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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF CEFIXIME AND CEFOTAXIME IN HUMAN PLASMA

Dragica Zendelovska[1], Trajče Stafilov[2*], Petar Miloševski[1]

[1]*Institute of Preclinical and Clinical Pharmacology and Toxicology, Medical Faculty, The "Sv. Kiril i Metodij" University, 50 Divizija bb, MK-1000 Skopje, Republic of Macedonia*
[2]*Institute of Chemistry, Faculty of Natural Sciences and Mathematics, The "Sv. Kiril i Metodij" University, POB 162, MK-1001 Skopje, Republic of Macedonia*
email: trajcest@iunina.pmf.ukim.edu.mk

A simple high-performance liquid chromatographic method is reported for the simultaneous determination of plasma concentrations of cefixime and cefotaxime. Plasma samples were treated with trichloroacetic acid to precipitate protein. Investigated drugs were resolved on a C-8 reverse-phase column, utilizing a mobile phase of 0.025 mol/l KH_2PO_4 (pH 2.2) and methanol (75:25, *V/V*). Ultraviolet detection was carried out for cefixime at 280 nm and cefotaxime at 255 nm. The method was found to be reproducible with a relative standard deviation less than 5.0 % over the concentration range 0.2–12 μg/ml for cefixime and 0.2–50 μg/ml for cefotaxime in plasma samples. The limits of quantification for investigated drugs were 0.2 μg/ml.

Key words: Cefixime; cefotaxime; human plasma; determination; high-performance liquid chromatography

INTRODUCTION

Cefixime and cefotaxime are the third-generation cephalosporin antibiotics. Cefixime is given by mouth in the treatment of susceptible infections including gonorrhoea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary-tract infections. Cefotaxime is given by injection as the sodium salt in the treatment of infections due to susceptible organisms, especially serious and life-threatening infections. They include brain abscess, gonorrhoea, intensive care, meningitis, peritonitis, pneumonia, surgical infections and typhoid fever [1].

In order to monitor plasma levels of cefixime and cefotaxime in patient samples and to carry out pharmacokinetic studies of the drugs, a rapid, sensitive and reproducible method for their determination is required. There are several investigations concerning the determination of cefixime and cefotaxime in pharmaceutical preparations by a high performance liquid chromatography (HPLC) with UV detection [2] or with a high-performance thin layer chromatography [3]. Marrtinez et al. [4] reported a spectrophotometric method for determination of cefotaxime based on derivatization with 1,2-naphthoquinone-4-sulphonate into solid-phase cartridges and its application to pharmaceutical and urine samples.

Various high-performance liquid chromatographic (HPLC) methods have been reported for the determination of cefixime [5–7] and cefotaxime [8–14] in biological fluids. Liu et al. [5] investigated the possibility of determination of cefixime in plasma and urine using HPLC column switching technique. This method is sensitive but it takes time, great reagent consumption and HPLC system with two pumps.

Many authors have performed the determination of cefixime or cefotaxime in plasma or serum after protein precipitation [6–13] using trichloroacetic acid [6], acetonitrile [7, 9], 2-propanol [11] or mixtures of cold methanol-sodium acetate [12] and chloroform-acetone [13]. Some of these previ-

ously published methods yielding poor separation of cefixime and cefotaxime due to the plasma and serum endogenous interference require an evaporation step and they are time consuming. On the other hand, Kraemer et al. [14] have performed the separation of cefotaxime from plasma with a solid-phase extraction technique using ciprofloxacine as an internal standard. This method using solid-phase extraction technique is also sensitive but needs an internal standard and therefore they are more complicated for the analysis.

In this paper we report a simple, sensitive, and an easy HPLC method to operate using UV detection for the simultaneous determination of cefixime and cefotaxime in the human plasma. Our aim was to develop a method that allowed determination of each analyte without the need from developing separate and distinct methods for each agent. In order to fulfil the aim, the method was first developed for the separation and determination of cephalosporine concentrations by optimising the experimental parameters and determining linearity for the investigated drugs. Then we validated the method for the determination of cefixime and cefotaxime concentrations by evaluating recovery, selectivity, linearity, precision and accuracy. Finally, the method was used for determination of cefixime in the plasma samples obtained from healthy volunteers.

