Research Article   **Open Access**

Development and Validation of an Innovative and Ecological Analytical Method Using High Performance Liquid Chromatography for Quantification of Cephalothin Sodium in Pharmaceutical Dosage

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Abstract

Cephalothin is a first-generation cephalosporin, that shows great activity against Gram-positive microorganisms. Its effect is bactericidal, and due its action, it is the most efficient first-generation cephalosporin against resistant microorganism (β-lactamase producers). Although this drug has been clearly studied and researched about its antimicrobial activity, pharmacokinetics and pharmacodynamics, there are a few studies in literature regarding the development of analytical methodology for this cephalosporin. The aim of this work was to develop and validate a new method of analysis, using high performance liquid chromatography, resulting in an innovative method, quick and using solvents of low toxicity, minimizing, in this way, its toxic actions to the operators and leavings in the environment. The method developed and validated for the quantification of sodium cephalothin in lyophilized powder for injectable solution used high performance liquid chromatography (HPLC). The mobile phase consisted in water with 0.7% of glacial acetic acid and ethanol (70:30 v/v), wave-length of 237 nm, Zorbax Eclipse Plus C18 Agilent™ column and room temperature of 25°C, retention time of 4,20 minutes. The method was linear in the concentrations of 20, 40, 60, 80 e 100 μg/mL, selective, accurate and robust towards these modifications: ethanol brand, water source, mobile phase rate, glacial acetic acid proportion, flow rate, room temperature and wave length. The dosing for CET was of 106.72%.

Introduction

With the advance of the infection diseases and the increase in the world mortality rate, because of pathogenic microorganisms, there came the necessity of discovering substances that would be able to fight this. The last decades were dedicated to the search of new drugs, with great importance the period of 1950 a 1970, known as "The golden era" for the discovery of antimicrobials, emerging several classes of them [1]. However, after this period, there was a decrease in the development of new molecules, what brought the worry about resistant microorganisms. In this way, a new approach to fight bacterial infections was through the improvement of this molecules already used [2].

An important class of antimicrobials is the cephalosporins, originally produced by *Cephalosporium acremonium*. The cephalosporins are classified as beta-lactam antibiotics, however, they show a broader action spectrum when compared to penicillins, because they are resistant of penicillinases. Changes in its structure gives a higher potency to this substance [3].

Cephalothin was one of the first modification obtained from the 7-aminocephalosporanic acid, the pharmacological structure of cephalosporins, classifying as a first-generation cephalosporin. This drug shows higher activity against Gram positive and less against Gram negative [3]. Due its instability in acid, it is administered parenterally. Figure 1 shows the chemical structure of the drug.

The development and validation of analytical methods for the determination of the quality of final products is extremely important, mainly when related to pharmaceutical products [4,5]. The evaluation of quality will determine the efficacy of cephalosporin pharmaceutical products and avoid damage in the patient health [6-11]. Some studies relating the quality control of cephalothin were found in literature for its quantification in biological matrices [12-20], and for analysis of CET in pharmaceutical dosage form [21-24]. Its monograph is in pharmacopoeias like Brazilian [25], United States [26], United Kingdom [27], European [28] and Japanese Pharmacopoeia [29].

Besides the importance of quality control, a crescent worry about environment and the worker makes necessary the development of conscious methods and less pollutants, in this way, the aim of this work is to improve the already validated technic for HPLC with a green chemistry approach, using less toxic solvents and a decrease in formation of residues [21].
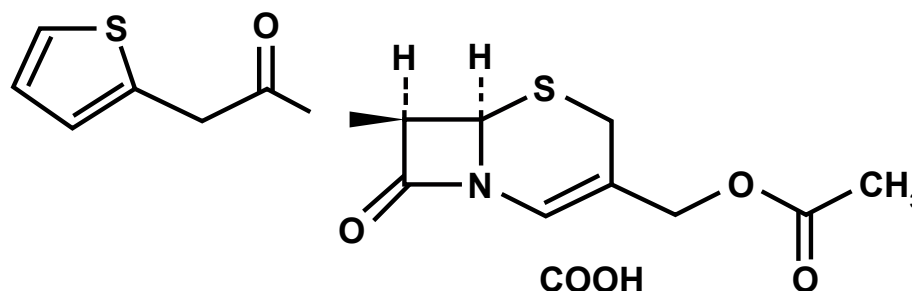
Experimental Details

Apparatus

The CLAE method was performed using a Waters system, model 1525 (Waters Chromatography Systems, California, USA), connected to a UV/VIS detector Waters 2487 and manual injector 7725i with 20 μL loop (Rheodyne Breeze™, California, USA), The separation was in isocratic form with a reversed phase column Zorbas Eclipse Plus C18 Agilent™ (150 × 4,6 mm; 5 μm) (Santa Clara, California, USA). It was used analytical balance model DV215CD (Discovery, Ohaus™, São Paulo, Brazil); ultrasound bath model USC2800A (Unique,

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

São Paulo, Brazil); purified water Milli-Q™ (Direct-Q™ 3, Merck Millipore, Germany); micropipette model Research™ Plus 100-1000 μL (Eppendorf, Hamburg, Germany) UV chamber with mirrors in the interior and UVC lamp (254 nm); UV-VIS spectrophotometer Shimadzu™ (Tokyo, Japan), model UV-1800, using quartz cells of 1 cm of optical path.

