Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 846 (2007) 355–358
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Analysis of cephalosporins in bronchial secretions by capillary electrophoresis after simple pretreatment

Melinda Andrási [a], Attila Gáspár [a,*], Álmos Klekner [b]

[a] Department of Inorganic and Analytical Chemistry, University of Debrecen, H-4010 Debrecen, POB 21, Hungary
[b] Department of Neurosurgery, University of Debrecen, H-4012 Debrecen, POB 7, Hungary

Received 1 May 2006; accepted 15 August 2006
Available online 15 September 2006

Abstract

The applicability of capillary zone electrophoresis (CZE) for analysis of cephalosporin antibiotics has been studied in bronchial secretion as highly viscous, thick and non-homogeneous samples. The lyophilization was found to be a simple but effective pretreatment of these samples to bring them into a form which is suitable for injection to CE capillary. The obtained good recovery data prove that the lyophilization/dissolution of bronchial secretion samples can be reproducibly performed.

Keywords: Cephalosporins; Capillary electrophoresis; Bronchial secretion

1. Introduction

Drug monitoring in the bronchial secretions (sputum) is essential to promote appropriate drug selection in case of intubated or mechanically ventilated patients, who produce large amount of bronchial secretion, which contains numerous pathogen bacteria. However, the therapy of the selected antibiotics often does not result in remarkable effect because of the slight penetration of the antibiotic from serum to sputum.

The sputum samples are often homogenized and liquefied with Sputolysin® in a ratio of at least 1:1 (v/v) [1], but then the sample will be largely diluted and the matrix can cause difficulties during measurements. Other [2–4] solid-phase extractions with C18 cartridges were used prior to the HPLC analysis of bronchoalveolar lavage fluid samples.

Nowadays the cephalosporins can be considered to be one of the most important and most frequently used groups of the antibiotics applied in medicine [5]. The cephalosporins are analyzed generally by liquid chromatography [6,7], and few cephalosporins have been analyzed also in sputum using HPLC [2,4,8,9] after different sample pretreatment procedures. In the last few years, some works on determination of cephalosporins by means of capillary electrophoresis (CE) have been published [10–16]. As the direct injection of untreated biofluid samples can be performed into the CE capillaries, considerable savings in analysis time and cost of consumables can be achieved [17]. In our recent works, cephalosporins have been analyzed in clinical samples, where no sample pretreatment was necessary [18,19].

In this work, we wanted to extend the applicability of CE for the analyis of highly viscous, thick, and non-homogeneous samples like sputum using simple pretreatment. Additionally, the CE needs to be first applied for fast and economical monitoring of drugs (cephalosporins) in the sputum to assist in checking the effectiveness of the applied antibiotic.

2. Experimental

2.1. Instrumentation

The capillary electrophoresis instrument was a HP 3DCE model (Agilent, Waldbronn, Germany). Separations were performed using a polyimide-coated fused-silica capillaries of 48 cm × 50 μm i.d. (effective length: 40 cm) (Polymicro Technology, USA). The applied voltage was +20 kV. The sampling tray was thermostated at 14 °C. The photometric detection was carried out at 270 nm.

* Corresponding author. Tel.: +36 52 512900; fax: +36 52 489677.
E-mail address: gaspara@tigris.unideb.hu (A. Gáspár).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.08.025

2.2. Chemicals and samples

Reagents of analytical grade were obtained from various distributors. The sodium tetraborate, sodium dodecyl sulfate (SDS), and sodium hydroxide for preparing buffer electrolytes were purchased from Spectrum-3D (Hungary). The buffer electrolyte of pH 9.1 included 25 mM borate and 50 mM SDS. The sample solutions were prepared by dissolving the solid salts of cefazolin, cefepim (Bristol–Myers), cefamandol (Human), cefuroxim, ceftazidim (Glaxo), and ceftriaxon (Roche) in water just before the analyses. The concentration of the stock solutions of all cephalosporins were 0.15 mg/mL. In some experiments the sputum was liquefied by Sputolysin (dithiothreitol, Calbiochem., La Jolla). In the case of analysis of biofluid samples after each run the capillary was post-conditioned by flushing 1 M NaOH (5 min), 0.1 M SDS (5 min) and the running buffer (5 min). The samples were stored in refrigerator at +4 °C. The electrophoretic runs were performed not later than 4 h after the solution preparation.

