

Development and Validation of a Modern and Stability-Indicating Method for the Quantification of Ceftriaxone Sodium in Powder for Injection by Infrared Spectroscopy

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Abstract This paper proposed the validation of a modern and useful analytical method for the quantification of ceftriaxone sodium in powder for injection, using Fourier-transform infrared (FT-IR) transmission spectroscopy. This technique has great advantage because it does not use organic solvents and it is in accordance with the International Conference on Harmonisation guidelines (ICH), contributing to the reduction of the generation of waste of organic solvents by the industry and minimizing the impact of its activities on the environment. The method was quantified by measuring the absorbance of the band corresponding to the carbonyl present in the drug molecule, in the region between 1800 and 1700 cm^{-1} , showing to be linear, accurate, precise, selective and robust. It was linear over the concentration range of 0.4-1.2 mg with correlation coefficient of 0.9983 and limits of detection and quantification of 0.016 and 0.049 mg, respectively. The validated method is able to quantify ceftriaxone sodium powder for injection preparation, it is an environmentally-friendly and low cost method and can be applied in the routine quality control of the pharmaceutical industry.

Keywords Ceftriaxone sodium, Validation, Infrared, Quantitative, Green Method

1. Introduction

Since 1970 cephalosporins are among the most potent and widely used anti-infective agents [1]. Ceftriaxone sodium (Figure 1) is a semi-synthetic antibiotic belonging to the class of the third generation cephalosporins. It is administered only by the parenteral route and has the capacity to overcome the blood-brain barrier [2-4].

It has bactericidal action; its main activity is related to the Gram-negative bacteria, being also able to act against Gram-negative bacilli resistant to the first and second generations cephalosporins [1, 4, 6, 7].

It is indicated in cases of septicemia, meningitis, disseminated Lyme borreliosis (early and late stages of the disease) (Lyme disease), intra-abdominal infections (peritonitis, gastrointestinal and biliary tract infections), bone, joint, soft tissue, skin and wound infections, infections in immunocompromised patients, kidney and urinary tract infections, infections of the respiratory tract, particularly pneumonia and otolaryngological infections, genital infections, including gonorrhea, perioperative prophylaxis of

infections [8-10].

There are several analytical methods for the quantification of ceftriaxone sodium, such as high performance liquid chromatography [5, 11-17]; ultra performance liquid chromatography [18, 19]; UV absorption spectroscopy [2, 20-23] and spectrofluorimetry [24]. Most of the proposed methods involve the use of organic solvents and buffer solutions, which contribute to the generation of these residues by the pharmaceutical industry.

The concern with a reduction of toxic solvents, as well as buffer solutions in order to reduce the impacts of their activities on the environment, is a tendency to be acquired by pharmaceutical industries. Therefore, it is necessary to develop innovative and environmentally-friendly methods [25-34].

Infrared spectroscopy is a relevant technique when the intention is to contribute to the environment, as it does not use toxic solvents, which allows the quantification through the measurements of the absorbances corresponding to a selected group present in the molecule [26, 30-33].

Based on all the advantages that this technique provides the aim of this study was to develop and validate an environmentally-friendly method for the quantification of ceftriaxone sodium powder for injection preparations by Fourier-transform infrared (FT-IR) transmission spectroscopy.

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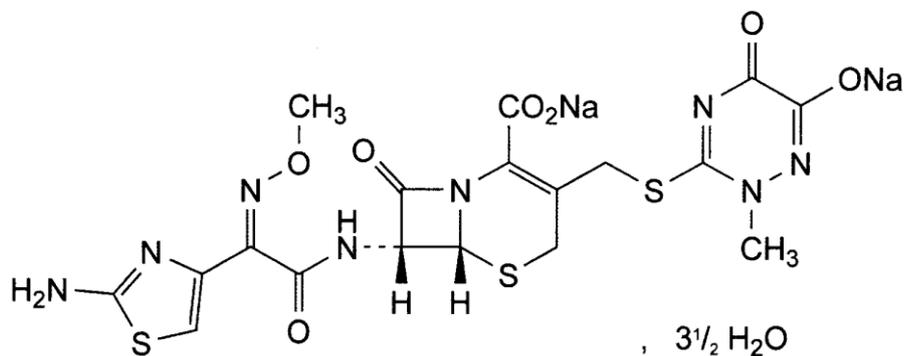