EXPERIMENTAL

Materials

All chemicals and reagents used were of a HPLC grade or an analytical grade. Cefixime was kindly supplied by De Graaf sa (Switzerland). Cefotaxime was purchased from Sigma (Germany). Methanol was obtained from Acros Organics (Belgium). Other chemicals of the reagent grade and solvents of analytical and the HPLC grade were purchased from Merck (Germany).

Instrumentation

HPLC was performed using a Perkin-Elmer liquid chromatography system (USA) consisting of a pump PE LC series 200, autosampler PE LC ISS Series 200, diode array detector PE LC 235 C and a column oven PE model 101. The chromatographic system is controlled by software package Turbochrom Version 4.1 plus and UV-spectrometric data are produced by the program TurboScan Version 2.0.

Chromatography

With respect to the location and shape of the peaks of cefixime and cefotaxime in the corresponding chromatograms, a series of parameters, including composition and pH of mobile phase (from 2 to 7), column packing, flow rate and detection wavelength, were tested. A set of column packing including C8, C18 and RP-select B with different lengths and particle sizes were tested. The final choice of the stationary phase giving satisfying resolution and run time was Hibar Lichrospher 100 RP 8 (250 × 4 mm) with 5 μm particle size, protected by a guard column Lichrospher 100 RP 8 (5 μm). A series of aqueous mobile phases containing buffer solutions with different pH values in combination with different modifiers including acetonitrile, methanol and triethylamine with different volume fractions were also tested. The results were most satisfactory when the mobile phase consisted of 0.025 mol/l KH_2PO_4 with pH 2.2 and methanol in volume fractions 75:25. pH of buffer solution (2.2) was adjusted by adding a very small amount of concentrated ortho-phosphoric acid. The mobile phase was filtered and degassed with helium. The column was termostated at 25°C. A flow-rate of 1.2 ml/min was employed with ultraviolet detection at 255 nm and 280 nm.

Stock solutions and plasma samples

Working stock solutions for cefixime and cefotaxime were prepared at a concentration of 1 mg/ml by dissolution of appropriate amount of cefixime in methanol and cefotaxime in water. These solutions were prepared monthly and stored at +4 °C. No change in stability over the period of 1 month was observed. The working solutions were prepared by diluting appropriate portions of these solutions with distilled water.

Typical calibration curves were constructed with six blank plasma samples spiked with appropriate amounts of the standard solutions. The calibration range was 0.2–12.0 μg cefixime and 0.2–50.0 μg cefotaxime per ml of plasma. The standard

samples were prepared according to the procedure as unknown samples. The calibration curves were obtained by plotting the peak height of cefixime and cefotaxime versus the concentration of cefixime and cefotaxime in μg/ml. The regression equations were calculated by the least-squares method.

Quality control (QC) samples were prepared at low, medium and high levels in the same way as plasma samples for calibration.

Sample preparation

Plasma samples were thawed at 20 °C for about 10 minutes. A 250 μl volume of the sample was transferred into a vial and vortexed with 250 μl of 6 % trichloroacetic acid for 30 s. After centrifugation (5 min, 4000 rpm), a 100 μl portion was injected on to the HPLC column.

RESULTS AND DISCUSSION

A typical chromatogram of standard solutions with concentrations of 3.0 μg/ml of cefixime and cefotaxime produced by the developed HPLC method is shown in Fig. 1 (b). The elution sequence and retention times were 7.5 min for cefotaxime and 9.4 min for cefixime.

During assay development, several solvents namely, trichloroacetic acid, perchloric acid, and acetonitrile were used to precipitate protein in plasma samples in order to obtain satisfactory values for recovery of cefixime and cefotaxime and chromatograms without any interfering peaks from the matrix. Results from this investigation show that the satisfactory values for the recovery of cefixime and cefotaxime (89.6 – 95.2 %) were obtained when plasma samples were prepared using trichloroacetic acid (6 %). It was found that preparation of plasma samples with trichloroacetic acid provides the cleanest chromatogram.

Under the chromatographic conditions described, cefixime and cefotaxime peaks were well resolved. Endogenous plasma components did not give any interfering peaks. Typical chromatograms of blank plasma in comparison to spiked samples are shown in Fig. 1 (a, c).

