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Capillary electrophoresis with field-amplified sample stacking for rapid and sensitive determination of sulfadiazine and sulfamethoxazole

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A new capillary electrophoresis method with field-amplified sample stacking (FASS) was developed for the analysis of sulfadiazine and sulfamethoxazole. After optimization of the separation and concentration conditions, the two compounds can be separated within 7 min and quantified with high sensitivity, with detection limits of 0.48 ng/mL for sulfadiazine and 0.76 ng/mL for sulfamethoxazole. This resulted in a 300–1500-fold improvement in concentration sensitivity relative to conventional capillary electrophoresis methods. The method was useful for qualitative and quantitative analysis of sulfadiazine and sulfamethoxazole in their preparations with recovery of 99.0%∼102% for sulfadiazine and 99.5% ∼ 99.7% for sulfamethoxazole.

1. Introduction

Sulfonamides are a class of antibacterial drugs with a fairly wide antibacterial spectrum and rapid absorption. However, research suggests that they could be a cause of cancer (Littlefield 1988). When they are present as residues in animal products or tissues, they could present a risk to consumer safety. Thus, simple, rapid, and sensitive methods for the separation and determination of these compounds are of great interest.

Capillary electrophoresis (CE) has become one of the most useful techniques for the monitoring of drugs (Hu et al. 2006; Rao et al. 2006; Hamoudová and Pospíšilová 2006; Chen et al. 2006). However, a shortcoming of CE is the high detection limit resulting from the limited optical path length for on-line UV detection and the small amount of sample loaded into the capillary. Overcoming the problems associated with the poor sensitivity of CE is currently the emphasis of many investigations. One approach is to use highly sensitive detectors (Engstrom et al. 1995; Ozaki et al. 1995; Zhang et al. 2005), but these have not been widely applied because of their high cost. Another useful method is sample concentration before the CE separation, especially on-line concentration by sample stacking or sweeping (Liu et al. 2002; Zhang et al. 1995; Chien and Burgi 1991; Deforce et al. 1996; Quirino and Terabe 2000; Quirino et al. 2002; Shihabi 1996; Shihabi and Friedberg 1998; Xiong et al. 1998; Hadwiger et al. 1996).

In recent years, several CE methods have been documented for the analysis of sulfonamides (Teshima et al. 2004; Berzas Nevado et al. 2005; Fan et al. 2003; Berzas Nevado et al. 2001; Fan et al. 2005), but these methods have relatively low sensitivity. It is therefore desirable to establish approaches that offer higher sensitivity than the existing methods.

In this work, a new CE method with field-amplified sample stacking is presented for the analysis of SD and SMZ. After optimization of the separation and concentration conditions, the two compounds can be quantified within 7 min with detection limits of 0.48 ng/mL for SD and 0.76 ng/mL for SMZ. The method was applied to the analysis of SD and SMZ in their three preparations with recoveries of 99.0%∼102% for SD and 99.5%∼99.7% for SMZ.

2. Investigations, results and discussion

2.1. Optimization of the separation conditions

To achieve satisfactory separation using CE, the effects of buffer pH, concentration, and voltage on the resolution and peak heights were investigated.

In CZE, the pH of the buffer plays an important role in the separation. In this study, the effect of buffer pH on peak heights and resolution was investigated over the pH range 5.5–9.0 with 30 mM NaH_2PO_4 and 25 kV applied voltage. From Fig. 1, it appears that the best resolution and sensitivity were achieved at pH 6.0, so pH 6.0 was preferred for further studies.

Buffer concentration also has an obvious influence on separation because it can influence the EOF and the viscosity of the electrolyte. The effect of the concentration of NaH_2PO_4 at pH 6.0 on separation and peak heights was studied in the range from 10 to 80 mM with a 25 kV applied voltage. As seen in Fig. 2, the resolution increased almost linearly with increasing buffer concentration. The best sensitivity was achieved at 50 mM, and so this concentration of NaH_2PO_4 was chosen.

The effect of varying the separation voltage from 20 to 30 kV was investigated under the conditions selected above. The migration time and resolution decreased significantly with increasing separation voltage; however, when it exceeded 27.5 kV, the peak heights of the analytes decreased. So a separation voltage of 27.5 kV was considered to be a good compromise between sensitivity and resolution.

Based on the investigation above, optimal separation was obtained with an electrolyte containing 50 mM NaH_2PO_4 (pH 6.0) and an applied voltage of 27.5 kV (current was 93.4 μA).

