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Determination of ethyl sulfate in human serum and urine by capillary zone electrophoresis

Balthasar Jung, Jitka Caslavska, Wolfgang Thormann*

Department of Clinical Pharmacology, University of Bern, Murtenstrasse 35, CH-3010 Bern, Switzerland

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ABSTRACT

The use of capillary zone electrophoresis (CZE) with indirect absorbance detection for the analysis of ethyl sulfate (EtS) in serum and urine was investigated. EtS is a direct metabolite of ethanol employed as marker for recent alcohol consumption. Fused-silica capillaries of 60 cm total length were either coated with cetyltrimethylammonium bromide (CTAB, 50 μm I.D. capillary) or poly(diallyldimethylammonium chloride) (PDADMAC, 100 μm I.D. capillary) to allow CZE analyses to be performed with reversed polarity. At pH 2.2 with a maleic acid/phthalic acid background electrolyte, both approaches provided reliable EtS serum levels down to 0.2 mg L^{-1} (1.6 μM) for the analysis of solid-phase extracts that were prepared after chloride precipitation. Analysis of urines diluted to a conductivity of 5 S m^{-1} and analyzed in the two capillary formats resulted in limits of quantification (LOQs) of 2 and 1 mg L^{-1}, respectively. With urines adjusted to 10 S m^{-1} via dilution or condensation, an LOQ of 0.6 mg L^{-1} (4.8 μM) was obtained in the CTAB coated capillary whereas in the PDADMAC-coated capillary of equal length not all matrix components were resolved from EtS. The developed assays are robust and suitable to monitor EtS in samples of individuals who consumed as little as one standard drink of an alcoholic beverage containing about 14 g of ethanol.

1. Introduction

Ethyl sulfate (EtS, sulfovinic acid, for structure refer to insert in Fig. 1) is a non-volatile, water soluble, stable upon storage, direct metabolite of ethanol. Less than 0.1% of the ingested ethanol is excreted as EtS in urine [1–3]. For an ethanol intake during a short time period, it was reported that EtS reaches its maximum concentration in serum about 3 h after the beginning of alcohol consumption [1,3–5]. Thus, EtS can be detected for an extended time period in serum (in the range of hours) and in urine (in the range of hours to days) after complete elimination of ethanol such that it can be employed as marker substance for recent alcohol consumption. Together with ethyl glucuronide (EtG, [3,4,6,7]), 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 (CDT, [8–11]).

The analysis of EtS in body fluids has attracted considerable attention in the past few years. Methods based upon LC–MS and LC–MS–MS with negative electrospray ionization have been developed for the determination of EtS in urine [1–5,12–15] and serum [4]. These efforts revealed that a calibration range for EtS in serum should be between 0.1 and 10 mg L^{-1} (0.8–79.3 μM). For urinary concentrations of EtS the range between 0.5 and 100 mg L^{-1} (4.0–792.8 μM) is of interest. To the knowledge of the authors no cut-off level has been proposed yet for EtS. Esteve-Turrillas et al. reported a capillary zone electrophoresis (CZE) method with indirect UV absorption detection for urinary EtS with a lower limit of quantitation (LOQ) of 5 mg L^{-1} [16]. No CZE-based assay for EtS in serum has been reported thus far. CZE with optical detection is an attractive technology that is less expensive than instrumentation featuring a MS and has successfully been employed for the monitoring of other alcohol markers, namely CDT [8,10] and EtG [6,7] in serum. The CZE conditions of Esteve-Turrillas et al. [16] for analysis of EtS in urine were adopted and optimized for better resolution and sensitivity using different capillary coatings, capillary inner diameters and urine concentrations. Furthermore, the determination of EtS in human serum after chloride precipitation and solid-phase extraction (SPE) was studied in the same CZE buffer. Data obtained with urinary and serum EtS assays using fused-silica capillaries coated with cetyltrimethylammonium bromide (CTAB) and

* Corresponding author. Tel.: +41 31 632 3288; fax: +41 31 632 4997.
E-mail address: wolfgang.thormann@ikp.unibe.ch (W. Thormann).

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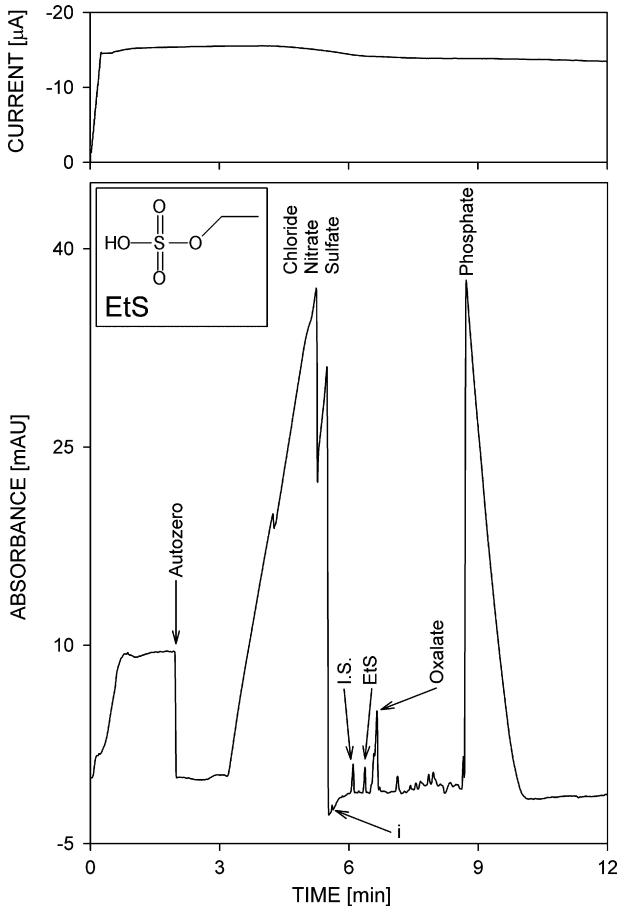

Fig. 1. Typical electropherogram and temporal behavior of the current for the analysis of a blank urine (10 S m^{-1}) that was spiked with EtS and I.S. (8 mg L^{-1} each) in the CTAB-coated capillary. Key: i refers to an impurity of the I.S. Insert: Chemical structure of EtS.

poly(diallyldimethylammonium chloride) (PDADMAC) are compared and shown to be suitable to monitor EtS on the mg L^{-1} level in both body fluids.

