Martina Petsch[1, 2]
Bernhard X. Mayer-Helm[1]
Robert Sauermann[1]
Christian Joukhadar[1]
Ernst Kenndler[2]

[1]Department of Clinical Pharmacology, Medical University Vienna
[2]Institute of Analytical Chemistry, University of Vienna, Vienna, Austria

Capillary electrophoresis analysis of fosfomycin in biological fluids for clinical pharmacokinetic studies

A feasible capillary zone electrophoresis (CZE) method with indirect UV and contactless conductivity detection was developed for the determination of fosfomycin, an antibiotic, in human plasma and microdialysis samples. Samples were collected from test persons during a clinical trial. The background electrolytes used consisted of 25 mM benzoic acid and 0.5 mM hexadecyltrimethylammonium bromide, adjusted with tris(hydroxymethyl)aminomethane solution to pH 6.95 for plasma, and to pH 8.05 for microdialysis samples. CZE separations of the anionic analyte were carried out with reversed electroosmotic flow directed towards the anode. The limit of detection was between 0.6 and 2 μg/mL, depending on the matrix and the detection method. No sample preparation was needed for microdialysis samples; for plasma samples, proteins were precipitated with methanol (1+2, v+v), and the supernatant was analyzed. The yield determined with spiked samples was about 100%, the reproducibility of the entire method, expressed by the RSD% of three independent determinations of fosfomycin in triplicate after spiking Ringer's solutions and plasma samples, respectively, was better than 8%. The method is thus well-suited for clinical studies for the determination of the antibiotic in biological fluids.

Keywords: Capillary zone electrophoresis / Conductivity detection / Fosfomycin / Indirect UV detection
DOI 10.1002/elps.200305860

1 Introduction

Fosfomycin, (−)-(1*R*,2*S*)-(1,2-epoxypropyl)phosphonic acid (Fig. 1), is an antibiotic that inhibits the synthesis of bacterial cell walls by preventing the peptidoglycan synthesis [1]. Fosfomycin as its disodium salt is widely used for treatment of infections of the central nervous system, of urinary trace infections, and of other infections that are caused by Gram-positive and -negative strains, like those of the soft tissues [2]. Due to its unique structure, which differs from other antibiotics, and its activity on the synthesis of bacterial cell walls, which are unaffected by other agents [1], hardly any cross resistance to other antibiotics is observed. Characteristics of fosfomycin are a very low molecular mass (181.8 g/mol as the disodium salt), pK_{a1} 2.0 [3] and pK_{a2} 6.4 [4], low plasma protein binding (< 5%) [1, 3], and a plasma half-life time of 1.5–2 h [1].

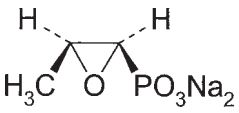

Figure 1. Structural formula of fosfomycin (as disodium salt).

Flow injection spectrophotometric detection after thermal-induced digestion has been proposed for the determination of fosfomycin in biological samples, *e.g.*, in urine; the urine samples had only to be diluted with water prior to analysis. However, the achieved limit of detection (138 μg/mL) was considerably high [5]. Several papers report the determination of this antibiotic in biological samples based on gas chromatographic methods *via* its silylated derivative [6–8]. Compared to commonly used molecular biological methods, *e.g.*, the determination of the inhibitory potency of the drug on bacterial growth in cell culture experiments (cup method), GC-MS determination showed a higher sensitivity [9]. Although offering sufficient sensitivity, the GC methods are often laborious due to the fact that especially biological samples necessitate clean-up steps and derivatization prior to analysis. In contrast, HPLC or capillary electrophoresis (CE) were described for the determination of fosfomycin without need for time-consuming derivatization steps. In this con-

Correspondence: Dr. Bernhard X. Mayer-Helm, Department of Clinical Pharmacology, Medical University Vienna, Waehringer Guertel 18–20, A-1090 Vienna, Austria
E-mail: bernhard.mayer@univie.ac.at
Fax: +43-1-40400-2998

Abbreviations: CCD, contactless conductivity detector; **i. v.**, intravenous

text, Robins *et al.* [10] reported a CE application with direct UV detection of aqueous organophosphonic acid solutions via *in situ* formation of a borate-phosphate ester.