Figure 1. Chemical structure of ceftriaxone disodium hemieptahydrate (CAS 104376-79-6) [5]

Table 1. Preparation of pellets for the test of recovery of infrared spectroscopy method for ceftriaxone sodium

	Ceftriaxone sodium sample (mg) (dilution 1:10, w/w in KBr)	Ceftriaxone sodium RCS (mg) (dilution 1:10, w/w in KBr)	%	Final theoretical concentration (mg/pellets)	KBr amount (mg)
Sample	4.0	---		0.4	146.0
R1	4.0	2.4	80	0.64	143.6
R2	4.0	4.0	100	0.80	142.0
R3	4.0	5.6	120	0.96	140.4
Standard	---	4.0		0.4	146.0

2. Material and Methods

2.1. Chemicals

The chemicals used were ceftriaxone sodium reference chemical substance (RCS) (assigned purity 99.6%) and ceftriaxone sodium in powder for injection, each flask contains 1.193 g of ceftriaxone sodium, equivalent to 1.0 g of ceftriaxone, it has no excipients, this was kindly donated by the pharmaceutical company ABL Antibióticos do Brasil® (Cosmópolis, SP, Brazil).

The potassium bromide (KBr) (Neon®, Brazil) was of analytical grade and was used for the preparation of pellets. Before use, the KBr was ground and dried at 120°C until constant weight.

2.2. Instrumentation and Analytical Conditions

2.2.1. Equipments

The FT-IR spectrometer (Shimadzu®, Kyoto, Japan, IR Prestige-21 model) was used and the spectral region was consisted of 4000 - 500 cm^{-1} at intervals of 2 cm^{-1} . 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 ceftriaxone sodium molecule, and this band had its height analyzed in terms of absorbance.

2.2.2. Obtaining the Analytical Curve

Equivalent amounts of 0.4, 0.6, 0.8, 1.0 and 1.2 mg of ceftriaxone sodium RCS were previously diluted in KBr (1:10 w/w) and after diluted in KBr again to obtain sufficient

quantities of 150 mg of pellets. The powders were mixed until a homogeneous mixture was obtained, this mixture was submitted to mechanical pressing for 10 minutes to obtain translucent pellets.

2.2.3. Preparation of Ceftriaxone Sodium RCS Pellets

Aliquots of powder equivalent to 0.8 mg of ceftriaxone sodium RCS (8.0 mg diluting 1:10 in KBr) were properly homogenized in 142 mg potassium bromide, to form pellets of 150 mg. The determinations of the substance were performed in triplicate absorbance.

2.2.4. Preparation of Ceftriaxone Sodium Sample Pellets

The contents of twenty vials of ceftriaxone sodium in powder for injection solution were mixed. From this mixture, amounts of 0.8 mg of ceftriaxone sodium (8.0 mg of the 1:10 dilution in potassium bromide) were taken and well homogenized with 142 mg of potassium bromide, comprising the total pellet weight of 150 mg. The determinations were performed in triplicate.

2.3. Method Validation

The method was validated following ICH specifications showing accuracy, linearity, precision, robustness, selectivity, detection limit and quantitation limit.

2.3.1. Selectivity

Selectivity was evaluated by the analysis of the spectra of the ceftriaxone sodium RCS and powder for injection (Figure 2) and also through the analysis of the ceftriaxone sodium in powder for injection was performed by submitting

the photolytic degradation by exposure of the diluted sodium ceftriaxone sample (1:10) to UVC light (254 nm) for 3 hours (Figure 3) and thermal degradation by exposing the diluted sample (1:10) in an oven at 105°C for 2 hours (Figure 4).