2.3. Sample collection

Serum and sputum were collected from 38 chronic intubated patients having purulent bronchopneumonia treated with cephalosporin in the intensive care unit (ICU, Department of Neurosurgery, University of Debrecen) by procedures that are in accordance with Hungarian ethical rules. Twenty seven females and 11 males were investigated; mean age 55.2 ± 12.0 years, range 44–68 years, body weight 67.6 ± 14.5 kg, range 53–82 kgs. All patients were treated in the ICU because of serious intracranial lesion and 11 of them were mechanically ventilated. Five patients received 65 mg/kg/24 h cefuroxime, six patients received 60 mg/kg/24 h ceftriaxone, four patients received 60 mg/kg/24h ceftazidime, five patients received 85 mg/kg/24 h cefepime, 14 patients received 65 mg/kg/24 h cefazolin and four patients received 45 mg/kg/24 h cefamandol intravenously in three equal doses per day. The serum and sputum samples were collected on the second day of treatment 6 h after the last dose of antibiotics. Sputum samples were sucked out from the trachea by a sterile sonda through the nasotracheal tube. The serum, cerebrospinal fluid and sputum samples were stored at −18 °C prior to analysis.

2.4. Pretreatment of sputum

The serum, cerebrospinal fluid samples could be directly injected to the capillary without any sample pretreatment. As it was shown in our earlier work, the applied method with three-step postconditioning and the cooled sampling tray allowed reproducible measurements [18]. The direct injection of sputum was only slightly reproducible because of its high viscosity and inhomogenity. Both hydrodynamic (100 mbar s) and electrokinetic (5 kV, 4 s) injecton modes were tried to be applied, however the capillary was often clogged. It was found that in the case of sputum samples the direct injection cannot be applied for reliable, reproducible capillary electrophoretic assay. The vortex mixing of the sputum spiked with water or methanol did not result in a less viscous, more homogeneous liquid. Some sputum liquefying agents have been reported, but those are often chemically incompatible with beta-lactames [2]. The often applied liquefying agent, Sputolysin was mixed with an equal amount of sputum sample. The mixture was vortexed until liquefaction occurred and then it was injected. Although the injection of this homogeneous sample was reproducible, the components of Sputolysin show large UV absorbance at the detection wavelengths of cephalosporins (both at 200 nm and 270 nm) resulting in difficulties to obtain well-resolved peak for some cephalosporins.

In our practice the lyophilization was found to be a simple but effective pretreatment of sputum to bring it into a form which is suitable for injection to CE capillary. The known amount of sputum samples stored at −18 °C were put directly (without melting) into lyophilizator (Lyovac GT2, Leybold) for 12 h under vacuum. The obtained porous material was crushed to small pieces and stirred with a glass stick before its dissolution in methanol–water (1:1) mixture. It was found that 0.01 g lyophilized sputum can be dissolved in 300 μL solvent. The used polar solvent was capable of dissolving the total amount of hydrophilic cephalosporins from the lyophilized sputum during 5 min vortex mixing. The obtained solutions have been centrifuged for 20 min at 9000 rpm at +4 °C. The supernatant was stored at +4 °C prior to CE analysis (not later than 2 days).

3. Results and discussion

It was reported in numerous works that serum or cerebrospinal fluid samples could be directly injected to the capillary without any sample pretreatment. As it was shown in our earlier work the applied method with a three-step postconditioning and the cooled sampling tray allowed reproducible measurements for such samples [18]. Although the direct injection of viscous sputum is also possible, often clogging of the capillary happens and different amounts of sample (analyte) may be transferred into the capillary because of the inhomogenity of sputum sample. Fig. 1 shows the electropherograms obtained by direct injection and CE analyis of serum, cerebrospinal fluid, and sputum samples obtained from the same patient who received ceftriaxone intravenously (60 mg/kg/24 h ceftriaxone). The ceftriaxon could be well resolved from the other components in each sample. It could be observed that before the large peaks of different proteins (after 6 min) numerous components could be detected at 270 nm. These components have quite characteristic UV spectra, but the target of this work was not to identify them. These samples either include these components “naturally” (components from blood), or can be a drug(s) administrated or its metabolites. When the peak areas of ceftriaxon were compared to the other unknown peaks, the highest ratio was obtained in serum, smaller in cerebrospinal fluid and quite small in sputum, according to the expected distribution/penetration tendency. The selectivity of the method is made even higher by the applied detection wavelength (270 nm), because most of the components of clinical samples (proteins, peptids, inorganic compounds) have no absorbance in this range.

