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Methods for Qualitative Analysis of Cefazolin Sodium Raw Material and Pharmaceutical Product

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Abstract In this work we studied qualitative methods for analysis and identification of cefazolin sodium, β-lactam antimicrobial for parenteral use, belonging to the group of first-generation cephalosporin, used as a therapeutic agent and for the surgical prophylaxis, in the pharmaceutical form of powder lyophilized for injectable solution. Qualitative analysis was performed by solubility, melting point, pH determination, thin layer chromatography, ultraviolet spectroscopy, infrared spectroscopy and high performance liquid chromatography, allowing the identification of sample. These methods were reproducible and quick to identify cefazolin sodium, which can be used routinely in analysis of quality control in pharmaceutical laboratories.

Keywords Cefazolin Sodium, Cephalosporin, Analytical Method, Qualitative Analysis, Quality Control

1. Introduction

The characterization of active substances for quality control is essential for obtaining effective and stable drugs, thus ensuring its content in the pharmaceutical specialties and its conservation throughout the period in which it is marketed. The quality in the manufacture of medicines is directly related to the promotion and maintenance of health, thereby performing analytical techniques for assessing the purity of the raw materials used in the manufacture of medicaments, quality specifications are necessary to verify that the product is suitable for use in the population. Failure to comply with these criteria can interfere with biopharmaceutical characteristics, in this sense, the analytical techniques are essential for the safe use of medicines.

Cephalosporins are a class of antibiotics widely used and therapeutically important. Clinical studies have shown that cephalosporins are prophylactic and therapeutic effective agents against a broad spectrum of microbial agents; they have low toxicity and are used to treat many types of bacterial infections[1-3].

Its mechanism of action consists of inhibition of synthesis of bacterial cell wall in a similar manner to that of penicillin, with the advantage to be active against microorganisms resistant to penicillin, in some cases[1]. Because of the large number of cefalosporisnas available, it should be viable to adopt a classification system. Although the can be classified based on chemical structure, clinical pharmacology, resistance to β-lactamase or antimicrobial spectrum, the classification system is the most usual for "generations" and is divided into 5 generations: first, second, third, fourth generation and recently, the fifth generation, in which the compounds are grouped according to the general characteristics of antimicrobial activity[3-5].

Cefazolin sodium is a semi synthetic antimicrobial which has no absorption orally, being found for parenteral use[6]. It shows good activity against bacteria Gram-positive and relatively moderate activity against micro-organisms, Gram-negative[3]. Its molecular formula is $C_{14}H_{13}N_8Na_4S_3$, its molecular weight is 476.5 g/mol with a sodium content of 48.3 mg per gram of cefazolin sodium. It is identified chemically as (6R,7R)-3-[[(5-methyl-1,3,4-thia-dizol-2-yl) sulfanyl]methyl]-8-oxo-7-[(1H-tetrazol-1-yl)acetyl]amide]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxy-late. The chemical structure of cefazolin sodium is shown in Figure 1.

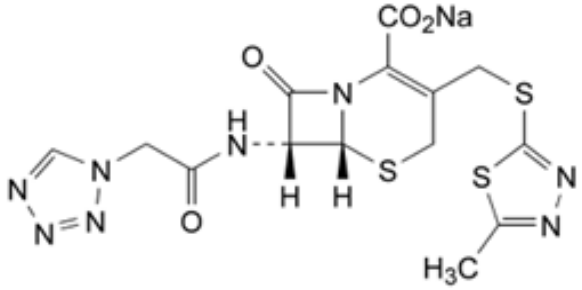

Figure 1. Chemical structure of cefazolin sodium (CAS 27164-46-1)

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Cefazolin is marketed worldwide since 1970, and are available in several countries such as ***South Africa***: Cefacidal™; Izacef™; Kefzol™; Ranzol™; ***Germany:*** Basocef™; Elzogram™; ***Argentina***: Cefalomicina™; Cefamezin™; ***Australia*** : Kefzol™; ***Austria***: Kefzol™; Servazolin™; Zolicef™, ***Belgium:*** Cefacidal™; Kefzol™; ***Brazil:*** Ceftrat™; Cellofarm™; Cellozina™; Cezolin™; Duocef™, Fazolon™; Zolin™; Kefazol™; ***Canada***: Kefzol™; ***Chile***: Kefzol™; ***Spain***: Areuzolin™; Brizolina™; Camil™; Caricef™; Cefa™; Resan™; Cefacene™; Cefadrex™; Dacovo™; Fazoplex™; Filoklin™; Gencefal™; Intrazolina™; Kefol™; Kurgan™; Neofazol™; Tasep™; Tecfazolina™; Zolival™; ***U.S.:*** Ancef™; Zolicef™; ***Philippines***: Cifoxim™; Cizo™; Cloviz™; Fazol™; Fonvicol™; Ilozef™; Lupex™; Maxcep™; Megacef™; Oryant™; Samarial™; Stancef™; Zofadep™; Zolival™; ***France***: Cefacidal™, ***Greece***: Biozolin™; Vifazolin™; ***Netherlands:*** Cefacidal™; Cefamezin™; Kefzol™; Servazolin™; ***Hong Kong:*** Cefamezin™; ***Hungary***: Totacef™, ***India***: Azolin™; Reflin™; Zolfin™; ***Indonesia***: Biozolin™; Cefazol™; ***Israel:*** Cefamezin™; Kefazin™; Kefzol™; Totacef™, ***Italy:*** Acef™; Cefabiozim™; Cefamezin™; Cefazil™; Cromezin™; Nefazol™; Recef™; Sicef™; Silzolin™; Totacef™; ***Japan:*** Cefamezin™; Otsuka™; Cez™; ***Mexico***: Cefacidal™; ***New Zealand***: Kefzol™; Zepilen™; ***Poland***: Biofazolin™; Tarfazolin™; ***Portugal***: Cefamezin™; Kurgan™; ***Czech Republic***: Kefzol™; Orizolin™; Vulmizolin™; ***Russia:*** Cefamezin™ (Цефамезин); Ifizol™ (Ифизол); Intrazoline™ (Интразолин); Kefzol™ (Кефзол); Orizolin™ (Оризолин); Reflin™ (Рефлин); ***Switzerland:*** Kefzol™; ***Thailand:*** Cefali™; Cefamezin™; Cefazillin™; Cefazol™; Cefzolin™; Fazolin™; Zefa™; Zepilen™; Zolicef™; Zolimed™; ***Turkey***: Cefamezin™; Cefozin™; Equizolin™; Iespor™; Maksiporin™; Sefamax™; Sefazol™ and ***Venezuela***: Cefacidal™; Cefarizon™; Cellozina™; Kefzol™ [7].