2.3.2. Linearity

To assess linearity of the method, five doses of ceftriaxone sodium RCS (0.4, 0.6, 0.8, 1.0 and 1.2 mg) were used. The method was evaluated by linear regression analysis, which is calculated by the square minimum method and statistical analysis was by ANOVA. The method was performed on three different days.

2.3.3. Accuracy

Accuracy was attained by the recovery assay, to which was added a known amount of standard in known quantity of sample [35]. The method was performed at three different levels (R1, R2, R3), being prepared in triplicate on three different days. Table 1 shows the preparation of the pellets for the recovery test method.

2.3.4. Precision

Precision was evaluated respecting both repeatability (intraday) and intermediate precision (interdays). The intraday precision was evaluated by the preparation and the analyses of six pellets ceftriaxone sodium at a concentration of 0.8 mg/150 mg on the same day and at about the same working conditions. Intermediate precision (among analysts) was assessed by performing the assay for a second analyst under the same experimental conditions. Six replicates at a concentration of 0.8 mg/150 mg were prepared and assayed. At the end of testing, the standard deviations of the measurements were calculated.

2.3.5. 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, that is, the validity of the method is maintained even with small variations in its working conditions. The following parameters were varied: compression of the pellets time two minutes down and above the usual working time compression; compressive strength, ranging four kN down and above the usual working compression and potassium bromide trade mark. The obtained responses were evaluated according to factorial design 2^3 and Table 3 shows the parameters of the evaluation of the robustness by factorial design 2^3 of the analytical method for ceftriaxone sodium analysis.

2.3.6. Detection and Quantitation Limits

The detection (LOD) and quantification (LOQ) limits were calculated based on the intercept standard deviation and the curve slope [35]. Three different curves were performed to obtain the data necessary to calculate. The values were

calculated by the equations 1 and 2.

$$\text{Equation 1.} \quad LD = 3.3 \times (SD/a)$$

$$\text{Equation 2.} \quad LQ = 10 \times (SD/a)$$

Where:

a = inclination of the analytical curve

SD = intercept of standard deviation

3. Results and Discussion

Quality Control ensures the safety and efficacy of pharmaceutical products. The validation of analytical and bioanalytical methods is indispensable in the process of good manufacturing practices.

The search for methods with low environmental impact is a tendency to be applied in routine analysis in the pharmaceutical industry, the replacement of analytical methods that employ organic solvents for others that do not employ them are viable alternatives for this objective [36].

Infrared spectroscopy is an excellent alternative because it does not use organic solvents, requires minimal or no pre-treatment of the sample and it is a suitable technique for drugs with solubility problems, once they can be prepared in the form of pellets [25-34].

Therefore the quantification of ceftriaxone sodium samples is very important for conducting pharmacokinetic studies, bioavailability, bioequivalence and consequently for the therapeutic monitoring of this substance. In this paper, a single reagent was chosen in order to obtain a low cost, simple and environmentally friendly infrared spectroscopy method for quantification of this medicine.

The method can be quantified by measuring the absorbance of the band corresponding to the carbonyl present in the drug molecule, in the region between 1800 and 1700 cm^{-1} , this band is specific and useful in the determination of ceftriaxone sodium in powder for injection, the values of these bands/peaks were provided in absorbance presenting accuracy, linearity, precision, robustness and selectivity.

3.1. Selectivity

The selectivity was determined by the overlap of the spectra of sodium ceftriaxone RCS and powder for injection as shown in Figure 2, the band used for the quantification is highlighted showing no interferences in the spectrum obtained for the quantification of the drug. Also it was determined by photolytic degradation, presenting a decrease of 19.82% of the band referring to carbonyl after 3 hours of exposure to the diluted ceftriaxone sodium in powder for injection (Figure 3). In the thermal degradation, there was a decrease of 19.88% of the band referring to carbonyl after 2 hours (Figure 4). It is noted that there was no presence of new bands in the spectro, only the decrease of the existing ones.