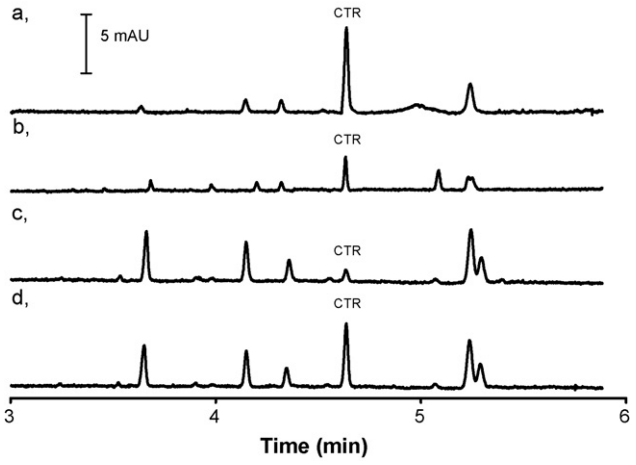

Fig. 1. Direct injection and CE analysis of clinical samples obtained from the same patient received ceftriaxone (CTR) intravenously (60 mg/kg/24 h ceftriaxone). (a) serum, (b) cerebrospinal fluid, (c) sputum injected without sample pretreatment (lyophilization), (d) sputum injected without sample pretreatment (lyophilization) and spiked with ceftriaxon (20 μg/mL) (separation conditions: electrolyte: 25 mM phosphate, 50 mM SDS, pH 9.1, applied voltage: +20 kV, injection: 50 mbar, 2 s) (detection: UV absorption at 270 nm).

Although, according to the electropherograms of Fig. 1, the direct injection of sputum is often possible, it cannot be applied for reliable, reproducible capillary electophoretic assay. Reproducible analysis could be achieved by lyophilization and dissolution of the sputum samples. There was no remarkable difference in the patterns of the electropherograms obtained for samples either lyophilized or not. In the migration window between 3 and 6 min, the anionic, non-proteinous, hydrophile components can be seen, which can be completely transferred to the supernatant after lyophilization.

In order to verify the good quantitation of analysis with the lyophilization/dissolution pretreatment procedure, the calibrations obtained for standards of ceftriaxon in water and sputum matrices were compared (Fig. 2). The calibration graphs (regression cofficients were 0.9989 and 0.9983 in water and sputum, respectively) were not considerably deviated: the equations of regressions were $y = 0.307x + 0.011$ and $y = 0.296x + 0.016$. The intercepts did not significantly differ from zero.

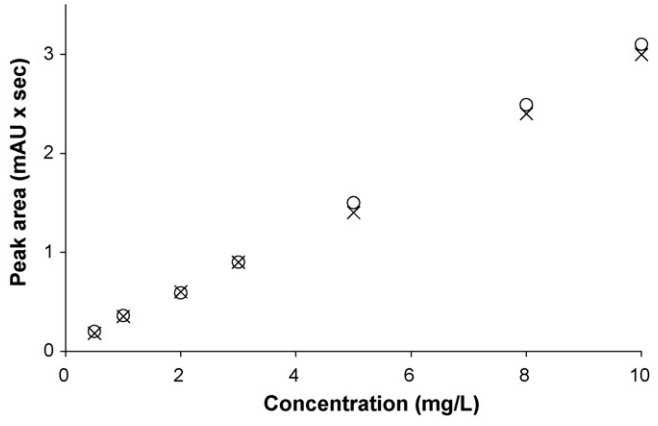

Fig. 2. Calibration diagram obtained for standards of ceftriaxon in water (◯) and sputum (×) matrices.

