Zhi Wang[1]
Hong Wan[1]
Magnus S. Anderson[1]
Mohamed Abdel-Rehim[2]
Lars G. Blomberg[1]

[1]Department of Chemistry, Karlstad University, Sweden
[2]AstraZeneca R&D, Södertälje, Sweden

Separation of lidocaine and its metabolites by capillary electrophoresis using volatile aqueous and nonaqueous electrolyte systems

The separation of the basic drug lidocaine and six of its metabolites has been investigated both by using volatile aqueous electrolyte system, at low pH and by employing non-aqueous electrolyte systems. In aqueous systems, the best separation of the compounds under the investigated conditions was achieved by using the electrolyte 60 mM trifluoroacetic acid (TFA)/triethylamine (TEA) at pH 2.5 containing 15% methanol. With this electrolyte, all seven compounds were well separated with high efficiency and migration time repeatability. The separations with bare fused-silica capillaries and polyacrylamide-coated capillaries were compared with higher separation efficiency with the latter. On the other hand, near baseline separation of all the seven compounds was also obtained by employing the non-aqueous electrolyte, 40 mM ammonium acetate in methanol and TFA (99:1, v/v), with comparable migration time repeatability but lower separation efficiency relative to the aqueous system.

Keywords: Capillary electrophoresis / Trifluoroacetic acid / Volatile electrolyte systems / Lidocaine and metabolites / Basic drugs EL 4547

1 Introduction

The basic drug lidocaine (LID) is commonly administered as a local anaesthetic and also used for the investigation of hepatic function. In addition, LID has antiarrhythmic properties and is frequently used as a therapeutic agent in the treatment of cardiac disorders [1–3] . LID is rapidly and extensively metabolised in humans. Its major metabolites are 4-hydroxy-2,6-xylidine (4-OH-XYL), 2,6-xylidine (XYL), glycinexylidide (GX) and monoethylglycinexylidide (MEGX). Low concentrations of 3-hydroxy-lidocaine (3-OH-LID) and 3-hydroxy-monoethylglycinexylidide (3-OHMEGX) have also been reported [4, 5] . MEGX and GX have pharmacological effects, both as antiarrhytmics and in terms of toxicity [6] . It is thus of interest to monitor the concentrations of the parent drug and its metabolites in plasma. The structures of LID and its metabolites are shown in Fig. 1. The determination of LID alone or together with its metabolites has been performed by HPLC and by GC [7–20] . In previous work, Abdel-Rehim *et al.* [21] reported GC separations of underivatized LID together with its metabolites using different fused silica capillary columns. Capillary electrophoresis (CE) methods for separation of LID and drugs with similar structure have been presented [22, 23]. Recently, our group has reported the CE separation of LID together with its metabolites by using the buffer system 35 mM phosphate/Tris (pH 3.6) containing 6 mM cetyltrimethylammonium bromide (CTAB) and 9% v/v methanol [24] . In terms of sensitivity and identification power, especially for the analysis

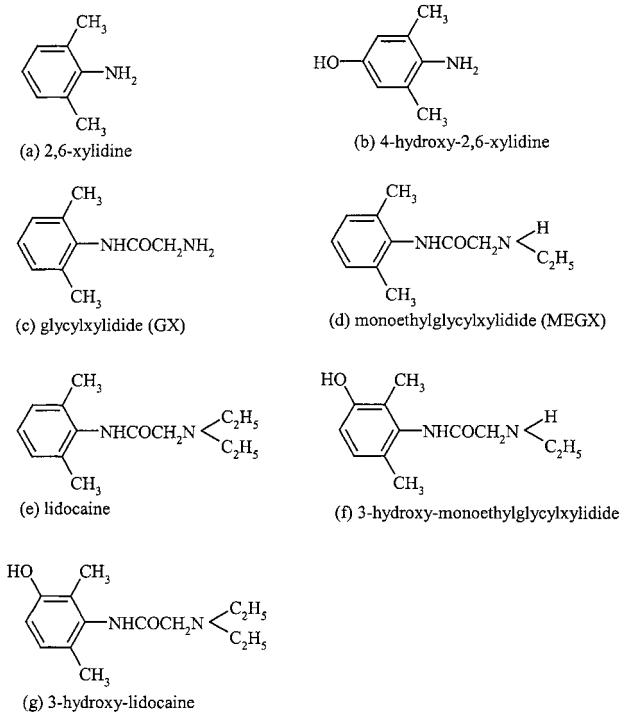

Figure 1. Structures of lidocaine and its metabolites.

Correspondence: Professor L. G. Blomberg, Department of Chemistry, Karlstad University, SE-651 88 Karlstad, Sweden
E-mail: lars.blomberg@kau.se
Fax: +46-54-7001424

Abbreviations: **GX**, glycinexylidide; **LID**, lidocaine; **MEGX**, monoethylglycinexylidide; **3-OH-LID**, 3-hydroxy-lidocaine; **3-OH-MEGX**, 3-hydroxy-monoethylglycinexylidide; **4-OH-XYL**, 4-hydroxy-2,6-xylidine; **TEA**, triethylamine; **XYL**, 2,6-xylidine

of biological samples, mass spectrometric (MS) detection has advantages over other commonly used CE detectors [25]. To take the best advantage of MS detection, CE separations with volatile electrolyte and without the involvement of surfactants are favorable since the accumulation of surfactants can cause fouling of the MS ion source and suppression of analyte signals [26, 27]. In addition, buffer constituents such as trifluoroacetic acid (TFA) can lead to decreased MS response by the formation of a strong ion pair with positively charged analytes [28, 29]. However, post-column addition of propanoic acid and 2-propanol, "TFA fix", can displace the TFA on the basis of volatility resulting in enhanced MS sensitivity [29, 30]. In the present work, the CE separation of LID and all its metabolites was investigated by using a volatile aqueous electrolyte as well as a nonaqueous electrolyte system to facilitate subsequent MS detection.