Beside such direct UV detection, for chromatographic determination of small nonabsorbing ions indirect detection methods are more common. These detection methods are based on the addition of a UV absorbing chromophore (probe) to the buffer or the eluent, and the measured signal is generated due to the replacement of the probe by the analyte [11–14]. In this way, fosfomycin was indirectly detected in serum and aqueous humor after separation by ion-exchange chromatography [15] as well as by CE [4, 16]. In both CE papers the accuracy, precision, and linear range are satisfying, but for both methods the background electrolyte (BGE) composition is quite complex, as well as elaborate serum clean-up procedures are needed.

Mass spectrometry in combination with CE was applied for the determination of phosphonic acids in tap water, and the results were compared to an LC-MS method. Characteristic fragment ions were screened by tandem MS and allowed accurate identification of the phosphonic acids [17]. One paper reported the application of flame photometric detection, normally used for GC analysis, in combination with CE, for the determination of alkylphosphonic acids in water [18]. Good detection limits of 0.1–0.5 μg/mL were achieved.

During the last years the application of conductivity detection gained more importance as an alternative detection method for small ions (see *e.g.*, a recent review by Zemann [19]). As described in [20], signal-to-noise ratios for some anions achieved with indirect UV can be enhanced by conductivity detection, provided that optimized electrolyte conditions are used. However, simultaneous optimization for both detection methods seems to be sophisticated [20, 21]. Application of conductivity detection for ion-exchange chromatographic determination of some alkylphosphonic acids and fosfomycin is reported in [22].

To determine the extracellular concentration of drugs or antibiotics, microdialysis techniques were established as very advantageous clinical methods [23–25]. A probe, consisting of a semipermeable membrane with a well-defined molecular mass cutoff, is implanted into the tissue, *e.g.*, skeletal muscle, subcutaneous adipose, brain or lung, and is perfused with a physiological fluid, like Ringer's solution, at a constant flow. The analytes diffuse through the membrane into the perfusate and are collected during time periods of 20–30 min. Microdialysis samples are characterized by lack of proteins, low analyte concentrations, aqueous medium, high ionic strength, and small sample volumes of 2–30 μL, for which CE methods have an advantage over HPLC methods, as only small injection volumes of several nL are needed.

The aim of the present paper was to develop a fast and feasible CE method for the determination of fosfomycin in biological samples, using indirect UV and contactless conductivity detection (CCD). A screening of different experimental conditions, *e.g.*, the type of chromophore used in the BGE and its concentration, the pH and the concentration of EOF reverser, was carried out to derive appropriate separation conditions for the matrices that had to be analyzed, namely human plasma and microdialysates. This method should be more sensitive than the previously reported ones [4, 16], and a more simple and rapid method for plasma pretreatment should be implemented.

2 Materials and methods

2.1 Chemicals

Fosfomycin disodium salt "Biochemie 8 g Trockenstechampulle" was kindly provided by Biochemie (Kundl, Austria). Tris(hydroxymethyl)aminomethane (Tris), benzoic acid (both analytical grade), and methanol (HPLC grade) were purchased from E. Merck (Darmstadt, Germany). Cetyltrimethylammonium bromide (CTAB, >99.0% purity) was purchased from Fluka (Buchs, Switzerland). Ringer's solution ÖAB (concentration: Na^+ 154 mM; Ca^{2+} 2.74 mM; K^+ 4.02 mM; Cl^- 163 mM) was from Mayrhofer Pharmazeutika (Linz, Austria). Ultrapure water with resistance > 18 MΩ·cm was prepared by the use of a Millipore Milli-Q apparatus (Bedford, MA, USA).

2.2 Apparatus

All experiments were performed with a ^{3D}CE apparatus (Agilent Technologies, Palo Alto, CA, USA). For separation an uncoated fused-silica capillary (50 μm ID, total length 64.5 cm, effective length 56.0 cm; Agilent Technologies) with a bubble cell was used. Indirect detection of fosfomycin was performed using a 25 mM benzoate solution with 0.5 mM CTAB added, adjusted with 1 M Tris solution to pH 6.95 for plasma and 8.05 for microdialysis samples, respectively. The background electrolyte solution was degassed by ultrasonication prior to use. The CCD cell (not commercially available for the ^{3D}capillary electrophoresis system used) was kindly donated by B. Gaš (Faculty of Science, Charles University, Prague, Czech Republic). Its construction and design has been described in [26]. This detection cell was already applied for the determination of, *e.g.*, essential amino acids [27] or haloacetic acids

