Validation of Cefazolin Sodium by UV-Spectrophotometric Method

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Abstract A simple, fast and reproducible UV Spectrophotometric method was developed and validated for quantification of cefazolin sodium in pharmaceutical form powder for injectable solution, the method proved effective, easy applicability, low cost, besides it does not generate toxic wastes to the operator and the environment, corroborating with the routine analysis of quality control to ensure the therapeutic efficacy of the drug already marketed. The method presented being capable to detect and quantify the drug obtaining satisfactory results regarding specificity, precision, accuracy and robustness, linear range of 8 to 28 μg/mL, showing correlation coefficient of 0.9999 when analyzed in the wavelenght λ=270 nm spectrophotometer.

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

1. Introduction

Cephalosporins are antibacterial antibiotics belonging to the group β-lactam antibiotics often used as a therapeutic agent, for being broad spectrum, and low toxicity[1]. Since their discovery in 1948 by Brotzu[2] cephalosporins are widely used because they have significant activity against Gram-positive and Gram-negative bacteria by inhibiting synthesis of bacterial cell wall in a manner to similar penicillin[3-4]. Moreover they can be used in treating allergic patients to penicillin[3, 5].

Cefazolin sodium (CFZ) is classified as a first-generation cephalosporin for injectable use; its use in clinical practice is distinguished by its effectiveness as a therapeutic agent and surgical prophylaxis. The antibacterial spectrum of cefazolin is typical of another first-generation cephalosporins, but stands out for showing activity against some *Enterobacter* species and have a longer half-life and can be administered at less frequent intervals, making it be preferred among the first-generation cephalosporins[4].

Cefazolin sodium is officially recognized in the British Pharmacopoeia, 2010; European Pharmacopoeia, 2005; Pharmacopoeia, 2005, Japanese Pharmacopoeia, 2006 and in The United States Pharmacopeia, 2010[6-10], in which methods by liquid chromatography with UV detection are employed.

Studies focused on the development and refinement of analytical methodologies is crucial for optimization of laboratory tests carried out in the pharmaceutical industry, for quality assurance. Some analytical methods are described in the literature for analysis of cefazolin sodium in raw material and finished product. Among these are found bromometry methods[11]; Colorimetry,[12]; highperformance liquid chromatography[13-20]; capillary electrophoresis[21-22]; ultraviolet (UV) and visible (VIS) spectrophotometry[23-27]; fluorimetry[28]; refractive index[29]; polarography[30]; chemiluminescence[31]; radiosensibility[32] and voltammetry[33]. However, many of these methods require pre-treatment of the sample, formation of metal complexes, drug degradation using sulfuric acid, among other time-consuming complex procedures, which require sophisticated equipment, high costs or use of large amounts of organic solvents, which are toxic for the operators and the environment. Furthermore, most of the methods described quantifies the drug in biological fluids.

On the other hand, the quality control in the pharmaceutical industry requires reliable analytical methods capable of ensuring therapeutic efficacy of the marketed drug, but that is simple, fast, versatile, that does not require complex or costly procedures, does not generate toxic wastes, so that, in this way it can be employed in laboratory tests. The method by ultraviolet absorption spectrophotometry has been employed as a proof of identification, by comparison of the spectral profile; this feature is important for characterization purposes of the substance from obtaining the wavelengths of greatest absorbance[34]. The use of this method for quantification of drugs also has distinguished itself by offering favourable characteristics for applications

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in quality control. The inclusion of this instrumental technique is highly recommended for official textbooks, because the methods developed by UV spectroscopy have shown results also accurate, reproducible and accurate [35-41]. In this sense, the objective of this study was to develop and validate a simple, fast, reproducible and viable method by spectrophotometry in UV region, for quantification of cefazolin sodium in pharmaceutical form powder for injectable solution, to be applied in routine analysis in pharmaceutical industries, ensuring therapeutic efficacy of the drug already marketed globally.

2. Methodology

It were used commercial samples of cefazolin sodium (generic) pharmaceutical form powder for injection solutions in ampoules containing 1 g vial (batch: 203674C v. 02/2013) and reference substance (batch: F366391 v. 11/2013) with a purity of 98.2% declared, kindly provided by ABL antibiotics of Brazil Ltda. (Cosmópolis-SP). Spectrophotometric readings were held in UV-Vis spectrophotometer Shimadzu UV-mini 1240 model, using quartz cuvettes 1 cm optical path, under controlled temperature 25° C. An equivalent of 20.0 mg of cefazolin sodium reference standard (RS) substance, previously dried for 1 hour at 105 ^{o}C, was weighed and transferred to a 100 mL volumetric flask, and the volume was completed with purified water, in order to obtain a stock solution of 200 µg mL^{-1}.

For qualitative analysis of 1 mL aliquots of stock solution were transferred into 10 mL volumetric flask and the volume was completed with different solvents to obtain solutions of 20 µg/mL in purified water (Millipore ®); hydrochloric acid 0.1 M PA (Synth); sodium hydroxide 0.1 M PA (Merck); methanol PA (Synth); 1% phosphate buffer pH 6 and 1% phosphate buffer pH 8. All solvents used were of analytical grade, the absorption spectra were recorded in the wavelength range between 200 and 400 nm, the same was carried out for cefazolin sodium lyophilized powder and the spectra were compared.

