Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1081 (2005) 2–8
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Analysis of ethyl glucuronide in human serum by capillary electrophoresis with sample self-stacking and indirect detection

L. Křivánková[b], J. Caslavska[a], H. Malášková[b], P. Gebauer[b], W. Thormann[a,*]

[a] *Department of Clinical Pharmacology, University of Bern, Murtenstrasse 35, CH-3010 Bern, Switzerland*
[b] *Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, CZ-61142 Brno, Czech Republic*

Available online 18 December 2004

Abstract

Ethyl glucuronide (EtG), a metabolite of ethanol, is a marker of recent alcohol consumption. In the past few years, its analysis in body fluids has attracted considerable attention because it closes a gap between short time and long time alcohol markers such as ethanol and carbohydrate-deficient transferrin, respectively. The capillary zone electrophoresis (CZE) analysis of EtG in model mixtures and human serum is reported using uncoated and coated fused-silica capillaries together with acidic buffers in the pH range between 3.2 and 4.4 and indirect detection. In these approaches, separation of EtG from endogenous macro- and microcomponents (anionic serum components of high and low concentration, respectively) is based upon transient isotachophoretic stacking referred to as sample self-stacking. The selection of a favorable buffer co-ion and pH is shown to be crucial for optimized sensitivity. A buffer composed of 10 mM nicotinic acid and ε-aminocaproic acid (pH 4.3) is demonstrated to provide a detection limit for EtG in serum of 0.1 μg/ml, a value that is relevant for clinical and forensic purposes.

Keywords: Ethyl glucuronide; Capillary electrophoresis; Serum; Alcohol marker

1. Introduction

Ethyl-β-D-glucuronide (ethyl glucuronide (EtG) for structure refer to Fig. 1) is a non-volatile, water soluble, stable upon storage, direct metabolite of ethanol that can be detected in body fluids and hair. EtG in serum peaks 2–3.5 h after ethanol has reached its maximum and can be detected in body fluids for an extended time period after complete elimination of ethanol (up to at least 8 h in serum) such that it can be employed as marker substance for recent alcohol consumption. It covers a clinically and forensically important time window between short term markers, such as ethanol itself, and long term markers, such as carbohydrate-deficient transferrin [1–5].

The analysis of EtG in body fluids and hair has attracted considerable attention in the past few years. Methods developed are based upon GC–MS after derivatization and LC–MS or LC–MS/MS with negative electrospray ionization, approaches that require sample pretreatment such as protein precipitation or solid phase extraction [5–7]. No capillary zone electrophoresis (CZE) assay for the determination of EtG has been reported thus far. We investigated the analysis of EtG in human serum via injection of neat or diluted serum and its visualization by indirect, on-column UV absorbance detection. The chosen approach is based on transient isotachophoretic solute stacking within a hydrodynamically injected, short sample pulse, i.e. sample self-stacking that was investigated in our laboratories in great detail using theoretical approaches and computer simulation [8–11]. Sample self-stacking of trace analytes in presence of macrocomponents of like charge (components of 100- to 1000-fold higher concentration than the trace analytes) has successfully been employed for analysis of acetoacetate, malate and citrate in human serum [11] and of nitrate in seawater [12].

The concentration of EtG in serum that should be properly recognized is between 0.1 and 5 μg/ml (0.45–22.5 μM) [2] and this weakly acidic compound has to be analyzed in

* Corresponding author. Tel.: +41 316323288; fax: +41 316324997.
E-mail address: wolfgang.thormann@ikp.unibe.ch (W. Thormann).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.11.099

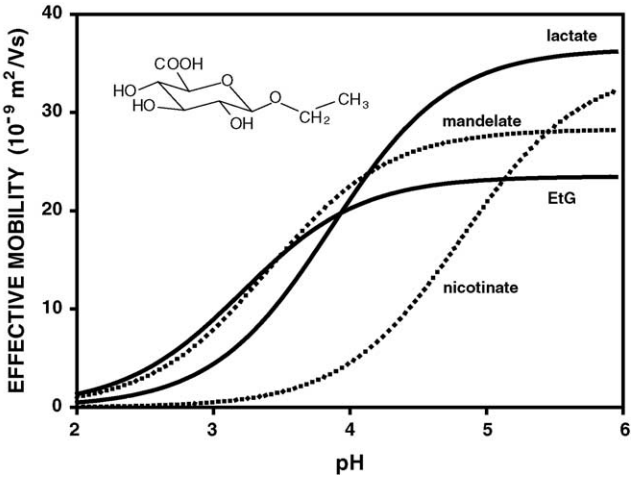

Fig. 1. Effective electrophoretic mobility of EtG, lactate, mandelate and nicotinate as function of pH. The insert depicts the chemical structure of EtG.

presence of several macrocomponents in mM concentrations, including chloride, phosphate, lactate and acetate. The work presented here includes: (i) the CZE characterization of EtG leading to the estimation of ionic mobility and p*K* value of this compound; (ii) a strategy to search for a buffer co-ion that is suitable for optimized sample self-stacking and indirect detection of EtG in presence of the endogenous macrocomponents; and (iii) the CZE determination of EtG in serum using sample self-stacking with buffers whose pH ranged between 3.2 and 4.4.

