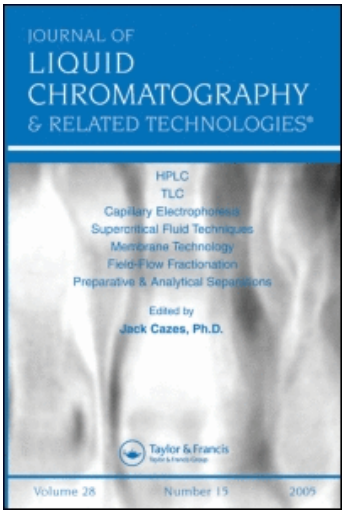


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# Simple Method for the Determination of Cefaclor in Human Plasma Samples by HPLC

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## ABSTRACT

A rapid and simple method for determination of cefaclor in human plasma samples, by high-performance liquid chromatography (HPLC), was developed. This method includes a deproteinization with perchloric acid

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as extraction procedure. Plasma extracts were analyzed on a reversed phase column eluted with a mixture of acetonitrile and 0.01 M sodium dihydrogen phosphate solution, adjusted to pH 6.5 with potassium hydroxide (7 : 93, v/v), and detected by absorbance at 260 nm. Retention times for cefaclor and the internal standard were 5.2 and 7.2 min, respectively. The method was linear in the range of 0.5–30 μg/mL and the detection limit of the method was 100 ng/mL. This method was useful and suitable for determination of cefaclor after oral administration in healthy volunteers, and could be used for bioavailability and bioequivalence studies of the drug.

*Key Words:* Cefaclor; Pharmacokinetics; HPLC; Human plasma.



## INTRODUCTION

Cefaclor is a second generation cephalosporin, which also displays features of a first generation cephalosporin antibiotic. Cefaclor has an important therapeutic efficacy in several infections of respiratory and urinary tract, soft tissues, and other community-acquired infections.[1,2] In addition, cefaclor is well tolerated. In vitro studies have shown that this drug is active against *E. coli*, *K. pneumoniae*, *P. mirabilis*, *H. influenzae*, gonococci, and staphylococci.[3,4]

To our knowledge, there are several reported methods for determination of cefaclor in human samples.[5–11] In some of them, a microbiologic determination was done,[5–8,11] whereas in others, a high-performance liquid chromatography (HPLC) procedure for determination of one or several cephalosporins was carried out.[10,12,13] Microbiological assay quantifies antibiotics according to their antimicrobial activity. However, those methods cannot be considered as specific as HPLC assays. Since the method of Signs et al.[10] was developed for determination of several cephalosporins, it was necessary to use mobile phase gradients to elute the different cephalosporins. In others,[12,13] a solid phase extraction was performed and cefaclor was determined using UV or mass spectrometry detection. In one case, sensitivity of one method is not good enough to follow time course for more than 4 h,[12] whereas, although the other method[13] has a very good sensitivity, it requires the use of expensive equipment not available in most of the laboratories.

In the present study, we have developed a simple, cheap, and rapid method to detect cefaclor in human plasma samples by HPLC. In addition, the method has been tested for determination of cefaclor after administration of therapeutic doses of this antibiotic to 6 volunteers.



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## EXPERIMENTAL

### Reagents and Solutions

Cefaclor (Fig. 1) was provided by Merck Mexico S.A. (Mexico City), whereas, 2-acetamidophenol (internal standard) was purchased from Sigma Chemical Co. (St. Louis, MO). Methanol and acetonitrile were chromatographic grade (Merck, Darmstadt, Germany). Deionized water was obtained through a Mill-Q system (Continental Water Systems, El Paso, TX). All other reagents were of analytical grade.

Stock solutions of cefaclor corresponding to 1 mg/mL were prepared by dissolving cefaclor in water and the internal standard (2-acetamidophenol) in methanol. Standard solutions were prepared by diluting the stock solution, ranging from 0.5 to 30 μg/mL in water. A standard solution of internal standard was prepared at a fixed concentration of 25 μg/mL in water. The 0.1 M sodium acetate (pH 5.2) and 0.01 M sodium dihydrogen phosphate solutions, adjusted to pH 6.5 with potassium hydroxide were prepared in deionized water.

### Extraction Procedure

Human plasma samples (0.5 mL) were pipetted into conical glass tubes of 15 mL and were mixed with 100 μL (25 μg) of 2-acetamidophenol (internal standard). After addition of 600 μL of a mixture of methanol–sodium acetate

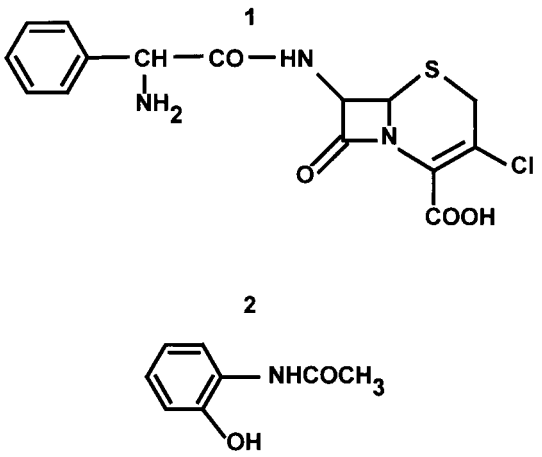


***Figure 1.*** Chemical structure of cefaclor (1) and the internal standard (2), 2-acetamidophenol.





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0.1 M pH 5.2 (70 : 30, v/v) and 20 μL of perchloric acid, samples were agitated in a Vortex (Supermixer) for 1 min at maximal speed and incubated at −20°C during 10 min. Two phases were separated by centrifugation at 4500 *g* for 5 min. The upper layer was directly injected (100 μL) into the chromatographic system.