Table 1
Intra-day reproducibility of ceftriaxon ($N = 10$, $c = 40$ μg/mL) in water, serum and sputum

	RSD (%)		Recovery (%)
	Migration time	Peak area	
Water	0.38	0.81	
Serum	0.77	1.41	103.1
Sputum	0.52	0.84	99.6
Sputum spiked[a]	0.76	1.78	97.8

[a] 2 g of sputum was spiked with 100 μL 1.2 mg/mL ceftriaxon. After homogenization the sample was divided into 10 parts and they were lyophilized. Each residue (9–10 mg) was dissolved in 300 μL methanol–water (1:1).

Table 1 contains the intra-day reproducibility and accuracy data which were obtained by ten replicate injections for water, serum and sputum samples. The precision of migration times and peak areas was smaller than 0.8 and 1.8 RSD% that are usual in CE analysis of real samples using well-optimized separation and pre/postconditioning steps. The reproducibility and the recovery achieved were not poor even in the case when the whole lyophilization/dissolution process and CE analyis were repeated 10 times (2 g of sputum was spiked with 100 μL, 1.2 mg/mL ceftriaxon. After homogenization the sample was divided into 10 parts and they were lyophilized. Each residue (9–10 mg) was dissolved in 300 μL methanol–water (1:1).) The obtained good recovery data prove that the lyophilization/dissolution of sputum samples can be reproducibly performed, and thus the analysis of sputum samples can be done with good precision and accuracy. It was found that the limit of detection (LOD) values did not significantly deviate in samples of pure solutions and sputum samples. The LOD values (S/N = 3) of the cephalosporins in sputum samples were in the range of 0.42–0.84 μg/mL.

3.1.1. Clinical application

Purulent bronchopneumonia is a frequent complication in patients with chronic intratracheal intubation. In spite of the targeted antibiotic treatment, production of abundant bronchial secretion containing pathogen bacteria often tends to be chronic and so mortality drastically increases. This problem indicates the importance of the investigation of the penetration of six cephalosporin antibiotics into the sputum. It was found that only the ceftriaxon could be detected in the sputum samples of those patients who received one of the six selected cephalosporins (Fig. 1c, and Table 2). Cephalosporins other than ceftriaxon did not reach a detectable concentration level in sputum, therefore we could study the determination of these cephalosporins in sputum, only if it was spiked with the cephalospin. The electropherograms of Fig. 3 demonstrate that the different cephalosporins are well resolved from the other components of the sample and they can be quantified. The concentrations of six cephalosporins in the serum in 6 h after intravenous drug administration are summarized in Table 2. The level of cefazolin, cefamandol, cefuroxime, ceftazidime, and cefepime in the sputum remained under 0.4–0.8 μg/ml and they could not be detected, but concentration of ceftriaxone was 1.9 ± 1.3 μg/ml.

Table 2
The concentration of six cephalosporins in serum and sputum from patients 6 h after intravenous drug administration

	No. of samples	Concentration (μg/mL)	
		Serum	Sputum
Cefazolin	14	79.5 ± 13.8	<0.5
Cefamandol	4	41.5 ± 10.6	<0.6
Cefuroxim	5	26.9 ± 4.9	<0.4
Ceftazidim	4	9.5 ± 3.4	<0.8
Ceftriaxon	6	64.9 ± 28.9	1.9 ± 1.3
Cefepim	5	20.7 ± 4.1	<0.5

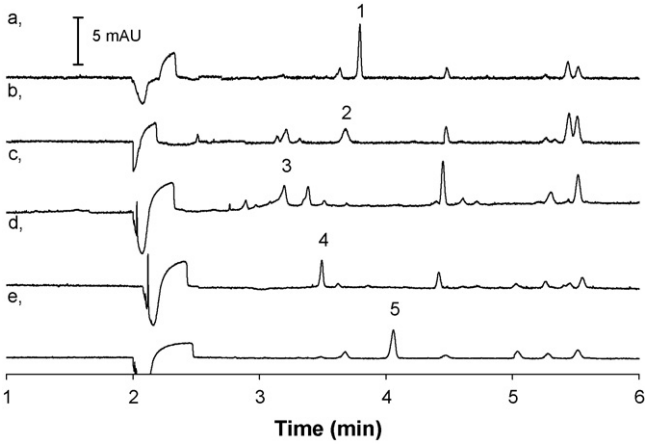

Fig. 3. Electropherograms of sputum samples obtained from patients and spiked different cephalosporins (a) cefuroxim (1); (b) cefamandol (2); (c) cefepime (3); (d) ceftazidim (4); (e) cefazolin (5). Separation conditions were the same as for Fig. 1.

