Validation of analytical methodology for quantification of cefazolin sodium pharmaceutical dosage form by high performance liquid chromatography to be applied for quality control in pharmaceutical industry

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A reversed-phase high performance liquid chromatography method was validated for the determination of cefazolin sodium in lyophilized powder for solution for injection to be applied for quality control in pharmaceutical industry. The liquid chromatography method was conducted on a Zorbax Eclipse Plus C_{18} column (250 x 4.6 mm, 5 μm), maintained at room temperature. The mobile phase consisted of purified water: acetonitrile (60: 40 v/v), adjusted to pH 8 with triethylamine. The flow rate was of 0.5 mL min^{-1} and effluents were monitored at 270 nm. The retention time for cefazolin sodium was 3.6 min. The method proved to be linear (r^2=0.9999) over the concentration range of 30-80 μg mL^{-1}. The selectivity of the method was proven through degradation studies. The method demonstrated satisfactory results for precision, accuracy, limits of detection and quantitation. The robustness of this method was evaluated using the Plackett–Burman fractional factorial experimental design with a matrix of 15 experiments and the statistical treatment proposed by Youden and Steiner. Finally, the proposed method could be also an advantageous option for the analysis of cefazolin sodium, contributing to improve the quality control and to assure the therapeutic efficacy.

Uniterms: Cefazolin sodium/determination. High performance liquid chromatography/reverse-phase/ quantitative analysis. Pharmaceutical industry/quality control. Cefalosporine/quality control. Medicines/ quality control.

Um método cromatográfico em fase reversa foi validado para a determinação de cefazolina sódica em pó liofilizado, a ser aplicado no controle de qualidade em indústrias farmacêuticas. O método por cromatografia líquida foi conduzido em coluna Zorbax Eclipse Plus C18 (250 × 4,6 mm, 5 μm) mantida à temperatura ambiente. A fase móvel consistiu de água purificada: acetonitrila (60 : 40 v/v), com o pH ajustado para 8 com trietilamina. A vazão usada foi de 0,5 mL min^{-1} e os analitos de interesse foram monitorizados a 270 nm. O tempo de retenção da cefazolina sódica foi de 3,6 min. As áreas dos picos de cefazolina sódica foram lineares na faixa de concentração de 30-80 μg mL^{-1} (r^2 = 0,9999). A seletividade do método foi demonstrada através de estudos de degradação. O método demonstrou resultados satisfatórios para precisão, exatidão, limites de detecção e de quantificação. A robustez do método foi avaliada utilizando o esquema fatorial de Plackett-Burman com uma matriz de 15 experimentos simultâneos, e analisados por tratamento estatístico proposto por Youden e Steiner. Finalmente, o método proposto pode ser também uma opção de êxito para a análise de cefazolina sódica, contribuindo para o controle de qualidade e para garantir a eficácia terapêutica.

Unitermos: Cefazolina sódica/determinação. Cromatografia líquida de alta eficiência/fase reversa/ análise quantitativa. Indústria farmacêutica/controle de qualidade. Cefalosporina/controle de qualidade. Medicamentos/controle de qualidade.

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INTRODUCTION

Since their discovery by Brotzu in 1948, cephalosporins maintain themselves as a class of antimicrobials that stands out for their high therapeutic importance and frequency of use, because of their safety and very effective use against a large variety of microbial agents (Brunton *et al.*, 2012). Cefazolin sodium (CFZ) is a β-lactam semi synthetic antibiotic classified as a first-generation cephalosporin; it has been commercially available since 1970, only in the form of pharmaceutical powder for injectable solution, not having oral absorption (Bolós *et al.*, 1987). Its use in clinical practice is distinguished for being used worldwide and keeping more than 40 years as a drug of choice for preoperative prophylaxis of various surgical procedures (Kusaba, 2009). Considering that it can be administered less frequently because of its longer half-life, a single dose of cefazolin immediately before surgery is the most widely used prophylaxis (Brunton *et al.*, 2012), and reduces the incidence of infections that may pose serious complications in patients undergoing surgery (Wang *et al.*, 2004). Although the antibacterial spectrum of cefazolin sodium is the typical of other first-generation cephalosporins, it prevails by demonstrating activity against *Enterobacter,* which makes it favorite among cephalosporins from this group (Kusaba, 2009; Brunton *et al.*, 2012).

