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Separation of sulphonamides and determination of the active ingredients in tablets by micellar electrokinetic capillary chromatography

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ABSTRACT

The separation and determination of seven sulphonamides and trimethoprim by micellar electrokinetic capillary chromatography were successfully achieved, employing sodium dodecyl sulphate (SDS) as a micellar phase and tetrabutylammonium bromide as additive. The effects of surfactant and modifier concentrations, pH and applied voltage on the retention behaviour of the solutes and the column efficiency were studied. The migration time of sulponamides increase with increasing SDS concentration and decreasing the applied voltage, but varies only slightly with pH. There is an optimum applied voltage at which a higher theoretical plate number is achieved, in contrast to the sulphonamides, the retention behaviour of trimethoprim gave a more obvious response to changes in the experimental conditions. The determination of three active ingredients in tablets was performed using sulphathiazole as an internal standard with good results. The theoretical plate number ranged between $2.0 \cdot 10^5$ and $2.8 \cdot 10^5$ with a 50-cm capillary.

INTRODUCTION

Micellar electrokinetic capillary chromatography (MECC) was first reported by Terabe *et al.* in 1984 [1]. Several reports and reviews of the theory and application of MECC have been published [2–4]. Chiral separations of some amino acid derivatives [5] and drugs [6] have also been performed by MECC with a micelle of sodium dodecyl sulphate (SDS) and a chiral additive or with chiral cholate micelles. The determination of drugs in pharmaceutical preparations by application of MECC has been achieved by using an internal standard method [7,8]. Purity testing of diltiazem has also been reported [9].

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In this paper, the separation and determination of seven sulphonamides and trimethoprim in pharmaceutical preparations by MECC with SDS is reported. The effects of pH, concentrations of surfactant and modifier and applied voltage are discussed. The application of this technique to the quantitative analysis of commercial tablets using an internal standard method is also described.

EXPERIMENTAL

Apparatus

Experiments were performed with a Bio-Rad Labs. (Richmond, CA, USA) HPE 100 apparatus equipped with a UV detector adjusted to 240 nm and a power supply able to deliver up to 12 kV D.C. Electrokinetic sampling was used to introduce samples into the capillary. A Bio-Rad Labs. 148-3014

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HPE capillary cartridge (50 cm × 50 μm I.D., uncoated) was employed and a Chromatopac C-R3A (Shimadzu, Kyoto, Japan) was used for data processing.

Drugs and reagents

The bulk drugs analysed are listed in Table I.

SD–SMZ–TMP tablets (nominally containing 200 mg of SD, 200 mg of SMZ and 80 mg of TMP per tablet) and SMZ–TMP tablets (nominally containing 400 mg of SMZ and 80 mg of TMP per tablet) were purchased from Beijing Pharmaceutical Factory (Beijing, China) and Xian Pharmaceutical Factory (Xian, Shaanxi, China), respectively. SDS was obtained from Nacalai Tesque (Kyoto, Japan). Tetrabutylammonium bromide (TAB) of analytical-reagent grade was a product of Beijing Chemical Factory (Beijing, China).

The buffer solutions were prepared by mixing 0.01 *M* sodium tetraborate solution with appropriate volume of 0.5 *M* sodium dihydrogenphosphate solution to give pH 9, 8, and 7, or with dilute hydrochloric acid to give pH 6 and 5. The appropriate amount of SDS was dissolved in the buffer solutions to obtain carrier solutions. The solutions were filtered through a 0.45-μm membrane filter and degassed by ultrasonication prior to their use.

Test solutions and procedure

Owing to the low solubility of TMP in water, all test solutions were prepared by shaking the solutes, after shaking them with an appropriate amount of methanol first, with a mixture of two volumes of methanol and eight volumes of pH 8.5 buffer solution that was added later. A resolution test mixture was prepared by dissolving the solutes to obtain a solution containing 100 μg/ml each of SPZ, SDM,SD, SMZ, ST and TMP and 50 μg/ml each of SN and SG. For the determination of the linear range and response factor, five solutions were prepared to give a series of concentrations ranging from 45 to 225 μg/ml of SD and SMZ and from 12 to 60 μg/ml of TMP. After preparation as above, the concentrations of the solutions for the recovery tests were 36 μg/ml of SD and SMZ and 14 μg/ml of TMP and other two concentrations levels equivalent to 80% and 120% of these concentrations. The powder of SD–SMZ–TMP tablets or SMZ–TMP tablets was dissolved at a concentration of 14 μg/ml of TMP. All the solutions contained 90 μg/ml of ST as internal standard.