For the development of the UV spectrophotometer method, the Ringbom curve was constructed to determine the range in which the drug has a increased linearity. Aliquots of stock solutions corresponding to 0.4; 0.6; 0.8; 1.0; 1.2 and 1.4 mL were transferred to 10 mL volumetric flasks, and the volume was completed with purified water to obtain solutions in concentrations of 8, 12, 16, 20, 24 and 28 µg/mL, respectively. Purified water was used as blank to reset the appliance; the dilutions were individually analyzed in UV spectrophotometer at a wavelength more than 270 nm.

3. Method Validation

The method was validated according to the parameters established in guidelines as Brazil, 2003; FDA, 2004; ICH, 2005; INMETRO, 2011[42-45].

3.1. Linearity

The linearity of the method was obtained through the analysis of three analytical curves on three different days. The results obtained were analyzed to obtain the equation of the straight by the least squares method and verification of the linearity was detected by statistical tool of analysis of variance (ANOVA).

3.2. Precision

The precision of the method was performed for repeatability and intermediate accuracy tests, which were evaluated by calculating the RSD sample content and statistical tools such as Student's t-test and ANOVA.

3.3. Calculation of Cefazolin Sodium Lyophilized Powder

The solutions were prepared in triplicate and sample readings were held in spectrophotometer at a wavelength of 270 nm. The calculation of the concentration of cefazolin sodium lyophilized powder was accomplished through the equations (1-2).

$$C_S = A_S \times \frac{C_{RS}}{A_{RS}} \qquad (1)$$

Where:
C_S = concentration of sample (µg/mL)
A_S = absorbance of the sample
C_{RS} = concentration substance reference
A_{RS} = absorbance of the chemical reference
The percentage of cefazolin sodium in the samples was calculated by equation 2.

$$C_S\% = \frac{(C_S \times 100)}{C_T} \qquad (2)$$

Where:
$C_S\%$ = percentage concentration of sample
C_S = concentration of cefazolin sodium in the sample (µg/mL)
C_T = theoretical concentration of cefazolin sodium in the sample (µg / mL)

3.4. Repeatability

The repeatability was determined by analysis of seven solutions of SQR cefazolin sodium in a concentration of 20 µg/mL prepared in the same day. Thus, results were obtained using the same experimental conditions and evaluated by the relative standard deviation.

3.5. Intermediate Precision

The intermediate precision was evaluated through analysis of solutions of cefazolin sodium RS and cefazolin sodium lyophilized powder at a concentration of 20 µg/mL, and executed by different analysts in different days. The analyses were performed in seven replicates.

3.5.1. Interday

Seven solutions of cefazolin sodium RS and cefazolin sodium lyophilized powder at a concentration of 20 μg/mL were prepared and analyzed daily for three consecutive days under the same experimental conditions and the results were evaluated by the relative standard deviation and variance analysis (ANOVA).

3.5.2. Between-Analysts

The accuracy among analysts was checked by preparing seven solutions of cefazolin sodium and cefazolin sodium RS lyophilized powder at a concentration of 20 μg/mL by two analysts, under the same experimental conditions, the results were evaluated by the relative standard deviation of the levels and the Student's t test.

3.5.2.1. Accuracy

The accuracy was determined by addition method in which a known quantity of standard is added to the sample, and is mathematically calculated according to the amount recovered. Samples were prepared in triplicate according to Table 1.

Table 1. Preparation of test solutions for recovering the spectrophotometric method in the UV region

	Cefazolin Sodium sample (20 μg/mL) (mL)	Cefazolin sodium RS (20 μg/mL) (mL)	Theoretical concentration (μg/mL) *
Sample	0.6	-	12
R1	0.6	0.2	16
R2	0.6	0.4	20
R3	0.6	0.6	24
RS	-	0.6	12

*10 mL volumetric flask

The percentage of recovered cefazolin sodium was calculated by equation 3.

$$\%R = \left[\frac{(C_A - C_S)}{C_T} \right] \times 100 \qquad (3)$$

Where:

C_A= concentration of the sample solution found added to RS (μg/ml)

C_S= concentration found in the sample (μg/mL)

C_T= theoretical concentration of the solution added RS (μg/mL)

3.5.2.2. Limit of Detection (LD)

The detection limit was calculated using the formula described in the literature[44], based on the standard deviation of the intercept and the slope of the analytical curve. The calculation was performed according to equation 4.

$$LD = \frac{3.3 \times \sigma}{IC} \qquad (4)$$

Where:

σ= standard deviation of the intercept
IC= slope of the analytical curve

3.5.2.3. Limit of Quantification(LQ)

The quantification limit was calculated using the formula described in the literature[44], based on the standard deviation of the intercept and the slope of the analytical curve. The calculation was performed according to equation 5.

$$LQ = \frac{10 \times \sigma}{IC} \qquad (5)$$

Where:

σ= standard deviation of the intercept
IC= slope of the analytical curve

3.5.2.4. Robustness

The robustness of the method was determined by comparing the contents obtained by varying the wavelength of absorption and evaluated by the relative standard deviation and by the Student's t test.