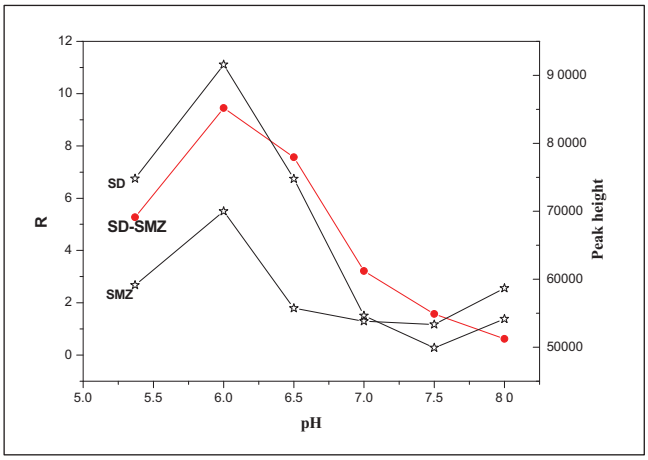

Fig. 1: Effect of pH on the resolution and peak heights of **SD** and **SMZ**. Conditions: Capillary 50 cm (42.4 cm to detector) × 75 μm i.d. Buffe 50 mM NaH_2PO_4, pH 5.37–8.0. Separation voltage was 27.5 kV, the detection wavelength was 214 nm, cartridge temperature was 25 ± 0.1 °C. Sample was injected − 8.0kV × 3.0 s. ●-Resolution, ☆ -Peak height

2.2. *Optimization of concentration conditions*

2.2.1. *Choice of solvent plug*

In FASS of the analytes, injection of a solvent plug of lower conductance has a great effect on the concentration efficiency. The effect of ethanol and water plugs on the concentration efficiency of the analytes was investigated. From Fig. 3, it is evident that the concentration efficiency of the water plug was better than that of ethanol, so the water plug was chosen.

2.2.2. *Choice of water-plug injection time*

In FASS of the analytes, length of the water plug has a great effect on the concentration efficiency. The length of a water plug is in direct proportion to its injection time. Therefore, the effect of water plug injection times from 3 s to 20 s on concentration efficiency of the analytes was investigated. The results of these experiments are presented in Fig. 4 and clearly indicate that the peak heights and areas of SD and SMZ were strongly increased when the water plug was injected for 12 s and that the peak heights and areas decreased when the injection time was further prolonged. So an injection time of 12 s was selected for the water plug.

In addition, it was found that the peak area (A) of the analyte was a function of the injection time (t) of the water plug. By applying

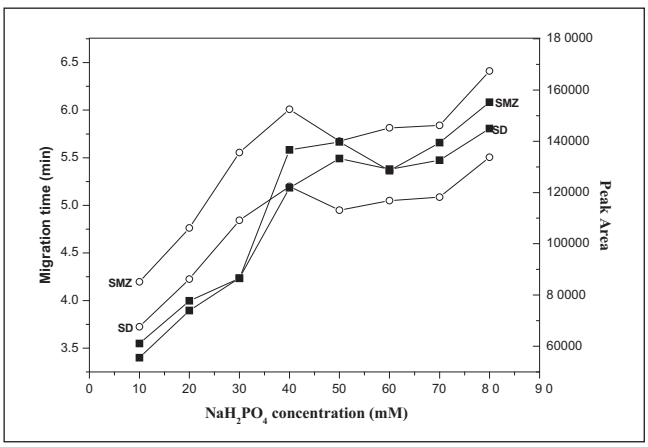

Fig. 2: Effect of NaH_2PO_4 concentration on the migration time and the peak area of **SD** and **SMZ**. NaH_2PO_4 concentration was from 10–80 mM, pH 6.0. Other conditions are same as Fig. 1. ◯-Migration time, ■-Peak area

