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

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ABSTRACT

The separation and determination of seven active ingredients in theophylline tablets by micellar electrokinetic capillary chromatography is described. On a 35 cm $\times 50\,\mu m$ I.D. capillary, baseline separation is possible with a carrier solution containing 0.05 *M* sodium dodecyl sulphate (SDS) in 0.02 *M* borate buffer (pH 9.2) solution. The migration time of solutes increased markedly with increasing SDS concentration and slightly with increasing pH. With consecutive injections of samples, slight decreases in the migration times of the solutes occurred, but they were in parallel, owing to the temperature rise of the capillary with time. The column efficiency was influenced by the micellar concentration and applied voltage, and optimum values at which the highest theoretical plate number was achieved were established. The determination of the active ingredients was performed using hydrochlorothiazide and levamisole hydrochyloride (for ephedrine hydrochloride only) as internal standards, with good linearity with correlation coefficients from 0.9965 to 0.9999 and recoveries from 94.1% to 101.1%. For quantitative information, measurements of peak height were better than peak area.

INTRODUCTION

Micellar electrokinetic capillary chromatography (MECC) was first reported by Terabe *et al.* [1] nearly 10 years ago, since when these have been several studies on the theory and application of MECC [2–4] and separations of chlorinated phenols [5], aspoxicillin [6], β-lactam antibiotics [7], sulphonamides [8], etc., have been successfully achieved. MECC has also been applied to the determination of active ingredients in pharmaceutical preparations and body fluids [6,9,10]. Generally, these determinations were performed on single-ingredient preparations or a few components of the same kind.

In the paper, we describe the separation and determination of seven active ingredients in theophylline tablets, irrespective of whether these ingredients are the same kind of compound or not. Although the molecular structures of the ingredients are completely different from each other, except for three derivatives of xanthine, the separation was successfully achieved simply by adjusting and selecting the pH value and the concentration of surfactant. The simultaneous determination of the seven active ingredients was completed within 8 min employing hydrochlorothiazide and levamisole hydrochloride (for ephedrine hydrochloride only) as internal standards. The application of a second internal standard improved the precision of the determination of ephedrine hydrochloride.

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EXPERIMENTAL

Apparatus

A Bio-Rad (Richmond, CA, USA) HPE 100 apparatus equipped with a UV detector set at 210 nm, a power supply able to deliver up to 12 kV d.c. and a Bio-Rad 148-3014 HPE capillary cartridge (35 cm × 50 μm I.D., uncoated) were employed. A Chromatopac C-R3A (Shimadzu, Kyoto, Japan) was used for data processing. Electrokinetic sampling was used to introduce samples into the capillary.

Drug and chemicals

Three derivatives of xanthine and phenobarbital were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Amidopyrine, phenacetin, ephedrine hydrochloride, hydrochlorothiazide and levamisole hydrochloride were authentic specimens, obtained from the Shaanxi Institute for Drug Control (Xian, Shaanxi, China). Theophylline tablets (three batches) were purchased from Xian Pharmaceutical Factory (Xian, Shaanxi, China).

Sodium dodecyl sulphate (SDS) was obtained from Nacalai Tesque (Kyoto, Japan). All other reagents and solvents, of analytical-reagent grade, were products of the Beijing Chemical Factory (Beijing, China). A carrier solution was obtained by dissolving a suitable amount of SDS in a buffer solution prepared by mixing 0.02 A4 sodium tetraborate solution with 0.2 A4 sodium dihydrogenphosphate solution to given pH 8.0, 8.5 and 9.0, or adding 0.1 *M* sodium hydroxide solution to sodium tetraborate solution to give pH 9.5 and 10.0. The solutions were filtered through a 0.45-μm membrane filter and degassed by ultrasonication before use.

