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High-performance liquid chromatographic method for the determination of cefpodoxime levels in plasma and sinus mucosa

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Abstract

A selective HPLC method is described for the determination of cefpodoxime levels in plasma and sinus mucosa. Sample preparation included solid-phase extraction with a C_8 cartridge. Cefpodoxime and cefaclor (internal standard) were eluted with methanol and analyzed on an optimised system consisting of a C_{18} stationary phase and a ternary mobile phase (0.05 *M* acetate buffer pH 3.8–methanol–acetonitrile, 87:10:3, v/v) monitored at 235 nm. Linearity and both between- and within-day reproducibility were assessed for plasma and sinus mucosa samples. Inter-assay coefficients of variation were lower than 13.6% ($n = 10$) for plasma (0.2 μg/ml) and lower than 12.4% ($n = 5$) for sinus mucosa (0.25 μg/g). The quantification limit was 0.05 μg/ml for plasma and 0.13 μg/g for tissue. The method was used to study the diffusion of cefpodoxime in sinus mucosa.

1. Introduction

The prodrug cefpodoxime proxetil is the esterified form of cefpodoxime, an active third-generation cephalosporin. The low dosages used (200 mg) make blood and tissue levels of cefpodoxime difficult to measure. Conventional extraction and analysis methods are not sensitive enough to determine third-generation cephalosporins in plasma in pharmacokinetic studies, which require detection levels as low as 0.1 μg/ml [1,2]. Liquid–liquid extraction techniques are not readily applicable, because cefpodoxime is a highly polar compound (due to the presence of carboxylic and amino-thiazolyl groups), while extraction methods based on protein precipitation are not specific and can only be used to determine concentrations over 0.16 μg/ml [3]. A method involving deproteinization with a subsequent clean-up extraction step has been proposed [4,5], but in our experience its performance is limited by the lack of internal standard. Liquid–solid extraction combined with liquid chromatography [6] or on-line column switching [7], is highly sensitive but requires sophisticated and expensive equipment.

We have developed an accurate and sensitive high-performance liquid chromatographic (HPLC) assay to measure plasma and tissue levels of cefpodoxime. It includes a solid-phase

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extraction step, an internal standard, and an optimized C_{18} stationary phase–ternary mobile phase system. The method was used for pharmacokinetic studies.

2. Experimental

2.1. Materials

Cefpodoxime (sodium salt of the free acid, RU 51763) was obtained from Roussel Uclaf (Romainville, France). The internal standard (cefaclor) was obtained from Lilly (Saint-Cloud, France). Stock solutions (100 mg/l) of both compounds were prepared in water and frozen at −20°C in glass tubes. UV-grade acetonitrile and methanol were obtained from Merck (Nogent sur Marne, France) and Carlo Erba (Rueil Malmaison, France), respectively. Analytical grade sodium acetate, glacial acetic acid, phosphoric acid (85%), and perchloric acid were obtained from Prolabo (Paris, France). Acetate buffer pH 3.8 was prepared from 0.05 *M* acetic acid and 0.05 *M* sodium acetate.

2.2. Chromatography

The HPLC system consisted of a Shimadzu LC 6A pump (Touzart et Matignon, Vitry sur Seine, France), an Ultra Wisp 715 injector (Waters-Millipore, Saint Quentin en Yvelines, France), and a Model 481 Lamda Max UV detector set at 235 nm (Waters-Millipore). Data were collected using Baseline Maxima 810 software (Waters-Millipore). Chromatography was performed on a Supelcosil LC 18 column (250 × 4.6 mm I.D., 5 μm particle size; Supelco, Saint Germain en Laye, France) protected by a C_{18} precolumn (20 × 4.6 mm I.D., 5 μm particle size; Shandon, Eragny, France). The mobile phase was 0.05 *M* acetate buffer (pH 3.8)–methanol–acetonitrile [87:10:3 (v/v) for plasma analysis and 86:12:2 (v/v) for tissue analysis]. The flow-rate was 1 ml/min. Separation was performed at room temperature.

2.3. Calibration

Calibration curves for tissue and plasma were constructed using cefpodoxime standards prepared daily. Plasma standards were prepared by adding aliquots of an aqueous solution of cefpodoxime (either 1 or 10 mg/l) to cefpodoxime-free human plasma to give final concentrations of 0, 0.1, 0.2, 0.5, 1.0, 2.0 and 3.0 μg/ml.

Tissue standards were prepared by adding aliquots of an aqueous solution of cefpodoxime to 20 mg of cefpodoxime-free sinus mucosa to give final concentrations of 0.25, 0.5, 0.75, 1 and 2 μg/g.