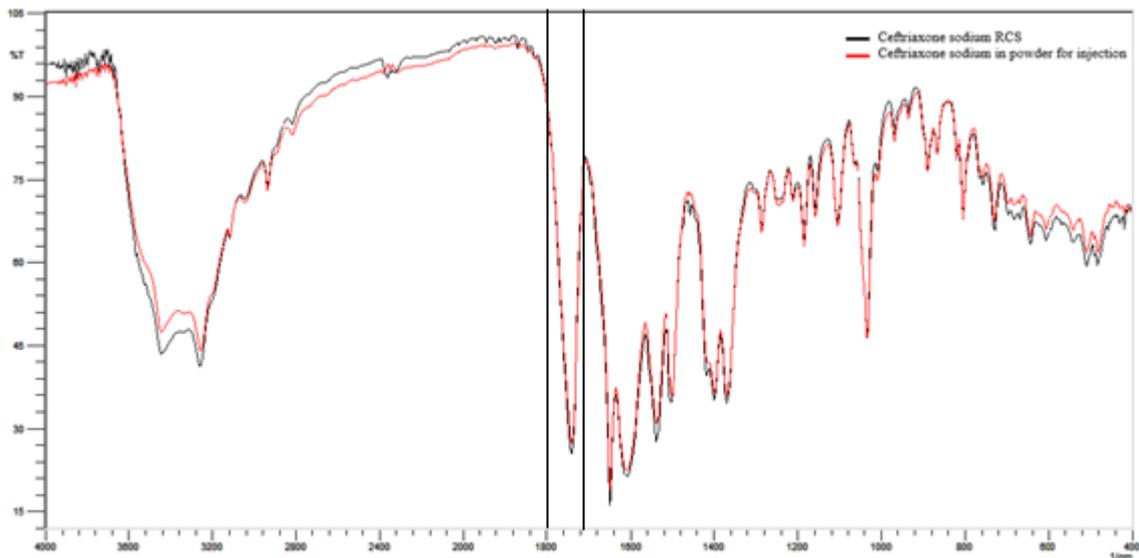


Figure 2. Overlap absorption spectra in the infrared region ceftriaxone sodium RCS and powder for injection with the band used for quantification

