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DETERMINATION OF IONIC METABOLITES FROM ETHYLENE GLYCOL IN HUMAN BLOOD BY ISOTACHOPHORESIS

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SUMMARY

Conditions for isotachophoretic determination of anionic metabolites in blood from ethylene glycol in poisoned humans were established. Leading electrolytes with 5 m*M* chloride and a pH between 2.3 and 2.5 gave good separation. Optimal separation was found with leading electrolytes at pH 2.5, and 10 m*M* acetic acid as terminating electrolyte. Separation and quantification of four out of six metabolites were possible. The four were glycolic acid, glyoxylic acid, oxalic acid and formic acid. Besides these compounds, citric acid, lactic acid, and α- and β-hydroxybutyric acid were separated and quantified. The formation of mixed zones did not give any serious problems, although in samples with high amounts of glycolic acid we had to reduce the maximum injected amount from 3 to 1 μl. This method might be valuable in further studies of the mechanism of ethylene glycol toxicity and as an important supplement in the diagnosis of late stages of ethylene glycol poisoning in which the glycol has been metabolized to glycolic acid.

INTRODUCTION

Ethylene glycol is a chemical widely used both in industry and among the public. It is best known as a component in many antifreeze solutions [1]. Ethylene glycol has a moderate oral toxicity [2] and has occasionally been drunk as a substitute or mistaken for ethanol. Ethylene glycol represents a danger to human health both among the public and in occupationally exposed workers.

Ingestion of ethylene glycol is followed by development of metabolic acidosis

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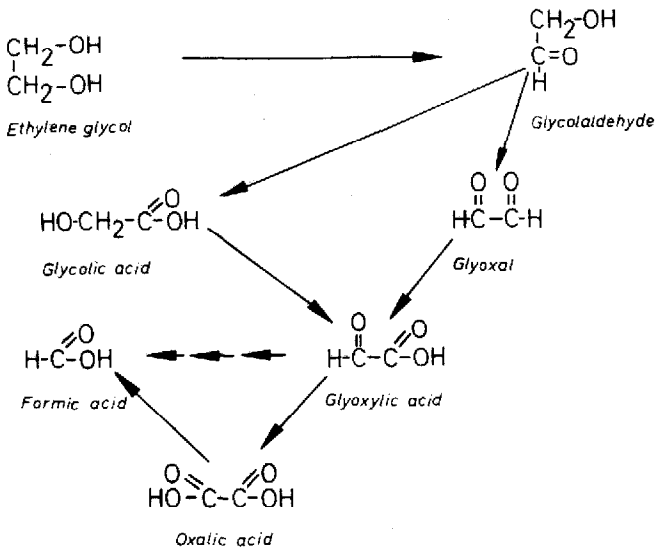

Fig. 1. Biotransformation of ethylene glycol. Several arrows mean intermediate products not shown here.

[3,4] due to an accumulation of organic anions, which is found both in experimental animals [2,3,5] and poisoned humans [6]. However, the need for easier and more accurate analytical methods is demonstrated by recent case reports and studies of ethylene glycol toxicity where measurements of metabolites are missing [8–11]. This is particularly striking since it is agreed that the metabolites are responsible for the main toxic effects [3,4,6,7].

Ethylene glycol gives rise to several biotransformation products (Fig. 1). The ionic metabolites are difficult to extract quantitatively with organic solvents, which is a practical way to concentrate and prepurify biological samples, e.g. plasma and serum. Gas chromatographic methods devised for determination of ethylene glycol metabolites include both laborious extraction steps and a derivatization step [3,5]. Extraction difficulties limit the choice of analytical methods and, furthermore, the metabolites have no absorbance at 254 nm, which is often used for detection in analytical methods, e.g. high-performance liquid chromatography (HPLC).

Analytical isotachophoresis represents an alternative method and has been shown in several studies to be well suited for direct analysis in crude biological samples, such as plasma, serum and urine [12–14]. The conductivity detector used in isotachophoreses is an universal detector for ionic compounds.

The present study describes an isotachophoretic method for direct quantification of ions found in plasma from ethylene glycol-poisoned patients.