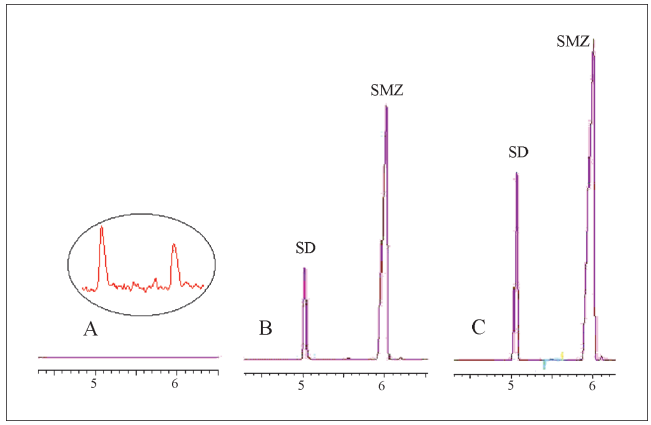

Fig. 3: Effect of solvent plug on concentration efficiency. **A** No injection any solvent plug; **B** Injection ethanol plug 0.5psi × 3 s; **C** Injection water plug 0.5psi × 3 s before injection sample (− 8.0kV × 3.0 s). Other conditions are same as Fig. 1

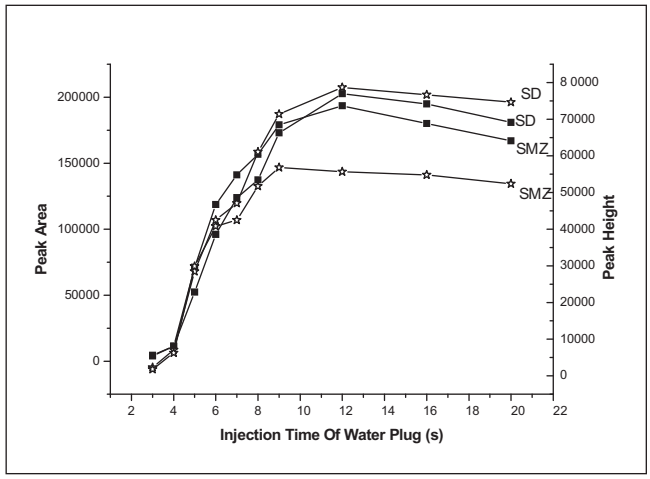

Fig. 4: Effect of injection time of water plug on peak areas and heights of SD and SMZ. Injection time of water plug was from 3–20 s with 0.5 psi before injection sample (−8.0kV × 3.0 s). Other conditions are same as Fig. 1. ☆ -Peak height, ■-Peak area

nonlinear regression to the experimental results (Fig. 5A-B), Eq. (2) is proposed to depict the influence of injection time on the peak area of SD and SMZ. The Eq. (2) is as follows:

$$A = \frac{A_1 - A_2}{1 + e^{(t-t_0)/dt}} + A_2 \qquad (2)$$

where A_1 and A_2 are the peak area of the analytes at the minimum and maximum injection times of the water plug, t is the injection time of the water plug and t_0 and dt are constants. In Fig. 5A-B, the scattered dots and the solid line represent the experimental and simulation results, respectively. The nonlinear regression parameters are listed in Table 1. An excellent correlation (correlation coefficients > 0.98) between the experimental and nonlinear regression results was obtained. As Eq. (2) indicates, two extremes (maximum or minimum) of the peak area for the analytes were reached at the minimum and maximum injection times of the water plug. The peak area of each analyte varied over the range of the maximum and minimum peak area values

Table 1: Nonlinear regression parameters of Eq. (2)

Analyte	A_1	A_2	t_0	dt	R^2
SD	−29419	1.9304E5	5.8418	1.4972	0.9858
SMZ	−23729	1.793E5	5.2530	1.0541	0.9828

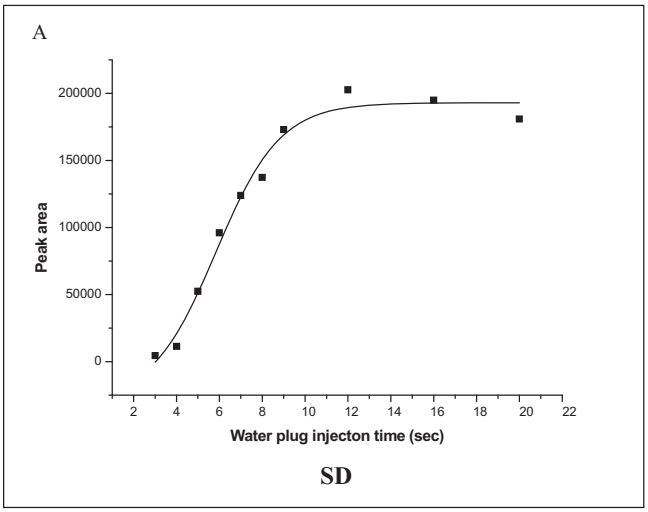

Fig. 5: Effect of injection time of water plug on peak areas of SD and SMZ. Other conditions are same as Fig. 2. ■- Experiment results; — nonlinear regression results