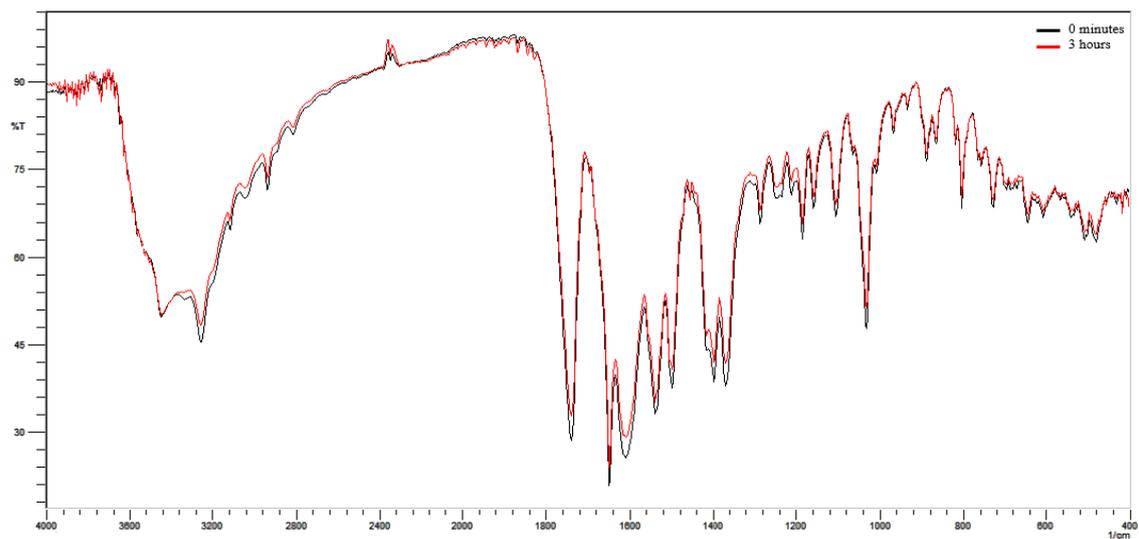


Figure 3. Overlap absorption spectra in the infrared region of the photolytic degradation of ceftriaxone sodium at times zero and 3 hours

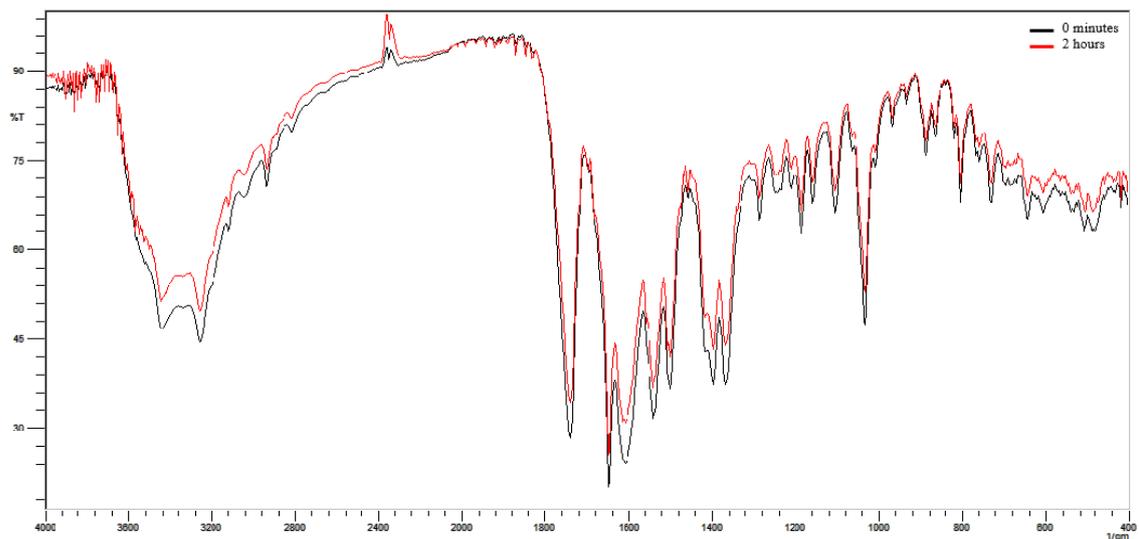


Figure 4. Overlap absorption spectra in the infrared region of the thermal degradation of ceftriaxone sodium at times zero and 2 hours

3.2. Linearity

The analytical curve was generated on three consecutive days by plotting the main absorbance values of spectra in the region of 1800-1700 cm^{-1} in relation to concentrations between 0.4 to 1.2 mg/pellet as shown in Figure 5 along with the error bars (in red). The correlation coefficient (R) was of 0.9983, ANOVA was used for statistical analysis and the calculation showed ($p < 0.05$), F calculation (3.54) lower than F critical (3.71), determining excellent linearity of the method, fact that reinforces the good linearity of the method.

3.3. Precision

The precision of the method was evaluated by parameters: intra-day or repeatability, inter-days or intermediate (among analysts). The results were expressed by the relative standard deviation (R.S.D.).

