ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
16 (1998) 1251–1260

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Choice of capillary electrophoresis systems for the impurity profiling of drugs[1]

M.J. Hilhorst, G.W. Somsen, G.J. de Jong *

Department of Analytical Chemistry and Toxicology, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, Netherlands

Received 11 June 1997; accepted 26 August 1997

Abstract

In order to develop a strategy for the impurity profiling of drugs, the possibilities of some capillary electrophoresis systems were investigated. A mixture containing a drug and some of its possible impurities has been used as a model problem. The test compounds were investigated by capillary zone electrophoresis (CZE) and by micellar electrokinetic chromatography (MEKC). The pH of the CZE buffer was varied, but the two stereoisomers could not be separated. Moreover, CZE is not suitable for neutral compounds. In MEKC, two different types of surfactants, sodium dodecyl sulphate (SDS) and cetyltrimethylammonium bromide (CTAB), have been used and the effect of type and concentration modifier on the separation and the elution window was studied. In the SDS system, both the resolution and the elution window could be increased considerably by the addition of modifier. The use of two MEKC systems of different selectivity seems to be a combination with high potential for the impurity profiling of drugs.

Keywords: Impurity profiling; Capillary zone electrophoresis (CZE); Micellar electrokinetic chromatography (MEKC); Elution window; SDS; CTAB

1. Introduction

The determination and identification of impurities is an important aspect of drug analysis. An accurate analytical profile of a drug substance and its formulation does not only fulfil the requirements of regulatory agencies, but is also essential from the development of a (potential) drug to the quality control of a marketed pharmaceutical product [1]. Impurity profiling is commonly performed by liquid chromatography (LC), which is an established methodology and has highly automated instrumentation available. Next to LC, capillary electrophoresis (CE) is becoming a routine analytical technique for the analysis of pharmaceutical samples [2]. Its speed, high efficiency, ease of operation and low consumption of chemicals make CE an interesting complementary and alternative technique to LC. In CE different modes of operation can be distinguished. The separation of charged compounds by capillary

* Corresponding author. Fax: +31 50 3637582; e-mail: g.j.de.jong@farm.rug.nl

[1] Presented at the Eighth International Symposium on Pharmaceutical and Biomedical Analysis (PBA '97), Orlando, FL, USA, 4–8 May, 1997.

0731-7085/98/$19.00
PII S0731-7085(97)00205-7

zone electrophoresis (CZE) is based on differences in electrophoretic mobility. In order to separate neutral compounds by CE, a charged surfactant should be added to the CE separation buffer so that the overall mobility of a compound is both a function of charge and phase partitioning between micelles and the aqueous solvent. This type of CE is called micellar electrokinetic chromatography (MEKC) and was first described by Terabe et al. [3].

Quite a number of papers have been published on impurity profiling by CE [4–9]. Most of them deal with the optimisation of one particular separation problem, i.e. in principle these studies do not formulate general experimental conditions, which are also suited for profiling of other samples. The goal of the present study was to find a set of CE systems which can be used as a more general approach. Because MEKC is more generally applicable than CZE, emphasis was put on MEKC systems. Although quantification is a significant part of impurity profiling, this paper mainly focuses on the problem of separating unknown compounds. The present selection of the appropriate separation conditions is based on the analysis of a test mixture consisting of a main component, the antidepressant fluvoxamine containing a primary amine group, and three of its possible impurities. Two impurities are quite different in nature, i.e an addition product with two acidic groups and a neutral compound, and one is very similar to the main compound (a stereoisomer). The CZE experiments were carried out with three buffers of different pH viz. 2.5, 7.0 and 9.3. In MEKC sodium dodecyl sulphate (SDS) and cetyltrimethylammonium bromide (CTAB) were used as an anionic and cationic surfactant, respectively. Moreover, the influence of modifier type and concentration on the migration behaviour of the test compounds and on the elution window was studied.

2. Theoretical aspects

In CZE the effective mobility (μ_{eff}) of a solute is based on its net charge and the frictional drag only, and consequently CZE is not suited for the separation of neutrals. In MEKC, however, a surfactant is added to the background electrolyte in concentrations higher than the critical micelle concentration so that analyte molecules can differentially distribute themselves between the aqueous and micellar phase. Since the phases move with a different speed through the capillary, selectivity is promoted [3]. Clearly, in MEKC the effective mobility of charged compounds is the result of phase partitioning and electrophoretic mobility in the aqueous phase and therefore the overall effective mobility μ_{f} is introduced [10].

