Characterization of Lidocaine and its Metabolites in Human Plasma Using Capillary Electrophoresis

Magnus S. Anderson,[1] Hong Wan,[1] Mohamed Abdel-Rehim,[2] Lars G. Blomberg[1]
[1]*Department of Chemistry, Karlstad University, SE-651 88 Karlstad, Sweden*
[2]*Astra Pain Control AB, SE-151 85 Södertälje, Sweden*

Received 23 April 1999; revised 19 June 1999; accepted 6 July 1999

Abstract: A method for the separation of lidocaine and six of its metabolites in plasma has been developed. Separation was performed using a buffer consisting of 35 mM phosphate/Tris pH 3.0, 6 mM cetyltrimetylammoniumbromid (CTAB) and 9% (v/v) methanol. The key variables, CTAB and methanol concentrations, were optimized by the application of a factorial design. Rinsing the capillaries with ethanol between runs resulted in highly reproducible migration times, relative standard deviation 0.69%. Under the conditions applied, the seven analytes could be separated with high efficiency and resolution within 8.1 min. Overall performance, as demonstrated in validation experiments, was excellent. © 1999 John Wiley & Sons, Inc. J Micro Sep 11: 620–626, 1999

Key words: *capillary electrophoresis; CTAB; lidocaine and metabolites; basic drugs; validation*

INTRODUCTION

Lidocaine is a basic drug commonly administered as a local anesthetic. It is also used for the investigation of hepatic function. Moreover, lidocaine has antiarrhythmic properties and is frequently used as a therapeutic agent in the treatment of cardiac disorders [1–3]. Lidocaine (LID) is rapidly and extensively metabolized in man, the major metabolites are 4-hydroxy-2,6-xylidine (4-OH-XYL), 2,6-xylidine (XYL), glycinexylidide (GX), monoethylglycinexylidide (MEGX). Low concentrations of 3-hydroxy-lidocaine (3-OH-LID) and 3-hydroxy-monoethylglycinexylidide (3-OH-MEGX) 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 lidocaine and its metabolites are shown in Figure 1.

The determination of lidocaine alone or together with its metabolites has been performed by high-performance liquid chromatography (HPLC) and by GC [7–13]. In previous work, Abdel-Rehim et al. reported GC separations of underivatized lidocaine together with its metabolites using different fused silica capillary columns [14]. Capillary electrophoresis methods for separation of lidocaine and drugs with similar structure have been presented [15,16], but CE analysis of lidocaine together with its metabolites has not yet been reported.

Our approach was to use a low pH CE system for simultaneous separation of lidocaine and its metabolites. This was because of the instability at higher pH which has been reported for one of the metabolites: 4-OH-XYL [17].

One of the main problems with the separation of positively charged analytes is the adsorption of the analytes at the fused silica capillary wall. Such interactions may cause band broadening and zone distortion. As a result, poor repeatability and low separation efficiency are often obtained. Several approaches have been employed to reduce the interactions with the wall. These include the use of basic buffer additives, e.g., hexylamine [18] and chemical modification of the capillary wall by means of a neutral hydrophilic coating, e.g., polyacrylamide [19]. Charge reversal of the capillary surface will also reduce the adsorption of positively charged analytes, which thereby will be electrostatically repelled. Such a reversal can be accomplished dynamically by the

Presented at the 21st International Symposium on Capillary Chromatography and Electrophoresis, Park City, Utah, June 20–24, 1999.

Correspondence to: L. G. Blomberg; e-mail: lars.blomberg@kau.se

© 1999 John Wiley & Sons, Inc.