2. Experimental

2.1. Chemicals and samples

All chemicals used were of highest analytical purity. Sodium EtS was purchased from TCI Europe (Zwijndrecht, Belgium). Vinylsulfonic acid sodium salt [25%, w/w aqueous solution; internal standard (I.S.)] and PDADMAC (typical molecular mass 200–350 kDa, 20%, w/w aqueous solution) were from Aldrich (Milwaukee, WI, USA). Maleic acid, phthalic acid, sodium chloride and sodium oxalate came from Fluka (Buchs, Switzerland), sodium sulfate, ammonium nitrate, disodium hydrogenphosphate, isopropanol, sodium hydroxide (NaOH), formic acid and ammonia solution (32%, w/w) were from Merck (Darmstadt, Germany) and CTAB was from Sigma (St. Louis, MO, USA). Silver acetate (SAC) was obtained from Acros Organics (Geel, Belgium). Acetonitrile (ACN), methanol (MeOH) and dichloromethane were from VWR (Leuven, Belgium). Bidistilled water was used throughout. Sera and urines used in this work stemmed from members of our department which gave their consent. Three individuals ingested known amounts of ethanol within about 30 min and urines were collected immediately before and up to 28 h after alcohol consumption. All others were asked to roughly estimate the quantity and time of their last intake of alcoholic beverages.

2.2. CZE Instrumentation and methods

CZE measurements were performed using the instruments P/ACE MDQ and ProteomeLab PA 800 (Beckman Coulter, Fullerton, CA, USA) with the UV detectors set to indirect detection at 214 nm (filter) and 215 nm (diode array detector), respectively. The cartridge temperature was set to 15 °C, the sample storage to 5 °C. Fused-silica capillaries were from Polymicro Technologies (Phoenix, AZ, USA) and had a total length of 60.2 cm (50 cm to the detector). The I.D.s were 50 μm (for experiments using CTAB) and 100 μm (for experiments using PDADMAC) whereas the outer diameters were 375 μm in both cases. Bare fused-silica capillaries were treated prior to their first use with 1 M NaOH, 0.1 M NaOH and water (50 μm I.D. capillaries: 30 min at 20 psi each (1 psi = 6894.76 Pa); 100 μm I.D. capillaries: 10 min at 30 psi each). Solute quantitation was based upon multi-level internal calibration using corrected peak areas (peak areas divided by detection time) for data evaluation.

2.2.1. CZE in 50 μm I.D. capillaries coated with CTAB

The background electrolyte (BGE) consisted of 15 mM maleic acid, 1 mM phthalic acid and 25 μM CTAB and is similar to that used previously by Esteve-Turrillas et al. [16]. The pH was set to 2.20 by addition of NaOH. At the beginning of every working day, the capillary was rinsed with the BGE for 10 min at 20 psi followed by stabilization with application of −19 kV for 90 min. Before each run, the capillary was flushed with the BGE for 5 min at 30 psi. Sample injection was effected with vacuum for 29 s at 0.5 psi. A plug of BGE was added behind the injected sample (0.02 psi min). A voltage of −19 kV (reversed polarity) was applied for the separation. At the end of each working day the capillary was washed with water for 20 min at 30 psi and the ends of the capillary were left in vials filled with water. Electroosmotic flow (EOF) was measured by injecting diluted BGE (1:1 with water) as sample.

2.2.2. CZE in 100 μm I.D. capillaries coated with PDADMAC

The PDADMAC coating [17–25] was applied after conditioning with NaOH by flushing the capillary for 5 min at 30 psi with a solution containing 0.1% (w/w) PDADMAC in BGE. The BGE was composed of 15 mM maleic acid and 1 mM phthalic acid that was adjusted to pH 2.20 by addition of NaOH. Following the application of the coating, it was stabilized under a voltage of 12.5 kV for 180 min, a procedure that was performed once and not repeated at the beginning of a new working day. Before each run, the capillary was flushed with 0.1% (w/w) PDADMAC in BGE for 30 s at 20 psi followed by a flush of BGE without the cationic polymer (2 min at 20 psi). Sample was injected by pressure for 10 s at 0.5 psi. A plug of BGE was added behind the injected sample (0.01 psi min) before application of −9 kV (reversed polarity). At the end of each run the capillary was washed with water for 30 s at 40 psi. At the end of a working day the capillary was washed with water for 20 min at 30 psi and dried with air for 10 min at 30 psi. EOF was measured by injecting diluted BGE (1:1 with water) as sample.

2.3. Sample preparation

2.3.1. Urine samples

Two strategies for normalization of the urine concentration were investigated. Initially, urines were diluted to a conductivity of 5 S m^{-1}, mixed 1:1 with the I.S. solution (8 mg L^{-1}) and analyzed by CZE. In a second approach, the conductivity of the urine

was adapted to a specific conductivity employing a urine aliquot according to

$$V = \frac{\kappa_a}{\kappa} V_a \quad (1)$$

where V is the urine aliquot in μL, V_a is the sample volume after reconstitution in μL, κ is the conductivity of the urine in S m^{-1} and κ_a is the conductivity of the adjusted sample after reconstitution in S m^{-1}. If not stated otherwise, κ_a was 10 S m^{-1}. The urine aliquot, 20 μL of I.S. solution (100 mg L^{-1}) and 250 μL ACN were mixed and evaporated to dryness with air at a maximum temperature of 45 °C. If needed, additional ACN was added in order to ensure fast evaporation of the fluid (10–60 min). If not stated otherwise, the residue was reconstituted in 250 μL water and analyzed by CZE.