2. Experimental

2.1. Chemicals and samples

All chemicals used were of the highest analytical purity. EtG was purchased from Medichem (Steinenbronn, Germany). Mesityl oxide (MO), L-histidine, potassium persulfate, [3-(methacryloyloxy)propyl]trimethoxysilane, tetramethylenediamine, acrylamide, acetic, L-malic, L-mandelic, benzoic, maleic, acetoacetic, ε-aminocaproic (EACA), L-lactic, β-hydroxybutyric, D-glucuronic, glyceric, glycolic acids were from Sigma (St. Louis, USA). Hydroxypropylcellulose (HPC) was from Ega (Steinheim/Albuch, Germany) and hydroxypropylmethyl cellulose was from Sigma. Salicylic, hydrochloric, picric, phenylacetic, nicotinic, glutamic acids, sodium chloride, sodium hydroxide, and sodium hydrogenphosphate were from Lachema Chemapol (Brno, Czech Republic). β-Alanine was from Loba Feinchemie (Fischamend, Germany), aspartic acid was from Reanal (Budapest, Hungary), citric, DL-mandelic and cinnamic acids were from Fluka (Buchs, Switzerland). Deionized water was employed for the preparation of all solutions.

Sera used in this work encompass a standard lyophilized serum (Sigma, St. Louis, USA) that was reconstituted in deionized water, our own sera and selected patient sera. Prior to analysis, all sera were diluted with deionized water in the ratio 1:1 and, throughout this manuscript, the concentrations given refer to the diluted sample. A mixture of 50 mM chloride, 1 mM phosphate, 0.05–0.32 mM citrate, 0.034 mM malate, 0.038 mM acetoacetate and 1 mM lactate was used as a protein-free model sample.

2.2. Instrumentation and procedures

CZE measurements were performed using the instruments P/ACE MDQ, P/ACE 5000 and P/ACE 5510 (Beckman, Fullerton, CA, USA) with the UV detectors set to indirect detection at 214 nm and having the cartridge temperature set to 25 °C. Fused-silica capillaries were purchased from Composite Metal Services (The Chase, Hallow, Worchester, UK) and had a total length of 60.2 cm (50 cm to the detector) in the P/ACE MDQ system, and of 47, 57 or 67 cm (40, 50 and 60 cm, respectively, to the detector) in the P/ACE 5000 and 5510 instruments. The inner diameters were 0.075 mm (all instruments) and 0.1 mm (P/ACE 5000). Bare fused-silica capillaries were treated prior to their first use and otherwise when necessary by washing at 20 psi (1 psi = 6894.76 Pa) with 1 M HCl for 20 min, washed with deionized water for 10 min, treated with 1 M NaOH for 20 min, washed again with water for 10 min and finally conditioned with the background electrolyte (BGE) for 10 min. Between runs, the capillary was washed with 1 M HCl for 5 min, washed with deionized water for 3 min, treated with 1 M NaOH for 5 min, washed again with water for 3 min, treated with air for 5 min and finally conditioned with the BGE for 5 min. Capillaries coated with polyacrylamide were prepared by the modified Hjertén method [13] as was described in [14]. Prior to measurements and between runs, polyacrylamide coated capillaries were washed at 20 psi with deionized water for 5 min and conditioned with BGE for 5 min. The voltage applied varied from −10 to −30 kV. Sampling was performed at a pressure of 0.5 psi (3.45 kPa), the time of sampling is shown in the conditions of individual experiments. Electroosmotic flow (EOF) was measured with MO. Its value both in bare capillaries (pH of BGE used was 3.2 and 3.5) and in coated capillaries (pH of BGE was 4.0–6.0) was lower than 1.3×10^{-9} m^2 V^{-1} s^{-1}.

3. Results

3.1. Determination of pK and ionic mobility of ethyl glucuronide

EtG is a glycoside of D-glucuronic acid with ethanol. For glucuronic acid, the ionic mobility (-26.7×10^{-9} m^2 V^{-1} s^{-1}) and the p*K* value (3.68) can be found in the table of isotachophoretic indices [15], see also Table 1. The corresponding parameters for EtG were estimated from ionic and effective mobilities that were determined by CZE using buffers of ionic strength 6 mM at pH 6

Table 1
Physicochemical parameters used for calculations [15]

Compound	Mobility (10^{-9} m^2 V^{-1} s^{-1})	pK
Chloride	−79.1	−2.00
Cinnamate	−28.3	4.44
Lactate	−36.5	3.86
Phosphate	−35.1; −61.5; −71.5	2.12; 7.47; 12.36
Phenylacetate	−31.7	4.41
Nicotinate	−34.6	4.82
Glucuronate	−26.7	3.68
Benzoate	−33.6	4.20
Mandelate	−28.3	3.41
Citrate	−28.7; −54.7; −74.4	3.13; 4.76; 6.40
Picrate	−31.5	0.71
Acetate	−42.4	4.76
Aspartate	−31.6; −51.8	3.9; 10.0
3-Hydroxybutyrate	−34.3	4.52
ε-Aminocaproate	28.8	4.37
β-Alanine	36.7	3.55