2 Materials and methods

2.1 Instrumentation and equipment

CE was performed on a Beckman (Fullerton, CA, USA) P/ACE 5510 capillary electrophoresis system equipped with a high voltage supply (0-30 kV), an automatic injector, a diode array UV detector and a liquid cooled capillary cartridge. Data were collected by means of Beckman P/ACE station software (Version 1.0). Chemometric experimental design for the optimisation experiments was done in Codex (Sum IT System AB, Sollentuna, Sweden). Untreated fused-silica capillaries (50 μm ID, 375 μm OD) obtained from Polymicro Technologies (Phoenix, AZ, USA) were used as separation capillaries. Polyacrylamide-coated capillaries were prepared according to Hjertén [31] with small modifications. Sample introduction was performed using pressure (0.5 psi) and on-column detection was made with UV at 200 nm.

2.2 Chemicals

Lidocaine and its metabolites were supplied from AstraZeneca (Department of Medicinal Chemistry, Södertälje, Sweden). Deionized water (18.2 MΩ) was from a Millipore Milli-Q plus water purification system (Molsheim, France). Ammonium acetate ($>$99% pure) and formic acid (90%) were purchased from Fluka (Stockholm, Sweden). (TFA $>$99% pure) was obtained from Aldrich (Stockholm, Sweden). Acetic acid (more than 99.8% pure) was from Ridel-de Haen (Stockholm, Sweden). Acros Organics (New Jersey, USA) supplied triethylamine (TEA) (99% pure). Other chemicals used in this work were of analytical grade.

2.3 Electrolyte and sample preparation

Stock electrolyte solutions of 100 mM TFA/TEA (pH 2.5) were prepared by first adding 0.77 mL of TFA to 100 mL of deionized water, and then adjusting the pH to 2.5 by adding an appropriate amount of TEA (the pH was measured with a pH meter, MeterLab, Radiometer Analytical, Lyon, France). The buffer solutions were degassed by vacuum and prior to analysis, they were filtered through a 0.45 μm membrane Millex-hv Millipore (Molsheim, France). Lower concentration of buffers with certain percentage methanol were obtained by mixing the appropriate amounts of methanol and water with appropriate amounts of the above buffer directly in the electrophoresis buffer vial. Stock solutions of lidocaine and its metabolites of 1 mg/mL were prepared in methanol. The solution was diluted with water to 0.05 mg/mL before use.

2.4 Capillary pretreatment and rinsing

For bare fused silica capillaries, new ones were pretreated by sequentially washing with 0.1 M NaOH for 30 min, H_2O for 10 min and running buffer for 10 min respectively before first use. Between runs, they were rinsed with running buffer for 5 min. For polyacrylamide-coated capillaries, rinsing was with H_2O for 20 min and running buffer for 10 min in succession before first use. Between runs, the capillaries were rinsed with running buffer for 5 min.

3 Results and discussion

3.1 Separation in volatile aqueous electrolyte systems

3.1.1 Scouting

The goal of this study was to find a suitable volatile electrolyte system for the separation of the drug lidocaine and its metabolites to facilitate further MS detection. At first, separations using 50 mM ammonium formate at pH 2.6 and 3.5 were screened. As a result, with this electrolyte system at pH 3.5 (close to its pK_a 3.74), only two broad split peaks were obtained for the seven compounds (Fig. 2A) while at pH 2.6, six peaks appeared with low separation efficiency, with two of them comigrating (Fig. 2B). This demonstrates that low pH was necessary to protonate the analytes so that they could be separated as cations. Consequently, a stronger volatile acid, TFA, which had been used as acid to adjust pH for the separation of some peptides in capillary electrophoresis [32], was chosen for further study. Volatile TEA, which was expected to decrease the interaction between the cationic analytes and the capillary wall at low pH, thus increas-

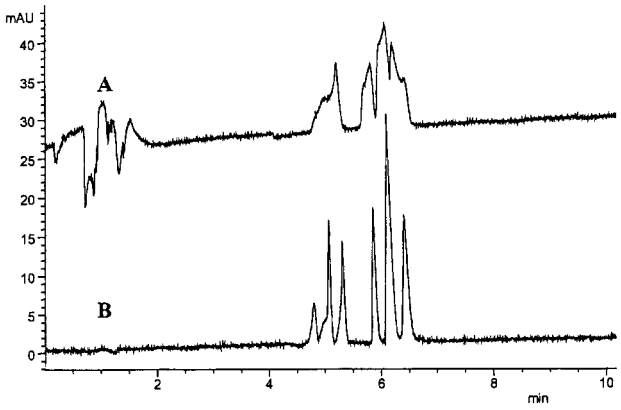

Figure 2. Effect of pH on resolution of lidocaine and its metabolites. Conditions: UV detection at 200 nm. Separation capillary, fused silica 57 cm (50 cm effective length) × 50 μm ID. Voltage 30 kV; temperature 25°C. Background electrolyte: (A) 50 mM ammonium formate (pH 3.5); current, 26 μA. (B) 50 mM ammonium formate (pH 2.6); current, 17.5 μA.

ing separation efficiency and improving separation, was selected as buffer cation. The initial experiments were performed using a TFA/TEA buffer at pH 2.5. Compared to the result indicated in Fig. 2B, this buffer gave much better separation with higher efficiency for all the compounds investigated. Some organic modifiers have been reported to modify separation in CE [33–35]. In our present work, methanol was proven to be effective. Further preliminary experiments showed that both the electrolyte concentration and methanol influenced the separation of the drugs.