Reagents

Cephalothin reference substance (CET RS), with declared content of 99.6% were kindly provided by the laboratory União Química Farmacêutica Nacional S/A (São Paulo, Brazil).

The samples used was commercial sodium cephalothin (generic) in lyophilized powder for injectable solution in ampoule containing 1000 mg of active substance. The samples have an adjuvant, sodium bicarbonate. The samples were kindly provided by the laboratory ABL Antibióticos do Brasil Ltda (Cosmópolis, Brazil).

All solutions and the mobile phase used in this method were prepared from ultrapure water obtained through Milli-Q (Direct-Q™ 3, Merck Millipore, Germany) equipment. HPLC grade ethanol and glacial acetic acid was used for the mobile phase and its brand was Baker JT (Mexico). For selectivity, it was used: 0.1 M hydrochloric acid solution (Synth™, São Paulo, Brazil), 0.01 M sodium hydroxide solution (Vetec Química Fina™, São Paulo, Brazil) and 0.3% hydrogen peroxide solution (Vetec Química Fina™, São Paulo, Brazil).

Methods

Preparation of CET SQR and CET sample solutions: The CET SQR solution was prepared weighting 5.02 mg and transferring to a 25 mL volumetric flask and adding purified water that will give 200 μg/mL stock solution. All other solutions used in the tests were prepared by this stock solution, taking the necessary volume to obtain the desired concentration and transferring to a 10 mL volumetric flask.

The content of 5 vials of CET sample in lyophilized powder for injectable solution were mixed and 5.27 mg was weighted and transferred to a 25 mL volumetric flask that was added purified water. It was obtained a 200 μg/mL stock solution. All solutions used in the tests were prepared taking the necessary volume from the stock solution, to obtain the desired concentration, and transferring to a 10 mL volumetric flask.

HPLC method: The method was performed in isocratic mode and at room temperature of 25°C. The mobile phase consisted in ethanol and acidified water with 0.7% glacial acetic acid (30:70, v/v) that was degassed by ultrasonic bath for 30 minutes before use. The injection volume was of 20 μL at flow rate of 1.0 mL/minute, using UV detection at 237 nm. The solutions tested were filtered through 0.45

μm membrane (Pall Corporation, Michigan, USA) before the injection.

Method validation

The parameters evaluated for the validation of the HPLC method were: system suitability, linearity, selectivity, precision (repeatability and intermediate precision), accuracy, robustness and limits of detection (LOD) and quantification (LOQ). The method was validated according to what is recommended by ICH guidelines literature [30].

System suitability: A 60 μg/mL CET sample solution was prepared and injected in sextuplicate. All chromatograms were analyzed, and the parameters evaluated such as retention time (t_R), peak area, number of plates (N), peak asymmetry (As), retention factor (k) and tailing factor (TF). It was calculated the relative standard deviation (RSD). The results are shown in Table 1.

Linearity: It was prepared a 200 μg/mL CET RS solution from whom was taken aliquots to prepare solutions of 20, 40, 60, 80 e 100 μg/mL and perform injections in triplicate. The equation of the line was determined by linear regression study, by the least squares method, analysis of variance (ANOVA) and residues analysis.

Precision: It was determined by repeatability precision and intermediate. The repeatability precision consisted in six injections of CET RS solution in the concentration of 60 μg/mL. It was done in the same day and carried by the same analyst. The intermediate was done in two ways. The first was performed by the same analyst but in three different days and following the same experimental conditions. In the second way, the analyst was changed, and the six injections were done in the same day and in the same experimental conditions. Statistical analysis was performed for each test through RSD values.

Accuracy: The accuracy of the method was performed by contaminated placebo, in which known amounts of a CET SR solution were added to a solution prepared with sodium bicarbonate. All injections were made in triplicate for each concentration. First, it was injected a 30 μg/mL solution of CET SR in water, and then, three different concentrations of the contaminated placebo that corresponded to 80, 100 and 120% respectively. Aliquots of 4.8; 6.0 e 7.2 mL of CET SR solution were added to an excipient solution to determine the accuracy in the feedstock. The solutions were prepared as shown in Table 2.

Robustness: The robustness was evaluated by small variations in seven parameters organized in eight experiments and followed the Youden and Steiner method. To determine the robustness, it was used CET SR and sample solutions in the concentration of 60 μg/mL and performed in triplicate. Table 3 shows the parameters and the variations for each one, where the capital letter represents the conditions used in

the method and the lower case when there was a variation and in Table 4 there is the range of variation.

The difference between the normal values and the ones changed in module should be lower than the value resulted from $\sqrt{2} x S$ in order to infer that the effects achieved with the variations of the parameters were not significant and therefore the method is robust for all selected factors.

Specificity: Specificity can be accessed by different analysis which can be easily found in the literature [31-36]. CET sample solution in the concentration of 60 µg/mL was submitted to forced degradation in acid, alkaline, oxidative, photolytic and neutral conditions. This parameter was performed to evaluate if there was any interference of degradation products in the quantification of CET sample. The solutions used as degradation solvents were: 0.1 M HCl, 0.01 M NaOH, 0.3% H_2O_2 and purified water, used in acid, basic, oxidative and neutral/photolytic degradation, respectively. The acid, oxidative and neutral conditions were heated to 60°C while basic and photolytic conditions were maintained at 25°C and, the photolytic degradation was induced by exposure to ultraviolet light (UVC, 254 nm). Aliquots were taken from 10 to 10 minutes until degradation above 10%.