(BGE: 6 mM salicylic acid + His) and pH 3.5 (BGE: 10 mM mandelic acid + β-Ala), a coated capillary of 50 cm effective length and 0.075 mm i.d., and having an applied voltage of −15 kV. As reference analytes, chloride, acetate, lactate and picrate were used with ionic mobilities of −79.1, −42.4, −36.5, and -31.5×10^{-9} m^2 V^{-1} s^{-1}, respectively ([15], see also Table 1). Calculations of ionic and effective mobilities were performed using three equations: (i) with one reference analyte using the relation for the ratio of mobilities, $u_X/u_A = t_A/t_X$; (ii) with one reference analyte using the relation involving the difference of mobilities [16]; and (iii) with two reference analytes using the relation published by Vespalec et al. [17]. The obtained average value for the ionic mobility of EtG was thereby determined to be -23.5×10^{-9} m^2 V^{-1} s^{-1}, the average effective mobility at pH 3.5 was -16.7×10^{-9} m^2 V^{-1} s^{-1}. With these data and the relationship $K = u_{eff}\,[H^+]/(u - u_{eff})$ that was derived from the relation between effective mobility of a weak acid and pH [18] and where u and u_{eff} are the ionic and effective mobilities, respectively, the pK value of EtG was calculated to be 3.21.

3.2. Separation of EtG from macro- and microcomponents in serum

For the separation of EtG from anionic macro- and microcomponents of serum, weakly acidic separation media were selected such that a number of substances are excluded from migration due to insufficient dissociation [11]. The remaining endogenous components contain macrocomponents, i.e. components with concentrations higher than 1 mM (chloride, phosphate, lactate and acetate, see Table 2), and microcomponents, such as malate, acetoacetate, citrate, oxalate and oxaloacetate whose concentrations are expected to be in the order of 10^{-6} M. EtG belongs to the second group. Depending on the chosen conditions, macrocomponents can act as stacker or destacker [10,11]. Chloride and phosphate dissociated to the first order are strong anions. Lactate with a pK of 3.86 [15] is the most interesting anion in the remaining group of macrocomponents as it changes strongly its effective mobility in the important pH range between 3 and 5 (Fig. 1). The data depicted in Fig. 1 represent effective mobilities of EtG, lactate, mandelate and nicotinate in the pH range 2–6 which can be covered with BGEs having mandelate (below pH 4) or nicotinate (above pH 4) as co-ions. In the pH range of 3.5–4.0, EtG migrates between mandelate as leader and lactate when present at sufficiently high concentration as terminator. At pH above 4, lactate becomes much faster than EtG and can act as transient leader. Mandelate is faster than EtG and does not fulfill the conditions of a terminator. Thus, another UV absorbing BGE co-ion which is slower than EtG has to be employed. Nicotinate, cinnamate, phenylacetate and benzoate fulfill the condition (Table 1) and can play the role of a terminator for EtG. Depending on the parameters of the analyte of interest, chloride as the main macrocomponent in the sample can act either as stacker or also against stacking, see [11].

Table 2
Concentration of selected macrocomponents in serum [23–25]

Compound	Concentration (mM)
Chloride	100
Lactate	0.5–10
Phosphate	0.5–2
Acetate	1

3.3. Determination of EtG in buffers with mandelate as co-ion

At pH 3.8 with a BGE composed of 5 mM mandelic acid and EACA, the system studied in detail elsewhere for analysis of acetoacetate and other minor anions in serum [11], EtG was found to co-migrate with lactate, a fact that is in agreement with the mobility data presented in Fig. 1. Reduction of pH was found to permit an interference-free detection of EtG in fortified blank serum and selected patient samples (Fig. 2). The patient sample whose data are shown in Fig. 2B was determined to contain a high amount of EtG (>5 μg/ml). The LOD of about 2 μg/ml (9 μM) obtained with this configuration is insufficient for clinical and forensic purposes. Thus, efforts were undertaken to analyze and optimize sample self-stacking in the mandelic acid system. For that purpose, EtG was analyzed in a number of mandelate buffers with β-Ala as counter component (Table 3). EACA with a pK of 4.37 (Table 1) was replaced by β-Ala because the pK of β-Ala (3.55, see Table 1) better matches the pH range encountered.