Sample solution was loaded by the electrokinetic method at the positive end of the capillary using a 10-kV constant voltage and a 10-s loading time. It is useful to rinse the internal wall of the capillary between individual injections. This was effected by injecting a flow of the carrier solution used into the capillary with a 100-μl syringe (Hamilton, Reno, NV, USA) at the negative end.All experiments were performed at ambient temperature.

RESULTS AND DISCUSSION

A typical electropherogram of the eight ingredients separated is shown in Fig. 1; each compound is completely resolved.

TABLE I

TEST SAMPLE

Peak No.	Compound	Abbreviation	Origin
1	Sulphanilamide	SN	British Pharmacopoeia,
2	Sulphaguanidine	SG	authentic specimen
3	Sulphaphenazole	SPZ	
4	Sulphadimethoxine	SDM	
7	Sulphathiazole	ST	
5	Sulphadiazine	SD	Bulk drugs, Chinese
6	Sulphamethoxazole	SMZ	Pharmacopoeia grade
8	Trimethoprim	TMP	

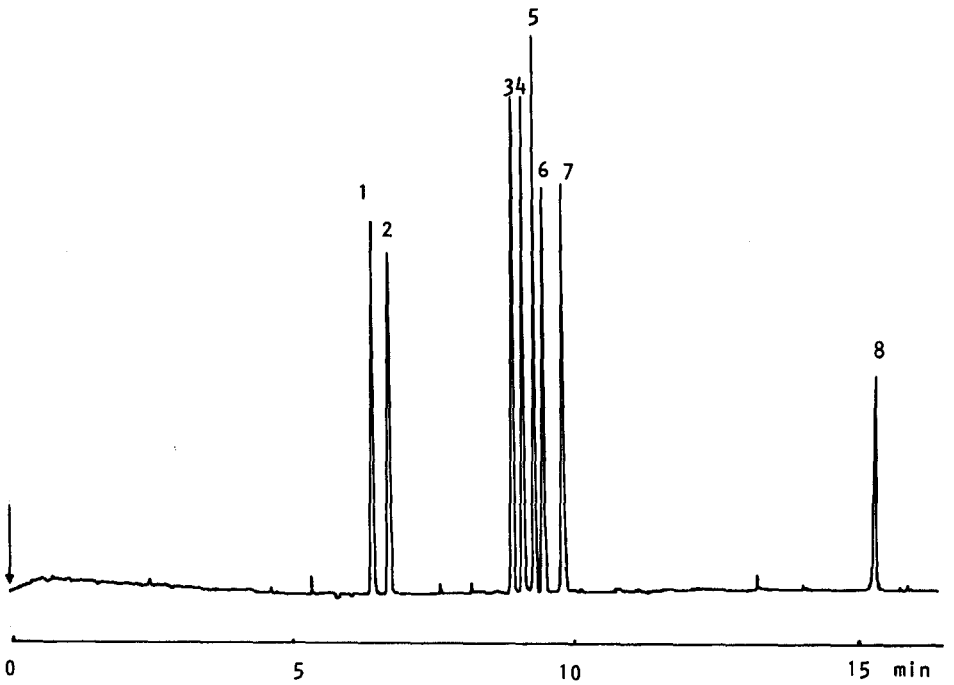

Fig. 1. Typical separation in MECC. For peak identifications, see Table I. Carrier, 0.025 *M* phosphate–borate solution (pH 8.5) containing 0.1 *M* SDS; applied voltage, 12 kV.