3.1.2 Optimization

Based on the results achieved at the scouting stage, electrolyte and methanol concentrations were optimized by a central composite design. The applied levels were low: electrolyte concentration 20 mM TFA/TEA (pH 2.5) and methanol 5% v/v, high: electrolyte concentration 60 mM TFA/TEA (pH 2.5) and methanol 15% v/v. For the design, 13 experiments were performed, including five runs in the centre point. Responses studied were the resolutions between analytes XYL and 4-OH-XYL, 4-OH-XYL and GX, and LID and 3-OH-MEGX. These pairs were the most difficult to separate in the previously performed scouting experiments. The influence of the variables on the migration time for the lastly eluted peak 3-OH-LID was also studied. The goal of the optimization was to find out a set of experimental conditions to obtain a rapid baseline separating system. The results obtained from optimization (Fig. 3) indicated that electrolyte concentration had a positive influence on the separation of the first

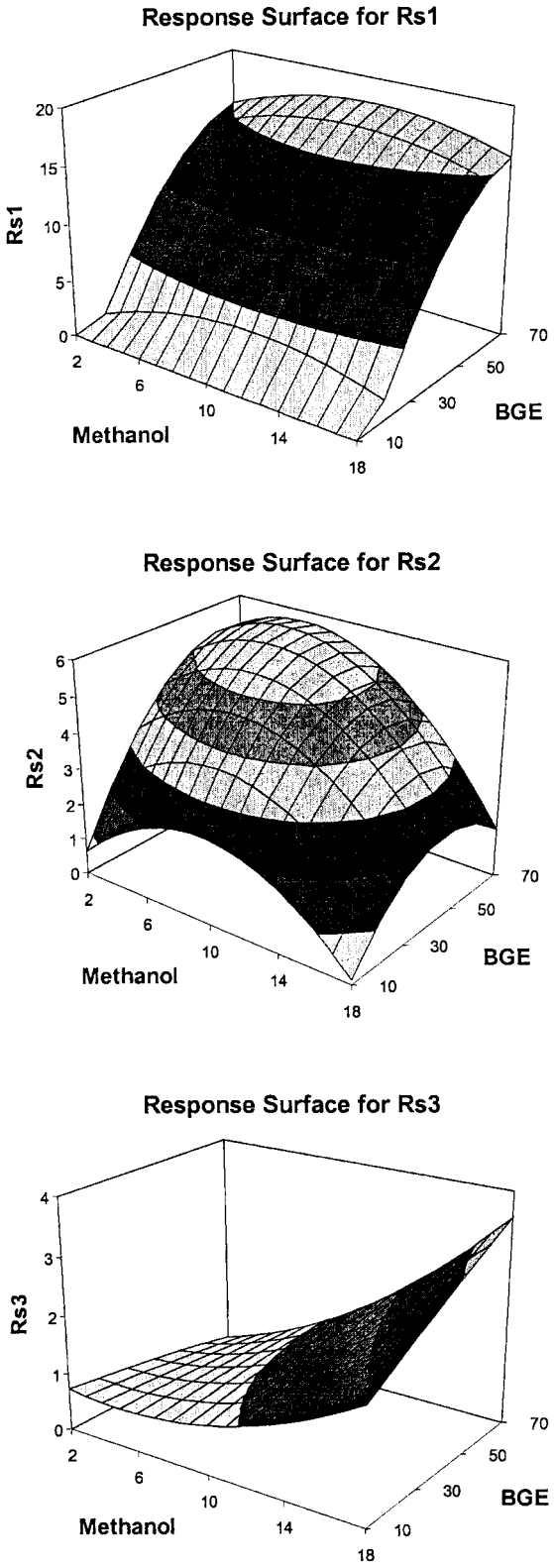

Figure 3. Effects of TFA/TEA concentration (BGE, mM) and methanol (%, v/v) on the resolution (Rs1) of analytes XYL and 4-OH-XYL, (Rs2) of 4-OH-XYL and GX, (Rs3) of LID and 3-OH-MEGX.

and third pairs, while the second pair showed an optimum in the experimental domain. On the other hand, increased methanol concentration improved the third pair separation to a large extent and there is an optimum methanol concentration for the first and second pairs. Among them, the third pair was most difficult to separate.

According to the contradicting optimum points for the three pairs obtained during the optimisation and taking the separation of all analytes into account, a final optimum condition was chosen as 60 mM TFA/TEA buffer at pH 2.5 containing 15% v/v methanol, capillary 47 cm (total length), potential 30 kV and temperature 25°C. An electropherogram is shown in Fig. 4A. The separation efficiency with this electrolyte system ranged from about 2.0×10^5 to 4.0×10^5 theoretical plates/m, depending on compounds.

