Comparison of HPLC and UV Spectrophotometric Methods for the Determination of Cefuroxime Sodium in Pharmaceutical Products

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Abstract

This paper describes the development and evaluation of a HPLC and UV spectrophotometric methods to quantify cefuroxime sodium in injectables. HPLC analysis were carried out using a C18 Wat 054275 column and a mobile phase composed of methanol and water (70:30), with a flow rate of 0.8 mL/min and UV detection at 280 nm. For the spectrophotometric analysis, water was used as solvent and the wavelength of 280 nm was selected for the detection. Both methods were found to quantify cefuroxime sodium in injectables accurately. Therefore HPLC and UV methods presented the most reliable results for the analyses of injectables.

Introduction

Cefuroxime (CAS 56238-63-2) (Figure 1) is a second generation cephalosporin with high antibacterial activity; it has enhanced in vitro activity against clinically important Gram-positive and Gram-negative microorganisms (1). The chemistry of cephalosporins has been widely explored because of their extensive medical applications (2). Several analytical procedures are available in literature for the analysis of antimicrobial. These methods are spectrophotometry (3–13), high performance liquid chromatography (14–19), capillary electrophoresis (20), fluorimetry (21–24), polarography (25–29), titrimetry (30), and bioassay (31–32). Spectrophotometric assay for determination of other cephalosporins as ceftazidime has been described (33) but no method for cefuroxime sodium had been previously described.

The purpose of this study was to develop and validate analytical methods to quantify cefuroxime sodium in injectables, using HPLC and UV spectrometry. The results obtained by these methods were statistically compared, by using analysis of variance (ANOVA). In addition, the reliability and feasibility of them were evaluated focusing on routine quality control analysis.

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Experimental

Reagents and materials

Cefuroxime sodium reference standard was kindly donated by Glaxo Smithkline. The injectables were purchased from Cellofarm Farmacêutica. Water was purified by using a Millipore system (Bedford, MA). Methanol (HPLC grade) was obtained from Merck (Fairfield, OH).

Instruments and analytical conditions

All HPLC measurements were made on a Waters 1525 Binary HPLC Pump, consisting of a 7725i manual injector with a 20 µL loop (Rheodyne, Torrance, CA), integrated UV detector UV–vis (Milford, MA). The system employed a 150 mm × 4.6 mm C18 column Wat 054275 (Milford, MA) and particle size of 5 µm guard column. The detector was utilized at 280 nm and UV spectra from 200 to 400 nm were recorded on-line for peak identification. The mobile phase consisted of methanol and water (70:30), at a flow rate of 0.8 mL/min. The injection volume was 20 µL. Ultraviolet spectrophotometric analyses were carried out on a UV-vis Shimadzu UV-mini 1240 (Shimadzu, Kyoto, Japan) spectrophotometer, in a 1 cm quartz cubette. The wavelength of 280 nm was selected for the quantitation of cefuroxime sodium and the measurements were obtained against water as a blank.

Preparation of standard and sample solutions

The standard stock solutions were prepared by dissolving 10 mg of cefuroxime sodium reference standard in 10 mL of water to get a concentration of 1 mg/mL. An aliquot of 120 µL of the

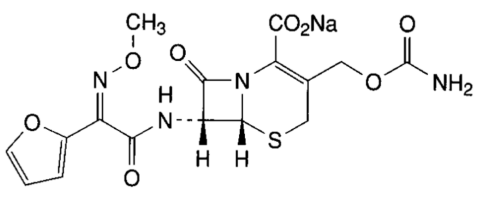

Figure 1. Structure of cefuroxime sodium.

obtained solution was transferred to a 10 mL volumetric flask. The volume was adjusted with water for spectrophotometric analysis or mobile phase for chromatographic analysis, resulting in solutions of 12 µg/mL.

The sample solutions were prepared by dissolving 10 mg of cefuroxime sodium powder for injection in 10 mL of water to get a concentration of 1 mg/mL. An aliquot of 120 µL of this solution was transferred to a 10 mL volumetric flask. The volume was adjusted with water for spectrophotometric analysis or mobile phase for chromatographic analysis, to obtain a solution at 12 µg/mL of cefuroxime.

Validation

The optimized spectrophotometric and chromatographicmethods were completely validated according to the procedures described in ICH guidelines Q2(R1) for the validation of analytical methods (34).