High performance liquid chromatography is a very common separation technique and broadly used for antibiotic quantification. That a large number of methods using high performance liquid chromatography is available in the literature stems from the numerous advantages that the method provides (Salgado *et al.*, 2005; Tozo, Salgado, 2006; Moreno, Salgado, 2008; Lopes, Salgado, 2009; Bonfilio *et al.*, 2009; Cazedey *et al.*, 2011; Corrêa *et al.*, 2012; Silva, Salgado, 2012). Many methods described for CFZ up to the moment are directed to its quantification in biological matrices (Wold, 1977; Bayoumi *et al.*, 1986; Nahata, 1990; Liang *et al.*, 1994; Bompadre *et al.*, 1998; Al-Rawithi *et al*, 2000; Samer, *et al.*, 2000; Tsai, Chen, 2000; Arayne *et al.*, 2007; Farthing *et al.*, 2008), requiring pretreatment of the sample, among other slow procedures, requiring sophisticated equipment and high costs and using large amounts of organic solvents which are toxic for the operators and for the environment. Few studies were validated for quantification of CFZ in pharmaceutical form (Moore *et al.*, 1991; Pinto *et al.*, 1995; Farag, 1998; Shinde *et al.*, 1998; Lalith *et al.*, 2010), for the control of drug quality post-production, to be performed by the industry providing security for the user.

Studies focused on the development and refinement of analytical methodologies are crucial for optimization of laboratory tests carried out in the pharmaceutical industry, for adjusting the dosage and thus avoiding toxicity and development of resistant microorganisms, to thereby ensure the efficacy and safety of the consumer. In this context different chromatographic systems were tested in order to find favorable conditions to develop a new analytical method, for the quantification of CFZ injectable solution, capable of ensuring therapeutic efficacy of the marketed drug globally.

The validated HPLC method is simple, fast and versatile, therefore, it can be employed in routine quality control analyses of the pharmaceutical industry, reducing the analysis time, reducing the use of solvents due to low retention time, reducing residue generated and consequently presenting lower costs is which justifies the strategy adopted in this study.

MATERIAL AND METHODS

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 did 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). HPLC grade acetonitrile used in the preparation of the mobile phase was purchased from J.T. Baker (Mexico). All other Chemicals were of analytical grade.

Apparatus

The HPLC method was performed on a Waters LC system (Waters Corporation, Milford, MA) equipped with a Waters 1525 binary pump, a Rheodyne Breeze 7725i manual injector and a Waters 2487 UV detector. The peak areas were integrated automatically by a computer using the Empower software program.

The chromatographic separation was carried out under isocratic reversed phase conditions on an Agilent™ Zorbax Eclipse Plus C_{18} (250 x 4.6 mm, 5 µm particle size) column.

HPLC Method

The procedure was performed isocratically at room

temperature. The mobile phase consisted of purified water and acetonitrile with 60:40 v/v, pH adjusted to 8, with drops of triethylamine (Merck). The volume of the injection was 10 µL at a flow rate of 0.5 mL min^{-1}, using UV detection at 270 nm. The solutions were filtered through a 0.45 µm filter (Pall Corporation, Michigan, USA) before being injected, and the mobile phase was degassed by ultrasound bath before being used.

Preparation of standard and sample solutions

A quantity of 10 mg of CFZ were accurately weighted and transferred to the 50 mL volumetric flask, and the volume was completed with mobile phase in order to obtain a stock solution of 200 µg mL^{-1}. Aliquots of this solution were transferred to 10 mL volumetric flasks, whose volumes were completed with mobile phase, in order to obtain the working solutions of 30.0, 40.0, 50.0, 60.0 and 80.0 µg mL^{-1}, which were used in the assay.

Method validation

The method was validated following the International Conference Harmonization (ICH, 2005) guidelines for validation of analytical methods.

System Suitability
The system suitability test was conducted to evaluate the resolution and reproducibility of the system to ensure that the complete testing system was suitable for the intended application. In order to obtain the required data, six solutions of CFZ reference standard at a concentration of 60.0 µg mL^{-1} were prepared and analyzed by HPLC. The parameters such as peak area, retention time (t_R), plate number (N), tailing factor (TF), and asymmetry factor (A_S) and their respective relative standard deviation (RSD) were analyzed.

