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Journal of Chromatography B, 690 (1997) 321–326

JOURNAL OF CHROMATOGRAPHY B

Application of capillary zone electrophoresis in cephalosporin analysis

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Received 20 May 1996; revised 27 August 1996; accepted 9 September 1996

Abstract

Cephalosporins have structures and antibiotic activity similar to those of penicillins which represent a class of compounds with closely related structures. Most of the cephalosporins contain aromatic groups and show distinctive UV spectra. Separating the different types of cephalosporins is a challenging task for HPLC, but the resolving power of capillary zone electrophoresis (CZE) makes this separation fast and simple. The present study reports the application of CZE for cephalosporin analysis and the separation of cephalosporins from plasma. Both field strength and temperature were shown to influence the plate number. The influence of injection time on the peak height was studied. Furthermore, the influence of pH value on the separation of cephalosporins by CZE was investigated. The low sample amount required and the relatively short analysis time are the main advantages of this method.

Keywords: Cephalosporins

1. Introduction

With the development of novel antibiotics, more and more compounds have been found that cannot be absorbed orally and must be administered intravenously or intramuscularly. Cephalosporins such as cefpirom, cefotaxim, cefodizim and cefuroxim are examples of such compounds (Fig. 1). Therefore, interest has been focused on the improvement of the bioavailibility of these drugs [1]. However, first of all, efficient assays have to be developed to analyze these drugs both in pharmaceutical formulations and in biological media. Until now, cephalosporins were only measured using HPLC [2,3] and micellar electrokinetic capillary chromatography (MECC) [4,5]. In biological fluids the determination of cephalosporin antibiotics is frequently performed by microbiological assay procedures [6,7]. Capillary zone electrophoresis (CZE) may be used particularly for hydrophillic compounds. Therefore, it may be well suited for highly water soluble cephalosporins [8]. The aim of the present study was to apply CZE analytic methods to separate and identify cephalosporins such as cefpirom, cefuroxim, cefotaxim and cefodizim, respectively in both water and plasma. The mobilities of cephalosporins were determined at different pH values.

The performance of the CZE approach for the

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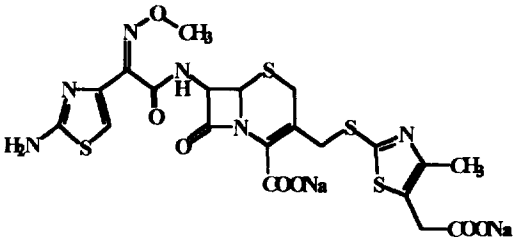
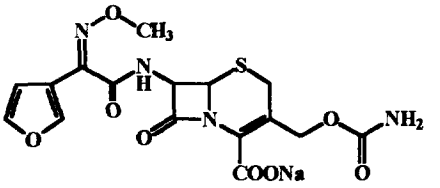
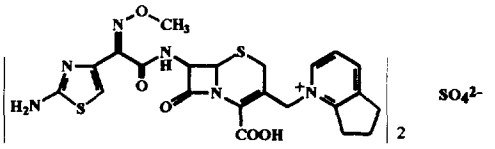
PII S0378-4347(96)00425-2



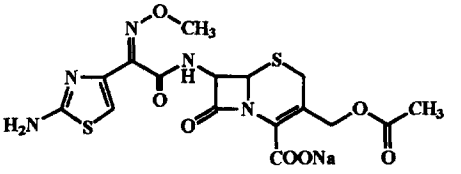
Cefodizim (Cd)



Cefuroxim (Rx)



Cefpirom (Cp)



Cefotaxim (Tx)

Fig. 1. Chemical structures of the compounds.

determination of cephalosporins was tested by measuring the detection limit and standard deviation.