Quantitative analysis

The solutions for the determination of the linear range and relative responsive factor were chromatographed using 0.025 *M* phosphate–borate buffer solution (pH 8.5) containing 0.1 *M* SDS and 0.01 *M* TAB; a 12-kV voltage was applied in the constant-voltage mode. The results were calculated by the peak-area ratio method, and the calibration plots of A_i/A_s *vs.* W_i/W_s, where A_s and A_i are peak areas and W_s and W_i are weights of the sample(s) and internal standard (i), showed excellent linearity in the ranges 30–150 μg/ml for SD and SMZ and 12–60 μg/ml for TMP. Response factors relative to ST were calculated by a conventional method (Table II).

TABLE II

RESULTS OF THE DETERMINATION OF LINEAR RANGE AND RESPONSE FACTORS (n = 5)

Parameter	SD	SMZ	TMP
Correlation coefficient (r)	0.9999	0.9995	0.9958
Intersection at area ratio axis	0.0764	0.0206	−0.3720
Slope	2.174	1.144	3.555
Response factor	0.4288	0.8353	0.5645
Relative standard deviation (R.S.D.)	2.77%	2.62%	5.88%

Recoveries were examined under the experimental conditions described above. The results and their relative standard deviations are given in Table III.

The average recovery for each ingredient, with three levels and five repeated injections per level, was close to the stated composition value, and the relative standard deviation (R.S.D.), especially for SD and SMZ, was small, demonstrating that this method is sufficiently accurate and reproducible.

Determinations of each ingredient in SD–SMZ–TMP tablets and in SMZ–TMP tablets were performed according to the procedure described above. Typical chromatograms are shown in Fig. 2.

The assay results are summarized in Table IV. The results suggest that micellar electrokinetic capillary chromatography may be a useful technique in pharmaceutical analysis.

TABLE III

RESULTS OF THE DETERMINATION OF RECOVERIES

Ingredient	Amount added (μg/ml)	Amount found (μg/ml)	Recovery (%)	Average (%)	R.S.D. (%)
SD	28.5	28.5	100.0	100.3	1.27
	36.1	35.8	99.2		
	41.8	42.5	101.7		
SMZ	28.8	29.3	101.7	101.6	0.50
	35.3	35.7	101.1		
	42.3	43.2	102.1		
TMP	11.5	11.1	96.5	98.6	4.26
	14.2	13.6	95.8		
	17.7	18.3	103.4		

Retention behaviour

The retention behaviour of solutes depends on several properties, including hydrophobicity and degree of dissociation in the solution. The dependence of the migration times of seven sulphonamides and TMP on pH was examined with 0.1 *M* SDS solutions in the pH range 5–9 and the results are shown in Fig. 3. The migration times of all the sul-