in water [28]. Samples were injected by applying pressure at the cathodic end of the capillary at 50 mbar for 9 s. The capillary cassette was thermostated to 25°C. The applied voltage was set to −25 kV with a ramp of 0.5 min after injection. Indirect UV absorbance detection was carried out using the built-in diode array detector (DAD) operating at 254 nm. New capillaries were conditioned before use with 1 M and 0.1 M sodium hydroxide at 45°C for 15 min each, followed by rinsing with water and buffer solution at 25°C, 15 min each. Before each run the capillary was flushed with 0.1 M sodium hydroxide for 4.5 min, followed by flushing with water for 2 min and buffer solution for 6 min. To prevent buffer depletion during a sequence run, the inlet and outlet buffer vial and the buffer supply vial were replenished before each run by the built-in replenishment system. For storage overnight the capillary was flushed with water, 0.1 M sodium hydroxide followed by water for 3 min each. A similar procedure is also described in [29].

2.3 Procedures

A stock solution of the antibiotic (10 mg fosfomycin/mL) in Ringer's solution was prepared by dissolving approximately 670 mg of fosfomycin disodium salt in 50 mL Ringer's solution. This stock solution was stable for 4 months when stored at −80°C. Standard solutions for the analysis of microdialysis samples or plasma samples were prepared every day by diluting suitable amounts of this stock solution in Ringer's solution. Standards for microdialysis samples were prepared in a concentration range of 0–1000 μg/mL. The calibration standards and microdialysis samples were directly injected without further sample preparation. For generating a calibration curve for plasma samples, drug-free plasma collected in lithium heparinized tubes was spiked 1:10 with fosfomycin in Ringer's solution, leading to a final concentration range of 0–1000 μg/mL. 80 μL of plasma from probands or plasma standards were mixed with 160 μL methanol, vortexed, and centrifuged at 15 000 × *g* for 2 min at room temperature. The supernatant was injected into the CE system. For the quantitation of the analyte the peak areas, for the determination of the LOD and the LOQ the peak heights were taken.

3 Results and discussion

3.1 Selection of the separation condition

As the method is intended to be applied for routine analysis, which means that a large number of samples have to be treated, the analysis time becomes an important parameter, in addition to the robustness, selectivity, sensitivity, reproducibility, and accuracy of the method. In order to shorten the analysis time, the reversed electroosmotic flow (EOF) mode was selected for the anionic analyte under consideration. The EOF can be reversed by addition of a cationic detergent to the BGE like the commonly used CTAB. Using this additive, a number of BGEs with different constituents and/or different pH were investigated concerning their applicability for the analysis of fosfomycin in microdialysates and plasma. In a first step, fosfomycin was spiked to the sample matrices, namely to Ringer's solutions on the one hand, and to the plasma of healthy test persons on the other hand.

Without going into details, it became clear that some of the BGEs were lacking detection performance for both sample types, *e.g.*, 50 mM phthalic acid/Tris, 0.5 mM CTAB, pH 7.8. Others did not fulfil the requirements in precision and accuracy (15 mM phthalic acid/NaOH, 0.5 mM CTAB, pH 8.1; 50 mM phosphate, 15 mM phthalic acid/NaOH, 0.5 mM CTAB, pH 7.5), or a high peak distortion was observed therein (50 mM HEPES, 15 mM phthalic acid/NaOH, 0.5 mM CTAB, pH 7.7). Buffers consisting of 25 mM benzoic acid/Tris, 0.5 mM CTAB, turned out to exhibit good quantitative performance data, but no single pH could be found for the analysis of both sample matrices with both detectors. Therefore, a BGE with pH 8.05 and 6.95 was used for microdialysis and plasma samples, respectively.