2.3.2. Serum samples

Serum (500 μL) and I.S. (50 μL at 15 mg L^{-1}) were mixed. Then 1 mL of SAC (50 mM) was added, vortexed and the sample was put on ice for 10 min prior to centrifugation (10 min at about 1000 × g). The silver chloride precipitate was disposed. 10 μL of concentrated formic acid was added to the supernatant prior to its application on the SPE column (Strata X-AW 33 μm Polymeric Weak Anion, 60 mg/3 mL; Brechbühler, Schlieren, Switzerland) which was previously conditioned with 1 mL MeOH and equilibrated with 1.5 mL 50 mM formic acid at pH 4.0 (adjusted with NaOH). The column was washed with 1 mL water and 1 mL MeOH and dried at 100 mbar vacuum for 1 min. The sample was eluted using 1 mL 5% (v/v) ammonia in MeOH. The eluent was evaporated to dryness with air at maximum 35 °C (about 20 min) and reconstituted in 125 μL water.

2.4. Determination of pH and conductivity

The pH was measured using a pH meter model 1.744.0010 from Metrohm (Herisau, Switzerland) and the conductivity was determined using a conductivity meter model 101 from Orion Research (Cambridge, MA, USA) equipped with a model PW 9510/65 cell from Philips (Eindhoven, The Netherlands).

3. Results and discussion

3.1. CZE of EtS in capillaries coated with polycations

With the pH 2.5 BGE used by Esteve-Turrillas et al. [16], which was composed of 15 mM maleic acid, 1 mM phthalic acid, NaOH and 50 μM CTAB, matrix peaks were detected close to (urine) or within (serum extract) EtS. The same was found to be true for an unidentified impurity of the I.S. Thus, the pH of the BGE was reduced to 2.2 which provided a good separation between EtS, I.S., oxalate and the unidentified peaks. Furthermore, the EOF was adapted via a reduction of the CTAB concentration to 25 μM. This provided data with a low EOF towards the anode (electroosmotic mobility: -8.8×10^{-9} m^2 V^{-1} s^{-1}). Peaks were identified by spiking with standards and reanalysis of the spiked sample. A typical electropherogram and the temporal behavior of the current are presented in Fig. 1. Furthermore, in order to reach a better sensitivity, the use of a 100 μm I.D. capillary was tested with the optimized BGE. These efforts, however, were abandoned as no reproducible results were obtained with the dynamic CTAB coating.

The 25 μM concentration of CTAB in the BGE is within a critical range in which a small change in the concentration of the polycation strongly influences the resulting EOF [26,27]. Thus, the search for a capillary coating which is less prone to EOF deviations and which does not require a long stabilizing time interval at the beginning of a working day was undertaken. Capillaries of 100 μm I.D. coated with the cationic polymer PDADMAC [17,18] were tested and compared with the performance of the dynamic coating of 50 μm I.D. capillaries using CTAB. The capillary was rinsed with the BGE containing 0.1% (w/w) PDADMAC whereas the electrophoretic run was executed without PDADMAC in the BGE. The applied voltage was set to −9 kV (current: about −28 μA) in order to obtain comparable detection times for EtS in both configurations as well as to keep the resulting current and hence joule heating reasonably low. With PDADMAC, the resulting positive charge of the capillary wall led to a strong EOF in the direction of the anode (electroosmotic mobility: -36.6×10^{-9} m^2 V^{-1} s^{-1}). The noncovalently bound layer of PDADMAC was reconditioned before each run (cf. Section 2.2.2) and provided reproducible results (see below). It is also imaginable to coat the capillary with successive ionic-polymer layer coatings in order to save time by getting rid of the regeneration step of the coating [18,21,22,25,28].

Reproducibility of detection times was assessed for the I.S. Independent of sample matrix (adjusted urine or serum extract) interday RSD (n = 5) values for the CTAB and PDADMAC approaches were <4.3 and <7.3%, respectively. Relative detection times of EtS (ratio of detection times of EtS and I.S.) varied less than 1% in all cases which demonstrates the robustness of the approaches used in this work. The calibration curves were calculated with five different concentrations for serum (0.2, 0.5, 1.5, 3.5 and 10 mg L^{-1}) and urine (2, 5, 15, 35 and 100 mg L^{-1}). Since most of the expected EtS concentrations in real samples are around the lowest calibration points, the suitability of different weighted linear regression models (based upon x, $1/x$ and $1/x^2$) were compared according to Karnes and March [29]. The relative concentration residuals for each calibration point were calculated as

$$\%\mathrm{RCR} = 100 \times \frac{\mathrm{RC} - \mathrm{NC}}{\mathrm{NC}} \quad (2)$$

where RC represents the interpolated concentration (using the particular calibration curve) and NC is the nominal concentration. For both coated capillaries, as well as for analysis of serum and urine, the sum of all %RCR was found to be smallest for the $1/x^2$ weighted linear regression and largest for the x weighted linear regression. Thus, the $1/x^2$ weighted linear regression was used in this work.

3.2. Urine assays

Esteve-Turrillas et al. [16] proposed to dilute every urine sample 1:5 with water. This sample preparation is very simple and convenient. It does, however, not account for the large range of urine concentrations which can differ up to 100-fold [30,31]. A wide concentration range of the 29 urine samples studied during the present work was also observed. Conductivity was determined to range from 3.09 to 31.2 S m^{-1}. Thus, urine should be normalized to a urine property, such as the creatinine concentration [30] or conductivity [32]. The studied CZE methods require samples which contain a similar amount of ions. Particularly the fast migrating anions, such as chloride, sulfate and nitrate, which cover a large part of the anions present in urine, should be present in similar amounts in the samples in order to guarantee comparable electropherograms. Thus, as the conductivity of urine samples was found to correlate with the creatinine level [32], the urines were adjusted to a specific conductivity which led to comparable electropherograms.

