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Separation of oxalate, formate and glycolate in human body fluid samples by capillary electrophoresis with contactless conductometric detection

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ABSTRACT

A new method for rapid determination of toxic metabolites after methanol and ethylene glycol intoxication – oxalate, formate and glycolate in various body fluid samples (blood serum, saliva, urine, exhaled breath condensate) by capillary electrophoresis with contactless conductometric detection was developed. A selective separation of the three target analytes from other constituents present in the analyzed biological matrices was achieved in less than 6 min in a fused silica capillary of 25 μm I.D. using an electrolyte comprising 50 mM L-histidine and 50 mM 2-(N-morpholino)ethanesulfonic acid at pH 6.1. The only sample preparation was dilution with deionized water. The limits of detection were 0.4, 0.6 and 1.3 μM and limits of quantitation 1.3, 1.9 and 4.2 μM for oxalate, formate and glycolate, respectively. The method provides a simple and rapid diagnostic test in suspected intoxication and is able to distinguish the ingested liquid, based on its metabolite trace. The method presents a fast screening tool that can be applicable in clinical practice.

1. Introduction

Intoxication by short chain alcohols belongs to the most common intoxication that occurs repeatedly worldwide. It is primarily due to easy availability of these chemicals and their wide application as industrial solvents, household cleaners, automobile coolants, heat transfer fluids, hydraulic brake fluids, de-icing solutions, etc. [1]. Among the most commonly encountered intoxications are those caused by methanol and ethylene glycol [2]. Intoxication by methanol typically occurs on a large scale as a result of unknowingly ingesting adulterated alcoholic beverages. Many cases have been reported worldwide (Kenya 2000, Estonia 2001, Norway 2002, India 2009, Czech Republic 2012, Libya 2013) with a large number of casualties. Separate incidents of methanol ingestion also occur on a regular basis. On the other hand, intoxication by ethylene glycol does not typically occur in a large scale, but has been reported to be even more frequent than methanol intoxication [3]. It usually involves cases of intentional poisoning, suicidal attempts, but also accidental ingestion by small children and pets. Methanol and ethylene glycol ingestion is a medical emergency that requires prompt diagnosis and intervention. Both methanol and ethylene glycol are metabolized in the human body by alcohol dehydrogenases to their respective aldehydes and further by aldehyde dehydrogenases into formic and glycolic acids. Formic and glycolic acids are the primary toxic metabolites that cause severe problems if untreated [3]. Glycolic acid is further metabolized to oxalate which rapidly precipitates with calcium cation in various tissues, notably in kidney and in urine [4] and is associated with nephropathy in delayed cases of ethylene glycol poisoning [4,5]. A correct diagnosis of intoxicated patients is a significant challenge, because methanol and ethylene glycol poisonings share many characteristics both clinically and biochemically. The initial stage of intoxication is typically asymptomatic. During later stage serious symptoms caused by the more toxic metabolites occur. The symptoms specific to elevated levels of formic acid include visual impairment and damage to the optical nerve [6,7], abdominal discomfort, nausea, headache and dizziness. If untreated, the high concentration of formic acid can cause serious respiratory failure [8], renal failure [9], coma, cerebral edema, seizures and eventual death from cardiorespiratory arrest [10]. While ethylene glycol poisoning does not typically lead to visual disturbances, the symptoms include central nervous system depression [3] followed by development of cardiopulmonary and renal failure [11,12] due to the metabolic transformation of ethylene glycol to glycolic acid and