In both CZE and MEKC, sample compounds, charged or uncharged, migrate with a velocity (v) depending on the electro-osmotic mobility μ_{eof}, the (overall) effective mobility $\mu_{\text{eff}}^{\text{ov}}$ and the field strength E according to:

$$v = (\mu_{eof} + \mu_{\text{eff}}^{\text{ov}})E \tag{1}$$

The (overall) effective mobility can be calculated from the observed migration times according to:

$$\mu_{\text{eff}}^{\text{ov}} = \frac{l_{\text{d}} l_{\text{t}}}{t_{\text{s}} V} - \frac{l_{\text{d}} l_{\text{t}}}{t_{\text{eof}} V} \tag{2}$$

in which l_{t} and l_{d} are the total length of the capillary and the distance from the injection end to the detection window, respectively. V is the applied voltage, and t_{s} and t_{eof} are the migration time for the solute and the time that the electro-osmotic flow (EOF) marker requires to reach the detector, respectively.

In MEKC the migration time difference between the micelle marker and the EOF marker is defined as the elution window which is an important parameter for optimization [11]. Enlarging the elution window results in a longer residence time of the compounds in the capillary, so that components that exhibit only a slightly different interaction with the micelles can be separated. The effect on the resolution is quantitatively described by Terabe et al. for neutral compounds [12] and by Peterson et al. for charged compounds [13]. An increase of the elution window also increases the peak capacity n of the window which depends on the efficiency N and the ratio of t_{mc} and t_{eof} according [14]:

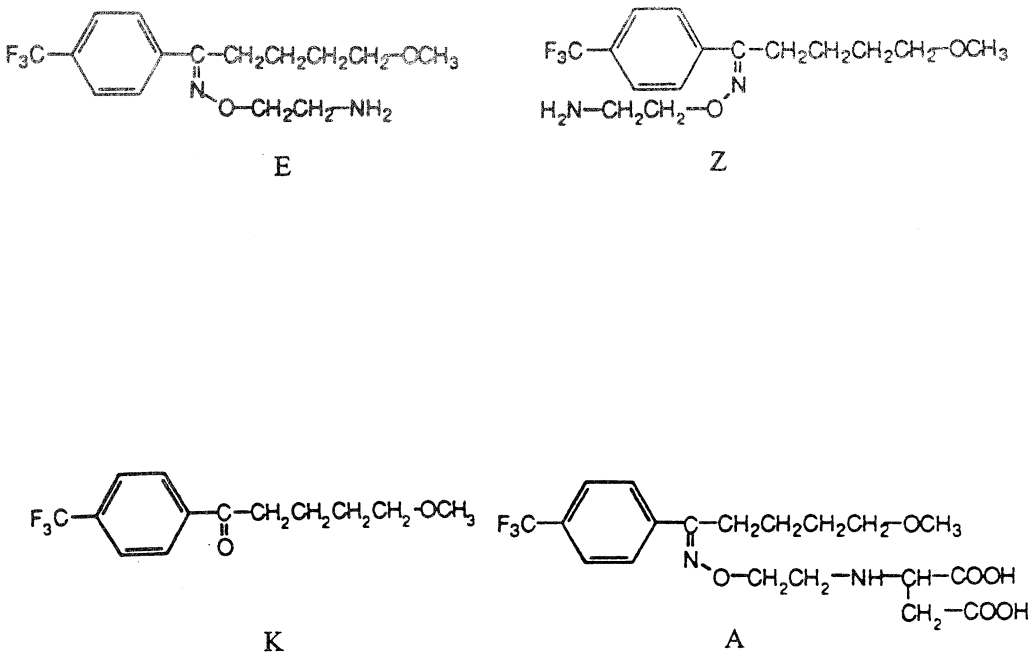

Fig. 1. Chemical structures of the test compounds. E, fluvoxamine (E isomer); Z, Z isomer; K, fluvoxketone; A, maleic acid addition product.

$$n = \frac{\sqrt{N}}{4} \ln\left(\frac{t_{mc}}{t_{eof}}\right) \quad (3)$$

Generally an increase of the ratio involves an enlargement of the elution window. In MEKC the observed velocity of the micellar phase (v_{mc}) is determined by its electrophoretic velocity ($v_{eff,mc}$) and by the velocity of the EOF (v_{eof}); v_{eof} and $v_{eff,mc}$ have an opposite sign and commonly the absolute value of v_{eof} is considerably larger than the absolute value of $v_{eff,mc}$. The ratio t_{mc}/t_{eof} can be expressed in terms of velocities according [14]:

$$\frac{t_{mc}}{t_{eof}} = \frac{\frac{l_d}{v_{mc}}}{\frac{l_d}{v_{eof}}} = \frac{v_{eof}}{v_{mc}} = \frac{v_{eof}}{v_{eof} + v_{eff,mc}} \quad (4)$$