In the BGE composed of 5–10 mM mandelic acid and β-Ala, pH 3.2 (electrolyte systems nos. 1–3 of Table 3), EtG was separated from serum microcomponents and migrated between malate and acetoacetate (Fig. 3). The LOD for EtG in serum was determined to be 1×10^{-5} M (Table 4). Due to a fluctuating baseline and protein clotting, the pH was increased up to 3.5 which resulted in slower migration of EtG and EtG became well separated from other microcom-

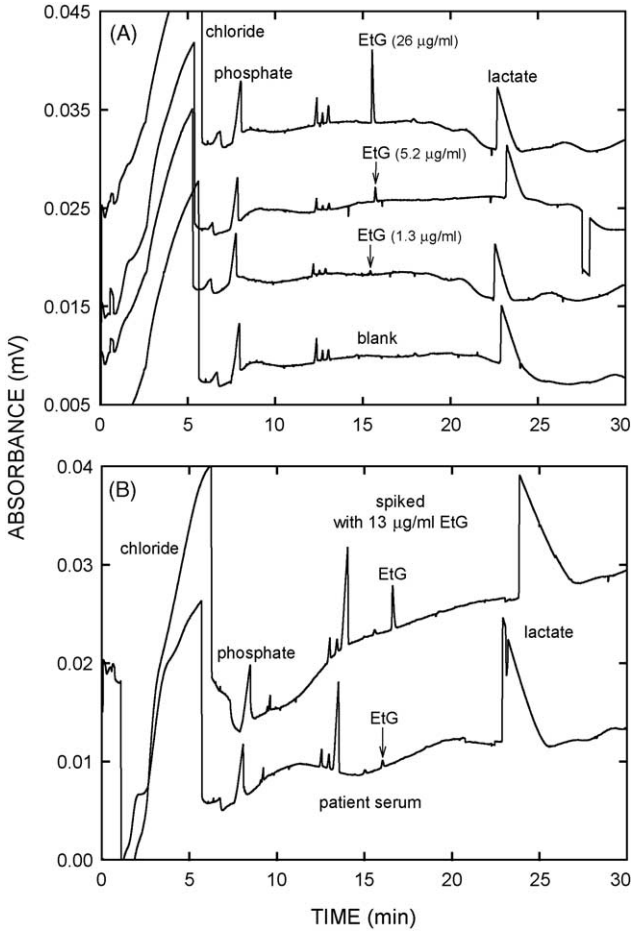

Fig. 2. Electropherograms obtained with 1:1 diluted serum of: (A) blank and fortified blank serum; and (B) a patient serum without and with addition of EtG analyzed in a BGE composed of 5 mM DL-mandelic acid and 1.96 mM EACA (pH about 3.2) containing 0.05% HPMC. Instrumental conditions: P/ACE 5510 with an uncoated 75 μm i.d. capillary of 67 cm total length, −30 kV (about 7 μA) and 5 s injection.

ponents (Fig. 3). In this system, EtG is stacked by lactate and destacked by chloride and phosphate. The concentration of chloride in serum is high but constant, the concentration of phosphate is relatively low (Table 2). The concentration of lactate varies in a broad range (0.5–10 mM, Table 2) and has the highest impact on EtG stacking. The negative (destacking) effect of chloride on EtG peak height was assessed with a protein-free model sample without lactate and using a 10 mM mandelic acid BGE at pH 3.5 and a coated, 0.1 mm i.d. capillary. One hundred percent peak height was adjudged to EtG stacked by intrinsic stacking when EtG was dissolved in water. At 50 and 100 mM chloride concentration in the sample, the relative peak height (RPH, the ratio of peak height to peak area) of EtG (2.7×10^{-5} M in the sample) was found to be 88.7 and 75.8%, respectively. The impact of lactate on the RPH of EtG (2.7×10^{-5} M) was investigated in the same experimental configuration using a sample containing 50 mM chloride, 1 mM phosphate, 0.05 mM citrate, 0.034 mM malate, 0.038 mM acetoacetate, i.e. in a sample

Table 3
Composition of used BGEs

No.	BGE	pH[a]
1	5 mM L-mandelic acid + β-Ala	3.2
2	5 mM L-mandelic acid + β-Ala + 0.05% HPC	3.2
3	10 mM L-mandelic acid + β-Ala	3.2
4	5 mM L-mandelic acid + β-Ala	3.3
5	5 mM L-mandelic acid + β-Ala	3.4
6	5 mM L-mandelic acid + β-Ala	3.5
7	5 mM L-mandelic acid + β-Ala + 0.02% HPC	3.5
8	10 mM L-mandelic acid + β-Ala	3.5
9	10 mM L-mandelic acid + β-Ala + 0.02% HPC	3.5
10	6 mM salicylic acid + histidine	6.0
11	7 mM cinnamic acid + EACA	4.1
12	7 mM cinnamic acid + EACA	4.2
13	7 mM cinnamic acid + EACA	4.4
14	5 mM cinnamic acid + EACA	4.3
15	10 mM phenylacetic acid + EACA	4.4
16	10 mM benzoic acid + EACA	4.4
17	10 mM nicotinic acid + EACA	4.0
18	10 mM nicotinic acid + EACA	4.2
19	10 mM nicotinic acid + EACA	4.3
20	10 mM nicotinic acid + EACA	4.4

[a] Counter components (β-Ala, histidine, EACA) were added until the desired pH was reached.