Linearity
Linearity was determined by the construction of three independent analytical curves. For this, aliquots of the stock solution were transferred to other volumetric flasks with the same solvent, to obtain six final concentrations: 30-80 µg mL^{-1}. The results were subjected to regression analysis by a least-squares method to calculate the calibration equation in which the determination coefficient and validity of the statistical parameters were evaluated.

Precision
Precision was assessed at different levels: Repeatability (intra-assay), by testing six evaluations of the same concentration sample of CFZ of 60.0 µg mL^{-1}, all on the same day and under the same experimental conditions and intermediate precision (inter-assay) by conducting the analysis on three different standard solution concentrations (high, intermediate and low), on three different days under the same experimental conditions (inter-days), and also by different analysts performing the analysis in the same laboratory (between analysts). Precision was evaluated by % RSD.

Detection and quantification limits
The LOD and LOQ of CFZ by the proposed method were determined using calibration standards. LOD and LOQ were calculated as 3 and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation.

Accuracy
Accuracy was attained via the recovery assay, in which known quantities of CFZ reference standard were added to a known quantity of the sample (ICH, 2005). The accuracy of the method was evaluated in triplicate at three concentration levels (R1 = 80, R2 = 100 and R3 = 120%), and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of CFZ in the drug product. This test was carried out by adding known amounts of CFZ-RS (0.90, 1.50 and 2.10 µg mL^{-1}) to the samples. The study was carried out in triplicate. Samples were prepared according to Table I.

TABLE I - Determination of the accuracy of the analytical method for the analysis of CFZ by HPLC

	CFZ sample (100 µg mL^{-1}) (mL)	CFZ RS (100 µg mL^{-1})(mL)	Theoretical concentration (µg/mL)[a]
R1	1.5	0.9	48
R2	1.5	1.5	60
R3	1.5	2.1	72

[a]10 mL volumetric flask

The percentage of recovered cefazolin sodium was calculated by equation 1.

$$\%R = \left[\frac{(C_A - C_S)}{C_T}\right] \times 100 \qquad (1)$$

where: C_A= concentration of the sample solution found added to RS (μg mL^{-1}); C_S= concentration found in the sample (μg mL^{-1}); C_T= theoretical concentration of the solution added RS (μg mL^{-1}).

Selectivity and study of forced degradation

The selectivity of the method was determined by subjecting a CFZ sample solution (200 μg mL^{-1}) to accelerated degradation by acidic, basic, neutral and photolytic conditions. The samples were evaluated at a concentration of 60 μg mL^{-1}. The sample was degraded in solutions of 0.001 mol L^{-1} HCl, 0.1 mol L^{-1} NaOH, and in purified water by acid, basic and neutral hydrolysis respectively, all maintained at 60 °C for 48 h. Photodegradation was induced by exposing the samples in a photostability chamber to 200 W h/m^2 of near UV light for 48 h. After the procedures, the samples were diluted with the mobile phase and were injected in the HPLC at a concentration of 60 μg mL^{-1}.

Robustness

The robustness of the method was demonstrated by the Plackett–Burman design, to evaluate the reliability of the analysis concerning small variations in its working parameters. Table II shows the factorial combination used in the Plackett-Burman test, where the letters A-G represent the parameters selected; 1-15 is the factors changed, where the level (0) is the optimal values of the procedure, whereas levels (1) and (−1) are respectively upper and lower values with regard to the selected one (0).

The choice of variables (factors) and levels that must be tested is very important to obtain a reliable test of robustness. The variables must be consistent with reality and practice levels should reflect the variation that can be generally observed. The factors and varying levels tested are shown in Table III.

TABLE III - Selected Factors and levels of variability

Factors	Unit	Limit	Condition varied (-1)	Condition Normal (0)	Condition varied (1)
A. Column brand	-----	-----	Waters	Agilent	Waters
B. Mobile phase flow rate	mL/min	± 2	0.48	0.5	0.52
C. Acetonitrile brand	-----	-----	Tedia	J. T. Baker	Tedia
D. Proportion of FM	%	± 2	58:42 v/v	60:40 v/v	62:38 v/v
E. Volume of injection	μL	± 2	8	10	12
F. Wavelength	λ	± 2	268 nm	270 nm	272 nm
G. Room temperature	°C	± 2	23	25	27

-1= Lower level; 0= Normal Level and +1 Upper Level.