Good repeatability of migration time is of importance and rinsing strategies between runs influence it [36]. In this study, two different rinsing procedures between runs

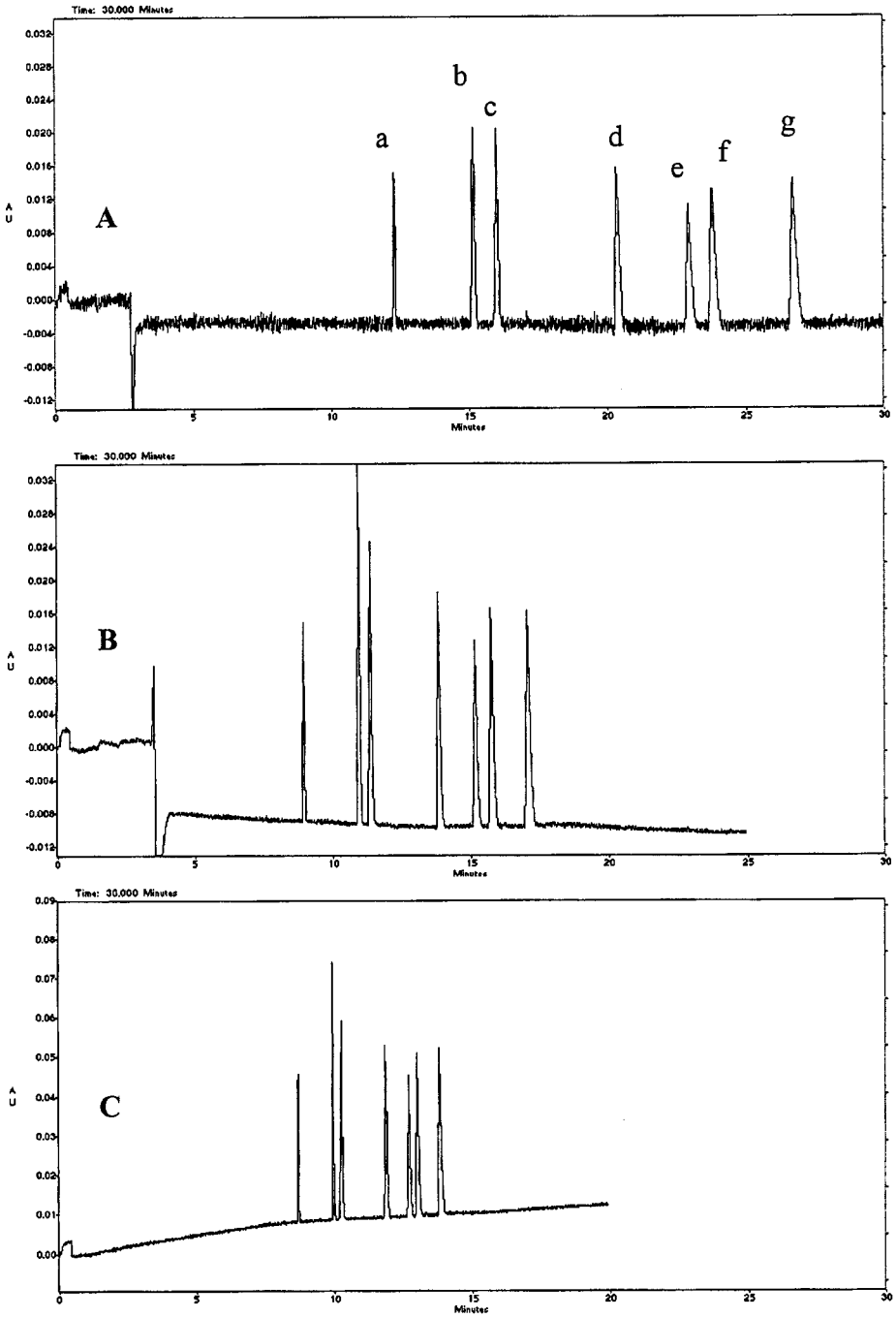

Figure 4. Electropherogram lidocaine and its metabolites under optimized electrolyte conditions: Background electrolyte, 60 mM TFA/TEA at pH 2.5, 15% v/v methanol. Other conditions: UV detection at 200 nm; temperature, 25°C; separation capillary: (A) fused silica, 57 cm (50 cm effective length) × 50 μm ID; voltage, 30 kV (526 V/cm). (B) Fused silica, 47 cm (40 cm effective length) × 50 μm ID; voltage, 25 kV (532 V/cm), current, 34 μA. (C) Polyacrylamide-coated, 57 cm (50 cm effective length) × 50 μm ID; voltage, 30 kV (526 V/cm); current, 34 μA. Peaks, see Fig. 1.

were examined after the capillary was conditioned by washing with 0.1 M NaOH for 30 min followed by H_2O for 15 min and running buffer for 10 min prior to its first use. The first strategy involved rinsing with 0.1 M NaOH for 3 min, H_2O for 3 min and running buffer for 5 min in succession while in the second strategy, only rinsing with running buffer for 5 min was involved. The separation profiles were similar with these two rinsing strategies. However, the first rinsing strategy sometimes resulted in anomalous baseline drift. On the other hand, no such baseline drifts were observed with the second rinsing strategy. Therefore, the simpler and timesaving second rinsing method was adopted in this study. The repeatability of migration times was evaluated with this rinsing method under the selected optimised separation conditions. The repeatability of migration time for the first and last eluting peaks for seven consecutive runs with the same electrolyte was 0.67 and 1.51%, respectively. By definition, a buffer is made up of a mixture of a weak acid and its conjugate base. Although our current electrolyte system consists of a strong acid, TFA, the above repeatability result shows that the current electrolyte with TFA as acid and TEA as basic cation at pH 2.5 is sufficiently good for CE use.

