A fully validated microbiological assay to evaluate the potency of ceftriaxone sodium

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Ceftriaxone (CFTX) sodium is a third-generation, broad-spectrum cephalosporin that is resistant to beta-lactamases. An alternative bioassay for the assessment of the potency of this drug in pharmaceutical formulations has not been previously reported. Thus, this paper reports the development and full validation of a 3 x 3 agar diffusion bioassay using a cylinder-plate method to quantify CFTX sodium in pharmaceutical samples. The strain *Staphylococcus aureus* ATCC 6538P was used as the test microorganism, and the results of the proposed bioassay displayed high linearity, precision, accuracy, specificity and robustness. All potency results were statistically analyzed using an analysis of variance (ANOVA) and were found to be linear (r=0.99999) in the range of 16–64 μg/mL, accurate (100.5%), and precise [repeatability: relative standard deviation (RSD)=1.4%; intermediate precision: between-day RSD=2.1% and between-analyst RSD=2.5%]. The specificity of the bioassay was determined by evaluating a degraded sample (50 ºC) at 0, 24 and 48 hours as compared against the results from the pharmacopeial liquid chromatography method for CFTX. The results validated the proposed microbiological assay, which allows reliable quantitation of CFTX in pharmaceutical samples. Moreover, it is a useful, simple and low-cost alternative method for monitoring the quality of this medicine.

Uniterms: Ceftriaxone/agar diffusion assay. Ceftriaxone/quality control. Ceftriaxone/microbiological assay. Drugs/quality control.

A ceftriaxona sódica é uma cefalosporina de terceira geração de uso parenteral, com amplo espectro de atividade e resistente a β-lactamases. Este estudo apresenta o desenvolvimento e validação de um bioensaio por difusão em ágar usando o método de cilindros em placas para determinação da potência deste antibiótico. A validação desenvolvida apresentou bons resultados em termos de linearidade, precisão, exatidão, especificidade e robustez. Empregou-se o *Staphylococcus aureus* ATCC 6538P como micro-organismo teste. Os resultados dos ensaios foram tratados estatisticamente utilizando-se análise de variância (ANOVA). O método apresentou linearidade (r=0,99999) na faixa de doses selecionada (16-64 μg/mL), precisão (repetibilidade: DPR=1,4%; precisão intermediária: inter-dias DPR=2,1% e inter-analistas: DPR=2,5%) e exatidão de 100,5%. A especificidade do bioensaio foi avaliada através da análise comparativa, por cromatografia líquida de alta eficiência, de amostras degradadas a 50 ºC nos tempos zero, 24 e 48 h. Os resultados encontrados demonstraram a validade do bioensaio proposto, o qual permite a quantificação confiável de ceftriaxona sódica em produtos farmacêuticos comerciais. Por ser metodologia simples e de baixo custo constitui-se em alternativa para a análise de rotina do controle de qualidade de medicamentos.

Unitermos: Ceftriaxona/ensaio de difusão em ágar. Ceftriaxona/controle de qualidade. Ceftriaxona/ensaio microbiológico. Fármacos/controle de qualidade.

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INTRODUCTION

Ceftriaxone (CFTX) sodium is a semisynthetic antibiotic that can effectively treat several types of bacterial infections. Unlike the other 3rd generation cephalosporins, CFTX has a long plasma half-life, up to 4- to 10-times longer than the other antibiotics in this class (Neu *et al.,* 1981; Stoeckel, 1981). This cephalosporin shows a broad spectrum against Gram-positive and Gram-negative bacteria, including enterobacteria, *Haemophilus influenzae* and *Streptococcus pneumoniae* (Rebuelto *et al.,* 2002).

Regarding the official quality control of CFTX, as raw material or in pharmaceutical preparation, the pharmacopeias recommend employing reversed phase liquid chromatography (RP-HPLC) with UV detection and a mobile phase composed of water, phosphate buffer and acetonitrile (British Pharmacopoeia, 2009; European Pharmacopoeia, 2005; Japanese Pharmacopeia, 2006; United States Pharmacopeia, 2011).