In a first step, all samples and calibrators were diluted to 5 S m^{-1} and mixed 1:1 with the I.S. solution (8 mg L^{-1}). Typical electropherograms obtained in CTAB- and PDADMAC-coated capillaries are presented in Fig. 2 (lower set of graphs) and Fig. 3, respectively. Electropherograms of samples which were taken after a dinner accompanied by many alcoholic beverages are shown as top graphs of the triplets. Calibration graphs were found to be linear and characterized with small y-intercepts (Table 1) for both coating

Table 1
Calibration data ($n = 5$)[a]

Capillary coating	Sample	Slope		y-Intercept		r		F	
		Mean	SD	Mean (mg L^{-1})	SD (mg L^{-1})	Mean	SD	Mean	SD
CTAB	Urine (5 S m^{-1})	0.095	0.001	−0.021	0.020	0.999	0.001	3005	3235
	Urine (10 S m^{-1})	0.099	0.004	0.006	0.020	0.999	<0.001	28792	42199
	Serum	0.542	0.028	0.002	0.031	0.998	0.002	1819	1312
PDADMAC	Urine (5 S m^{-1})	0.096	0.007	−0.007	0.038	0.998	0.003	2546	2014
	Serum	0.546	0.031	−0.025	0.019	0.999	<0.001	3564	2070

[a] Except for the serum analyses in the PDADMAC-coated capillary, data were monitored on the P/ACE MDQ. Ratio of corrected peak area and concentration were taken as y and x values, respectively, and employed for linear regression analysis.

Table 2
Validation data ($n = 5$) of the assays[a]

Capillary coating	Sample	EtS (mg L^{-1})	Precision (RSD (%))		Accuracy (% of nominal value)	
			Intraday	Interday	Intraday	Interday
CTAB	Urine (5 S m^{-1})	10	3.3	3.8	99.5	99.8
		40	2.5	2.7	97.8	98.7
		80	0.9	2.3	99.1	99.1
	Urine (10 S m^{-1})	10	1.0	4.2	91.4	98.7
		40	1.5	2.9	94.4	99.0
		80	1.4	2.7	96.7	99.6
	Serum	1	2.2	6.4	101.2	105.5
		4	1.4	4.5	104.5	106.4
		8	1.9	6.4	101.2	103.3
PDADMAC	Urine (5 S m^{-1})	10	0.5	0.8	98.9	99.1
		40	1.3	2.5	102.1	99.6
		80	2.6	1.2	102.1	100.5
	Serum	1	4.2	1.7	94.8	99.3
		4	2.4	2.0	100.8	101.2
		8	1.1	1.7	105.3	103.7

[a] Except for the serum analyses in the PDADMAC-coated capillary, data were monitored on the P/ACE MDQ.

methods. Using three concentration levels, the assay performance in CTAB- and PDADMAC-coated capillaries was found to be good and comparable (Table 2). The LOQ's were determined to be 2 and 1 mg L^{-1}, respectively (Table 3), values that are somewhat too high for patient screening. The same is true for the 5 mg L^{-1} LOQ reported by Esteve-Turrillas et al. [16].

With the CTAB coating, 7 out of 29 urines revealed an EtS peak that could be quantitated. Using the PDADMAC coating, EtS could be quantitated in 5 additional urines (EtS values between 1.0 and 2.0 mg L^{-1}). Four of these urines revealed a tiny detectable EtS peak in the CTAB-coated capillary. Using the PDADMAC coating, one real sample with a conductivity higher than 5 S m^{-1} did not reveal an expected EtS peak. Furthermore two urines had a conductivity lower than 5 S m^{-1}. The concentration of these urines was not altered and the I.S. was added directly. The result had to be calculated considering the actual conductivity of the sample, so that the amount of EtS could be estimated in respect to a conductivity of 5 S m^{-1}. The samples with a lower salt concentration than 5 S m^{-1} resulted in slight shifts to shorter detection times.

The electrophoretic method was found to work well for urines up to 10 S m^{-1} (Fig. 1). From the 29 samples analyzed, however, 7 had a conductivity lower than 10 S m^{-1}. Thus, a second approach comprising evaporation of a urine aliquot (cf. Eq. (1)) to dryness and reconstitution in 250 μL water (final conductivity of 10 S m^{-1}) was investigated (Fig. 2, upper set of graphs). In order to accelerate evaporation, 250 μL of an organic solvent was added. Tests were performed with isopropanol, ACN, dichloromethane and MeOH. ACN was found to be best suited to accelerate the evaporation. Furthermore, it is interesting to note that the analysis of 10 S m^{-1} samples revealed sharpest peaks for EtS when compared with data generated in the 2.5–15 S m^{-1} range (data not shown). Assay calibration (Table 1) and validation (Table 2) were executed for 10 S m^{-1} samples using the CTAB coating. Calibration graphs were found to be linear and characterized with small y-intercepts (Table 1). Precision and accuracy data were determined to be comparable to

Table 3
LOQ and LOD ($n = 3$) data of the assays[a]

Capillary coating	Sample	LOQ			LOD (mg L^{-1}) s/n ≈ 3
		(mg L^{-1})	RSD (%)	(% of nominal value)	
CTAB	Urine (5 S m^{-1})	2.0	1.3	101.7	1.0
	Urine (10 S m^{-1})	0.6	8.7	104.4	0.4
	Serum	0.2	3.2	99.9	0.1
PDADMAC	Urine (5 S m^{-1})	1.0	5.2	90.5	0.5
	Serum	0.2	4.0	98.2	0.1

[a] Except for the serum analyses in the PDADMAC-coated capillary, data were monitored on the P/ACE MDQ.