In Fig. 2 the electropherograms obtained with 25 mM benzoic acid/Tris, CTAB, pH 8.05 for samples of Ringer's solution spiked with fosfomycin, are shown. The difference

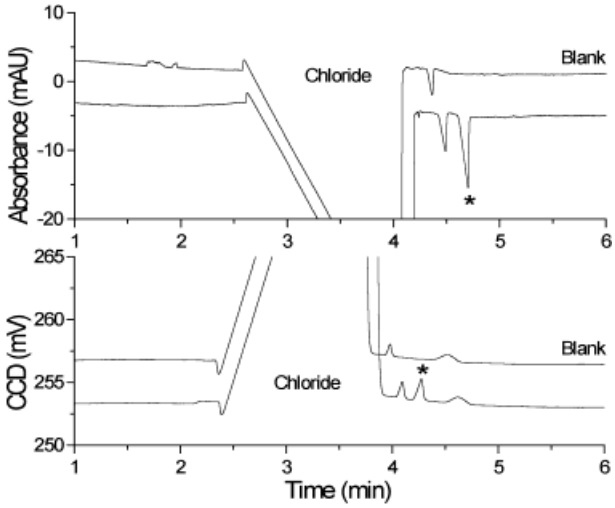

Figure 2. Electropherogram of fosfomycin in Ringer's solution at pH 8.05 with indirect UV absorbance detection (upper trace) and CCD (lower trace). BGE, 25 mM benzoic acid + 0.5 mM CTAB, adjusted with 1 M Tris to pH; fosfomycin concentration, 125 μg/mL; capillary, uncoated fused-silica capillary (50 μm ID, total length 64.5 cm, effective length to UV 56.0 cm; effective length to CCD, 50.5 cm) with bubble cell; voltage, −25 kV; temperature, 25°C. Fosfomycin peak is marked with an asterisk.

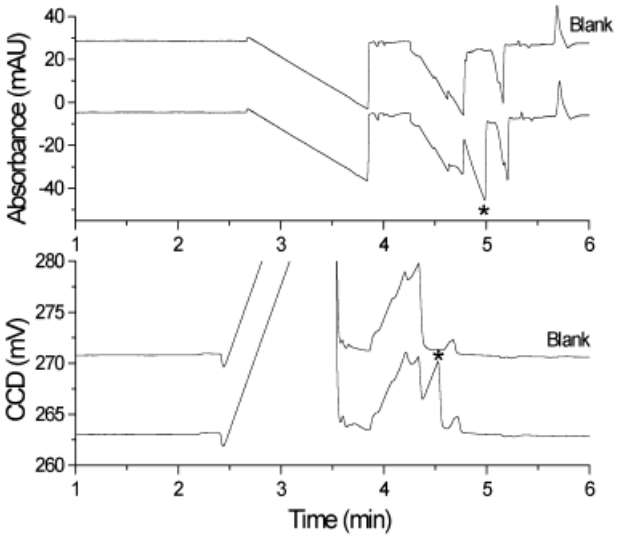

Figure 3. Electropherogram of human plasma blank and plasma spiked with fosfomycin at pH 8.05 with indirect UV absorbance detection and CCD. BGE, 25 mM benzoic acid + 0.5 mM CTAB, adjusted with 1 M Tris to pH. Fosfomycin concentration 1000 μg/mL. Other conditions as in Fig. 2. Fosfomycin peak is marked with an asterisk.

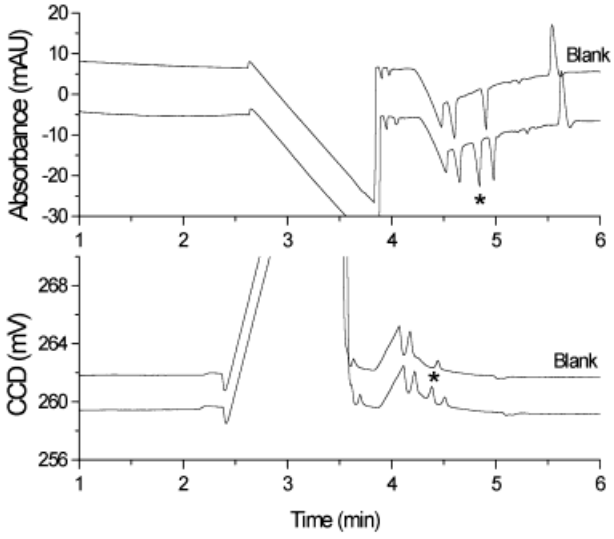

Figure 4. Electropherogram of human plasma blank and plasma spiked with fosfomycin at pH 6.95 with indirect UV absorbance detection and CCD. Fosfomycin concentration, 125 μg/mL; BGE, 25 mM benzoic acid + 0.5 mM CTAB, adjusted with 1 M Tris to pH. Other conditions as in Fig. 2. Fosfomycin peak is marked with an asterisk.