2. Experimental

2.1. Apparatus

Capillary electrophoresis experiments were performed on a Hewlett-Packard Model G1600A ^{3D}CE system (Waldbronn, Germany) with a diode-array detector from 190 to 600 nm. CE ChemStation equipped with a HP Ergo Ultra VGA monitor, and a HP Vectra 486/66U workstation were used for instrument control, data acquisition and data analysis. The system was controlled by Windows software, which was modified to the HP system. The detection wavelengths were 200 and 270 nm. Capillaries (fused-silica) from Hewlett-Packard with a total length of 48.5 cm, length to detector of 40 cm and internal diameter of 50 μm were used for the determination of the mobilities of cephalosporins.

2.2. Chemicals

Cefpirom, cefotaxim, cefuroxim and cefodizim were obtained from Hoechst (Frankfurt/M., Germany). Acetone for chromatography was obtained from Merck (Darmstadt, Germany). CAPS-buffer [3-(cyclohexylamino)-1-propanesulfonic acid] (20 m*M*, pH 10), 20 m*M* phosphate buffer (pH 7–9) and 20 m*M* citrate buffer (pH 2.5–6) were of the highest grade possible and were purchased from Fluka Chemie (Switzerland).

2.3. Sample and buffer preparation

The cephalosporins were dissolved in water. Concentrations ranged from 2 μg/ml up to 100 μg/ml. Samples and buffers were filtered through a 0.2-μm syringe filter and injected immediately into the apparatus. Buffers were degassed by ultrasound for at least 10 min before use.

2.4. Analysis conditions

The capillaries were preconditioned for 10 min with 1.0 *M* NaOH before the first run. Before each run, the capillary was flushed with 0.1 *M* NaOH for 2 min and with running buffer for 5 min. The temperature was kept at 25°C, a separation potential of 30 kV was used and the electrophoretic current was in the range of 40–100 μA. Acetone was used as a marker substance for electroosmosis. The samples [water–acetone (99:1)] were injected at a 50 mbar pressure for 9 s (hydrodynamic injection) with a sample volume of 18.8 nl. Detailed experimental conditions are listed in Figs. 1–6.

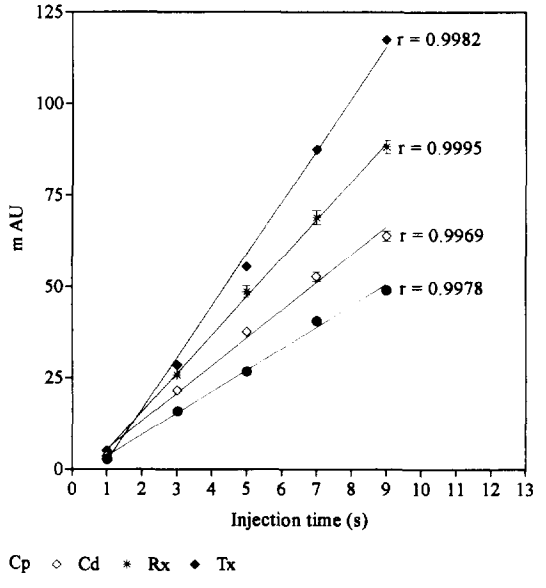


Fig. 2. Influence of the injection time on peak height. Buffer, pH 7.2, 20 m*M* phosphate; capillary, 48.5 (40 cm to detector)×50 μm I.D.; field strength, 30 kV; temperature, 25°C; pressure injection, 9 s at 50 mbar; detection, 200 nm.

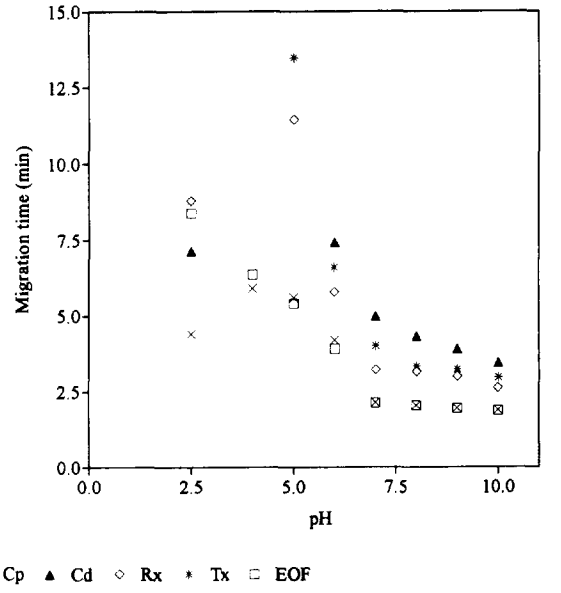


Fig. 3. Influence of pH on the separation of cephalosporins. Buffers: pH 2.5–6, 20 m*M* citrate; pH 7–9, 20 m*M* phosphate; and 20 m*M* CAPS, pH 10. Conditions as in Fig. 2.