Linearity

Standard solutions containing 1000 µg/mL of cefuroxime sodium in water were prepared, in triplicate. Aliquots of these solutions were diluted in water (for UV analysis) or mobile phase (for HPLC analysis), to six different concentrations, corresponding to 10, 11, 12, 13, 14, and 15 µg/mL of cefuroxime. Calibration curves with concentration versus peak area or absorbance were plotted for each method and the obtained data were subjected to regression analysis using the least squares method.

Precision

The intra-day precision was evaluated by analyzing six samples (n = 6), at the test concentration of 12 µg/mL, using the UV and the HPLC methods. Cefuroxime sodium contents and the relative standard deviations (RSD) were calculated.

Accuracy

Cefuroxime sodium reference standard was accurately weighed and added, at three different concentrations. At each concentration, samples were prepared in triplicate and the recovery percentage was determined by UV and HPLC methods.

Robustness

The robustness of the method was determined by the variation of the analyst and mobile phase flow rate. The flow rate was checked in 0.8 mL to 1.0 mL.

Analysis of cefuroxime sodium powder for injection

Samples of Zencef were analyzed by the validated HPLC and UV methods. The sample solutions for the HPLC and UV analyses were prepared as described previously. The cefuroxime sodium contents were determined by using the two methods and the obtained results were statistically compared by using ANOVA test and Tukey's multiple comparison test, applied at 0.05 significance level.

Results and Discussion

During the chromatographic method development, methanol showed to be a more adequate organic solvent than acetonitrile, regarding the cefuroxime sodium retention. A typical chromatogram obtained is as shown by Figure 2.

After the evaluation of the cefuroxime sodium UV spectrum in various solvents (water, phosphate buffer pH 6.0, methanol, hydrochloric acid 0.1M and sodium hydroxide 0.1 M), and in the range of 200–400 nm (Figure 3), the wavelength of 280 nm was chosen due to the adequate molar absorptivity of cefuroxime sodium in this region and to minimize possible interference from other compounds and solvents in the samples.

Validation

A linear relationship was found between the cefuroxime sodium concentrations and the response of both HPLC and UV methods. The regression analysis data are presented in Table I. High regression coefficient (r^2) values were obtained (0.9992 and 0.9996, respectively). A random pattern of the regression residues was found and no significant deviation of linearity was detected in the assayed range.

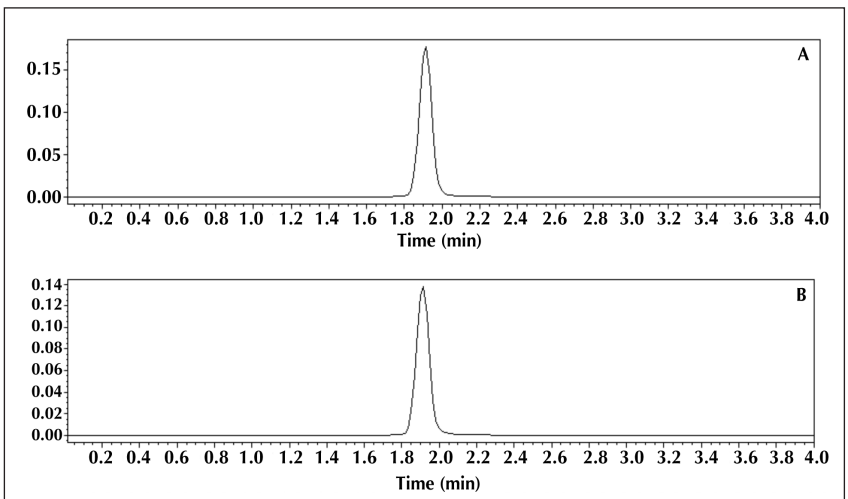

Figure 2. A typical chromatogram showing the separation of cefuroxime sodium (14 µg/mL) standard solution (A) and sample solution (B).

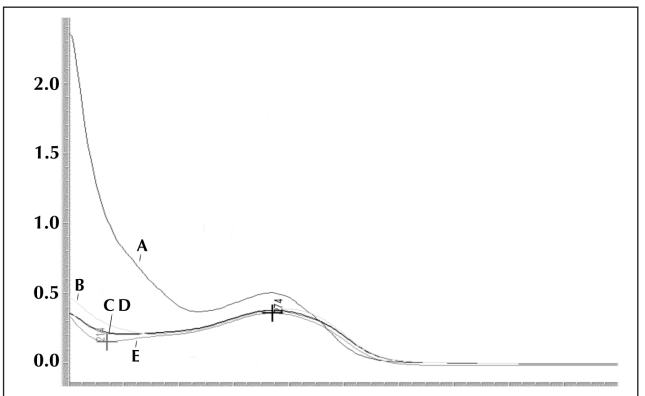

Figure 3. Ultraviolet region spectrum in the of cefuroxime sodium reference substance at 10 mg/mL in: methanol (A), phosphate buffer pH 6.0 (B), water (C), hydrochloric acid 0.1 M (D), and sodium hydroxide 0.1 M (E).