with $e^{(t-t_0)/dt}$. This can be explained on the basis that the correct water plug provides a higher electric field at the tip of the capillary, which will in turn improve the sample stacking producedure (Chien and Burgi 1991). However, the result of the proposed model in Fig. 5A-B was on the low side compared to Chien's model (Chien 1991). This might be explained if Chien's model did not take into account the effect of a strong laminar flow, such that when the water plug was too long, a strong laminar flow was generated as a result of the mismatch of EOF velocity in the sample and background buffer zones (Liu et al. 2002).

2.2.3. Choice of sample injection voltage

The effects of sample injection voltage on peak intensity were tested. The results showed that the peak intensity increased with increasing sample-injection voltage from –4 to –10 kV. However, when the injection voltage was too high the analytes would move through the boundary of the water plug and the running buffer into the running buffer, which resulted in incomplete stacking of the analytes and a decrease in separation efficiency. In the present case, an injection voltage of − 10 kV was used in order to achieve high detection sensitivity without sacrificing separation efficiency.

2.2.4. Choice of sample injection time

The effects of sample injection time on the peak intensity are shown in Fig. 6. The peak heights and area of SMZ increased

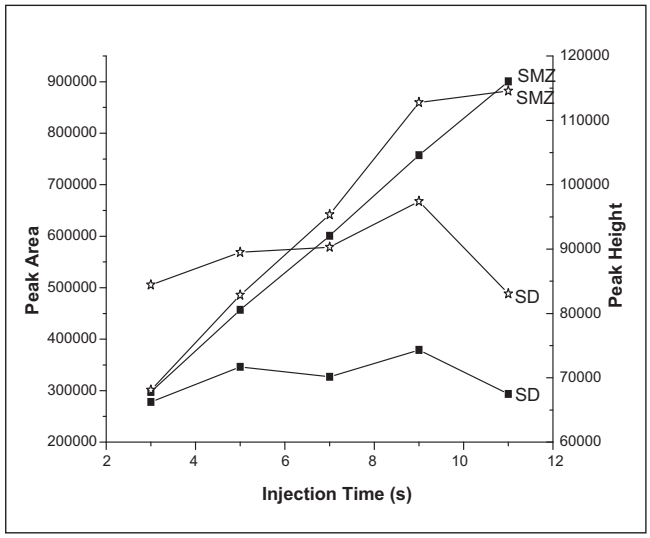

Fig. 6: Effect of injection time on peak areas and heights of SD and SMZ. Injecting sample 3–11 s with –10 kV after injection 0.5 psi × 12 s water plug. Other conditions are same as Fig. 1. ☆ -Peak height, ■-Peak area

with increasing injection time from 3 to 11 s; but the peak heights and area of SD increased with increasing injection time from 3 s to 9 s. With further increases in injection time, the peak height and area of SD began to decrease, which may be due to sample overloading that resulted in SD moving into the running buffer through the boundary of the water plug and the running buffer, and incomplete stacking of SD. Therefore, the injection time was chosen as 9 s as a compromise between SD and SMZ.

In accordance with the results above, SD and SMZ were injected at −10 kV for 9 s after injection of a 12 s-water plug and separated with 50 mM NaH_2PO_4 at pH 6.0. A typical electropherogram of the standard mixture under the optimum separation and concentration conditions is shown in Fig. 7A. The two analytes were well separated and concentrated within 7 min. The stacking efficiency was investigated with a solution of 125 μg/mL SD and 125 μg/mL SMZ, the results are shown in Table 2.

2.3. Linearity, detection limit and repeatability

A six-point calibration curve for each analyte was established by plotting peak area against analyte concentration. The corresponding regression equations, as well as other characteristic parameters are listed in Table 3. The calibration curves exhibit excellent linear behavior over the concentration range. The limits of detection (LODs) were estimated from the calibration curve of peak height versus standard concentration and are based on the concentration necessary to yield a net height equal to three times the standard deviation of the baseline noise, i.e. LOD = 3 *s*/*S* (*s* is the standard deviation of the baseline noise, *S* the slope of the regression equation of peak height versus sample concentration). The baseline noise was evaluated by recording

Table 2: Concentration multiples (CM_{height} and CM_{area}) of the analytes[a]