When v_{eof} is reduced to values close to the absolute value of $v_{eff,mc}$, large ratios and thus large elution windows can be obtained. Reduction of the EOF can be achieved by the addition of organic modifiers [15,16], which induce changes in viscosity, permittivity and ζ potential. A modifier may also influence the distribution of the analytes between the micellar and aqueous phase. In other words, in MEKC modifiers can be used to improve resolution and selectivity.

When a cationic surfactant is used, the surfactant molecules form a positively charged double layer on the capillary wall [17]. As a consequence, the EOF is opposite in comparison with normal CZE. The relation between the direction of the bulk flow and that of the micelles is preserved, however, the applied electric field should be reversed in order to keep the flow of the bulk solution towards the detector.

3. Experimental

3.1. Materials and methods

Fluvoxamine maleate (E isomer) and three of its possible impurities viz. the Z isomer, an addition product (adduct) and fluvoxketone (ketone) (Fig. 1) were donated by Solvay Duphar B.V. (Weesp, Netherlands). The main component (E

isomer) and the Z isomer are primary amines, both with a pK_a of about 9.2. Fluvoxketone is a neutral compound and the addition product contains both basic and acidic groups. A test mixture was prepared in water containing 2.0×10^{-4} M of both the ketone and the adduct, 0.71×10^{-4} M of the E isomer and 1.3×10^{-4} M of the Z isomer. Methanol and acetonitrile were purchased from Labscan (Dublin, Ireland), acetophenone, propiophenone, butyrophenone, valerophenone, hexaphenone and octanophenone from Sigma (St Louis, USA) and acetone (EOF marker), potassium-dihydroxyphosphate, boric acid, phosphoric acid, sodium dodecyl sulphate (SDS) and cetyltrimethylammonium bromide (CTAB) from Merck (Darmstadt, Germany). All chemicals were of analytical grade. Deionized water was obtained from a Elga ultra pure water system (Salm en Kipp BV, Netherlands). For the CZE experiments the following buffers were prepared as background electrolyte: phosphate (25 mM, pH 2.5), phosphate (25 mM, pH 7.0) and borate (25 mM, pH 9.3). All buffers were adjusted to the proper pH by addition of 2.0 M sodium hydroxide. The buffers used in the MEKC experiments contained borate (25 mM, pH 9.3) with 50 mM SDS or phosphate (25 mM, pH 7.0) with 10 mM CTAB. In order to study the effect of modifiers, methanol or acetonitrile were added to the buffers up to 20% (v/v) in 5% increments. The separation buffers were filtered through 0.45 μm membrane filter before use. An iterative procedure using a homologue series of alkylphenones was used for the determination of the migration time of the micelles (t_{mc}). A common micelle tracer such as Sudan III was not used, because when organic modifiers are present in the separation buffer the marker might not be entirely included in the micelles [18].

3.2. CE-system

The experiments were performed with a HP^{3D} Capillary Electrophoresis system (Hewlett Packard, Waldbronn) equipped with a on-column diode array detector (DAD). An uncoated fused silica capillary of 64.5 cm $\times$ 50 μm i.d. with an effective length of 56 cm was used for the CZE experiments. In the MEKC experiments a 62 cm $\times$ 50 μm i.d. capillary with an effective length of 54.0 cm when SDS was used as surfactant and a 50 cm $\times$ 50 μm i.d. with a effective length of 41.5 cm for CTAB. Before use, the capillaries were rinsed with 1 M NaOH (15 min), followed by deionized water (15 min) and separation buffer (30 min). Between runs, the capillary was flushed with the buffer for 2 min. After change of the separation buffer, the capillary was flushed with the new buffer for 30 min. The capillary was thermostated to 30°C and samples were hydrodynamically injected by applying a pressure of 50 mbar for 2 s. Electrophoresis was performed at a constant voltage of 30 kV. The data were collected and interpreted using HP chemstation software version 04.02.

4. Results and discussion

4.1. CZE

First, the potential of CZE for the separation of the test mixture was studied. The data on the free zone mobilities of the test compounds are also helpful for the explanation of the behaviour of the charged compounds in the MEKC systems.