Table 2. Determination of precision for test-F and t-test between analysts of spectroscopy method in the infrared region

Test-F: Two samples for variance	Test-t: Two samples assuming equal variances
$F_{\text{cal}} 2.53 < 6.39 F_{\text{crit}}$	$t_{\text{cal}} 0.78 < 2.23 t_{\text{crit}}$
$P_{\text{value}} 0.20 > 0.05$	$P_{\text{value}} 0.45 > 0.05$

The repeatability showed provided the R.S.D. value of 2.64%. Intermediate precision was demonstrated on different

days by two analysts, table 2 shows the comparative analysis and statistical findings.

3.4. Robustness

The robustness of the method was evaluated through the absorbance values found through eight trials. Sixteen translucent pellets with concentration referring to 0.8 mg ceftriaxone sodium were read (Table 3). Then, the analysis of variance for each contrast was determined then the analysis of variance was determined for each contrast (Table 4), it is noticed that the changes did not present significant interference in the proposed method, where all values (p -value) are greater than (0,05), showing the appropriate robustness of the method.

The Pareto graph (Figure 6) shows the effects values in order to evaluate the possible interactions between factors. From the data presented it is possible to verify that there were no statistically significant interactions for one (p -value) of 0.05, therefore, none of the altered factors interfered significantly in the quantification of the medicine. Although the demonstration of robustness of the method was adequate for the analysis, it is important to note that the factor 2 represented by the compressive force and the combination of factors 1 and 3 represented by the compression time and KBr mark presented greater variations in the results.

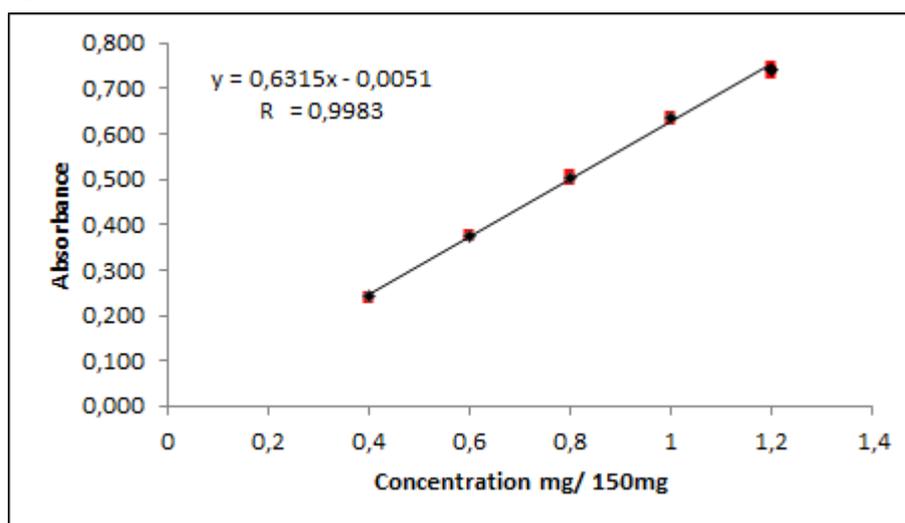


Figure 5. Graphical representation of the calibration curve of ceftriaxone sodium by spectroscopy method in the infrared region

Table 3. Parameters of the robustness evaluation by factorial design 2^3 of the analytical method for ceftriaxone sodium analysis by spectroscopy in the infrared region

Test	Time (minutes)	Strength (kN)	Mark KBr	Abs. I	Abs. II	Average	RSD (%)
1	8	76	Neon®	0.544	0.514	0.529	4.01
2	12	76	Neon®	0.516	0.518	0.517	0.27
3	8	84	Neon®	0.511	0.525	0.518	1.91
4	12	84	Neon®	0.515	0.513	0.514	0.28
5	8	76	Synth®	0.532	0.512	0.522	2.71
6	12	76	Synth®	0.545	0.551	0.548	0.77
7	8	84	Synth®	0.515	0.528	0.522	1.76
8	12	84	Synth®	0.530	0.511	0.521	2.58

