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Short communication

Adaptation of capillary electrophoresis to the determination of selected cephalosporins for injection

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Abstract

The migration behaviour of cephazolin, cefuroxime sodium, ceftriaxone sodium, cefoperazone sodium and ceftazidime in a mixture was studied. Phosphate–borate buffer pH 5–8 alone and with addition of sodium dodecylsulfate (SDS) was used. In capillary zone electrophoresis of all research compounds separation was not achieved. It was observed that supplementation buffer pH 6.5 with SDS (10 g/l) improved resolution of cephalosporins, but addition of pentanesulfonic acid (17.4 g/l) to the running buffer at pH 6.5 results in separation of each cephalosporin. In this condition good repeatability of migration times as well as repeatability of peak area were confirmed.

Keywords: Pharmaceutical analysis; Cephalosporins; Antibiotics

1. Introduction

The cephalosporins – semisynthetic antibiotics – were derived from cephalosporin C, found among the fermentation products of *Cephalosporium acremonium* over fifty years ago. The active nucleus, 7-aminocephalosporanic acid, is very closely related to the penicillin nucleus, 6-aminopenicillanic acid, and consists of a β-lactam ring fused with a 6-membered dihydrothiazine ring. Methods of cephalosporin antibiotics analysis include microbial and chromatographic assays. Recently capillary electrophoresis (CE) has proven to be a significant and versatile technique for the analysis of this group of compounds [1–4].

In the previous works we elaborated and adapted the capillary electrophoresis (CE) method to assay penicillin preparations: Augmentin, Unasyn, Piperacillin, Tazocin and carbapenem Tienam [5–9].

The aim of this study was to elaborate conditions, using the same phosphate–borate buffer, to assay the selected cephalosporins in preparations for injection.

The results of migration behaviour in pH range 5–8 of cephazolin, cefuroxime sodium, cefotaxime sodium, ceftriaxone sodium, cefoperazone sodium and ceftazidime in capillary zone electrophoresis (CZE), and in micellar electrokinetic capillary chromatography (MECC) in presence of sodium dodecylsulfate (SDS) and pentanesulfonic acid sodium salt are presented.

2. Experimental

2.1. Apparatus

Capillary electrophoresis experiments were carried out on Waters Quanta 4000 E CE System, equipped with 30 kV power supply, a UV spectrophotometric detector connected to a data collection system and able to perform both hydrodynamic injection and

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voltage injection. The detection wavelength was 214 nm. Separations were performed in a 60 cm×75 μm I.D. fused-silica capillary coated with polyimide (AccuSep capillaries, Waters) thermoregulated at 25°C, with a voltage of 18 kV applied. Hydrodynamic injection was used.

2.2. Samples and reagents

Cephalosporin preparations for injections from Polish market: Biofazolin (cephazolin sodium), IBA-Bioton; Cefobid (cefoperazone sodium), Pfizer; Claforan (cefotaxime sodium), Roussel Uclaf; Fortum (ceftazidime), Glaxo; Rocephin (ceftriaxone sodium), Roche; and Zinacef (cefuroxime sodium), Glaxo were analysed.

Disodium hydrogenphosphate, sodium tetraborate, sodium hydroxide, phosphoric acid were of reagent grade. SDS was from Sigma, pentanesulfonic acid sodium salt and dimethyl sulfoxide (DMSO) from Merck. Water used to prepare sample solutions and running buffers was obtained from a Labconco system.

The CE electrolyte contained constant concentrations of disodium hydrogenphosphate (3.12 g/l) and sodium tetraborate (7.63 g/l) and different contents of SDS (7, 10 and 14 g/l) adjusted with sodium hydroxide or phosphoric acid to pH 5, 6, 6.5, 7 and 8.

All cephalosporins were dissolved in water at concentrations of 0.1–0.5 mg/ml each.