4. Results

4.1. Qualitative Analysis

The molecular absorption in the ultraviolet region of the spectrum depends on the electronic structure of the molecule; thereby the identification of the drug can be carried out by determining its absorption characteristics in different solvents[46]. The spectra of cefazolin sodium SQR and lyophilized powder at a concentration of 20.0 μg/mL in many solvents were analyzed, among these solvents, water solvent came up with results similar to phosphate buffer solutions 1% pH 6 phosphate buffer and 1% pH 8, with a maximum absorption wavelength at 271 nm and absorbance around 0.5585 (Figure 1) while using methanol the maximum absorption observed was 0.6521 at 273 nm in an acid medium it was of 267 nm with absorbance of 0.4417 and basic medium it was 285 nm and absorbance of 0.5221.

After analyzing the spectra, purified water was chosen to use as a solvent, by presenting appropriate features in the spectra and economic and environmental advantages, such as being easy acquisition and disposal and low cost.
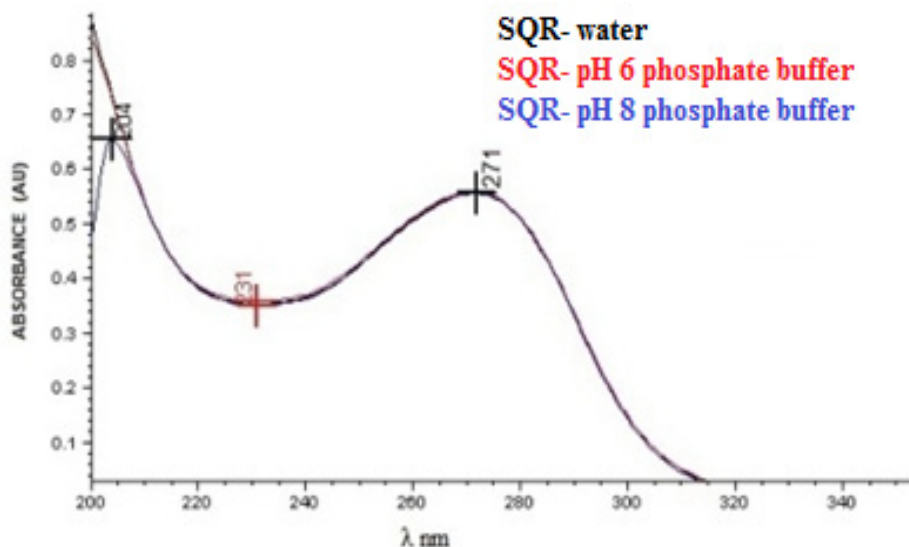
Figure 1. Absorption spectrum in the ultraviolet region, solution of cefazolin sodium RS in a concentration of 20 µg/mL, using as solvent: water, pH 6 phosphate buffer and pH 8 phosphate buffer

The spectral profiles of cefazolin sodium RS and lyophilized powder in various solvents are presented in Figure 2.
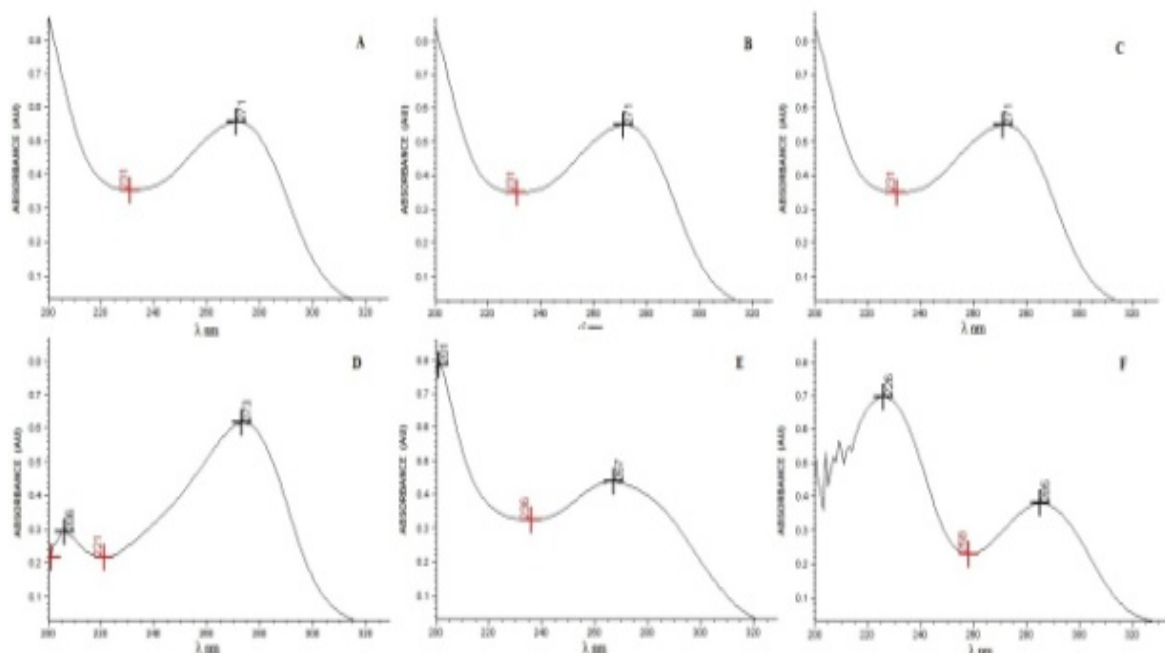