in migration times of fosfomycin of approximately 0.5 min between UV and CCD detection is caused by the arrangement of the detectors. It is built into the cassette, positioned 5.5 cm in front of the UV detection window, as described in [26]. It can be seen that the analyte peak can clearly be distinguished from other sample constituents. Even the extreme excess of chloride in the solution, which forms the large triangular peak typical for concentration overload, does not interfere.

The BGE with pH 8.05 is not suitable for the determination of fosfomycin in spiked plasma samples, as can be seen from Fig. 3, showing the electropherograms of human plasma blank and spiked plasma. With this matrix the analyte peak is found on the rear flank of a large peak, and quantitation will be hampered thus. However, reduction of the BGE to pH 6.95 shows the clear separation of the analyte from all other peaks present (Fig. 4).

The BGE with pH 6.95, suitable for the separation of fosfomycin in the spiked plasma samples is, in contrary, not fully suitable for Ringer's solution as sample matrix, because at this pH one contaminant is migrating closely after the analyte, from which it is not fully resolved (Fig. 5). This is observed especially with the CCD record. It can thus be concluded that there is no single BGE, which can be used for the determination of fosfomycin in both types of matrices. We have to apply two different buffers, with best conditions at pH 6.95 for the plasma samples, and at pH 8.05 for the microdialysates. These BGEs will be further used for the analysis of real samples. Exact pH adjustment is important due to pH sensitive migration behavior.

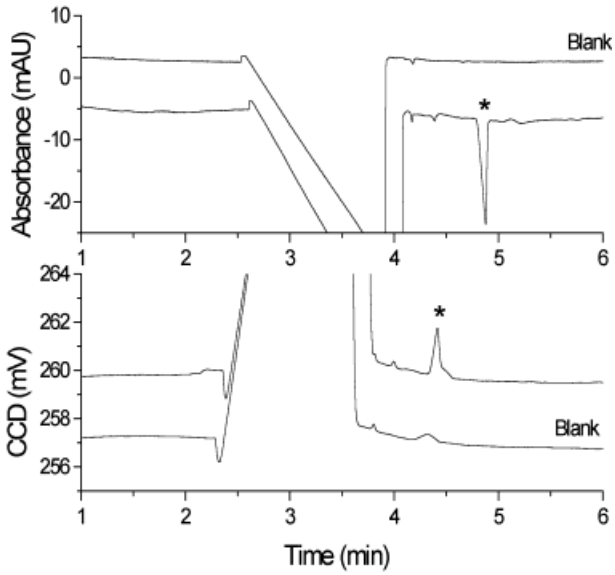

Figure 5. Electropherogram of fosfomycin in Ringer's solution pH 6.95 with indirect UV absorbance detection and CCD. BGE, 25 mM benzoic acid + 0.5 mM CTAB, adjusted with 1 M Tris to pH; fosfomycin concentration, 125 μg/mL. Other conditions as in Fig. 2. Fosfomycin peak is marked with an asterisk.

Table 1. Characteristics of fosfomycin determination in spiked Ringer's solution and plasma, respectively

Parameter		Ringer's solution		Plasma	
		UV (254 nm)	CCD	UV (254 nm)	CCD
LOD (μg/mL)[a]		0.86	0.62	0.86	2.0
LOQ (μg/mL)[b]		3.5	2.0	3.5	6.8
Instrument precision, RSD%[c]		3.7	4.5	3.2	5.6
% Yield (RSD %)	c = 10 μg/mL	123.8 (6.3)	123.8 (3.6)	90.6 (3.8)	96.8 (3.8)
	c = 100 μg/mL	106.8 (6.2)	107.7 (6.9)	101.2 (5.7)	99.2 (10.4)
	c = 300 μg/mL	100.6 (1.5)	102.2 (1.7)	94.8 (7.8)	88.1 (17.5)

a) (S/N = 3)
b) (S/N = 10); Number of replicates for yield: 3
c) n = 10, 100 μg/mL level