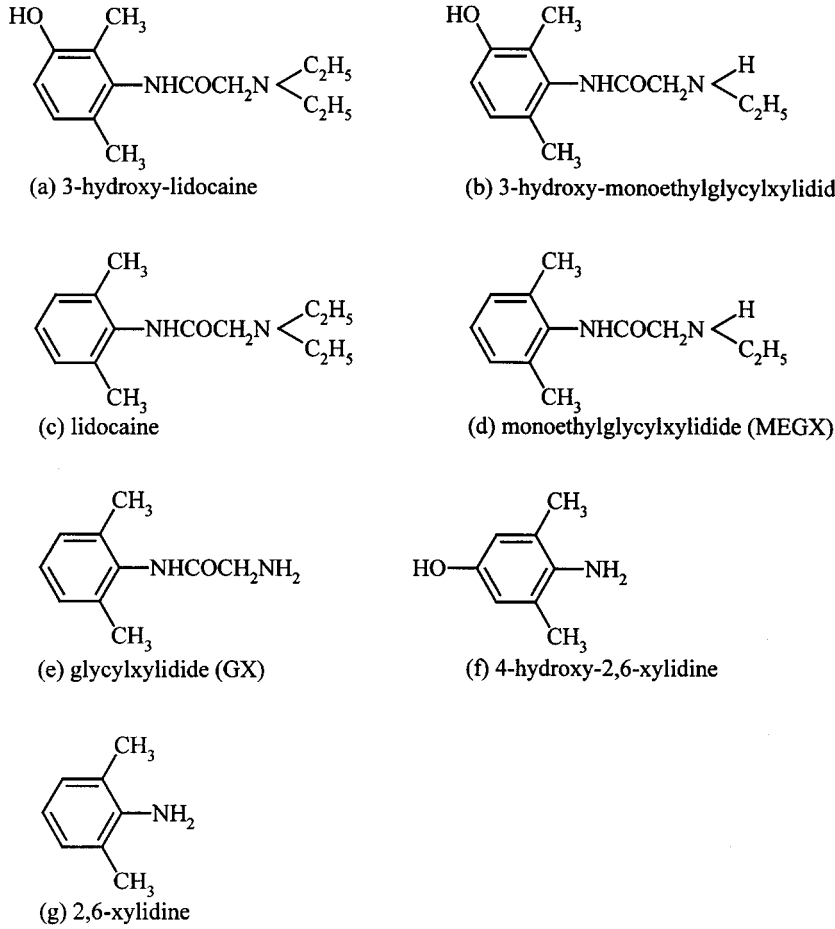

Figure 1. *Structures of lidocaine and its metabolites.*

addition of a cationic surfactant to the running buffer, e.g., CTAB [20].

In this paper we present an optimized CE method for the separation of lidocaine and its metabolites. CTAB was used to dynamically coat the fused silica capillary to prevent analyte wall interactions. The method was optimized by a full factorial design and validated for the analytes in human plasma.

EXPERIMENTAL

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 cartridge. Data were collected by means of Beckman P/ACE station software (version 1.0). Chemometric experimental design for the optimization experiments was done in the computer program Codex (Sum IT System AB, Sollentuna, Sweden).

Untreated fused-silica capillaries (50 μm inner diameter (i.d.), 375 μm outer diameter (o.d.)) obtained from Polymicro Technologies (Phoenix, AZ, USA) were used as separation capillaries. All electrophoresis runs were carried out with reversed polarity at 25° C. Sample introduction was performed using pressure (0.5 psi). On-column detection was made with UV at 200 nm.

Chemicals. Lidocaine and its metabolites were supplied by Astra Pain Control, Södertälje, Sweden. Deionized water (18.2 MΩ) was from a Millipore Milli-Q plus water purification system (Molsheim, France). Phosphoric acid-85%, CTAB, and Tris(hydroxymethyl)aminomethane (Tris) were obtained from Sigma-Aldrich (Gillingham-Dorset, UK). Other chemicals used in this work were of analytical grade.

Electrolyte preparation. The running buffers were prepared by adding appropriate aliquots of phosphoric acid to deionized water. The pH was measured with a pH meter, MeterLab, Radiometer Analytical (Lyon, France) and adjusted as required by addition of aliquots of Tris. 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). A freshly made solu-

tion could be used for at least 3 days. Additives such as organic modifiers were mixed with the buffer directly in the electrophoresis buffer vial.