Figure 2. Absorption spectrum in the ultraviolet region, solution of cefazolin sodium RS in a concentration of 20 µg /mL, using as solvent: purified water (A); phosphate buffer pH 6 (B); phosphate buffer pH 8 (C) methanol (D) hydrochloric acid 0. 1 M (E); 0.1 M sodium hydroxide (F)

Samples of cefazolin sodium RS and lyophilized powder were compared, both kept maximum absorption wavelength at 271 nm, with a small difference in absorbance of 0.5585 to cefazolin sodium SQR and 0.5554 to cefazolin sodium lyophilized powder, when dissolved in water at a concentration of 20 µg/mL (Figure 3).

The results show that the spectral profiles demonstrated for cefazolin sodium RS and the sample of lyophilized powder, dissolved in various solvents, are consistent and can be used as proof of identification by comparison. The method for routine qualitative analysis performed in the quality control laboratory, and can be used for identification of cefazolin sodium in powder form for pharmaceutical injectable solution, through its absorption characteristics. The method has the advantages of being simple, rapid, reproducible, inexpensive, and using purified water as a solvent, which has adequate characteristics in the spectra, and it is easy to purchase and disposal, it does not generate toxic waste to the operator and the environment being able to ensure the pharmaceutical composition of the product already marketed.
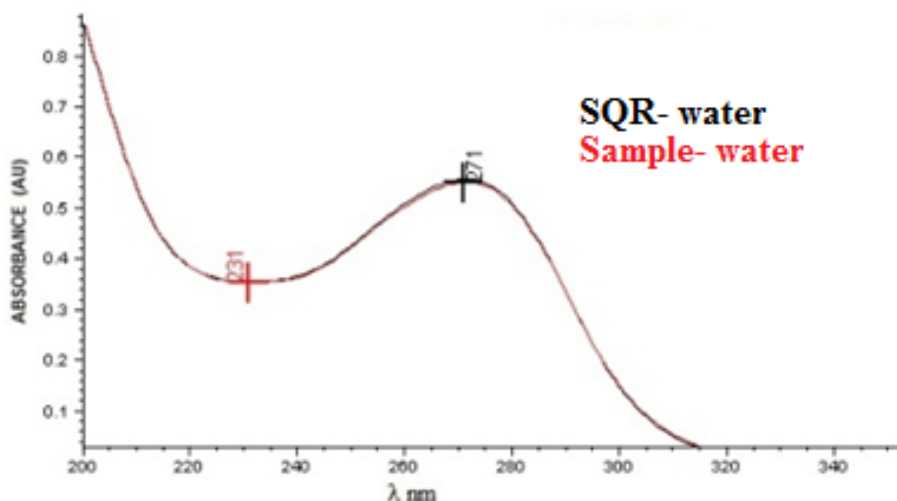
Figure 3. Absorption spectrum in the ultraviolet region of cefazolin sodium RS compared to cefazolin sodium lyophilised powder, both at a concentration of 20 μg/mL using purified water as solvent

4.2. Quantitative Analysis

Quantitative spectrophotometric analysis in the ultraviolet region has as its principle the direct relationship between the amount of light absorbed and the concentration of the substance, also known Lambert-Beer law, which is the mathematical basis for measuring radiation absorption at a given wavelength when undergoing a sample since there is an exponential relationship between the light transmission and thickness of absorbent means. The absorbance given by electromagnetic spectra is obtained using equation 6[34,47].

$$A = \log(Io / I) = \varepsilon.b.c \qquad (6)$$

Where:

A= Absorbance

Io= intensity monochromatic radiation acting on the sample

I= intensity of radiation emerging from the sample

ε = molar absorptive, one characteristic quantity of the absorbing species which magnitude depends on the wavelength of the incident radiation

b= distance traveled by the beam through the sample (cm).

c= concentration of the absorbing species (mol/ L)

The spectrophotometry in the ultraviolet region occurs by absorption of energy that, when quantized, leads to the passage of electrons from ground state orbitals to orbitals in a higher-energy excited state, and thus depends on the electronic structure of the molecule, the solvent, the temperature and the length of the wave radiation[46].

This technique is widely used in quantitative analysis due to the ease of implementation, good sensitivity and accuracy, coupled with the low cost and wide applicability of routine quality control. Therefore, the development and validation of methods using ultraviolet spectrophotometry for analysis of cefazolin sodium is highly relevant for routine analysis during manufacturing by pharmaceutical industries