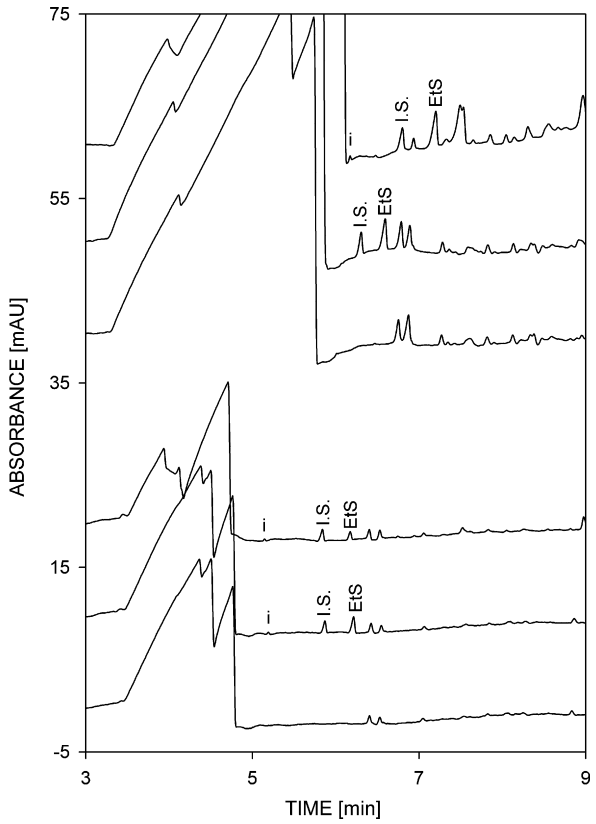

Fig. 2. Analysis in a CTAB-coated capillary of urines diluted to a conductivity of 5 S m^{-1} (lower triplet of graphs) and urines adjusted to a conductivity of 10 S m^{-1} (upper triplet of graphs). From bottom to top: blank urine, blank urine spiked with EtS (15 mg L^{-1}) and I.S. (8 mg L^{-1}), and a real sample (EtS: 6.71 mg L^{-1} in the 5 S m^{-1} sample). Key as for Fig. 1.

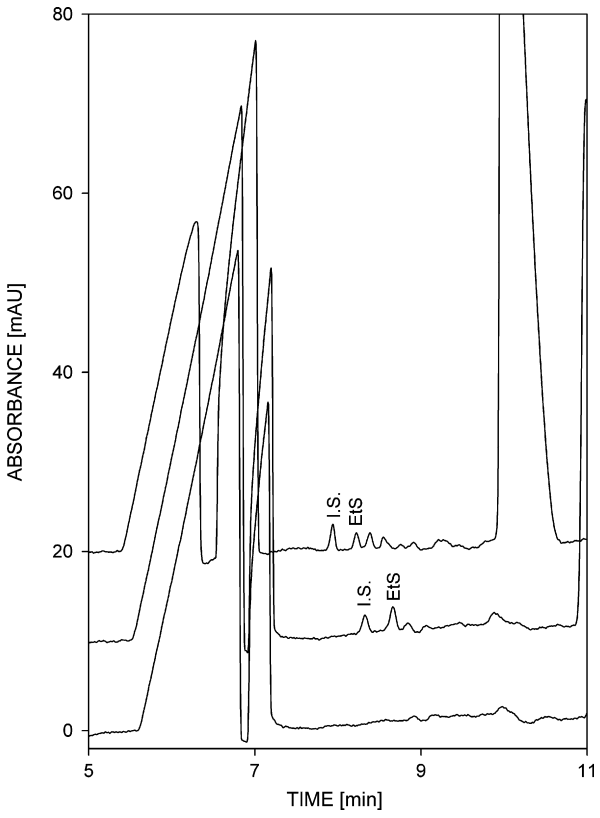

Fig. 3. Analysis in a PDADMAC-coated capillary of urines diluted to a conductivity of 5 S m^{-1}. From bottom to top: blank urine, blank urine spiked with EtS (15 mg L^{-1}) and I.S. (8 mg L^{-1}), and a real sample (EtS concentration: 5.91 mg L^{-1}).

those obtained with the first approach (Table 2). LOQs and limits of detection (LODs), however, were somewhat better (Table 3). This improvement is essential as many positive urines have EtS levels around 1–2 mg L^{-1}. It is important to realize that the urine adjustment to a uniform conductivity level is done prior to the analysis. With assays using post-analysis normalization, it is possible that intentional urine dilution obtained via drinking of water provides a urinary analyte concentration below the LOD, this resulting in a false-negative outcome [33]. Using the PDADMAC approach, the EtS peak was not baseline separated from a peak of the urine matrix. Thus, this coating was not evaluated with urine adapted to 10 S m^{-1}. Longer capillaries or capillaries with a smaller I.D. would have to be employed for complete resolution.

With the adjustment of the urine to 10 S m^{-1}, 15 out of the 29 analyzed real urine samples revealed an EtS peak that could be quantitated. The consumption of a small amount of ethanol, such as a glass of wine (approximatively 14 g of ethanol), and urine collection 2–3 h after the beginning of the ethanol ingestion, resulted in EtS levels between 2.5 and 4.5 mg L^{-1} (urines of three test persons). In the urines of a subject consuming 0.875 g ethanol per kg body mass, the highest EtS amount found was 38.69 mg L^{-1} (urine collected after 7 h) and EtS could be detected in urines that were collected up to 24 h after the start of ethanol consumption (Fig. 4). No EtS was detected in the sample collected thereafter. Lower EtS levels were monitored after ingestion of 0.462 g ethanol per kg body mass (Fig. 4). Note that an insufficient number of samples was collected for proper description of the excretion peak of EtS. For that example, EtS could be quantitated up to the urine collected 11.5 h after the beginning of ethanol intake, whereas a tiny EtS peak was detected