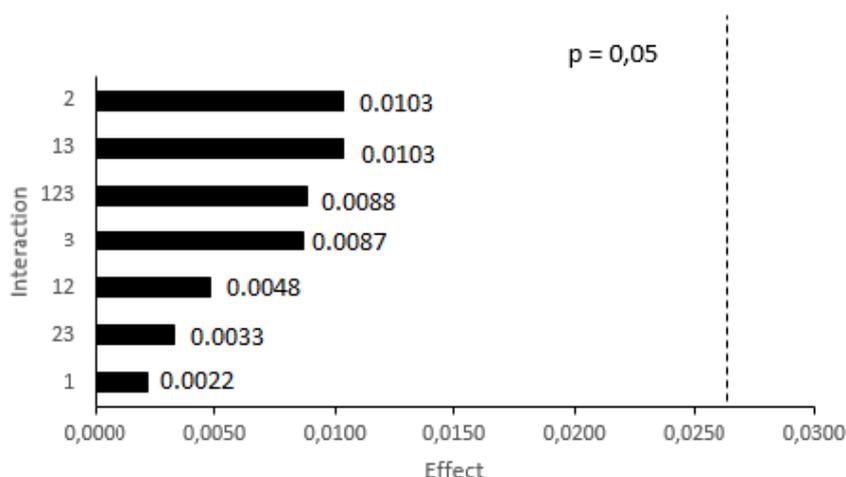
Table 4. Analysis of variance for robustness spectroscopy in the infrared region

Source of variation	df	SS	SA	F	F critical	value-P
Factor 1	1	0.00002	0.00002	0.1565	5.3177	0.7026
Factor 2	1	0.00044	0.00044	3.4087	5.3177	0.1019
Factor 3	1	0.00029	0.00029	0.6975	5.3177	0.4277
Factor interaction 1 and 2	1	0.00009	0.00009	2.2338	5.3177	0.1732
Factor interaction 1 and 3	1	0.00042	0.00042	3.2483	5.3177	0.1090
Factor interaction 2 and 3	1	0.00005	0.00005	0.3787	5.3177	0.5552
Factor interaction 1, 2 and 3	1	0.00031	0.00031	2.3672	5.3177	0.1623
Treatments	7	0.00162	0.00023	1.7844	3.5005	0.2173
Residue	8	0.00103	0.00013	-	-	-

Factor 1 = time of compression (minute) / factor 2 = Strength (kN) / Factor 3= mark KBr
 df= degrees of freedom / SS= Sum of Squares/ SA= square average / F= test statistic

Table 5. Determination of the accuracy of the analytical method for analysis of ceftriaxone sodium by spectroscopy in the infrared region

	Ceftriaxone sodium RCS added (mg)	Ceftriaxone sodium RCS found (mg)	Recovery ¹ (%)	RSD ² (%)	Average recovery (%)
R1	0.240	0.241	100.41	1.09	
R2	0.400	0.401	100.33	0.84	100.44
R3	0.560	0.563	100.57	2.13	

**Figure 6.** Pareto graph of the interactions of the robustness variable for the spectroscopy method in the infrared region

3.5. Accuracy

The accuracy of the method is in accordance with fixed limits of 98.0 to 102.0%, as shown in table 5, indicating the adequacy of the method developed in the quantification of the ceftriaxone sodium in powder for injection.

3.6. Detection and Quantitation Limits

The value found for the detection limit was 0.016 mg and the quantitation limit was 0.049 mg. These values indicate the reliability of the method for the determination and quantification of ceftriaxone sodium in powder for injection.

4. Conclusions

The method presented for the quantification of ceftriaxone in powder for injection presented linearity, selectivity, precision, accuracy, robustness and adequate limits of

detection and quantification in concentrations ranging from 0.4 to 1.2 mg. The proposed method used only one reagent with minimum procedures of sample preparation, encouraging its application in the routine analysis and contributing to minimize the environmental impact of pharmaceutical industries. Therefore, the validated method is an acceptable alternative method for quality control and can be easily applied in the routine analysis of ceftriaxone sodium.

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