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# Determination of structurally related phenothiazines by capillary zone electrophoresis and micellar electrokinetic chromatography

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## Abstract

Fourteen structurally related basic phenothiazines were determined by capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC), applying electrolyte systems containing the fluorinated cationic surfactant FC 135. Effective mobilities of the phenothiazines were determined as a function of pH by CZE in the anionic mode with a reversed electroosmotic flow (EOF). Larger differences in overall effective mobilities were obtained in MEKC, applying the cationic surfactant cetyltrimethylammonium bromide as micelle forming agent. The quantitative abilities and matrix effects of CZE with reversed EOF were studied, and as an example the concentrations of thioridazine and promethazine in pharmaceutical preparations were determined.

*Keywords:* Capillary electrophoresis; Micellar electrokinetic chromatography; Phenothiazines; Antihistamines

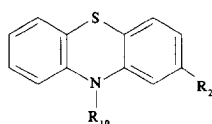
## 1. Introduction

Phenothiazine and its derivatives are a group of basic drugs used as antihistamines, antipsychotics and neuroleptics in the therapy of different diseases. So far, pharmaceutical preparations containing phenothiazine compounds have been analyzed by spectrophotometric methods [1], gas chromatography [2–4], isotachopheresis [5] and liquid chromatography [6].

Since its introduction capillary zone electrophoresis (CZE) [7,8] has developed into a highly

efficient separation method for the analysis of charged compounds. The application field of capillary electrophoresis was extended to neutral compounds with the introduction of micellar electrokinetic chromatography (MEKC) by Terabe et al. [9,10]. Amongst others, many pharmaceutical substances have been analyzed using these separation techniques. Although MEKC was initially developed for the determination of neutral compounds, it has proved to be also a powerful analytical tool for the separation of compounds with almost identical electrophoretic mobilities [11,12] and for mixtures containing both charged and uncharged compounds [13,14].

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No. compound	R <sub>10</sub>	R <sub>2</sub>	pK <sub>a</sub>
1 propiomazine	CH <sub>2</sub> CH(CH <sub>3</sub> )N(CH <sub>3</sub> ) <sub>2</sub>	COC <sub>2</sub> H <sub>5</sub>	-
2 ethopropazine	CH <sub>2</sub> CH(CH <sub>3</sub> )N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub>	H	9.58
3 promethazine	CH <sub>2</sub> CH(CH <sub>3</sub> )N(CH <sub>3</sub> ) <sub>2</sub>	H	9.10
4 acetopromazine	(CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>	COCH <sub>3</sub>	-
5 triflupromazine	(CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>	CF <sub>3</sub>	9.41
6 promazine	(CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>	H	9.40
7 trimeprazine	CH <sub>2</sub> CH(CH <sub>3</sub> )CH <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>	H	9.00
8 chlorpromazine	(CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>	Cl	9.30
9 trifluoperazine		CF <sub>3</sub>	8.10
10 perphenazine		Cl	-
11 thioridazine		SCH <sub>3</sub>	9.50
12 prochlorperazine		Cl	8.10
13 fluphenazine		CF <sub>3</sub>	-
14 methotrimeprazine	CH <sub>2</sub> CH(CH <sub>3</sub> )CH <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>	OCH <sub>3</sub>	-

Fig. 1. Structural formulae and pK<sub>a</sub>-values of phenothiazines.

In this work we studied the applicability of CZE and MEKC for the separation of fourteen structurally related phenothiazines. In Fig. 1 the structural formulae and the pK<sub>a</sub>-values of the phenothiazine derivatives are given.

## 2. Experimental

### 2.1. Instrumentation

All experiments were carried out on a BioFocus 3000 capillary electrophoresis system (BioRad, Hercules, CA, USA). The temperature was kept constant at 25°C and the wavelength of the UV detector was set at 240 nm. Pressure injection was carried out with an injection constant of 13 789.52 Pa s (2 p.s.i. s). A 50 μm I.D. fused-silica capillary from Polymicro Technologies (Phoenix, AZ, USA) was used, total length 70.0 cm, distance between injection and detection, 65.4 cm. All experiments were carried out with a constant voltage of 20 kV. In the anionic mode the cathode is placed at the inlet

side and the anode at the outlet side of the capillary, respectively, and vice versa in the cationic mode.

