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Development and validation of a new analytical method based on UV-visible spectroscopy for quantification of ceftaroline fosamil in powder for injection

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Quantification of drugs is an essential part for ensuring quality, safety and efficacy of pharmaceutical formulations. For this, validated methods must be used in order to guarantee analytical reliability during monitoring in clinical studies and quality control. In the present study, we aim to develop a reliable, accurate, accessible and rapid UV-VIS spectrophotometry method for quantification of ceftaroline fosamil in powder for intravenous administration. Each analyzed solution was prepared individually and the only reagent used was Milli-Q water, as a solvent, making the method eco-friendly. The absorbances were measured at 242 nm, the peak absorbance found for the drug. The developed method was validated according to ICH and ANVISA guidelines, proving to be specific and demonstrating good linearity in the concentration range of 5 $\mu g.mL^{-1}$ to 15 $\mu g.mL^{-1}$, with determination (r^2) and correlation coefficients (r) equal to 0.9999. The limits of detection and quantification found were acceptable (0.51 $\mu g.mL^{-1}$ and 1.55 $\mu g.mL^{-1}$, respectively). The method exhibited excellent intermediate precision and repeatability, with relative standard deviation values of 0.87% and 0.98%, respectively. Furthermore, the method's accuracy was confirmed, showing a mean recovery of 100 ± 2.67%. Additionally, the robustness was assayed and confirmed using Plackett-Burmann design. In conclusion, the method can easily be applied for routine quality control analysis.

Keywords: *ceftaroline fosamil; UV spectrophotometry; validation; quality control.*

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Introduction

Ceftaroline fosamil (Figure 1), the prodrug of active metabolite ceftaroline, belongs to the cephalosporin antibacterial class and it is available in powder form for intravenous administration. It is relatively recent and firstly approved for use in the U.S. in 2010 (1). Since 2014, ceftaroline fosamil has also been approved for use in Brazil (2). In both countries, the drug is indicated to treat adult and pediatric patients affected with acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia. It is considered a broad-spectrum cephalosporin and it is effective against several Gram-negative and Gram-positive bacterial species, including methicillin-resistant *Staphylococcus aureus* (MRSA), a current worldwide public health concern (1-6).

The development of a reliable and accurate method for quantifying drugs, such as ceftaroline fosamil, is extremely important for quality control and clinical studies, for example. Several chromatography-based methods for quantification of ceftaroline fosamil and its active form ceftaroline can be found in literature, such as LC-MS/MS and HPLC-UV methods for quantification of ceftaroline in biological samples, and RP-HPLC and HPLC-UV methods for measurement of ceftaroline fosamil in powder for injection (6-11).

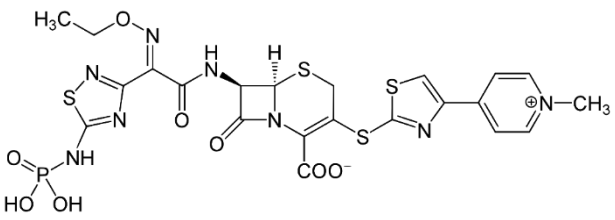

Figure 1. Chemical structure of ceftaroline fosamil.

UV-VIS spectroscopy, a reliable alternative to chromatography, works with the ability of molecules to absorb light energy at particular wavelengths, measuring the amount of light absorbed, which is proportional to the concentration of the absorbing molecules or atoms in solution (12). The UV-VIS method for quantification of drugs, based on Beer's law, has been available commercially for over 70 years and is still used extensively in the pharmaceutical industry, being present in more than two hundred monographs found at the United States Pharmacopeia (USP), mainly for quantitative analyzes (13). It has many advantages over other methods used for the same purpose, being, in general, relatively low-cost and simple, when compared

to HPLC-based validation methods. Frequently, non-toxic solvents, as water, can be used, in contrast to methods developed using HPLC that often utilize organic solvents that are not eco-friendly.
In consideration of the points described previously, this paper depicts the development and validation of an UV-VIS spectrophotometry method for quantification of ceftaroline fosamil in powder for injection, an accessible and cost-effective alternative to methods currently found in literature.

Experimental

Equipment and materials

The method was developed using a UV-1800 Spectrophotometer Shimadzu (Kyoto, Japan) and 1 cm quartz cells. Furthermore, a ME204 analytical weighing scale (Mettler Toledo, Switzerland) and an ultrasonic bath USC-2850 (Unique, Brazil) with a frequency of 25 kHz were used to prepare the solutions. Milli-Q Plus system (Millipore, Bedford, USA) was used to obtain purified water. Ceftaroline fosamil powder for injection (Zinforo® 600 mg) with known content (Lot: 0008D9; 102.7% according to certificate of analysis) was purchased form Wyeth pharmaceutical industry. This sample was used as standard. Another sample of ceftaroline fosamil (Zinforo® 600 mg; Lot: 0009E0) was purchased and worked as sample.