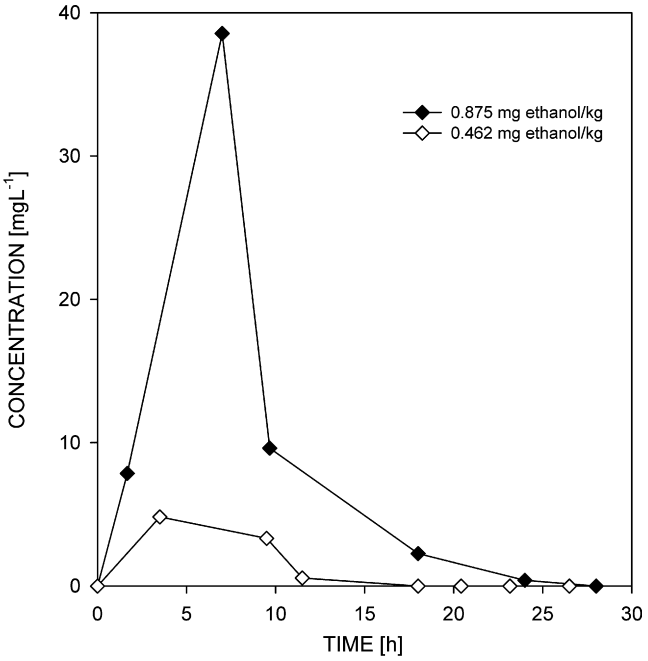

Fig. 4. EtS concentration in urines collected from a male person who ingested 0.875 g ethanol per kg body mass and a female individual who consumed 0.462 g ethanol per kg body mass. The urines were adjusted to a conductivity of 10 S m^{-1} and analyzed in the CTAB-coated capillary.

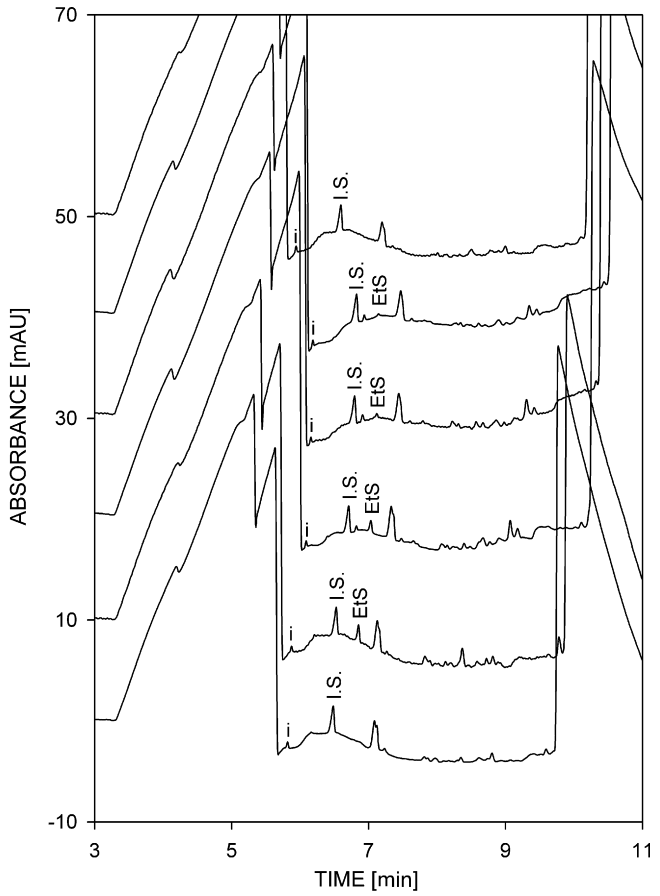

Fig. 5. Electropherograms obtained with the urines of a female individual who consumed 0.462 g ethanol per kg body mass. Urines were sampled immediately before the ethanol intake, and 3.5, 9.5, 11.5, 18 and 20.5 h after the beginning of ethanol intake (from bottom to top, respectively, and displayed with a *y*-axis offset). The urines were adjusted to a conductivity of 10 S m^{-1} and analyzed in the CTAB-coated capillary.

at 18 h as is shown with the electropherograms presented in Fig. 5. These data suggest that the urine adaptation to a conductivity of 10 S m^{-1} is best suited for clinical purposes. Sample preparation is simple, fast and cost-effective. Moreover, the creatinine level of the urine or any other property allowing a normalization of the concentration of the samples (e.g. osmolality, density and specific gravity [30,32]) could be used. For that purpose, however, Eq. (1) must be adapted accordingly. In principle, both capillary coatings can be employed. Practical criteria, such as the time interval required for capillary equilibration at the beginning of the day and the buffer volume needed for capillary rinsing, should be considered for the selection of the coating method. The use of the 100 μm I.D. capillary provides a somewhat better detection limit which is certainly valuable for patient screening. With the PDADMAC coating, however, a longer capillary would have to be employed for proper analysis of urines adjusted to 10 S m^{-1}.

3.3. Serum assays

In order to get rid of parts of the serum's matrix and to concentrate the samples to reach lower LODs, it was necessary to simplify the sample matrix. Attempts using ultrafiltration or protein precipitation with ACN followed by evaporation to dryness and reconstitution did not provide satisfying CZE data. SPE using a weak anion exchange column provided a good recovery of the analytes. However, the high chloride content of the extract did not allow a concentration of the sample. Thus chloride was precipitated with SAC and removed prior to SPE. As the chloride concentration in serum is very stable around 100 mM, an equal amount of SAC was added to the sample. Using the conditions given in Section 2.3.2 provided EtS recoveries (n = 5) of 88.4, 86.5 and 83.7% for serum spiked with 1.0, 4.0 and 8.0 mg L^{-1} of EtS, respectively (about a 3.5-fold concentration). Analyses were executed in capillaries coated with CTAB and PDADMAC and selected electropherograms are presented in Figs. 6 and 7, respectively.