### 2.2. Chemicals

Propiomazine maleate salt, ethopropazine hydrochloride, promethazine hydrochloride, acetopromazine maleate salt, triflupromazine hydrochloride, promazine hydrochloride, trimeprazine hemi-(+)-tartrate salt, chlorpromazine hydrochloride, trifluoperazine dihydrochloride, perphenazine, thioridazine hydrochloride, prochlorperazine dimaleate salt, fluphenazine dihydrochloride and methotrimeprazine maleate salt were obtained from Sigma (St. Louis, MO, USA), cetyltrimethylammonium bromide (CTAB) was obtained from Merck (Darmstadt, Germany) and the fluorinated cationic surfactant FC 135 was obtained from Fluorad/3M (Leiden, Netherlands). The critical micelle concentration (CMC) of FC 135 is ca. 2 g/l, determined from surface tension data provided by the manufacturer. Phenergan (Pharma Chemie, Haarlem, Netherlands) and Melleril (Sandoz Pharma, Basel, Switzerland) tablets were obtained at a local pharmacy.

### 2.3. Standard solutions

Stock solutions of the phenothiazines were prepared by weighing accurately 10.0 mg of the compounds and dissolving them in 10.0 ml deionized water. Perphenazine and prochlorperazine dimaleate salt were dissolved in 10.0 ml methanol. All solutions were diluted ten times with deionized water to give sample solutions with a final concentration of 0.1 mg/ml. For the calibration graphs, dilutions of the stock solutions were used at concentrations of 0.1, 0.08, 0.06, 0.04, 0.02, and 0.01 mg/ml.

### 2.4. Sample preparation

Phenergan and Melleril tablets, labelled to contain 25 mg promethazine and 100 mg thioridazine, respectively, were pulverized and dissolved in 100 ml deionized water by sonication for 30 min. These solutions were diluted 5 times

and 20 times, respectively, so that the concentration of the sample is near the middle of the linear range of the calibration graph. These dilutions were used for injection without further pretreatment.

### 3. Results and discussion

#### 3.1. Determination of phenothiazines by capillary zone electrophoresis with reversed electroosmotic flow

Phenothiazines are basic compounds with  $pK_a$ -values between 8 and 10. They migrate in the downstream mode [15] at intermediate pH in

normal CZE experiments, carried out in the cationic mode. However, these analyses are hampered by adsorption of the basic compounds on the negatively charged capillary surface, resulting in very bad peak shapes and a low resolution. This is illustrated in Fig. 2A, where an electropherogram is shown for the separation of six phenothiazines, applying a background electrolyte of 0.02 M Tris–formic acid at pH 3.5. This adsorption can be efficiently reduced by a dynamical modification of the capillary surface with the fluorinated cationic surfactant FC 135, resulting in a charge reversal of the capillary wall and a reversed electroosmotic flow (EOF) [16–18]. Therefore these experiments are carried out in the anionic mode. Moreover, in these experiments the phenothiazines migrate in the up-

