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Identification and separation of five cephalosporins by micellar electrokinetic capillary chromatography

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Abstract

The cephalosporins are a group of structurally related, broad spectrum β-lactam antibiotics isolated from the mold *Cephalosporium*. Methods of analysis of cephalosporin antibiotics include microbiological, titrimetric and chromatographic assays. Chromatographic techniques, including high-performance liquid chromatography, have been extensively utilized for specific and sensitive assays of β-lactam antibiotics in a variety of matrices, *i.e.* clinical and pharmaceutical. Several of the drawbacks of HPLC in the analysis of cephalosporins in food and biological samples include matrix interferences and low resolution due to column adsorption. Recently, the applicability of capillary electrophoresis to the resolution of β-lactam antibiotics has been demonstrated in the literature. In this paper we employed sodium borate and an anionic surfactant, sodium dodecyl sulfate (SDS), in a separation technique called micellar electrokinetic capillary chromatography with UV detection, to resolve a mixture of five cephalosporins —cefuroxime, cephalexin, cephapirin, cefamandole nafate and cephalothin. The presence of SDS in the running buffer above the critical micelle concentration, creates a pseudostationary phase enabling high-efficiency chromatographic separations. The effect of the ion-pairing reagent, pentanesulfonic acid sodium salt, on the resolution of the cephalosporin mixture in conjunction with SDS was also examined.

1. Introduction

The cephalosporins are a group of β-lactam antibiotics similar in structure and action to the penicillins. Isolated from the mold *Cephalosporin*, these antibiotics have a broad anti-bacterial spectrum, are resistant to β-lactamase, and exhibit less allergic cross-reactivity with penicillins [1]. The cephalosporins are extensively employed in treating Gram-positive and Gram-negative infections. Methods of analysis of cephalosporin antibiotics include microbiological [2] and chromatographic assays [4–7]. Microbiological methods include the cylinder-plate, turbidometric and disk assays. The cylinder-plate method is based upon the diffusion of antibiotics from a vertical cylinder through an agar layer [2]. The turbidometric method is contingent upon the suppression of growth of a microbial culture. Both methods are very sensitive but are non-selective, frequently imprecise, and do not provide detection of decomposition products. The disk assay is a qualitative detection method for β-lactam antibiotics [3]. Liquid chromatographic methods in contrast, are sensitive, specific and stability indicating [8–11].

Recently, capillary electrophoresis (CE) has proven to be a significant and versatile technique for the analysis of a wide range of biomolecules

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[12–14]. The application of CE in the analysis of β-lactam antibiotics was previously demonstrated with the use of anionic surfactants, including sodium dodecyl sulfate (SDS) and pentanesulfonic acid [15]. This CE method, involving micellar electrokinetic capillary chromatography (MECC), utilizes micellar buffer solutions which exhibit a differential partitioning effect specifically with neutral and weakly ionic molecules, such as cephalosporin antibiotics. We previously reported the separation of a mixture of penicillins by MECC with UV detection [16].

In this paper, we report the separation of a mixture of a solution of five cephalosporin antibiotics including cefuroxime, cephalexin, cefamandole nafate and cephalothin by MECC with UV detection. The utilization of different CE conditions *i.e.* buffer system, applied voltage and general applicability to pharmaceutical antibiotics is presented.

2. Experimental

2.1. Instrumentation

A commercially available CE instrument (270A-HT, Applied Biosystems, Foster City, CA, USA) was used. Data acquisition was controlled by a Macintosh IIci (Apple, Cupertino, CA, USA). A 72 cm × 50 μm I.D. fused-silica capillary (Applied Biosystems) was used for all analyses. Analyses were carried out using Model 600 software (Applied Biosystems) Data integration was carried out using a Laser Jet III (Hewlett-Packard, Boise, ID, USA). Fused-silica capillaries were conditioned by flushing capillaries 30 min with 1 *M* NaOH followed by a rinse with deionized water for 10 min. CE conditions for all experiments were carried out at 30°C. Hydrostatic injection times was 5 s. The applied voltage was 306 V/cm. The UV detector was set