EXPERIMENTAL

All chemicals were pro analysi. In all reagents we used deionized water. Hydroxypropylmethylcellulose (HPMC) was obtained from Dow (Midland, MI, U.S.A.) and Triton X-100 from Sigma (St. Louis, MO, U.S.A.).

TABLE I

OPERATIONAL SYSTEMS

System	Leading ion	Counter-ion	pH	Terminating electrolyte
A	5 m*M* Cl^*	H_3O^+	2.30	10 m*M* Acetic acid
B	5 m*M* Cl^{**}	H_3O^+	2.52	10 m*M* Acetic acid
C	5 m*M* Cl^{***}	β-Alanine	2.85 and 3.14	10 m*M* Acetic acid
D	5 m*M* Cl^{***}	β-Alanine	2.85 and 3.14	5 m*M* *n*-Caproic acid

*5 m*M* Hydrochloric acid.
**3 m*M* Hydrochloric acid and 2 m*M* sodium chloride.
***Adjusted to the desired pH with β-alanine.

Blood samples were collected in Venoject vacutainer tubes (Terumo, Haasrode, Belgium).

Analytical isotachophoresis analysis was performed with a LKB (Bromma, Sweden) 2127 Tachophor equipped with both a conductivity and a UV detector. The volume injected varied from 1 to 15 μl. The PTFE capillary was between 24 and 26 cm long (measured from point of injection to the UV detector) with an I.D. of 0.5 mm. The capillary and electrolyte chambers were often washed with detergents. In most cases the analysis was started with a driving current of 200 μA, which was decreased to 100 μA prior to detection. The effect applied was kept below 1.2 W.

Operational systems used are shown in Table I. To all electrolytes was added 0.2–0.4% HPMC. In some experiments 0.1% Triton X-100 was included in addition to HPMC.

RESULTS

Effect of Triton X-100 in leading electrolytes

When measurements were performed with the leading electrolyte at a pH value below 3.0, the applied power was high and small air bubbles often became trapped, leading to narrowing of the capillary and local overheating. This was avoided by adding Triton X-100, which wetted the surface and prevented the bubbles sticking to the PTFE capillary. Unfortunately Triton X-100 interfered with both α- and β-hydroxybutyric acid, so quantification of these anions was impossible with Triton X-100 in the leading electrolyte.

Analysis of plasma, blood and serum at different operational systems and pH

Fig. 1 shows the biotransformation of ethylene glycol. Two metabolites, glycolaldehyde and glyoxal, are not ionic and therefore cannot be determined by analytical isotachophoresis.

The isotachophoretic analysis of a plasma sample with two different operational systems is shown in Fig. 2. One operational system, leading pH 2.3, but different matrices (Fig. 3), shows greater variations in zone length. External

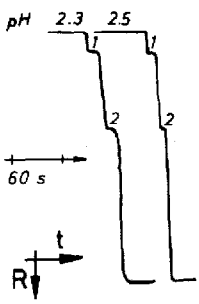

Fig. 2. Isotachopherograms for the separation of anions in heparinized plasma at three different pH values of leading electrolyte. The operational systems are (from left) A and B (Table I). R = Increasing resistance; t = increasing time; i = 100 μA. Peaks: 1 = phosphate; 2 = lactate.

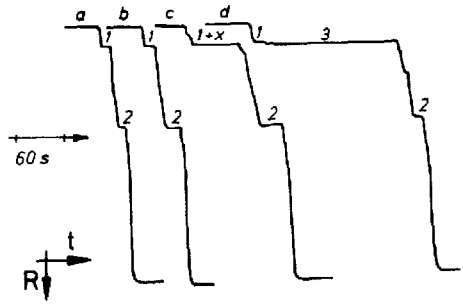

Fig. 3. Isotachopherograms for the separation of anions in blood-derived samples, operational system A (Table I). The following samples were analysed: a = heparinized plasma; b = serum; c = hemolysed blood; d = EDTA plasma. R = Increasing resistance; t = increasing time; i = 100 μA. Peaks: 1 = phosphate; 2 = lactate; 3 = EDTA; x = unknown.

ionic additives used to prepare plasma are oxalate, citrate and EDTA. These compounds are normally added in such large amounts that the final concentrations in the sample are several millimoles per litre. These additives disturb the analysis and should therefore be avoided. Oxalate is in addition a metabolite of ethylene glycol and should not be used for preparing plasma. Use of citrate and EDTA also increased the occurrence of mixed zones. An example of analysis of EDTA plasma is given in Fig. 3d. Heparinized plasma and serum are best to use since no external anionic compounds are added.