Detection (LOD) and Quantification (LOQ): According to the ICH, the LOD and the LOQ are studies based on the standard deviation of intercept and in the slope of the analytical curve. After obtaining three analytical curves, LOD and LOQ were calculated as:

$$LOD = 3,3 x \frac{\sigma}{S} \qquad (1)$$

$$LOQ = 10 \frac{\sigma}{S} \qquad (2)$$

Where σ is the standard deviation and S is the slope of the calibration curve.

Results and Discussion

Different chromatographic conditions were tested to develop a quantification method for CET SR e CET samples. The choice of the chromatographic column was based in the peaks resolution. The Zorbax Eclipse Plus C18 Agilent™ (150 × 4.6 mm; 5 µm) showed a better peak symmetry and lower system pressure.

For the determination of the mobile phase there was made several tests varying the concentration of the glacial acetic acid in water and the proportion of ethanol. All mobile phases tested showed appropriate peaks, but just one of them covered all parameters settled in system suitability. The mobile phase used was water with 0.7% glacial acetic acid and ethanol (70:30, v/v). The use of water and a less toxic organic solvent reduced the formation of waste and damage for the chromatographic system. The chromatogram obtained in the method conditions is shown in the Figure 2.

Linearity

Table 5 shows the area values obtained for each concentration used for the determination of linearity. The residue analysis showed that the regression model used is appropriate. The area values were plotted in each concentration and linearity was observed in the range of 20 to 100 µg/mL. The results were analyzed using test of variance (ANOVA) that showed no deviation from linearity and the regression model is appropriate. The ANOVA calculated is in Table 6. The analytical curve

	Parameters				
	Retention factor (>2.0)	Retention time (min)	Peak asymmetry (≤ 2.0)	Number of plates (>2000)	Area
	2.23	4.018	1.06	3675.20	2427956
	2.25	4.046	1.14	3545.79	2346598
	2.34	4.155	1.10	3739.41	2580807
	2.34	4.160	1.16	3482.35	2420956
	2.38	4.207	1.18	3981.51	2479499
	2.39	4.219	1.19	3669.72	2476334
Mean	2.32	4.13	1.14	3682.33	2455358
RSD%[a]	2.88	2.02	4.38	4.72	3.18

[a]RSD%: Relative Standard Deviation

Table 1: Parameters obtained for system suitability for the developed method for CET samples.

CET SR		
Volume added of CET SR solution (100 µg/mL) (mL)	Volume added of placebo solution (mL)	Nominal concentration (µg/mL)
4.8	5.2	48
6.0	4.0	60
7.2	2.8	72

Volumetric flask: 10 mL

Table 2: Procedure to determine the accuracy.

Variations	Parameters	Experiments							
		1	2	3	4	5	6	7	8
A/a	Ethanol brand	A	A	A	A	a	a	A	a
B/b	Water source	B	B	B	b	B	B	B	b
C/c	Proportion of mobile phase	C	C	C	c	C	c	C	c
D/d	Proportion of glacial acetic acid (%)	D	D	D	d	d	d	D	D
E/e	Flow rate (mL/min)	E	E	E	e	e	E	E	E
F/f	Room temperature (ºC)	F	F	F	F	F	f	F	F
G/g	Wavelength (nm)	G	G	G	g	g	G	G	g

Table 3: Parameters selected for the determination of robustness, using Youden and Steiner.

and residue analysis are on Figure 3.

Precision

The precision of the method was determined by repeatability, when six solutions of CET sample in the concentration of 60 μg/mL were injected by the same analyst, in the same day and under the same experimental conditions, providing a RSD of 1.91%. For the determination of interday precision, analysis was performed on three consecutive days, and RSD% was 4.90%. For precision between analysts it was made six solutions of CET sample in the same concentration as before, under same experimental conditions, in the same day, bust with a different analyst. The value of RSD% was 1.90. All results for precision are shown in Tables 7 and 8.

The results obtained in interday precision was statistically evaluated by analysis of variance and according to ANOVA there was no significant deviation, as shown in Table 9.

Accuracy

The accuracy of the method was made by the contaminate placebo method by adding a known quantity of a CET SR solution to the placebo solution. It was determined in three different concentrations predetermined and resulted in 95.38%, lower than what is recommended in literature [37-39]. The average percentage is shown in Table 10.

Robustness

The robustness was evaluated by the Youden and Steiner method that consists in small variations in seven parameters organized in eight experiments, previously shown on Tables 3 and 4. The effects resulting from the changed parameters were evaluated in comparison to the values obtained as reference for the test 1+=5.39 and 1-=7.83. All effects are shown in Table 11.

The results of method validation for analysis of CET showed that the high-performance liquid chromatography method is appropriate to quantify this cephalosporin [40].