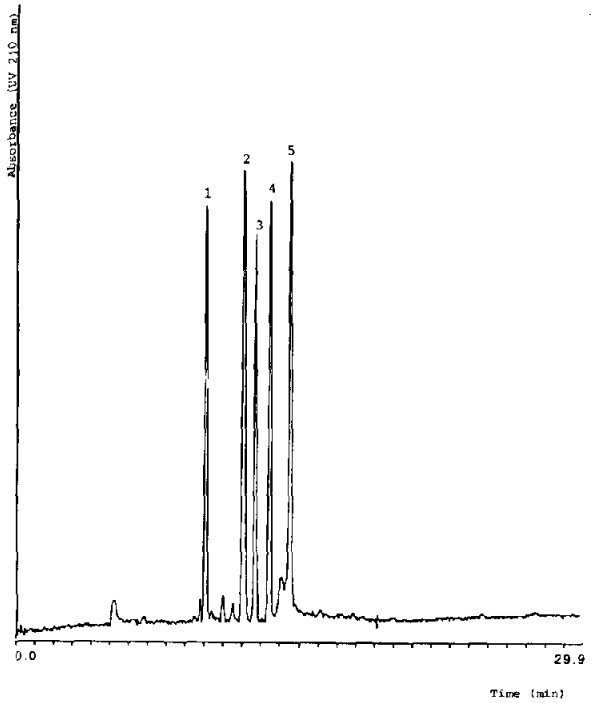


Fig. 1. Typical electropherogram of a mixture of five cephalosporins, concentrations ranging from 100–200 μg/ml. CE conditions: 20 m*M* sodium borate, 200 m*M* SDS, 306 V/cm, 210 nm. See Table 1 for peak identification.

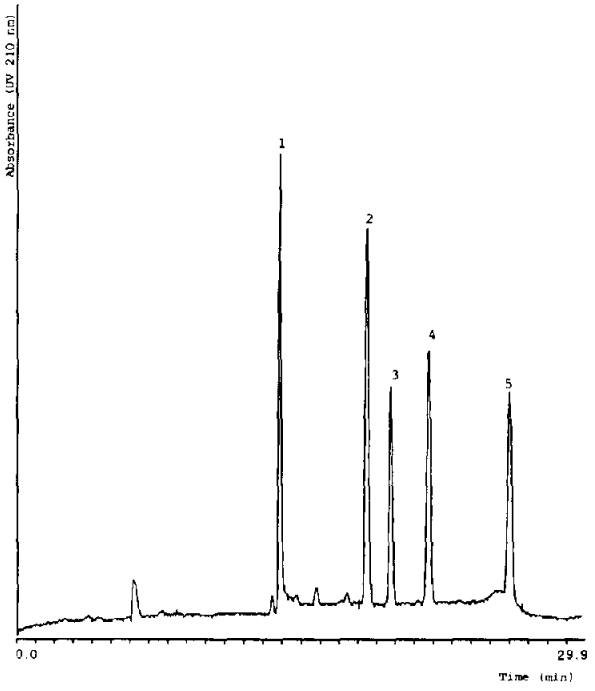


Fig. 2. Typical electropherogram of a mixture of five cephalosporins, concentrations ranging from 100–200 μg/ml. CE conditions: 20 m*M* sodium borate, 200 m*M* SDS, 100 m*M* pentanesulfonic acid, 306 V/cm, 210 nm.

at 210 nm and the rise time and range were 0.1 s and 0.05, respectively. Capillary distance to the detector was 50 cm.

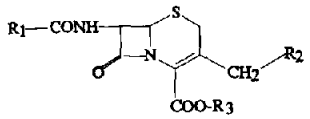
2.2. Reagents and materials

All buffers, reagents, standards and samples were prepared with deionized water (Milli-Q grade; Millipore, Marlborough, MA, USA). In-house working standards were used in this study. Sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) was purchased from Mallinckrodt (Paris, KY, USA). SDS was purchased from Sigma (St. Louis, MO, USA). Pentanesulfonic acid sodium salt was purchased from Fisher Scientific (Pittsburgh, PA, USA). A mixed cephalosporin standard was prepared by weighing 10 mg of each working standard into a 100-ml volumetric flask, diluting to volume with deionized water and mixed.

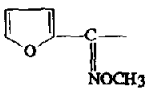
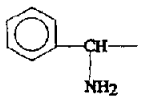
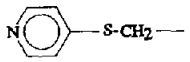
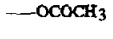
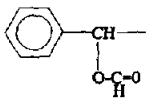
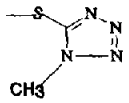
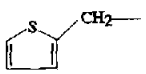
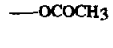
2.3. Sample preparation

Weigh amount of capsule composite equivalent to one capsule and dissolve suitable quantity of deionized water to obtain a concentration of *ca.* 100 μg/ml of cephalosporin antibiotic (a composite is a mixture of the contents of 20 capsules. The average mass of a capsule is determined from the total mass of the composite divided by the number of capsules). Filter solution through a Gelman acrodisc or appropriate filter prior to injection.

