Journal of Chromatography A, 1281 (2013) 142–147

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Capillary electrophoresis with contactless conductometric detection for rapid screening of formate in blood serum after methanol intoxication

Petr Kubáň[a,*], František Foret[a], Robert Bocek[b]

[a] Bioanalytical Instrumentation, CEITEC MU, Veveri 97, 602 00 Brno, Czech Republic
[b] Department of Anaesthesiology, Resuscitation and Intensive Care, Hospital and Policlinics Havířov Dělnická 1132/24, 73601 Havířov, Czech Republic

ARTICLE INFO

Article history:
Received 26 November 2012
Received in revised form 4 January 2013
Accepted 7 January 2013
Available online 15 January 2013

Keywords:
Capillary electrophoresis
Contactless conductivity detection
Formate
Methanol intoxication

ABSTRACT

A new method for rapid, direct determination of formate in blood serum samples by capillary electrophoresis with contactless conductometric detection is presented. A selective separation of formate was achieved in approximately 1 min using an electrolyte system comprising 10 mM L-histidine, 15 mM glutamic acid and 30 μM cetyltrimethylammonium bromide at pH 4.56. The only sample preparation was dilution (1:100) with deionized water. The limit of detection and limit of quantitation was 2.2 μM and 7.3 μM, respectively, which corresponds to 0.22 mM and 0.73 mM in undiluted blood serum. The method provides a simple and rapid diagnostic test in suspected methanol intoxication cases. The method has been successfully tested on determination of formate in blood of a patient admitted to the hospital under acute methanol intoxication. The peak concentration of formate detected in the patient blood serum was 12.4 mM, which is 10- to 100-fold higher than the normal values in healthy population. The developed method presents the fastest test currently available to detect formate in blood samples.

1. Introduction

Intoxication by industrial chemicals such as methanol, formaldehyde or ethylene glycol is occurring regularly worldwide. Sometimes, the scale of such a poisoning can be similar to common acts of terrorism and have thus a significant impact on society. For instance, in documented cases of methanol poisoning in Kenya 2000 [1], Tunisia 2003 [2], India 2009 [3], and even in Europe: Estonia 2001 [4], Norway 2002 [5] and most recently the Czech Republic (September 2012), the number of fatalities was comparable to Matsumoto city incident 1994 [6], Tokio Sarin attack 1995 [7] or Moscow theater hostage crisis 2002, in all of which, highly toxic nerve gases have been used.

Rapid analysis and identification of suspected toxic chemical compounds in body fluids during acute intoxication is important, especially with respect to the administration of an efficient antidote and initiation of a subsequent medical treatment. Often, the toxicity of the metabolites may be higher than the toxicity of the originally ingested compound. This is for instance true for methanol poisoning: the toxicity of methanol is much lower than the toxicity of its metabolic products, formaldehyde and formate. Initial symptoms of methanol intoxication are difficult to diagnose and are generally non-specific. At a later stage of intoxication, the concentration of methanol in blood may already be significantly lowered, as the major part is converted to formate. This occurs typically between 12 and 24 h after methanol ingestion. The symptoms specific to elevated levels of formate include abdominal discomfort, nausea, headache, dizziness, blurring or complete loss of vision, large anion gap and deep metabolic acidosis leading to hyperventilation [8]. If untreated, the high concentration of formate can cause serious damage to the optical nerve [9,10], respiratory failure [11], renal failure [12], coma, cerebral edema, seizures and eventual death from cardiorespiratory arrest [13]. In healthy individuals, typical concentration levels of formate in blood range from 0.07 to 1.2 mM [14,15]. Formate concentration can be significantly elevated during acute methanol intoxication. From the available literature data, the concentration of formate in identified methanol poisoning cases was found to be between 0.2 and 32 mM [15–17]. Blood formate concentrations above 10 mM (0.5 g/L) have been associated with severe toxicity due to methanol ingestion, permanent tissue damage, or fatality [18,19]. Therefore, the determination of formate rather than methanol should be used as a marker for acute methanol intoxication. A simple, but fast, screening method for formate able to quantify its concentrations above the maximum normal levels (1.2 mM) would be of great importance in clinical practice. Further, monitoring of levels of formate in blood with high temporal resolution during subsequent medical treatment (hemodialysis, antidote administration [20]) is also important as it may be used to identify the time point when patient's condition has been stabilized.

* Corresponding author. Tel.: +420 532290201; fax: +420 541212113.
E-mail address: petr.kuban@ceitec.muni.cz (P. Kubáň).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.01.035

Currently, the methods for formate determination in blood samples used in clinical laboratories are either time consuming, require difficult sample preparation/derivatization and/or may not be sufficiently sensitive. Typically, headspace GC-FID [21–26] or GC–MS [27–29] is used after conversion of formate into its volatile esters. The described procedures often involve several pretreatment steps and take between 30 and 60 min. HPLC has also been used for formate analysis [30] but there is no significant time saving during sample preparation compared to GC. Enzymatic essays based on conversion of formate to carbon dioxide by a specific enzyme and subsequent spectrophotometric detection of the reaction by-product have been used as well [31–34]. Unfortunately, none of the conventional methods offers the required simplicity and rapidity needed for fast screening purposes.