2.4. Controls

Plasma and tissue controls were prepared by adding aliquots of cefpodoxime stock solution to give final concentrations of 0.2, 1, and 2.5 μg/ml for plasma and 0.25 and 1 μg/g for tissue. These samples were stored at −20°C until use.

2.5. Pretreatment of samples

Plasma

Solid-phase extraction of plasma samples was performed on Bond Elut C_8 cartridges (3 ml, 500 mg) (Varian, Harbor City, CA, USA), pretreated with methanol (3 ml) followed by phosphoric acid (2 ml of a 1% solution). Plasma (0.5 ml) was diluted with 1 ml of a solution containing cefaclor (2 μg/ml) in phosphoric acid (1%), and applied to the extraction column. Each cartridge was rinsed with 3 ml of perchloric acid (1%), and eluted with 750 μl of methanol. A 50-μl volume of the methanol eluent was injected onto the HPLC column.

Tissue

Solid-phase extraction was performed on 1-ml Bond Elut C_8 cartridges pretreated with methanol (1 ml) followed by phosphoric acid (1 ml of a 1% solution). Tissues samples were first finely chopped using a scalpel. Then 20 mg was incubated in 500 μl of phosphate buffer (10^{-2} *M*, pH

7.0) and rotated overnight (12 h, +4°C). The tissue suspension in buffer was centrifuged for 10 min at 800 *g*. An aliquot of the supernatant (400 μl) was mixed with 1 ml of internal standard (50 μg/l cefaclor) and applied to the extraction cartridge. The cartridge was rinsed with perchloric acid (1%), and eluted with 150 μl of methanol; 75 μl of the methanol eluent were injected onto the HPLC column.

3. Results

3.1. Plasma

Fig. 1 shows representative chromatograms of drug-free plasma, and plasma from a healthy volunteer who had received cefpodoxime proxetil. Cefpodoxime and cefaclor had retention times of 16.8 and 18.2 min, respectively. No interference from endogenous compounds was observed.

The efficiency of cefpodoxime extraction from plasma was estimated as 95.1% from the mean recovery of cefpodoxime standards (0.2, 0.5, 1, and 3 μg/ml); values obtained in 10 analytical runs are shown in Table 1.

Linear calibration curves were obtained in the 0.1–3 μg/ml range. Standard curves were fitted to a first degree polynomial $y = 0.602x - 0.017$ by weighted linear regression ($1/c$), where y represents the concentration and x the cefpodoxime/cefaclor peak-height ratio. The correlation coefficient was 0.999.

Between-day variation was estimated by analysis of three controls. Coefficients of variation were in the range 3.5–13.6% for cefpodoxime concentrations between 0.2 and 2.5 μg/ml (Table 2).

The detection limit defined as the concentration resulting in a signal-to-noise ratio of 3, was 0.01 μg/ml. The quantification limit, estimated by using a statistical method which fitted the between-day standard deviation (S.D.) of the calibration curve intercept, and defined as the ratio between 3 S.D. and the mean slope, was 0.05 μg/ml.

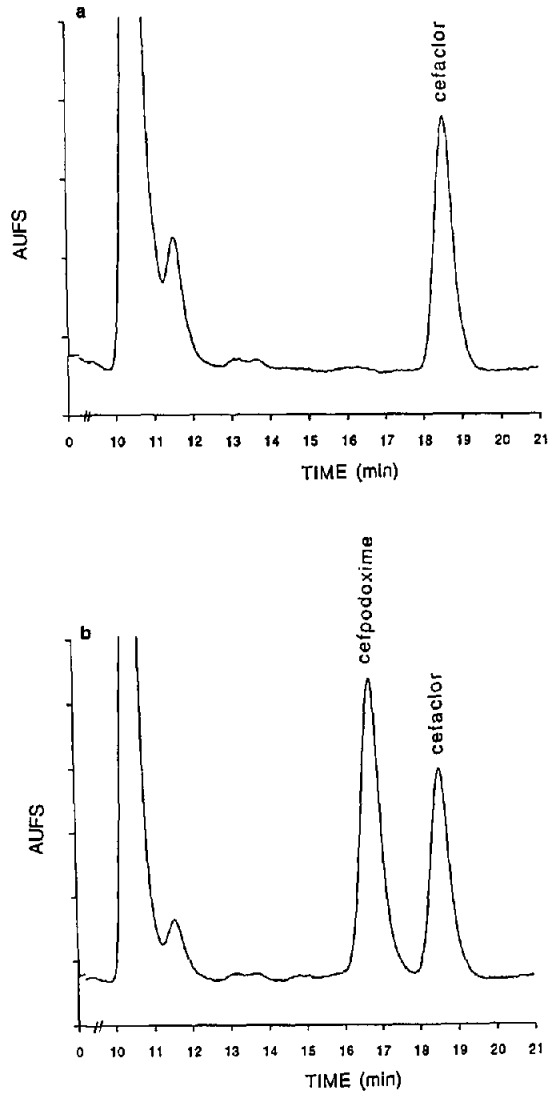

Fig. 1. Chromatograms of plasma extracts. (a) Cefpodoxime-free plasma; (b) plasma from a healthy volunteer who had received an oral dose of 200 mg of cefpodoxime proxetil (observed concentration 2 μg/ml).