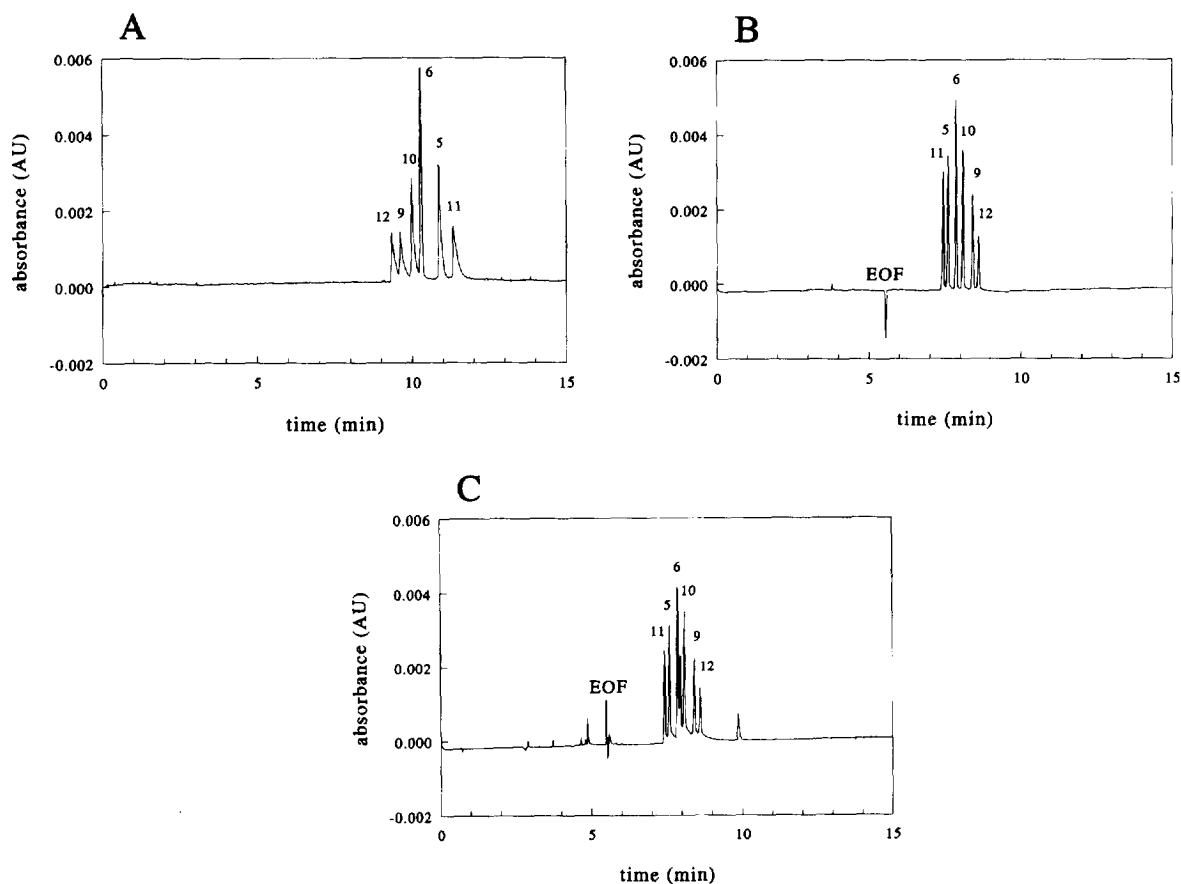


Fig. 2. Electropherogram of the separation of thioridazine (11), triflupromazine (5), promazine (6), perphenazine (10), trifluoperazine (9) and prochlorperazine (12) (all 0.1 mg/ml) dissolved in (A and B) deionized water and (C) 10-fold diluted human urine. Background electrolyte, 20 mM Tris–formic acid at pH 3.5, containing (A) 0 and (B and C) 50 mg/l FC 135.

Table 1  
Compositions of background electrolytes at different pHs

Cation <sup>a</sup>	Buffering counter species <sup>a</sup>	pH
0.02 M Tris	formic acid	3.5
0.02 M Tris	acetic acid	5.0
0.02 M Tris	MES	6.2
0.02 M Tris	MOPS	7.2

All background electrolytes contain the fluorinated cationic surfactant FC 135, unless otherwise noted.

<sup>a</sup>Tris = tris(hydroxymethyl)aminomethane; MES = 2-(N-morpholino)ethanesulphonic acid; MOPS = morpholinopropanesulphonic acid.

stream mode, which makes a better separation possible [8,19].

The effective mobilities of the phenothiazines were determined as a function of pH. In Table 1 the compositions of the different background electrolytes are given and in Table 2 all effective mobilities are listed. In CZE the highest resolution is generally obtained at a pH near the  $pK_a$ -values of the analytes. However, for these solutes at higher pH deteriorating peak shapes

were observed. This is due to a less effective shielding of the negatively charged surface silanol groups with increasing pH [18], resulting in adsorption of the basic phenothiazines on the capillary surface. To check the influence of FC 135 on the effective mobilities of the analytes, experiments were carried out in the cationic mode, applying a background electrolyte of 0.02 M Tris–formic acid at pH 3.5 without FC 135. As can be seen from the results, listed in Table 2, almost identical effective mobilities were obtained, showing that the migration of the analytes is not affected by the presence of the cationic surfactant FC 135 in the background electrolyte.