Table 1



Chemical structures of five cephalosporins

No.	Cephalosporin	R_1	R_2	R_3
1	Cefuroxime			H
2	Cephalexin		H	H
3	Cephapirin			Na
4	Cefamandole nafate			Na
5	Cephalothin			Na

3. Results and discussion

The ability of MECC to resolve a mixture of five cephalosporins is demonstrated by Fig. 1. The chemical structures of the cephalosporins in the standard mixture are provided in Table 1. In MECC, an ionic surfactant, such as SDS, is added to the buffer solution above the critical micelle concentration (CMC) forming self-aggregating micelles. Neutral and weakly ionic analytes, such as the cephalosporins, will become partially incorporated into the micelle, and therefore migration will be altered by the velocity of the micelle. The greater the solubility of the analyte in the micelle, the slower it will migrate. Cephalosporin migration order will generally be controlled by the ionization state and hydrophobicity of the different side chains and functional groups of each molecule. Cefuroxime migrates faster toward the cathode due to the overall positive charge, whereas cephalothin undergoes the slowest migration by virtue of its anionic character. Intermediate migration velocities are attributable to electrostatic and hydrophobic interactions.

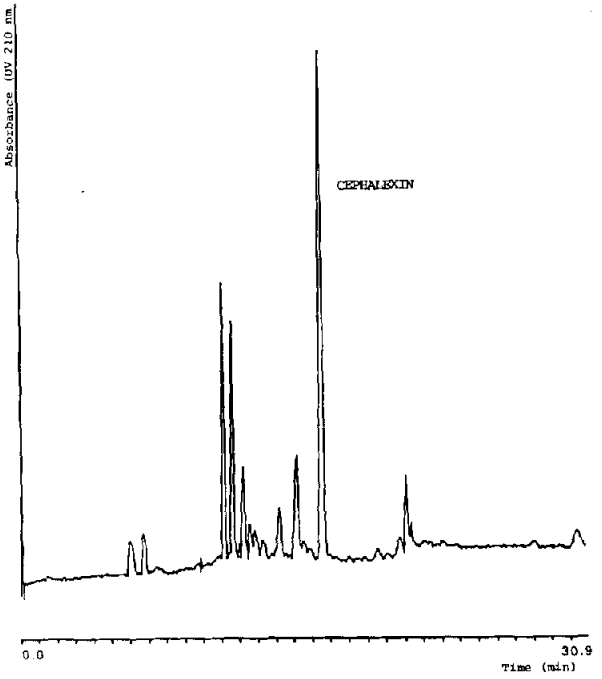


Fig. 4. Electropherogram of degraded cephalexin (1 mg/ml), pharmaceutical capsule form, other peaks unidentified. CE conditions as in Fig. 2.

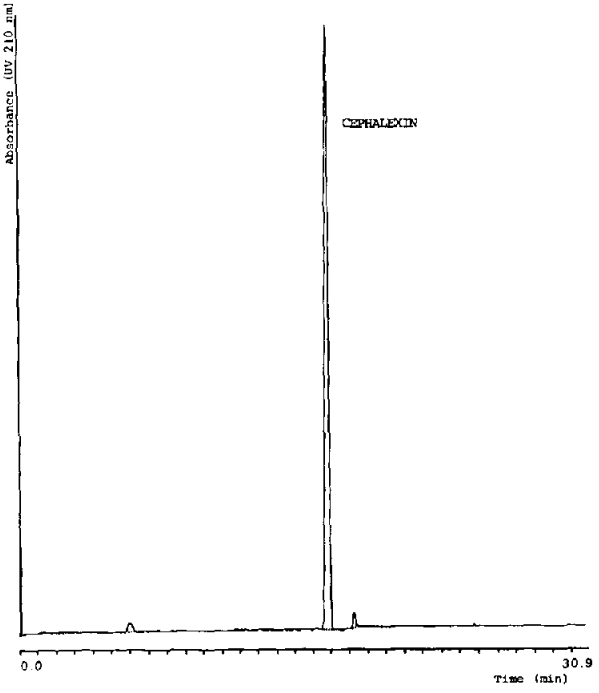


Fig. 3. Typical electropherogram of cephalexin (1 mg/ml), pharmaceutical capsule form. CE conditions as in Fig. 2.

The migration times of each cephalosporin were significantly increased with the addition of an ion-pairing reagent, pentanesulfonic acid to the running buffer, as demonstrated in Fig. 2. At relatively higher concentrations, pentanesulfonic acid tends to be a secondary micelle-former that alters the pseudostationary phase of the running buffer, thus resulting in higher resolutions. Analysis of cephalosporin antibiotics using the same buffer concentrations is demonstrated in Figs. 3 and 4. It appears that this application may be used for identification and as a stability-indicating assay for cephalosporin pharmaceuticals.

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