Procedure for the quantitative analysis

A test solution for separation studies was prepared by shaking the solutes with an appropriate amount of methanol first, owing to the low solubility of phenacetin in water, then with a mixture of methanol and pH 9.2 buffer solution that was added later, the final concentration of methanol in the solution being 20%. Five solutions were prepared as above to give a series of concentrations in the ranges 8–10 μg/ml of phenobarbital and ephedrine hydrochloride, 12-60 μg/ml of caffeine. 20–100 μg/ml of theophylline and theobromine and 80–400 μg/ml of amidopyrine and phenacetin for the determination of the linear range and response factors. After preparation as described above, the concentrations of the solutions for the recovery test were about 20 μg/ml of phenobarbital and ephedrine hydrochloride, 30 μg/ml of caffeine. 50 μg/ml of theophylline and theobromine and 200 μg/ml of amidopyrine and phenacetin and two other concentration levels equivalent to 80% and 120% of these concentrations. An appropriate amount of finely ground powder of theophylline tablets was dissolved to give a solution at the same concentrations as above. The concentrations of internal standards in all the solutions were about 70 μg/ml of hydrochlorothiazide and about 15 μg/ml of levamisole hydrochloride.

Sample solutions were introduced into the capillary by the electrokinetic method at the positive end of the capillary using a constant voltage of 5 kV and a loading time of 5 s. All the experiments were completed at ambient temperature.

Procedure for capillary rinsing

In order to obtain good working conditions and reproducible data, it is helpful to wash the internal wall of the capillary between each individual injection. The capillary was washed with carrier solution after each analysis and, when the carrier solution was replaced, was washed first with water, then with 0.1 *M* sodium hydroxide solution, with water again and finally washed and filled with carrier solution to be used for the next run. These procedures were carried out by injecting the appropriate fluid into the capillary with a 100-μl syringe (Hamilton, Reno, NV, USA) at the negative end.

RESULTS AND DISCUSSION

Migration characteristics

The seven active ingredients in theophylline tablets and the two internal standards were successfully separated by MECC. A typical electropherogram is shown in Fig. 1; each compound was baseline resolved.

Experimental conditions such as pH value, SDS concentration and the order of priority of consecutive runs could affect the migration behaviours of the solutes. When the concentration of SDS was 0.05 *M* and the applied voltage was 10 kV, the effect

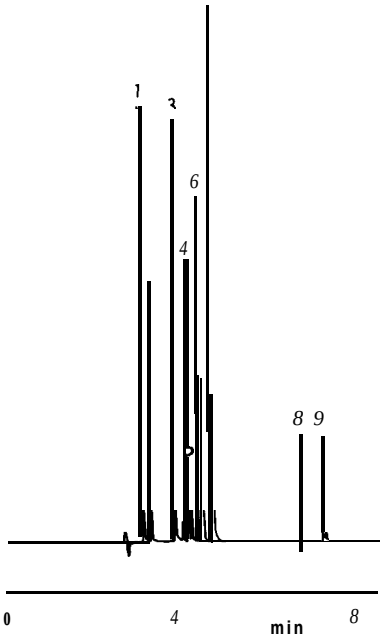

Fig. 1. Separation of seven ingredients by MECC. Carrier, 0.02 *M* borate-phosphate buffer (pH 9.2) containing 0.05 *M* SDS. Applied voltage, 10 kV. Peaks: 1 = theobromine; 2 = caffeine; 3 = hydrochlorothiazide (I.S. 1); 4 = theophylline; 5 = phenobarbital; 6 = amidopyrine; 7 = phenacetin; 8 = levamisole hydrochloride (IS. 2); 9 = ephedrine hydrochloride.

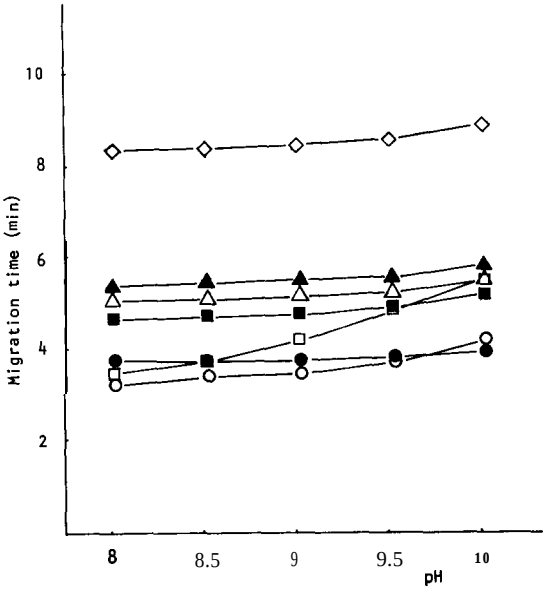

Fig. 2. Effect of pH on the migration times of (0) theobromine, (●) caffeine, (0) theophylline, (■) phenobarbital, (△) amidopyrine, (A) phenacetin and (0) ephedrine hydrochloride. Other conditions as in Fig. 1.