From the foregoing it can be concluded that phenothiazines can be determined by CZE with reversed EOF, applying an electrolyte system in the pH range 3.5–7.2 containing 50 mg/l FC 135. However, a complete separation of the fourteen drugs is not possible in this pH range, as the differences in effective mobility are too small. In Fig. 2B an electropherogram is shown for the separation of six phenothiazines, applying a background electrolyte of 0.02 M Tris–formic acid at pH 3.5 containing 50 mg/l FC 135. Notice

Table 2  
Effective mobilities for 14 phenothiazines as a function of pH

Compound	System I <sup>a</sup>	System II <sup>b</sup>			
	pH 3.5	pH 3.5	pH 5.0	pH 6.2	pH 7.2
Propiomazine	18.33	18.41	17.72	16.95	17.03
Ethopropazine	19.17	19.10	18.73	17.74	17.86
Promethazine	20.50	20.43	20.22	18.98	18.79
Acetopromazine	19.02	19.16	19.01	17.83	17.59
Triflupromazine	18.44	18.89	18.82	17.88	18.92
Promazine	20.73	20.39	20.39	19.17	19.06
Trimeprazine	19.84	19.60	19.57	18.37	18.36
Chlorpromazine	19.51	19.64	19.49	18.32	18.23
Trifluoperazine	22.92	23.59	17.12	15.88	16.69
Perphenazine	21.53	21.76	16.28	14.56	12.22
Thioridazine	17.39	17.58	17.61	16.30	16.65
Prochlorperazine	24.27	24.57	17.54	15.71	14.61
Fluphenazine	20.73	20.83	15.76	14.41	13.09
Methotrimeprazine	18.78	18.58	18.64	17.33	17.07

<sup>a</sup> Electrolyte system I containing 0 mg/l FC 135.

<sup>b</sup> Electrolyte system II containing 50 mg/l FC 135.

Table 3  
Migration time,  $t$  (min), and effective mobility,  $m_{\text{eff}}$  ( $10^{-5}$  cm<sup>2</sup>/V s) with standard deviations (in parentheses) for six phenothiazines dissolved in (I) water and (II) ten-fold diluted human urine

Compound	I		II	
	$t$	$m_{\text{eff}}$	$t$	$m_{\text{eff}}$
EOF	5.44 (0.06)	-70.11 (0.77)	5.54	-68.86
Thioridazine	7.26 (0.10)	17.58 (0.09)	7.44	17.59
Triflupromazine	7.42 (0.10)	18.69 (0.10)	7.60	18.67
Promazine	7.67 (0.11)	20.39 (0.10)	7.87	20.39
Perphenazine	7.88 (0.12)	21.71 (0.07)	8.10	21.76
Trifluoperazine	8.19 (0.13)	23.51 (0.07)	8.43	23.61
Prochlorperazine	8.37 (0.13)	24.51 (0.08)	8.62	24.61

Background electrolyte, 20 mM Tris–formic acid at pH 3.5 containing 50 mg/l FC 135;  $n = 5$ .

that the migration order of the six phenothiazines is reversed compared to Fig. 2A. In Table 3 all migration times and effective mobilities are listed for five experiments, showing a good repeatability. In order to study the influence of the composition of the sample matrix, experiments were carried out with ten-fold diluted human urine, spiked with the same sample compounds (all 0.1 mg/ml). In Fig. 2C the electropherogram for this separation is shown. As can be seen from the results, listed in Table 3, comparable migration times and effective mobilities were obtained for both sample mixtures.