Analyte	Pharameter	No Stacking (N_1)	Stacking (N_2)	N_2/N_1
SD	Peak height	117	56311	481
	Peak area	387	123323	319
SMZ	Peak height	106	95809	904
	Peak area	322	469481	1458

[a] Concentration of SD and SMZ: 125 μg/mL

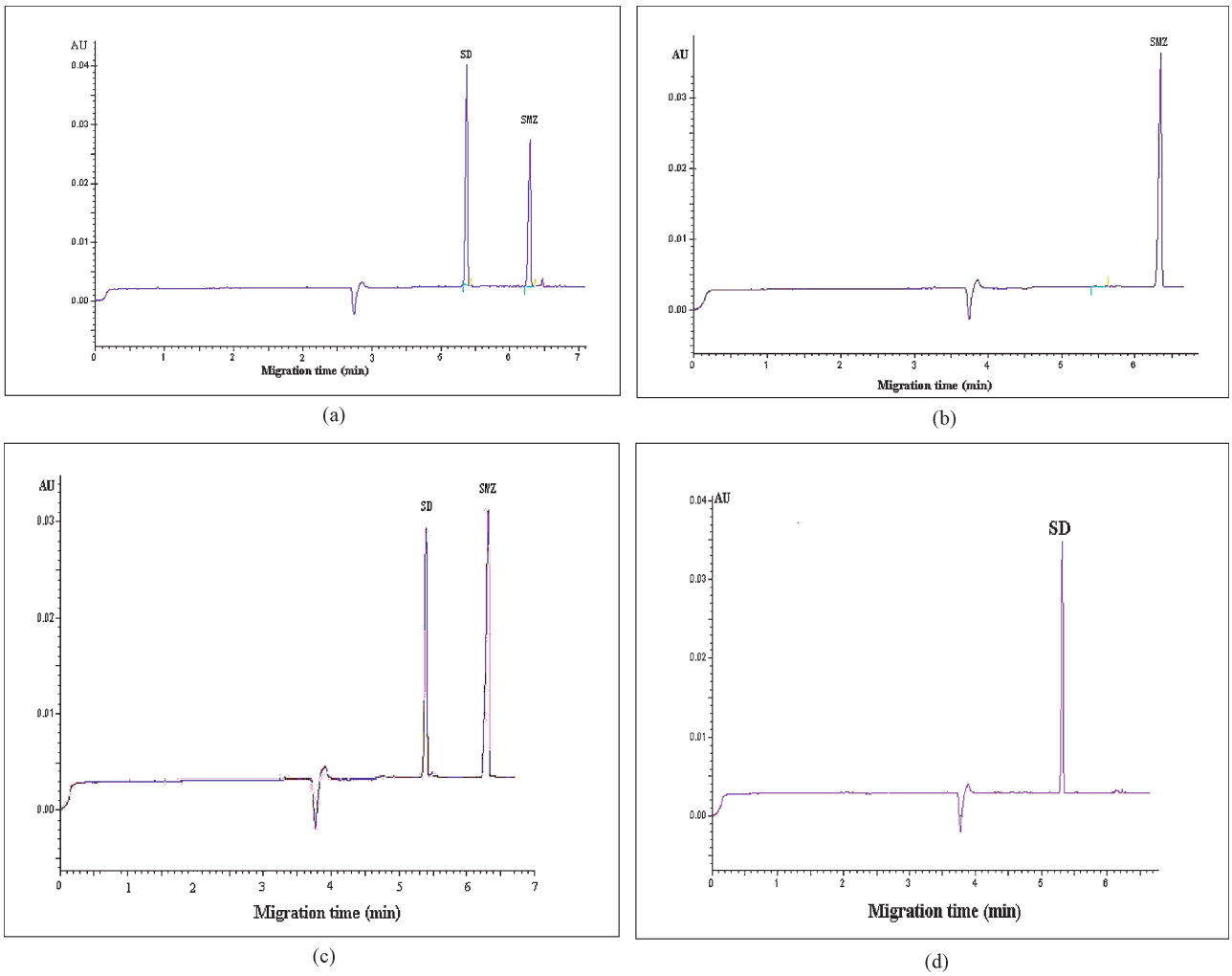

Fig. 7: The electropherograms of the standards mixture solution and the real samples. **A** The standards mixture; **B** TMP-SMZ tablet; **C** TMP-SMZ-SD tablet; **D** SD tablet. Conditions: Injection sample 9 s with –10 kV after injection 0.5 psi × 12 s water plug. Other conditions are same as Fig. 1

the detector response every 1 min over a period of about 7 min. The LODs of SD and SMZ are also given in Table 3.