The CZE experiments were carried out at pH 2.5, 7.0 and 9.3, which yielded an EOF of 6.6×10^{-9}, 6.6×10^{-8} and 8.6×10^{-8} $m^2V^{-1}s^{-1}$ respectively. The small value at pH 2.5 is due to the lack of protonation of the silica groups on the capillary wall. The CZE results are summarized in Fig. 2. At pH 2.5 the isomers and the adduct are

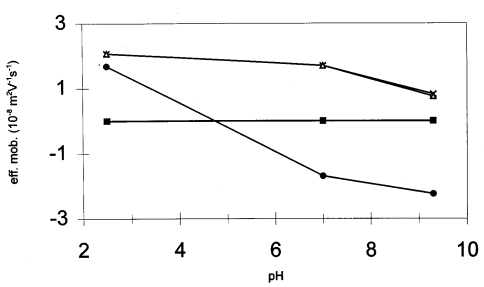

Fig. 2. Effective mobility versus pH. △, fluvoxamine (E isomer); X, Z isomer; ■, fluvoxketone; •, addition product.

positively charged and migrate before t_{eof}. The Z and E isomer migrate as a single peak. The peak shape of fluvoxketone was not satisfactory because it co-migrates with the EOF, which gives a small baseline disturbance at t_{eof} . At pH 7.0 the Z and E isomer again co-migrate before t_{eof}, indicating a positive charge. In this case the adduct migrates after t_{eof} indicating a negative charge. At pH 9.3 the pH of the buffer is close to the pK_a of fluvoxamine and the Z and E isomer are partly separated, possibly due to a small difference in their pK_a values. The relatively high EOF at pH 9.3 results in an analysis time of only 3.5 min.

Overall, plate numbers in the range 2×10^5–$8 \cdot 10^5$ per meter were obtained except for fluvoxketone. The Z and E isomers show similar behaviour in CZE and cannot be separated entirely. Moreover, the neutral compound cannot be separated from the EOF disturbance of the baseline. Due to a small EOF a buffer with pH 2.5 is only suited for profiling of positively charged compounds.

4.2. MEKC

4.2.1. Type of surfactant

For MEKC analysis of the test mixture, SDS and CTAB were used as surfactant and their selectivity was studied. These two surfactants were selected because of their different selectivity as has been pointed out by linear solvent energy relationships [19,20] as well as retention indices studies [21]. Because of the positively charged double layer, CTAB may prevent unwanted adsorption of basic compounds on the capillary wall [22]. With SDS as surfactant a buffer of pH 9.3 is used in order to obtain a sufficiently high EOF. For CTAB a pH 9.3 was found to give an elution window of 2 min only, which is too small for useful impurity profiling. Therefore, a separation buffer with a pH 7.0 was used. The concentration of surfactant was chosen well above (factor 5–10) the critical micelle concentration. In practice the optimum surfactant concentration will depend on the separation problem at hand. When a low concentration of micelles is used, the separation between hydrophobic analytes is improved, while a high concentration of micelles supports the separation of relatively hydrophilic compounds. However, the nature of the impurities is, at least partly, unknown, and therefore an average concentration of micelles was selected according to the strategy described by Terabe [23].

For each test compound plate numbers above 8×10^5 per meter were obtained in the MEKC system containing 50 mM SDS (Fig. 3). Fluvoxamine and its Z isomer could not be separated and migrated with about the same velocity as the micelles. This is somewhat surprising since the isomers are (partly) positively charged at pH 9.3 and therefore will migrate in the direction opposite of the negatively charged micelles. This was expected to result in migration times shorter than t_{mc}. Apparently, regardless of their charge, the isomers exhibit a strong affinity towards the micelles probably involving hydrophobic and ionic interactions. An increase or decrease of the SDS concentration did not result in a separation of the Z and E isomer. The neutral fluvoxketone is distributed between the aqueous and micelle phase and is, therefore, separated from the baseline disturbance at t_{eof} yielding a sharp peak. The adduct has the shortest migration time of the test compounds. Probably the affinity of the negatively charged adduct for the micelles will be relatively low because of electrostatic repulsion.

For the CTAB system the peaks, in particular the ketone and the adduct, are somewhat broader, but the Z and E isomers are completely separated without the addition of a modifier and all analytes migrate within 7 min (Fig. 4). The peak order is reversed in comparison with the system containing sds. Although the isomers and the micelles have a positive charge the isomer separation indicates that some interaction occurs. The negatively charged adduct nearly co-migrates with the micelles indicating a strong interaction. Clearly the SDS and CTAB system exhibit a quite different selectivity. The relative bad peak shape of the adduct may be due to adsorption to the CTAB double layer on the capillary wall [22].