### 3.2. Determination of phenothiazines by micellar electrokinetic chromatography

As phenothiazines possess hydrophobic properties, MEKC experiments were carried out with the cationic surfactant CTAB, thus introducing a second separation mechanism based on micellar solubilization. For these experiments the migration of the compounds can be described by an overall effective mobility,  $m_{\text{OV}}$ , which is a weighted average of the effective mobility in the aqueous phase,  $m_{\text{eff}}$ , and the effective mobility of the micelles,  $m_{\text{MC}}$ , according to [20]:

$$m_{\text{CV}} = \frac{1}{1+k} m_{\text{eff}} + \frac{k}{1+k} m_{\text{MC}}$$

In Fig. 3 the influence of the CTAB concentration on the overall effective mobility,  $m_{\text{OV}}$ ,

is illustrated. The electrolyte system consisted of 0.02 M Tris–acetic acid at pH 5.0 containing 50 mg/l FC 135. As can be seen from Fig. 3,

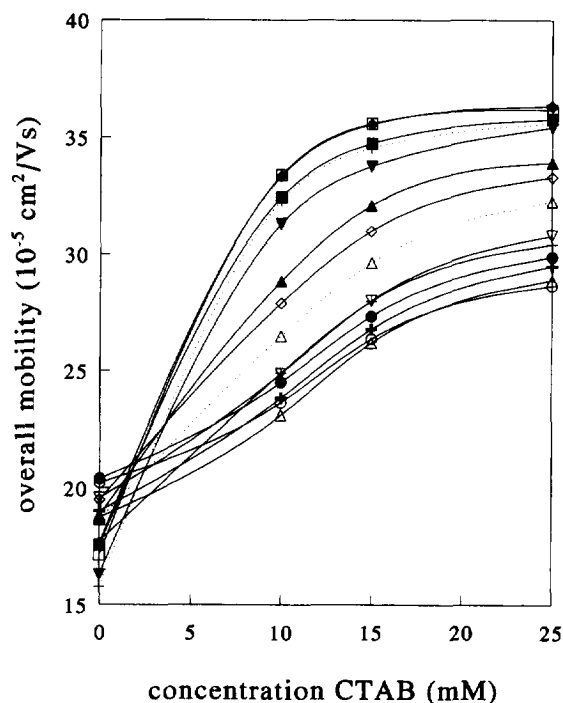


Fig. 3. Overall effective mobility,  $m_{\text{OV}}$ , versus concentration of CTAB for (full lines) (+) 1, ( $\Delta$ ) 2, ( $\circ$ ) 3, ( $\diamond$ ) 4, ( $\blacktriangle$ ) 5, ( $\bullet$ ) 6, ( $\nabla$ ) 7, ( $\diamond$ ) 8, ( $\square$ ) 9, ( $\blacktriangledown$ ) 10, ( $\blacklozenge$ ) 11, ( $\blacksquare$ ) 12 and (dotted lines) (+) 13 and ( $\Delta$ ) 14. See Fig. 1 for the names of the compounds. Background electrolyte, 20 mM Tris–acetic acid at pH 5.0 containing 50 mg/l FC 135 and different concentrations CTAB.

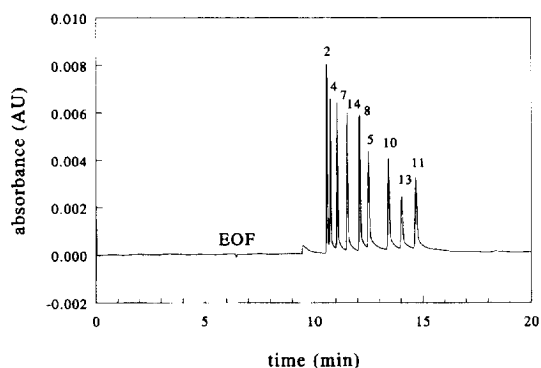


Fig. 4. Electrokinetic chromatogram of the separation of (2) ethopropazine, (4) acetopromazine, (7) trimeprazine, (14) methotrimeprazine, (8) chlorpromazine, (5) triflupromazine, (10) perphenazine, (13) fluphenazine and (11) thioridazine (all 0.1 mg/ml). Background electrolyte, 20 mM Tris-acetic acid at pH 5.0 containing 50 mg/l FC 135 and 10 mM CTAB.

optimum resolution is obtained at a CTAB concentration of 10 mM, although not all compounds can be separated with this electrolyte system. In Fig. 4 an electrokinetic chromatogram is shown for the separation of nine phenothiazines, applying a background electrolyte of 0.02 M Tris-acetic acid at pH 5.0 containing 50 mg/l FC 135 and 10 mM CTAB. The slightly tailing peaks are probably due to interactions between