3. Results and discussion

The influence of pH on the migration times of six cephalosporins mixture in electrolyte solution adjusted to pH 5, 6, 7 and 8 was examined. Two systems of electrophoresis were used – classical CZE (Fig. 1) and MECC (with addition of 10 g/l SDS to running buffer), (Fig. 2). Such SDS concentration was chosen according to our previous experiences from elaboration of Piperacillin ini. assay [7]. In case of CZE the migration times of all analysed cephalosporins increased with decreased pH value of the buffer. In pH range 6 to 7 the migration times were almost constant for all investigated compounds. In case of MECC the migration times of

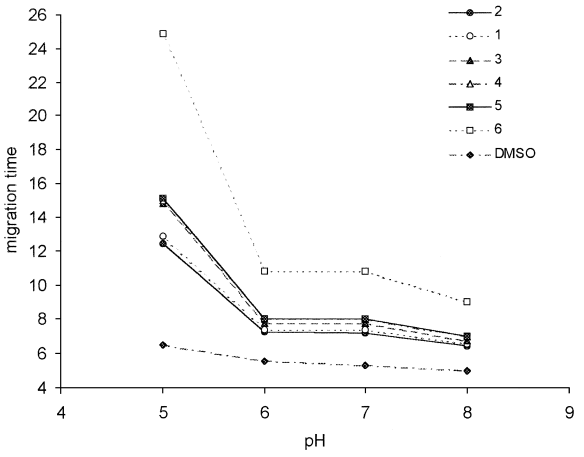

Fig. 1. Dependence of the migration times of cephalosporins mixture on pH of phosphate–borate buffer; 1=ceftazidime, 2=cefoperazone, 3=cefotaxime, 4=cefuroxime, 5=cephazolin, 6=ceftriaxone.

the solutes increased gradually along with decreasing pH of running buffer and do not significantly differ from CZE experiments. In both conditions all analysed cephalosporins had negative electrophoretic mobility and ceftriaxone migrated much slower than the other antibiotics, probably because of the characteristic highly acidic heterocyclic system on the 3-thiomethyl group attached to C-3 position of cephem structure. It was also noticed in both systems that

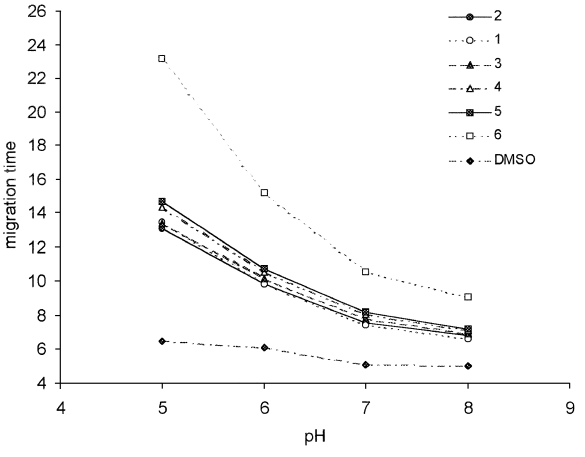

Fig. 2. Dependence of the migration times of cephalosporins mixture on pH of phosphate–borate buffer supplemented with 10 g/l SDS. 1=ceftazidime, 2=cefoperazone, 3=cefotaxime, 4=cefuroxime, 5=cephazolin, 6=ceftriaxone.