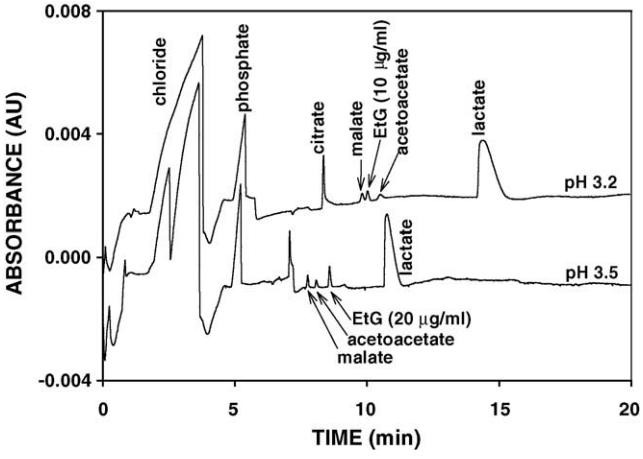

Fig. 3. Electropherograms obtained with a model mixture of anions (50 mM chloride, 1 mM phosphate, 1 mM lactate, 0.25 mM acetate, 0.16 mM citrate, 0.034 mM malate, 0.038 mM acetoacetate and 20 or 10 μg/ml EtG) analyzed in BGEs composed of 5 mM L-mandelic acid and β-Ala at pH values of 3.5 (lower graph) and 3.2 (upper graph). Instrumental conditions: P/ACE MDQ with an uncoated 75 μm i.d. capillary of 60.2 cm total length, −30 kV (about 6 μA) and 5 s injection.

Table 4
LOD of EtG determination in 1:1 diluted serum in selected BGEs[a]

BGE no.	pH_{BGE}	LOD (μg/ml)	LOD (M)
2	3.2	2.222	1.0×10^{-5}
9	3.5	2.200	9.90×10^{-6}
16	4.4	0.087	3.9×10^{-7}
15	4.4	0.084	3.78×10^{-7}
19	4.3	0.079	3.56×10^{-7}
12	4.2	0.047	2.12×10^{-7}

[a] Data obtained on P/ACE 5000 with a coated 0.1 mm i.d. capillary of 57 cm total length, detection at 214 nm, voltage −15 kV, injection 5 s/0.5 psi and having fortified 1:1 diluted serum.

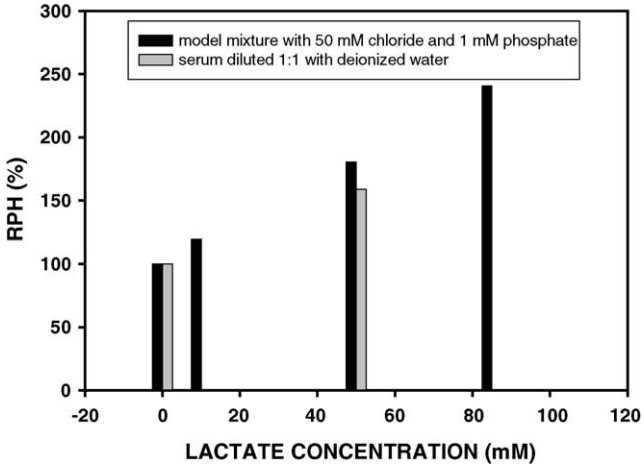

Fig. 4. RPH values as function of lactate concentration obtained in a BGE composed of 10 mM L-mandelic acid and β-Ala (pH 3.5). A model mixture comprising 6 μg/ml (27 μM) EtG, 50 mM chloride, 1 mM phosphate, 0.05 mM citrate, 0.034 mM malate, 0.038 mM acetoacetate and lactate and a 1:1 diluted serum sample fortified with EtG (6 μg/ml) and lactate were analyzed. Instrumental conditions: P/ACE 5000 with a coated 0.1 mm i.d. capillary of 57 cm total length, −15 kV (about 6 μA) and 5 s injection.

that simulates the composition of serum diluted 1:1 with water [11], and varying amounts of lactate. Stacking by lactate was thereby found to be evident (Fig. 4). With 85 mM lactate, the RPH increased more than twice. As can be seen from Fig. 5, presence of the high lactate concentration not only sharpens the peak of EtG but also decreases its detection time, which is an univocal evidence of terminating type of stacking [19–21]. Lactate has a stacking effect on EtG but, as can be seen from the trace in the presence of 85 mM lactate (Fig. 5), the zone of citrate becomes totally deteriorated as for citrate lactate acts as destacker [11]. The highest physiological concentration (10 mM) that can be expected in serum of patients with diabetes mellitus after a physical effort [22],

