Analysis of Sulfonamide Antibiotics by Capillary Electrophoresis

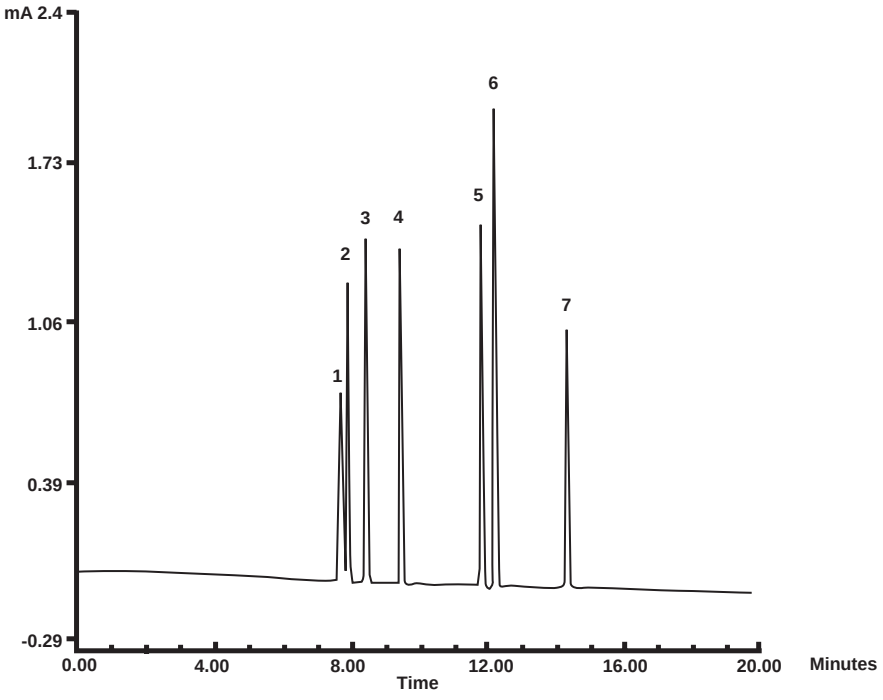

Fig. 1. Separation of sulfonamides by CZE. Peak identity: 1, sulfanilamide; 2, sulfamethazine; 3, sulfamerazine; 4, sulfadiazine; 5, sulfamethizole; 6, sulfamethoxazole; 7, sulfisoxazole. Sample concentration was 10 ppm each.

Analysis Conditions

Instrument	BioFocus® 3000 system
Polarity	positive to negative
Capillary	50 µm x 50 cm, uncoated (catalog number 148-3040)
Run buffer	50 mM NaH_2PO_4 + 50 mM $Na_2B_4O_7$ (pH 6.4) + 2 mM β-cyclodextrin
Injection	1psi * sec
Run voltage	15 kV
Detection	200 nm
Cartridge temperature	20 °C
Autosampler temperature	20 °C

Introduction

Sulfonamides are an important class of antibiotics used in the treatment of microbial infections in humans and animals. The antimicrobial activity of these drugs results from their inhibition of the biosynthesis of folic acid. This vitamin, which is essential for nucleic acid metabolism, is synthesized by enzymatic conversion from *p*-aminobenzoic acid (PABA), a reaction blocked by the sulfonamides. Bacteria which depend upon endogenous conversion of PABA to folic acid are sensitive to sulfonamides, while other organisms (such as humans), which derive folic acid from alternative biosynthetic pathways or from dietary sources, are unaffected.

Analysis of sulfonamides and other antibiotics is generally performed by HPLC, but recently separation of sulfonamides by capillary zone electrophoresis[1, 2] and by micellar electrokinetic capillary chromatography[3] has been described. In this study, sulfonamides were separated by capillary zone electrophoresis using β-cyclodextrin as an additive.