The precision data obtained for the evaluated methods are demonstrated in Table II. Both methods presented RSD values lower than 2.0%, assuring a good precision.

Accuracy (Table II) was investigated by means of a standard addition experiment. Both chromatographic and spectrophotometric methods exhibited mean recoveries (n = 9) close to 100% demonstrating an adequate accuracy.

The difference in the retention time, the peak area and the analyst (for a given cefuroxime sodium concentration) caused by the aforementioned minor alterations were insignificant (Table II).

Analysis of injectable cefuroxime sodium

The validated chromatographic and spectrophotometric methods were applied to the analysis of cefuroxime sodium in Zencef (Table III). ANOVA test revealed a statistically significant difference between the results obtained for injectable samples, from the distinct methods, at a confidence level of 0.05. Chromatographic analysis showed to be the most sensitive and selective method, and might be applied successfully for cefuroxime sodium trace analysis and quantitation in biological matrices. We cannot discharge, however, the analyses time and cost. The spectrophotometric method is clearly less expensive and requires shorter analysis time, besides the ease of handling and lower residues generation.

Since the use of cefuroxime sodium as a potent antimicrobial drug is widespread, the development and validation of simple and reliable methods are essential to assure the quality of the raw materials and pharmaceutical formulations marketed nowadays. A simple method to identify and precisely quantify these drugs may be an important tool to avoid treatment inefficacy and development of resistance due to the exposition to sub therapeutic doses (35).

Table I. Overview of the Linearity Data Obtained for Cefuroxime Sodium by the Chromatographic and Spectrophotometric Methods

Regression parameters	HPLC	UV
Regression coefficient (r^2)	0.9992	0.9996
Slope ± standard error	114396 ± 0.24	–0.0328 ± 0.0027
Intercept ± standard error	67334 ± 0.15	0.0412 ± 0.0019
Relative standard error (%)	1.04	1.40
Concentration range (µg/mL)	5.0-14.0	5.0–14.0
Number of points	6	6

Table II. Validation Paramaters of the Evaluated Methods for Cefuroxime Sodium Determination

Validation parameters	HPLC	UV
Intra-day precision, n = 6 (RSD%)	1.04	1.40
Accuracy, n = 9 (mean recovery, %) (12 µg/mL)	100.10	100.82

Table III. Robustness of the HPLC Method for Cefuroxime Sodium by Varying the Analyst

Analyst	Area	Mean ± SEM	RSD (%)
1	691545		
	682258		
	685912	688479 ± 0.27	0.72
	691089		
	699896		
	680178		
2	691563		
	613157		
	599899	634243 ± 1.25	3.31
	651955		
	630085		
	618799		

RSD = relative standard deviation
SEM = standard error mean

Conclusion

HPLC and UV spectrophotometry were found to be adequate methods to quantify cefuroxime sodium in injectable solutions; the chromatographic and spectrophotometric methods presented the most reliable results. Since these methods are fast and simple, they may be successfully applied to quality control analyses, with the aim of quantifying and identifying cefuroxime sodium in pharmaceutical products.

Table IV. Robustness of the HPLC Method for Cefuroxime Sodium by Varying the Mobile Phase Flow Rate

Flow (mL/min)	Area	Mean ± SEM	RSD (%)
0.8	691545		
	682258		
	685912		
	691089		
	699896	688479 ± 0.27	0.72
	680178		
1.0	699595		
	692278		
	695982		
	698189		
	699396	696003 ± 0.14	0.38
	690578		

RSD = relative standard deviation.
SEM = standard error mean.

Table V. Cefuroxime Sodium Contents in Injectable Samples Obtained by HPLC and UV (n = 6)

	Cefuroxime sodium content (%) ± S.D.	
Sample	HPLC	UV
Injectable	99.84 ± 0.24	99.49 ± 0.62

S.D.: standard deviation.

Acknowledgements

Authors thank Glaxo Smithkline Ltda for providing cefuroxime reference substance. This work was supported by PACD-FCFAr-UNESP-Brazil, FUNDUNESP-Brazil, FAPESP-Brazil and CNPq-Brazil.

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Manuscript received April 26, 2010;
Revision received July 20, 2010.