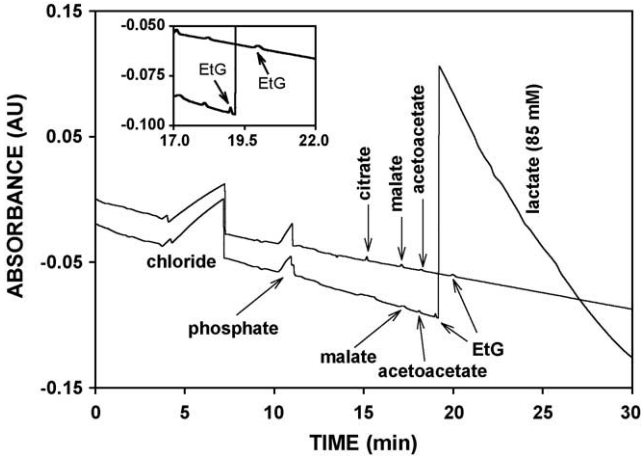

Fig. 5. Electropherograms of model mixtures of anions containing 85 mM lactate (lower graph) and no lactate (upper graph) analyzed in a BGE composed of 10 mM L-mandelic acid and β-Ala (pH 3.5). The insert depicts the EtG peaks at elongated *y*- and *x*-axis scales. Other conditions as for Fig. 4.

however, does not improve sensitivity significantly (Fig. 4). Attempts to add lactate to the serum samples to reach the concentration of about 80 mM were not successful. Addition of 50 mM lactate to the serum samples improved RPH similarly as in the model mixture (Fig. 4). However, no sensitivity improvement was reached (Table 4). Thus, the further investigation of the mandelate system was abandoned.

3.4. Determination of EtG in buffers with nicotinate and other co-ions

For enhancement of sensitivity, the use of the pH 4.0–4.4 range was evaluated. For that purpose, coated capillaries with 0.1 mm i.d. were employed and BGEs with four different co-ions that are slower than EtG were assessed (systems 11–20 of Table 3). Best results in terms of stacking and thus LOD were obtained in 5 mM cinnamic acid with EACA, pH 4.2 (Fig. 6). EtG was well resolved from other serum microcomponents and the LOD was determined to be 2.1×10^{-7} M, a value that is much better than that obtained with the mandelate system (Table 4). The only problem of this system was the low solubility of cinnamic acid so that no stock solution could be prepared. Similar results were also obtained with phenylacetate (BGE no. 15, Fig. 7) but this system was avoided because of its offensive odor. With the benzoate co-ion (BGE no. 16, Fig. 8), the LOD was found to be comparable (Table 4) but an unknown serum microcomponent migrated close to EtG. The use of nicotinate was found to be most convenient when employed at pH 4.3 (BGE no. 19 of Table 3, Fig. 9). For the four co-ions tested, the impact of the presence of lactate was investigated with the protein-free model sample. In all cases, the ratio of peak height to peak area for EtG was determined to increase with increased addition of lactate (up to about 30% with 5 mM lactate, data not shown).

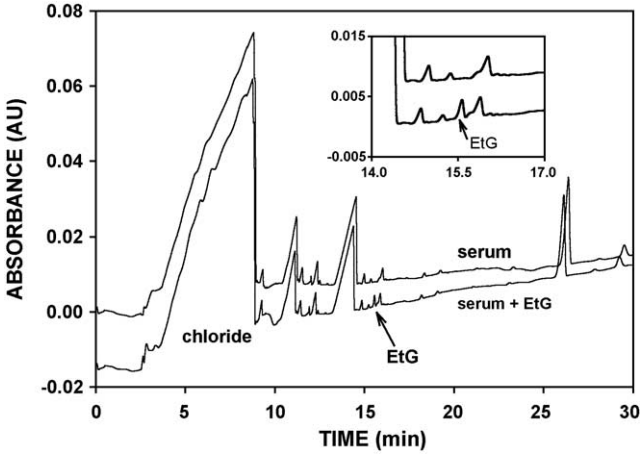

Fig. 6. Electropherograms of 1:1 diluted blank serum and blank serum fortified with 6 μg/ml (27 μM) EtG obtained in a BGE composed of 7 mM cinnamic acid and EACA (pH 4.2). The insert depicts the EtG peak at elongated *y*- and *x*-axis scales. Instrumental conditions: P/ACE 5000 with a coated 0.1 mm i.d. capillary of 57 cm total length, −15 kV (about 6 μA) and 5 s injection.

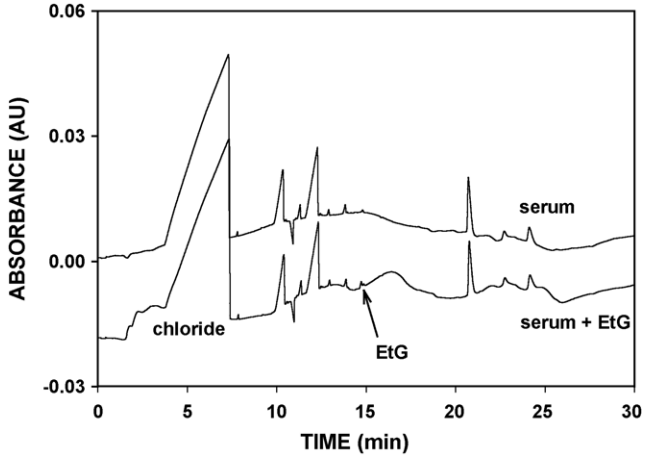

Fig. 7. Electropherograms of 1:1 diluted blank serum and blank serum fortified with 2.52 μg/ml (11.35 μM) EtG obtained in a BGE composed of 10 mM phenylacetic acid and EACA (pH 4.4). Instrumental conditions: P/ACE 5000 with a coated 0.1 mm i.d. capillary of 57 cm total length, −15 kV (about 7.7 μA) and 5 s injection.