Several alternative physicochemical methods to assay CFTX in pharmaceutical formulations are described in the literature, such as spectrophotometric methods (El-Walily *et al.,* 2000; Al-Momani, 2001; Amim, Ragab, 2004; Sankar *et al.,* 2006; Okoye *et al.,* 2007; Adegoke, Quadri, 2012), flow injection analysis (FIA) with chemiluminescence detection (Yinhuan, Jiuru, 2006), spectrofluorometry (Shah *et al.,* 2011), reversed-phase high-performance liquid chromatography (RP-HPLC) (Hecq *et al.,* 2006; Jane *et al.,* 2006; Tippa, Singh, 2010; Akl *et al.,* 2011), high performance thin layer chromatography (HPTLC) (Eric-Jovanovic *et al.,* 1998) and differential pulse polarography (DPP) (Sengün *et al.,* 1985).

To assess the concentration of CFTX in biological matrices such as plasma, serum, cerebrospinal fluid and bile, several physicochemical methods have been reported, such as HPLC (Patel *et al.,* 1981; Trautmann, Haefelfinger, 1981; Ascalone, Dal Bò, 1983; Bowman *et al.,* 1984; Chan *et al.,* 1986; Granich, Krogstad, 1987; Bompadre *et al.,* 1998; Kohlhepp *et al.,* 1998; Tsai *et al.,* 1999; Nemutlu *et al.,* 2009; Mcwhinney *et al.,* 2010), spectrofluorometric assays (Omar *et al.,* 2009), and capillary zone electrophoresis (CZE) (Quaglia *et al.,* 1997). Microbiological techniques are used to evaluate the bioavailability of CFTX in biological fluids (Rebuelto *et al.,* 2002; Ismail *et al.,* 2005) and to study the susceptibility of several microorganisms to CFTX (Beskid *et al.,* 1981; Eickhoff, Ehret, 1981; Baumgartner, Glauser, 1983; Emmerson *et al.,* 1985; Dias *et al.,* 1998).

The use of microbiological assays to evaluate the potency of CFTX in pharmaceutical formulations is uncommon. The literature reports only one method which uses a 5 point calibration curve with paper discs in a bi-layer agar diffusion assay using *Bacillus subtilis* (ATCC 6633) as the test microorganism (Cantón *et al.,* 1993). However, the original method was not validated and the quantification does not follow the procedure recommended for a 5 x 1 assay (Esteban *et al.,* 1990).

Physicochemical techniques are recognized to be fast, precise and accurate in quantifying cephalosporin antibiotics. However, some disadvantages are inherent to these methods, as the interference of the excipients, tedious extraction steps and lack of selectivity complicate the performance (Ahmed *et al.,* 2011; Adegoke *et al.,* 2012). Furthermore, most of these procedures are not simple in the routine analyses, they require dedicated or sophisticated equipment and expensive reagents, which are often not available in quality control laboratories (El-Walily *et al.,* 2000; Souza *et al.,* 2006).

Alternative methods to evaluate the potency of antibiotics, such as the fully validated microbiological agar diffusion assay, are simple and operationally inexpensive. Furthermore, such bioassays are suitable for quality control laboratories that do not have specialized and sophisticated instruments (Souza *et al.,* 2006; Schmidt *et al.,* 2008,2009).

Therefore, the aim of the present study was to develop and validate a low-cost, simple, specific, accurate and reproducible microbiological agar diffusion assay using a cylinder-plate method and propose it as a useful alternative to the physicochemical methods described in the literature for quantitation of ceftriaxone sodium as raw material and injectable formulation.