4.3. Ringbom's Curve

For the development of the UV spectrophotometric method, a Ringbom curve was constructed in order to establish the concentrations of work to which the drug has linearity. Aliquots of the stock solution of 200/mL in SQR were transferred with the aid of a micropipette to 10 mL volumetric flasks, and the volume was completed with purified water, to obtain increasing concentrations 2-176 μg/mL, then analyzed individually. The readings were carried out at a wavelength of 270 nm, the absorbance of each concentration was converted to transmittance and the graphic of transmittance versus concentration is shown in Figure 4.
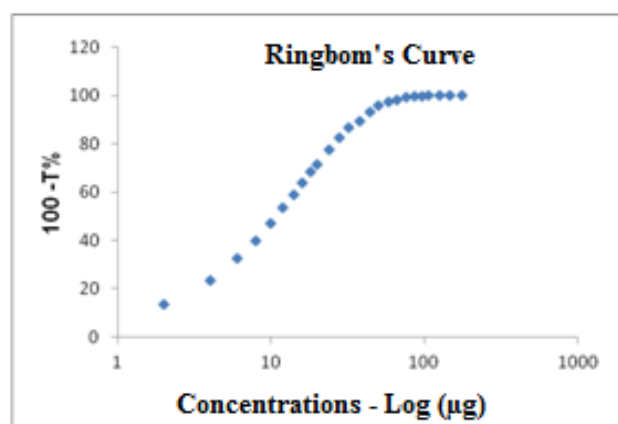

Figure 4. Ringbom's curve obtained by the spectrophotometric method in the UV region to cefazolin sodium RS using water as solvent at a wavelength of 270 nm

4.4. Calibration Curve

The content of cefazolin sodium powder of 20 vials for the injectable solution was weighed and mixed; amount equivalent to 20 mg of RS was weighed and transferred to a 100 mL volumetric flask, which was dissolved and diluted with purified water to obtain stock solution at a concentration of 200/mL. From this solution, volumetric dilutions were prepared by transferring aliquots of 0.4, 0.6,

0.8, 1.0, 1.2 and 1.4 mL to 10 mL volumetric flasks and the volume was completed with purified water, for obtaining solutions with concentrations of 8, 12, 16, 20, 24 and 28 µg/mL, respectively, in which Beer's statement is obeyed. Each concentration was prepared in triplicate. The spectrophotometric readings were measured at a wavelength of 270 nm by using purified water as blank. The calibration curve was constructed with the average absorbance of three determinations versus the concentrations of cefazolin sodium RS. The equation of the straight line was obtained by linear regression (Table 2).

The calibration curve of cefazolin sodium RS (Figure 5) was built with the average absorbance values of three analytical curves obtained during the tests of linearity.

The calibration curve of cefazolin sodium was calculated by analysis of variance (ANOVA) and the results obtained are shown in Table 3.
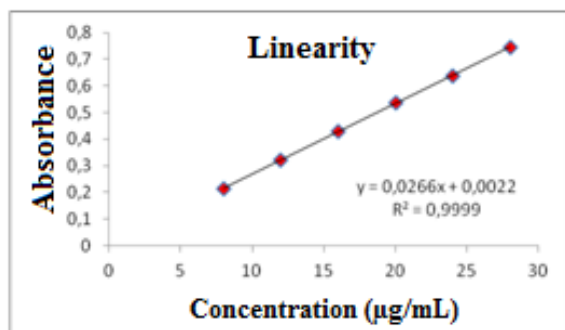

Figure 5. Calibration curve of cefazolin sodium RS obtained by the spectrophotometric method in the UV region, using water as solvent at 270nm

Table 2. Calibration curve of cefazolin sodium RS by the spectrophotometric method in the UV region, at 270 nm

Concentration (µg/mL)	Absorbance[a]	Average absorbance ± DP[b]	RSD[c] (%)
8	0.2177 0.2098 0.2172	0.2149 + 0.0044	2.02
12	0.3242 0.3113 0.3243	0.3199 + 0.0075	2.29
16	0.4318 0.4224 0.4292	0.4278 + 0.0049	1.13
20	0.5422 0.5261 0.5403	0.5362 + 0.0088	1.62
24	0,6327 0,6520 0,6297	0.6381 + 0.0121	1.91
28	0.7439 0.7572 0.7381	0.7464 + 0.0098	1.33

[a]Average value of three determinations / [b]Standard deviation / [c]Relative standard deviation

Table 3. Analysis of variance of absorbance values determined in the obtaining of the calibration curve of cefazolin sodium RS using the spectrophotometric method in the UV region

Sources of variation	Degree of freedom	Sum of squares	Variability	F calculated	F tabulated
Between concentration	5	0.59322	0.11864	1702.03*	3.11
Linear regression	1	0.59319	0.59319	8509.72*	4.75
Deviation of linearity	4	0.00003	0.0000075	0.11	3.26
Residue	12	0.000836	0.00006970		
Total	17	0.594060			

* Significant at $p < 0.05\%$

4.5. Precision

The precision of the method was determined by repeatability (intraday), expressing the results on the basis of relative standard deviation. Seven cefazolin sodium RS solutions prepared at a concentration of 20 µg/mL were submitted to successive and the data obtained on the same day under the same experimental conditions, and laboratory analyst provided RSD of 0.32%.

Intermediate precision was evaluated through interday precision and the between-analysts. The values of content of cefazolin sodium in lyophilized powder, determined during the assessment of the accuracy of the proposed method are shown in Table 4. The interday precision and between-RSD analysts showed 0.81 and 1.01%, respectively.

The interday precision was evaluated by analysis of variance while the between-analyst precision was evaluated by Student's t test, as shown in Tables 5 and 6.