Separation conditions for ethylene glycol metabolites

Separation of a standard solution containing metabolic products from ethylene glycol and anions normally found in blood is shown in Fig. 4. This separation was achieved with 5 mM chloride as leading electrolyte at both pH 2.3 and 2.5. With n-caproic acid as terminating ion we did not obtain separation at pH 2.85 but at pH 3.14 (system D). We injected at least 3 nmol of each of the ten chemicals shown in Fig. 4.

An example of the analysis of plasma from an ethylene glycol-poisoned patient is shown in Fig. 5. In plasma from unpoisoned persons, glycolic acid (zone 2) is not found and the presence of β-hydroxybutyric acid (zone 4) depends on other external factors. Phosphate and lactic acid are always present in plasma. For a description of the clinical picture see ref. 6.

The low pH used in these experiments resulted in long analysis times but stable zones. As can be seen in Fig. 6, the zone length per nmol was longer at low pH and therefore also gave lower detection limits.

Leading electrolyte with pH 2.5 and 2.3 was the best operating system for determination of the following acids in blood: oxalic acid, phosphoric acid, glyoxylic acid, citric acid, formic acid, glycolic acid, lactic acid, and α- and β-hydroxybutyric acid. We recommend pH 2.5.

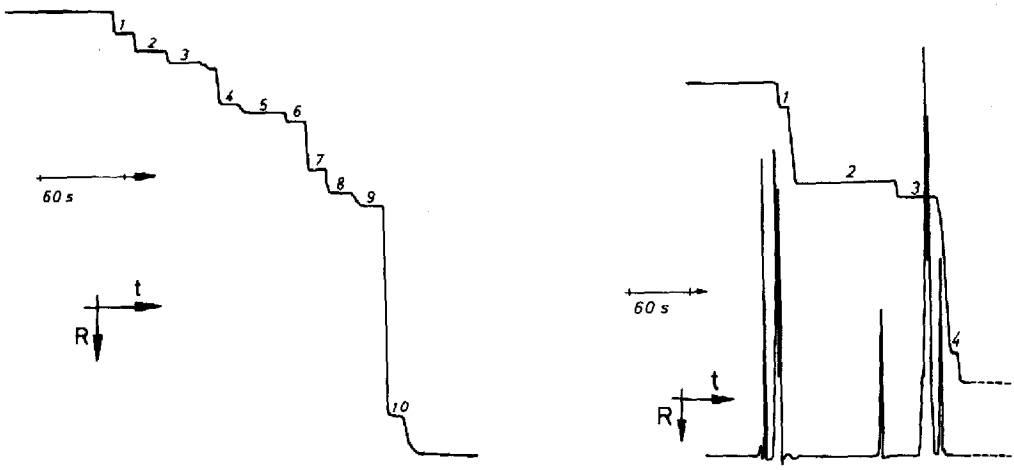

Fig. 4. Isotachopherogram for the separation of ten standard acids in water, operational system B (Table I). R = Increasing resistance; t = increasing time; i = 100 μA. Peaks: 1 = oxalic acid; 2 = phosphate; 3 = EDTA; 4 = glyoxylic acid; 5 = citric acid; 6 = formic acid; 7 = glycolic acid; 8 = lactic acid; 9 = α-hydroxybutyric acid; 10 = β-hydroxybutyric acid.

Fig. 5. Isotachopherogram for the separation of anions in heparinized plasma from an ethylene glycol-intoxicated patient, operational system B (Table I). R = Increasing resistance; t = increasing time; i = 100 μA. Peaks: 1 = phosphate; 2 = glycolate; 3 = lactate; 4 = β-hydroxybutyric acid.

