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

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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

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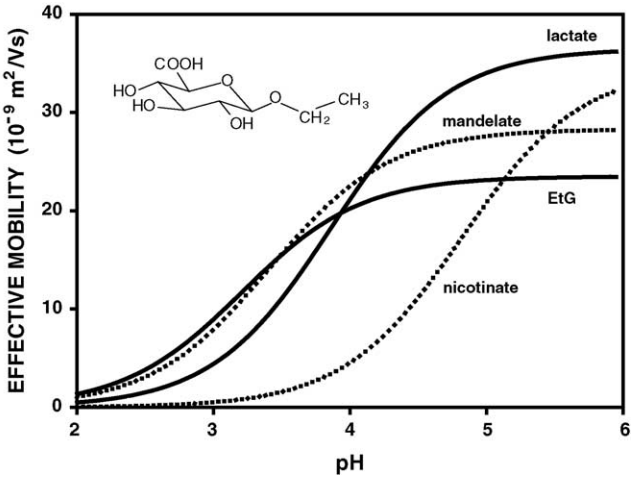

Fig. 1. Effective electrophoretic mobility of EtG, lactate, mandelate and nicotinate as function of pH. The insert depicts the chemical structure of EtG.

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