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oxalic acid. The diagnostic hallmark of any alcohol poisoning is a combination of a high anion gap, metabolic acidosis and osmolal gap. These indicators are unfortunately highly non-specific and can be caused by various other conditions, for instance diabetic ketoacidosis [13], multiple organ failure [14] or renal failure [15] that are not directly related to alcohol poisoning. Simple laboratory tests may sometimes fail to reveal the real cause of the clinical findings. For instance, an enzymatic kit for measuring serum lactate can show elevated lactate values, which are caused by the presence of glycolic acid [16–18]. Therefore, clinical samples are often sent for analysis to a specialized laboratory with more selective analytical techniques. When alcohol intoxication is suspected, GC is the method of choice in most clinical laboratories. While the determination of methanol is easy and fast by GC [19], the same is not true about ethylene glycol because of its high polarity and high boiling point [20–22]. The GC analysis of toxic metabolites (formic, glycolic and oxalic acid) is equally if not more challenging than that of ethylene glycol, because their analysis requires difficult sample derivatization, is time consuming and in some cases may not be sufficiently sensitive. Headspace GC with flame ionization detection [19,20,23–28] or GC–MS [22,29–33] is the most commonly used GC techniques. HPLC and ion chromatography (IC) provide a viable alternative to analysis of metabolites [34–37], but there is no significant time saving during sample preparation compared to GC. For instance a simultaneous separation of glycolic and oxalic acids in body fluid samples by IC [37] required the use two different separation columns, which makes this approach instrumentally complicated. Enzymatic essays have been used as well [38–42] but simultaneous analysis of more than one metabolite requires parallel analysis involving different chemistries, making such an approach expensive. CE is becoming popular in analysis of samples of biological origin, mostly because these samples are often unavailable in large quantities and have rather complicated matrix composition. Another major advantage of CE is the analysis speed. The analysis of biological samples by CE has been extensive, as documented by several review articles [43–45]. Biological samples that were analyzed by CE with contactless conductivity detection (C4D) include saliva [46], blood plasma [47–52] and urine [53–55], but to our best knowledge there is no CE-C4D method available to simultaneously determine formate, glycolate and oxalate in various body fluid samples. The goal of this study was therefore to develop a CE-C4D method for fast screening of three major toxic metabolites of methanol and ethylene glycol (formate, glycolate, oxalate) in various body fluid samples. Previously, the lack of such methods during the occurrence of large number of cases of methanol poisoning in 2012 in the Czech Republic lead us to develop a fast (<1 min) CE-C4D method for screening of formate in blood serum samples [56]. Other, similar CE methods were also developed for formate analysis after supported liquid membrane preconcentration [57,58]. When patients are brought to the emergency unit at late stage of the intoxication, whether caused by methanol or ethylene glycol, the concentrations of parent toxic alcohol may already be low or not detectable. No methanol was detected in blood serum in several patients intoxicated with methanol in 2012 in the Czech Republic (personal communication). Similar observations were also made for ethylene glycol intoxications [59]. For example, ethylene glycol was undetected in three patients with glycolic acid concentration higher than 9 mM [60,61], in two patients who died from documented ethylene glycol poisoning [62], and in a patient with ethylene glycol-associated renal failure [12]. These findings underscore the importance of multiple metabolite detection and analysis. In this work a fast method for simultaneous separation of formate, glycolate and oxalate – the major metabolic products of methanol and ethylene glycol intoxication – was developed. The method may be of particular significance, when used with a portable CE instrument on site. However, as such instruments are still only available in research laboratories a commercial CE instrument with a C4D that is custom-made to fit the capillary cartridge of the CE instrument was used to simplify eventual transfer of the developed method into current clinical practice. The method shows an application potential not only to blood samples but also to a wide number of other, non-invasively taken samples (urine, saliva, exhaled breath condensate) that may be particularly appealing for fast clinical diagnosis.

2. Experimental

2.1. Instrumentation

An Agilent HP CE system (Model G1600AX, Agilent Technologies, Waldbronn, Germany) was used for all analyses. The separations were performed at a potential of −15 kV applied at the injection side of the separation capillary. Separation capillary used was a fused silica (FS) capillary (25 μM I.D./375 μM O.D., 33/18 cm total/effective length, Microquartz, GmbH, Munich, Germany). Capillary temperature was maintained at 20 °C and injections were performed hydrodynamically at 50 mbar for 20 s. Between the runs the capillary was flushed by the background electrolyte (BGE) at a pressure of 950 mbar for 3 min. The instrument was equipped with a custom made C4D, ADMET (Version 5.06, ADMET s.r.o., Prague, Czech Republic) operating at a frequency of 1.8432 MHz and voltage 50 V_{pp}. The analog signal from C4D was processed through an Agilent Interface 35900 E and the resulting electropherograms were analyzed using 3D CE Chemstation (Rev. A 10.01, Agilent).

2.2. Chemicals

2.2.1. Reagents, standards, electrolytes

All chemicals were of reagent grade and double distilled (DD) water (Heraeus Quarzschmelze, Hanau, Germany) was used for stock solution preparation and dilutions. 10 mM stock solutions of inorganic anions were prepared from their sodium salts (chloride, nitrate, nitrite, sulfate (Pliva – Lachema, Brno, Czech Republic), thiocyanate (Sigma Aldrich, Steinheim, Germany)). Organic acids (formic, oxalic, glycolic, fumaric, tartaric, malonic, maleic, citric, acetic, lactic) were prepared from reagent grade chemicals (Sigma–Aldrich, Steinheim, Germany, Pliva-Lachema, Brno, Czech Republic). BGE for CE measurements was prepared daily by diluting 200 mM stock solutions of L-histidine (HIS, Sigma–Aldrich) and 2-(N-morpholino)ethanesulfonic acid (MES, Sigma-Aldrich) to the required concentration. Cetyltrimethylammonium bromide (CTAB) was prepared as 10 mM stock solution.

2.3. Sample preparation

The only sample preparation was dilution with DD water. When not in use all the samples were stored at −20 °C. For quantitative analysis, an aliquot of internal standard (fumarate) to yield the final concentration of 50 μM was added to all samples.

2.3.1. Exhaled breath condensate (EBC)

Exhaled breath condensate was obtained by 1 min breathing into a home made EBC sampler described in [63]. The sample was used as is with no dilution.

2.3.2. Saliva and urine

Saliva and urine samples were collected from volunteers at the university into the plastic containers and diluted 1:100 (saliva) or 1:500 (urine) before analysis.