MATERIAL AND METHODS

Chemicals

The ceftriaxone sodium reference standard from Brazilian Pharmacopeia (assigned purity 847.3 µg/mg) and the sample were commercially obtained. The sample (batch 97728B) was within its shelf-life and claimed to contain 500 mg of ceftriaxone sodium sterile powder for injection (Eurofarma, Brazil). All reagents used were analytical grade (Difco, USA; Merck, Germany). Ultrapure and bidistilled water were used in the experiments.

Ceftriaxone sodium reference solution

Ten milligrams of the ceftriaxone sodium reference standard was transferred into a 100 mL volumetric flask

and dissolved in sterile phosphate buffer solution pH 7 [K_2HPO_4 1.36% (w/v) and KH_2PO_4 0.4% (w/v)]. Aliquots of this solution were diluted in the identical buffer yielding working solutions with final concentrations of 16, 32 and 64 µg/mL (S1, S2 and S3, respectively).

Preparation of the sample solutions

Five-hundred milligrams of the CFTX sample was transferred to a 250 mL volumetric flask and dissolved with sterile phosphate buffer solution pH 7. Five milliliters of this solution were transferred to a 25 mL volumetric flask and dissolved to obtain a final concentration of 400 µg/mL. Aliquots of this solution were further diluted in the identical buffer solution to obtain the concentrations of 16, 32 and 64 µg/mL (T1, T2 and T3, respectively), which were tested against S1, S2 and S3.

Microorganism and inoculum standardization

The strain *Staphylococcus aureus* ATCC 6538P was selected as the test microorganism because of its susceptibility to ceftriaxone sodium, yielding sharply defined zones of growth inhibition, which allows more precise measurements. The culture of *Staphylococcus aureus* ATCC 6538P (INCQS - National Institute for Health Quality Control, Brazil), after reconstitution, were cultivated and maintained on Grove-Randall's 1 culture medium (Difco). The microorganism standardization was prepared according to the procedure described in the Brazilian and USP Pharmacopeias (Farmacopeia Brasileira, 2010; United States Pharmacopeia, 2011). Prior to use, the microorganism was grown in a slant medium (Grove-Randall's 1, for 24 h at 35 ± 2 °C). Using aseptic techniques, the growth was suspended in a 0.9% NaCl sterile solution and diluted to give a suspension with 25 ± 2% turbidity (transmittance) at 580 nm using a 10 mm absorption cell, with 0.9% NaCl sterile solution as blank. The Grove-Randall's 1 culture medium at 48 °C was inoculated with the standardized suspension at 1% (v/v) to compose the upper layer in the plate.

Agar diffusion bioassay

The bioassay followed the 3 × 3 parallel line design (3 doses of standard and 3 doses of sample in each plate), with 6 plates/assay, in accordance with the European and Brazilian Pharmacopoeias (European Pharmacopoeia, 2005; Farmacopeia Brasileira, 2010). For the base layer agar, 21 mL of Grove-Randall's 2 culture medium (Difco) in a 100 × 20 mm Petri dish was used. After

solidifying, 5 mL of inoculated Grove-Randall's 1 medium was poured onto the base layer. In each plate, 6 stainless steel cylinders (8 × 6 × 10 mm – external diameter x internal diameter x height) were placed on the surface of the inoculated medium. Three alternated cylinders were filled with 150 µL of reference solutions (S1, S2 and S3), and the other three cylinders were filled with the concentrations of the sample solutions (T1, T2 and T3) (Figure 1). The plates were incubated at 35 ± 2 ºC aerobically for 16 h. The growth inhibition zone diameters (mm) were carefully measured with an electronic caliper. All experiments were performed in a biological safety cabinet and the infected material was decontaminated before being discarded.
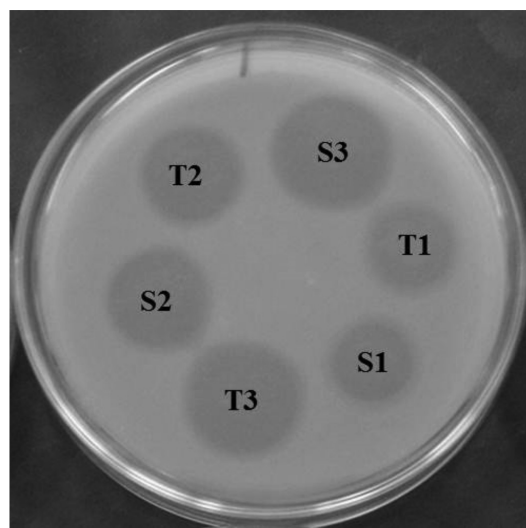