Selectivity

The selectivity of the method was evaluated by forced degradation observing the chromatograms of CET SR to make clean if there would be any degradation substance. The chromatograms on Figures 4 and 5 shows that the degradation products have negligible interference with CET peak.
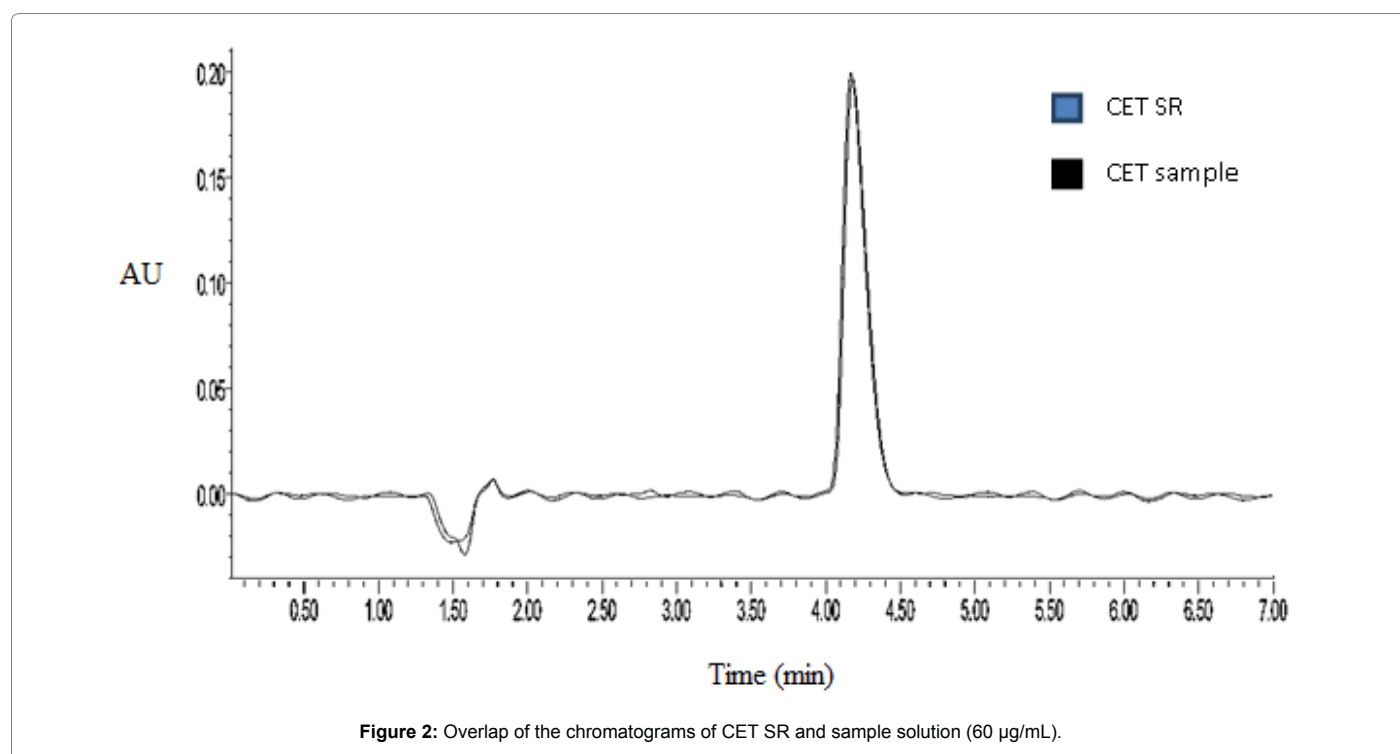
Limit of detection and quantification

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 1.95 μg/mL. In turn, the calculated LQ was 5.90 μg/mL. The calculated values for the LOD and LOQ indicated the ability of the method to detect and quantify reliably CET.

Conclusion

The qualitative analysis of CET sample in lyophilized powder for injection solution was performed by the organoleptic characteristics and high-performance liquid chromatography (HPLC) that demonstrated that these methods are appropriate to identification.

The mainly objective of this work was the development and validation of an analytical method for the quantification of CET sample, with a green chemistry approach. The developed analytical method used ethanol as organic solvent reducing, in this way, the toxicity to the professional and environment. Moreover, it was used a few quantities of organic solvent and no buffering solutions, reducing the waste [41].