3.2. Tissue

A chromatogram of a sinus mucosa sample is shown in Fig. 2. The efficiency of cefpodoxime extraction was around 55% (Table 1).

Linear calibration curves were obtained in the 0.25–2 μg/g range ($y = 1.139x + 0.021$; $r = 0.998$). Between-day coefficients of variation were lower than 12.4% (Table 2), while the

Table 1
Recovery of cefpodoxime from plasma and sinus mucosa samples

Concentration	Recovery (mean ± S.D.) (%)
Plasma (n = 10)	
0.2 μg/ml	99.0 ± 12.9
0.5 μg/ml	93.7 ± 10.1
1.0 μg/ml	93.6 ± 13.6
3.0 μg/ml	94.3 ± 11.8
Sinus mucosa (n = 5)	
0.5 μg/g	56.6 ± 6.3
0.75 μg/g	54.3 ± 8.1
1.0 μg/g	63.4 ± 4.7

quantification limit was 0.13 μg/g for a 20 mg sample.

4. Discussion

Endogenous components of plasma or tissue can interfere with the chromatographic cefpodoxime peak. Reliable measurements require optimisation of the extraction procedure, as well as of the chromatographic and detector systems. Steenwyk *et al.* [7] proposed the use of an automated injector (Advanced Automated Sample Processor, Varian). However, the cartridges in this system suffer from desiccation, and the elution yield may decrease by as much as 20% within 12 h [6]; this makes the system unsuitable for pharmacokinetic studies. Moreover, the method could not be automated using the ASPEC system (Gilson, Villiers le Bel, France), because the internal standard was not stable for more than 12 h in the presence of phosphoric acid (1%). We handled this problem by applying the samples to Bond Elut C_8 columns and eluting with methanol. The introduction of a perchloric acid washing step resulted in improved purification of the plasma samples by eliminating the protein, without affecting the overall yield. The extraction yield from tissues was low. This did not result from poor stability of cefpodoxime during the incubation step but from the small volume of methanol used for elution. The use of a small volume avoided the problem of reproducibility due to the subsequent evaporation step. However, not all interfering compounds were initially eliminated, necessitating optimisation of the stationary phase used in the chromatographic procedure. The Δ2 isomer of cefpodoxime was not tested for interference as

Table 2
Within-day and between-day reproducibility of the method

Target concentration	*n*	Within-day reproducibility		*n*	Between-day reproducibility	
(μg/ml or μg/g)		mean	C.V. (%)		mean	C.V. (%)
Plasma						
0.2	5	0.21	2.0	10	0.21	13.6
0.5	5	0.51	2.8	–	–	–
1.0	5	1.00	2.4	10	1.03	8.3
2.0	5	1.98	2.6	–	–	–
2.5	–	–	–	10	2.55	3.5
3.0	5	3.01	1.1	–	–	–
Sinus mucosa						
0.25	5	0.24	22.3	5	0.24	12.4
0.5	5	0.50	11.2	–	–	–
0.75	5	0.74	6.4	–	–	–
1.0	4	1.03	10.9	5	1.02	7.8
2.0	5	1.99	2.3	–	–	–