4.2.2. Addition of modifiers

In order to study the effect of organic modifiers on the separation and the elution window, acetonitrile and methanol have been added in four

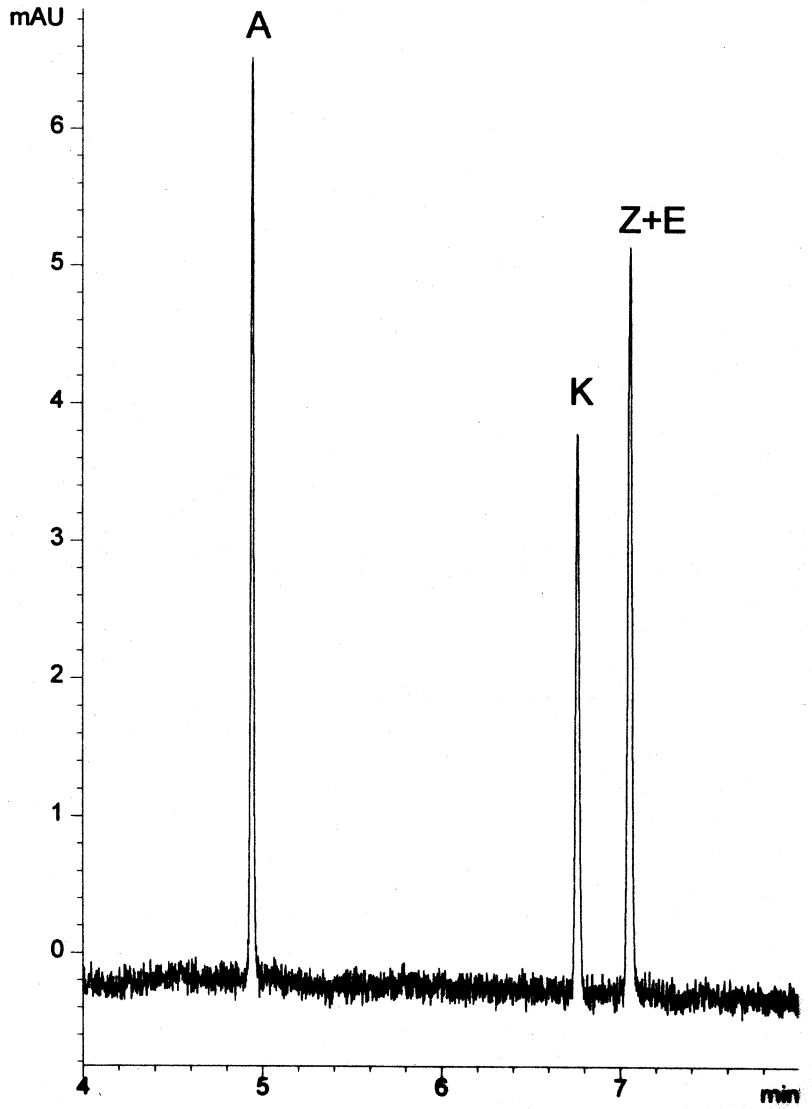

Fig. 3. Electrokinetic chromatogram of the test mixture in the 50 mM SDS MEKC system. Peak denotation, see Fig. 1.

different concentrations (5,10,15 and 20% v/v) in both the SDS and CTAB system. Fig. 5 and Fig. 6 show the overall effective mobility of the test compounds in the studied MEKC systems. Since all the test compounds migrate after t_{eof} their overall effective mobilities have a negative sign. The overall effective mobility of the analytes becomes less negative if the modifier concentration increases. For the SDS system the change in overall effective mobility is largely the same for all compounds so that the mutual differences in μ_f are constant. Nevertheless, because the addition of modifiers a decrease of the EOF is induced and a corresponding increase in the elution window. The separation of the test components is still enhanced when the modifier is added. In the CTAB system, the overall effective mobilities only increase moderately when modifier is added (Fig.