FIGURE 1 - CFTX potency evaluation by agar diffusion assay using the cylinder-plate method with a 3 x 3 experimental design. Zones of growth inhibition observed for doses 16, 32 and 64 µg/ml of the CFTX reference substance (*S1, S2, S3*) and sample (*T1, T2, T3*) using the strain *S. aureus* ATCC 6538P.

HPLC

In addition to the bioassay, the remaining CFTX after hydrolysis degradation was assessed by a LC method using the chromatographic conditions described in the USP monograph for CFTX sodium (United States Pharmacopeia 2011). Briefly, 20 µL sample volumes were injected into a LC system (Shimadzu LC-10AD, Japan) equipped with a 270-nm detector and a C18 column (4 x 150 mm). Each sample was run in triplicate. The mobile phase (water, acetonitrile, buffer solution pH 7, buffer solution pH 5 – 552:400:44:4) containing 0.32% of tetraheptylammonium bromide was used at a flow rate of 2 mL/min.

Calculations

In all experiments, the CFTX potency was statistically calculated using the parallel-line model for a 3 x 3 assay design. The regression, parallelism and linearity of the response were evaluated by an analysis of variance (ANOVA). To complement the statistical validation, the confidence interval (IC95) of each assay was considered (Farmacopeia Brasileira, 2010; Hewitt, 2003; European Pharmacopoeia, 2005; ICH, 2005; Schmidt *et al.,* 2009).

Validation of the method

All experimental conditions of the proposed method were tested and adjusted prior to the validation to ensure the best assay conditions. The method was validated according the International Conference on Harmonization (ICH 2005) and USP guidelines 2011). The following operational characteristics were evaluated:

Range – Assessed by the selected doses for the calibration curve and confirmed by determination of the accuracy, precision and linearity of the method.

Linearity – Evaluated through 12 independent assays using linear regression analysis and calculated by a least-squares method for three doses of the reference substance.

Precision – Assessed through the repeatability and intermediate precision and expressed as the relative standard deviation (RSD). The repeatability was examined by assaying 6 different test solutions of the CFTX sample against the reference standard. The assays were performed by the same analyst, under identical experimental conditions in one day (intraday). The intermediate precision was evaluated by performing the analysis in the same laboratory on 2 separate days (interday) with different analysts (between-analysts).

Accuracy – The test was repeated in three consecutive days. Three concentration levels, covering 80% to 120% of the selected range of 16, 32 and 64 µg/mL, were tested each day. Forty mg of CFTX was transferred to a 100 mL volumetric flask and dissolved in a phosphate buffer solution pH 7.0 to obtain a stock solution with a concentration of 400 µg/mL. The 3 concentration levels at 100% were prepared from aliquots of 1, 2 and 4 mL of the stock solution transferred into 50 mL volumetric flasks and diluted with a phosphate buffer solution pH 7 to give final concentrations of 16, 32 and 64 µg/mL, respectively. The doses at 80% nominal concentration were prepared from aliquots of 0.8, 1.6 and 3.2 mL of the stock solution transferred into 25 mL volumetric flasks to give final concentrations of 12.8, 25.6 and 51.2 µg/mL, respectively. The doses at 120% nominal concentration were obtained from aliquots of 1.2, 2.4 and 4.8 mL of the stock solution transferred into 25 mL volumetric flasks and diluted with phosphate buffer solution pH 7 to give solutions with final concentrations of 19.2, 38.4 and 76.8 µg/mL, respectively. These working solutions were assayed against the 3 concentration levels of the reference standard solution at 100% nominal concentration.