## Chromatographic System

The chromatographic system consisted of a Model 515 solvent delivery system (Waters Assoc., Milford, MA), a Model 717 autosampler (Waters), a Model 2487 detector (Waters), and the Millenium data acquisition system (Version 2010, Waters).

Elution of the compounds was performed on a 150 × 4.6 mm ID reverse-phase Xterra$^{R}$ $C_8$ column (5 μm particle size, Waters), using a mixture of acetonitrile and 0.01 M sodium dihydrogen phosphate solution, adjusted to pH 6.5 with potassium hydroxide (7 : 93, v/v) as mobile phase at flow rate of 1 mL/min. Effluent from the column was detected by absorbance at 260 nm. All analyses were carried out at room temperature.

## Assay Calibration

Calibration of the method was carried out by addition of known amounts of cefaclor and the internal standard, to drug-free plasma samples that were extracted as described above.

Calibration curves were established in the range of 0.5–30 μg/mL. The internal standard was used at a fixed concentration of 25 μg/mL. The actual sample concentrations were calculated by determination of the peak-height ratios of cefaclor and the internal standard (2-acetamidophenol).

## RESULTS

Typical chromatograms, obtained after injection of plasma extracts into the chromatographic system, are shown in Fig. 2. Retention times for cefaclor and the internal standard were 5.2 and 7.2 min, respectively. No interfering peaks occurred at these times. Any endogenous contaminants remaining in the extracts were eluted before the elution of both the internal standard and cefaclor, and samples could be injected immediately after elution of the internal standard.





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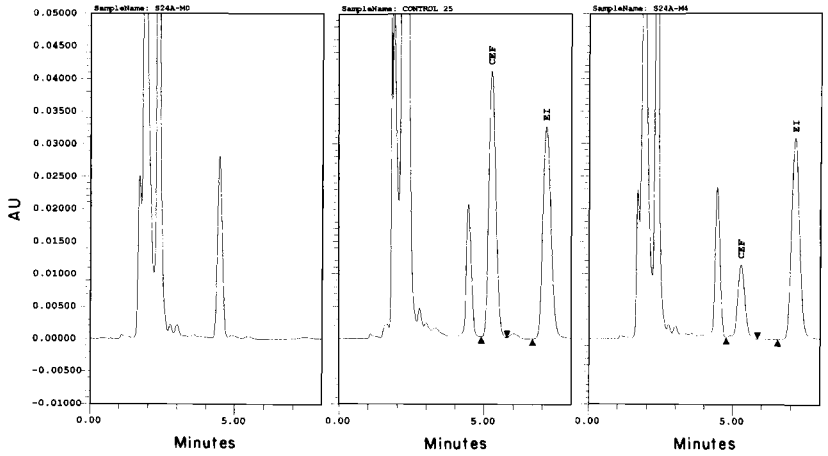


***Figure 2.*** Typical chromatograms resulting after the injection of blood extracts into the chromatographic system. Left—drug-free plasma; center—plasma spiked with 25 μg/mL of either cefaclor (CEF) and the internal standard (EI); and right—plasma obtained from a healthy volunteer 1 h after cefaclor administration of 500 mg.

A linear relationship was obtained when height ratios of cefaclor to internal standard were plotted against cefaclor concentration ranging between 0.5 and 30 μg/mL ($r = 0.9997$), as shown in Fig. 3.

The recovery of cefaclor and the internal standard from plasma samples was similar for both compounds, $94.7\% \pm 3.2\%$ and $96.3\% \pm 2.5\%$ for cefaclor and the internal standard, respectively, by comparison of peak heights from plasma extracts with those from standard solutions. The intra-day accuracy and precision were evaluated, by sextuplicate analysis of drug-free plasma samples to which cefaclor had been added, at concentrations of 2, 8, and 25 μg/mL (Table 1). Inter-day accuracy and precision were evaluated by the same analyst, in duplicate, during three different days (six samples) for the same concentrations employed in the intra-day evaluation (Table 1). In both cases, coefficient of variation was between 2.35% and 9.08%. Detection limit of the method with a signal-to-noise ratio of 3 was 0.1 μg/mL.

Stability of cefaclor and 2-acetamidophenol (internal standard) was assessed in solution and plasma samples. Both drugs were stable after 1 month of freezing at $-40^{\circ}$C in both solution and plasma samples. Stability of extracted samples was evaluated at room temperature in the autosampler, and no degradation of compounds was observed after 24 h.