Calibration graphs were found to be linear and characterized with small y-intercepts (Table 1). Intraday and interday precision data (n = 5) for the experiments with CTAB and three EtS concentrations (1.0, 4.0 and 8.0 mg L^{-1} in serum) were lower than 2.2 and 6.4%, respectively (Table 2). Corresponding values for the PDADMAC-coated capillary were 4.2 and 2.0%, respectively (Table 2). All accuracy values were between 94.8 and 106.4% (Table 2). The LOQ (0.2 mg L^{-1}) and the LOD (0.1 mg L^{-1}) were the same for both methods (Table 3). It is important to note that the analyses in the PDADMAC-coated capillary were performed on the PA 800 instrument which featured a diode array detector set to 215 nm whereas those in the CTAB-coated capillary were generated on the MDQ which was equipped with a 214 nm bandpass filter. A potential difference in the sensitivity of the two detector assemblies was not evaluated. Looking at the data obtained with the 5 S m^{-1} urines (Table 3), however, it can be assumed that the sensitivity obtained with the MDQ is somewhat better. For serum samples both methods were characterized by very similar

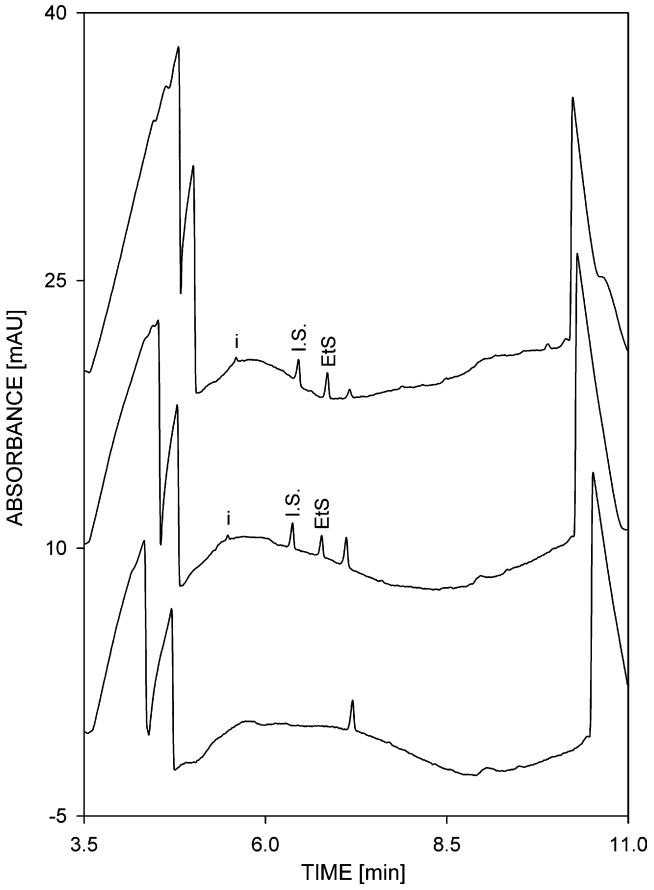

Fig. 6. Analysis in a CTAB-coated capillary of serum extracts prepared from blank serum, blank serum spiked with EtS (1.5 mg L^{-1}) and I.S. (1.5 mg L^{-1}), and a real sample with an EtS serum concentration of 1.86 mg L^{-1} (bottom to top graphs, respectively).

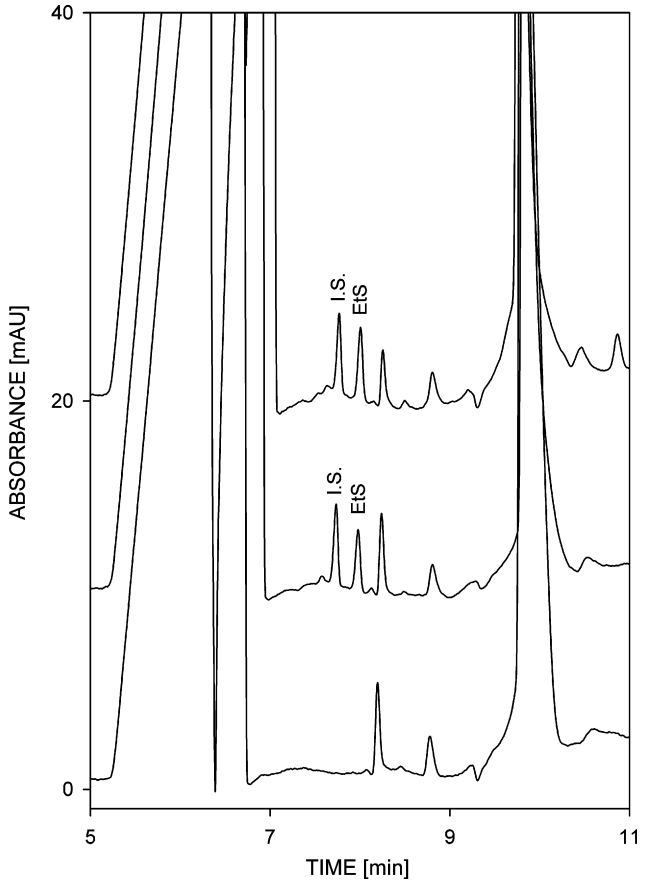

Fig. 7. Analysis in a PDADMAC-coated capillary of serum extracts prepared from blank serum, blank serum spiked with EtS (1.5 mg L^{-1}) and I.S. (1.5 mg L^{-1}), and a real sample with an EtS serum concentration of 1.70 mg L^{-1} (bottom to top graphs, respectively).

performances. Based on the method validation data, no method can be considered as being superior.