The minimal inhibitory concentration (MIC) values of the bacteria isolated from the sputum cultures of the investigated 38 patients for the investigated six cephalosporins were higher than 2 μg/mL, that is they exceeded the mean concentration of cephalosporins in the sputum.

4. Conclusion

In this work, the CE was applied for the first time for fast and economical monitoring of drug (different cephalosporins) in the sputum as highly viscous, thick and non-homogeneous samples to assist in the checking of the effectiveness of the applied antibiotic. The lyophilization was found to be a simple but effective pretreatment of these samples, the used polar solvent was capable of dissolving the total amount of hydrophilic cephalosporins from the lyophilized sputum. The obtained good recovery data prove that the lyophilization/dissolution of bronchial secretion samples can be reproducibly performed.

The obtained results have great significance in medical treatment. The simple application of capillary electrophoresis for human biological samples offers a possibility for evaluating actual effectiveness of antibiotics that can promote optimization of individual antibiotic therapy. According to the results, the proposed CE method and sample pretreatment procedure are capable for quantitative determination of cephalosporins in bronchial secretions.

Acknowledgement

Our work was supported by the Economic Competitiveness Operative Programme of Hungary (GVOP-3.2.1.-2004-04-0032/3.0. and GVOP-2005-3.3.1).

References

[1] M. Kolak, F. Karpati, et al., Int. J. Med. Microbiol. 293 (2003) 309.
[2] M. Ehrlich, F.D. Daschner, K. Kümmerer, J. Chromatogr. B 751 (2001) 357.
[3] M.T. Rosseel, R. Peleman, et al., J. Chromatogr. B 689 (1997) 438.
[4] B. Barroso, M. Didraga, R. Bischoff, J. Chromatogr. A 1080 (2005) 43.
[5] E. Bergogne-Bérézin, Curr. Ther. Res. 57 (1996) 87.
[6] L.G. Martínez, P.C. Falcó, A.S. Cabeza, J. Pharm. Biomed. Anal. 29 (2002) 405.
[7] V.F. Samanidou, A.S. Ioannou, I.N. Papadoyannis, J. Chromatogr. B 809 (2004) 175.
[8] A.A. Vinks, R.W. Brimicombe, et al., J. Antimicrob. Chemother. 40 (1997) 125.
[9] M. Kuwabara, H. Sasaki, H. Fukuhara, et al., Jpn. J. Antibiot. 42 (1989) 921.
[10] K.D. Altria, Analysis of Pharmaceuticals by Capillary Electrophoresis, Vieweg, Braunschweig, 1998.
[11] H. Fabre, P.G. Penalvo, J. Liq. Chromatogr. 18 (1995) 3877.
[12] Y. Mrestani, R. Neubert, J. Schiewe, A. Härtl, J. Chromatogr. B 690 (1989) 321.
[13] H. Nishi, N. Tsumagari, T. Kakimoto, S. Terabe, J. Chromatogr. 477 (1989) 259.
[14] A. Gáspár, M. Andrási, Sz. Kardos, J. Chromatogr. B 775 (2002) 239.
[15] M. Hernández, F. Borrull, M. Calull, Trends Anal Chem. 22 (2003) 416.
[16] Y. Mrestani, A. Härtl, R. Neubert, Int. J. Pharm. 309 (2006) 67.
[17] D.K. Lloyd, H. Watzig, J. Chromatogr. B 663 (1995) 400.
[18] A. Gáspár, Sz. Kardos, M. Andrási, Á. Klekner, Chromatographia 56 (Supp.) (2002) S–109.
[19] Á. Klekner, A. Gáspár, Sz. Kardos, et al., J. Neurosurg. Anesthesiol. 15 (2003) 249.