3. Results and discussion

3.1. Setup of the method

In this paper we studied the influence of injection time on the peak height. It could be observed that increasing of the injection time led to lowering of the detection limit.

Plots of linearity of the peak height versus in-

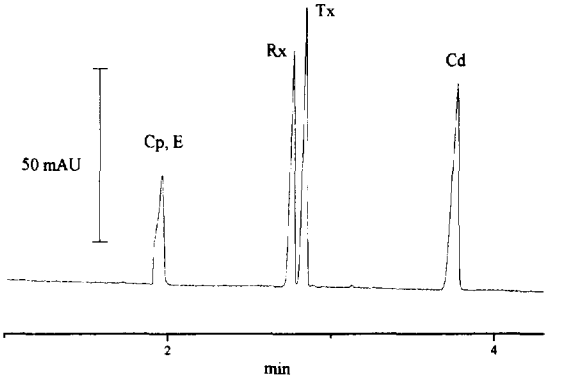


Fig. 4. Electropherogram of a mixture of cephalosporins in 20 m*M* phosphate buffer at pH 7.2, other conditions as in Fig. 2.

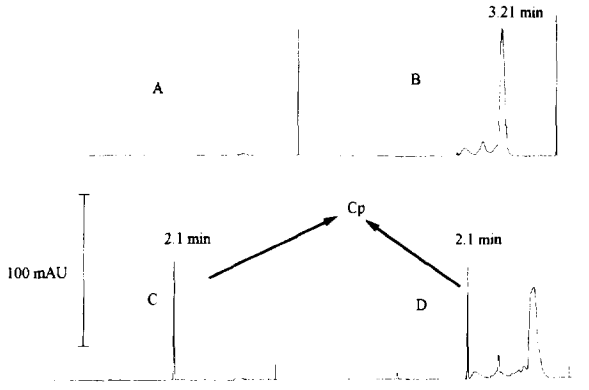


Fig. 5. Electropherogram of plasma. (A,B) Blank plasma at 270 and 200 nm, (C,D) plasma containing 15 μg/ml of cefpirom at 270 and 200 nm, pH 7.2, 20 m*M* phosphate. Conditions as in Fig. 2.

jection time over the range of 1–9 s were made for the four compounds. The correlation coefficients are presented in Fig. 2.

The influence of the pH on the separation of a cephalosporin mixture shown in Fig. 3 was investigated from pH 2.5 to pH 10. The cephalosporins exhibited different separation behavior at various pH values. They could all be detected at pH 2.5 and from pH 6. Concerning separation of the four solutes best results were obtained at pH 6 and 7.2, respectively. Furthermore, it could be observed that at both

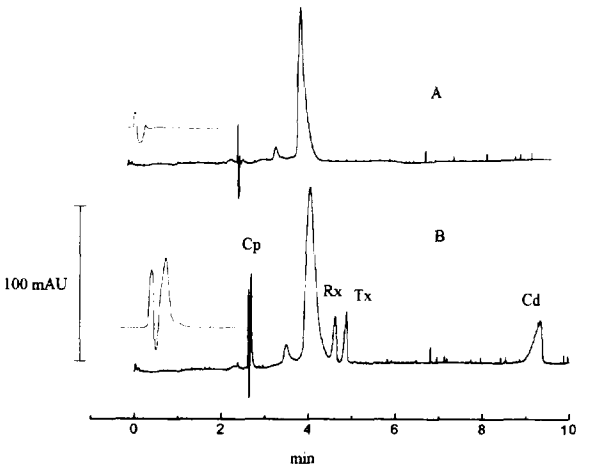


Fig. 6. Electropherogram of plasma. (A) Blank plasma, (B) plasma containing 15 μg/ml each of Cp, Rx, Tx and Cd. Buffer: pH 6, 20 m*M* citrate; detection: 200 nm; conditions as in Fig. 2.