Capillary electrophoresis (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 that could be important in the fast screening, such as the one for formate. The analysis of biological samples by CE has been extensive, as documented by several review articles [35–37] and the variety of analyzed biological fluids is not limited to blood samples but includes also cerebrospinal fluid, tear fluid, saliva and urine. The biological samples are predominantly analyzed for their protein and biomolecular content, however, analysis of small organic molecules, including organic acids is becoming also important [38]. The analysis of formate by CE has however been rare [39,40] and its determination has not been associated with methanol intoxication.

The introduction of capacitively coupled contactless conductivity detection (C4D) [41,42] has opened another dimension for the analysis of low molecular weight compounds because their relatively high equivalent conductance provides a sensitive response in C4D. It is thus not surprising that the analysis of these compounds in biological samples by CE-C4D has recently obtained significant attention. The samples analyzed include saliva [43], blood plasma [44–49] and urine [50–52]. None of the presented methods described the analysis of formate, except a recent study by Tůma et al. [52]. In their work urine has been analyzed for a variety of organic acids in metabolic disorder screening, including formate. Unfortunately, formate has not been separated from fumarate and tartrate as it has not been the target analyte.

In this work we present a new method and background electrolyte (BGE) system in which fast and selective separation of formate is achieved in blood serum samples. The method is the fastest available, selective screening for formate, which can readily be applied for analysis of formate during the suspected methanol intoxication. The method was applied for the screening of formate during the recent methanol intoxication cases in the Czech Republic in September 2012.

2. Experimental

2.1. Material and methods

2.1.1. Electrophoretic system

A purpose-built CE instrument was employed for all electrophoretic separations. The separation voltage of −18 kV was provided by a high voltage power supply unit (Spellman CZE2000R, Spellman, Pulborough, UK). The separation capillaries used were fused silica (FS) capillaries (50 μm I.D., 375 μm O.D., 40 cm total length, 20 cm effective length, Polymicro Technologies, Phoenix, AZ, USA). Prior to the first use, the separation capillary was preconditioned by flushing with 0.1 M NaOH for 30 min, deionized (DI) water for 10 min and background electrolyte (BGE) solution for 10 min. Between two successive injections, the capillary was flushed with BGE solution for 1 min. At the end of a working day, the capillaries were washed with DI water for 10 min, followed by applying a vacuum for 5 min to remove any liquid from inside and stored dry overnight. All CE experiments were performed at ambient temperature.

2.1.2. Injection

Injection of standard solutions and blood serum samples was carried out hydrodynamically. The injection capillary end was immersed in a sample vial and elevated to a height of 10 cm for 20 s.

2.1.3. Detection system

A C4D was used for the detection of the separated analytes. It consisted of an external function generator (GW Instek GFG-8219A, New Taipei City, Taiwan) providing a sinusoidal excitation signal (frequency: 290 kHz, amplitude: 20 V peak-to-peak) to an in-house built detector cell [53] with a pre-amplifier (OPA655, Burr Brown, TX, USA). The amplified cell current was led to an external detector circuitry for further processing. Data were collected using Panther 1000 AD convertor.

2.2. Chemicals

2.2.1. Reagents, standards, electrolytes

All chemicals were of reagent grade and DI water (Purite, Neptune, Watrex, Prague, CR) was used for stock solution preparation and dilutions. 10 mM stock solutions of inorganic anions were prepared from their sodium salts (chloride, nitrate, sulfate, phosphate all from Pliva-Lachema, Brno, Czech Republic). Organic acids (formic, fumaric, tartaric, malonic, maleic, malic, succinic, acetic) were prepared from reagent grade chemicals (Sigma–Aldrich, Steinheim, Germany, Pliva-Lachema, Brno, Czech Republic). Lithium lactate was from Pliva-Lachema, Brno, Czech Republic. BGE for CE measurements was prepared daily by diluting 100 mM stock solutions of L-histidine (HIS, Sigma–Aldrich) and 2-(*N*-morpholino)ethanesulfonic acid (MES, Sigma–Aldrich) or 50 mM glutamic acid (GLU, Sigma–Aldrich) to the required concentration. Cetyltrimethylammonium bromide (CTAB, Sigma–Aldrich) was prepared as 10 mM stock solution in 5% acetonitrile and was added to the BGE to yield the final concentration of 30 μM.

2.2.2. Sample preparation

Blood serum samples were obtained from Department of Anaesthesiology, Resuscitation and Intensive Care, Hospital and Policlinics Havířov. Before injection into the CE instrument, the serum samples were diluted 1:100 with DI water. No other treatment was necessary. The lyophilized serum samples were purchased as lyophilized powders from Sigma and prepared according to supplier's instructions. All samples were stored at −20 °C.

3. Results and discussion

3.1. Optimization of the electrolyte system

Low molecular weight organic acids were previously separated using a MES/HIS electrolyte with pH 6 by Law et al. [54]. At this pH, comigration of several acids exists due to their similar electrophoretic mobilities. To separate the comigrating acids, MES/HIS electrolyte was modified with 0.025% HP-β-CD and 10% methanol. 16 organic acids were separated in 14 min, but the separation system was not applied to the analysis of biological samples. Tůma et al. [52] have separated 29 organic acids in urine, however the critical analytes for this study (formate, fumarate and tartarate) were not resolved. In here, we have optimized the background electrolyte (BGE) composition to achieve fastest possible separation of