A fast, cost-saving and sensitive method for determination of cefuroxime in plasma by HPLC with ultraviolet detection

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Abstract
Cefuroxime (CFX) is a broad-spectrum second-generation cephalosporin and one of the best choices for antibiotic prophylaxis. However, when used in critically ill patients, it may present changes in its pharmacokinetic properties. Therefore, therapeutic drug monitoring of CFX is necessary for effective dosing strategies. A simple, rapid and sensitive liquid chromatographic method with UV detection was developed and validated for the quantification of CFX in plasma. The method involved a single-step precipitation of proteins with methanol and trifluoroacetic acid. Cefuroxime was analyzed on a Brisa LC2C18 column in isocratic mode consisting of 0.1% trifluoroacetic acid in water and acetonitrile (75:25) with UV detection at a wavelength of 280 nm. The retention times of CFX and cephazolin (internal standard) were 9.8 and 7.4 min, respectively. The calibration curve was linear over a concentration range of 0.25–50 μg/ml. The limits of detection and quantification were 0.1 μg/ml and 0.25 μg/ml, respectively. The accuracy and precision were always <10%. The mean recovery was 93.52%. This fast and simple method could be applied in routine analysis and pharmacokinetic studies.

KEYWORDS
cefuroxime, cephalosporins, HPLC, pharmacokinetics, plasma

1 INTRODUCTION

Antibiotic resistance has become a serious global problem and is steadily increasing worldwide in almost every bacterial species treated with antimicrobial drugs (Sengupta et al., 2013). Cefuroxime (CFX) is a broad-spectrum second-generation cephalosporin. Its pharmacological properties make it the best choice for antibiotic prophylaxis, such as its safety, low cost, good efficacy against the most common intraoperative microorganisms and ability to be maintained both in plasma and in tissues throughout surgery (Aalbers et al., 2015; Knoderer et al., 2011). However, when this antibiotic is used in critically ill patients, it may present changes in distribution, metabolism and excretion (Bodenham et al., 1988; Paepe et al., 2002; Seyler et al., 2011), and during some procedures, such as surgeries with extracorporeal circulation, CFX concentrations may vary significantly and cause several complications, including wound infections (Ridderstolpe et al., 2001). Because of this, it is very complex to predict pharmacokinetic parameters in critically ill patients, whose pathological conditions account for a great inter- and intra-variability of these parameters. Therefore, therapeutic drug monitoring of CFX in biological fluids is necessary to validate current dosing strategies, as well as to investigate the relevance of alternative dosing regimens adjusted to each individual patient in order to obtain clinical efficacy outcomes minimizing the risk of bacterial resistance emerging during therapy, especially in critically ill patients.
Various methods to quantify CFX in human plasma using high-performance liquid chromatography with UV detection have been developed and validated. One of them provides high sensitivity with low limit of quantification values, but the extraction step included solid-phase extraction, which is a relatively expensive method (Denooz & Charlier, 2008; Piva et al., 2000; Qureshi et al., 2013). For that reason, the aim of the present study was to propose a simple, isocratic and rapid HPLC method with UV detection for quantifying CFX in human plasma samples.

2 | EXPERIMENTAL

2.1 | Chemicals, solvents and reagents

Cefuroxime sodium salt and cephalosporin sodium salt (internal standard) were purchased from Merck (Madrid, Spain). The reagents were stored at 2–8°C. Trifluoroacetic acid (99%) was obtained from Panreac AppliChem (Barcelona, Spain). Acetonitrile, methanol and water were of HPLC grade (Merck, Madrid, Spain).

2.2 | Instrumentation

The LC system consisted of an Agilent series 1220 Infinity (Agilent Technologies Spain, Madrid, Spain) with a dual gradient pump, a manual injector, a thermostatic column compartment and a variable wavelength detector, all belonging to the 1220 series. The system mentioned above was connected to a Gilson 234 Autoinjector for HPLC systems (Gilson Incorporated, Middleton WI, USA). The chromatograms were recorded using Open Lab ChemStation software for LC system (Agilent, Spain).

2.3 | Chromatographic conditions

Chromatographic separation was achieved using a Brisa LC² C18 column (250 x 4.6 mm i.d. x 5 μm) from Teknokroma (Barcelona, Spain), with an Eclipse XDB C18 (4.6 mm x 12.5 mm) Agilent (Madrid, Spain) guard column. The mobile phase consisted of 0.1% trifluoroacetic acid in water–acetonitrile (75:25) using an isocratic elution with a flow rate of 1.0 ml/min. The injection volume was 50 μl and detection was performed at a wavelength of 280 nm at room temperature (−22°C). The run time was 13 min.

2.4 | Standard solutions

Stock solutions of CFX (200 μg/ml) and cephazolin (200 μg/ml), as internal standard (IS), were prepared in water. Working solutions of CFX at 0.1, 1, 10, 20 and 50 μg/ml were prepared by corresponding dilution of the stock solution with water. Working solutions were stored at −80°C.