Sample preparation during method development and optimization. Stock solutions of lidocaine and its metabolites were prepared by dissolving appropriate aliquots of the analytes in a water–ethanol solution (1:1), acidified to pH 3.0 with phosphoric acid. The analyte solutions were magnetically stirred for 10 min and filtered through a 0.45 μm membrane filter before use.

Sample preparation during validation in human plasma. To 1000 μL of human plasma contained in an ultracentrifugation tube, Sartorius centristart 1, appropriate aliquots of the analyte solution (see above) were added. The analyte-plasma solution was acidified with 40 μL of phosphoric acid (1 M) and finally diluted to a total volume of 2000 μL with distilled water. The final concentration of the analytes ranged from 1–1000 μM. After centrifugation (25 min, 2900 rpm) the supernatant was injected into the CE system.

RESULTS AND DISCUSSION

Scouting. A standard solution of lidocaine and its metabolites was prepared for scouting and optimization of the separation conditions. The approach was to use a running buffer sufficiently acidic to protonate the analytes so that they could be separated as cations. The first experiments were performed using a phosphate/Tris buffer at pH 3.0. As might be expected the peaks were tailing, the cationic analytes were obviously interacting with the capillary wall.

To improve peak shape, CTAB was added to the buffer. This positively charged detergent is dynamically adsorbed at the capillary wall. As a result, a reversal of the electroosmotic flow (EOF) takes place. Preliminary experiments showed that reasonably good separations could be obtained using a low concentration of CTAB in the phosphate/Tris buffer. To improve the resolution further, a number of organic modifiers were examined. Addition of organic solvents to the running buffer sometimes extends the migration-time window, improves the resolution, or sometimes modifies the selectivity. The effects of organic modifiers on CE have been extensively studied, e.g., [21–26]. In the present work, the best efficiencies and peak shapes were obtained when using methanol. Finally, experiments were performed to evaluate the key factors that govern the separation and the selection of the low and high limits for the factorial design.

Optimization. For the separation of lidocaine and its metabolites, two key factors, CTAB and methanol concentrations were optimized by a full factorial design. The applied levels were low: CTAB 2 mM and methanol 5% (v/v); high: CTAB 8 mM and methanol 15% (v/v). For the design, 13 experiments were performed, including five runs in the center point. Responses studied were the resolution between analytes 3-OH-MEGX and LID and analytes 4-OH-XYL and XYL. 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 last eluted peak XYL was also studied. The responses were chosen to obtain a rapid baseline separated system. The results obtained from optimization indicated that methanol concentration was the main factor for resolution as well as for the migration time, Figure 2. For the resolution between analytes 3-OH-MEGX and LID a high concentration of methanol resulted in an enhanced resolution. In contrast, a low concentration of methanol had positive effects on the resolution between analytes 4-OH-XYL and XYL. The experiments showed that CTAB had minor ef-