of pH on migration time of solutes is as shown in Fig. 2. The migration times of all the solutes except theobromine and theophylline increased slightly with increasing pH from 8 to 10. In contrast, the migration times of theobromine and theophylline increased considerably. Xanthine molecules contain an acidic functional group owing to the tautomeric shift of hydrogen from nitrogen to keto oxygen (enolization), a weakly acidic H being formed on the resulting OH group [11]. Hence theobromine and theophylline gradually dissociated, having a negative charge within the above pH range, and migrated slowly towards the cathode owing to an opposite electrophoretic migration. Having no NH group to participate in enolization, caffeine is an exception. Because the acid dissociation constant (pK_a = 8.77) of theophylline is stronger than that of theobromine (pK_a = 10.04) [12], theophylline migrated more slowly than theobromine.

Phenobarbital is also an acidic compound, with the strongest dissociation constant (pK_1 = 7.3, pK_2 = 11.8) [13] of these compounds, but its migration time did not change much. Possibly phenobarbital dissociated and achieved a sufficiently slow migration velocity prior to pH 8, also owing to its electrophoretic migration to the anode, and consequently the migration time of phenobarbital did not increase with increase in pH from 8 to 10. Also, we observed that for MECC separation a high pH is always better than a low pH.

The dependence of the migration times of the seven active ingredients on SDS concentration was examined at pH-9.2 with surfactant concentrations in the range 0–0.1 A4 (Fig. 3). Theobromine, caffeine, amidopyrine and phenacetin were not resolved by electrophoresis without SDS. The migration times of all the the ingredients increased gradually with increasing SDS concentration. The changes in the migration time of amidopyrine, phenacetin and ephedrine hydrochloride were larger, indicating that they were more readily solubilized in the surfactant, presumably owing to their higher hydrophobicity at pH 9.2.

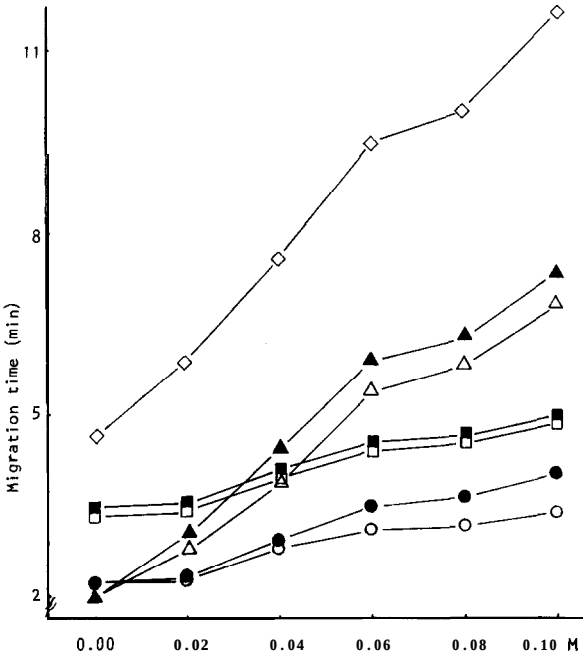

Fig. 3. Effect of SDS concentration on the migration times of solutes. Other conditions as in Fig. 1. Compound identifications as in Fig. 2.

When consecutive runs were made under constant chromatographic condition (pH 9.2, 0.05 *M* SDS), the migration times of the ingredients decreased with increasing number of injections. The results indicated that the changes in the migration times of the solutes are parallel and tended gradually to become stable. This may be related to the temperature inside the capillary, which increased with the time during which the electrical field was applied, and accordingly, the migration times of the ingredients decreased slightly.

Efficiency of separation

The results of a study of the effect of surfactant concentration and applied voltage on the efficiency of separation are shown in Figs. 4 and 5. The plate number (N) was calculated according to 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 [14]. The results indicated that both of them influenced the column efficiency in MECC.

Fig. 4. shows that the separation efficiency, except for phenacetin, increased with increasing SDS concentration, but after solute-specific levels had been reached it decreased with increasing micellar concentration. The optimum surfactant concentration for the separation efficiency of most of the compounds was cu. 0.06 *M*; an SDS concentration of 0.05 A4 was selected for subsequent determinations.

