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Research Article

A Green and Sustainable Method by Infrared for Quantitative Determination of Sodium Cephalothin

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Abstract

Cephalothin sodium is a semi-synthetic first-generation cephalosporin used in clinics for the treatment of skin and soft tissues infectious diseases, mainly caused by *Staphylococcus aureus*, and on surgical prophylaxis. The quality control of drugs is one of the most important parts in the production process, in this way, the development of new analytical methods makes necessary. So, a spectrophotometric method by infrared was proposed for the quantitation of cephalothin sodium in lyophilized powder. This method was chosen because it is simple, it does not use organic solvents and the generation of waste is very low, what makes this method greener, clean, of low cost and sustainable. All parameters recommended on International Conference on Harmonization guidelines were successfully accomplished. It showed linearity in the range of 0.4-0.8mg with correlation coefficient of 0.9985 and it was selective when compared to the reference substance, sample and placebo. It was also robust, when changes in time and strength of compression were performed; it was accurate using the method of recovery of standard with mean recovery of 99.97%. The limits of detection and quantification were, respectively, 0.05 and 0.16mg, therefore, this method is also sensitive. A greener, cleaner and ecologically correct method for the analysis of cephalothin in lyophilized powder. In this way, the method proved to be an ecological alternative that can be applied in the routine analyzes of this drug.

Keywords: Cephalothin Sodium; Ecological Alternative; Lyophilized Powder; Quality Control; Spectrophotometric Method by Infrared

Introduction

The cephalosporins belong to the beta-lactam antibiotic class and was first isolated as cephalosporin C from a filtrate of *Cephalosporium acremonium*. This substance showed a weak antimicrobial action, but, it was resistant to penicilinases and, with changes in the lateral chains many other substances were produced and are still used in clinical treatments [1].

Cephalothin sodium (Figure 1) is a semi-syntheticfirst-generation cephalosporin that shows bactericidal activity against Gram positive microorganisms. Its mechanism of action is by inhibition of the synthesis of cell wall of bacterias, in this way, the microorganism is more susceptible to death [2,3]. Nowadays it is used for infections in soft tissues and as post-surgical prophylaxis [4,5].

The quality control of drugs is an important part of the process to guarantee its efficacy and safety. There are many studies on literature of analytical methods to quantify cephalothin sodium in biological matrices [6-9] and in its dosage form and raw material [10-12] by High Performance Liquid Chromatography (HPLC) and IR-Raman spectroscopy. Cephalothin is also present in official summaries like the [13-17], that include HPLC as main technique, and microbiological assay, however all of them use organic solvents that are harmful to the environment and the operators. The green analytical chemistry is an idea that has, nowadays, growing because of the need to protect the environment by reducing the generation of waste, to maintain the operator's health and costs of analysis and with residues. It is possible to develop a method with this characteristic, and it has already been done by ecologically correct adjustments [18-28]. So, the aim of this paper is to propose aspectrophotometric method by infrared with a green chemistry approach.

Experimentals

Equipment

Spectrophotometric analysis was performed in IR Prestige-21 (Shimadzu®, Kyoto, Japan). The pellets were prepared in KBr previously dried in a heater model 315 SE (Fanem®, São Paulo, Brazil) at 105°C during 24 hours. An analytical balance model DV215CD (Ohaus®, São Paulo, Brazil) was used to weight the powders.

Chemicals and reagents

All chemicals used during the analysis are: Potassium Bromide (KBr) (Neon®, São Paulo, Brazil), analytical reagent; acetone (Qhemis®, Indaiatuba, São Paulo, Brazil), analytical grade; cephalothin sodium reference (RCS), content of 99.6%, provided by União Química Farmacêutica Nacional S/A (São Paulo, Brazil) and dosage form content 1g of cephalothin sodium (lyophilized powder for injectable solution), provided by ABL-Antibióticos do Brasil LTDA (Cosmópolis, São Paulo, Brazil).

Before the preparation of the pellets, it was performed a dilution, in proportion of 1:10, with KBr and cephalothin (RCS or dosage form), and this was the stock mixture. It is necessary in order to decrease errors during weighting. The pellets were prepared weighing

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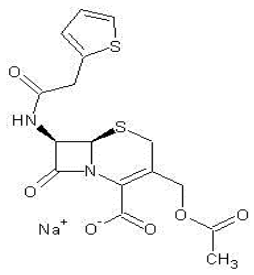

Figure 1: Chemical structure of cephalothin sodium (CAS 58-71-9).