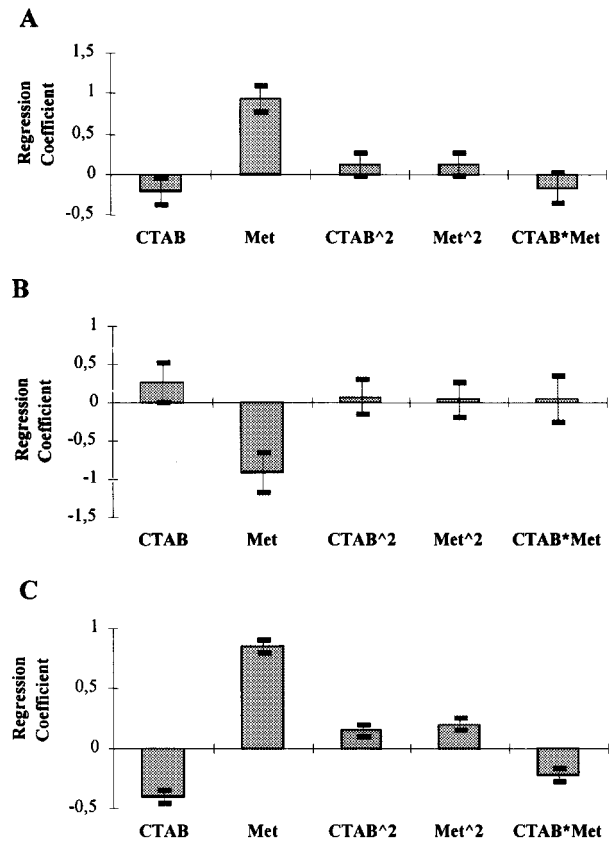

Figure 2. *Effects of CTAB and methanol on (A) resolution between analytes 3-OH-MEGX and LID, (B) resolution between analytes 4-OH-XYL and XYL, (C) migration time for the last eluted analyte.*

fects on the resolution, thus indicating that the major role of CTAB, in the present system, is to cover the capillary inner surface. According to the two antagonist optimum points obtained during the optimization, a final optimum condition, in the center of the design, was chosen.

Optimum separation conditions of analytes 4-OH-XYL and XYL and analytes 3-OH-MEGX and LID are shown in Figure 3(A) and (B), respectively. Figure 3(C) shows an electropherogram obtained using the proposed optimum conditions, i.e., 35 mM phosphate/Tris buffer pH 3.0, CTAB 6 mM and methanol 9% (v/v), potential −25 kV and temperature 25° C.

Purging conditions. A high degree of repeatability is of crucial importance and this may be difficult to achieve when using CTAB as a dynamic coating reagent [27]. In the present system the choice of purging conditions was crucial to achieve high-efficiency and reproducible separations. Two different purging conditions were examined. First, capillary preconditioning and rinsing using NaOH (0.2 M). Second, capillary preconditioning and rinsing using ethanol. Prior to use, new separation capillaries were preconditioned with NaOH or ethanol for 30 min followed by water for 10 min and finally running buffer for 30 min. To ensure reproducible separations the capillary was, before each injection, rinsed with NaOH or ethanol for 3 min, water for 1 min, and running buffer for 3 min.

The separation profiles were similar after preconditioning and rinsing with NaOH and ethanol, but the migration times were approximately two times longer when using a NaOH pretreated capillary, Figure 4(A). Further, using NaOH an obvious trend was observed indicating even longer migration times. The repeatability of migration times was poor, the relative standard deviation, R.S.D., for LID was 6.6%. The efficiency Figure 4(B), was approximately 200,000 theoretical plates/m and after a few consecutive separations, peak shapes, and efficiencies were even worse. A prolonged flush with running buffer