Table 4. Determination of interday precision and between-analysts spectrophotometric method in the UV region for analysis of cefazolin sodium in lyophilized powder

	Interdays precision		Between-analysts precision		
Day	Content[a] (%)	RSD[b] (%)	Analist	Content[a] (%)	RSD[b] (%)
1	99.90		A	99.066	
2	98.95	0.75			1.01
3	98.44		B	100.5	

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

Table 5. Data obtained from analysis of interday precision spectrophotometric method in the UV region for determination of cefazolin sodium, by analysis of variance (ANOVA)

Source of variation	GL	Sum of squares	Average squares	F calculated	F tabulated
Between groups	2	1.53038E-05	7.6519E-06	2.82	3.55
Within groups	18	4.85086E-05	2.69492E-06		
Total	20	6.38124E-05			

*Significantat p<0,05%

Table 6. Data obtained from analysis of the accuracy of analyst spectrophotometric method in the UV region for analysis cefazolin sodium RS, by Student's t test

t test: two samples assuming equal variances – Significant at p<0,05%						
Source of variation	Average	Degree of freedom	variancy	T calculated	P value	T tabulated
Analyst 1	0.5346	6	1.66191E-05			
Analyst 2	0.5277	6	6.33239E-05	2.03	0.06	2.17

Table 7. Recovery values obtained for each level of concentration tested by the spectrophotometric method using water as solvent at 270 nm

Cefazolin sodium RS added (µg/mL)	Cefazolin sodium SQR found[a] (µg/mL)	Recuperation (%)	Average Recuperation (%)	RSD[b] (%)
4	3.99	99.82		
8	8.14	101.74	100.56	1.03
12	12.01	100.11		

[b]RSD= relative standard deviation

4.6. Limit of detection (LD) and Limit of Quantification (LQ)

The sensitivity of the spectrophotometric method was determined by the limits of detection (LD) and quantification (LQ). The calculated values for LD and LQ were 0.2229 µg/mL and 0.6754 µg/mL, respectively.

4.7. Robustness

Table 8. Evaluation of the robustness of the analytical method for analysis of cefazolin sodium by spectrophotometry in the UV region.

Sample	Length of wave (nm)	Content[a] (%)	RSD[b] (%)
	269	101.10	
1	271	101.88	0.9390
	273	100.00	

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

Changes in the wavelength of absorption were performed to evaluate the robustness of the method. During the analysis of the pharmaceutical product, the levels found no significant difference and RSD were below 0.93%, demonstrating the robustness of the proposed method, compared to the measured parameter, as shown in Table 8.

5. Discussion

The method was developed and validated using water as solvent, based on the good absorption intensity of the functional chromophore groups present on the drug with this solvent, and presents advantages such as simplicity in sample preparation, low cost and easy disposal.

By analyzing the Ringbom's curve, it was determined the best concentration range was selected for the method. The results obtained in the evaluation of linearity demonstrated that the solutions of cefazolin sodium SQR showed a linear

correlation between absorbances and concentrations in the range used. Plotted graphs of absorbance versus concentration were used, which demonstrated a linearity in the range 8-28 μg/mL. The equation of the line for the method was: y= 0.0266 x 0.0022, with a correlation coefficient of 0.9999.

The limits of detection and quantification were calculated based on the calibration curves generated on different days. The values found for detection and quantitation limits were 0.22 μg/mL and 0.67 μg/mL, respectively, which demonstrates that the range used is above the limits and, thus, with appropriate values of concentration.

The precision of the method was demonstrated by repeatability (intraday precision) and intermediate precision (interday and between-analysts). The experimental value obtained from intraday analysis showed RSD of 0.32% for analysis of seven replicates of cefazolin sodium SQR performed on the same day under the same conditions. In interday precision, the analyses were performed on different days yielding an average content of 99.103%, and RSD of 0.75%. Statistical tools (ANOVA) were used to confirm that the significance level of 5%, variances in absorbance between analyzed days, did not differ statistically, giving an F 2.83 calculated that therefore is less than the tabulated F observed 3.55. The accuracy between analysts showed an RSD of 1.01% and was evaluated by t test which confirmed the effectiveness of the method, because the value found for t calculated 2.03 was less than the tabulated t value (2.17), showing that the significance level of 5% averages are not statistically different, therefore the results obtained demonstrate that the method is validated accurate.

The accuracy was evaluated by the addition method and calculated mathematically by recovery. The results obtained are in the range of 99.82 to 101.74%, which is consistent with the recommended range for the accuracy of analytical methods (98-102%)[42].

The parameter chosen to evaluate the robustness of the method was the wavelength. The results obtained by varying the wavelength of two units above and below two units showed no significant difference in the quantification of the sample. The test was evaluated using the ANOVA statistical tool featuring F calculated to be 0.14 which is less than F Tabulated of 3.55.

The proposed spectrophotometric method met all requirements required by the official codes agency guidelines[10, 34, 44), making it adequate, presenting simplicity, specificity, linearity, precision, accuracy and robustness, and can be used for assay of cefazolin sodium in lyophilized powder.

6. Conclusions

In this paper, an analytical method by UV spectrophotometry was developed and validated for the quantification of cefazolin sodium in pharmaceutical preparations, which shows advantages such as simplicity, speed, versatility, easy to apply, inexpensive and does not use toxic reagents and pollutants, satisfactory validation parameters, with highly reproducible results indicating linearity, selectivity, precision, proving to be suitable for routine analysis in pharmaceutical industries.