3.2 Method characteristics

The values of the analytical characteristics, LOD (for a signal-to-noise ratio of 3), LOQ (signal-to-noise ratio of 10), accuracy, and instrument precision are given in Table 1. Guidelines for definition were taken from [30]. It can be seen that values for LOD and LOQ for microdialysis samples are slightly lower with the CCD than with indirect UV detection, whereas both achieved values for plasma samples are slightly higher. However, the LOD is in the low μg/mL range or below, which is similar or slightly better than described in [4, 16]. Linear ranges were from 3.5 or 6.8 μg/mL, respectively, to 1000 μg/mL for both matrices, with a correlation coefficient $R > 0.9994$ for both detection methods. Instrument precision is described by the relative standard deviation, RSD%, obtained from ten replicate injections at a concentration level of the analyte of 100 μg/mL. It is between 3.2 and 5.6%. It should be pointed out that no suitable internal standard could be found, as there are almost no free positions in the electropherograms.

The accuracy of the method is expressed by the yield determined at three different concentration levels of the analyte spiked to Ringer's solution and plasma, respectively. It is about 100% and higher for the microdialysis samples, and about 100% and lower for the plasma samples. Also given is the RSD% of the yields, obtained from three independent replicate determinations at the three concentration levels each. Note that the confidence intervals for the yield (Student's factor, t, for *e.g.*, 99% probability and 2 degrees of freedom is 9.92) are always larger than those for the injection (t for 99% probability and 9 degrees of freedom is 3.25).

3.3 *In vivo* samples and pharmacokinetics of fosfomycin

Blood and microdialysis samples were taken *in vivo* from 12 healthy volunteers, to which 8 g of fosfomycin was administered. The microdialysis samples were directly injected into the CE instrument, whereas the plasma samples were treated with methanol (1+2, v+v), and centrifuged for 2 min at 15 000 × g before injection. Typical electropherograms of subcutaneous and muscular microdia-

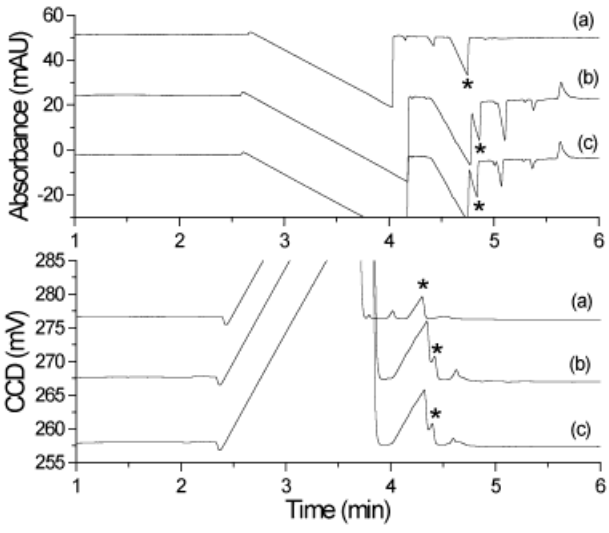

Figure 6. Microdialysis samples taken from one representative proband after i.v. administration of fosfomycin. (a) Fosfomycin standard in Ringer's solution given for comparison; microdialysis samples from (b) muscle tissue, and (c) subcutaneous. BGE, 25 mM benzoic acid + 0.5 mM CTAB, adjusted to pH 8.05 with 1 M Tris. Other conditions as in Fig. 2. Fosfomycin peak is marked with an asterisk.

lysis samples from one representative test person, taken 1 h after administration of fosfomycin, are shown in Fig. 6. It can be seen that some additional peaks appear in such microdialysis samples (Figs. 6b and c) compared to fosfomycin in Ringer's solution (Fig. 6a). As seen from the record of a fosfomycin standard (Fig. 6a), the analyte peak is more asymmetrical due to its higher concentration and the accompanied overload. However, even the large peak migrating just before the analyte in the real microdialysis samples (see Figs. 6b and c) does not hinder quantitation with UV detection, whereas the smaller analyte peak in the CCD record results in a higher LOQ. Analyte quantitation is better for the real plasma samples, as can be seen from Fig. 7 for samples taken 1 h after administration. Here the analyte peaks are well separated from all other matrix elements.