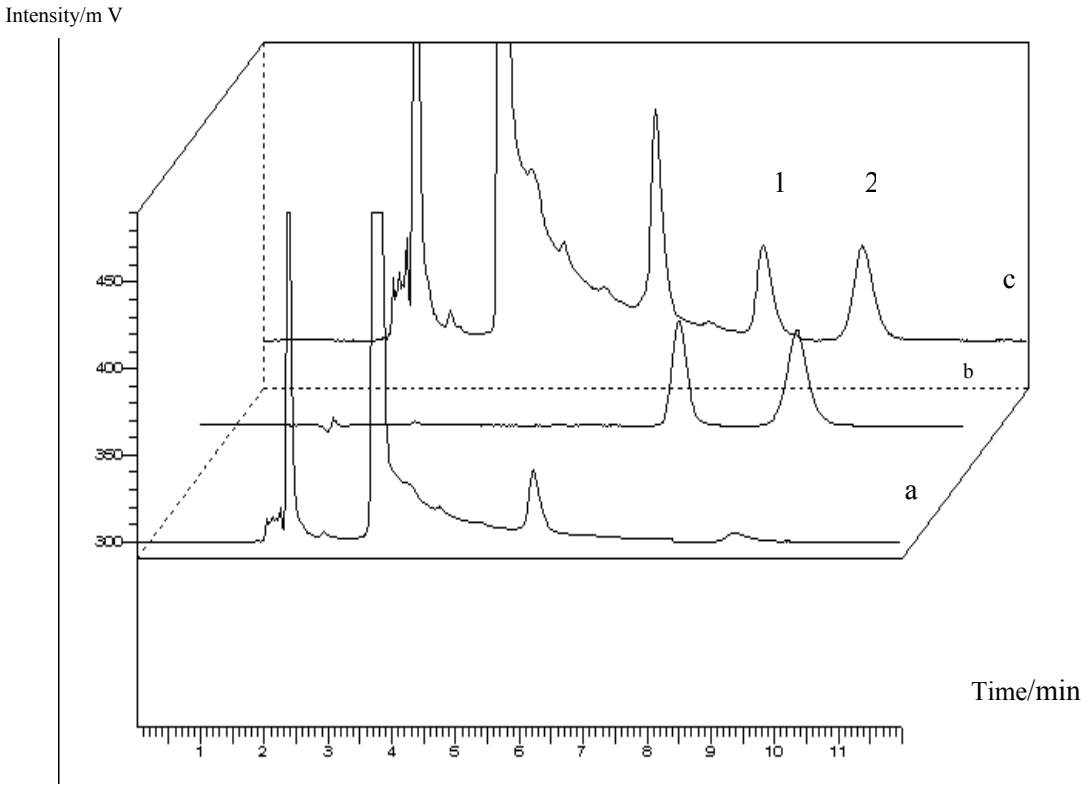

Fig. 1. Chromatograms of blank plasma sample (a), standard solution of cefixime and cefotaxime (b) and spiked plasma sample (c) containing 4.0 μg/ml cefotaxime (1) and cefixime (2)

The developed HPLC method was used for the analysis of plasma samples from healthy volunteers after oral administration of cefixime. Typical chromatograms of plasma samples from healthy volunteers before (a) and after (b) administration of 400 mg cefixime are shown in Fig. 2.