The nicotinate system at pH 4.3 was found to provide reliable results together with an attractive LOD (Table 4). Electropherograms obtained with small amounts of EtG were clean (Fig. 10, serum sample spiked with 0.11 μg/ml EtG) and reproducible. The LOD for this system was determined to be 3.56×10^{-7} M (Table 4). Repeatability was assessed for $n = 6$ with a serum sample spiked with 0.67 μg/ml (3 μM) EtG. The obtained R.S.D. values for detection time, peak area and peak height were 1, 7.5 and 0.72%, respectively. Furthermore, calibration for fortified serum in the range between 0 and 0.89 μg/ml (0 and 4 μM) EtG resulted in a linear calibration graph expressed as y (AU × min) = 2556.8 x (μM) − 82.94 and a regression coefficient r of 0.9996. Thus, this assay should be suitable for the determination of EtG in human serum.

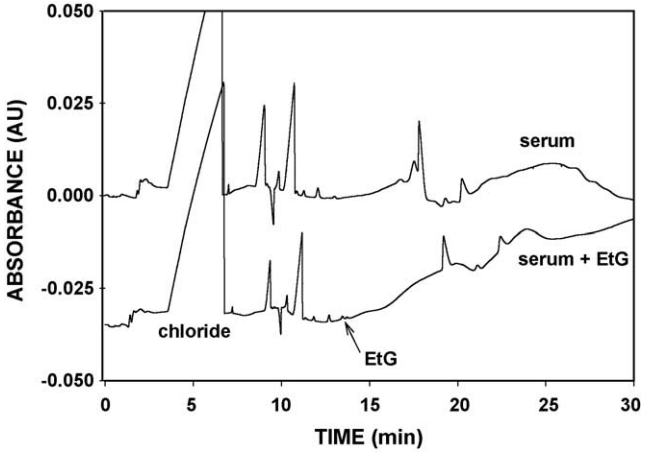

Fig. 8. Electropherograms of 1:1 diluted blank serum and blank serum fortified with 2.52 μg/ml (11.35 μM) EtG obtained in a BGE composed of 10 mM benzoic acid and EACA (pH 4.4). Instrumental conditions: P/ACE 5000 with coated 0.1 mm i.d. capillary of 57 cm total length, −15 kV (about 8.5 μA) and 5 s injection.

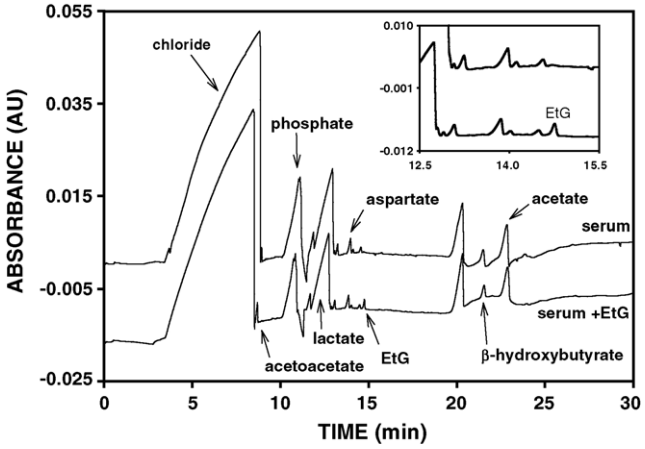

Fig. 9. Electropherograms of 1:1 diluted blank serum and blank serum fortified with 2.52 μg/ml (11.35 μM) EtG obtained in a BGE composed of 10 mM nicotinic acid and EACA (pH 4.3). The insert depicts the EtG peak at elongated y- and x-axis scales. Instrumental conditions: P/ACE 5000 with coated 0.1 mm i.d. capillary of 57 cm total length, −15 kV (about 5 μA) and 5 s injection.

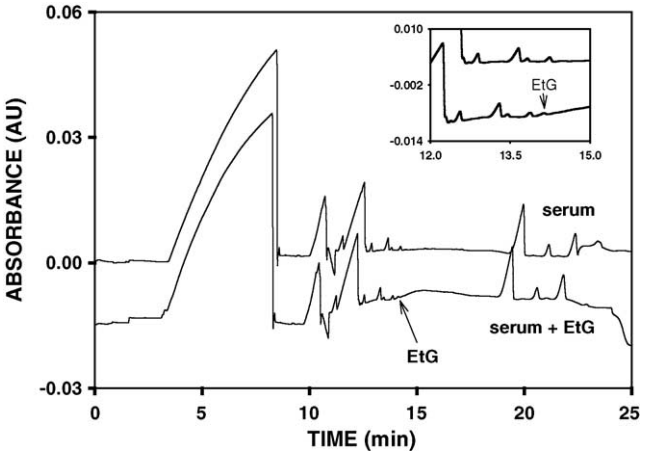

Fig. 10. Electropherograms of 1: 1 diluted blank serum and blank serum fortified with 0.11 μg/ml (0.5 μM) EtG obtained in a BGE composed of 10 mM nicotinic acid and EACA (pH 4.3). The insert depicts the EtG peak at elongated y- and x-axis scales. Instrumental conditions as for Fig. 9.