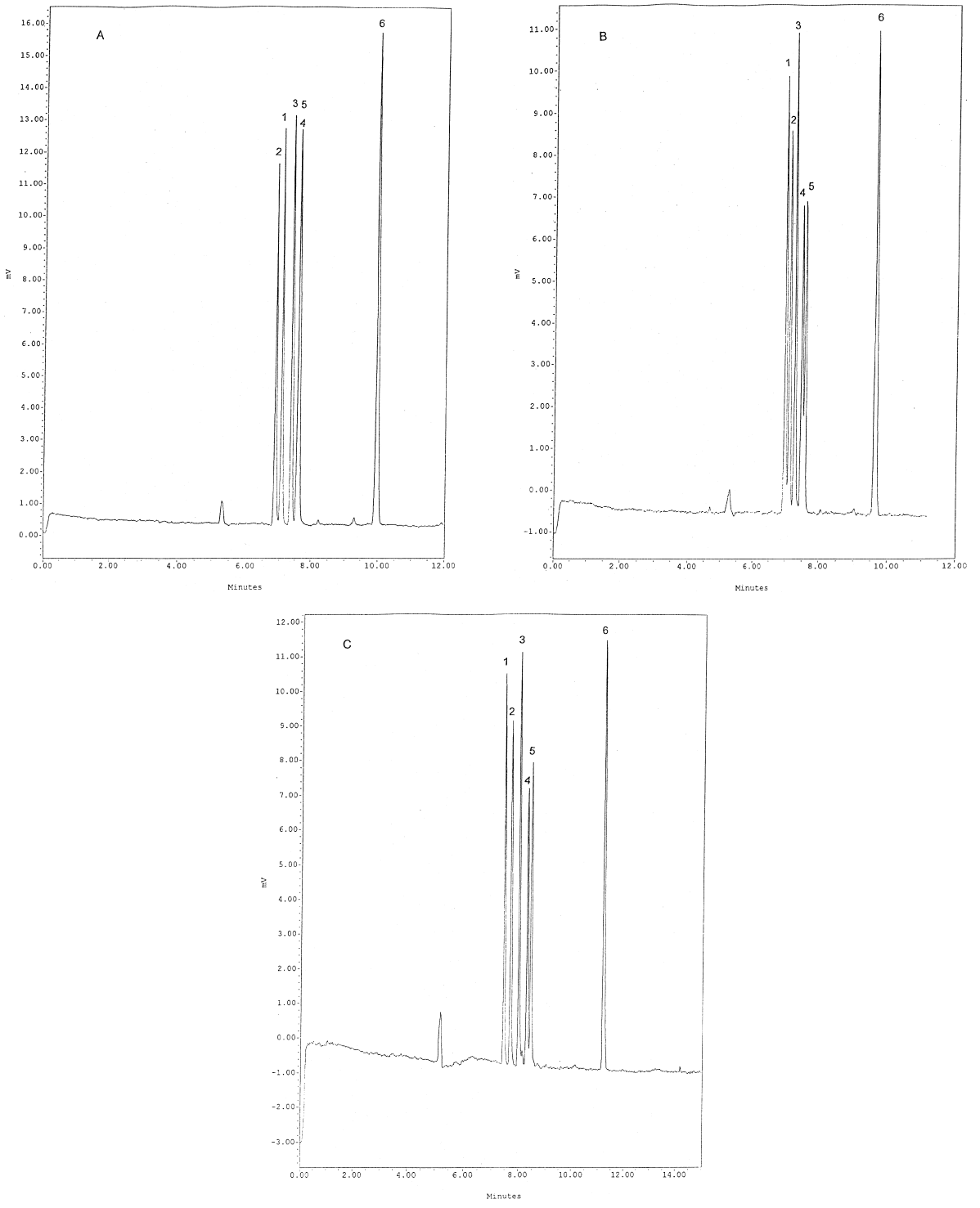

Fig. 3. Typical electropherogram of a cephalosporins mixture: CE conditions: 18 kV, 214 nm; A: buffer phosphate–borate pH 6.5; B: buffer phosphate–borate pH 6.5 containing 10 g/l SDS; C: buffer phosphate–borate pH 6.5 containing 10 g/l SDS and 17.4 g/l pentanesulfonic acid; 1=ceftazidime, 2=cefoperazone, 3=cefotaxime, 4=cefuroxime, 5=cephazolin, 6=ceftriaxone.

ceftazidime and cefoperazone had shorter migration times than the other cephalosporins. In classical CE it was noticed that independently of the buffer pH value, cephazolin and cefuroxime created a single peak on the electropherogram. Ceftazidime and cefoperazone had the same migration time at pH 8. It was observed that addition of SDS and performance MECC improved resolution of cephalosporins in mixture and separated cephazolin from cefuroxime in the whole pH range of experimental conditions, but not always to the basis. In MECC experiments in buffer of pH 6 and pH 7, six peaks were obtained on electropherograms of cephalosporins mixture; however, cefoperazone and ceftazidime in pH 6 as well as cefuroxime and cephazolin in pH 7 were close to each other. Migration times for ceftazidime, cefoperazone, cefotaxime, cefuroxime and cephazolin during MECC in comparison to CZE were only slightly longer.