In this work, the cefazolin sodium was analysed by solubility, melting point, pH determination, thin layer chromatography, ultraviolet spectroscopy and high performance infrared spectroscopy liquid chromatography analysis for identification of cefazolin sodium. The present study aims to gather and propose new analytical methods for identification and structural characterization of cefazolin sodium, aiming shorter time of analysis, low cost, feasibility and ease of implementation in the pharmaceutical industry, to ensure the quality of the product already sold.

2. Chemicals

CFZ reference substance (purity 98.2 %) and CFZ lyophilized injectable form containing 1000 mg of the active component, were kindly donated by the Laboratory ABL Antibióticos do Brasil Ltda (Cosmopolis-SP, Brazil). The vials do not present excipients. All solutions and mobile phases used in this assay were prepared from ultrapure water obtained from a Milli-Q Plus (Millipore, USA). All other chemicals were of analytical grade.

3. Methods

a) Analysis of drug appearance: The appearance of the drug was assessed visually and then it was compared with official compendiums [8-12].

b) Moisture content: The moisture content was determined by Karl Fischer Orion™ model AF8, using Karl Fischer reagent free from pyridine methanol VETEC with at most 0.005% of water and sodium tartrate dihydrate (volumetric standard for standardization of Karl Fischer reagent contains 15.66 + 0.05% H_2O). The method of loss on drying was carried out in infrared moisture analyser, Gehaka™ model 2000 IV- the scales with infrared radiation heating, which allows to assess the humidity of the sample.

c) Solubility: The solubility test was performed at 25°C in test tubes with 25 mm diameter x 150 mm height and analytical grade solvents. The solubility was indicated according to the recommendations in official compendia designated by descriptive terms wherein the term part refer to dissolving 1g of a solid in the number of solvent milliliters, provided the number of parts.

d) Determination of the melting range: the melting range was determined in capillary tubes with thickness of 1.6 mm and 7.5 cm in length, three capillaries tests being performed simultaneously inserted in automated equipment (Stuart Scientific SMP3 - Staffordshire, UK) with a heating rate of 1.0°C per minute.

e) Determination of pH: The pH potentiometric determination was obtained at pH meter device model B474™ brand Micronal, previously calibrated solution of cefazolin sodium, subjecting lyophilized powder 100 mg/mL, prepared in purified water. The test was conducted in a controlled temperature of 25ºC.

f) Spectrophotometry in the ultraviolet region: Ultraviolet spectra were obtained on a Shimadzu UV-Vis spectrophotometer; model UV-mini-1240, using quartz cuvettes with a 1 cm optical path. Readings were taken between 200 and 400 nm, being determined in solutions prepared in various solvents such as purified water (Milli-Q™), 0.1 *M* hydrochloric acid PA (Synth™), 0.1 *M* sodium hydroxide PA (Merck™), methanol PA (Synth™), phosphate buffer pH 6, and 1% phosphate buffer pH 8 containing 20.0 µg/mL.

g) Infrared absorption spectrophotometry: The infrared spectra were obtained with a Shimadzu spectrophot ometer Fourier Transform Infrared Spectrophotometer Model: IR Prestige-21 IR Affinity-1 FTIR-8400S/Kyoto - Japan, with digitization of the spectra (region 500-4000 cm^{-1} and 2 cm^{-1} interval). The tablets were prepared by mixing 1% of sample (reference substance and lyophilized powder) weighing 1.5 mg of cefazolin sodium in 150 mg KBr previously pulverized in an agate mortar and dried at 105 °C until constant weight, they were placed in special molds and then pressed under vacuum pressure of 80.000 kN to form transparent discs.

h) Thin-layer chromatography: The plates used for identification of the drug were silica gel 60 F254 DC-Fertigfolien alugram™ 20 x 20 cm (Macherey-Nagel) with a thickness of 0.25 mm, purchased commercially, and activated at 105 °C for 1 h. The mobile phase was placed in a vat chromatographic until saturation. The mobile phase used was methanol or absolute ethyl alcohol: water (90:10, v/v). Aliquots of 10 µL of solutions containing cefazolin sodium (sample and reference substance) freshly prepared in purified water at a concentration of 5 mg/mL (w/v), were applied to the stationary phase consisting of silica gel impregnated in aluminum and held to the test. After the end of the 10 cm elution, the plate was removed from the glass vessel and dried under a stream of air, the spots were detected by exposing the plate in the UV (365 nm) and soon afterwards the exposure to iodine atmosphere, which were analyzed for size, shape, position and R_f values.

i) High Performance Liquid Chromatography (HPLC): The reverse phase chromatographic method was performed on Waters system consisting of binary pump gradient chromatographic Waters 1525™, manual Rheodyne injector and detector Breeze 7725i UV-Vis Waters 2487™. The analysis of the drug under study was performed isocratically on Zorbax Eclipse Plus columns 5 mm C18 (250 mm x 4.6 mm) Agilent™ and 5 mm Symmetry C18 (4.6 x 250 mm) Waters™ kept at room temperature. The mobile phase consisted of water and acetonitrile (60:40 v/v), pH was adjusted to 8 with drops of triethylamine and the test was performed at a flow rate of 0.5 mL/min using UV detector at 270 nm, the peak areas were integrated using the program Empower software™. The suitability of the chromatographic system was determined based on conformance testing of the chromatographic system *(system suitability)* to ensure the performance of the chromatograph for analysis performance, injecting solutions in seven working concentration of 60 µg/mL. The relative standard deviation of the parameters of asymmetry of the peak broadening factors, number of plates, retention time and peak area was analyzed as recommended by the FDA in 2004[13].