TABLE II - Robustness test using the experimental model of Plackett-Burman

Parameter analytical	Combination factorial														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	1	1	1	0	1	0	0	0	-1	-1	-1	0	-1	0	0
B	0	1	1	1	0	1	0	0	0	-1	-1	-1	0	-1	0
C	0	0	1	1	1	0	1	0	0	0	-1	-1	-1	0	-1
D	1	0	0	1	1	1	0	0	-1	0	0	-1	-1	-1	0
E	0	1	0	0	1	1	1	0	0	-1	0	0	-1	-1	-1
F	1	0	1	0	0	1	1	0	-1	0	-1	0	0	-1	-1
G	1	1	0	1	0	0	1	0	-1	-1	0	-1	0	0	-1

A–G: selected factors; 1–15: N (number of experiments) = 2n + 1 where n = number of factors; −1, 0, +1: levels for the factors

The robustness was determined in triplicate from injections of standard solutions containing 60 µg mL^{-1} CFZ, under the same established experimental conditions.

To determine the influence of changes in each parameter on the final result, the average of dosage performed in triplicate assay corresponding to normal ranges was compared to the average of dosage corresponding to the modified levels.

The average effect of each variable is the average difference among the observations made at the modified levels and those made at the optimum level. The deviation of each factor was calculated using the methodology Youden and Steiner.

Equation 2 illustrates the effect evaluation of the changing parameter A-brand column, likewise the other parameters were also evaluated (Berzas *et al.,* 2004).

$$\sqrt{2S} > |DA| \qquad (2)$$

where:

$$S = \sqrt{\frac{2}{7}(DA^2 + DB^2 + DC^2 + DD^2 + DE^2 + DF^2 + DG^2)}$$

Since the deviation of each factor changed (DA, DB, DC etc) should be less than the value resulting from √2S to infer that the effects obtained with the variations of the parameters were not significant and therefore the method is robust for all selected factors.

RESULTS AND DISCUSSION

Preliminary tests were carried out in order to obtain a reliable method so as to avoid damage to the chromatographic column and try to decrease formation of residue. Many mobile phase systems were tested in different chromatographic columns. Still to establish the best working conditions, the distribution coefficient (log D) was determined using ACD/Labs 6.0 software, considering that the distribution coefficient is the evaluation of the partition coefficient (log P) at different pHs. The results indicate that the pH where the drug solubility is more stable is between 7 and 11 (Figure 1). However this chromatographic column can be used over a pH range of 2-9. Therefore, the pH range from 7 to 9 is indicating to perform the analysis. The mobile phase was adjusted to pH 8 using drops of triethylamine.

The CFZ chromatogram obtained by the proposed method showed good resolution and peak symmetry. The retention time observed (3.6 min) allows a rapid determination of the CFZ, which is desirable for a routine analysis (Figure 2).
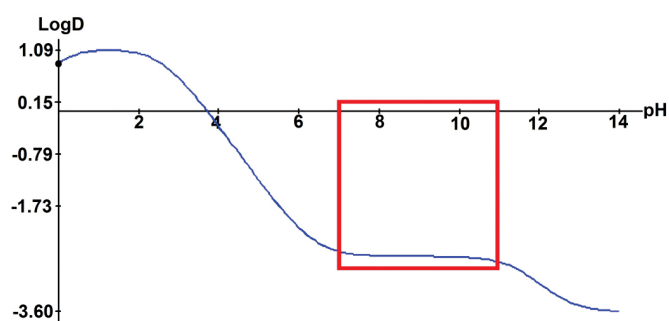