Selectivity – The ability of the proposed method to assess the content of CFTX sodium in the presence of impurities and degraded substances was tested by comparing the results obtained in the bioassay to the results from the pharmacopeial LC method for the identical degraded sample. A CFTX sodium commercial sample was reconstituted with water for injection and heated to 50 ºC for 2 days. The sample was analyzed by both methods at 0, 24 and 48 h. The conditions of the LC method were in accordance with the USP monograph for CFTX sodium (United States Pharmacopeia, 2011).

Robustness – Several method parameters were modified in assaying a CFTX sample. The considered factors were the inoculum concentration (1.3%), incubation temperature (32 °C), volume of the inoculated layer (thickness – 4 mL) and the solvent used for the standard and sample dilution (sterile water for injection).

RESULTS AND DISCUSSION

The use of a suitable analytical method is fundamental in the quality control of active substances in either the pharmaceutical or raw material form. The choice of the method is normally based on several factors such as the drug source; its complexity; purity and sample quantity; and the qualitative, semi-quantitative or quantitative purpose of the method. Furthermore, the availability of equipment and reagents should be considered in the development of accessible and useful methodologies.

Taking in account that the potency of an antibiotic may be assessed through the comparison of the inhibition of growth of a susceptible microorganism induced by known concentrations of the antibiotic and its respective reference standard (European Pharmacopoeia, 2005; United States Pharmacopeia, 2011), a 3 x 3 microbiological assay was proposed for determining the CFTX sodium concentration in injectable pharmaceutical dosage forms.

The range of the doses selected (16 to 64 µg/mL) for the bioassay was shown to be most appropriate for this assay system for the following reasons: the susceptibility of the microorganism to lower concentrations on the curve, the linear relationship between the logarithm of the dose and the observed response, and the significant slope of the curve. The linearity study involved 12 independent assays with

6 plates/assay. The experimental average zone diameters (mm) and RSD values (%) for the standard solutions are presented in Table I. The difference between the average size of the inhibition zones for doses 16-32 µg/mL, and 32-64 µg/mL was approximately 3 mm, showing good linearity in the response obtained by the method. Furthermore, the RSD values showed low variability in the response (intradose) obtained in this bioassay.

TABLE I - Mean diameters of the growth inhibition zones obtained for the CFTX standard curve for 16, 32 and 64 µg/mL solutions

Concentration (µg/mL)	Mean diameter ± SD[a] (mm)	RSD (%)
16	16.3 +/- 0.3	1.9
32	19.4 +/- 0.2	1.0
64	22.4 +/- 0.5	2.0

[a] n= 12 independent assays with 6 Petri dishes each

The logarithm of the concentrations (µg/mL) and the mean diameter of the inhibition zones (mm) were used to calculate the calibration curve for CFTX. The method showed good linearity for the range of 16–64 µg/mL. The representative linear equation was $y = 10.176x + 4.046$. The determination coefficient ($r^2=0.99998$) and correlation coefficient ($r=0.99999$) were highly significant. The bioassays were validated using an analysis of variance (ANOVA). In all experiments, the regression was highly significant ($P<0.05$) and no deviation was found in either the parallelism or the linearity ($P>0.05$). Moreover, all bioassays gave potency results within a confidence interval of 90–115% (IC95), indicating that the experiments performed during the validation were well executed. Simultaneously, the developed method was found to have significant response differentiation between doses and significant sensitivity to the selected doses.

The bioassay precision, in terms of repeatability (intra-assay), was evaluated by analyzing, on identical days, six different test solutions of CFTX sodium powder for injection with identical theoretical concentrations. The CFTX activity ranged from 99.2% to 102.8%, with an RSD value of 1.4% (Table II).