The proposed method can be considered as innovative because it wasn´t found in the literature any approach for the quantification of CET sample with the view of waste and toxic solvents reduction.
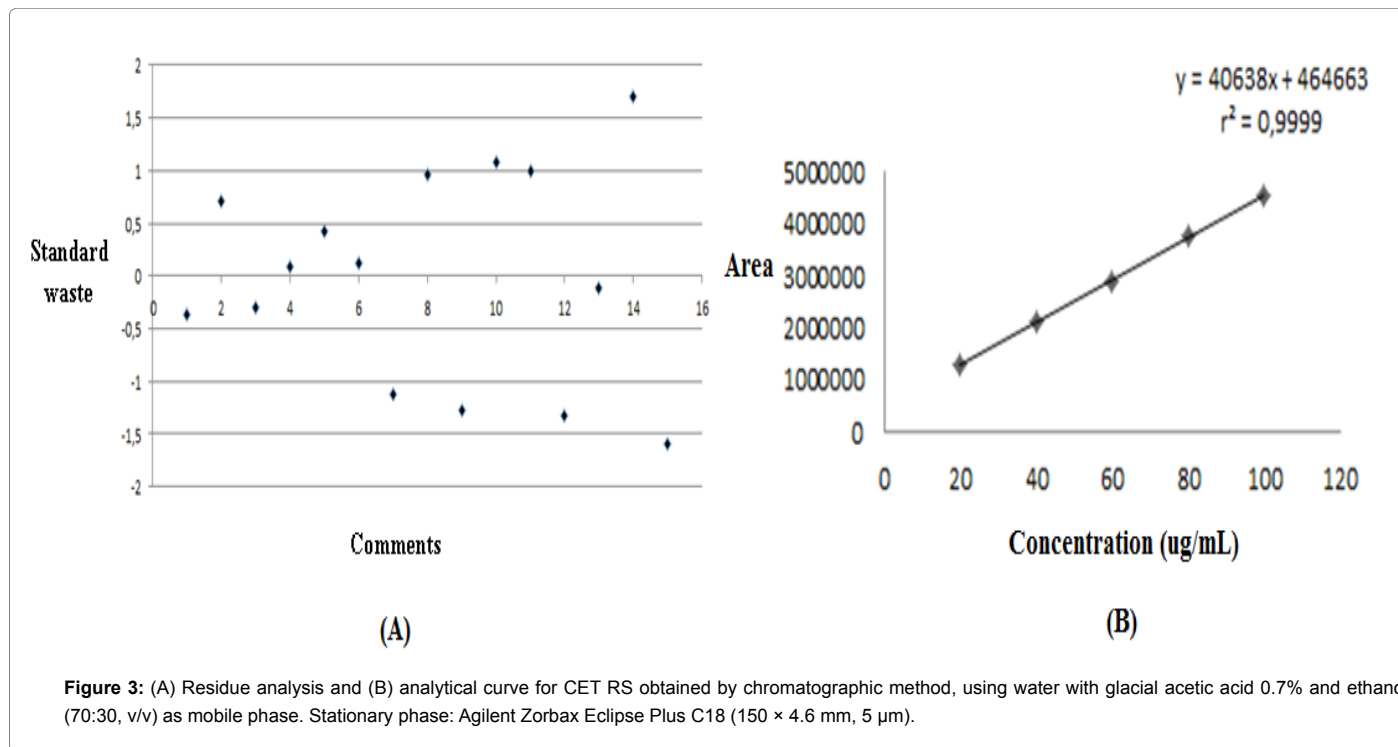

Figure 2: Overlap of the chromatograms of CET SR and sample solution (60 μg/mL).