FIGURE 1 - CFZ distribution coefficient determined by ACD/Labs 6.0 software.
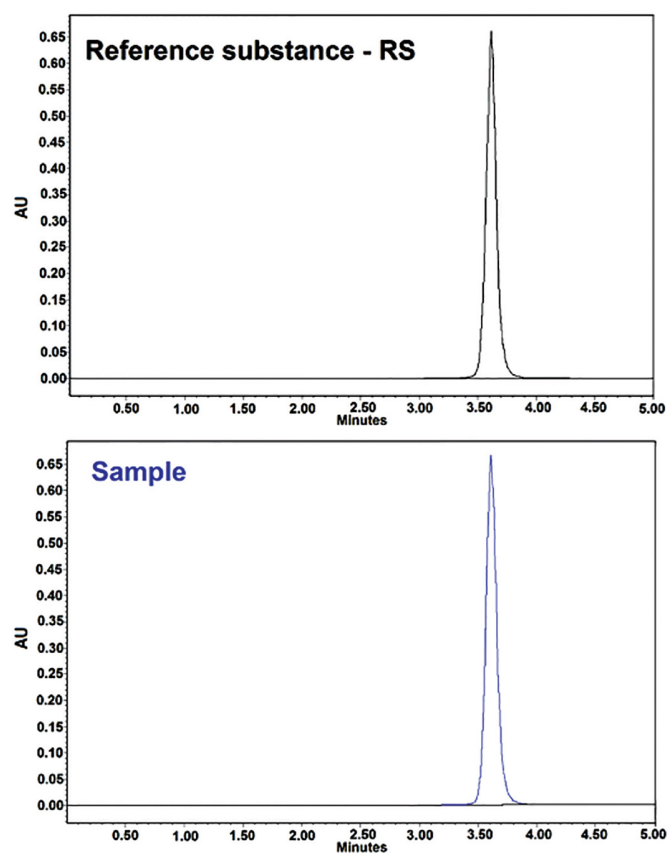

FIGURE 2 - Chromatogram of CFZ (60 µg mL^{-1}). Chromatogram standard - black, sample – blue.

System suitability

The results of *system suitability* indicate that the selected chromatographic parameters are able to identify and quantify the drug analyzed. The results are consistent with the literature (FDA, 2004). The *system suitability* was checked by injecting six replicate solutions of CFZ RS in with concentration of 60 µg mL^{-1}. The parameters measured in *system suitability* were peak area, retention time, resolution, peak purity, tailing factor, capacity factor and theoretical plate. In all measurements the peak area

TABLE IV - Parameters evaluated in system suitability test

	Parameters evaluated					
	Asymmetry (≤ 2.0)	Tailing Factor (≤ 2.0)	Capacity Factor (> 2.0)	Plate numbers (>2000)	Retention Time (min)	Area
	1.0	0.36	2.27	7803.61	3.603	4063634
	1.0	0.36	2.27	7833.96	3.610	4094335
	1.0	0.36	2.27	7838.30	3.611	4077877
	1.0	0.36	2.27	7868.73	3.618	4097063
	1.0	0.38	2.27	7807.94	3.604	4090641
	1.0	0.36	2.27	7855.68	3.615	4085460
average	1.0	0.36	1.0	7834.70	3.61	4084835
[a]RSD	0.0	1.69	0.0	0.33	0.16	0.30

[a]RSD= Relative standard deviation

varied less than 2.0%, the average retention time was 3.6 minutes (Relative Standard Deviation = 0.16%), the retention factor was more than 2, plate number was more than 7803 and tailing factor was less than 2, for the CFZ peak. The proposed method offers good detectivity and CFZ can be detected accurately. The results obtained for the parameters of *System Suitability* are presented in Table IV.

Linearity

Table V shows the values of areas obtained by the different concentrations of CFZ RS.

Residue analysis indicated that the regression model used is appropriate. Each average value of absolute areas obtained in determining the analytical curve was plotted in relation to its concentration, observing linearity in the range of 30-80 µg mL^{-1}, as shown in Figure 3.

The results for linearity were statistically analyzed using tests of variance (ANOVA); according to ANOVA there is no deviation from linearity in calibration curve and the regression model is appropriate. The ANOVA calculated for the data from the analytical curve of CFZ is presented in Table VI.