2.5 | Preparation of calibration curve and quality controls

Calibration curve (CC) and quality control (QC) samples were spiked by the addition of 20 μl of an appropriate working solution of CFX to 180 μl of drug-free plasma. Plasma drug-free samples were obtained from blood donors supplied by Murcia Hemodonation Center (Spain). After mixing, 20 μl of IS was added. Eight levels of concentration (0.25, 0.50, 1, 2, 5, 10, 20 and 50 μg/ml) were used by diluting appropriate working solutions. Four levels of concentration were prepared for QCs (0.25, 2, 10 and 50 μg/ml).

2.6 | Sample preparation

First, 20 μl of IS solution (200 μg/ml) was added to 200 μl of plasma. After mixing, 100 μl of methanol and 100 μl of a 1:2 solution (trifluoroacetic acid, 99%–methanol) were added to precipitate plasma proteins. After this, the sample was vortexed for 10 s and sonicated during 5 min. Then, the sample was centrifuged for 10 min at 8000 g. Supernatant (300 μl) was transferred into an autosampler vial, and 50 μl of the sample was injected into the HPLC system.

2.7 | Method validation

The validation of the LC with UV detection method was performed according to the guidelines for bioanalytical method validation of the US Food and Drug Administration (2018). The following parameters were evaluated: linearity, limit of detection (LLOD), limit of quantification (LLOQ), accuracy, precision, recovery, selectivity, specificity and carry-over.

2.7.1 | Linearity, detection and quantification limits

The linearity of the proposed chromatographic method was examined by analyzing a series of eight concentrations of CFX plus IS in human plasma. Calibration curves were obtained by plotting the ratio peak-area compound/IS against known concentrations of CFX. Two replicates of each level were assayed. The LLOD of CFX was established as the concentration that provides a signal to noise ratio of 3. The LLOQ was accepted as the lowest concentration on the calibration curve that can be determined with acceptable precision (coefficient of variation ≤ 20%).
2.7.2 | Recovery

Recovery tests were analyzed at LLOQ, low, mid and high QCs of 0.25, 2, 10 and 50 μg/ml, respectively, by comparing the analytical results of the extracted samples with extracted blank plasma spiked with the analyte post extraction. Five replicates were tested at each level. Recovery (%) was calculated as mean peak area of extracted plasma samples/mean peak area of extracted blank plasma spiked with the analyte post-extraction (representing 100% recovery) × 100.

2.7.3 | Accuracy and precision

Accuracy and precision were estimated by measuring samples spiked with IS at four concentrations: LLOQ, low, medium and high. Intra-day precision and accuracy were calculated on a single day using five replicates at each concentration level. Inter-day precision and accuracy were evaluated using five replicates at each concentration level over five consecutive days. The accuracy was calculated with the bias expression as (measured concentration – nominal concentration)/nominal concentration) × 100. The results of accuracy should be ±15% of nominal concentration, except for ±20% at the LLOQ. For precision values, the results should be ±15% CV (variation coefficient) and ≤20% for LLOQ.

2.7.4 | Selectivity, specificity and carryover

The selectivity of this method was studied by analyzing six samples of drug-free plasma that were free of interference at the retention times of CFX and the internal standard. The specificity was examined by analyzing blank samples of drug-free plasma that were free of interference at the retention times of CFX and IS. The selectivity of this method was studied by analyzing six samples of drug-free plasma that were free of interference at the retention times of CFX and IS. The selectivity of this method was studied by analyzing six samples of drug-free plasma that were free of interference at the retention times of CFX and IS. The selectivity of this method was studied by analyzing six samples of drug-free plasma that were free of interference at the retention times of CFX and IS.

The peaks corresponding to CFX and IS were obtained at 9.8 and 7.4 min, respectively (Figure 1). The total run time of analysis was 13 min. This analysis time was shorter than those published previously (Denooz & Charlier, 2008; Szlagowska et al., 2010). Calibration curves were plotted by the ratio of area of CFX/IS vs. the nominal concentration (y = 0.00009x) (Table 1). The LLOD and LLOQ values were 0.1 and 0.25 respectively (Table 1), which were lower than those published by other authors using HPLC/UV detection (Barbour et al., 2009; Denooz & Charlier, 2008; Skhirtladze-Dworschak et al., 2019). These values indicate that the proposed method is suitable and has adequate sensitivity for determining CFX concentrations in plasma. The recovery tests were determined at LLOQ, low, mid and high QCs of 0.25, 2, 10 and 50 μg/ml. Five replicates were tested at each level. Mean recovery (± SD) was 93.52 ± 6.23% (CV 6.67%), this value is remarkable for this antibiotic (Table 2) and higher than that reported in another study with a similar sample procedure (Denooz & Charlier, 2008; Szlagowska et al., 2010). Accuracy and precision results are shown in Table 3. Intra- and inter-day precision were evaluated at four levels of reagents and using equipment available in most analytical laboratories. This enhanced and rapid HPLC assay for plasma CFX will allow individual levels of determination in order to rapidly adjust dosing regimens based on pharmacokinetic/pharmacodynamic breakpoints of CFX in critically ill patients. For that purpose, some chromatographic conditions were investigated. Various HPLC analytical methods of CFX have been reported previously using different buffers as mobile phase (Alberellos et al., 2015; Al-Said et al., 2000; Can et al., 2006; Cios et al., 2017; Foord, 1976; Grupta & Stewart, 1986; Signs et al., 1984; Szlagowska et al., 2010; Vercheval et al., 2018; Wolff et al., 2013). However, trifluoroacetic acid/acetonitrile as mobile phase has not been used for CFX determination, although it is a useful eluent previously tested by our research group (Escudero et al., 2011). The advantages of this mobile phase are its fast and simple preparation, and a reduced tendency to precipitate and clog inside the chromatographic system, in comparison with buffer solutions.