Precision and recovery

Although no buffer was added to the leading electrolyte at pH 2.5 and 2.3, the quantification was quite accurate. Addition of glycolic acid to a plasma sample from an unpoisoned subject giving a final concentration of 7 mM gave a recovery of 106 ± 3% and a coefficient of variation (C.V.) of 2.9% (n = 10). Determination of oxalic acid and glyoxylic acid was possible with the method, but in samples

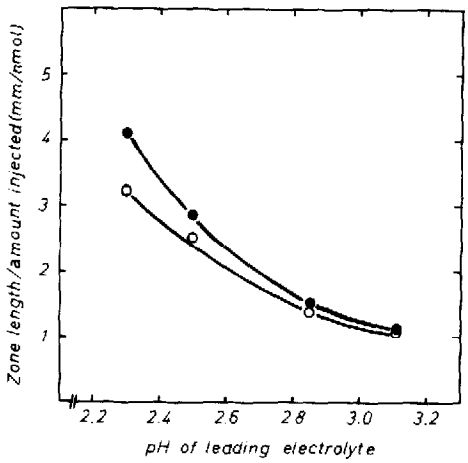

Fig. 6. Zone length as a function of pH of the leading electrolyte. All experiments performed with 5 mM chloride in the leading electrolyte at the indicated pH, operational system A, B and C (Table I) respectively. Current at determination was 100 μA. (●) Glycolic acid; (○) formic acid.

TABLE II

STANDARD CURVES

$y = kx + a$; r = correlation coefficient. Measured with leading electrolyte 5 mM hydrochloric acid at pH 2.5 and 10 mM acetic acid as terminating electrolyte, operational system B (see Table I).

Ion determined	k* (mm/nmol)	a (mm)	r
Oxalic acid	3.10	0.25	0.9998
Citric acid	2.36	−0.40	0.9995
Formic acid	2.50	1.46	0.9999
Glycolic acid	2.86	−0.14	0.9988
Glyoxylic acid	2.60	0.45	0.9918
Lactic acid	3.38	1.63	0.9985
α-Hydroxybutyric acid	2.89	0.59	0.9989
β-Hydroxybutyric acid	3.70	−0.19	0.99999
EDTA	5.12	1.52	0.9990

*Current 100 μA and chart recorder at 0.5 mm/s, which means that 1 mm/nmol = 2 s/nmol.

from ethylene glycol-poisoned patients values below 0.2–0.3 mM are found [6]. Concentrations that are low are near the detection limit for the method, and therefore the accuracy is not good.

Characteristics of calibration graphs in the range 1–7 nmol are given in Table II.

Formation of mixed zones

The following anions are normally found in blood in concentrations less than 1 mM: oxalate, citrate, formate and α- and β-hydroxybutyrate. At such low concentrations no mixed zones are normally formed. At pH 2.5 in system B, mixed zones were formed between citrate and glyoxylic acid when more than 5 nmol of each were present, a situation not found in real samples. Lactic acid and glycolic acid also formed mixed zones if more than 15 nmol of each were present. Since high concentrations of glycolic acid and lactic acid were found in samples from poisoned patients, this problem was encountered when 3 μl were injected. No mixed zones were found when a 1-μl sample was used. Experiments with EDTA plasma gave mixed zones between EDTA and phosphate.

Other detector systems

Only oxalic acid has absorbance at 254 or 206 nm, so there was no advantage in using UV absorbance at these wavelengths instead of the conductivity detector.

DISCUSSION

The method described has been used to study metabolites in blood from ethylene glycol-poisoned humans, and it was found to be sufficiently sensitive for toxicological studies [6].

We propose that analytical isotachophoresis can be used as a first-hand ana-

lytical tool in the diagnosis of metabolic acidosis of unknown origin. First, the method is simple and relatively rapid since it requires no pretreatment or extraction of the plasma samples. Second, in late stages of methanol and ethylene glycol poisoning the original toxic agent ingested may be completely metabolized. In previous studies we have reported cases where high concentrations of formate and glycolate were observed although no methanol and very low ethylene glycol concentrations were found [6,15]. Hence only identification of the metabolite will provide the definitive diagnosis in such cases. Third, the clinical symptoms are related to accumulation of metabolites. Methanol and ethylene glycol would be relatively non-toxic if they were not metabolized. Therefore the prognosis and clinical course can be better assessed after measurement of the toxic metabolites.

A future use of isotachophoresis is in the study of metabolism of glycols. Of special interest should be the glycol ethers, lately shown to have toxic effects on *reproduction [16]*.

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