4. Results

Qualitative analyzes are essential for the identification of raw materials and securing the content of active substance in marketed pharmaceutical form; in this context, this work proposes some analytical methods to identify reproducible, reliable and easy performance.

Cefazolin sodium aspect powder had presented a crystalline odorless and hygroscopic white or almost white. The physical aspects of cefazolin sodium corresponded to those described in official compendia[8-12].

The moisture content by Karl Fischer presented to cefazolin sodium in lyophilized powder was 1.6%, and IR was 2.5% as shown in Table 1.

Table 1. Moisture cefazolin sodium powder for injectable solution determined by Karl Fischer and by infrared moisture analyzer

	Karl Fischer	Infrared Scale
Test[a]	Moisture content (%)	Moisture content (%)
1	1.63	2.50
2	1.54	2.40
3	1.62	2.60
Average[b]	1.59	2.50
SEM[b]	0.02	0.05

[a] Each essay is the average of three determinations on different days
[b] Standard error of the mean

The moisture content was presented by Karl Fischer method (1.6%) and by infrared moisture analyzer (2.5%). The method by infrared is described in Brazilian pharmacopoeia, 2010[14] to evaluate the moisture content, this method is capable of detecting volatile substance and not just the presence of water; however the Brazilian Pharmacopoeia, 2010 does not present the cefazolin sodium in this compendium. The USP Pharmacopoeia, 2010 [11] presents water determination in titrimetric by Karl Fischer method and the result found for cefazolin sodium are consistent with the recommended (not more than 2%). It is interesting to remember that each water molecule has a molecular weight equivalent to 18.015 g/mol, so excess moisture can interfere on weighing the exact amount of drug, leading to dubious results of content or potency, in quantitative analysis. The moisture content specified above may also indicate poor conservation of raw materials and can lead to deterioration. These events make the moisture determination of extreme importance.

Solubility is a qualitative method which characterizes the sample according to their polarity, being useful in the identification and purity of the drugs. Among the tests, although the solubility test cannot be taken in the strict sense of physical constant, it complements and supports with other assays being predictive for determining the best solubility being necessary for determining the solvents to be employed in quantitative tests. The results are shown in Table 2 and the terms for solubility are described according to Table 3.

Table 2. Solubility of cefazolin sodium powder for injectable solution in different solvents at 25°C

Solvents	Descriptive term
Ethyl acetate	Very slightly soluble
acetone	slightly soluble
0.1 M acetic acid	Very slightly soluble
0.1M hydrochloric acid	practically insoluble
water	very soluble
butanol	practically insoluble
chloroform	practically insoluble
dichloromethane	practically insoluble
ethanol	Very slightly soluble
ethyl ether	practically insoluble
Sodium hydroxide 0.1 M	easily soluble
methanol	slightly soluble

Table 3. Solubility Descriptive terms and their meanings required to solubilise 1 g solid

Descriptive term	Solvent
very soluble	Less than 1 part
easily soluble	1 to 10 parts
soluble	10 to 30 parts
slightly soluble	30 to 100 parts
slightly soluble	100 to 1000 parts
very slightly soluble	From 1000 to 10,000 parts
practically insoluble or insoluble	Over 10,000 parts

Source: FARMACOPEIA BRASILEIRA, 2010[14]

Samples of cefazolin sodium lyophilized powder showed being easily soluble in water and very slightly soluble in ethanol[8-11], slightly soluble in methanol[12] and practically insoluble in chloroform and ether[11, 15]. So the results are within specification.

In determining the melting range, cefazolin sodium RS presented early degradation viewed around 172ºC by the color change of the substance, progressively increasing in intensity until its complete carbonization, from white to yellow-orange-brown-black. However, bubble formation due to the melting of the liquefying samples was evidenced from 180°C with the sample elution due to boiling observed up to 200°C. The same was carried out for cefazolin sodium lyophilized powder, which had the same characteristics; the results are in Table 4.

Table 4. Values of the melting range obtained for a sample of cefazolin sodium RS

Test[a]	Color change (ºC)	Beginning of melting range (ºC)	Find melting range(ºC)
1	172.90	180.20	190.00
2	172.60	181.00	190.00
3	172.40	180.60	190.00
Average	172.63	180.60	190.00
RSD[b]	0.22	0.14	----

[a]Each essay is the mean of three determinations on different days; [b]Relative standard deviation

The determination of the melting range is simple, economical and rapid, often used in the characterization of a compound, and is an important indicator of purity, when compared to reference standards, because a small amount of impurity may cause lower point melting or extend the melting range of a particular compound[16]. In the official compendia, the melting point of cefazolin sodium is not displayed. For ZAPPALA et al. 1975[17] melting point of cefazolin is 190 °C. Reports of analyses of suppliers as West-Ward Pharmaceuticals[18] headquartered in Eatontown, New Jersey, USA indicate that the melting range for the drug under study is 180 ºC to 200 ºC and in the reports of the Chinese supplier ChemKoo[19] the melting point for cefazolin sodium is 190 ºC, therefore concludes that the results are consistent.