pH 4 and 5 not all of the cephalosporins were detected, indicating that cefodizim, cefuroxim and cefotaxim might have a stronger electrophoretic migration than the electroosmotic flow. Cefuroxim and cefotaxim had similar ionic mobility at pH 2.5 and hence were not resolved at this pH value. On the basis of these results the following conditions were chosen to standardize the separation procedure at pH 7.2 for standard solution and at pH 6 for plasma: 25°C, 30 kV voltage, and 9-s injection time, respectively. Using these conditions all peaks of the cephalosporin mixture could be well resolved at a comparatively short analysis time.

The effective mobilities of cephalosporins were determined at different pH values (Table 1) using the following equation [9]:

$$\mu_e = \frac{L_g L_l}{V}\left(\frac{1}{t_m} - \frac{1}{t_0}\right) \quad (1)$$

where μ_e is the effective mobility, V the applied voltage, L_g the effective capillary length (to the detector), L_l the total capillary length, t_m the migration time of the solute and t_0 the migration time of neutral marker. Cefpirom and cefodizim had a positive electrophoretic mobility at pH 2.5. Assuming the amino groups were ionized in this case, the positive electrophoretic mobility of cefpirom and cefodizim was in the same direction as the electroosmotic flow. At pH 6 cefpirom had a negative electrophoretic mobility. At pH 7 to pH 10 this molecule exhibited a zero electrophoretic mobility and moved with the electroosmotic flow. Cefuroxim and cefotaxim had negative electrophoretic mobilities and migrated in the direction of the cathode.

3.2. Analytical parameters

Tables 2–4 summarize the estimated qualitative and quantitative analytical parameters of the free zone electrophoretic separation of a mixture of water-soluble cephalosporins in aqueous solution and in plasma. Each analyte could be specified by its ionic mobility and its characteristic UV spectrum. The cephalosporins showed two absorption maxima at 200 and 270 nm. To determine the linearity, plots of peak area versus concentration over the range of

Table 1
Effective mobility of cephalosporins at various pH values

pH	μ_e			
	Cp	Rx	Tx	Cd
2.5	11.60±0.20	−1.70±0.065	−1.70±0.065	2.32±0.23
4	1.31±0.02	–[a]	–[a]	–[a]
5	−0.66±0.04	−10.34±0.460	−11.80±0.23	–[a]
6	−1.35±0.08	−17.09±0.991	−18.70±1.460	−24.80±0.843
7	0.0	−16.70±0.875	−20.60±0.854	−28.40±0.675
8	0.0	−18.34±1.500	−20.27±0.604	−27.58±0.210
9	0.0	−19.26±0.032	−21.69±1.090	−27.30±0.697
10	0.0	−17.14±0.776	−20.80±1.590	−26.09±1.01

[a] Not determined (migration times longer than 45 min).
μ_e: Effective mobility (10^{-5} cm^2 V^{-1} s^{-1}). For conditions see Fig. 2.

Table 2
Analytical parameters for the determination of cephalosporins studied at pH 7.2

Compound	μ_e (10^{-5} cm^2 V^{-1} s^{-1})	R.S.D. (f) (%)	Detection limit (μg ml^{-1})
Cp	0	2.40	2
Rx	−17.74±0.12	1.62	0.4
Tx	−18.74±0.22	1.81	0.4
Cd	−27.80±0.40	3.30	1

R.S.D. (f): Relative standard deviation of the peak areas.
Conditions as in Fig. 4.