Results

Figure 1 shows the optimal separation conditions for seven sulfonamides by CZE at pH 6.4, with 2 mM ß-cyclodextrin as modifier. Lower pH runs resulted in increased separation times due to reduced osmotic flow. In the absence of ß-cyclodextrin, slight changes in pH affected the separation adversely and migration time reproducibility was very poor, especially for the slower migrating peaks sulfamethizole, sulfamethoxazole, and sulfisoxazole. Addition of ß-cyclodextrin, a neutral cyclic oligosaccharide with polar hydroxy groups, improved reproducibility. This additive probably stabilized the separation by selectively forming inclusion complexes with the various sulfonamides in its hydrophobic cavity. Increasing the

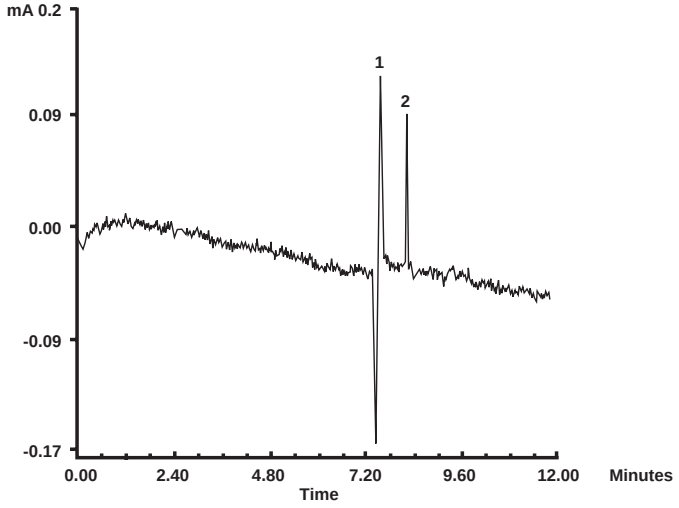

Fig. 2. Detection limit for sulfonamides by CZE. Peak identity: 1, methanol; 2, sulfamerazine at 1 ppm.

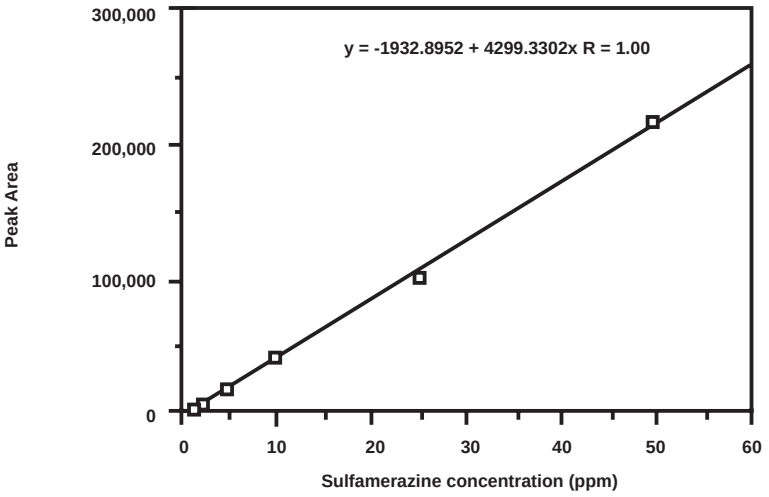

Fig. 3. Linearity plot for sulfamerazine.

Table 1. Reproducibility of migration times and peak areas for sulfonamides analyzed in Figure 1.

Compound	Migration Time (%RSD)	Peak Area (%RSD)
Sulfanilamide	0.41	1.71
Sulfamethazine	0.44	1.15
Sulfamerazine	0.48	2.78
Sulfadiazine	0.56	2.71
Sulfamethizole	0.89	3.13
Sulfamethoxazole	0.82	3.43
Sulfisoxazole	1.04	4.26

Analysis conditions were as in Figure 1, with the exception of capillary dimensions (50 µm ID x 75 cm L, uncoated) and applied voltage (20 kV). Number of analysis=6.

ß-cyclodextrin concentration to 5 mM did not improve the peak shape for sulfanilamide, and adding 10% MeOH to the run buffer at pH 6.4 only increased the migration times.

As shown in Figure 2, the limit of detection for sulfamerazine with this analysis method is about 1 ppm (S/N=5) and the response is linear in a concentration range from 1 to 50 ppm (Figure 3). Migration time and peak area reproducibilities for seven sulfonamides over six runs are listed in Table I. Values ranged from 0.4% to 1.04% for migration times and 1.15 to 4.26% for peak areas. These results demonstrate that free zone capillary electrophoresis provides satisfactory separation selectivity, detection sensitivity, and quantitative precision to serve as an analytical tool for identification of sulfonamides in pharmaceutical preparations and in the quality control of sulfonamide formulations.

References

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