Preparation of samples

Each sample and standard used was prepared individually by weighing the equivalent of 5 mg of ceftaroline fosamil, transferring the mass to a 50 mL volumetric flask and adding 30 mL of ultrapure water. Subsequently, the solutions were submitted to ultrasonic extraction for 15 minutes, after which the volumetric flask was completed with ultrapure water. Afterward, the solutions prepared were diluted by transferring different aliquots, according to the desired final concentration required for each specific experiment, to 10 mL volumetric flasks and completing them with ultrapure water. When completed, each of the samples was immediately analyzed.

Validation

The proposed method was developed and validated in accordance to ICH and ANVISA guidelines (14,15). Specificity, linearity, limits of detection and quantification, precision, accuracy and robustness were analyzed employing the absorbances generated by the working solutions in the wavelength of 242 nm (correspondent to the peak absorption of the drug) using UVProbe® software.

Specificity

Specificity was determined by analyzing the potential interference of arginine, the only excipient of ceftaroline fosamil formulation currently available, in the resultant absorbances. For that matter, a solution containing the same amount of the excipient found in a 10 $\mu g.mL^{-1}$ solution of ceftaroline fosamil was prepared. The absorbances generated were compared to those produced by a 10 $\mu g.mL^{-1}$ solution of ceftaroline fosamil.

Linearity

For evaluation of linearity, three stock standard solutions of concentration 100 $\mu g.mL^{-1}$ were prepared. For each stock solution, five distinct concentrations (5 $\mu g.mL^{-1}$, 8 $\mu g.mL^{-1}$, 10 $\mu g.mL^{-1}$, 12 $\mu g.mL^{-1}$ and 15 $\mu g.mL^{-1}$) were obtained by dilution of specific volumes (0.5 mL, 0.8 mL, 1.0 mL, 1.2 mL and 1.5 mL) of the primary solutions with ultrapure water in 10 mL volumetric flasks. The samples were immediately processed and the absorbances generated by the solutions were collected and analyzed. Cochran C test was used to evaluate homoscedasticity. ANOVA test was performed through Microsoft Excel ® for statistical analysis of the results.

Limits of detection (LOD) and quantification (LOQ)

Both of the limits were calculated using data obtained from the calibration curve. The following formulas were used:

$LOD = (3.3 * \sigma)/S$ Eq. 1

$LQD = (10 * \sigma)/S$ Eq. 2

Being σ the residual standard deviation of the regression line and S the slope of the calibration curve.

Precision

The precision was evaluated through analysis of intraday and interday repeatability using the same instrument and similar conditions. Six standard solutions of concentration 10 $\mu g.mL^{-1}$ were prepared separately and analyzed on the same day for evaluation of intraday repeatability and the relative standard deviation (RSD %) was calculated using the obtained measurements. The interday precision was evaluated through the determination of the content of six standard solutions of theorized concentration of 10 $\mu g.mL^{-1}$ on three distinct days and the RSD % was calculated based on the measurements. Analysis of variance was also used for evaluation of precision.

Accuracy

Accuracy was determined through recoveries assays. A stock sample solution with concentration of 100 $\mu g.mL^{-1}$ was prepared and diluted into three different concentrations (6 $\mu g.mL^{-1}$, 8 $\mu g.mL^{-1}$ and 10 $\mu g.mL^{-1}$). Then, 0.4 mL aliquots of a solution 2 $\mu g.mL^{-1}$ prepared using the ceftaroline fosamil reference standard were spiked to the solutions, generating final solutions with total concentration of ceftaroline fosamil equivalent to 8 $\mu g.mL^{-1}$, 10 $\mu g.mL^{-1}$ and 12 $\mu g.mL^{-1}$. The experiment was repeated three times for each concentration level and the

results obtained were used to calculate and average percentage recovery.

Robustness

For robustness evaluation, the Plackett-Burman method was used for designing the experiment. The evaluation was performed by variation of four factors: water purity, extraction time, wavelength reading and filter type. A total of fifteen experiments, in duplicate, were made with random variations of the chosen factors. The generated absorbances from each were analyzed and used for calculating the content of each solution. Consequently, it was possible to determine the influence of the factors on the method performance. The Plackett-Burman design helped reducing the number of experiments, allowing multiple factors to be analyzed at the same time, therefore making the validation more efficient and lowering the costs (16). The design was obtained through MiniTab 21 ® statistical software, which was also used to process the data. In Table 1, the chosen factors and varying levels can be observed.

Table 1. Selected factors and levels of variability used for robustness testing during validation of UV-VIS spectrophotometry for quantitation of ceftaroline fosamil in powder for injection.