Figure 3: (A) Residue analysis and (B) analytical curve for CET RS obtained by chromatographic method, using water with glacial acetic acid 0.7% and ethanol (70:30, v/v) as mobile phase. Stationary phase: Agilent Zorbax Eclipse Plus C18 (150 × 4.6 mm, 5 μm).
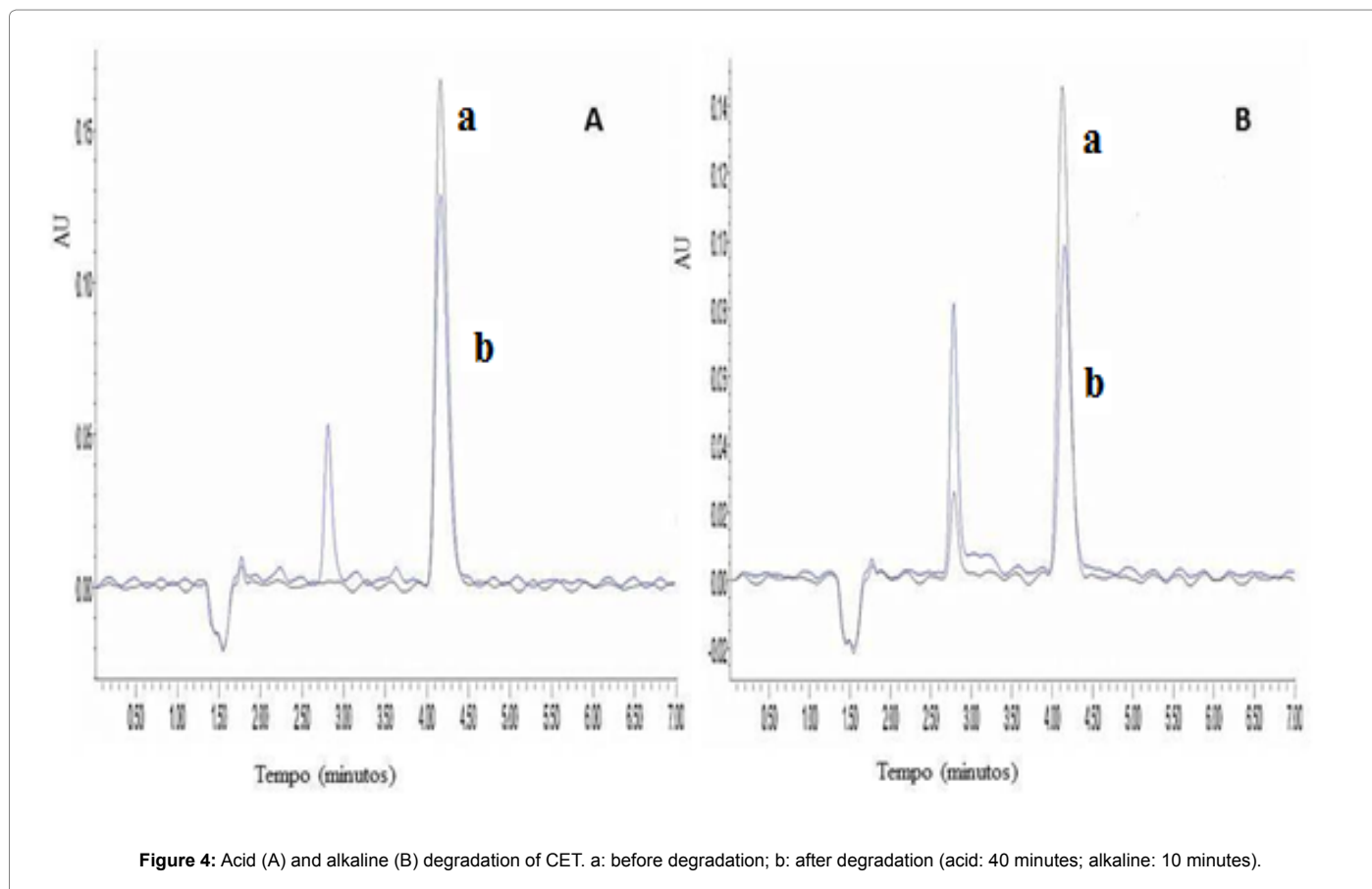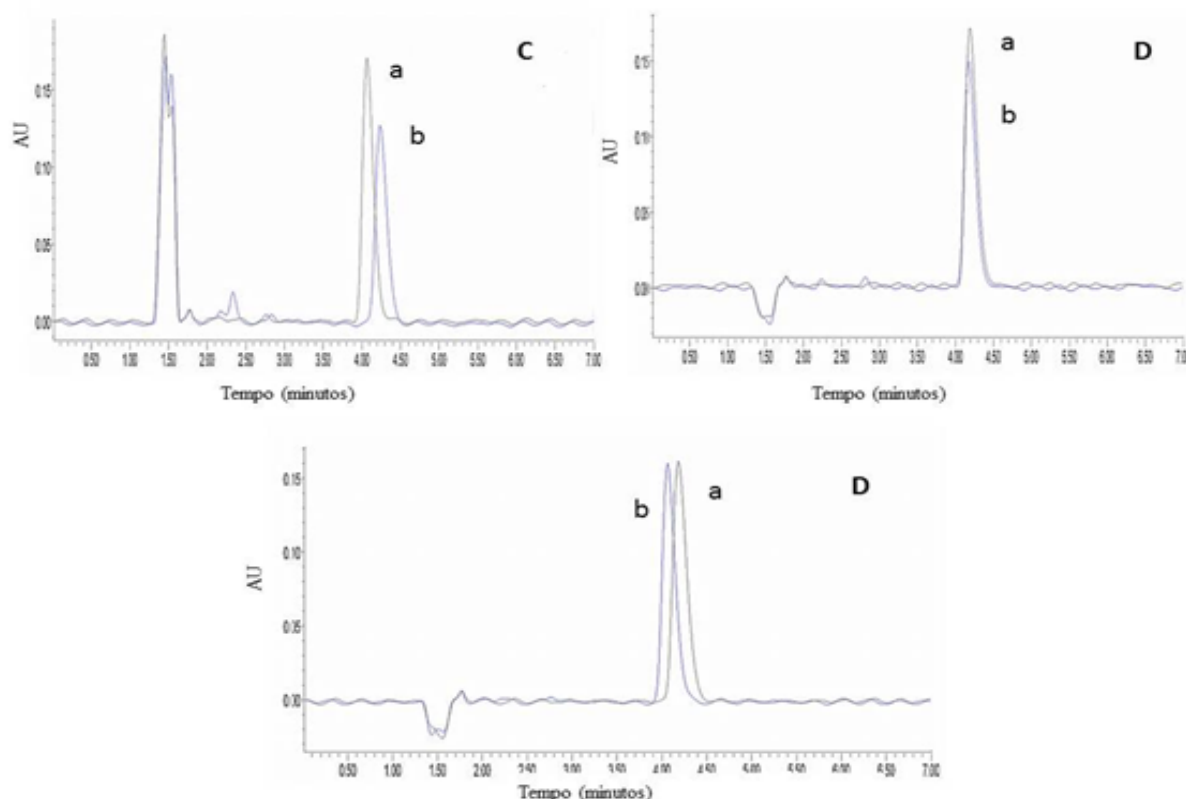

Figure 4: Acid (A) and alkaline (B) degradation of CET. a: before degradation; b: after degradation (acid: 40 minutes; alkaline: 10 minutes).

Figure 5: Oxidative (C), neutral (D) and photolytic (E) degradation of CET. a: before degradation; b: after degradation (oxidative and neutral: 20 minutes; photolytic: 7 hours).

S. No	Factors	Unit	Limit	Varied Condition (1)	Normal Condition (0)	Varied Condition (-1)
A	Ethanol brand	-	-	Scharlau	J. T. Baker	Scharlau
B	Water source	-	-	CFQ	CB	CFQ
C	Proportion of mobile phase	%	2	68:32 (v/v)	70:30 (v/v)	72:28 (v/v)
D	Proportion of glacial acetic acid	%	0.1	0.8%	0.7%	0.6%
E	Flow rate	mL/min	0.1	1.1	1.0	0.9
F	Room temperature	°C	2	27	25	23
G	Wavelength	nm	2	239	237	235

Table 4: Range of variations for the determination of cephalothin.

Concentration (µg/mL)	Areas[a] (AU)	Average area	RSD%[c]
20	1258759 1314090 1262120	1278323	2.43
40	2094694 2111862 2096859	2101138	0.44
60	2845181 2952666 2836871	2878239	2.24
80	3771901 3766990 3647539	3728810	1.89
100	4522205 4616382 4446401	4528329	1.88

[a]: Average value of three determinations; [c]RSD%: Relative Standard Deviation

Table 5: Peak area obtained for each concentration of CET SR solution.