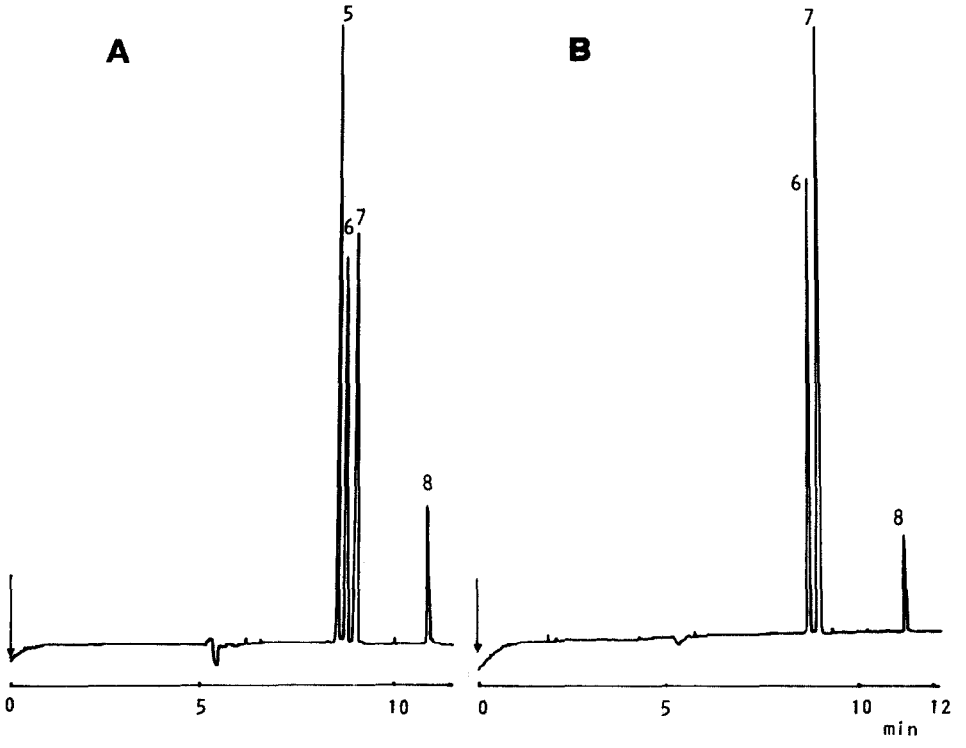

Fig. 2. Chromatogram obtained in the assay of (A) SD–SMZ–TMP tablets and (B) SMZ–TMP tablets. Conditions as in Fig. 1, with addition of 0.01 *M* TAB to the micellar solution. For peak identifications, see Table I.

TABLE IV

ASSAY RESULTS FOR THE PREPARATIONS OF SULPHONAMIDES ($n=8$)

Sample	Batch No.	Ingredient	Amount found (mg)	Label claim (mg)	Percentage of label claim	R.S.D. (%)
SD–SMZ–TMP tablets	900676	SD	202.5	200	101.3	0.31
		SMZ	205.2	200	102.6	0.99
		TMP	77.2	80	96.5	4.34
	900877	SD	196.6	200	98.3	0.72
		SMZ	201.2	200	100.6	0.82
		TMP	74.2	80	92.7	3.46
SMZ–TMP tablets	900853	SMZ	395.5	400	98.9	1.28
		TMP	79.3	80	99.1	4.91
	900811	SMZ	392.9	400	98.2	1.27
		TMP	79.6	80	99.5	3.93

phonamides except sulphathiazole increased only slightly with increasing pH, but that of trimethoprim changed dramatically. At lower solution pH, it may be possible that the sulphonamides are undissociated, and most of them are distributed in the micellar phase. At higher pH, although they are dissociated, having a negative charge owing to their acidic properties, they migrate slowly toward the cathode with an opposite electrophoretic migration giving a net migration time that is little influenced by pH. Trimethoprim can be dissolved not only in acidic media [10] but also in basic media [11]; therefore, when the carrier solution pH changed from acidic (pH 5) to basic (pH 9), trimethoprim underwent a variation from cationic to neutral to anionic. Accordingly, the retention of trimethoprim varied from rapid (in low-pH medium) to slow (in neutral medium) and again to rapid (in high-pH medium).

In MECC, solutes partition between the aqueous and micellar phases in order of increasing hydrophobicity. The effect of SDS concentration on the retention time is shown in Fig. 4.

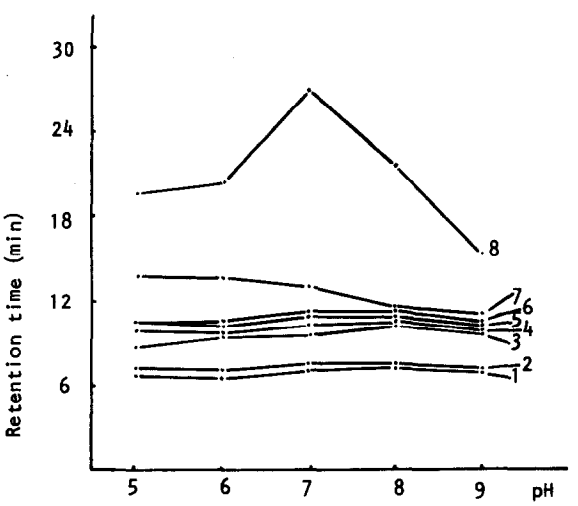

Fig. 3. Effect of pH on retention time. Other conditions as in Fig. 1. For compound identifications, see Table I.