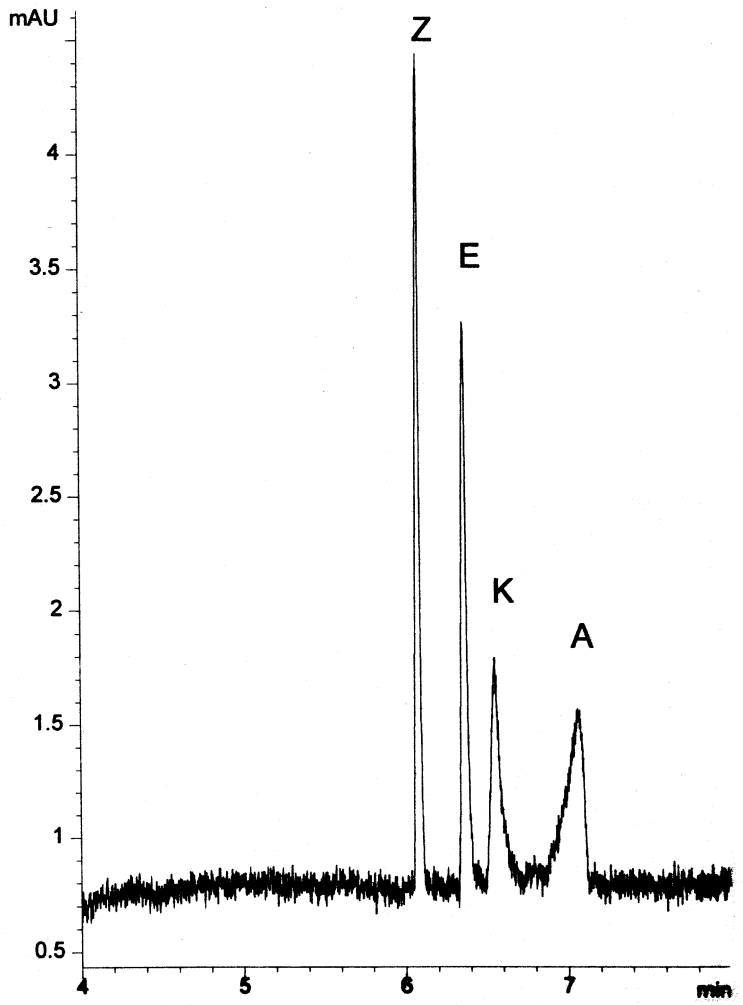

Fig. 4. Electrokinetic chromatogram of the test mixture in the 10 mM CTAB system. Peak denotation, see Fig. 1.

6). Furthermore, the slope of the curves for fluvoxketone and the charged compounds is not the same, resulting in a peak reversal at different concentrations of modifier. Interestingly, the peak order of the isomers is the same in both MEKC systems.

In contrast to the CTAB systems, with SDS as a surfactant complete separation of the Z and E isomer can only be achieved if a relatively high percentage of modifier is added. In Fig. 7 the resolution of the Z and E isomer obtained in the SDS system is plotted versus the percentage modifier in the buffer. In order to achieve a baseline separation between Z and E, the buffer should contain at least 15% methanol or about 8% acetonitrile. Two reasons can be put forward for the improved resolution at higher concentration modifier. Firstly, the EOF decreases when modifier is added, which results in a larger elution window and thus a higher resolution. Secondly, the modifier induces a selectivity change in distribution of the isomers between the micellar and the aqueous phase. For compounds migrating closely to t_{mc}, such as the Z and E isomer, the contribution of the larger window on their resolution will be small compared to the effect of a selectivity change.

The t_{mc}/t_{eof} ratio is depicted in Fig. 8 as a function of the percentage and type of modifier for both MEKC systems. For the SDS system the quotient increases considerably with increasing modifier percentage. Compared to 0% modifier, a gain in peak capacity of a factor 1.8 (methanol) and a factor 2.3 (acetonitrile) was obtained (Eq. (3)) when 20% modifier was added to the SDS buffer. Fig. 8B shows that for CTAB the increase of t_{mc}/t_{eof} is relativily small, yielding a gain in peak capacity of a factor 1.2 and 1.5 only for methanol and acetonitrile, respectively. Considering the small effect upon addition of modifier on both t_{mc}/t_{eof} and the mobilities of the analytes, it can be concluded that addition of modifier in the CTAB system does not involve a significant improvement.

5. Conclusions

The potential of CE, and MEKC in particular, for the analytical profiling of drugs has been demonstrated using fluvoxamine and three of its possible impurities as test compounds. With CZE complete separation of all components of the test mixture could not be achieved due to the similar behaviour of stereoisomers and the present of a neutral compound. However, a combination of MEKC systems offers a high separation power.