Precision

The precision of the method was determined by repeatability (intraday), expressing the results based on the RSD. Seven solutions of CFZ were prepared in a concentration of 60 µg mL^{-1} and were submitted to successive analyses and the date obtained on the same day under the same experimental conditions, by the same analyst and at the same laboratory, providing a RSD of 0.84%. For the determination of interday precision, analyzes were performed using three samples, on three consecutive days, yielding experimental values of contents of 100.29% 100.11% to 100.79%, with an average content

TABLE V - Values of the peak areas for the CFZ RS to the analytical curve

Concentration (µg mL^{-1})	Areas[a] (AU)	Average Area	RSD[b] (%)
30	2152162		
	2107217	2110833	1.87
	2073120		
40	2764639		
	2756486	2752218	0.55
	2735528		
50	3349823		
	3380328	3372399	0.58
	3387046		
60	3971999		
	3912803	3969727	1.40
	4024378		
70	4572851		
	4513790	4589643	1.86
	4682288		
80	5154763		
	5136111	5183200	1.27
	5258725		

[a] Average value of three determinations / [b]RSD = Relative standard deviation

of 100.39% and RSD was 0.35%. For precision between analysts, two samples were evaluated by two analysts, obtaining experimental values of 98.49% and 100.12%, with an average of 99.31% and RSD of 1.17, as shown in Table VII.

The precision showed RSD values lower than

recommended in the literature (ICH, 2005; FDA, 2004). The results obtained in interday precision and precision between-analyst were also statistically evaluated by analysis of variance and according to ANOVA there was no significant deviation, as shown in Table VIII and Table IX.
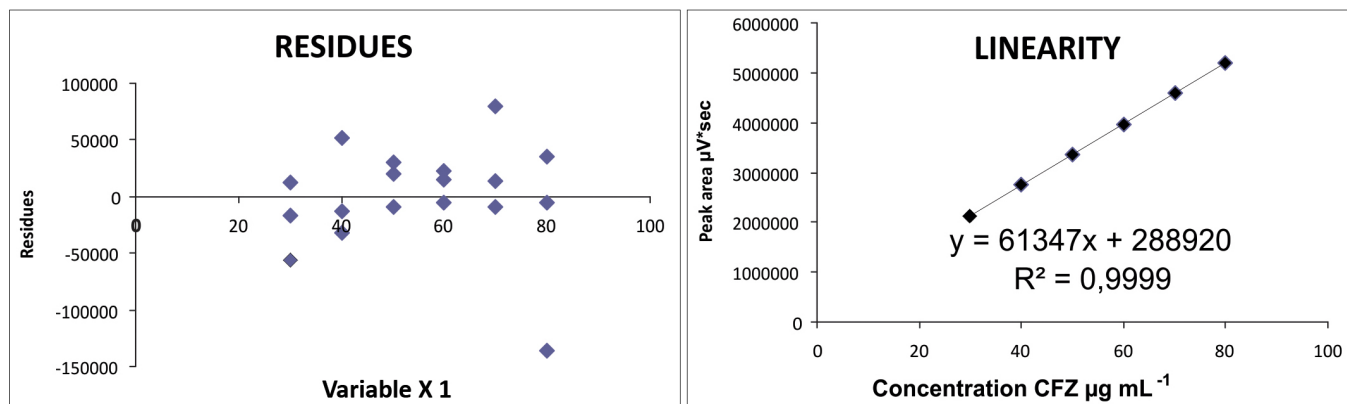

FIGURE 3- Residue analysis and analytical curve for CFZ RS obtained by chromatographic method, using purified water: acetonitrile (60:40 v/v) pH 8 adjusted with TEA, as mobile phase. Stationary Phase: Agilent Zorbax Eclipse Plus C_{18} (250 x 4.6 mm, 5 μm).

TABLE VI - Analysis of variance (ANOVA) for linearity

Source of variation	Degree of freedom	Sum of squares	Variability	F calculated	F critical
Between concentration	5	19760858787504.8	3952171757500.9	1396.43*	3.11
Linear regression	1	19760858787504.8	19760858787504.8	6982.14*	4.75
Deviation of linearity	4	0.00003	0.0000075	0.00	3.26
Residue	12	33962392533.3	2830199377.7	-	-
Total	17	19794821180038.2	-	-	-