To calculate the intermediate precision, the same sample was analyzed in triplicate on 2 separate days (between-day; Table III) and by 2 different analysts (between-analysts; Table IV), yielding RSD values of 2.1% and 2.5%, respectively. These low RSD values confirmed the capacity of the method to generate, with the same sample, reproducible results with a low response variation in independent assays.

TABLE II – Results of the repeatability evaluation of the microbiological assay of CFTX powder for injection

Theoretical amount (mg/fa)	Experimental amount (mg/fa)	Potency (%)	Confidence interval (IC95)	RSD (%)
500	514.2	102.8	95.4 - 110.9	1.4
	496.2	99.2	93.1 - 105.8	
	499.8	99.9	97.3 - 102.7	
	512.5	102.5	96.0 - 109.5	
	502.5	100.5	91.9 - 109.9	
	509.1	101.8	95.6 - 108.5	

fa – flask ampoule

TABLE III - Between-day precision data of the CFTX bioassay. Sample solutions at 100% theoretical concentration were tested in triplicate in two separate days

Day	Potency found (%)	Confidence interval (IC95)	RSD (%)
1	100.5	91.9 - 109.9	2.1
	101.8	95.6 - 108.5	
	102.8	95.4 - 110.9	
2	102.8	93.0 – 113.8	
	99.6	96.0 – 103.3	
	97.5	93.2 – 101.9	

TABLE IV - Between-analyst precision results obtained in the CFTX bioassay validation. Sample solutions at 100% theoretical concentration were tested in triplicate by two analysts

| Analyst	Potency found (%)	Confidence interval (IC95)	RSD (%)
1	99.6	96.0 - 103.3	2.5
	97.5	93.2 - 101.9	
	102.8	93.0 - 113.8	
2	96.2	91.5 – 101.1	
	97.1	91.9 – 102.5	
	97.3	90.8 – 104.2	

The accuracy of the method was evaluated at 80, 100 and 120% of the range selected for the bioassay (16-64 µg/mL), covering the specific range of 12.8-76.8 µg/mL. The mean accuracy was 100.5% with an RSD of 1.6% (Table V). Thus, the results obtained in the bioassay were close to the true concentration values of the tested samples. Moreover, it shows the capacity

TABLE V - Accuracy of the proposed microbiological assay assessed by the analysis, in triplicate, of CFTX sample solutions diluted to 80%, 100% and 120% of the theoretical concentration

Theoretical potency (%)	Day	Potency found (%)	Average potency (%)	Accuracy (%)	RSD (%)
80	1	81.9	81.8		
	2	81.5			
	3	81.9			
100	1	99.1	99.0	100.5	1.6
	2	99.3			
	3	98.5			
120	1	120.3	120.4		
	2	120.1			
	3	120.7			

of the proposed method to accurately quantify or detect samples containing low or high concentrations of CFTX, displaying that the bioassay is able to detect samples that do not meet the assay requirements recommended by the Pharmacopeias. Generally, this essential parameter for quantitative methods is not tested in agar diffusion assay validation studies.

To evaluate the selectivity of the proposed method, a CFTX commercial sample reconstituted to 10 mg/mL was exposed to dry heat (50 ºC) for 48 h. The concentration of the remaining CFTX in the sample was assessed by both the LC pharmacopeial method (United States Pharmacopeia, 2011) and the proposed bioassay at 0, 24 and 48 h. At time zero, the CFTX potency was 103.1% ± 0.6 and 107.3% ± 0.6 in the bioassay and HPLC, respectively. The CFTX showed low thermal stability, as shown in Figure 2, in which similar decreasing concentration curves were registered by both methods. However, the microbiological assay
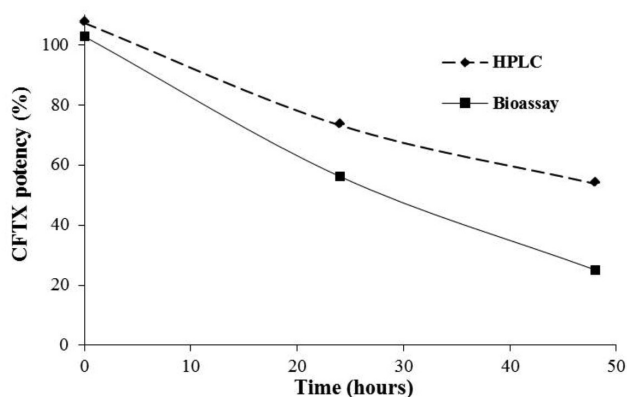
FIGURE 2 - Bioassay selectivity: the remaining content of CFTX at time 0, 24 and 48 h of exposure to dry heat at 50 ºC as assessed by HPLC and the biological assay.