Source of variation	Degree of freedom	Sum of squares	Variability	F calculated	F critical
Between concentration	4	19820486162242.30	4955121540560.57	1421.21*	3.48
Linear regression	1	19815971578889.40	19815971578889.40	5683.54*	4.96
Deviation of linearity	3	4514583352.89	1504861117.63	0.43	3.71
Residue	10	34865555714.66	3486555571.46	-	-
Total	14	19855351717956.90	-	-	-

*Significant at $p < 0.05\%$

Table 6: Analysis of variance (ANOVA) for linearity.

Intraday precision	Average	RSD%[b]
Day 1	2891854.67	1.91
Day 2	2885353.00	3.03
Day 3	2655204.17	2.14

[b]RSD%: Relative Standard Deviation

Table 7: Intraday precision for the analytical method developed for HPLC.

Interdays			Between analysts		
Day	Area[a]	RSD%[b]		Area[a]	RSD%[b]
1	2891855		1	2810803	
					1.90
2	2885353	4.80	2	2887266	
3	2655204				

Table 8: Interdays and between analysts precision for the analytical method developed for HPLC.

Source of variation	Sum of squares	DF	Average squares	F calculated	F critical	P-value
Between groups	12511752861.61	5	2502350572.32	0.109131	3.10	0.99
Within groups	275156900845.33	12	22929741737.11			
Total	287668653706.94	17				

*$p < 0.05$

Table 9: Analysis of variance (ANOVA) for interday precision.

Accuracy (days)	Recuperation (%)	Average recuperation (%)	RSD (%)[a]
1	96.72		
2	97.15	95.38	3.44
3	92.30		

[a]RSD%: Relative Standard Deviation

Table 10: Results for CET SR method accuracy.

Factor	(1)	Content (%)[a,b] Effects	(-1)	Content (%)[a,b] Effects
A- Ethanol brand	Scharlau	104.12-106.50=-2.38	Scharlau	103.14-98.93=4.21
B- Source of water	CFQ	105.53-105.08=0.45	CFQ	101.81-100.26=1.55
C- Proportion of mobile phase (v/v)	68:32	105.25-105.36=-0.10	72:28	102.28-99.79=2.49
D- glacial acetic acid concentration (%)	0.8	107.12-103.49=3.63	0.6	102.50-99.56=2.94
E- Flow rate (mL/min)	1.1	104.85-105.76=-0.91	0.9	102.65-99.42=3.23
F- Room temperature (ºC)	27	106.02-104.59=1.43	23	99.65-102.42=-2.77
G - Wavelength (nm)	239	104.37-106.24=-1.86	235	100.66-101.41=-0.75

[a]Average contents obtained in normal conditions - Average contents obtained in altered conditions; [b]Reference criteria calculated: 5.39 to test +1 e 7.83 to test -1

Table 11: Results for sodium cephalothin method robustness.

So, we can conclude that the developed and validated method can be used in quality control for CET sample in lyophilized powder for injection because it demonstrated to be effective to quantification.

Acknowledgements

Conflict of Interest

The authors report no declarations of interest.

References

1. Aminov RI (2010) A brief history of the antibiotic era: lessons learned and challenges for the future. Frontiers in Microbiology 1: 134.

2. Chopra I, Hesse L, O'Neill AJ (2002) Exploiting current understanding of antibiotic action for discovery of new drugs. Journal of Applied Microbiology Symposium Supplement 92: 4-15.

3. Wright PM, Seiple IB, Myers AG (2014) The evolving role of chemical synthesis in antibacterial drug discovery. Angewandte Chemie 53: 8840-8869.

4. Bonfilio R, Cazedey ECL, Araújo MB, Salgado HRN (2012) Analytical validation of quantitative high-performance liquid chromatographic methods in pharmaceutical analysis: a practical approach. Critical Reviews in Analytical Chemistry 42: 87-100.

5. Kogawa AC, Salgado HRN (2016) Analytical methods need optimization to get innovative and continuous processes for future pharmaceuticals. Scholars Acad J Pharm 5: 240-244.

6. Vieira DCM, Salgado HRN (2011) Comparison of HPLC and UV

spectrophotometric methods for the determination of cefuroxime sodium in pharmaceutical products. Journal of Chromatographic Science 49: 508-511.

7. Moreno AH, Salgado HRN (2012) Comparison of high performance liquid chromatography and three titrimetric methods for the determination of ceftazidime in pharmaceutical formulations. Advances in Analytical Chemistry 2: 6-13.

8. Pedroso TM, Salgado HRN (2014) Validation of analytical methodology for quantification of cefazolin sodium by liquid chromatography to be applied for quality control in pharmaceutical industry. Brazilian Journal of Pharmaceutical Sciences 50: 213-223.

9. Rodrigues DF, Salgado HRN (2016) Development and validation of a green analytical method of RP-HPLC for quantification cefepime hydrochloride in pharmaceutical dosage forms: simple, sensitive and economic. Current Pharmaceutical Analysis 4: 306-314.

10. Aléssio PV, Kogawa AC, Salgado HRN (2017) Quality of ceftrixone sodium in lyophilized powder for injection evaluated by clean, fast and efficient spectrophotometric method. Journal of Analytical Methods in Chemistry.

11. Consortti LP, Salgado HRN (2017) A critical review of analytical methods for quantification of cefotaxima. Critical Reviews in Analytical Chemistry 47: 359-371.