Sepaniak and Cole [3] studied the experimental factors that influence column efficiency and reported that there was an optimum applied voltage for the most efficient separation. Fig. 5 shows some

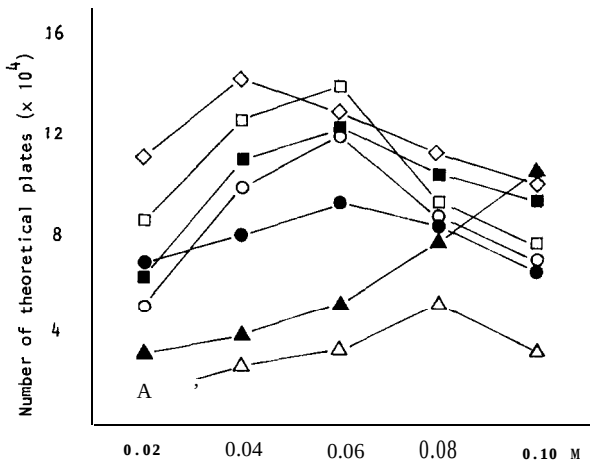

Fig. 4. Effect of SDS concentration on column efficiency. Other conditions as in Fig. 3. Compound identifications as in Fig. 2.

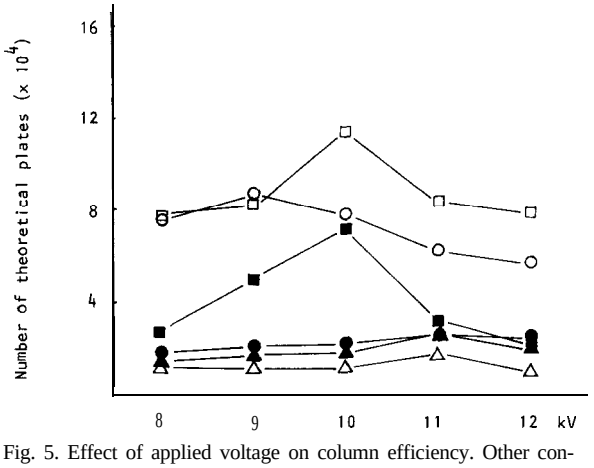

Fig. 5. Effect of applied voltage on column efficiency. Other conditions as in Fig. 1. Each point is the mean of six determinations and the R.S.D.s are between 3.7% and 6.2%. Compound identifications as in Fig. 2.

TABLE I

RESULTS OF THE DETERMINATION OF LINEAR RANGE AND RESPONSE FACTORS (n = 6)

Compound	Concentration range (μg/ml)	Correlation coefficient	Intercept	Slope	Response factor	R.S.D. (%)
Theobromine	20-100	0.9965	0.01483	0.6690	1.150	2.15
Caffeine	12-60	0.9998	0.01251	0.6958	1.026	3.30
Theophylline	20-100	0.9998	0.00694	0.5863	1.471	1.80
Phenobarbital	8–40	0.9999	0.00892	0.3076	1.690	4.28
Amidopyrine	80–400	0.9996	0.02781	0.2673	2.166	2.82
Phenacetin	80–400	0.9983	0.02708	0.3910	2.070	2.56
Ephedrine HCl	8–40	0.9990	0.00171	0.1408	6.838	3.24

plots of theoretical plate number vs. applied voltage, indicating that the optimum applied voltage for each of the ingredients was within the range 9–11 kV.

Quantitative analysis

Quantitative analyses were performed in three steps, determination of linearity and response factor, test of recovery, and assay of a preparation, using 0.02 *M* borate-phosphate buffer solution (pH 9.2) containing 0.05 *M* SDS and an applied voltage of 10 kV in the constant-voltage mode, the results were calculated by the peak-height ratio method. The solutions for the determination of the linear