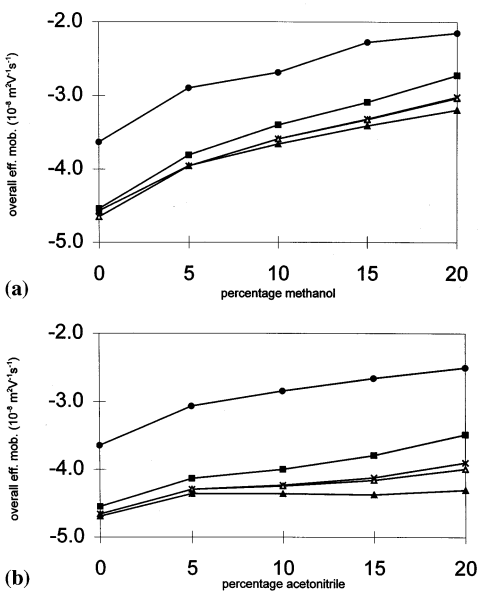

Fig. 5. Overall effective mobility versus percentage methanol (A) and acetonitrile (B) in the separation buffer containing 50 mM SDS. △, fluvoxamine (E isomer); X, Z isomer; ■, fluvoxketone; ●, addition product; ▲, micelle.

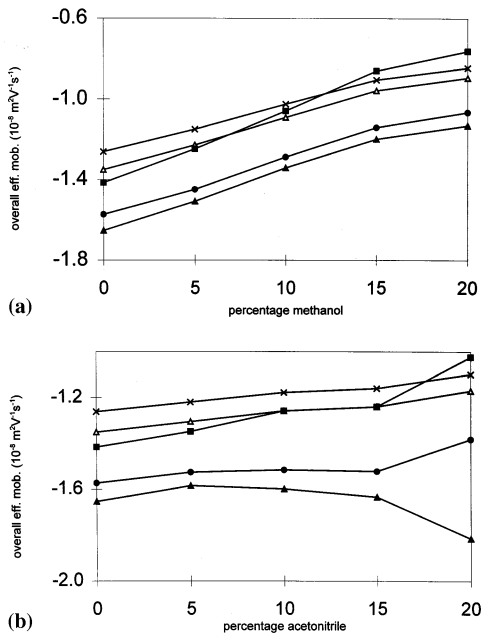

Fig. 6. Overall effective mobility versus percentage methanol (A) and acetonitrile (B) in the separation buffer containing 10 mM CTAB. △, fluvoxamine (E isomer); X, Z isomer; ■, fluvoxketone; ●, addition product; ▲, micelle.

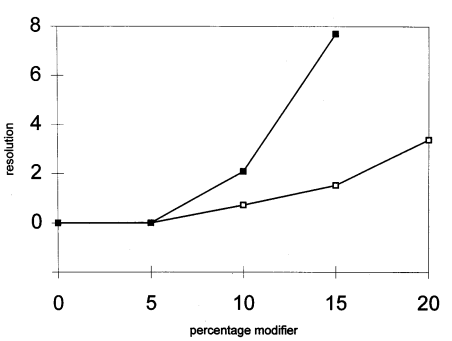

Fig. 7. Resolution of the Z and E isomer versus percentage methanol (□) and acetonitrile (■) in the separation buffer containing 50 mM SDS.

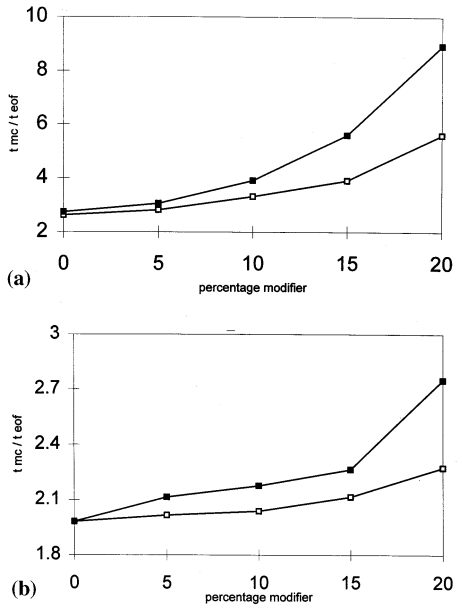

Fig. 8. Quotient of t_{mc} and t_{eof} versus the percentage methanol (□) and acetonitrile (■) in the separation buffer for the SDS (A) and the CTAB (B) system.