12. Chang YL, Chen YF, Chen CF, Tsai TH (2000) Determination of unbound cephalothin in rat blood by on-line microdialysis and microbore liquid chromatography. Journal of Chromatography 742: 125-130.

13. Matsuda Y, Hobo S, Naito H (1999) Transferability of cephalothin to the alveolar cavity in thoroughbreds. Journal of Veterinary Medical Science 61: 209-212.

14. Sakata Y (1980) The pharmacokinetic studies of cephalothin, cefazolin and cefmetazole in the neonates and the premature babies. The Kurume Medical Journal 27: 275-298.

15. Brisson AM, Fourtillan JB (1981) Determination of cephalosporins in biological material by reversed phase liquid column chromatography. Journal of Chromatography 223: 394-399.

16. Mcwhinney BC, Wallis SC, Hillister T, Roberts JA, Lipman J, et al. (2010) Analysis of 12 beta-lactam antibiotics in human plasma by HPLC with ultraviolet detection. Journal of Chromatography B 878: 2039-2043.

17. Buhs RP, Maxim TE, Allen N, Jacob TA, Wolf FJ (1974) Analysis of cefoxitin, cephalothin and their deacylated metabolites in human urine by high-performance liquid chromatography. Journal of Chromatography 99: 609-618.

18. Signs SA, File TM, Tan JS (1984) High-pressure liquid chromatography method for analysis of cephalosporins. Antimicrobial Agents and Chemotherapy 26: 652-655.

19. Rouan MC, Abadie F, Leclerc A, Juge F (1983) Systematic approach to the determination of cephalosporins in biological fluids by reversed phase liquid chromatography. Journal of Chromatography 275: 133-144.

20. Wold JS, Turnipseed SA (1977) The simultaneous quantitative determination of cephalothin and cefazolin in serum by high pressure liquid chromatography. Clinical Chimica Acta 78: 203-207.

21. Rugani KS, Salgado HRN (2015) Stability-indicating LC method for the determination of cephalothin in lyophilized powder for injection. Analytical Methods 6: 4437-4445.

22. Hagel RB, Waysek EH, Cort WM (1979) High pressure liquid chromatography analysis of antibiotics susceptibility disks. Antimicrobial Agents and Chemotherapy 16: 372-377.

23. Mitchell SM, Ullman JL, Teel AL, Watts RJ (2014) pH and temperature effects on the hydrolysis of three beta-lactam antibiotics: ampicillin, cefalotin and cefoxitin. Science of the Total Environment 466: 547-555.

24. Nilsson-Ehle I, Yoshikawa TT, Schotz MC, Guze LB (1979) Quantitation of antibiotics by high-pressure liquid chromatography: cephalothin. Antimicrobial Agents and Chemotherapy 13: 221-227.

25. Brazilian Pharmacopoeia (2010) Agência Nacional de Vigilância Sanitária. 5th edn, Brasília, DF.

26. United States Pharmacopeia Convention (2014) USP 37. The United States Pharmacopeia. The National Formulary (NF 32). 37th edn. Rockville.

27. British Pharmacopoeia (2012) London: The Stationery Office.

28. European Pharmacopoeia (2011) Council of Europe, 7th edn.

29. Japanese Pharmacopoeia (2011) Tokyo Society of Japanese Pharmacopoeia. 16th edn., pp: 557-560.

30. ICH (2005) International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use. Validation of analytical procedures: Text and Methodology Q2 (R1). ICH Steering Committee, Switzerland, 2005.

31. Marona HRN, Schapoval EES (1999) A High-performance liquid chromatographic assay of sparfloxacin. Journal of Pharmaceutical and Biomedical Analysis 20: 413-417.

32. Marona HRN, Zuanazzi JAS, Schapoval EES (1999) Determination of sparfloxacin and its degradation products by HPLC-PDA. Journal of Antimicrobial Chemotherapy 44: 301-302.

33. Lopes CCGO, Salgado HRN (2009) Development of a validated stability-indicating HPLC assay and stress degradation studies of linezolid in tablets. Chromatographia 69: S129-S133.

34. Totoli EG, Salgado HRN (2014) Development and validation of an economic, environmental friendly and stability-indicating analytical method for determination of ampicillin sodium for injection by RP-HPLC. World Journal of Pharmacy and Pharmaceutical Sciences 3: 1928-1943.

35. Totoli EG, Salgado HRN (2015) Development, optimization and validation of a green and stability-indicating HPLC method for determination of daptomycin in lyophilized powder. Journal of AOAC International 98: 1276-1285.

36. Pedroso TM, Medeiros ACD, Salgado HRN (2016) RP-HPLC x HILIC chromatography for quantifying the ertapenem sodium with a look at green chemistry. Talanta 160: 745-753.

37. Association of Official Analytical Chemists (2002) Official methods of analysis. 17th edn. AOAC: Gaithesburg.

38. AOAC (Association of Official Analytical Chemists) (2016) Official Methods of Analysis. 17th edn. Gaithesburg.

39. Horwitz W, Kamps LR, Boyer KW (1980) Quality assurance in the analysis of foods and trace constituents. J Assoc Off Anal Chem 63: 1344-1354.

40. Youden WJ, Steiner EH (1975) The association of official analytical chemistry. Washington DC, USA, p: 33.

41. Moreno AH, Salgado HRN (2008) Development and validation of HPLC method for determination of ceftazidime. Journal of AOAC International 91: 739-743.