3.1.3 Separation in polyacrylamide-coated capillaries

Polyacrylamide-coated capillaries have been extensively employed in CE. There are several reasons to use coated capillaries, for example, to decrease the adsorption of basic analytes at the capillary wall, to improve peak symmetry and to enhance separation efficiency [37]. Further, the coating can suppress the electroosmotic flow (EOF), which, for example, is important for the separation of peptides and proteins according to their isoelectric point [38]. Figure 4C presents the electropherogram obtained with a 57 cm polyacrylamide-coated capillary under the same experimental conditions as in Fig. 4A. The separation efficiency, ranging from 3×10^5 to 6×10^5 theoretical plates/m, was indeed much improved as compared to the uncoated capillary. The migration time repeatability for the first and lastly eluting peaks for seven consecutive runs using the same electrolyte was 1.59 and 0.56%, respectively. It should be noted that the migration time was shorter with polyacrylamide-coated capillaries than with uncoated ones.

3.2 Separation in non-aqueous electrolytes

3.2.1 Separation

Non-aqueous CE has been shown to be useful for many applications [39–43]. The method is advantageous when a sample is unstable in aqueous solution (in our case, 4-OH-XYL has been reported to be unstable at high pH in aqueous solution [44]) and when MS detection is expected to be used as some volatile organic solvents can facilitate the MS operation. In addition, nonaqueous conditions may be necessary for analytes that are poorly soluble in aqueous buffers. In this study, some nonaqueous separation conditions were investigated for the separation. First, the electrolyte system methanol(MeOH)-acetonitrile(ACN)-acetic acid-ammonium acetate(NH_4Ac), which has been so far the most widely used system for the separation of basic drugs in nonaqueous CE [43], was investigated by changing the relative content of methanol and acetonitrile and of acetic acid and ammonium acetate. However, poor separations were observed with this system. The choice of electrolyte has been reported to have a significant influence on the separation in nonaqueous CE [42]. It has been shown that pK_a values of many analytes are higher in acetonitrile than in water, and much higher in methanol than in water [45, 46]. This may give some explanations to the effect of different electrolyte composition on the separation [45, 46].

In the further study, TFA, instead of acetic acid, was chosen as the acid. Subsequent investigations demonstrated that better separation could be achieved with this system than with the previous one, indicating that the change from acetic acid to TFA, mainly an acidity change, could influence the selectivity of separation (Fig. 5). The relative concentration of methanol and acetonitrile and the content of TFA and ammonium acetate were optimised. The results showed that an increase of the content of MeOH relative to ACN influenced the separation. A change in the concentration of TFA in the range between 1 and 5% had little influence on migration time as well as on the separation. An increase of the concentration NH_4Ac resulted in increased migration times and changed selectivity, with the best overall separation at 40 mM. Figures 6A-C show the influence of the main factor, NH_4Ac concentration, on the separation. Under the investigated conditions, the best separation in the shortest time for the seven compounds was achieved at MeOH/ACN/TFA (99/0/1 v/v/v) containing 40 mM NH_4Ac (Fig. 6B). Here, near baseline separation for all the seven compounds was obtained. As mentioned above, methanol has a higher influence on the pK_a values than acetonitrile. Different salts have been used to adjust separation selectivity in nonaqueous CE systems [43]. To further improve the separation, the volatile TEA was investigated in place of NH_4Ac. The concentration of TEA was also optimised at 40 mM, and as above, the separation was only little influenced by the variation of TFA from 0.5 to 2%. The separation under optimised conditions is shown in Fig. 6D. The comparison of Figs. 6B and D show that the separation profiles are quite similar for the two systems, but with longer migration time with