The separation mechanism of MEKC for charged compounds seems complex. Modifiers, especially acetonitrile, have an important influence on the resolution and the elution window. A larger window improves the separation and the peak capacity, but also enlarges the time of analysis. In case of impurity profiling a large window is favourable because the number and nature of the impurities are unknown. Because commonly only a limited amount of samples has to be analysed, the relatively large analysis time often is not a real drawback. A buffer containing 10% acetonitrile and a micelle concentration of 50 mM SDS for the anionic MEKC system and 10 mM CTAB for the cationic system appears to be a good compromise between the elution window and the analysis time. Using these conditions in initial experiments, the concentration surfactant or modifier can further be optimised.

In principle, all analytes can be eluted when an experiment with a anionic surfactant is combined with an experiment with a cationic surfactant. Neutral components will migrate between t_{eof} and t_{mc}. Impurities that are not detected before t_{mc} in one system will have the same charge as the micelles, but a higher overall effective mobility. In the second system, where the polarity of the electrodes is reversed, this impurity will migrate before t_{eof} when no interaction with the micelles occurs, or between t_{eof} and t_{mc} when it interacts with the micelles. In other words, with selection of these two MEKC systems, probably all of the impurities present in the sample will be detected (assuming that they are UV active) whether or not separated from each other and regardless of their polarity. This concept will be further tested for other mixtures. Preferably, the pH should be the same in both systems in order to ensure that the charge of the analytes is unchanged. However, at pH 7.0 the EOF in the SDS system becomes to slow resulting in long analysis times, while at pH 9.3 the elution window in the CTAB system becomes to small for an useful profile. In other words, further experiments are required to find two suitable micelle systems that can be used at the same pH. Moreover, perhaps the use of other surfactants will also lead to a further increase of the separation power. In this respect the possibilities of cyclodextrins are also interesting [9] and their use for the impurity profiling of drugs has to be investigated.

Acknowledgements

The authors thank Dr T.K. Gerding of Solvay Duphar B.V. (Weesp, Netherlands) for the donation of the test compounds and for stimulating discussions.

References

[1] C.A. Johnson, J. Pharm. Biomed. Anal. 4 (1986) 565–571.
[2] K.D. Altria, J. Chromatogr. 646 (1993) 245–257.
[3] S. Terabe, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111–113.
[4] K.D. Altria, Y.L. Chanter, J. Chromatogr. A 652 (1993) 459–463.
[5] G. Castanda Penalvo, E. Julien, H. Fabre, Chromatographia 42 (1995) 159–164.

[6] B. Nickerson, S. Scypinski, H. Sokoloff, S. Sahota, J. Liq. Chromatogr. 18 (1995) 3847–3875.
[7] M. Gilges, Chromatographia 44 (1997) 191–196.
[8] B.R. Thomas, X.G. Fang, X. Chen, R.J. Tyrrell, S. Ghodbane, J. Chromatogr. B 657 (1994) 383–394.
[9] K.D. Altria, J. Chromatogr. A 735 (1996) 43–56.
[10] M.G. Khaledi, S.C. Smith, J.K. Strasters, Anal. Chem. 63 (1991) 1820–1830.
[11] A.T. Balchanus, M.J. Sepaniak, Anal. Chem. 59 (1987) 1466–1470.
[12] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834–841.
[13] A.G. Peterson, E.S. Ahuja, J.P. Foley, J. Chromatogr. B 683 (1996) 15–28.
[14] J. Vindevogel, P. Sandra, Introduction to Micellar Electrokinetic Chromatography, Chapter 11, Hüthig, Heidelberg, Germany, 1992.
[15] R. Weinberger, I.S. Lurie, Anal. Chem. 63 (1991) 823–827.
[16] A.E. Bretnall, G.S. Clarke, J. Chromatogr. A 716 (1995) 49–55.
[17] J.C. Reijenga, G.V.A. Aben, Th.P.E.M. Verheggen, F.M. Everaerts, J. Chromatogr. 260 (1983) 241–254.
[18] M.M. Bushey, J.W. Jorgenson, Anal. Chem. 61 (1989) 491–493.
[19] S. Yang, M.G. Khaledi, J. Chromatogr. A 692 (1995) 301–310.
[20] J.G. Bumgarner, M.G. Khaledi, J. Chromatogr. A 738 (1996) 275–283.
[21] P.G.H.M. Muijselaar, H.A. Claessens, C.A. Cramers, Anal. Chem. 69 (1997) 1184–1191.
[22] P.G.H.M. Muijselaar, H.A. Claessens, C.A. Cramers, J. Chromatogr. A 735 (1996) 395–402.
[23] S. Terabe, J. Pharm. Biomed. Anal. 10 (1992) 705–715.

.