The pH is the number that represents the concentration of hydrogen ions in an aqueous solution, for practical reasons, its definition is experimental. The test solution was prepared by weighing 1 g of cefazolin sodium lyophilized powder, added to 10 mL volumetric flask, which was supplemented with purified water. The test was performed in triplicate and the average of three determinations of pH, cefazolin sodium lyophilized powder was 4.88. The results obtained in the pH analysis are in accordance with specifications, which establish the range of pH 4-6[8-11] or pH of 4,5-6,5[12] for solution of cefazolin sodium in purified water 0.1 g/mL.

In the analysis by ultraviolet, solutions were tested in water, methanol, 0.1 M hydrochloric acid, sodium hydroxide, 0.1 M phosphate buffer and 1% pH 6 phosphate buffer pH 8, 1%, water solvent gave similar results to solutions phosphate buffer pH 6, and 1% 1% phosphate buffer pH 8, with a maximum absorption wavelength at 271 nm and absorbance of 0.5585 to cefazolin sodium dissolved in purified water to 20.0 µg/mL. The spectra were analysed and from these, we chose to use water as a solvent, by presenting appropriate features in the spectra and having economic and environmental advantages, such as being easy acquisition and disposal and have low cost (Figure 2). The spectral profiles of cefazolin sodium RS lyophilized powder were consistent in all tested solvents, showing equivalent absorption peaks, and therefore can be used as proof of identification by comparison. This parameter too allowed to quantify the drug in another paper[20] and was compared to reference spectrum in the ultraviolet region (Figure 3) available in official compendia - Japanese Pharmacopoeia 2011[12].

The spectrophotometry in the ultraviolet region is an identification method based on analyte concentration and the intensity of light absorbed. The ultraviolet absorption spectra are employed as proof of identification, by comparing the spectral profile, wherein the standard and sample readings are carried out simultaneously and under the same conditions. This feature is important for the characterization of substance from getting greater wavelengths absorbance corresponding to chromophores presented in each molecule of electronic structure and identification of the drug can be made by determining its absorption characteristics in different solvents[14, 21]. The presented method is able to identify samples of cefazolin sodium with the advantages of being simple, rapid, reproducible, not expensive and easily discarded without generating toxic waste to the operator and the environment, corroborating qualitative analysis of routine laboratory quality control in the characterization of cefazolin sodium in pharmaceutical form powder for injectable solution, ensuring the effectiveness of the product already sold. The spectra obtained for RS cefazolin and lyophilized powder were compared among them (Figure 2) and were compared to the spectra reported in the literature[12] (Figure 3) and no significant differences, confirming the purpose of this technique is the identification of molecule by comparison with a reference standard.

The absorption spectra in the infrared region in KBr pellets also showed similar spectra to cefazolin sodium RS and lyophilized powder sample as shown in Figure 4 and Figure 5 shows that the spectra of reference cefazolin sodium

are available in the data library of the equipment used and the spectrum shown by the Japanese Pharmacopoeia, 2011[12].

The absorption spectra in the infrared region in KBr pellets showed characteristic absorption bands of cephalosporin compounds, as shown in Table 5 and are in accordance with the foregoing cephalosporin nucleus in Figure 1.

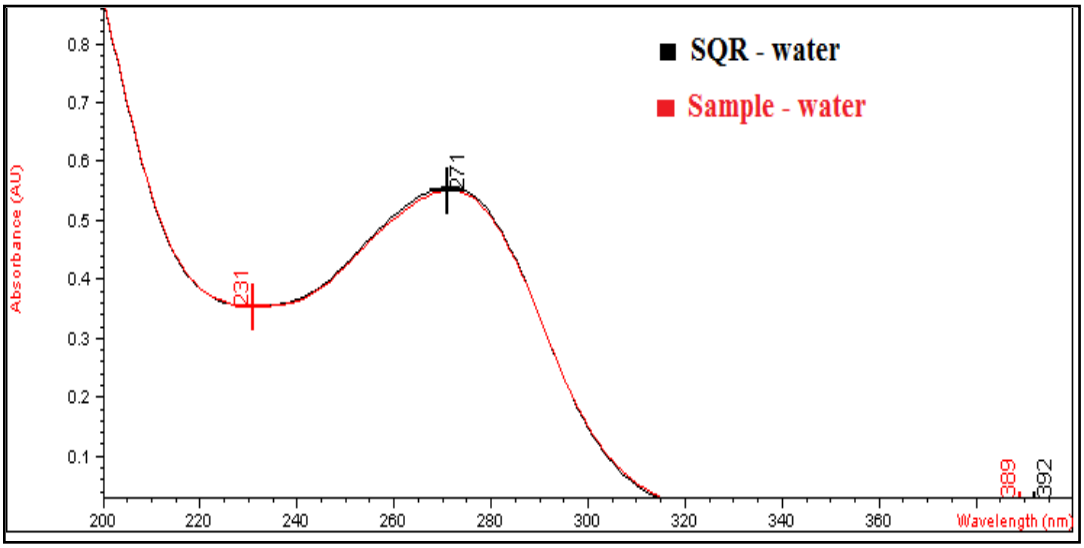

Figure 2. Absorption spectra of aqueous solutions of cefazolin sodium RS and cefazolin sodium lyophilized powder, both with a concentration of 20.0 µg/mL in the ultraviolet region