presented higher sensitivity, because it showed a decrease in potency of approximately 77%, as compared to 53% detected by HPLC for the same sample. This preliminary result shows that the decomposition products of CFTX were not microbiologically active. Therefore, the microbiological assay was shown to be specific, because the impurities and degradation products did not interfere in the ability of the method to assess the analyte.

Figure 3 displays the chromatograms of the CFTX sodium reference standard (A), the freshly prepared sample (B), and after 24 h (C) and 48 h (D) of exposure to dry heat at 50 ºC. The main peak at approximately 3.7 min corresponds to CFTX. In 3C and 3D, the secondary peaks increase, mainly at approximately 3.5 min.

Considering the quantitation purpose of this bioassay and its inherent response variability that is characteristic of all bioassays systems (United States Pharmacopeia, 2011), it was considered essential to test the influence of small variations in the analytical conditions initially proposed. Among several critical factors involved in an agar diffusion assay, 4 were selected. These are directly related to the substance diffusibility and the growth of the inoculum. Therefore, to assess the method robustness, the following parameters were modified: inoculum concentration (1.3%), incubation temperature (32 °C), volume (thickness) of the inoculated layer (4 mL) and the solvent used for standard and sample dilution (bidistilled water) as shown in Table VI. The results showed no significant influence on the mean potencies when an ANOVA was applied (P<0.05), supporting the robustness of the method. The robustness results were also close to the range of 96.2% to 102.8% obtained under normal conditions for the repeatability, between-day/analyst precision and accuracy at 100% of the nominal concentration.
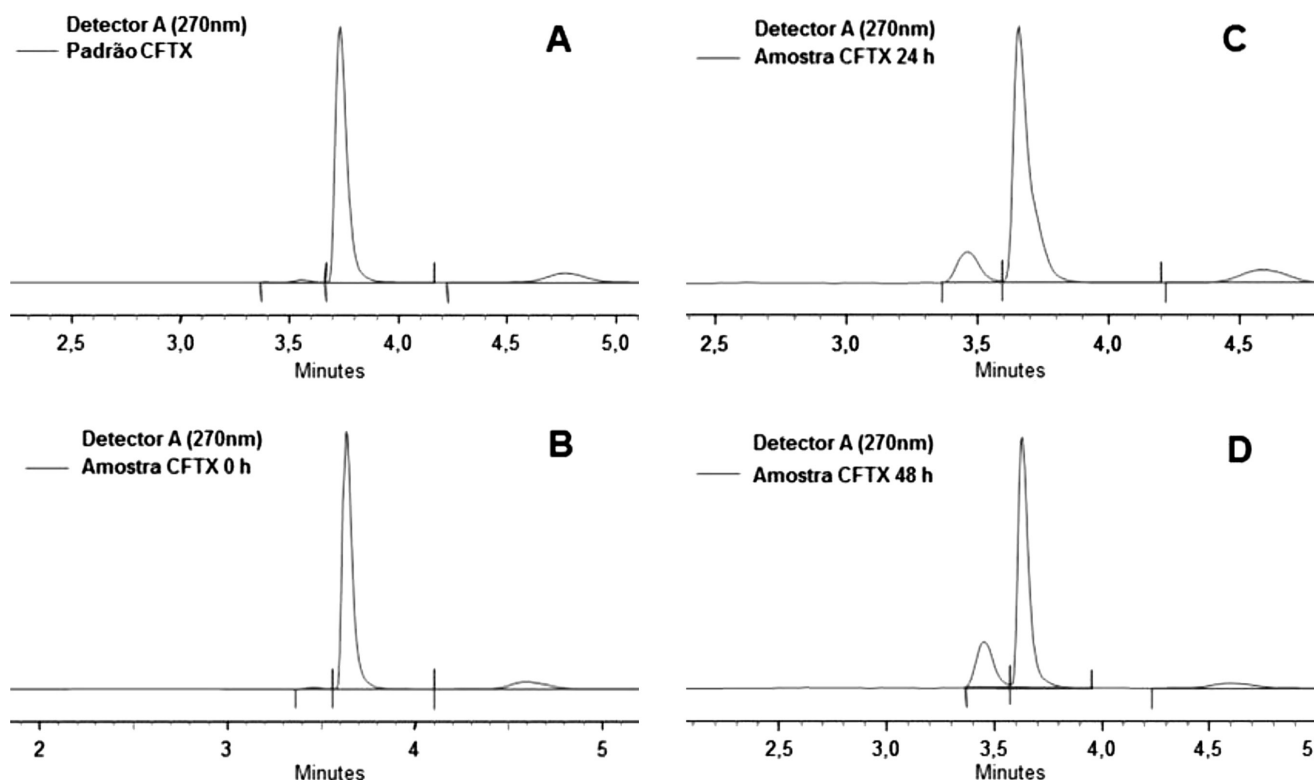
FIGURE 3 - Chromatograms of the CFTX reference standard (A). Freshly prepared sample (B). Sample after exposure to dry heat at 50 ºC for 24 h (C) and 48 h (D). The CFTX main peak is at 3.7 min. The chromatographic parameters were λ=270-nm, C18 column and mobile phase (water, acetonitrile, buffer solution pH 7, buffer solution pH 5 – 552:400:44:4) containing 0.32% of tetraheptylammonium bromide was used at a flow rate of 2 mL/min.