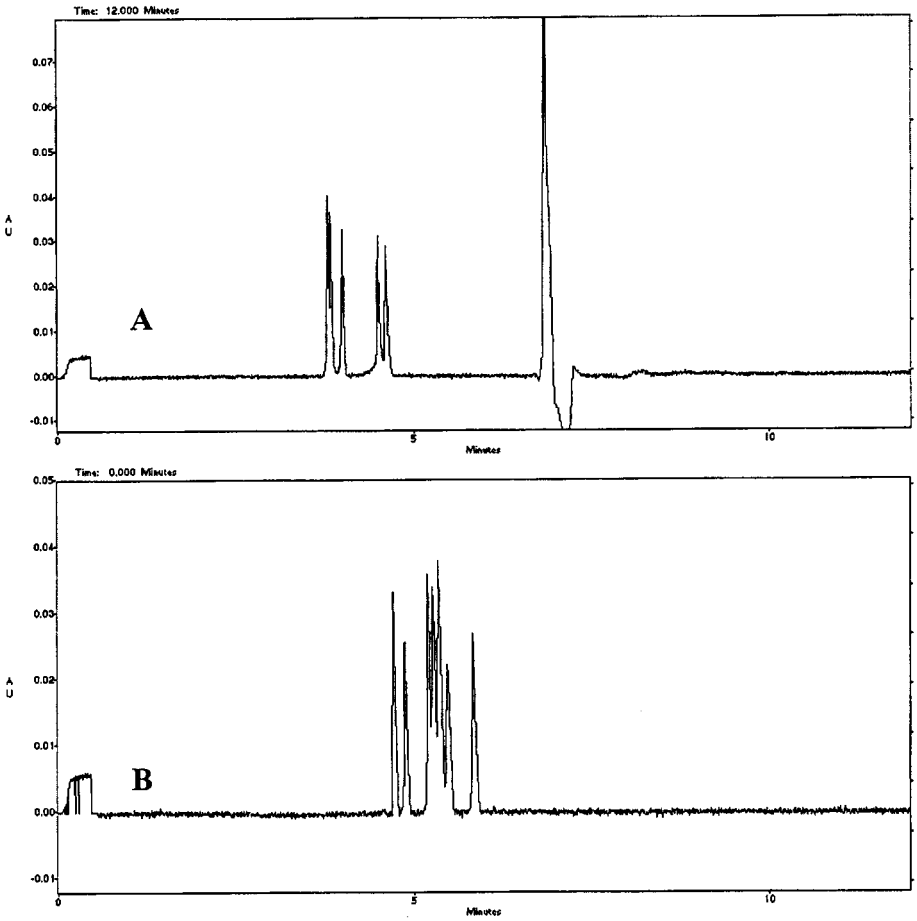

Figure 5. Effect of TFA on separation of lidocaine and its metabolites in nonaqueous buffer. Conditions: UV detection at 200 nm. Separation capillary, fused silica 57 cm (50 cm effective length) $\times$ 50 μm ID. Voltage, 25 kV; temperature 15°C. Background electrolyte: (A) 20 mM ammonium acetate in methanol, acetonitrile and acetic acid (50:49:1 v/v/v); current, 13 μA. (B) 20 mM ammonium acetate in methanol, acetonitrile and TFA (50:49:1 v/v/v); current, 18 μA.

the latter electrolyte. We expected that the separation would be improved by employing polyacrylamide-coated capillaries thus enhancing separation efficiency. However, no improvement of the separations as compared to that shown in Figs. 6B and D was achieved. Separation efficiencies were 0.13×10^5 to 0.75×10^5.

3.2.2 Repeatability of migration time

Due to the volatility of the organic electrolyte system, inferior repeatability was expected compared to the aqueous system, which possibly could influence later quantitative studies. In this work, seven consecutive replica runs evaluated the repeatability of migration time with the same electrolyte solution under the optimized conditions. Contrary to our expectation, the migration time repeatability was quite good with the RSDs of the first and fourth peaks at 1.45 and 1.65%, respectively, indicating that similar migration time repeatability could be achieved with nonaqueous CE as with the aqueous electrolyte system.

3.3 Comparison with reversed polarity CE

In an earlier study of LID and its metabolites we used a reversed polarity CE system with CTAB in the buffer for the separation [24]. Elution order was then from a to g (for legend see Fig. 1). In the present work a normal polarity was used and the elution order in an aqueous buffer was the opposite as before, Fig. 4. In the nonaqueous buffer, the elution order was somewhat different as compared to the aqueous buffer, peak c was thus eluted before peak e and d, Fig. 6. This difference must depend on selective interactions with the non-aqueous buffer. In general, resolution is slightly better in the present work than in our earlier system when we used CTAB in the buffer [24] but migration times are now somewhat longer.

4 Concluding remarks

The separation of LID and its metabolites has been investigated by using a volatile aqueous electrolyte and a non-aqueous electrolyte. Such electrolyte systems are