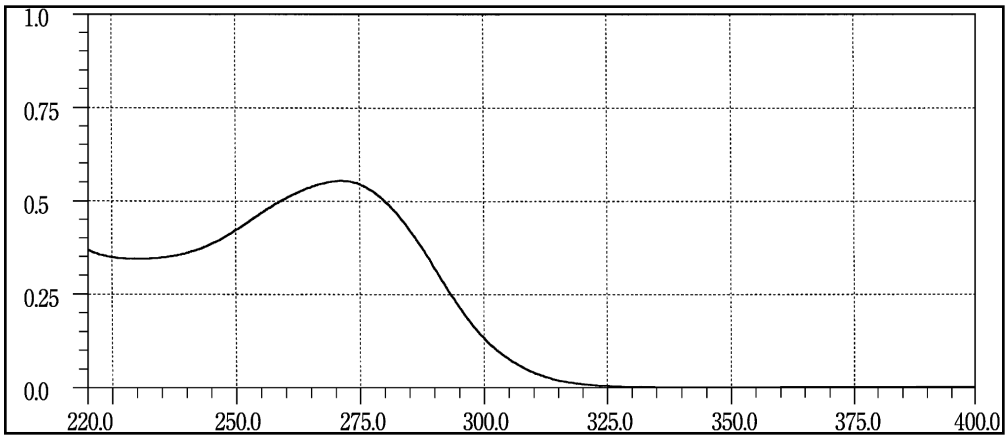

Figure 3. Reference spectrum in the ultraviolet region for cefazolin sodium available in official compendia - Japanese Pharmacopoeia 2011 [12]

Table 5. Frequency bands and groupings corresponding bands observed in the infrared spectra of cefazolin RS and lyophilized powder

Frequency Range (cm) Observed	Frequency Range (cm) Reference *	Responsible Groupings
3282; 3419	3500-3300	Stretch grouping N-H and C-H
3056-3229	3100-3000	Stretch grouping-C = N and -N = N
2900	3000-2850	Stretch grouping-C-H
1761	1760-1700	Stretch grouping carboxylate C = O
1671	1680-1630	Stretch grouping amide C = O
1600, 1386	1600-1400	Stretch grouping carboxylate –COO
1600, 1540, 1490	1600, 1580, 1500, 1450	Stretch grouping of aromatic C = C
1241, 1183, 1100, 1062	1350-1000	Stretch grouping C-N
1540	1640 – 1550	Folding grouping N-H amide secondary

Source: Pavia et al., 2010; Silverstein et al., 2006[21-22]

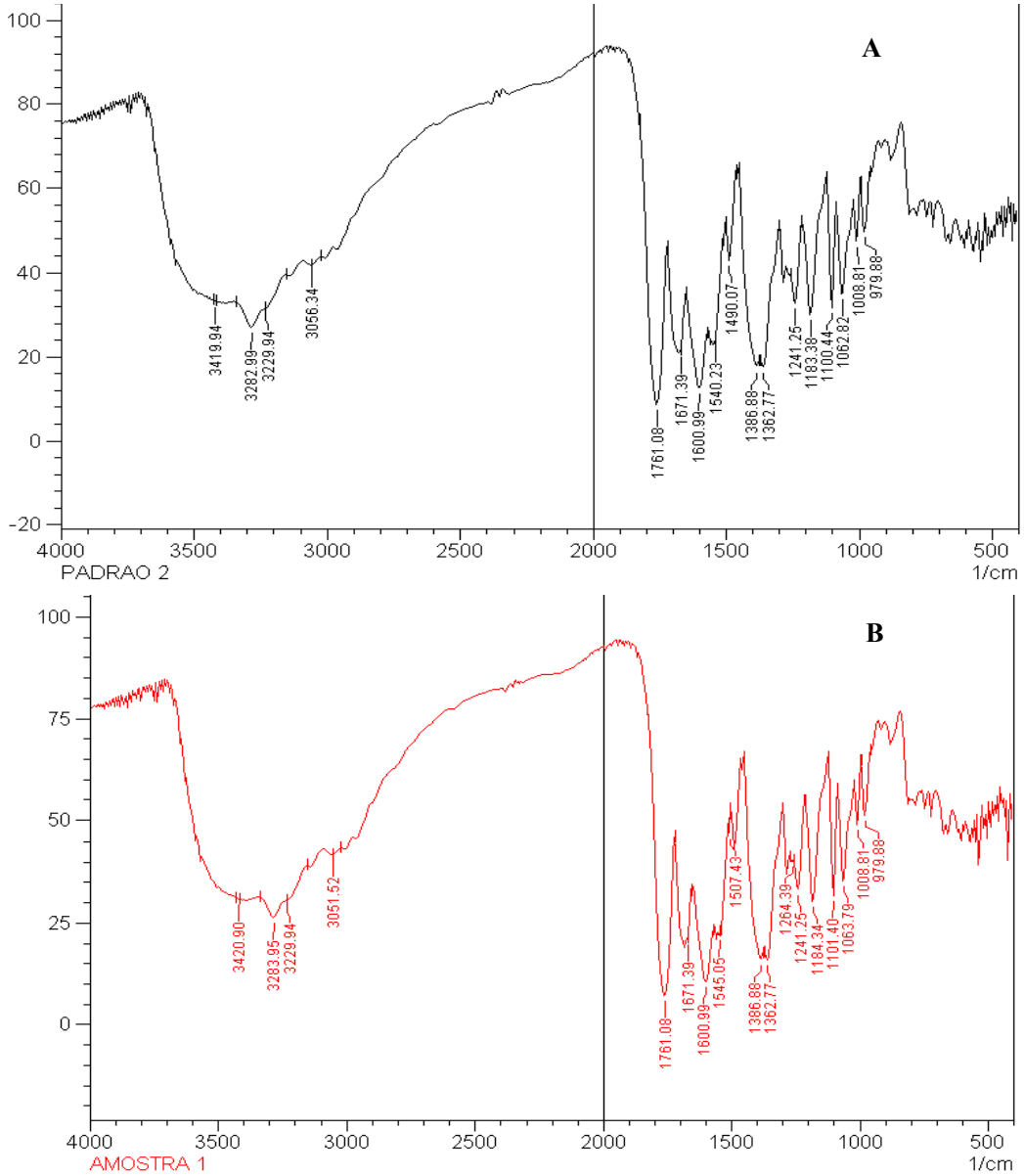

Figure 4. Spectrum in the infrared region of cefazolin sodium RS (A) and cefazolin sample of lyophilised powder (B) in KBr pellets