The repeatability of the migration time and peak area of SD and SMZ were determined at three concentration levels (n = 6) in the respective ranges given above. The relative standard deviations (RSD) of the migration time and peak area of each analyte are also shown in Table 3.

2.4. Application and recovery

The optimum conditions were applied to the separation and determination of SD and SMZ in three commercial preparations. A typical electropherogram obtained is shown in Fig. 7B-D. The peaks were identified by comparison with previously obtained migration times and standard additions.

Table 3: Analytical performance of CE testing system ($n = 6$)

Item	SD	SMZ
Migration time RSD (%)	1.50	1.74
Peak area RSD (%)	1.83	2.49
Liner range (μg/mL)	0.05 – 10.0	0.025 – 10.0
Regression equation [b]	Y = 16230x + 154.64	Y = 25804x – 710.29
Correlation coefficient (R^2)	0.9998	0.9990
LOD (ng/mL)	0.48	0.76

[b] Y and x stand for the peak area and the concentration (μg/mL) of the analytes, respectively

The contents of the analytes found in the samples SD and SMZ together with their relative standard deviations (RSD) are given in Table 4. Each showed agreement between the values claimed and found. The recoveries (Table 5) of the method were evaluated by the addition of suitable amounts of standard substances to actual samples with known SD and SMZ contents, and were satisfactory.

Results of the present method compared with those of other CE methods are listed in Table 6. From Table 6, it may be observed that the present method is very simple and sensitive for the analysis of SD and SMZ.

3. Experimental

3.1. Apparatus

All separations were performed on a P/ACE^{TM} MDQ system (Beckman Coulter Instrument, Fullerton, CA, USA) equipped with a P/ACE UV detector. The system was controlled by P/ACE Gold Station software. The separation was carried out on a 50.2 cm (40 cm to the detector) × 75 μm

Table 4: Contents of analytes in samples (n = 3)

Sample	Analyte	Content found (mg/tablet)	Scalar quantity (mg/tablet)
TMP-SMZ tablet	SMZ	395.2	400
TMP-SD-SMZ tablet	SD	194.6	200
	SMZ	197.2	200
SD tablet	SD	492.3	500

Table 5: Recovery of the two analytes (n = 5)

Sample	Analyte	Concentration spiked (μg/mL)	Concentration found (μg/mL)	Recovery (%)	Average (%)	RSD (%)
TMP-SMZ tablet		0.125	0.126	100.8		
	SMZ	0.250	0.248	99.20	99.5	1.19
		1.25	1.231	100.8		
TMP-SD-SMZ tablet		0.250	0.258	103.2		
	SD	0.500	0.496	99.20	102	2.15
		2.50	2.568	102.7		
		0.125	0.123	98.40		
	SMZ	0.250	0.257	102.8	99.7	2.70
		1.25	1.224	97.92		
SD tablet		0.250	0.251	100.4		
	SD	0.500	0.499	99.80	99.0	2.02
		2.50	2.417	96.68		

i.d. fused-silica capillary (Beckman Coulter Instrument, USA). The detection wavelength was 214 nm. The detection was cathodic. The capillary was maintained at 25 °C. The water plug was injected from the anode at 0.5 psi before the sample was reverse electrokinetically injected.
The capillary was treated prior to its first use by flushing with 0.1 M NaOH for 10 min, and distilled water for 10 min. Between two runs, a rinse-cycle of distilled water for 2 min, 0.1 M NaOH for 2 min, distilled water for 2 min and running buffer for 2 min was used.

3.2. Reagents and materials

SD and SMZ were purchased from the National Institute for the Control of Pharmaceutical and Biological Products, China. All the reagents used were of analytical grade and distilled water was used throughout the study.
Stock standard solutions of SD (200.0 μg/ml) and SMZ (200 μg/ml) were prepared in ethanol-water (1:1, v/v). Working standard solutions were obtained by diluting the corresponding, respectively stock standard solutions with ethanol-water (1:1, v/v) to the desired concentration.
Buffer solutions were prepared from 0.1 M NaH_2PO_4 stock solutions. The pH of the buffer was adjusted by 2 M NaOH to give the required pH. All buffer solutions were filtered through a 0.45-μm membrane before use.
TMP-SMZ tablets (scalar quantity: 80.0 mg/tablet of TMP and 400.0 mg/tablet of SMZ), SD tablets (scalar quantity: 500.0 mg/tablet of SD) and TMP-SMZ-SD tablets (scalar quantity: 80.0 mg/tablet of TMP, 200.0 mg/tablet of SMZ and 200.0 mg/tablet of SD) were purchased from a local drugstore.