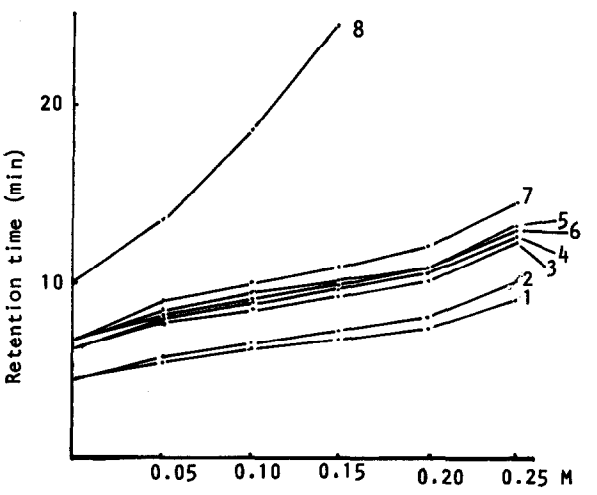

Fig. 4. Effect of SDS concentration on the retention time of ingredients. Other conditions as in Fig. 1. For compound identifications, see Table I.

The retention times of all the sulphonamides and trimethoprim increased gradually with increasing SDS concentration. SN and SG, SPZ and SDM, and SD, SMZ and ST were not resolved by electrophoresis without SDS. When the SDS concentration was above 0.05 *M* (above the critical micelle concentration), then they were readily separated from each other. TMP gave a longer retention time which increased faster than those of the sulphonamides (see Fig. 1), presumably owing to its higher hydrophobicity.

The dependence of the migration velocities of the solutes on the electrical field strength was examined under various applied voltages. The plots of retention time *vs.* applied voltage indicated, as expected, that the migration velocity of each solute increased with increasing applied voltage, but the relative retention time of each solute did not altere and did not influence the resolution of the solutes.

The addition of tetrabutylammonium salts to the SDS solution can improve the resolution of some compounds [12]. In order to optimize the separation of SD, SMZ and TMP for quantitative analysis, TAB was added to the SDS solution (pH 8.5) and the results are shown in Fig. 5. The migration times of SD, SMZ and ST gradually increased and the resolution between SD and SMZ was improved. This indicates that the addition of TAB enhances the interaction between the anionic solute and the micelle. The anionic solute and TAB tend to form ion pairs, which may be solubilized more easily by the anionic micelle than the free anionic solutes.

The addition of TAB to the micellar solution caused a considerable decrease in the migration time of TMP from that observed in the SDS solution alone. Cathionic TAB added to the SDS solution probably combines with the anionic SDS micelle. Consequently, the addtion of TAB to SDS solution could possibly prevent TMP from combining with the micelle.

Column efficiency

The theoretical plate number for the chromatographic peak of each ingredient that was obtained at different micellar concentrations and different applied voltages was calculated by the equation $N=2\pi(t_R h/A)^2$, where t_R, h and A are retention time, peak height and peak area, respectively [13]. The calculated results indicated that both the surfactant concentration and applied voltage influenced the column efficiency in MECC. The effect of the surfactant concentration on the theoretical plate number is illustrated in Fig. 6.

The theoretical plate number, except for SMZ and ST, increased with increasing surfactant concentration. However, it decreased with increasing micellar concentration after solute-specific levels

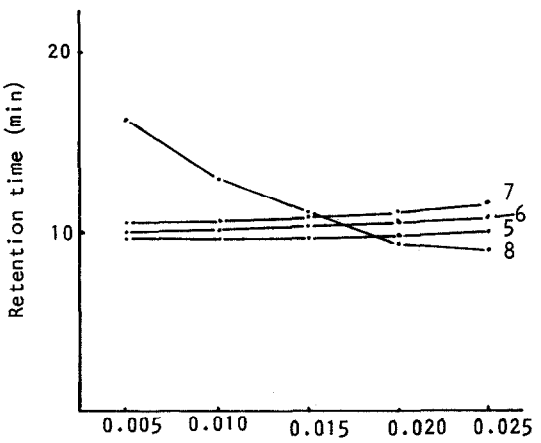

Fig. 5. Effect of TAB concentration on the retention times of (5) SD, (6) SMZ, (7) ST and (8) TMP. Other conditions as in Fig. 1.