* Significant at p <0.05 %

TABLE VII - Results for cefazolin sodium method precision

Interday precision				Between-analysts precision			
Day	**Content**[a] **(%)**	**Average Content (%)**	**RSD**[b] **(%)**	**Analist**	**Content**[a] **(%)**	**Average Content (%)**	**RSD**[b] **(%)**
1	100.29	100.39	0.35	A	100.12	99.31	1.17
2	100.11			B	98.49		
3	100.79						

[a]average of three determinations; [b]RSD= relative standard deviation

TABLE VIII - Analysis of variance (ANOVA) for interday precision

Source of variation	DF	Sum of squares	Average squares	F calculated	P-value	F critical
Between groups	2	269208531.8	134604265.9	0.10	0.89	3.55
Within groups	18	22465779885	1248098882			
Total	20	22734988417				

* p > 0.05%

TABLE IX - Analysis of variance (ANOVA) for between-analyst precision

Source of variation	DF	Sum of squares	Average squares	F calculated	P-value	F critical
Between groups	1	718684.57	718684.57	0.00026	0.98	4.74
Within groups	12	32952861485	2746071790			
Total	13	32953580170				

* p > 0.05%

TABLE X- Results for cefazolin sodium method accuracy

	CFZ RS added (µg mL^{-1})	CFZ RS found^a (µg mL^{-1})	Recuperation (%)	Average recuperation (%)	RSD^b (%)
R1	18	18.21	101.16	100.37	
R2	30	30.16	100.52		0.86
R3	42	41.76	99.44		

^{a}average of three determinations; ^{b}RSD= relative standard deviation

Accuracy

The accuracy showed good recovery between the true value and the value found. The accuracy of the method was determined by analysis of three concentrations in the pre-established range and experimental results corresponding to the average percentage of 100.37% are shown in Table X.

Limits of detection and quantification

The sensitivity of the method was determined by chromatographic detection (LOD) and quantitation (LOQ) limits. The value calculated for the lowest concentration detected by analytical procedure was 3.10 µg mL^{-1}. In turn, the calculated LQ was 9.41 µg mL^{-1}. The calculated values for the LOD and LOQ indicated the ability of the method to detect and quantify reliably CFZ.

Selectivity

Selectivity of the method was evaluated by forced degradation observing the chromatograms of the cefazolin sodium standard containing possible degradation products. The chromatograms depicted in Figure 4 show that the degradation products had negligible effect on to the cefazolin sodium peak. However in degradations in neutral or photolytic conditions showed no additional peaks, but showed a significant decrease of 62% and 68%, respectively, compared to the peak of drug at the time of preparation. Moreover, the resolution among cefazolin sodium and potential degradation products was higher than 1.5 indicating adequate separation of cefazolin sodium from other peaks (Snyder *et al.*, 2010).

This separation confirmed the method selectivity for degradation products.

Robustness

The robustness was evaluated by the Plackett-Burman factorial matrix, in which 15 experiments were performed with simultaneous changes, previously presented in Table II and Table III. The effects resulting from the changed parameters have been evaluated in comparison to the values obtained as reference for the test +1 1.47 and 1.54 for the test -1 (Equation 2). No effect showed significant results, indicating that the proposed method is robust. The robustness was confirmed by the Plackett-Burman model. The results indicated that the validity of the method is maintained even with small variations in its working conditions. Concentrations found in normal conditions and changed for each factor are shown in Table XI.

The results of method validation for the analysis of CFZ showed that the developed high performance liquid chromatography method is appropriate to quantify this cephalosporin in powder for injection.