TABLE II

RESULTS OF RECOVERY STUDY

Six injection at each level

Compound	Amount added (μg/ml)	Amount found (μg/ml)	Recovery (%)	Average (%)	R.S.D. (%)
Theobromine	40.68	41.60	102.3	101.1	1.34
	48.78	49.48	101.4		
	59.70	59.41	99.6		
Caffeine	23.78	23.39	98.4	95.8	2.41
	30.80	28.96	94.0		
	36.15	34.87	94.9		
Theophylline	39.95	40.94	102.5	100.1	3.05
	49.90	50.48	101.2		
	63.15	61.04	96.1		
Phenobarbital	17.06	17.26	101.2	91.6	3.64
	22.36	21.80	97.5		
	23.88	22.46	94.1		
Amidopyrine	160.0	160.8	100.5	91.6	2.13
	200.6	191.2	95.3		
	240.2	233.0	97.0		
Phenacetin	162.0	156.6	96.1	95.2	1.65
	196.0	186.4	95.1		
	238.1	223.1	93.6		
Ephedrine HCl	17.14	16.53	96.4	94.1	3.40
	19.98	18.07	90.4		
	25.22	24.04	95.3		

TABLE III

RESULTS OF THE DETERMINATION OF ACTIVE INGREDIENTS IN THEOPHYLLINE TABLETS (n = 6)

Compound	Label claim (mg)	Sample	Amount found (mg)	Percentage of label claim	R.S.D. (%)
Theobromine	25	A	26.7	106.6	1.64
		B	24.5	98.2	1.24
		C	24.8	99.2	2.47
Caffeine	15	A	14.4	95.7	2.18
		B	13.8	92.0	1.62
		C	14.0	93.0	2.17
Theophylline	25	A	25.0	99.9	3.05
		B	24.4	97.6	3.15
		c	25.9	103.4	1.30
Phenobarbital	10	A	10.1	101.1	4.46
		B	9.5	94.8	2.16
		C	9.8	97.8	4.07
Amidopyrine	100	A	96.8	96.8	1.20
		B	99.5	99.5	2.30
		C	98.8	98.8	2.50
Phenacetin	100	A	91.6	91.6	1.75
		B	97.7	97.7	2.88
		C	96.2	96.2	2.38
Ephedrine HCl	10	A	9.2	92.3	3.98
		B	9.2	91.9	3.86
		C	9.9	99.2	3.83

range and relative response factor were chromatographed and the calibration graphs of h_s/h_i *vs.* M_s/M_i, where h_s and h_i are peak heights and M_s and M_i are the masses of the sample (s) and internal standard (i), showed good linearity over a suitable range. Response factors relative to internal standards were calculated by a general method and all the results are given in Table I.

TABLE IV

COMPARISON OF THE PRECISION [SHOWN BY R.S.D. (n = 6)] BETWEEN PEAK-HEIGHT RATIOS AND PEAK-AREA RATIOS

Compound	R.S.D. (%)	
	Peak-height ratio	Peak-area ratio
Theobromine	3.78	5.36
Caffeine	3.21	5.86
Theophylline	2.12	5.79
Phenobarbital	2.28	4.57
Amidopyrine	3.28	5.01
Phenacetin	2.86	4.78
Ephedrine HCl	3.38	–

Under the chromatographic conditions described above, recoveries were checked at three concentration levels and six repeated injections per level. The results and their relative standard deviations are given in Table II and show that the average recoveries were fairly good.

The assay of theophylline tablets was performed according to the above procedure under the optimized conditions. The results are given in Table III and suggest that MECC is suitable for the analysis of multi-component preparations.

Reproducibility

The chromatographic results obtained by consecutive runs under the same experimental conditions indicated that peak-height ratios changed with increasing number of injections in addition to the change in migration time described above. The peak-height ratios of the ingredients that migrated rapidly tended to increase and those of the ingre-

dients that migrated slowly tended to decrease; the longer the migration time of an ingredient, the greater was its change. This is presumably due to different adsorption, relative to the internal standard, of the sample migrating at a different velocity on the internal wall of the capillary with increasing temperature inside the capillary.

In this work, the quantitative results were calculated using peak-height ratio method, as the precision of the results was better than that given by the peak-area ratio method (Table IV).

For the solutes that migrate slowly and are of low concentration in a pharmaceutical preparation, in addition to the unfavourable effects of the MECC technique itself, large differences in retention times and responses between a solute and internal standard may result in poor precision in quantitative analysis. In this work ephedrine hydrochloride, similarly to trimethoprim in a previous study [8], belonged to this category. The use of a second internal standard, levamisole hydrochloride, which was located near ephedrine hydrochloride on the chromatogram, improved the reproducibility for ephedrine hydrochloride.

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