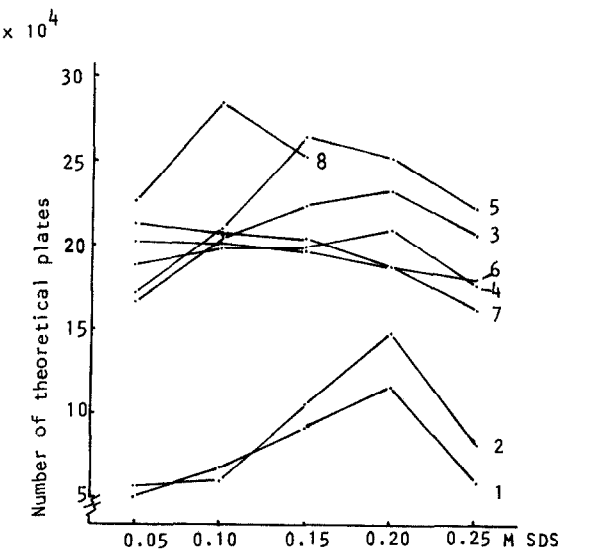

Fig. 6. Effect of SDS concentration on column efficiency. Other conditions as in Fig. 4. For compound identifications, see Table I.

TABLE V

VARIANCE ANALYSIS FOR THE TWO CONSTANT MODES

Solute	Variance of peak area ratios in constant-voltage mode ($n=6$)	Variance of peak area ratios in constant-current mode ($n=6$)	Ratio
SD	0.000437	0.00381	8.72
SMZ	0.000225	0.00127	5.64
TMP	0.000501	0.00271	5.41

had been reached. The results indicate that for the separation efficiency of each ingredient there is always an optimum surfactant concentration.

The applied voltage also influences the efficiency of separation. The results of a study of the effect of applied voltage on efficiency for the MECC system are shown in Fig. 7.

In this work, the efficiency for most solutes increased with increasing applied voltage. Sepaniak and Cole [3] reported that there was a Van Deemter-like relationship between plate height and applied voltage, and an optimum applied voltage exists for the highest separation efficiency. In this work, it may be that an optimum voltage for the highest theoretical plate number was not attained because the maximum voltage of the apparatus is 12 kV.

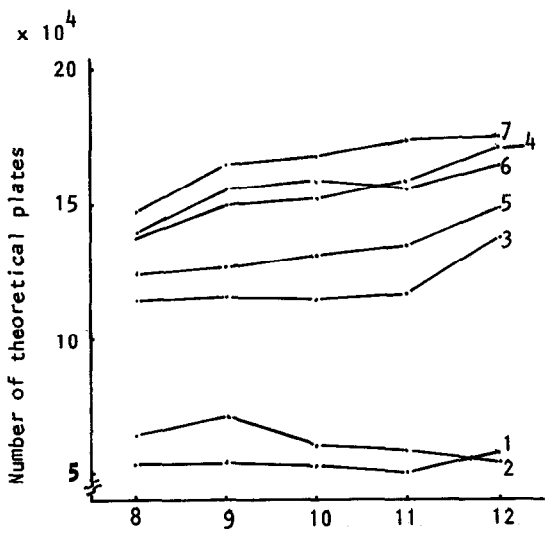

Fig. 7. Effect of applied voltage on column efficiency. Other conditions as in Fig. 1. Each point is the mean of six determinations; the R.S.D.s are between 3.6% and 5.7%. For compound identifications, see Table I.

Reproducibility of quantitative analysis

HPE-100 has two operational modes, constant voltage or constant current. The reproducibility of peak-area ratios (sample to internal standard) obtained in the constant-voltage mode for six replicates, was compared with that in the constant-current mode under identical experimental conditions. The results are given in Table V.

The reproducibility of peak-area ratios obtained in the constant-voltage mode is better than those obtained in the constant-current mode.

In this experiment, the reproducibility obtained was similar to that expected from a comparable high-performance liquid chromatographic method, especially for SD and SMZ. The reproducibility of the analytical results for TMP was slightly inferior. This may be due to adverse effects of the MECC technique itself, such as variation of peak area with migration time and adsorption of the sample on the internal wall of the capillary. It may also be due to the location of the TMP peak on the chromatogram. The TMP peak is far from the internal standard peak, and the difference in peak-area value between TMP and the internal standard was also large. In future work, it may therefore be advisable to employ another internal standard located near the TMP peak to improve the reproducibility with TMP.

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