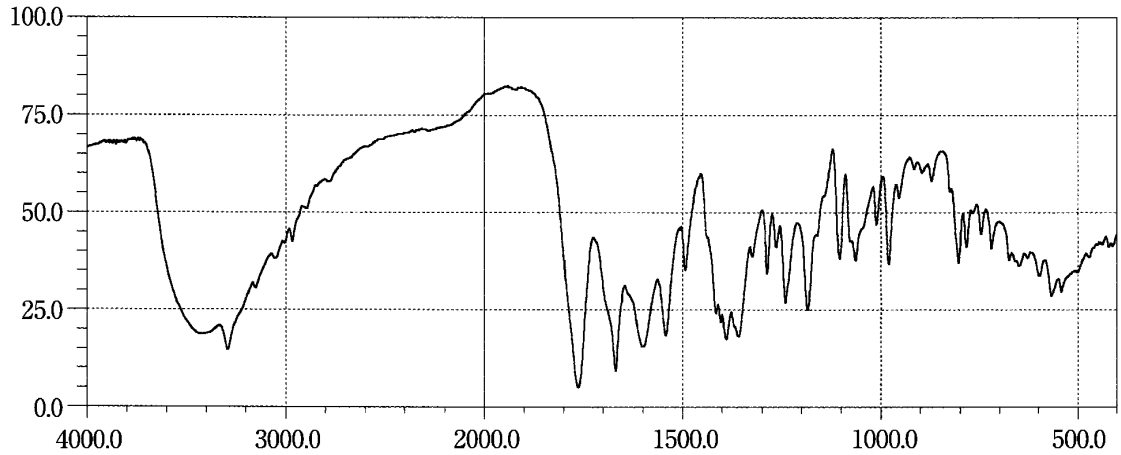

Figure 5. Reference spectrum of cefazolin sodium available spectrum in the infrared region in official compendia - Japanese Pharmacopoeia 2011 [12]

The absorption spectra in the infrared region in KBr pellets showed characteristic bands of cephalosporin compounds, such as stretching bands of the grouping NH been demonstrated that near 3419, 3282 cm^{-1}; at 3229 and

3056 cm^{-1} bands were presented by stretching the grouping = CH, whereas at 1761 cm^{-1} bands stretch appear C = O grouping carboxylate, around 1671 cm^{-1} are bands stretch C = O grouping of the amide and 1600, 1540, 1490 cm^{-1} are bands stretch C = C grouping of aromatic, it is also suggested that provided the bands at 1600 and 1386 cm^{-1} they refer to the stretch of carboxylate COO- grouping. The bands presented in 1241, 1183, 1100 and 1062 cm^{-1} represent the grouping CN stretch, while the bands 1540 cm^{-1} are related to the stretching of the secondary amide NH grouping. The thiadiazole and tetrazole rings presented in the molecule are composed of tertiary amines and therefore do not present characteristic bands in the infrared spectra. The spectra obtained for RS cefazolin and lyophilized powder (Figure 4) were compared to the spectra reported in the literature[12] (Figure 5) and show the same characteristic absorption bands', confirming the purpose of this technique is the identification of molecule by comparison with a reference standard. The technique of infrared spectrophotometry allows correlating the peaks appeared in the spectra, to thereby characterizing the analyte. It presents great evidence of identity of a structure being practical, fast and selective with the advantage of requiring small amounts of sample, having a viable cost benefit (referring to instrumentation), increase the ability to identify or characterize complex structures, and not generate toxic waste.

The thin layer chromatography is a method for characterization of pharmaceuticals, among analytical methods for identification, because of its easy, versatile and inexpensive implementation and understanding[2, 23]. For the study of thin layer chromatography, various systems eluents were tested in order to verify the behaviour of this cephalosporin against certain mobile phases, such as chloroform: methanol: formic acid (18:2:1) v/v/v; chloroform: methanol: formic acid (18:7:1) v/v/v, chloroform: methanol: formic acid (33:7:3) v/v/v, acetone: acetic acid: chloroform (1:0.5:3:5) v/v/v; *n*-butanol: acetic acid: water (4:1:5) v/v/v, dichloromethane: methanol: ammonium hydroxide: acetonitrile (6:4:2:2) v/v/v/v, dichloromethane: methanol: acetonitrile: 5% acetic acid (6:4:2:2) v/v/v/v; butane: sodium acetate buffer pH 4.5: butyl acetate: acetic acid (6:26:32:32) v/v/v/v, water: methanol (2:8) v/v: methanol: water: ethanol (3:7) v/v, water: ethanol (2:8) v/v water: ethanol (1:9) v/v; being all analytical grade solvents. The spots shown in chromatographic plates were evaluated for size, shape, position and R_f values. Good results have been obtained using as mobile phase methanol and water: ethanol (1:9) v/v, these systems exhibited R_fs 0.82 and were revealed in iodine atmosphere (Figure 6). The mobile phase methanol proved to be suitable to identify various cephalosporins, such as cefazolin sodium, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, although showing that near R_fs 0.82, 0.85, 0.83, 0.74 and 0.84 respectively, it was still possible to differentiate them by presenting stain characteristics. The chromatogram revealed in iodine atmosphere and UV light 365 nm (Figure 7).