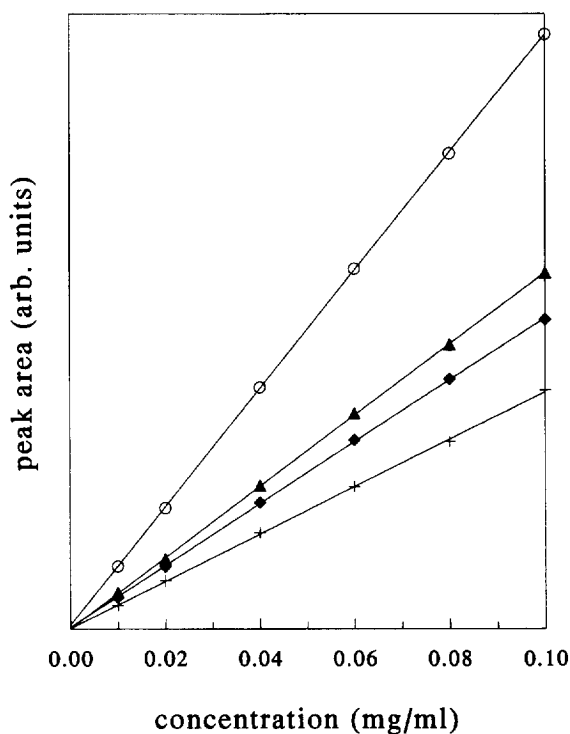


Fig. 5. Calibration graphs of measured peak area versus injected sample concentration for (+) fluphenazine, (◆) thioridazine, (▲) triflupromazine and (○) promethazine. Background electrolyte, 20 mM Tris-formic acid at pH 3.5 containing 50 mg/l FC 135.

Table 4

Migration time,  $t$  (min), and effective mobility,  $m_{\text{eff}}$  ( $10^{-5}$  cm<sup>2</sup>/V s) with standard deviations (in parentheses) for nine phenothiazines dissolved in (I) water and (II) ten-fold diluted human urine

Compound	I		II	
	$t$	$m_{\text{eff}}$	$t$	$m_{\text{eff}}$
EOF	6.41 (0.02)	-59.50 (0.20)	6.39	-59.70
Ethopropazine	10.52 (0.05)	23.25 (0.05)	10.53	23.47
Acetopromazine	10.68 (0.05)	23.78 (0.05)	10.69	24.02
Trimeprazine	10.99 (0.05)	24.78 (0.06)	10.99	24.99
Methotrimeprazine	11.45 (0.05)	26.18 (0.06)	11.45	26.38
Chlorpromazine	12.02 (0.06)	27.75 (0.06)	12.02	27.96
Triflupromazine	12.41 (0.06)	28.77 (0.08)	12.41	28.96
Perphenazine	13.36 (0.07)	30.95 (0.10)	13.30	31.02
Fluphenazine	13.96 (0.08)	32.17 (0.10)	13.88	32.22
Thioridazine	14.55 (0.09)	33.27 (0.05)	14.50	33.39

Background electrolyte, 20 mM Tris-acetic acid at pH 5.0 containing 50 mg/l FC 135 and 10 mM CTAB;  $n = 5$ .

Table 5  
Regression correlation coefficients,  $r$ , and limits of detection (LOD) ( $\mu\text{g/ml}$ ), for the calibration graphs shown in Fig. 5 ( $n = 6$ )

Compound	$r$	LOD ( $\mu\text{g/ml}$ )
Fluphenazine	0.99983	2.18
Thioridazine	0.99993	1.36
Triflupromazine	0.99996	1.08
Promethazine	0.99999	0.63

the sample compounds and a mixed micellar surfactant layer on the capillary wall. In Table 4 all migration times and effective mobilities are listed for five experiments, showing a good repeatability. For the MEKC system, also experiments were carried out with ten-fold diluted human urine, spiked with the same sample compounds (all 0.1 mg/ml). Comparable migration times and effective mobilities were obtained for both sample mixtures, which are also listed in Table 4.