5–180 μg ml^{-1} were made for a mixture of the four compounds. The regression equations of the curves and correlation coefficients presented in Table 4 were in good accordance. To control the reproducibility of the peak areas and of the migration times, five injections of a mixture of cephalosporins (10 μg ml^{-1}) were made for both media. The detection limits for all analytes were determined at 200 nm using Eq. (2) below (Tables 2 and 3):

$$L = 3N \cdot \frac{C}{h} \quad (2)$$

Table 3
Analytical parameters for the determination of cephalosporins in plasma at pH 6

Compound	μ_e (10^{-5} cm^2 V^{-1} s^{-1})	R.S.D. (t) (%)	R.S.D. (f) (%)	Detection limit (μg ml^{-1})
Cp	−0.70±0.02	4.3±0.74	10.3±4.44	6
Rx	−17.57±0.08	5.2±1.78	4.4±1.70	2
Tx	−18.30±0.82	4.1±1.60	2.3±0.32	2
Cd	−28.70±0.30	8.9±3.24	3.4±0.02	4

R.S.D. (t): Relative standard deviation of the migration times.
R.S.D. (f): Relative standard deviation of the peak areas.
Conditions as in Fig. 6.

Table 4
Regression equations and correlation coefficients for cephalosporins

Compound	Aqueous solution	Plasma
Cp	$y = 0.767x + 2.302$ ($r = 0.9998$)	$y = 0.787x + 0.347$ ($r = 0.9992$)
Rx	$y = 0.152x - 0.099$ ($r = 0.9999$)	$y = 0.154x - 0.729$ ($r = 0.9990$)
Tx	$y = 0.476x + 2.471$ ($r = 0.9994$)	$y = 0.389x + 2.120$ ($r = 0.9994$)
Cd	$y = 0.652x - 0.474$ ($r = 0.9990$)	$y = 0.656x - 0.501$ ($r = 0.9991$)

where N = noise, C = sample concentration, h = peak height.

3.3. Application

CZE is very suitable for analysis of cephalosporins due to their high UV absorption and their very good solubility in water. This is also the case for analysis of cephalosporins present in biological tissues and other body liquids in low concentrations. In aqueous solution the cephalosporins were well separated and determined, although they have similar structures. Fig. 4 shows a typical electropherogram of a mixture of cephalosporins using CZE.

In plasma the cephalosporins were also analyzed quantitatively. The cephalosporins had two UV absorption maxima both at 200 and 270 nm whereas the plasma components exhibited UV absorption only at 200 nm in that range where the cephalosporins were detected indicating that all cephalosporins could be determined at 270 nm (Fig. 5).

The determination of the cephalosporins in plasma was performed at a pH value of 6. The results of these measurements showed that at pH 6 all four cephalosporins were separated from plasma components and detected (Fig. 6). In Table 3 the standard deviation of the peak areas and of the migration times is reported. For measuring the cephalosporins in plasma, the samples for CZE were only diluted in water 1:5 and were injected into the apparatus without any further sample preparation. The direct cephalosporin determination in plasma using CZE has some advantages compared to HPLC [10,11], particularly concerning the sample preparation. Usually, for the determination of cephalosporins in plasma by HPLC, it is essential to precipitate the protein component prior to measurement. Improvement of reproducibility using CZE was achieved by flushing the capillary with washing solutions. Best results were obtained when a freshly prepared sample was used and the capillary was flushed first with 0.1 M NaOH for 5 min and then with separation buffers for 10 min.

4. Conclusion

In this investigation the qualitative and quantitative determination of cephalosporins using capillary zone electrophoresis was studied. It has been demonstrated that this technique is well suited for cephalosporin analysis and has several advantages compared to HPLC such as fast sample preparation and short analysis time. The resolution and the separation of all four cephalosporins in both water and plasma were shown to depend on pH. In water the cephalosporins were well separated at pH 6 and pH 7.2, respectively. In plasma they could be separated at pH 6. They possessed different ionic mobility at different pH values and always migrated in the direction of the cathode. Furthermore, in plasma the drugs could be quantified without protein precipitation what turned out to be the main advantage compared to HPLC.

Acknowledgments

The authors wish to thank the Deutsche Forschungsgemeinschaft for the financial support of the project NE 427/4-1.

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