ACKNOWLEDGEMENTS

REFERENCES

[1] C.Y. Chan, K. Chan, G.L. French, Rapid high performance liquid chromatographic assay of cephalosporins in biological fluids. Journal Antimicrobial Chemotherapy, v.18, n.4, p.537–545, 1986.

[2] G. Brotzu, Ricerche su di un nuovo antibiotico. Labori dezz 'Istituto d'lgiene di Cagliari, 1948.

[3] H.R.N. Salgado, G.C.G. Tozo, Microbiological assay for cefoxitin sodium in dosage form. Journal of AOAC, v. 90, n. 2, p. 2007.

[4] L.L. Brunton, B.A. Chabner, B.B Knollmann. As bases farmacológicas da terapêutica de Goodman & Gilman. 12. ed. São Paulo: AMGH; 2012. 2079p.

[5] A.H. Moreno, H.R.N. Salgado, Development of a new high-performance liquid cromatographic method for determination of ceftazidime. Journal of AOAC international, v.91, n.4, p.739-743, 2008.

[6] British Pharmacopoeia, London: The Stationery Office, 2010.

[7] European Pharmacopoeia. 5 th. Council of Europe, 2005. v.2, p.1220-1222

[8] Portuguesa Pharmacopeia. 6 th. Lisboa: Imprensa Nacional, 2000.

[9] Japanese Pharmacopoeia 15. th. Disponivel em: http://jpdb.nihs.go.jp/jp15e/ Acessado em 06 Nov. 2011.

[10] USP. THE UNITED STATES PHARMACOPEIA, 33. th. Rockville: The United States Pharmacopeial Convention, 2010.

[11] J.J. Kaminski, N. Bodor, Determination of penicillins and cephalosporins using 3-bromo 4,4-dimethyl-2-oxazolidinone. International Journal of Pharmaceutics, v.3, p.151-156, 1979.

[12] H. Salem, H. Askal. Colourimetric and AAS determination of cephalosporins using Reineck's salt Journal of Pharmaceutical and Biomedical Analysis, v.29, p.347-354, 2002.

[13] A.M. Brisson, J.B. Fourtillan, Determination of cephalosporins in biological material by reversed-phase liquid column chromatography. Journal of Chromaiogmphy, v.223, p.393-399, 1981.

[14] S.M. Bayoumi, J.J. Vallner, J.T. Dipiro, Quantitation of cefazolin sodium in plasma and tissues by high-performance

liquid chromatography. International Journal of Pharmaceutics, v.30, p.57-61, 1986.

[15] D. Liang, D. Chow, C. WHITE, High-performance liquid chromatographic assay of cefazolin in rat tissues. Journal of Chromatography B, v.656, p.460-465, 1994.

[16] S. Bompadre, L. Leone, L. Ferrante, F.P Alo, G. Ioannidis, Determination of cefazolin in human serum by high performance liquid chromatography with on-line solid phase extraction. Journal of Liquid Chromatography & Related Technologies, v.21, n.3, p. 417-426, 1998.

[17] S. Al-Rawithi, R. Hussein, D.A. Raines, I. Alshowaier, W. kurdi, Sensitive assay for the determination of cefazolin or ceftriaxone in plasma utilizing LC. Journal of Pharmaceutical and Biomedical Analysis, v.22, p.281–286, 2000.

[18] M. S. Arayne, N. Sultana, B. Z Bi, Simultaneous determination of cefazolin or ceftizoxime in presence of ascorbic acid from pharmaceutical formulation and human serum by RP-HPLC. Pakistan Journal of Pharmaceutical Sciences, v.20, n.1, p.56-61, 2007.

[19] C. Farthing, D. Farthing, D.F. Brophy, T. Larus, L. Maynor, I. Farkhry, T.W.B. Gehr, High-Performance Liquid Chromatographic determination of cefepime and cefazolin in human plasma and dialysate. Chromatographia, v.67, n.5/6, p.365-368, 2008.

[20] N. Lalitha, Stability-indicating reverse phase HPLC method for the determination of cefazolin. Tropical Journal of Pharmaceutical Research, v.9, n.1, p.45-50, 2010.

[21] B.X. Mayer, M. Petsch, E.M. Tschernko, M. Muller, Strategies for the determination of cefazolin in plasma and microdialysis samples by short-end capillary zone electrophoresis. Electrophoresis, v.24, p.1215–1220, 2003.

[22] H. Sum, L. Wei, Y. Zuo, Y. Wu, Effective separation and simultaneous detection of gatifloxacin, aminomethylbenzoic acid, cefazolin and cefminox in human urine by capillary zone electrophoresis. Journal of the Iranian Chemical Society, v. 8, n. 4, p.1043-1051, 2011.

[23] P. Papazova, P.R. Bontchev, M. Racarova, New spctrophotometric method for determination of cephazolin. Talanta, v.30, n.1, p.51-53, 1983.

[24] G. A. Saleh, H. F. Askal, M. F. Radwan, M. A. Omar, Use of charge-transfer complexation in the spectrophotometric analysis of certain cephalosporins. Talanta, v.54, p.1205–1215, 2001.