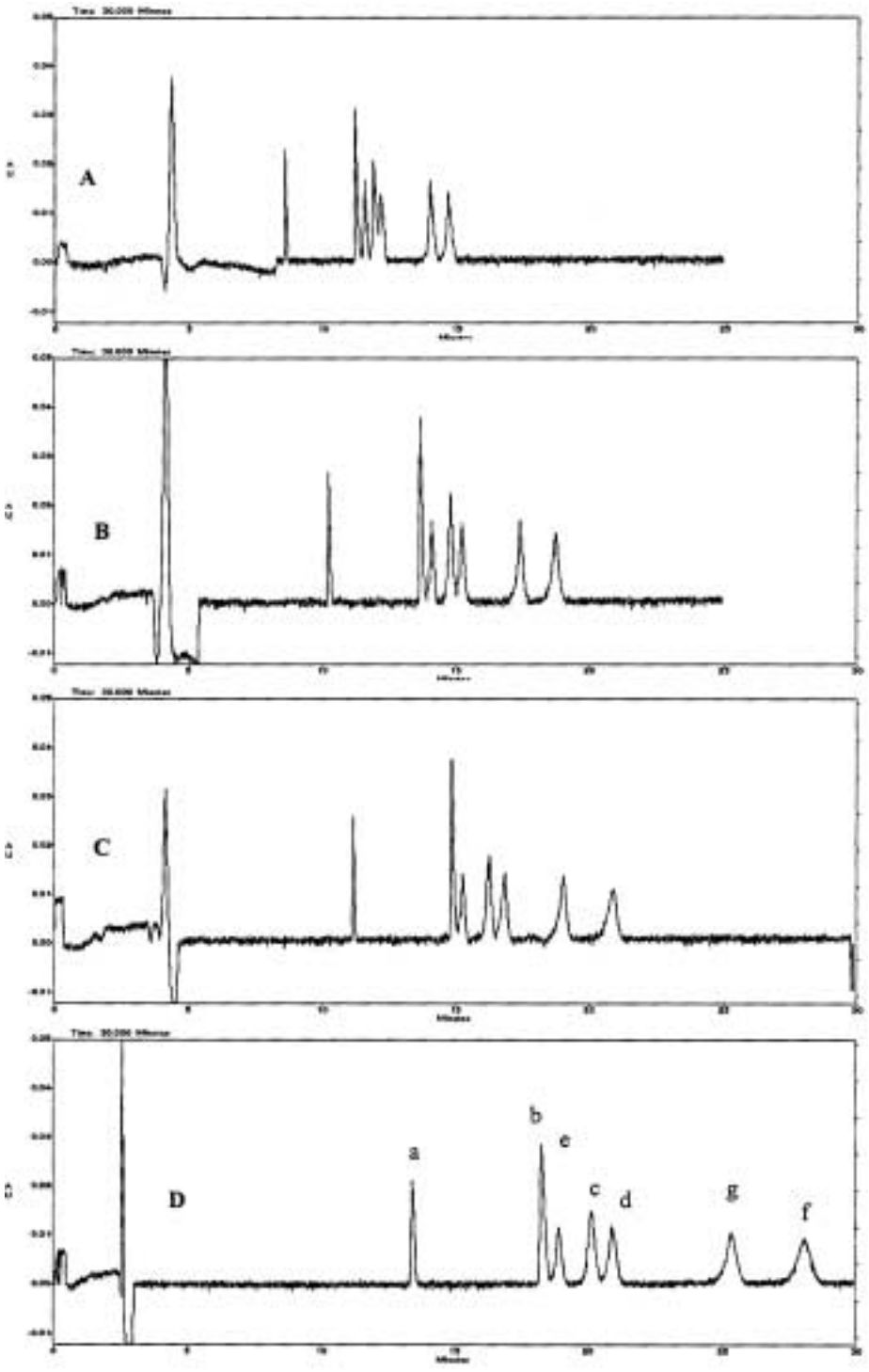

Figure 6. Effect of BGE composition on separation of lidocaine and its metabolites in nonaqueous CE. Background electrolyte: (A) 20 mM ammonium acetate in methanol and TFA (99:1 v/v); current, 15 μA. (B) 40 mM ammonium acetate in methanol and TFA (99:1 v/v); current, 25 μA. (C) 60 mM ammonium acetate in methanol and TFA (99:1 v/v/v); current, 32 μA. (D) 40 mM TEA in methanol and TFA (99:1 v/v); current, 22 μA. Other conditions as in Fig. 5. Peaks, see Fig. 1.

expected to be beneficial for MS detection. TFA/TEA at low pH was demonstrated to be a suitable volatile aqueous electrolyte system for CE separation of basic drugs with good migration time repeatability. On the other hand, non-aqueous CE could be another choice in basic drug analysis. TFA exerts a significant influence on separation selectivity of our test analytes. Apffel *et al.* [29, 30] reported that the observed decrease in MS response for some substances eluted in TFA containing buffers was due to strong ion pairing of positively charged analytes and negatively charged TFA ions. At our laboratories, GC, HPLC and CE have been applied for determi-

nation of lidocaine and metabolites. Comparing the limit of quantification (LOQ) obtained with the different methods, the LOQ achieved with GC [21], nitrogen/phosphorous detector, was in the nM range, also HPLC with an MS detection gave LOQ in the range [19]. For CE with UV-detection [24] and this work, LOQ was in the μM range. However, preliminary work indicates that detection with MS can improve concentration sensitivity by a factor of 100.

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5 References

[1] Griffith, M. J., Linker, N. J., Garratt, C. J., Ward, D. E., Camm, A. J., *Lancet* 1991, *336,* 670–673.

[2] Meyer-Wyss, B., Renner, E., Luo, H., Scholer, A., *J. Hepatol*. 1993, *19,* 133–139.

[3] Strichartz, G., *Anaesthesiology* 1995, *83,* 654–655.

[4] Gröningsson, K., Lindgren, J.-E., Lundberg, E., Sandberg, R., Wahlén, A., in: Florey, K. (Ed.), *Profiles of Drug Substances,* Academic Press Inc., Orlando, FL 1985, *14,* 207–243.

[5] Tam, Y. K., Tawfik, S. R., Coutts, R. T., *J. Chromatogr. B* 1987, *423,* 199–206.

[6] Narang, P. K., Crouthamel, W. G., Carliner, N. H., Fisher, M. L., *Clin. Pharmacol. Ther.* 1978, *24,* 654–662.

[7] Luzzi, F. A., Wenger, T. L., Klinger, J. K., Barchowsky, A., Strauss, H. C., *J. Chromatogr.* 1984, *311,* 291–299.

[8] Kushida, K. Oka, K., Suganuma, T., Ishizaki, T., *Clin. Chem.* 1984, *30,* 637–640.

[9] Björk, M., Pettersson, K-J., Österlöf, G., *J. Chromatogr. B* 1990, *553,* 229–234.