For further research according to the obtained results a running buffer at average pH 6.5 was chosen. Additionally this buffer was supplemented with various amounts of SDS (7 g/l, 14 g/l, and 28 g/l). Neither the increase nor decrease of SDS concentrations had improved the separation conditions of analysed cephalosporins. Finally, 17.4 g/l pentanesulfonic acid was added to the buffer pH 6.5 supplemented with 10 g/l SDS. Separation of each cephalosporin, especially cefuroxime and cephazolin, in this case was significantly better. Fig. 3A presents the electropherogram of a cephalosporin mixture at buffer pH 6.5 without supplementation, Fig. 3B with addition of 10 g/l SDS and Fig. 3C presents the separation of cephalosporins when the running buffer contained 10 g/l SDS and 17.4 g/l pentanesulfonic acid. At high concentrations, pentanesulfonic acid tends to be a secondary micelle-former, which alters the pseudostationary phase of running buffer, thus resulting in higher resolutions [1]. In Table 1 the results of cephalosporin analyses by CE in phosphate–borate buffer pH 6.5 containing 10 g/l SDS and 17.4 g/l pentanesulfonic acid are presented. The electrophoretic mobility of analytes was calculated from the observed migration times with the equation: $\mu_{ep} = \mu - \mu_{eo} = (L_d\ L_t/V)(1/t_m - 1/t_{eo})$, where μ_{ep} is the electrophoretic mobility of the analyte tested, μ is the apparent mobility, μ_{eo} is the electroosmotic mobility, t_m is the migration time measured directly from the electropherogram, t_{eo} is the migration time for uncharged solute (DMSO as neutral marker), L_t is the total length of capillary, L_d is the length of capillary between injection and detection and V is

Table 1
Results of cephalosporins analyses by CE in phosphate–borate buffer pH 6.5 containing 10 g/l SDS and 17.4 g/l pentanesulfonic acid

Cephalosporin	Electrophoretic mobility μ_{ep} (10^{-4} $cm^2/V\,s$)	Concentration (mg/ml)	Migration time (min)	Repeatability of migration time (RSD, %)	Repeatability of corrected area (RSD, %)
		0.10			2.98
Ceftazidime	−1.56	0.25	7.4	0.31	2.06
		0.50			0.67
		0.10			2.28
Cefoperazone	−1.69	0.25	7.7	0.37	2.14
		0.50			0.47
		0.10			2.41
Cefotaxime	−1.85	0.25	8.0	0.32	2.16
		0.50			0.97
		0.10			2.22
Cefuroxime	−2.00	0.25	8.3	0.32	2.18
		0.50			1.05
		0.10			2.40
Cephazolin	−2.03	0.25	8.4	0.31	1.87
		0.50			1.70
		0.10			2.86
Ceftriaxone	−2.89	0.25	11.10	0.40	3.01
		0.50			1.57

applied voltage. In this condition good repeatability of migration times as well as repeatability of peak area were confirmed. In the case of ceftriaxone, when the migration time was the longest (11.1 min), repeatabilities of migration time and corrected peak area expressed by RSD were slightly worse than other cephalosporins data.

Performed analyses of selected cephalosporins in different separation conditions increased knowledge about behaviour of these compounds, which could be helpful in identification and assay of cephalosporins by CE method.

4. Conclusions

Classical capillary electrophoresis did not allow for separation of ceftazidime from cefoperazone and cefuroxime from cephazolin, however addition of 10 g/l SDS and 17.4 g/l pentanesulfonic acid and performance micellar electrokinetic chromatography significantly improved separation of all analysed cephalosporins. Thus elaborated conditions of MECC can be of use for the identification and determination of cephalosporins.

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