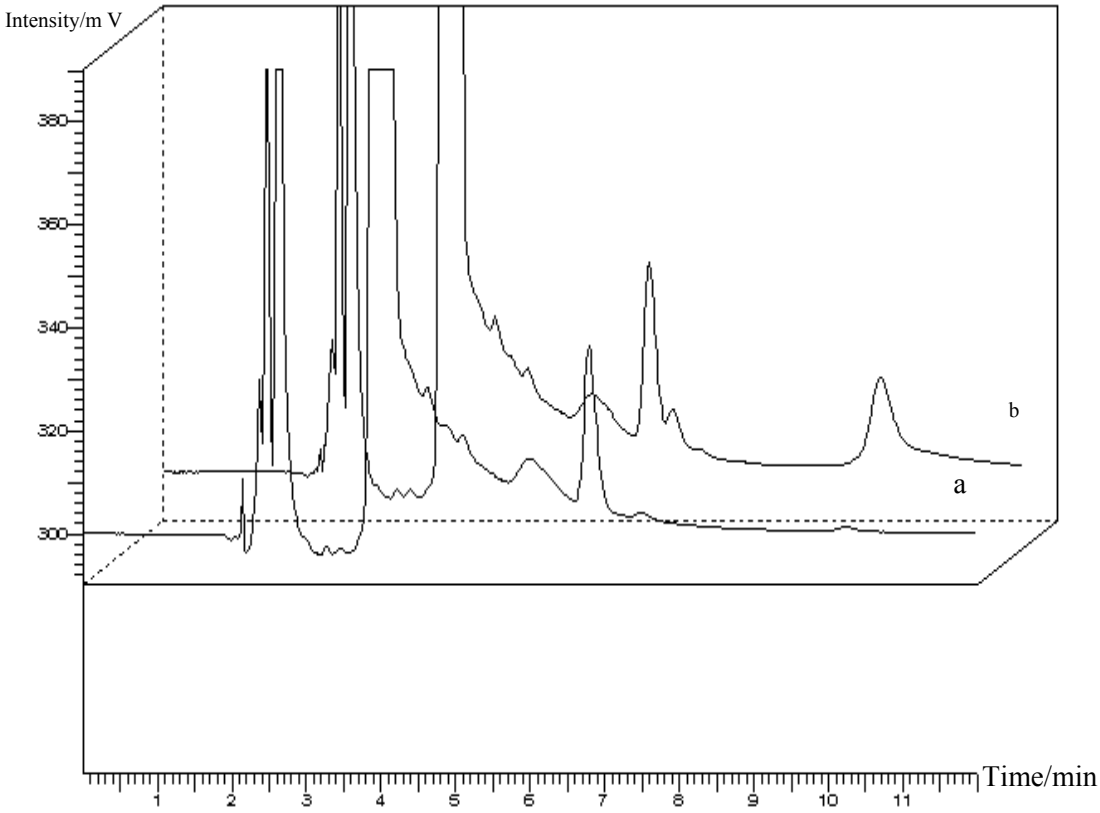

Fig. 2. Chromatograms of plasma samples from healthy volunteers before (a) and after (b) administration of 400 mg cefixime

Analytical performances

Calibration standards and spiked quality control samples of cefixime and cefotaxime were prepared by the spiking blank human plasma with known amounts of cefixime and cefotaxime. Linearity was tested in three different days at six concentration points ranged from 0.2 to 12.0 μg/ml of cefixime and 0.2 to 50.0 μg/ml of cefotaxime in plasma samples. Respective regression equations were: for cefixime $y = 4941.9 \cdot \gamma - 931.92$ and $y = 3120.6 \cdot \gamma - 225.93$ for cefotaxime. The correlation coefficients were 0.9975 and 0.9987, respectively.

The intra-day precision was determined by measuring individually prepared three series of spiked plasma samples at six different concentration levels of cefixime and cefotaxime and the results are given in Table 1. Relative standard deviations at all six concentrations studied were less than 4.55 %, illustrating the precision of the method for routine purposes. The inter-day precision was also determined by measuring three series of spiked plasma samples at six different concentration levels of investigated drugs in three different days. The inter-day variations of the method throughout the linear range of concentrations are shown in Table 1. As it can be seen from the results presented in Table 1, for the inter-day precision, RSDs ranged from 0.83 to 4.35 % for cefixime and 2.18 to 4.55 % for cefotaxime. These data indicate a considerable degree of precision and reproducibility for the method both during one analytical run and between different runs.

The intra- and inter-day accuracy was determined by measuring plasma quality control samples at low, middle and high concentration levels. An indication of accuracy was based on the calculation of the relative error of the mean observed concentration as compared to the nominal concentration. Accuracy data are presented in Table 2. The relative errors of –0.67 to 6.0 % for cefixime and –1.5 to –3.0 % for cefotaxime indicate that the method has sufficient accuracy for the plasma samples.

Table 1

Intra- and inter-day precision data

Nominal concentration	Intra-day		Inter-day	
	Mean (n = 3) observed concentration	Relative standard deviation	Mean (n = 9) observed concentration	Relative standard deviation
μg/ml	μg/ml	%	μg/ml	%
Cefixime				
0.2	0.22	4.55	0.23	4.35
1.0	0.97	2.06	0.98	3.06
2.0	1.96	1.02	1.99	2.51
4.0	3.98	1.01	3.96	3.03
8.0	7.94	1.39	7.93	1.89
12.0	12.11	0.99	12.11	0.83
Cefotaxime				
0.2	0.22	4.55	0.22	4.55
2.0	1.97	3.55	1.98	4.55
4.0	3.96	3.03	3.92	3.57
10.0	9.90	2.02	9.96	2.21
25.0	24.93	1.16	24.77	2.18
50.0	49.53	1.23	49.39	2.25