TABLE VI - Robustness data of the CFTX bioassay validation tested at 100% theoretical sample concentration

Condition challenged	Parameter	Potency found (%)	Confidence interval (IC95)	RSD (%)
Inoculum concentration	1.3%	99.3	93.7 - 105.4	
		99.2	94.2 - 104.6	
		100.2	93.0 - 107.2	
Incubation temperature	32ºC	101.0	94.3 - 108.2	
		102.7	96.3 - 109.6	
		102.7	95.2 - 110.8	2.3
Inoculated layer	4 ml	101.4	92.3 - 111.5	
		101.2	92.8 - 110.4	
		101.4	97.1 - 105.8	
Standard/sample solvent	Bidistilled water	95.4	86.1 - 105.5	
		98.9	89.9 - 108.8	
		99.5	90.6 - 109.2	

CONCLUSION

Analytical methods used for the quantitative determination of active substances must generate reproducible and reliable data. Therefore, in the routine quality control of medicine, it is mandatory to use well-characterized and fully validated analytical methods to yield reliable results that can be satisfactorily interpreted. The proposed microbiological assay for determining the potency of CFTX in pharmaceutical formulations was fully validated according to the ICH parameters and produced results which confirm its specificity, accuracy, robustness,

precision and significant linearity of response. Moreover, the bioassay produced results supporting those obtained by the pharmacopeial LC method for CFTX. However, the biological method has several advantages, including its simplicity and low cost, becoming increasingly appropriate when a LC system is not available for determining the potency of the antibiotic. Therefore, the proposed bioassay can be a useful method in the quality control of ceftriaxone sodium in pharmaceutical products and the raw material.

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