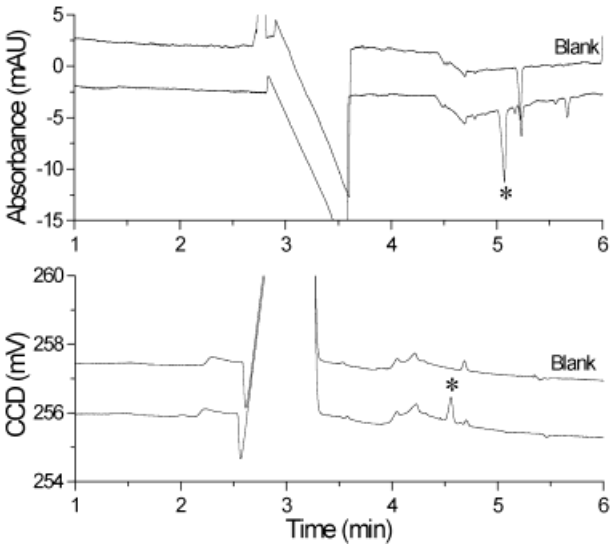

Figure 7. Plasma sample of one representative test person 1 h after *i.v.* administration of 8.0 g fosfomycin. BGE, 25 mM benzoic acid + 0.5 mM CTAB, adjusted with 1 M Tris to pH 6.95. Other conditions as in Fig. 2. Fosfomycin peak is marked with an asterisk.

One representative time *versus* concentration plot is shown in Fig. 8 to monitor the pharmacokinetic progress of fosfomycin. Maximum plasma concentrations of fosfomycin in all test persons ranged from 300–600 μg/mL, achieved approximately 40 min after administration of the antibiotic. Concentrations remained above the LOQ even after 6 h, dropping to concentrations between 50 and 150 μg/mL. Microdialysis concentrations, which were not recovery-corrected, were lower, with maximum concentrations in the range of 50–300 μg/mL, and were above the LOQ even after 6 h, with concentrations between 20–80 μg/mL. A detailed description and the results of the clinical trial will be published elsewhere.

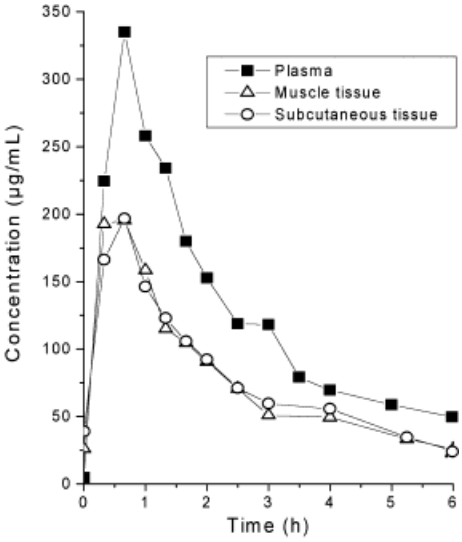

Figure 8. Pharmacokinetic profile of fosfomycin in plasma and microdialysis samples taken from one representative test person after i.v. administration of 8.0 g fosfomycin. Analysis were performed with indirect UV-detection.

4 Concluding remarks

The analytical method worked out for plasma and microdialysis samples enables the determination of fosfomycin in a wider concentration range than those described in [4] and [16] (there the upper concentration is 100 μg/mL), with comparable results for LOD and LOQ. Accuracy and precision of the present method fulfil the demands of the clinical study presented in this paper, and will be applied for two more clinical studies. The analysis time is considerably short, which is an advantage in clinical-analytical practice, due to the large number of samples to be analyzed. The other advantage, compared to the described methods, is the avoidance of complex sample preparation procedures.

For the given analytical problem, the CCD does not show a benefit compared to indirect UV detection. It has a slightly higher LOD and LOQ for plasma samples. The results showed that it can be seen as an alternative to indirect UV detection, but it is not complementary. In both matrices, plasma and microdialysates, the selection of an internal standard is problematic. Before the analyte peak no positions are free in the electropherograms, after the analyte peak an internal standard would increase the analysis time significantly. Therefore, the method of an external standard for quantitation is appropriate.

Received December 4, 2003

5 References

[1] Reeves, D. S., *J. Antimicrob. Chemother.* 1994, *34*, 853–858.