Table 2

Intra- and inter-day accuracy data

Nominal concentration	Intra-day		Inter-day	
	Mean (n = 3) observed concentration	Relative error	Mean (n = 9) observed concentration	Relative error
μg/ml	μg/ml	%	μg/ml	%
Cefixime				
0.5	0.52	4.0	0.53	6.0
3.0	2.98	– 0.67	2.96	– 1.33
7.0	6.91	– 1.29	6.90	– 1.43
Cefotaxime				
1.0	0.97	– 3.0	0.97	– 3.0
8.0	7.85	– 1.88	7.88	– 1.5
30.0	29.3	– 2.33	29.21	– 2.63

The limit of quantification was defined as the lowest amount detectable with precision of less than 15 % (n = 5) and accuracy of ±15 % (n = 5). The limits of quantification were found to be 0.2 μg/ml for cefixime and cefotaxime for the plasma samples.

The stability of cefixime and cefotaxime in plasma was investigated using spiked samples at two different concentration levels prepared in duplicate. Spiked samples were analyzed after different storage conditions: immediately, after staying in an autosampler for 2, 12 and 24 hours, after one and two freeze/thaw cycles and after 1 month stored at –20 °C. The results from this investigation show that cefixime and cefotaxime added to plasma samples are stable in different storage conditions.

Ruggedness

Ruggedness was tested on the second HPLC column of the same type by determining linearity, precision and accuracy. Linearity was performed at six concentration points for cefixime and cefotaxime in plasma in the concentration range from 0.2 to 12.0 μg/ml and 0.2 to 50.0 μg/ml respectively. The regression equations were: for cefixime $y = 4880.7 \cdot \gamma - 1015.9$ and for cefotaxime $y = 3258.6 \cdot \gamma - 133.75$. The correlation coefficients were 0.9961 and 0.9981 respectively. Intra-day precision and accuracy were determined by measuring three series of plasma quality control samples. Relative standard deviations at all three concentrations studied for cefixime and cefotaxime were less than 5.2 %. The relative errors ranged from 0.7 to 6.2 % of the nominal concentrations of investigated drugs. As it can be seen, the results of this assessment are very similar to those obtained by the previous investigation on the first HPLC column. That means that this HPLC method for determination of cefixime and cefotaxime in spiked human plasma samples is rugged.

CONCLUSION

In summary, this assay procedure provides an economical and accurate method for simultaneous determination of cefixime and cefotaxime in plasma samples. The validation data demonstrate good precision and accuracy, which prove the reliability of the proposed method. The method can also be used to monitor cefixime and cefotaxime levels in clinical samples.

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Р е з и м е

ПРИМЕНА НА ВИСОКОЕФИКАСНАТА ТЕЧНА ХРОМАТОГРАФИЈА ЗА СИМУЛТАНО ОПРЕДЕЛУВАЊЕ НА ЦЕФИКСИМ И ЦЕФОТАКСИМ ВО ПЛАЗМА

Драгица Зенделовска[1], Трајче Стафилов[2*], Петар Милошевски[1]

[1]Институт за претклиничка и клиничка фармакологија и токсикологија, Медицински факултет, Универзитет „Св. Кирил и Методиј“, 50 Дивизија бб, 1000 Скопје, Република Македонија
[2]Институт за хемија, Природно-математички факултет, Универзитет „Св. Кирил и Методиј“, п. фах 162, МК-1001 Скопје, Република Македонија
**e-mail: trajcest@iunona.pmf.ukim.edu.mk*

Клучни зборови: цефиксим; цефотаксим; плазма; определување; високоефикасна течна хроматографија

Предложен е едноставен метод за симултано определување на цефиксим и цефотаксим во хумана плазма со високоефикасна течна хроматографија. Примероците од плазма се третираат со трихлороцетна киселина за таложење на протеините. Испитуваните лекови се издвоени на реверзно-фазна колона С-8, со примена на мобилна фаза од 0,025 mol/l KH_2PO_4 (pH 2,2) и метанол (75:25, *V/V*). Детекцијата на ултравиолетовиот детектор е вршена на бранова должина од 280 nm за цефиксим и на 255 nm за цефотаксим. Утврдено е дека методот е репродуцибилен со релативна стандардна девијација помала од 5,0 % во концентрационо подрачје од 0,2 до 12 μg/ml за цефиксим и од 0,2 до 50 μg/ml за цефотаксим во примероци од плазма. Границата на квантификација на методот за испитуваните лекови изнесува 0,2 μg/ml.