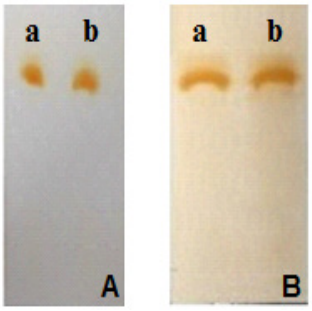

Figure 6. Chromatograms of cefazolin sodium RS (a) and cefazolin sodium lyophilized powder (b), methanol mobile phase (A) and water: ethanol (1:9 v / v) (B) disclosed in iodine atmosphere

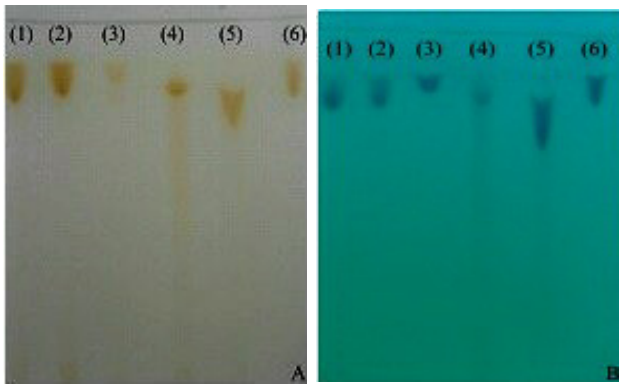

Figure 7. Comparison of chromatograms of cefazolin sodium RS (1), cefazolin sodium lyophilized powder (2), cefoxitin (3), ceftazidime (4), ceftriaxone (5) and cefuroxime (6). Revelation in iodine atmosphere (A) and a UV chamber (UVA 365 nm) (B), using methanol as solvent

To evaluate the behaviour of the drug in stress conditions, samples of cefazolin sodium were prepared under acid, basic, neutral and photolytic oxidation conditions. The elution of drug was performed on a vat containing chromatographic mobile phase consisting of water: ethanol (1:9) v/v and the chromatograms were revealed with ultraviolet light. Additional spots were visualized through time in which the drug has been exposed to stress conditions (Figure 8).

The thin layer chromatography (TLC) is a technique of solid-liquid absorption, which consists in the separation of compounds by migration of affinity substances for the stationary phase. It is a simple, fast, and economical visual aid, used for monitoring of organic reactions and compound identification[2, 23, 24]. The official compendia do not describe tests to identify cephalosporins by thin layer chromatography, only for related substances[8-11]. Whereas there are no methods for CCD for cefazolin sodium in pharmacopoeial compendia, we sought studies in the literature that describe[25-29]. However, many of the methods found generally used as the mobile phase, mixtures of various toxic and difficult revealing solvent disposals. Studies are identifying cephalosporins by TLC and a new method for identification of cefazolin sodium was proposed and considered.

Among the tested systems, mobile phase methanol showed satisfactory results with R_fs 0.82 allowing identification of the drug among other cephalosporins, as shown in the chromatogram in Figure 7. However, this

mobile phase eluted rapidly and was unable to clearly separate the products generated when the specimen was subjected to acidic, basic and photolytic and stress. Seeking alternatives to better separate these products, proportions of water: methanol and water-ethanol were tested in order to decrease the elution strength of the mobile phase. The system consists of water: ethanol (1:9) v/v showed R_f 0.75 and was the best that could separate these products. The results indicate that cefazolin sodium is easily identifiable, showing characteristic spot of purple colouring in chamber 365 nm UVA and brown in iodine atmosphere. The proposed new system has advantages over the methods described previously, the parameters were selected to propose a method with a simple mobile phase to minimize the cost and a decrease in consumption of organic solvents looking to generate less waste toxic. The method is consisted of solvent economically viable; it is safe for operators and for the environment. Moreover, it is practical and appropriate to identify cefazolin sodium in qualitative analyses of routine quality control of the product.

A new method for high performance liquid chromatography was studied for the identification of cefazolin sodium in order to reduce operating costs in order to avoid damage to the chromatographic column, reducing the analysis time and reducing the production of toxic waste. For that, different mobile phases were tested at different chromatographic columns, to find favorable conditions. The chromatogram of cefazolin sodium obtained by the proposed method showed good resolution and peak symmetry and a retention time of 3.6 minutes allowing its application in quality control, having the advantage of rapid determination of the drug (Figure 9).

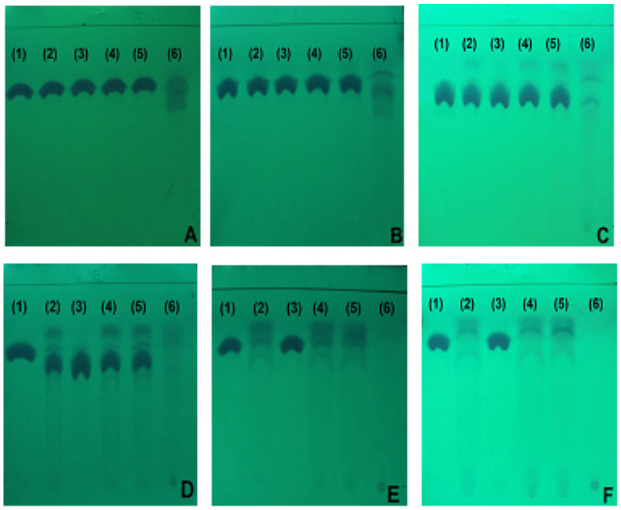

Figure 8. Chromatogram of cefazolin lyophilized powder intact (1) and in solution: neutral (2) photolytic (3), core (4), acidic (5) and oxidative (6) using a mobile phase water: ethanol (1: 9) v/v in camera revealed in UV (UVA 365 nm) immediately after preparation (a), after 0.30 h of degradation (B) after 6 h of degradation (C) after 24 h of degradation (D), after 96h of degradation (E) and after 168 h of degradation (F)