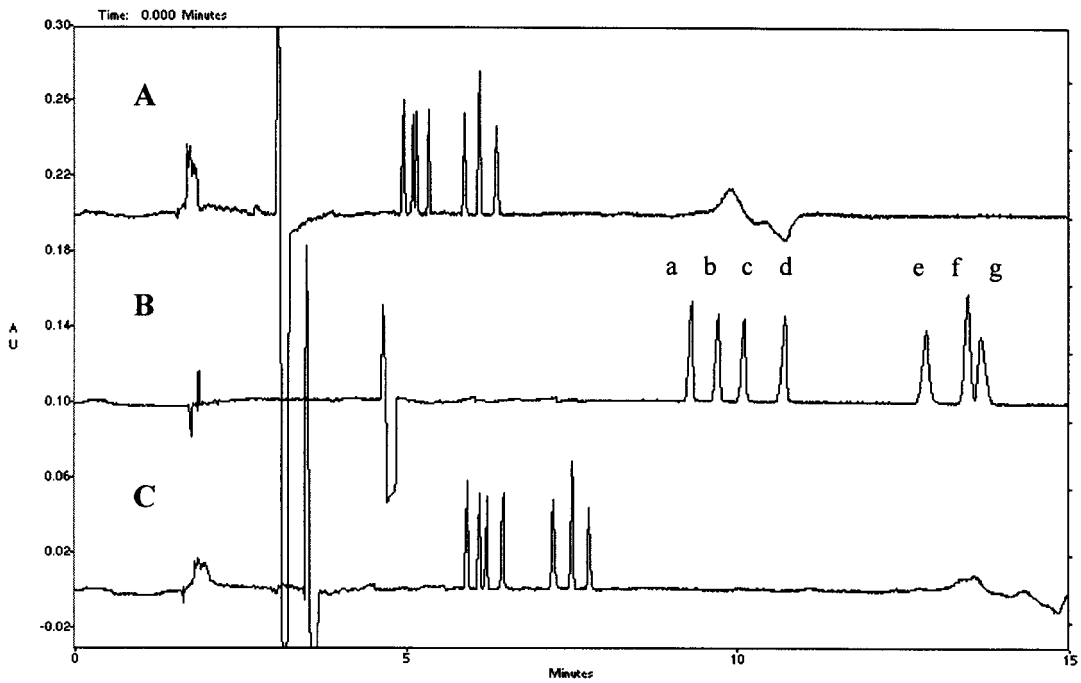

Figure 3. *Optimal separation conditions for lidocaine and its metabolites, UV detection at 200 nm. Separation capillary, fused silica 50 μm i.d., effective length 40.5 cm (total length 47.5 cm); voltage −25 kV; temperature 25° C. (A) Optimum separation condition for the resolution between analytes (3-OH-MEGX) and (LID). Background electrolyte; phosphate / Tris 35 mM pH 3.0, CTAB 8 mM, methanol 5% (v / v). (B) Optimum separation condition for the resolution between analytes (4-OH-XYL) and (XYL). Background electrolyte; phosphate / Tris 35 mM pH 3.0, CTAB 2 mM, methanol 15% (v / v). (C) Applied optimum condition for the separation of lidocaine and its metabolites. Background electrolyte; phosphate / Tris 35 mM pH 3.0, CTAB 6 mM, methanol 9% (v / v). Peaks: a = 3-OH-LID; b = 3-OH-MEGX; c = LID; d = MEGX; e = GX; f = 4-OH-XYL; g = XYL. Sample concentration: 100–150 μM.*

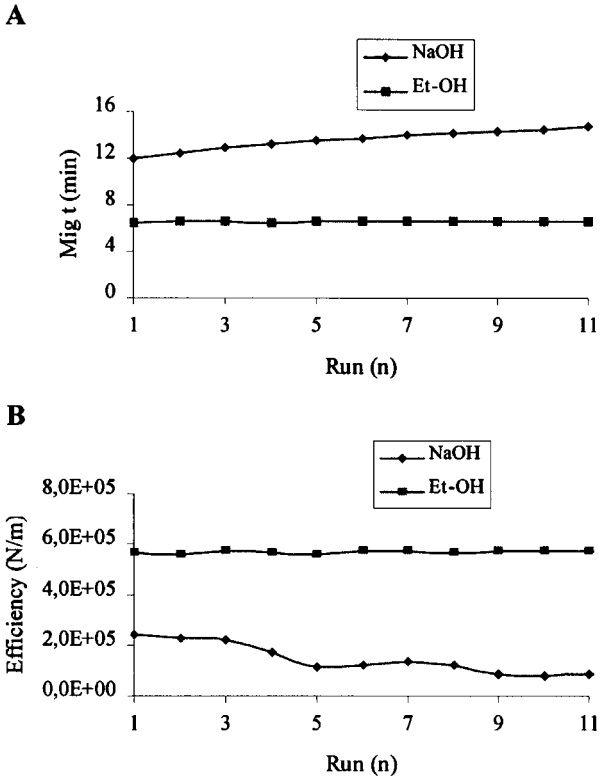

Figure 4. *Capillary purging: effects of sodium hydroxide and ethanol on (A) migration time and (B) separation efficiency.*

(8 min) was performed but no improvement of efficiency and repeatability of migration time was observed after such a treatment.