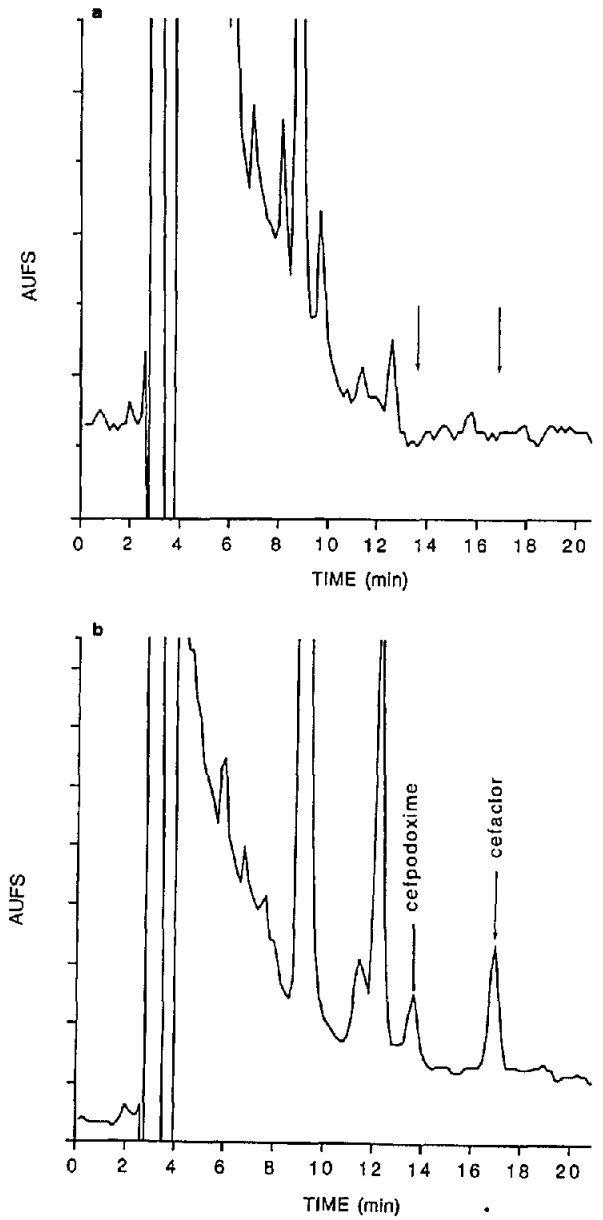

Fig. 2. Chromatograms of tissue extracts. (a) Cefpodoxime-free sinus mucosa; (b) sinus mucosa from a healthy volunteer who had received an oral dose of 200 mg cefpodoxime proxetil (observed concentration 0.4 μg/g).

it was not detectable in plasma samples. Among the seven analytical columns tested (same particle size: 5 μm, but differing the carbon percentage between 7 and 19%), the Supelcosil column gave the most symmetrical peaks, while it also gave satisfactory resolution (better than 1) of the cefpodoxime peak from that of another sample component eluting in the same fraction.

We used a ternary mobile phase as proposed by Steenwyk *et al.* [7], but with some modifications. We found that the pH of the mobile phase and the methanol concentration were the main factors influencing the selective elution of cefpodoxime, cefaclor and other sample components.

Fig. 3 shows the effects of the pH of the mobile phase on the elution of cefpodoxime and cefaclor. The most selective elution of cefpodoxime was found to occur at pH 6.0, but another plasma component coeluted at this pH.

Decreasing the buffer pH to 3.8 gave acceptable selectivity between cefpodoxime and cefaclor. This pH was used for subsequent studies of the influence of the acetonitrile and methanol content on peak separation. At concentrations above 8%, methanol reversed the elution order of cefpodoxime and cefaclor. Increasing the methanol concentration reduced retention times but increased the selectivity between cefpodoxime and cefaclor; acetonitrile had little effect on this latter parameter, but its use was justified by its lower elution strength relative to methanol. We took advantage of these properties to optimize the separation for a tissue matrix.

In conclusion, we have developed a reliable

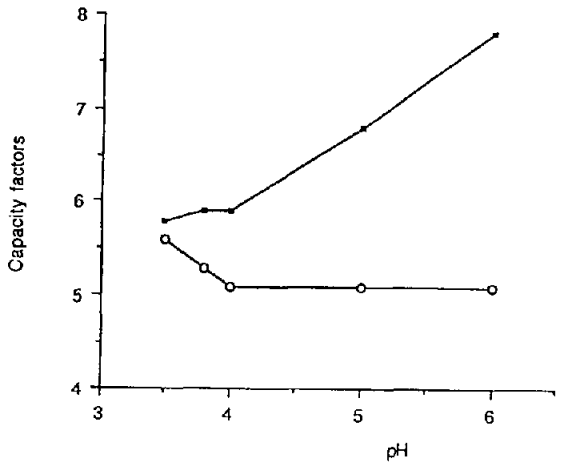

Fig. 3. Effect of the mobile phase buffer pH on the capacity factors of cefpodoxime (○) and cefaclor (■).

HPLC technique for the assay of cefpodoxime. Sensitivity was improved by using detection at 235 nm, *i.e.* the wavelength of the maximum absorbance of cefpodoxime in this mobile phase. Moreover, by studying the effects of various factors on the separation process we were able to adapt the method to meet the needs of pharmacokinetic studies of cefpodoxime in plasma and tissue.

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