[25] G.A. Saleh, H.F. Askal, I.A. Darwish, A.A. El-Shorbagi, Spectroscopic analytical study for the charge-transfer complexation of certain cephalosporins with chloranilic acid. Analytical Sciences, v.19, p.281-287, 2003.

[26] G.A. Saleh, S.R. El-Shaboury, F.A. Mohamed, A.H. Rageh, Kinetic spectrophotometric determination of certain cephalosporins using oxidized quercetin reagent. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, v.73, p. 946–954, 2009.

[27] A.H. Rageh, S.R. El-Shaboury, G.A. Saleh, F.A. Mohamed, Spectophotometric method for determination of certain cephalosporins using 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl). Natural Science, v.2, n.8, p.828-840, 2010.

[28] L.I. Bebawy, K. El-Kelani, L. Abdel-Fattah, Fluorimetric

determination of some antibiotics in raw material and dosage forms through ternary complex formation with terbium (Tb3+) Journal of Pharmaceutical and Biomedical Analysis, v.32, p.1219-1225, 2003.

[29] L. Weng, Y. Lu, L. Shi, X. Zhang, L. Zhang, X. Guo, J. Xu, In situ investigation of drug diffusion in hydrogels by the refractive index method. Analytical Chemistry, v.76, p.2807-2812, 2004.

[30] B. Ogorevc, V. Hudnik, S. Gomiscek, M.R. Smyth, J.G. Vos, A spectroscopic and polarographic investigation of the complexation of cefazolin with copper (H) ions. Inorganica Chimica Acta, v.108 p.L3-L6, 1985.

[31] Y. Li. J. Lu. Chemiluminescence flow-iniection analvsis of β-lactam antibiotics using the luminol–ermanganate reaction. Luminescence, v. 21, p.251-255, 2006.

[32] A.S. Crucq, C. Slegers, V. Deridder, B. Tilquin, Radiosensitivity study of cefazolin sodium. Talanta, v.52, p.873–877, 2000.

[33] H.S. El-Desoky, E.M. Ghoneim, M.M. Ghoneim, Voltammetric behavior and assay of the antibiotic drug cefazolin sodium in bulk form and pharmaceutical formulation at a mercury electrode. Journal of Pharmaceutical and Biomedical Analysis, v.39, p. 1051–1056, 2005.

[34] Brazilian Pharmacopeia, 5th. ed., Brasília, Brazil: Anvisa, 2010

[35] H.R.N. Marona, E.E.S. Schapoval, Spectrophotometric determination of sparfloxacin in tablets. Journal Antimicrobial Chemotherapy, v.44, n.1, p.136-137, 1999.

[36] H.R.N. Salgado, C.L.C.G. Oliveira, Development and validation of a UV spectrophotometric method for determination of gatifloxacin in tablets. Die Pharmazie, v.60, n.4, p.263-264, 2005.

[37] G.C. Gomes, H.R.N. Salgado, Validation of UV spectrophotometric method for determination of lomefloxacin in pharmaceutical dosage form. Acta Farmacéutica Bonaerense, v.24, n.3, p. 406-408, 2005.

[38] A.H. Moreno, H.R.N. Salgado, Spectrophotometric determination of ceftazidime in pharmaceutical preparations using neocuproin as a complexing agent. Analytical Letters, v.41, p. 1–10, 2008.

[39] A.H. Moreno, H.R.N. Salgado, Rapid and selective UV spectrophotometric method for the analysis of ceftazidime. Journal of AOAC international, v. 92, n. 3, p.820-823, 2009.

[40] R. Bonfilio, M.B. Araujo, H.R.N. Salgado, Development and validation of an UV-derivative spectrophotometric method for determination of glimepiride in tablets. Journal of Brazilian Chemical Society, v.22, n.2, p.292-299, 2011.

[41] F.A.M. Fiorentino, H.R.N. Salgado, Development and validation of a UV-spectrophotometric method for determination of flucloxacillin sodium in capsules.Current Pharmaceutical Analysis, v.7, p.268-276, 2011.

[42] Brazil, Ministério da Saúde. Resolução RE nº 899, de 29 de maio de 2003. "Guia para validação de métodos analíticos e bioanalíticos". Diário Oficial da União, Brasília, 02 june. 2003.

[43] FDA FOOD AND DRUG ADMINISTRATION. Validation

of chromatographic methods. Washington: Center of Drug Evaluation and Research, 2004.

[44] ICH International Harmonised Tripartite Guideline. Validation of analytical procedures: text and methodology Q2 (R1). Commission of the European Communities. Geneva, 2005.

[45] Inmetro. DOQ-CGCRE-008, revisão 04: orientação sobre validação de métodos analíticos. 2011. Online Available: http://pt.scribd.com/doc/13218567/DOQCGCRE802Orienta coes-Validacao-de-Metodos-Ensaio-Quimico. August 27 th, 2012.

[46] R.M. Silverstein, F.X. Webster, D.J. Kiemle, Identificação espectrométrica de compostos orgânicos. 7. ed. Rio de Janeiro: LTC; 2006. 490p.

[47] F.R.P. Rocha, L.S.G. Teixeira, Estratégias para aumento de sensibilidade em espectrofotometria UV-VIS. Química Nova, v.27, n.5, p.807-812, 2004.