[10] Bernal, J. L., del Nozal, M. J., Rosas, V., Villarino, A., *Chromatographia* 1994, *38,* 617–623.

[11] Lau, O. W., Chan, K., Wong, Y. C., *Methods Find. Clin. Pharmacol.* 1991, *13,* 475–481.

[12] Parker, R. J., Collins, J. M. Strong, J. M., *Drug Metab. Dispos.* 1996, *24,* 1167–1173.

[13] Rossi, S. S., Moore, A. C., Hagey, L. R., Yaksh, T. L., Chaplan, S. R., *Therap. Drug Monitor* 1997, *19,* 179–184.

[14] Escuder-Gilabert, L., Sagrado, S., Villanueva-Camanas, R. M., Medina-Hernandez, M. J., *Chromatographia* 1999, *49,* 85–90.

[15] Dal Bo, L., Mazzucchelli, P., Marzo, A., *J. Chromatogr. A* 1999, *854,* 3–11.

[16] Abdel-Rehim, M. Bielenstein, M., Arvidsson, T., *J. Microcolumn Sep.* 2000, *12,* 308–315.

[17] van Hout, M. W. J., de Zeeuw, R. A., de Jong, G. J., *J. Chromatogr. A* 1999, *858,* 117–122.

[18] Koster, E. H. M., Wermes, C., Morsink, J. B., de Jong, G. J., *J. Chromatogr. B* 2000, *739,* 175–182.

[19] Abdel-Rehim, M. Bielenstein, M., Askemark, Y., Tyrefors, N., Arvidsson, T., *J. Chromatogr. B* 2000, *741,* 175–188.

[20] Koster, E. H. M., de Jong, G. J., *J. Chromatogr. A* 2000, *878,* 27–33.

[21] Abdel–Rehim, M. Bielenstein, M., Arvidsson, T., *J. Microcolumn Sep.* 1998, *10,* 589–596.

[22] Bretnall, A. E., Clarke, G. S., *J. Chromatogr. A* 1996, *745,* 145–154.

[23] Chee, G. L., Wan, T. S. M., *J. Chromatogr. B* 1993, *612,* 172–177.

[24] Anderson, M. S., Wan, H., Abdel-Rehim, M., Blomberg, L. G., *J. Microcolumn Sep.* 1999, *11,* 620–626.

[25] Ding, J., Vouros, P., *Anal. Chem.* 1999, *71,* 378A–384A.

[26] Varghese, J., Cole, R. B., *J. Chromatogr.* 1993, *652,* 369–376.

[27] Lu, W., Poon, G. K., Carmichael, P. L., Cole, R. B., *Anal. Chem.* 1996, *68,* 668–674.

[28] Temesi, D., Law, B., *LC · GC Int.* 1999, *12,* 175–180.

[29] Apffel, A., Fischer, S., Goldberg, G., Goodley, P. C., Kuhlmann, F. E., *J. Chromatogr. A* 1995, *712,* 177–190.

[30] Apffel, A., Chakel, J., Udivar, S., Hancock, W. S., Souders, C., Pungor Jr., E., *J. Chromatogr. A* 1995, *717,* 41–60.

[31] Hjertén, S., *J. Chromatogr.* 1985, *347,* 191–198.

[32] Becklin, R. R., Desiderio, D. M., *Anal. Lett.* 1995, *28,* 2175–2190.

[33] Bretnall, A. E., Clarke, G. S., *J. Chromatogr. A* 1995, *716,* 49–55.

[34] Chen, N., Terabe, S., Nakagawa, T., *Electrophoresis* 1995, *16,* 1457–1462.

[35] Riekkola, M.-L., Wiedmer, S. K., Valkó, I. E., Sirén, H., *J. Chromatogr. A* 1997, *792,* 13–35.

[36] Wätzig, H., Degenhardt, M., Kunkel, A., *Electrophoresis* 1998, *19,* 2695–2752.

[37] Cifuentes, A., Canalejas, P., Diez-Masa, J. C., *J. Chromatogr. A* 1999, *830,* 423–438.

[38] Hjertén, S., Elenbring, K., Kilar, F., Liao, J., Chen, A. J. C., Siebert, C. J., Zhu, M., *J. Chromatogr.* 1987, *403,* 47–61.

[39] Bjørnsdottir, I., Tjørnelund, J., Hansen, S. H., *Electrophoresis* 1998, *19,* 2179–2186.

[40] Tomlinson, A. J., Benson, L. M., Naylor, S., *LC · GC Int.* 1995, *8,* 210–216.

[41] Porras, S. P., Jussila, M., Sinervo, K., Riekkola, M.-L., *Electrophoresis* 1999, *20,* 2510–2518.

[42] Hansen, S.H., Bjørnsdottir, I., Tjørnelund, J., *J. Chromatogr. A* 1997, *792,* 49–55.

[43] Riekkola, M.-L., Jussila, M., Porras, S. P., Valkó, I., *J. Chromatogr. A* 2000, *892,* 155–170.

[44] Tam, Y. K., Ke, J., Coutts, R. T., Wyse, G., Graye, M. R., *Pharm. Res.* 1990, *7,* 504–507.

[45] Porras, S. P., Riekkola, M.-L., Kenndler, E., *J. Chromatogr. A* 2001, *905,* 259–268.

[46] Porras, S. P., Riekkola, M.-L., Kenndler, E., *Chromatographia* 2001, *53,* 290–294.