The use of ethanol rinsing gave both high-efficiency and reproducible separations, Figure 4(B) and 4(A). The efficiency was consistently at 580,000 theoretical plates/m, the repeatability, R.S.D., of migration time was excellent 0.69%. A drawback of ethanol rinsing is that performance may vary for different batches of fused silica capillary tubing. Analysis was thus performed on capillaries obtained from three different batches, similar selectivity, efficiency, and repeatability were obtained. Further, it seems that type of rinsing required depends on the type of analytes. For example, for protein separations, rinsing with 0.1 M NaOH is needed in order to eliminate protein buildup on the capillary surface [28].

Selectivity–specificity. A blank plasma injection and a spiked plasma sample were analyzed according to the proposed method. The blank electropherogram, Figure 5, shows that no interfering peaks are eluted in the time window of interest. Further, the spiked plasma sample demonstrates baseline separation of all seven compounds within 8.1 min. Efficiencies and resolutions are given in Table I. The procedure was repeated using plasma from two more individuals and the results were not significantly different from those shown in Figure 5.

Limit of detection (LOD). A signal-to-noise ratio of approximately 3 is generally considered to be acceptable for estimating the detection limit. The detection limits obtained using normal injection (5 s, 0.5 psi) were 5.0 μM for 3-OH-LID, 3-OH MEGX, LID, MEGX and GX; 7.5 μM for 4-OH-XYL and 13 μM for XYL. To improve the sensitivity, the injected volume was increased (40 s, 0.5 psi), a five time enhancement of the sensitivity was achieved. The LOD values using normal and long time injection are presented in Table I. The relatively poor sensitivity, might be explained by the detection mode. In the development of the separation conditions, the detection was by UV, which, for many practical applications, has too poor LOD.

Linearity. Calibration curves were prepared in order to examine the linearity of the response. Drug-free plasma was thus spiked with standards in a concentration range between 5–500 μM. Six concentration levels were used (excluding blank) and two injections were made at each level. The calibration curves for each metabolite were constructed by plotting the peak-area versus the concentration of the metabolite. The procedure, including sample preparation, was performed four times using plasma from one individual. Two curves were made within 1 day (day 1). Further, one curve was made day 3 and another curve day 5. The linear regression correlation coefficients, r^2, were above 0.99 for all analytes. According to the equations and correlation coefficient r^2 no significant variations were observed between the different analysis occasions.

The linearity range was estimated to approximately 3 orders of magnitude when using 5 s injection time. However, when using 40 s injection time the linearity range was 2 orders of magnitude.

To verify and show the quality fit of the calibration curve, three more concentration levels were analyzed. They were chosen between the concentration points in the calibration curve: one near the LOD (20 μM), one in the center (150 μM), and one in the upper part of the standard curve (400 μM). The solutions were replicated two times and the amounts determined were compared to the theoretical amounts. The variations were within $\pm$15% deviation of the nominal value in the center and the upper part of the calibration curve, at 20 μM variations within $\pm$25% were obtained. For the unstable 4-OH-XYL larger variations were obtained, indicat-