A total of 14 sera were analyzed. Five were blank sera which did not reveal any EtS using the two methods. A small amount of ethanol intake, such as a glass of wine (approximatively 14 g of ethanol) and blood withdrawal about 2 h after the beginning of the ethanol ingestion, resulted in positive EtS detection in three out of four sera. The three EtS peaks were lower than the LOQ but were above the LOD. In one case EtS could not be detected with any of the two methods. In five samples which were taken after a dinner accompanied by many alcoholic beverages, higher amounts of EtS were found (for examples see top graphs in Figs. 6 and 7). EtS concentrations between 0.26 and 1.95 mg L^{-1} were monitored and the values obtained by the two methods were found to correlate well ($y = 0.96x + 0.02$, $r = 0.996$).

4. Conclusions

The data presented in this paper demonstrate robust approaches for the CZE determination of EtS in urine diluted to 5 S m^{-1}, urine adjusted to a conductivity of 10 S m^{-1} and extracted serum. The sample matrices were chosen to account for the variability of urine concentration, to enhance sensitivity and to be able to analyze all samples with the same CZE configuration which features reversed polarity and indirect UV detection. Two capillary coatings were evaluated, a 50 μm I.D. fused-silica capillary dynamically coated with CTAB (25 μM CTAB added to the BGE) and a 100 μm I.D. capillary coated with PDADMAC. Reproducible data down to mg L^{-1} EtS levels were obtained with both capillary treatments. The LOQ of the serum assays is 0.2 mg L^{-1}. With adjustment of the urine to 10 S m^{-1} the LOQ with the CTAB-coated capillary could be reduced to 0.6 mg L^{-1}. These sensitivities were found to be sufficient to detect EtS in samples of humans who consumed as little as one alcoholic drink.

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References

[1] F.M. Wurst, S. Dresen, J.P. Allen, G. Wiesbeck, M. Graf, W. Weinmann, Addiction 101 (2006) 204.
[2] H. Schneider, H. Glatt, Biochem. J. 383 (2004) 543.
[3] A. Helander, O. Beck, Clin. Chem. 50 (2004) 936.
[4] C.C. Halter, S. Dresen, V. Auwaerter, F.M. Wurst, W. Weinmann, Int. J. Legal Med. 122 (2008) 123.
[5] S. Dresen, W. Weinmann, F.M. Wurst, J. Am. Soc. Mass Spectrom. 15 (2004) 1644.
[6] L. Křivánková, J. Caslavska, H. Malášková, P. Gebauer, W. Thormann, J. Chromatogr. A 1081 (2005) 2.
[7] M. Mrázková, J. Caslavska, W. Thormann, L. Křivánková, Electrophoresis 27 (2006) 4772.
[8] J. Joneli, C. Lanz, W. Thormann, J. Chromatogr. A 1130 (2006) 272.
[9] A. Helander, J.P. Bergstrom, Clin. Chim. Acta 371 (2006) 187.
[10] C. Lanz, M. Kuhn, V. Deiss, W. Thormann, Electrophoresis 25 (2004) 2309.
[11] A. Helander, A. Husa, J.O. Jeppsson, Clin. Chem. 49 (2003) 1881.
[12] W. Bicker, M. Lämmerhofer, T. Keller, R. Schuhmacher, R. Krska, W. Lindner, Anal. Chem. 78 (2006) 5884.
[13] L. Morini, L. Politi, A. Zucchella, A. Polettini, Clin. Chim. Acta 376 (2007) 213.
[14] L. Politi, L. Morini, A. Groppi, V. Poloni, F. Pozzi, A. Polettini, Rapid Commun. Mass Spectrom. 19 (2005) 1321.
[15] A. Helander, H. Dahl, Clin. Chem. 51 (2005) 1728.
[16] F.A. Esteve-Turrillas, W. Bicker, M. Lämmerhofer, T. Keller, W. Lindner, Electrophoresis 27 (2006) 4763.
[17] N. Cohen, E. Grushka, J. Capillary Electrophor. 1 (1994) 112.
[18] T.W. Graul, J.B. Schlenoff, Anal. Chem. 71 (1999) 4007.
[19] J.S. Fritz, S.A. Steiner, J. Chromatogr. A 934 (2001) 87.
[20] M.E. Roche, M.A. Anderson, R.P. Oda, B.L. Riggs, M.A. Strausbauch, R. Okazaki, P.J. Wettstein, J.P. Landers, Anal. Biochem. 258 (1998) 87.
[21] T. Kelly, P. Doble, M. Dawson, Electrophoresis 28 (2007) 3566.
[22] P. Zakaria, M. Macka, P.R. Haddad, J. Chromatogr. A 985 (2003) 493.
[23] G. Danger, M. Ramonda, H. Cottet, Electrophoresis 28 (2007) 925.
[24] C. Stathakis, E.A. Arriaga, D.F. Lewis, N.J. Dovichi, J. Chromatogr. A 817 (1998) 227.
[25] R. Nehmé, C. Perrin, H. Cottet, M.D. Blanchin, H. Fabre, Electrophoresis, in press.
[26] Y. Yang, M.C. Breadmore, W. Thormann, J. Sep. Sci. 28 (2005) 2381.
[27] J.L. Beckers, P. Boček, Electrophoresis 23 (2002) 1947.
[28] H. Katayama, Y. Ishihama, N. Asakawa, Anal. Chem. 70 (1998) 2254.
[29] H.T. Karnes, C. March, J. Pharm. Biomed. Anal. 9 (1991) 911.
[30] C.S. Barbanel, J.W. Winkelman, G.A. Fischer, A.J. King, J. Occup. Environ. Med. 44 (2002) 407.
[31] D.B. Barr, L.C. Wilder, S.P. Caudill, A.J. Gonzalez, L.L. Needham, J.L. Pirkle, Environ. Health Perspect. 113 (2005) 192.
[32] A. Baldacci, R. Theurillat, J. Caslavska, H. Pardubska, R. Brenneisen, W. Thormann, J. Chromatogr. A 990 (2003) 99.
[33] H. Dahl, N. Stephanson, O. Beck, A. Helander, J. Anal. Toxicol. 26 (2002) 201.