3.3. Sample preparation

Ten tablets of each commercial preparation were weighed, powdered and the contents mixed thoroughly. Then a quantity of the powder equivalent to one tenth of one tablet was extracted with ethanol-water (1:1, v/v) for 1.0 h in an ultrasonic bath, and the supernatant was filtered through a 0.45–0.45-μm membrane. The solution was diluted with ethanol-water (1:1, v/v) to the desired concentration, and then was directly injected into the CE for the determination.

3.4. Field-amplified sample-stacking procedure

A water plug was pre-introduced into the capillary at the anodic end by application of a pressure of 0.5 psi, and then a voltage was applied with negative polarity for injection of the sample at the cathodic end. After sample injection, the voltage was turned off and the ends of the capillary were returned to buffer reservoirs; a positive voltage was applied for the separation.

3.5. Calculation of concentration multiple

The concentration multiple (CM) was calculated using the equations (1) $CM_{height} = H_{CM}/H_0$ and (2) $CM_{area} = A_{CM}/A_0$, where the numerator is the

Table 6: Comparison of the present to other CE methods

	Capillary length (cm)	Buffer conditions	Analysis time (min)	Line range (μg/mL)	Detection limit (ng/mL) λ (nm)	Analytes/Application
Teshima et al. (2004)	64.5 cm × 50 μm i. d. uncoated fused-silica capillary	15 mM phosphate, pH = 6.2	8	12.5–80	1000 for SMZ	SMZ and TMP/Human Plasma
Berzas-Nevado et al. (2005)	60.2 cm × 50 μm i. d. uncoated fused-silica capillary	20 mM borate (pH 9.3), 25 mM sodium dodecylsulfate, and 5% *v/v* acetonitrile	8	12.5–80	3000 for SMZ	SMZ, TMP and their metabolities/Human serum
Fan et al. (2003)	65 cm × 75 μm i. d. uncoated fused-silica capillary	30 mM phosphate, pH = 6.0	2	2.0–80	1050 for SD 1280 for SMZ 214	TMP, SD and SMZ/TMP-SMZ tablet and TMP-SD-SMZ tablet
Berzas-Nevado et al. (2001)	60.2 cm × 75 μm i. d. uncoated fused-silica capillary	15 mM phosphate, pH = 6.2	6	1–25	1000 for SMZ	SMZ, TMP/Preparations
Fan et al. (2005)	7.5 cm × 75 μm i. d. uncoated fused-silica capillary	30 mM phosphate, pH = 8.5	2	2.0–100	700 for SMZ 214	TMP and SMZ/TMP-SMZ tablet
The present method	60.2 cm × 75 μm i. d. uncoated fused-silica capillary	50 mM NaH_2PO_4, pH = 6.0	7	0.05–10.0 for SD 0.025–10.0 for SMZ	0.48 for SD 0.76 for SMZ 214	SD and SMZ/TMP-SMZ tablet, TMP-SD-SMZ tablet and SD tablet

peak height or peak area obtained with optimal stacking and the denominator is the peak height or peak area obtained from $-8.0\text{kV} \times 3.0\,\text{s}$ injection.

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References

Berzas Nevado JJ, Castañeda Peñalvo G, Guzmán Bernardo FJ (2001) Determination of sulfametoxazole, sulfadiazine and associated compounds in pharmaceutical preparations by capillary zone electrophoresis. J Chromatogr A 918: 205–210.

Berzas Nevado JJ, Castañeda Peñalvo G, Guzmán Bernardo FJ (2005) Micellar electrokinetic chromatography method for the determination of sulfamethoxazole, trimethoprim and their main metabolites in human serum. J Separation Sci 28: 543–548.

Chen G, Xu XJ, Zhu YZ, Zhang LY, Yang PY (2006) Determination of honokiol and magnolol in Cortex Magnoliae Officinalis by capillary electrophoresis with electrochemical detection. J Pharm Biomed Anal 41: 1479–1484.

Chien R-L (1991) Mathematical modeling of field-amplified sample injection in high-performance capillary electrophoresis. Anal. Chem 63: 2866–2869.