4. Conclusions

From the results obtained so far for the CZE analysis of EtG in 1:1 diluted human serum and without analyte extraction, the mandelate system is shown to provide insufficient stacking and thus sensitivity. Having nicotinate as co-ion, however, it is demonstrated to provide a LOD of 3.56×10^{-7} M (0.079 μg/ml), a value that is relevant for clinical and forensic purposes. More work is required to assess the suitability of this assay for the determination of EtG in a broad range of patient samples. The data illustrate that proper use of sample self-stacking can lead to about a 30-fold increase in analytical sensitivity with direct injection of a body fluid. For analysis of a microcomponent in a complex sample

matrix containing multiple macrocomponents of like charge, the knowledge of the effective mobilities as function of pH and the concentration of the macrocomponents permits the selection of a suitable system.

Acknowledgements

This work was partly supported by the Swiss National Science Foundation and grant no. IAA4031401 from the Grant Agency of the Academy of Science of the Czech Republic.

References

[1] G. Schmitt, R. Aderjan, T. Keller, M. Wu, J. Anal. Toxicol. 19 (1995) 91.
[2] G. Schmitt, P. Droenner, G. Skopp, R. Aderjan, J. Forensic Sci. 42 (1997) 1099.
[3] F.M. Wurst, C. Kempter, S. Seidl, A. Salt, Alcohol Alcohol. 34 (1999) 71.
[4] F.M. Wurst, C. Kempter, J. Metzger, S. Seidl, A. Salt, Alcohol 20 (2000) 111.
[5] W. Weinmann, P. Schaefer, A. Thierauf, A. Schreiber, F.M. Wurst, J. Am. Soc. Mass Spectrom. 15 (2004) 188.
[6] M. Nishikawa, H. Tsuchihashi, A. Miki, M. Katagi, G. Schmitt, H. Zimmer, T. Keller, R. Aderjan, J. Chromatogr. B 726 (1999) 105.
[7] I. Janda, A. Alt, J. Chromatogr. B 758 (2001) 229.
[8] P. Gebauer, W. Thormann, P. Boček, J. Chromatogr. 608 (1992) 47.
[9] P. Gebauer, W. Thormann, P. Boček, Electrophoresis 16 (1995) 2039.
[10] P. Gebauer, L. Křivánková, P. Pantůčková, P. Boček, W. Thormann, Electrophoresis 21 (2000) 2797.
[11] L. Křivánková, P. Pantůčková, P. Gebauer, P. Boček, J. Caslavska, W. Thormann, Electrophoresis 24 (2003) 505.
[12] C. Tu, H.K. Lee, J. Chromatogr. A 966 (2002) 205.
[13] S. Hjertén, J. Chromatogr. 347 (1985) 191.
[14] S. Krásenský, S. Fanali, L. Křivánková, P. Boček, Electrophoresis 16 (1995) 968.
[15] T. Hirokawa, M. Nishino, N. Aoki, Y. Kiso, Y. Sawamoto, T. Yagi, J. Akiyama, J. Chromatogr. 271 (1983) D1.
[16] G.M. Mc Laughlin, J.A. Nolan, J.L. Lindahl, R.H. Palmieri, K.W. Anderson, S.C. Morris, J.A. Morrison, T.J. Bronzert, J. Liq. Chromatogr. 15 (1992) 961.
[17] R. Vespalec, P. Gebauer, P. Boček, Electrophoresis 13 (1992) 677.
[18] F. Foret, L. Křivánková, P. Boček, Capillary Zone Electrophoresis, VCH, Weinheim, 1993.
[19] L. Křivánková, P. Gebauer, W. Thormann, R.A. Mosher, P. Boček, J. Chromatogr. 638 (1993) 119.
[20] L. Křivánková, P. Gebauer, P. Boček, J. Chromatogr. A 716 (1995) 35.
[21] L. Křivánková, P. Pantůčková, P. Boček, J. Chromatogr. A 838 (1999) 55.
[22] L. Křivánková, P. Boček, J. Microcol. Sep. 2 (1990) 80.
[23] Wissenschaftliche Tabellen Geigy, Teilband Hämatologie und Humangenetik, eighth ed., Ciba-Geigy, Basel, 1979.
[24] R.A. Chalmers, A.M. Lawson, Organic Acids in Man. Analytical Chemistry, Biochemistry and Diagnosis of the Organic Acidurias, Chapman and Hall, London, 1982.
[25] H.A. Harper, Review of Physiological Chemistry, Czech ed., Avicenum, Prague, 1977.