[2] Patel, S. S., Balfour, J. A., Bryson, H. M., *Drugs* 1997, *53*, 637–656.

[3] Vicente M. V., Olay, T., Quecedo, M. C. R., Rodriguez, A., *Chemotherapy* 1979, *25*, 329–335.

[4] Baillet, A., Pianetti, G. A., Taverna, M., Mahuzier, G., Baylocq-Ferrier, D., *J. Chromatogr.* 1993, *616*, 311–316.

[5] Tzanavaras, P. D., Themelis, D. G., *Anal. Biochem.* 2002, *304*, 244–248.

[6] Loste, A., Hernandez, E., Bregante, M. A., Garcia, M. A., Solans, C., *Chromatographia* 2002, *56*, 181–184.

[7] Webster, G. K., Bell, R. G., *J. AOAC Int.* 1999, *82*, 620–624.

[8] Dios-Vieitez, M. C., Goni, M. M., Renedo, M. J., Fos, D., *Chromatographia* 1996, *43*, 293–295.

[9] Longo, A., Di Toro, M., Pagani, E., Carenzi, A., *J. Chromatogr.* 1981, *224*, 257–264.

[10] Robins, W. H., Wright, B. W., *J. Chromatogr. A* 1994, *680*, 667–673.

[11] Macka, M., Johns, C., Doble, P., Haddad, P. R., *LC-GC Eur.* 2001, *19*, 38–47.

[12] Macka, K., Johns, C., Doble, P. Haddad, P. R., *LC-GC Eur.* 2001, *19*, 178–188.

[13] Steiner, F., Beck, W., Engelhardt, H., *J. Chromatogr. A* 1996, *738*, 11–23.

[14] Cousins, S. M., Haddad, P. R., Buchberger, W., *J. Chromatogr. A* 1994, *671*, 397–402.

[15] Pianetti, G. A., Moreira de Campos, L. M., Chaminade, P., Baillet, A., Baylocq-Ferrier, D., Mahuzier, G., *Anal. Chim. Acta* 1993, *284*, 291–299.

[16] Leveque, D., Gallion, C., Tarral, E., Monteil, H., Jehl, F., *J. Chromatogr. B* 1994, *655*, 320–324.

[17] Mercier, J. P., Morin, P., Dreux, M., *Chimia* 1999, *53*, 511–514.

[18] Kientz, C. E., Hooijschuur, E. W. J., Brinkman, U. A. T., *J. Microcol. Sep.* 1997, *9*, 253–259.

[19] Zemann, A. J., *Electrophoresis* 2003, *24*, 2125–2137.

[20] Mayrhofer, K. Zemann, A. J., Schnell, E., Bonn, G. K., *Anal. Chem.* 1999, *71*, 3828–3833.

[21] Katzmayr, M. U., Klampfl, C. W., Buchberger, W., *J. Chromatogr. A* 1999, *850*, 355–362.

[22] Pianetti, G. A., Baillet, A., Traore, F., Mahuzier, G., *Chromatographia* 1993, *36*, 263–267.

[23] Martinez Martinez, M. S., Gutierrez Hurtado, B., Colinio Grandarillas, C.-I., Martinez Lanao, J., Sanchez Navarro, A., *Anal. Chim. Acta* 2002, *459*, 143–150.

[24] Davies, M. I., *Anal. Chim. Acta* 1999, *379*, 227–249.

[25] Müller, M., Haag, O., Burgdorff, T., Georgopoulus, A., Weninger, W., Jansen, B., Stanek, G., Pehamberger, H., Agneter, E., Eichler, H.-G., *Antimicrob. Agents Chemother.* 1996, *40*, 2703–2709.

[26] Gaš, B., Zuska, J., Coufal, P., van de Goor, T., *Electrophoresis* 2002, *23*, 3520–3527.

[27] Coufal, P., Zuska, J., van de Goor, T., Smith, V., Gas, B., *Electrophoresis* 2003, *24*, 671–677.

[28] Lopez-Avila, V., van de Goor, T., Gaš, B., Coufal, P., *J. Chromatogr. A* 2003, *993*, 143–152.

[29] Pucci, V., Mandrioli, R., Raggi, M. A., *Electrophoresis* 2003, *24*, 2076–2083.

[30] Green, J. M., *Anal. Chem.* 1996, *68*, 305A–309A.