Factors	Varied condition (-1)	Normal condition (0)	Varied condition (1)
Filter type	Millipore Nylon	Sorbline PP	Whatman PVDF
Water type	Ultrapure Water	Milli-Q Water	Distilled Water
Wavelength	240 nm	242 nm	244 nm
Extraction duration	10 min	15 min	20 min

Results and Discussion

Method validation

The UV method for quantitation of ceftaroline fosamil in powder for injection was validated according to the validation parameters recommended by ICH and ANVISA guidelines (14,15). The presented method provides an accessible and simple way of quantification of ceftaroline fosamil in the pharmaceutical product. The summarized results can be observed at Table 2.

Specificity

Specificity test confirmed the absence of excipient's interference in the absorption spectra at 242 nm, as can be observed in Figure 2. Thus, the method can be considered specific for drug quantitation in the routine, considering eventual interference from samples stored following conditions indicated by laboratory producer.

Table 2. Method validation parameters obtained using UV-VIS spectrophotometry for quantification of ceftaroline fosamil in powder for injection.

Validation parameter	Result
Linearity	
Range	5 µg/mL – 15 µg/mL
Correlation coefficient (r)	0.9999
Determination coefficient (r^2)	0.9999
Regression equation	y = 0.0564x + 0.0091
Limit of Detection (LOD)	0.51 µg.mL^{-1}
Limit of Quantification (LOQ)	1.55 µg.mL^{-1}
Precision (RSD %)	
Repeatability	0.98
Intermediate precision	0.87
Accuracy (Recovery Rate %)	100 ± 2.67

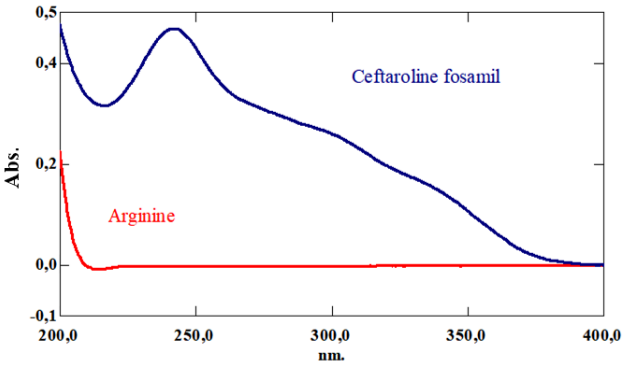

Figure 2. Absorption spectra of ceftaroline fosamil solution (in blue) and arginine solution (in red), in water at concentration of 10 µg.mL^{-1}, conducted during specificity tests for UV-VIS spectrophotometry applied to drug quantitation.

Linearity

It was observed the presence of linearity between the concentration of ceftaroline fosamil (in the range of 5 µg.mL^{-1} to 15 µg.mL^{-1}) and the absorbances, with high correlation and determination coefficients (r = 0.9999, .R^2 = 0.9999), indicating a strong linear relationship between the concentrations and their absorbances. The curve's representative equation found was y = 0.0564x + 0.0091, being the curve slope and the intercept found to be 0.0564 and 0.0091, respectively. Additionally, analysis of variance (ANOVA) was used for statistical test of the results, resulting in a significant linear regression ($F_{calculated}$ = 3740.34 > $F_{critical}$ = 4.96, α = 0.05) and a non-significant deviation of linearity ($F_{calculated}$ = 0.17 < $F_{critical}$ = 3.71, α = 0.05), as illustrated in Table 3. In addition, the data was confirmed to have homoscedasticity (equal variances) by Cochran's C test (C = 0.446 < $C_{critical}$ =

0.684, α = 0.05). Hence, the results demonstrated that the method displays strong evidence of a linear response over the desired concentration range.

Table 3. Analysis of variance (ANOVA) for linearity using UV-VIS spectrophotometry method for quantification of ceftaroline fosamil in powder for injection.

Source of variation	DF*	Sum of squares	Variability	F calculated	F critical
Between concentration	4	0.55	0.14	935.21	
Linear regression	1	0.55	0.55	3740.34	4.96
Deviation of linearity	3	$7.48E^{-5}$	$2.49E^{-5}$	0.17	3.71
Residue	10	$1.48E^{-4}$	$1.48E^{-4}$		
Total	14	0.56			

* Degrees of Freedom; Significance level: α = 0.05.

Limits of detection and quantification

Both limits were determined by applying the regression curve's standard deviation to the equations (Eq. 1 and Eq. 2) shown earlier. The limit of detection for ceftaroline fosamil, which represents the lowest concentration of an analyte that can be differentiated in a solution, was equal to 0.51 $\mu g.mL^{-1}$. Furthermore, the limit of quantification, which is the lowest amount of an analyte that can be quantified with good precision and accuracy, corresponded to 1.55 $\mu g.mL^{-1}$. These limits demonstrate the good sensibility of the method, capable of detecting and quantifying even small amounts of the drug with precision and accuracy.