Chien R-L, Burgi DS (1991) Field amplified sample injection in high-performance capillary electrophoresis. J Chromatogr A 559: 141–152.

Deforce DLD, Ryniers FPK, Van den Eeckhout EG (1996) Analysis of DNA adducts in DNA hydrolysates by capillary zone electrophoresis and capillary zone electrophoresis-electrospray mass spectrometry. Anal. Chem 68: 3575–3584.

Engstrom A, Andersson PE, Josefsson B (1995) Determination of 2-(9-anthryl)ethyl chloroformate-labeled amino acids by capillary electrophoresis and liquid chromatography with absorbance or fluorescence detection. Anal. Chem 67: 3018–3022.

Fan LY, Chen HL, Chen XG, Hu ZD (2003) Separation and determination of sulfonamides in pharmaceutical preparations by a microfluidic capillary electrophoresis system with a continuous sample introduction interface. J Separation Sci 26: 1376–1382.

Fan LY, Liu LH, Chen HL, Chen XG, Hu ZD (2005) Continuous on-line concentration based on dynamic pH junction for trimethoprim and sulfamethoxazole by microfluidic capillary electrophoresis combined with flow injection analysis system. J Chromatogr A 1062: 133–137.

Hadwiger ME, Torchia SR, Park S, Biggin ME, Lunte CE (1996) Optimization of the separation and detection of the enantiomers of isoproterenol in microdialysis samples by cyclodextrin-modified capillary electrophoresis using electrochemical detection. J Chromatogr B 681: 241–249.

Hamoudová R, Pospíšilová M (2006) Determination of ibuprofen and flurbiprofen in pharmaceuticals by capillary zone electrophoresis. J Pharm Biomed Anal 41: 1463–1467.

Hu Z, He LC, Zhang J, Luo GA (2006) Determination of three bile acids in artificial Calculus Bovis and its medicinal preparations by micellar electrokinetic capillary electrophoresis. J Chromatogr B 837: 11–17.

Littlefield N (1988) Technical Report. Chronic toxicity and carcinogenicity studies of sulfamethazine in $B6CF_1$ mice. National Center for Toxicological Research Jefferson. AR.

Liu SH, Chen XG, Hu ZD (2002) Field-amplified sample stacking in capillary electrophoresis for on-column concentration of alkaloids in Sophora flavescens Ait. Electrophoresis 23: 3392–3397.

Ozaki H, Itou N, Terabe S, Takada Y, Sakairi M, Koizumi H (1995) Micellar electrokinetic chromatography-mass spectrometry using a high-molecular mass surfactant on-line coupling with an electrospray ionization interface. J Chromatogr A 716: 69–79.

Quirino JP, Terabe S (2000) Sample stacking of cationic and anionic analytes in capillary electrophoresis. J Chromatogr A 902: 119–135.

Quirino JP, Kim JB, Terabe S (2002) Sweeping: concentration mechanism and applications to high-sensitivity analysis in capillary electrophoresis. J Chromatogr A 965: 357–373.

Rao YK, Chou C-H, Tzeng Y-M (2006) A simple and rapid method for identification and determination of cordycepin in Cordyceps militaris by capillary electrophoresis. Anal Chim Acta 566: 253–258.

Shihabi ZK (1996) Peptide stacking by acetonitrile-salt mixtures for capillary zone electrophoresis. J. Chromatogr A, 744: 231–240.

Shihabi ZK, Friedberg M (1998) Insulin stacking for capillary electrophoresis. J Chromatogr A 807: 129–133.

Teshima D, Otsubo K, Makino K, Itoh Y, Oishi R (2004) Simultaneous determination of sulfamethoxazole and trimethoprim in human plasma by capillary zone electrophoresis. Biomed Chromatogr 18: 51–54.

Xiong Y, Park S-R, Swerdlow H (1998) Base stacking: pH-mediated on-column sample concentration for capillary DNA sequencing. Anal. Chem 70: 3605–3611.

Zhang J, Fang Y, Hou JY, Ren HJ, Jiang R, Roes P, Dovichi NJ (1995) Use of non-cross-linked polyacrylamide for four-color DNA sequencing by capillary electrophoresis separation of fragments up to 640 bases in length in two hours. Anal. Chem 67: 4589–4593.

Zhang JY, Hu ZD, Chen XG (2005) Quantification of glutathione and glutathione disulfide in human plasma and tobacco leaves by capillary electrophoresis with laser induced fluorescence detection. Talanta 65: 986–990.