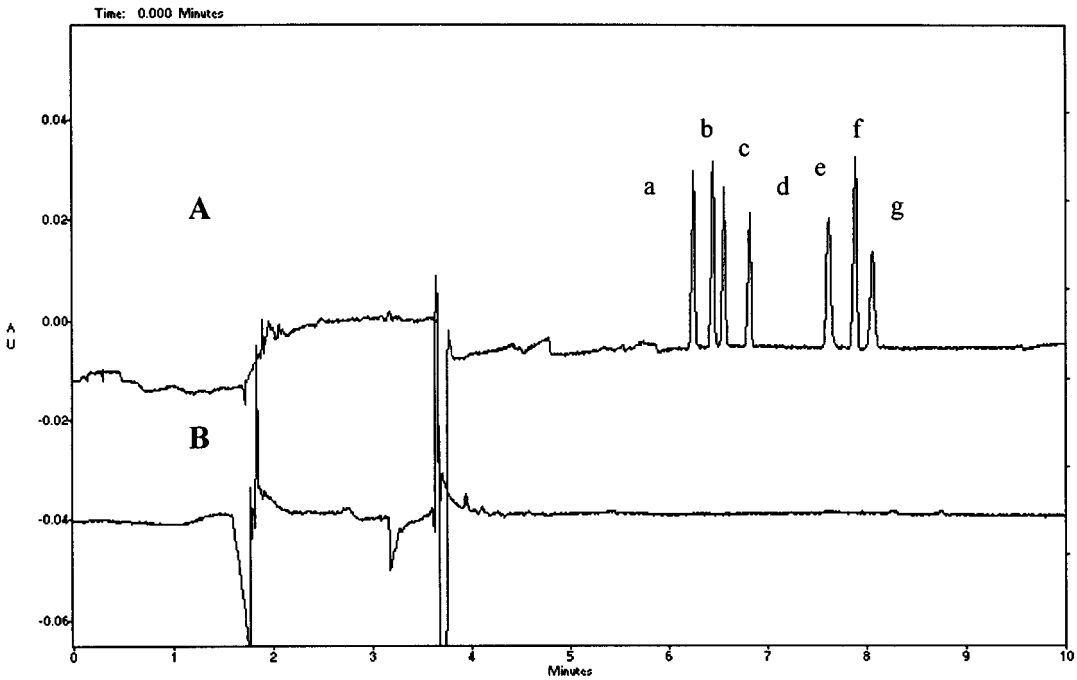

Figure 5. *Electropherogram of lidocaine and its metabolites, UV detection at 200 nm. Separation capillary, fused silica 50 μm i.d., effective length 40.5 cm (total length 47.5 cm); voltage −25 kV; temperature 25° C. Background electrolyte; phosphate / Tris 35 mM pH 3.0, CTAB 6 mM, methanol 9% (v / v). (A) A standard solution of lidocaine and its degradation products in plasma (100-150 μM), peaks as in Figure 3. (B) A blank injection of plasma.*

ing that a determination of this compound is somewhat uncertain.

Repeatability. Precision over a short time while keeping the operating conditions constant was checked by performing eight consecutive separations of a spiked plasma solution at a concentration of 150 μM. The relative standard deviation R.S.D for migration time of all analytes was in the range 0.67–0.89%. For all analytes the variations of the peak-area were in the range 1.48–3.21%, except for 4-OH-XYL 16.6%.

Within-day precision–between-day precision. Five sample preparations in plasma from one individual were analyzed on three different days to explore the variation between days and within-days. Three concentration levels were used: one near the LOD value 20 μM, one in the center 150 μM, and one near the upper boundary of the calibration curve 400 μM. The R.S.D. for the migration time was in the range 1.21–1.84% on the within-day basis and 3.14–4.24% on the between-day basis. The variations in area are presented in Table II.

Table I. *Separation data and detection limits for lidocaine and its metabolites in spiked human plasma.*

Elution order	Migration time (min)	Efficiency (N/m)	Resolution (Rs)	LODs[a] (μM)	LODs[b] (μM)
3-OH-LID	6.27	580,000		1.0	5.0
3-OH-MEGX	6.47	510,000	3.66	1.0	5.0
LID	6.58	570,000	2.00	1.0	5.0
MEGX	6.84	640,000	4.73	1.0	5.0
GX	7.63	470,000	12.8	1.0	5.0
4-OH-XYL	7.90	620,000	4.05	1.5	7.5
XYL	8.07	400,000	2.31	3.0	13

[a]Limit of detection (LODs) defined as signal-to-noise ratio of approximately 3, injection was performed using 40 s injection time and a pressure option of 0.5 psi.
[b]Limit of detection (LODs) defined as signal-to-noise ratio of approximately 3, injection was performed using 5 s injection time and a pressure option of 0.5 psi.