Precision

For determination of intraday repeatability, six solutions obtained from independent weightings of ceftaroline fosamil 10 $\mu g.mL^{-1}$ were prepared and analyzed, on the same day and conditions by the same analyst, yielding an RSD (%) of 0.98. For interday analysis, the experiment was repeated on three non-consecutive days, by the same operator and under the same conditions, generating average content values of 98.0%, 98.4% and 99.7% and an RSD (%) of 0.87, as can be observed in Table 4. Intraday repeatability and interday assays showed good results, with reproducible data in the three days of analysis. Therefore, both intraday and interday precision follows the literature recommendations, indicating high precision and minor variability in the measured absorbance values. ANOVA test was also performed to statistical analysis of the interday results. The $F_{calculated}$ (1.27, p = 0.309) was lower than the $F_{critical}$ (3.68), showing the absence of significant difference between the results obtained on the different days.

Accuracy

The accuracy was assessed by analysis of three different ranges from the method concentration (80 – 120%) in triplicate. The mean recovery was 100 ± 2.67%, demonstrating that the method has proper accuracy, as recommended by ICH and ANVISA guidelines (14,15). The results for each level concentration can be examined in Table 4.

Table 4. Precision and accuracy assays results for quantification of ceftaroline fosamil in powder for injection using UV-VIS spectrophotometry method.

Level % concentration	Content (%)[a]			Recovery[b]
	Day 1	Day 2	Day 3	
80	-	-	-	98.90
100	97.98	98.42	99.68	102.67
120	-	-	-	99.07
Average		98.69		100.21
RSD		0.87		2.12

[a]average of six determinations; [b]average of three determinations.

Robustness

The Plackett-Burman design was employed for simultaneous determination of the level of influence of the chosen parameters on the results of the performance of the method and, thus, of the robustness. Fifteen experiments were carried out with different conditions, as shown in Table 5. According to the results, it was not observed variations with statistically significant influence on the method, all presenting p-values greater than 0.05 in the ANOVA test, as illustrated by Figure 3. However, as can be noted in the same graph, some factors have shown higher impact on the drug content than others, such as duration of extraction (p = 0.083, α = 0.05) and wavelength reading (p = 0.239, α = 0.05). Nevertheless, the method can be considered robust.

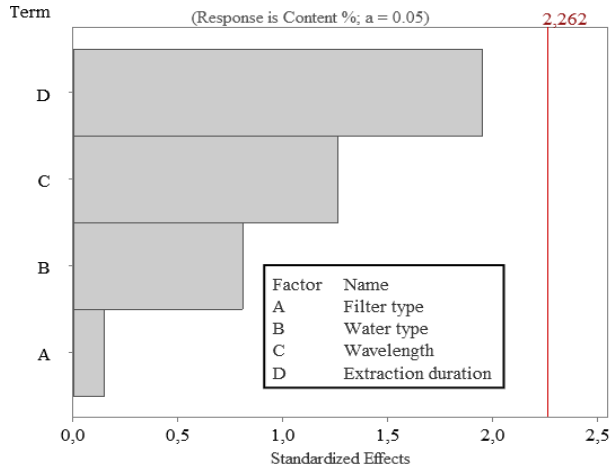

Figure 3. Pareto charts illustrating the variables' effects during robustness testing experiments, applied to UV-vis spectrophotometric method for quantitation of ceftaroline fosamil in powder for injection.

Table 5. Factorial design for robustness assay during experiments of quantification of ceftaroline fosamil in powder for injection, generated by MiniTab 21®.

Run	Factors				Content (%)[5]
	A[1]	B[2]	C[3]	D[4]	
1	-1	-1	1	1	101.12
2	-1	1	-1	-1	97.14
3	1	-1	1	-1	97.58
4	1	-1	1	1	100.97
5	-1	1	1	1	104.03
6	0	0	0	0	100.98
7	-1	-1	-1	-1	97.70
8	1	1	1	-1	103.49
9	0	0	0	0	99.99
10	-1	1	1	-1	97.68
11	0	0	0	0	100.26
12	1	1	-1	1	104.12
13	-1	-1	-1	1	101.14
14	1	1	-1	1	96.46
15	1	-1	-1	-1	97.46

[1]Filter type, [2]Water type, [3]Wavelength reading, [4]Extraction duration, [5]Mean of two determinations.

Conclusions

The present study described the development and validation of a method for quantification of ceftaroline fosamil in powder for injection using UV-VIS spectrophotometry, a promising alternative to the others currently published methods, due to its speed, simplicity and low cost. The method was validated according to ICH and ANVISA guidelines, meeting the acceptance criteria for parameters such as linearity, precision, accuracy and robustness (14,15). For these reasons, the method can be easily applied to quality control routines.

Conflict of interest

The authors declare no conflicts of interest.

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