### 3.3. Quantitative aspects

In order to determine the quantitative abilities of CZE separations with reversed EOF, calibration graphs were set up for promethazine, triflupromazine, thioridazine and fluphenazine, applying a background electrolyte of 0.02  $M$  Tris–acetic acid at pH 5.0 containing 50 mg/l FC 135. In Fig. 5 all calibration graphs are shown, and in Table 5 the regression parameters are presented, showing a linear relationship between peak area and injected sample concentration.

As an application, we determined promethazine and thioridazine in Phenergan and Melleril tablets, respectively. Both samples were measured three times. In Table 6 the determined concentrations and standard deviations for both compounds are listed. As can be seen from these results the labelled and measured values agree well.

## 4. Conclusions

Basic phenothiazine compounds can be successfully determined by capillary zone electro-

Table 6  
Labelled and measured amounts with standard deviations (in parentheses) for thioridazine and promethazine in Melleril and Phenergan tablets

Compound	Labelled	Measured
Thioridazine	100	102.06 (0.64)
Promethazine	25	28.86 (0.08)

$n = 6$ ,  $m = 3$ .

phoresis in the anionic mode with the addition of the cationic fluorinated surfactant FC 135 to the background electrolyte, resulting in a reversed wall charge and a reversed EOF. However, with micellar electrokinetic chromatography, combining both electrophoretic and chromatographic separation principles, a larger difference between the overall effective mobilities can be obtained, resulting in an improved resolution. Nine phenothiazines could be separated applying an electrolyte system of 0.02  $M$  Tris–acetic acid at pH 5.0 containing 50 mg/l FC 135 and 10 mM CTAB.

The quantitative abilities of CZE with reversed EOF were satisfactory and promethazine and thioridazine could easily be determined in pharmaceutical preparations without any sample pretreatment.

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## References

- [1] G. Morait, L. Petroniu and C. Popa, *Farmacia*, 26 (1978) 205.
- [2] A.P. De Leenheer, *J. Chromatogr.*, 77 (1973) 339.
- [3] E.C. Dinovo, L.A. Gottschalk, B.R. Nandi and P.G. Greddes, *J. Pharm. Sci.*, 65 (1976) 667.
- [4] H. Maurer and K. Pflieger, *J. Chromatogr.*, 306 (1984) 125.

- [5] I. Jelinek, J. Dohnal, J. Snopek and E. Smolková-Keulemansová, *J. Chromatogr.*, 464 (1989) 139.
- [6] S. Li and W.C. Purdy, *J. Pharm. Biomed. Anal.*, 49 (1991) 409.
- [7] F.E.P. Mikkers, F.M. Everaerts and Th.P.E.M. Verheggen, *J. Chromatogr.*, 169 (1979) 1.
- [8] J.W. Jorgenson and K.D. Lukacs, *Anal. Chem.*, 53 (1981) 1298.
- [9] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 113.
- [10] S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1985) 834.
- [11] J. Vindevogel and P. Sandra, *J. High Resolut. Chromatogr.*, 14 (1991) 795.
- [12] H. Nishi, N. Tsumagari and S. Terabe, *Anal. Chem.*, 61 (1989) 2434.
- [13] H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Pharm. Sci.*, 79 (1990) 519.
- [14] S. Fujiwara and S. Honda, *Anal. Chem.*, 59 (1987) 2773.
- [15] F.M. Everaerts, A.A.A.M. van de Goor, Th.P.E.M. Verheggen and J.L. Beckers, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 12 (1989) 28.
- [16] A. Emmer, M. Jansson and J. Roeraade, *J. Chromatogr.*, 547 (1991) 544.
- [17] W.G.H.M. Muijselaar, C.H.M.M. de Bruijn and F.M. Everaerts, *J. Chromatogr.*, 605 (1992) 115.
- [18] M.T. Ackermans, F.M. Everaerts and J.L. Beckers, *J. Chromatogr.*, 606 (1992) 229.
- [19] J.C. Giddings, *Sep. Sci.*, 4 (1969) 181.
- [20] M.G. Khaledi, S.C. Smith and J.K. Strasters, *Anal. Chem.*, 63 (1991) 1820.