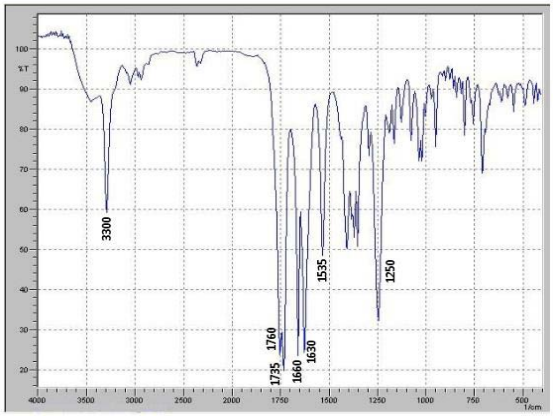

Figure 2: IR spectra of cephalothin sodium reference.

an amount from this stock mixture and KBr to obtain pellets in the desired concentrations. The spectra obtained were compared to a cephalothin's spectra present on literature.

The placebo pellets were prepared in the same way described above with sodium bicarbonate, the only excipient used in cephalothin sodium, and the spectra of placebo, cephalothin sodium RCS and dosage form were obtained and overlapped to verify if there was any interference of the placebo and if the dosage form matched to the RCS.

Method

The pellets used in the analysis were of 150mg. During development of the method it was obtained the spectra of cephalothin sodium, RCS and dosage form, and placebo. After overlapping, it was chosen the region of 1600-1500cm^{-1}, to quantify cephalothin sodium dosage form. The blank pellets consisted in potassium bromide.

Preparation of pellets: First of all, it was prepared the stock mixture by weighing exactly 51.3mg of cephalothin reference and 461.4mg of potassium bromide. From this mixture, it was performed the dilutions to obtain the desired pellets concentrations (0.4-1.2mg/15mg) using KBr as diluent.

Method validation: After the development of the method it was chosen five concentrations (0.4-0.8) that exhibited absorbance between 0.2-0.8, following the Lambert-Beer's law. The validation parameters followed were: selectivity, linearity, precision, accuracy, robustness and limits of quantitation and detection [29,30].

Selectivity: Pellets of the cephalothin sodium RCS and dosage

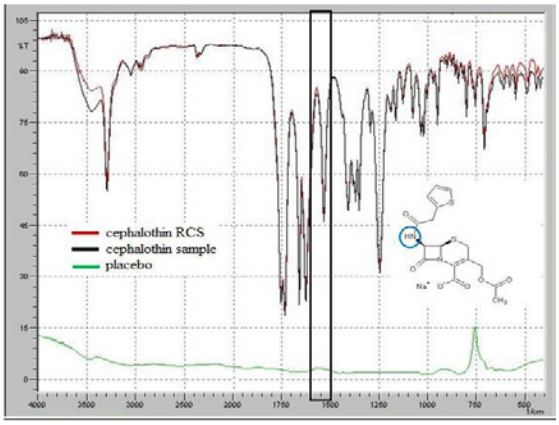

Figure 3: Overlap of the spectra obtained for cephalothin sodium standard, samples and placebo.

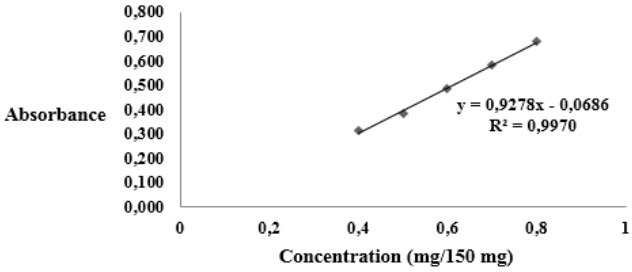

Figure 4: Linearity curve for cephalothin using the proposed method.

form were prepared and its spectra were compared to the spectra produced by the pellets of placebo (sodium bicarbonate). The placebo pellets were prepared in the same composition present in the commercial form of cephalothin.

Linearity: It was performed using the five concentrations established, in triplicate for each concentration, in three consecutive days. The linearity was evaluated by regression analysis and it was also calculated the correlation coefficient and analysis of variance (ANOVA).

Precision: This parameter was evaluated by repeatability, interday and intermediate precision. The repeatability was performed by using the same concentration (0.6mg/150mg) in six pellets in the same day. The interday precision was performed in the same conditions of repeatability but, in another day. The intermediate precision was performed by a different analyst in another day, under the same conditions of repeatability precision. The results were analyzed by Relative Standard Deviation (RSD).

Accuracy: The accuracy was measured by the recovery method in three levels (80, 100 and 120%) of the concentration used (0.6mg/150mg). The pellets were prepared using the stock mixture of cephalothin sodium, RCS and dosage form, and KBr as diluent. To prepare the three levels of concentrations it was always added 4mg of the sample mixture in all the pellets, just the quantity of reference mixture varied, being added 0.08, 2.0 and 3.2mg respectively. It was also prepared pellets with only the sample mixture (0.4mg/150mg) and only with reference mixture, in the same concentration. The

Table 1: Summary of linearity parameters for quantitation of cephalothin sodium and ANOVA.

Parameters	1600-1500 cm^{-1}
Range (mg)	0.4-0.8
Time of compression (min)	7
Pressure of compression (kN)	90
Temperature (°C)	20
Diluent	KBr
Correlation coefficient (r^2)	0.9985
Regression	660.39 (4.96)
Lack of fit	1.02 (3.71)

results were obtained in terms of percentage of recovery.