CONCLUSION

The present study involves development of HPLC method using simple mobile phase which contains no buffer in its constitution, which is sensitive and rapid, as well as subsequent validation of developed method according to ICH guidelines. Prioritizing shorter analysis can reduce the consumption of organic solvents making the method financially advantageous for quantification
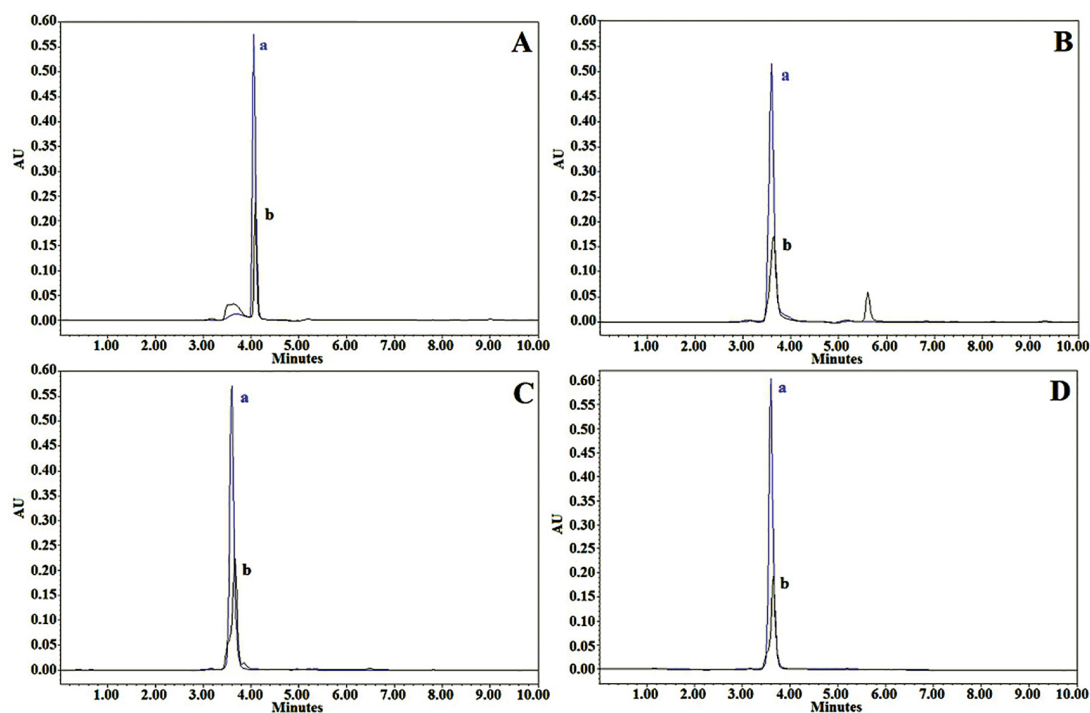
FIGURE 4 - CFZ Chromatogram of 60 µg mL^{-1} before degradation (a) and after 48 hours under degradation (b). In Basic (0.1 mol L^{-1} NaOH) at 60 °C (A); acid (0.001 mol L^{-1} HCl) at 60 °C (B) neutral (purified water) at 60 °C (C); and photolytic (UV) at room temperature (D).

TABLE XI - Results for cefazolin sodium method robustness

Factor	(+1)	Content (%)^{a,b}	(-1)	Content (%)^{a,b}
Column brand	A= Waters™	100.38–101.46= -1.08	A= Waters™	100.83 – 101.63 = -0.80
Mobile phase flow rate (µg mL^{-1})	B= 0.52	101.21–100.63= 0.58	B= 0.48	101.30 – 101.15 = 0.15
Acetonitrile brand	C= Tedia	100.89–100.95= -0.06	C= Tedia	100.71 – 101.75 = -1.04
Proportion of FM (%)	D= 62:38	101.00–100.83= 0.17	D= 58:42	101.19 – 101.27 = -0.08
Volume of injection (mL)	E= 12	100.92–100.92= 0.00	E= 8	100.85 – 101.60 = -0.75
Wavelength (nm)	F= 272	100.71–101.13= -0.42	F= 268	101.52 – 100.94 = 0.58
Room temperature (°C)	G= 27	101.70–101.14= -0.56	G= 23	101.25 – 101.20 = 0.05

^{a}Average contents obtained in normal conditions - Average contents obtained in the altered conditions; ^{b}Reference criteria calculated: 1.47 (Test +1) and 1.54 (Test-1)

of CFZ in pharmaceutical powder in the pharmaceutical industry. The method proved to be able to quantify the cefazolin sodium in its pharmaceutical form, fulfilling the necessary criteria such as system suitability and validation of system, as linearity, precision, accuracy and robustness. The method is financially advantageous for the pharmaceutical industry, because the analysis is fast, and it generates a smaller amount of organic solvent waste. Thus this method is indicated for be used in routine quality control analyses of the pharmaceutical industry.

ACKNOWLEDGEMENTS

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