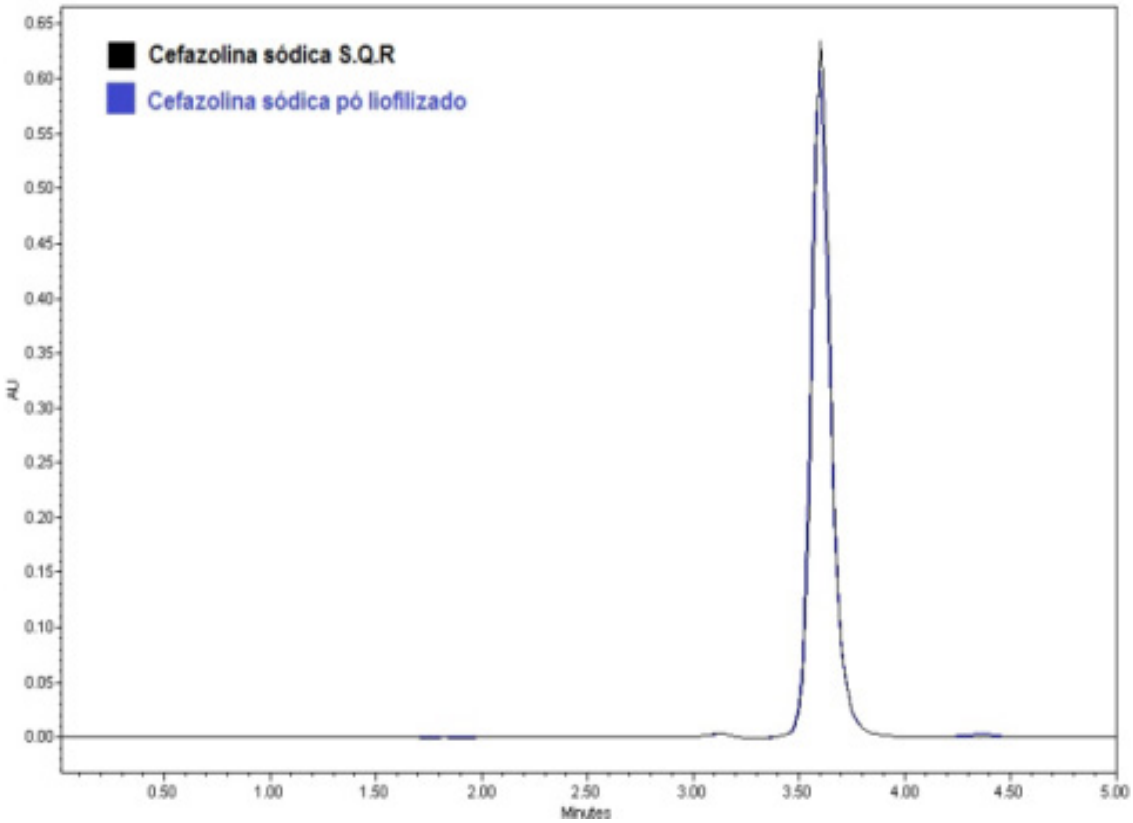

Figure 9. Chromatogram of cefazolin sodium RS - (black), cefazolin sodium lyophilized powder - (blue), both at a concentration of 60 µg/mL, obtained with mobile phase: water: acetonitrile (60:40 v/v), adjusted pH 8 with TEA and stationary phase: Agilent Zorbax Eclipse Plus C18 (250 x 4.6 mm) 5 mm

The results obtained in examining the suitability of the chromatographic system are described in Table 6.

Table 6. Parameters evaluated in analysing the suitability of the chromatographic system studied for analysis of cefazolin sodium

	Parameters evaluated				
	Asymetry (≤2,0)	Tailing Factor (≤2,0)	Plates (>2000)	Retention time (min)	Area
	1.0	0.36	7803.61	3.603	4063634
	1.0	0.36	7833.96	3.610	4094335
	1.0	0.36	7838.30	3.611	4077877
	1.0	0.36	7868.73	3.618	4097063
	1.0	0.38	7807.94	3.604	4090641
	1.0	0.36	7855.68	3.615	4085460
avarage	1.0	0.36	7834.70	3.61	4084835
[a]RSD	0.0	1.69	0.33	0.16	0.30

[a]Relative standard deviation

The results of system suitability indicate that the selected chromatographic parameters are able to identify the cefazolin sodium as recommended by ICH, 2005 [30]. The method for high efficiency liquid chromatography showed satisfactory results of system suitability. The data obtained in verifying the system suitability (Table 6) indicate that the system is safe and reliable, which is consistent with the recommendations by the FDA, 2004[13], in which the parameters are suggested: Asymmetry of the peak and enlargement factor ≤ 2, Number of plates > 2000; relative standard deviation among the test < 2%. Therefore, the proposed method fulfills the requirements recommended in the literature and can be used for analysis of cefazolin sodium in the pharmaceutical industry. Many methods described for CFZ up to the moment are directed to its quantification in biological matrix, but few studies are described for analysis of CFZ in pharmaceutical form [31-35]. The new method developed has the advantages of presents a simple mobile phase, not requiring preparation of buffer as a constituent, thereby achieving greater column shelf-life to minimize costs. Besides a decrease in analysis time and consumption of organic solvents, there is always a concern for the environment, looking for generating less waste. Therefore the method is appropriate for the control of drug quality post-production, to be performed by the industry providing security for the user and the quantitative analysis of this methodology will be presented in a future work.

5. Conclusions

With globalization, technological advances occur ever faster and provide different methods daily, targeting efficiency, reproducibility, lower uptime to fit routine pharmaceutical industries, where "time is money"; however, they are often complex and costly. Qualitative analyzes made possible the rapid characterization of cefazolin sodium with the advantages of being simple, fast, reproducible and inexpensive methods and can be used routinely in analyzes of quality control. It is worth mentioning that the Farmacopeia Brasileira (2010) still does not describe methods for qualitative analysis of cefazolin sodium, being at the discretion of the pharmaceutical industry adoption of the most convenient methods to prove the quality of medicines produced.

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