Table II. *Precision of peak area for determination of standard substances in plasma samples.*[a]

Compound	Within-day (R.S.D.%)			Between-day (R.S.D.%)		
	20 μM	150 μM	400 μM	20 μM	150 μM	400 μM
3-OH-LID	9.3	1.5	2.5	5.7	4.3	1.2
3-OH-MEGX	8.4	1.9	2.2	2.3	3.7	4.6
LID	7.9	1.7	1.6	3.9	6.8	6.7
MEGX	7.6	1.5	2.0	9.4	4.2	2.6
GX	6.4	2.2	2.6	6.3	4.7	4.0
4-OH-XYL	21.1	16.6	7.5	23.2	19.2	2.7
XYL	16.7	3.2	1.7	25.3	0.50	4.8

[a] Precision calculated for three concentration levels: 20, 150, and 400 μM, ($n = 3$). Background electrolyte: phosphate/Tris 35 mM, pH 3.0, CTAB 6 mM, methanol 9% (v/v). Separation capillary: fused silica 50 μm i.d., effective length 40.5 cm (total length 47.5 cm); voltage -25 kV, temperature 25°C.

Carry-over. Carry-over was examined by injection of a spiked plasma solution at a concentration of 500 μM. After separation and capillary rinsing; a blank injection of plasma was made. No analytes and sample matrix residues were detected after the rinsing step.

A method for the characterization of lidocaine and six of its metabolites in human plasma has been developed and optimized. A low concentration of CTAB in the running buffer resulted in improved peak shape and efficiency. The method developed is rapid, precise, and simple to perform. However, detection limits are too high for real analyses. The poor sensitivity may be overcome by the use of mass spectrometric detection. In a future publication, we will report the CE separation of lidocaine and its metabolites using coated columns and mass spectrometric detection.

ACKNOWLEDGMENT

The work was financially supported by the Swedish Natural Science Research Council, K-AA/KU 04404-315.

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. Anesthesiology 1995, 83, 654–655.
4. Gröningsson, K.; Lindgren, J.-E.; Lundberg, E.; Sandberg, R.; Wahlén, A. In Analytical Profiles of Drug Substances, Florey, K. Ed.; Academic Press: Orlando, FL 1985; Vol. 14, pp. 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. Meth 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. Abdel-Rehim, M.; Bielenstein, M.; Arvidsson, T. J Microcolumn Sep 1998, 10, 589–596.
15. Bretnall, A.E.; Clarke, G.S. J Chromatogr A 1996, 745, 145–154.
16. Chee, G.L.; Wan, T.S.M. J Chromatogr B 1993, 612, 172–177.
17. Tam, Y.K.; Ke, J.; Coutts, R.T.; Wyse, G.; Graye, M.R. Pharmacol Res 1990, 7, 504–507.
18. Lurie, I.S.; Conver, T.S.; Ford, V.L. Anal Chem 1998, 70, 4563–4569.
19. Hjertén, S. J Chromatogr 1985, 347, 191–198.
20. Kaneta, T.; Tanaka, S.; Taga, M. J Chromatogr A 1993, 653, 313–319.
21. Bretnall, A.E.; Clarke, G.S. J Chromatogr A 1995, 716, 49–55.
22. Chen, N.; Terabe, S.; Nakagawa, T. Electrophoresis 1995, 16, 1457–1462.
23. Riekkola, M.-J.; Wiedmer, S. K.; Valkó, I.E.; Sirén, H. J Chromatogr A 1997, 792, 13–35.
24. Vercammen, J.; Verleysen, K.; Sandra, P. J High Resolut Chromatogr 1998, 21, 605–607.
25. Liu, Z.; Zou, H.; Zhang, Y. J High Resolut Chromatogr 1998, 21, 234–240.
26. Schwer, C.; Kenndler, E. Anal Chem 1991, 63, 1801–1807.
27. Wätzig, H.; Degenhardt, M.; Kunkel, A. Electrophoresis 1998, 19, 2695–2752.
28. Fritz, J.S.; Ding, W. J High Resolut Chromatogr 1997, 20, 575–580.