Regarding the chromatographic columns, other authors have reported C8 and C6 columns (Toi et al., 2020; Vercheval et al., 2018; Zhang et al., 2014). In addition, C18 columns have been reported as well (Alberellos et al., 2015; Foord, 1976; Grupta & Stewart, 1986; Szlagowska et al., 2010). In our case, 150 and 250 mm length C18 columns were tested and the 250 mm length was chosen to achieve the best resolution. In addition to this, rapid sample preparation was optimized using protein precipitation to obtain the best recoveries. Different reagents were tested, like acetonitrile, methanol, mixtures of them and acidic solvents, but finally, a mixture of trifluoroacetic acid–methanol was chosen for the extraction procedure.

3 | RESULTS AND DISCUSSION

3.1 | Method development

The goal of this paper was to achieve a satisfactory method for determination of CFX in a short run time, consuming small volumes of
QCs concentration (0.25, 2, 10 and 50 μg/mL) and five replicates measurements were recorded. The values of CV precision were <6% for intra-day and <9% for inter-day, which indicate good precision for the proposed method. Intra- and inter-day accuracy were analyzed in the same way and these measurements are shown in Table 3. All of these were ≤12% for both intra- and inter-day accuracy. Good results were reported, showing that the method is reliable, according to previous reported methods (Szlagowska et al., 2010; Wolff et al., 2013).

Six blank plasma samples were analyzed and there were no endogenous interferences at the retention times of CFX and IS.

### TABLE 1 Linearity, detection and quantification limits parameters for cefuroxime in human plasma

| Validation parameters     | Cefuroxime          |
|---------------------------|---------------------|
| Linearity range           | 0.25–50 μg/ml       |
| Slope                     | 0.00009             |
| Intercept                 | 0.00                |
| Correlation coefficient   | 0.9998              |
| Limit of detection        | 0.1 μg/ml           |
| Limit of quantification   | 0.25 μg/ml          |

**FIGURE 1** Chromatograms of cefuroxime in plasma by HPLC: blank plasma (a); and blank plasma spiked with cefuroxime and cephalixin as internal standard (b)
Intra-day and inter-day accuracy (bias) and precision (CV) of cefuroxime (n = 5)

| Nominal concentration (µg/ml) | Mean recovery ± SD | Bias (%) | CV (%) |
|-----------------------------|-------------------|---------|-------|
|                            | Intra-day         |         |       |
| 0.25                       | 0.26 ± 0.01       | 4.97    | 5.87  |
| 2                          | 2.16 ± 0.12       | 8.41    | 5.50  |
| 10                         | 10.91 ± 0.47      | 9.18    | 4.28  |
| 50                         | 52.1 ± 0.87       | 4.37    | 1.66  |
|                            | Inter-day         |         |       |
| 0.25                       | 0.28 ± 0.01       | 11.7    | 4.59  |
| 2                          | 2.20 ± 0.19       | 10.0    | 8.75  |
| 10                         | 10.49 ± 0.58      | 4.9     | 5.6   |
| 50                         | 51.70 ± 1.08      | 2.15    | 2.10  |

TABLE 3 Intra-day and inter-day accuracy (bias) and precision (CV) of cefuroxime (n = 5)

(Figure 1). These chromatograms were compared with spiked plasma samples. Moreover, well-resolved peaks for CFX and IS were observed. There was no significant interference by other drugs that may be administered to these patients observed at the retention times of CFX and the IS. Satisfactory results were obtained indicating the high selectivity and specificity of the method. Finally, the carryover effects have not been reported because, after running a set of samples with high concentrations of CFX, there were no peaks at the same CFX retention time in six blank plasma samples.

4 | CONCLUSIONS

In this work a simple, rapid, economic and sensitive method for determination of cefuroxime by HPLC–UV was developed and satisfactory validated. Furthermore, one of the most important advantages of the proposed method is the fact that it could be applied to clinical studies, routine analyses and pharmacokinetic studies, with a short run time.

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