Robustness: The robustness of the method consisted in small changes in three parameters and evaluation if these changes would interfere in the results. The parameters chosen to be altered were pressure, time of compression and the potassium bromide brand. Pellets were prepared in the same concentration (0.6mg/150mg) and results were analyzed by F-Test and t-Test.

Limits of detection (LOD) and quantitation (LOQ): Both parameters were obtained using the three linearity curves and applied in these equations:

$$LD = (SDax\ 3.3)/AS \quad (1)$$

$$LQ = (SDa\ x\ 10)/AS \quad (2)$$

SDa: Standard deviation of the intercept to the Y axis of, at least, three analytical curves.

AS: average slope of analytical curve.

Results and Discussion

Method development

The spectra obtained for cephalothin standard (Figure 2) was analyzed and compared to the spectra present in literature [31], and it showed the cephalothin sodium characteristics band in: 3300cm^{-1} (N-H stretching band); 1760cm^{-1} (β-lactam carbonyl); 1735cm^{-1} (ester carbonyl); 1660-1535cm^{-1} (secondary amide carbonyl); 1630cm^{-1} (carboxyl carbonyl) and 1250cm^{-1} (C-O stretching band).

Method validation

Selectivity: The spectra overlap of cephalothin sodium reference, dosage form and placebo did not show interference of the excipient in the region of 1600-1500cm^{-1}, corresponding to the secondary amide carbonyl band, used to quantify cephalothin sodium (Figure 3).

Linearity: The three linearity curves, obtained by plotting concentration vs. mean absorbance, provided a correlation coefficient of 0.9985 (Figure 4). The analysis of variance (ANOVA) showed significant linear regression, because $F_{calculated}$ (660.39) was higher than $F_{critical}$ (4.96, $p > 0.05$). In Table 1 there is a summary of the results obtained for linearity of the method.

Precision: The intraday (repeatability), interday and intermediate precision were based in statistical analysis by Relative Standard Deviation (RSD). The results obtained for intraday precision showed RSD of 1.99%, interday presented RSD of 2.92% and, the intermediate precision, 4.86% (Table 2).

Accuracy: It was determined by the average recovery using the standard addition method. As explained before, three levels of

Table 2: Determination of intraday, interday and intermediate precision and accuracy of the method for cephalothin sodium.

Precision			Mean absorbance*	RSD (%)	
Intraday			0.49	1.99	
Interday	Day 1	0.494	0.492	2.92**	
	Day 2	0.49			
Intermediate	Analyst 1	0.494	0.507	5.10***	
	Analyst 2	0.522			
Accuracy	Concentration added (mg)	Recovered concentration (mg)	Recovery (%)	Mean recovery	RSD (%)
	0.08	0.08	99.84		
	0.2	0.201	100.44	99.97	0.88
	0.32	0.319	99.64		

*Average of 6 values
**t Test = tcalculated (0.37) < tcritical (2.23)
*** t Test = tcalculated (2.18) < tcritical (2.26)

Table 3: Robustness results for the developed method to quantify cephalothin sodium.

Tests result	Pressure of compression (kN)		Time of compression (minutes)		KBr brand
	88	92	5	9	Synth
Fcal	1.4	7.94	1	1.2	1.12
Fcrit	19	19	19	19	19
***t*cal**	0.4	3.71*	1	0.7	5.05*
***t*crit**	2.8	2.78	2.8	2.8	2.78

*Significative variation

concentration were used and it is on Table 2. The mean recovery obtained was 99.97%, very close to 100% and according to the limits specified in official summaries [32,33].

Robustness: The robustness was analyzed by small changes in three parameters: pressure and time of compression and the KBr brand. Test-F and test t were used as statistical tools and the normal condition was compared to each changed parameter, and it showed significant difference in two altered conditions, when the pressure of compression was higher than the condition used in the method and when the KBr brand was changed. The results of the tests are presented in Table 3.

Limits of detection (LOD) and quantification (LOQ): The LOD and LOQ were obtained by the three linearity curves and using the Equations 1 and 2. The values were, respectively, 0.05 and 0.16mg/150mg. These values were very close to zero, what means that this method is sensitive.

Conclusion

The methods present in literature for quantitation of cephalothin sodium evolves the use of organic solvents, mainly methanol and acetonitrile, as well as, buffer solutions, what leads to a great generation of waste. The proposed method in this paper is greener, of low cost and clean, mainly because of the absence of solvents during the analysis, so, there is the generation of a very small amount of waste, it is faster, uses only one reagent and it is very easy to perform.

The IR spectrophotometry is, by itself, greener when compared to other analytical methods and the method developed and validated in this work proved to be precise, accurate, selective and robust, and can applied in the quantification of cephalothin sodium in lyophilized powder for injection, being an alternative method to make the quality control of this drug.

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