	1	0.7512	100.16	0,60	A	0.7179	95,73	2.76
	2	0.7439	99.18		B	0.7473	99,64	
	3	0.7521	100.28					

[1]average of three determinations
[2]R.S.D: relative standard deviation

The calibration curve for cefuroxime RS (Figure 4) was constructed by the disposition of the average value of absorbances in relation to their respective concentrations, showing a good linearity in the range from 0.5 to 2.5 mg/pellet, as shown in Figure 3. The correlation coefficient (R) presented was 0.9991, a value close to 1.0, which shows the excellent linearity of the method.

The ANOVA was calculated for the analytical curve data of cefuroxime. The data showed that, statistically ($P < 0.05$), there was no linearity deviation, since the F calculated (1.19) was lower than the F tabulated (3.61), fact that reinforces the good linearity of the method.

3.1.2. Precision

The precision of the method was evaluated by three parameters: intra-day or repeatability, inter-days or intermediate precision, and between analysts. The results were expressed based on the R.S.D. The intra-assay precision provided the R.S.D. value of 0.60%. The R.S.D. values presented by the inter-assay and between-analysts were lower than 2.76%, and are represented in the Table 3. The data show a good precision of the method, since the R.S.D. values are close to 2% and below 5%.

3.1.3. Accuracy

The accuracy of the method was determined by the recovery assay, which was done in three concentration levels and the results are presented in the Table 4. The data shows that the method has the adequate accuracy, meaning that the experimental concentration values are very close to the real values, since in the range of 80% - 120%, the recovery rates were close to 100%.

Table 4. Experimental values obtained in the recovery test for cefuroxime by infrared spectroscopic proposed method

Recovery	Added, mg	Found, mg	Recovery, %	RDS
R_1	0.1	0.100	100.58	
R_2	0.2	0.197	98.69	2,01
R_3	0.4	0.400	100.23	

3.1.4. Robustness

The robustness was evaluated by small modifications, individually, in the following method parameters: total pellet weight, brand of potassium bromide and time compression. The results are represented in the Table 5. The R.S.D. values shown are smaller than 2%, showing the robustness of the analytical method for the analysis of the cefuroxime by IR spectroscopy.

Table 5. Parameters of the robustness evaluation of the analytical method for the analysis of cefuroxime by IR spectroscopy

Variable	Range	CFU content (g/vial)	CFU content (%)	R.S.D1. (%)
Total pellet weight	248 mg	0.754	100.53	0.80
	250 mg	0.742	98.93	
	252 mg	0.748	99,73	
KBr brand	Synth®	0.744	99.20	1.20
	Shimadzu®	0.757	100.9	
Time of compression	10 minutes	0.752	100.26	0.75
	15 minutes	0.744	99.20	
	20 minutes	0.755	100.66	

1R.S.D.: relative standard deviation
CFU: cefuroxime

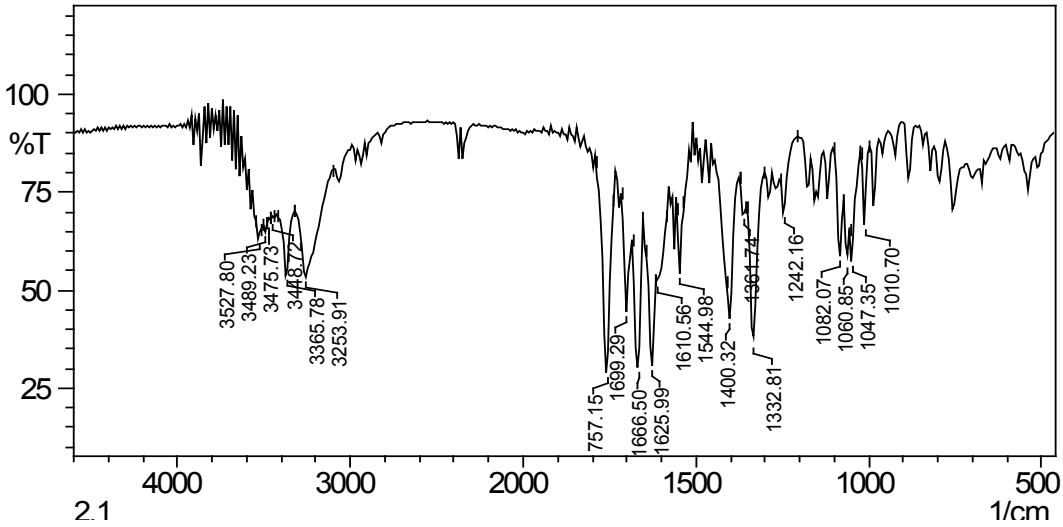

Figure 5. Graphical representation of the quantification limit of cefuroxime injectable pharmaceutical form

3.1.5. Detection and Quantification Limits

The detection and quantification limits were calculated in order to determine the sensibility of the method. The calculated value for the detection limit was of 0.15 mg, and the quantification limit was of 0.5 mg. The values obtained indicate the reliability of the method to detect and quantify the CFU powder for injectable preparation. The Figure 5 shows a graph of the limit of quantification.

4. Conclusions

The results showed that the infrared method for the cefuroxime in powder for injection preparation quantitation presented good linearity, precision, accuracy and robustness in the range of concentrations from 0.5 to 2.5 mg/pellet. Therefore, it is a method interchangeable with other methods already described for the same purpose and can be used in routine tests for quality control of a pharmaceutical industry. Furthermore, it has some advantages over other methods described in the literature for cefuroxime, and the main one is that do not use organic solvents, which contributes to the non-generation of this type of residue, contributing to minimize the environmental impact of pharmaceutical industries. In addition, this study opens the possibility of applying the IR spectroscopy to the quantification of other drugs.

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