To evaluate the usefulness of the method for determination of cefaclor after oral administration, 6 healthy men received 500 mg of cefaclor, and

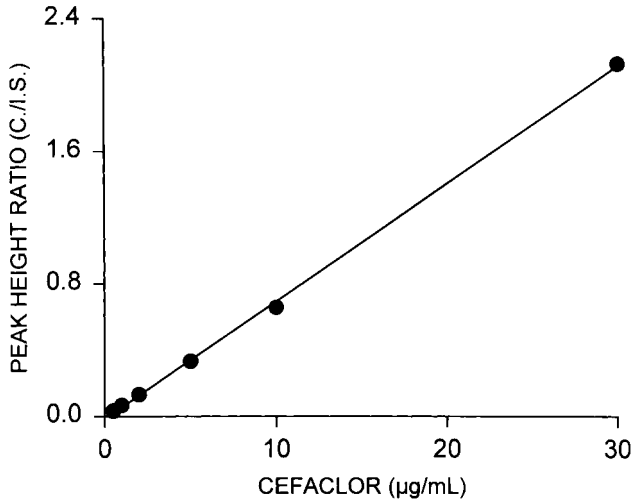


***Figure 3.*** Calibration curves of cefaclor in human plasma samples established in the range of 0.5–30 μg/mL. Data are expressed as the mean of 6 determinations ± SEM.

plasma samples were obtained at selected times. The time course of cefaclor concentrations is shown in Fig. 4. Pharmacokinetic parameters obtained in those subjects were maximal concentration ($C_{max}$) of 11.45 ± 1.6 μg/mL, time to reach $C_{max}$ ($t_{max}$) 0.87 ± 0.055 h, and area under the plasma levels against-time curve (AUC) of 14.93 ± 1.64 μg h/mL. These parameters are

***Table 1.*** Accuracy and precision of the method for determination of cefaclor in human plasma samples.[a]

| Added concentration (μg/mL) | Found concentration (μg/mL) | Accuracy (%) | Coefficient of variation (%) |
|---|---|---|---|
| Intra-day | | | |
| 2 | 1.986 ± 0.037 | 99.28 | 4.57 |
| 8 | 7.500 ± 0.255 | 93.75 | 8.34 |
| 25 | 24.506 ± 0.235 | 98.02 | 2.35 |
| Inter-day | | | |
| 2 | 2.088 ± 0.077 | 104.40 | 9.08 |
| 8 | 8.568 ± 0.190 | 107.10 | 5.42 |
| 25 | 25.725 ± 0.494 | 102.90 | 4.71 |

[a]Data are presented as the mean ($n = 6$) ± SEM.





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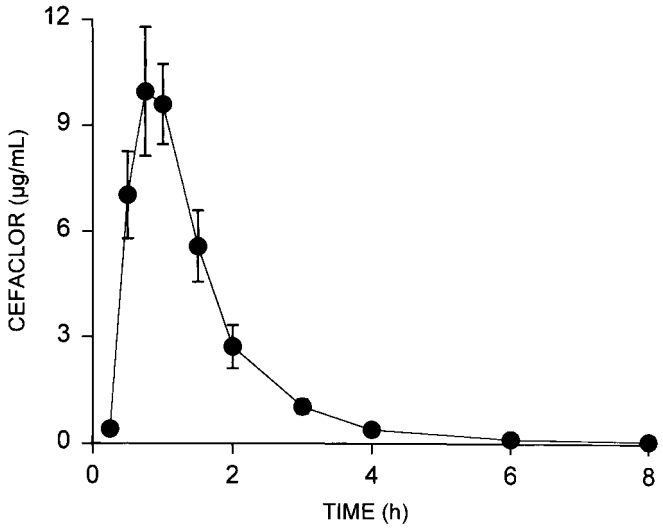


*Figure 4.* Time course of cefaclor plasma levels in healthy volunteers that received a single oral dose of 500 mg. Data are presented as the mean of 6 subjects ± SEM.

similar to those reported in pharmacokinetic studies carried out in other populations.[14]

## DISCUSSION

A new method for determination of cefaclor in plasma samples has been developed. This method is more simple and rapid than those previously reported by HPLC or microbiologic assay. Since a mixture of acetonitrile and 0.01 M sodium dihydrogen phosphate solution, adjusted to pH 6.5 with potassium hydroxide (7 : 93, v/v) was used, it was possible to reduce the pressure in the solvent delivery system. In contrast to other reports, no solvent gradients were used in this study. Deproteinization allowed us measurement of cefaclor without consideration of protein binding, which could affect microbiological assays.[15] Moreover, deproteinization with perchloric acid permitted us to get cleaner samples than those obtained with acetonitrile or methanol only. Additionally, the method was sensitive enough to determine 100 ng/mL. Results of this study show that the method reported here is sensitive and selective for cefaclor determination in human plasma samples. It is economical, as only a deproteinization is required as extraction procedure,





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and only standard UV equipment is required. Moreover, the method was sensitive enough and useful for determination of cefaclor in human plasma samples.

In conclusion, the method reported here is suitable to carry out pharmacokinetic studies in humans after administration of therapeutic doses of cefaclor.

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