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Short communication

Thiamine analysis in biological media by capillary zone electrophoresis with a high-sensitivity cell

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Abstract

A capillary zone electrophoresis method with high-sensitivity cell (Z-cell) has been developed for the determination of thiamine in biological media (plasma, urine, saliva). The urine samples were diluted (1:1, v/v) in water and were directly injected into the apparatus. For the quantitative assay of thiamine in plasma it is necessary to precipitate the protein component. Good results were achieved by treating the sample with acetonitrile (1:3, v/v). Using a capillary with high sensitivity cell led to an approximately nine-fold improvement of the detection limit compared to standard capillaries and four-fold improvement compared to capillary with bubble cell. The samples in the biological media were analysed using a calibration curve for thiamine concentrations between 0.1 and 200 μg ml^{-1}. The detection limit, the effective mobility and the relative standard deviation of the migration times and of the peak areas were determined. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Thiamine

1. Introduction

The determination of thiamine is often performed by reversed-phase high-performance liquid chromatography (RP-HPLC) [1–3]. Agostini and Godoy determined the thiamine concentration in various organisms ranging from plankton to fish in the lake Ontario (North America) ecosystem by HPLC with scanning fluorescence detection [4]. Hongmei et al. determined vitamins in suaeda salsa using HPLC [5]. Furthermore, thiamine was investigated in pharmaceutical preparation, in brown rice flour, in dried yeast and in baby foods using HPLC [6]. Several papers have demonstrated the application of high-performance electrophoretic techniques for the determination of water-soluble vitamin [7–9]. Fotsing et al. investigated the determination of water-soluble vitamins in pharmaceutical formulations by capillary zone electrophoresis (CZE) [10]. Using capillary electrophoresis (CE) thiamine was also determined in native citrus juice and fruits and in food [11,12]. The determination of thiamine in biological media using CZE has not yet been reported. CZE with high-sensitivity cell [13] turned out to be a new technique for thiamine analysis in biological media. The plasma samples were only diluted with water and then injected without any further sample preparation. The performance of the CZE approach for the

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determination of thiamine was tested by measuring the detection limit at 200 nm and the relative standard deviation (RSD) of the migration times and of the peak areas.

2. Experimental

2.1. Apparatus

CE experiments were performed on a Hewlett-Packard Model G1600A (Waldbronn, Germany) ^{3D}CE system with diode-array detector from 190 to 600 nm. A CE ChemStation equipped with a HP Vectra 486/66U workstation was used for instrument control, data acquisition and data analysis. The system was controlled by windows software, which was modified to the HP system. The detection wavelength was 200 nm. A standard capillary (fused-silica) of 80.5 cm (length to detector 72 cm)×75 μm I.D., a capillary with bubble cell (optical path length is 150 μm) and a capillary with high-sensitivity cell (Z-cell) obtained from Hewlett-Packard, with a 8.5 cm outlet, 72 cm effective length×75 μm I.D. were used for the determination of thiamine.

2.2. Chemicals

Thiamine (Fig. 1) was obtained from Fluka (Buchs, Switzerland). Acetone, methanol, ethanol, acetonitrile, potassium hydrogenphosphate and potassium dihydrogenphosphate were obtained from Merck (Darmstadt, Germany).

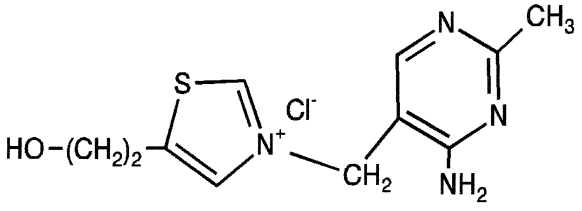

Fig. 1. Chemical structure of thiamine.

2.3. Sample preparation

Standard solutions of thiamine were prepared from 0.01 to 400 μg ml^{-1} in water. Human urine was diluted with standard solutions containing a known amount of thiamine. The urine samples were filtered through a 0.45-μm syringe filter and injected immediately into the apparatus. Plasma samples (1 ml) containing 0.05–600 μg ml^{-1} thiamine were mixed with acetonitrile (1:3, v/v). These mixtures were centrifuged at 2200 *g* for 10 min. The supernatant was filtered through a 0.45-μm syringe filter and injected.

2.4. Buffer preparation

For CE, a 10 m*M* phosphate buffer solution (pH 7.2) was prepared by dissolving 1.05 g potassium hydrogenphosphate and 0.53 g potassium dihydrogenphosphate in water, filling up to a volume of 1000 ml. The pH of the buffer was measured at 25°C using a HI 9321 microprocessor pH meter (Hana Instruments). The buffer solution was filtered through a 0.45-μm syringe filter and degassed by ultrasound for at least 10 min before use.

2.5. Recovery study

Solutions of plasma samples containing 10 and 100 μg ml^{-1} of thiamine were prepared. These plasma samples were mixed with acetonitrile and centrifuged (see Section 2.3). The resulting peak areas were compared with peaks resulting from aqueous solutions at the same concentrations.

2.6. Analysis conditions

A new capillary was washed for 10 min with NaOH (1.0 *M*) at 40°C, followed by washing for 10 min with water at the same temperature and for 5 min with water at 25°C. Before each injection, the capillary was flushed with 0.1 *M* NaOH for 5 min and with the actual buffer solution for 5 min. The temperature was kept at 25°C, a separation potential

of 30 kV was used. Acetone was used as a marker substance for the determination of the electroosmotic mobility. The samples (buffer–acetone, 99:1) were injected at a pressure of 50 mbar for 5 s (hydrodynamic injection). Detailed experimental conditions are given in the legends to the figures.

3. Results and discussion

In this paper we studied the separation and the determination of thiamine in aqueous solution and in biological media. The determination of thiamine in aqueous solution was performed using a standard capillary, a capillary with bubble cell and a capillary with a high-sensitivity cell. The results showed that the application of a capillary with high sensitivity cell led to an improvement of the detection limit of about four-fold over a capillary with bubble cell and of about nine-fold over a standard capillary (Fig. 2).

CZE utilising a high-sensitivity cell is very suitable for the analysis of thiamine due to its UV absorption and good solubility in water. This is also the case for the analysis of thiamine in biological tissues and other body liquids at low concentrations. In our studies, thiamine had a positive electrophoretic mobility ($+18.5\cdot10^{-5}$ cm^2 V^{-1} s^{-1}). The

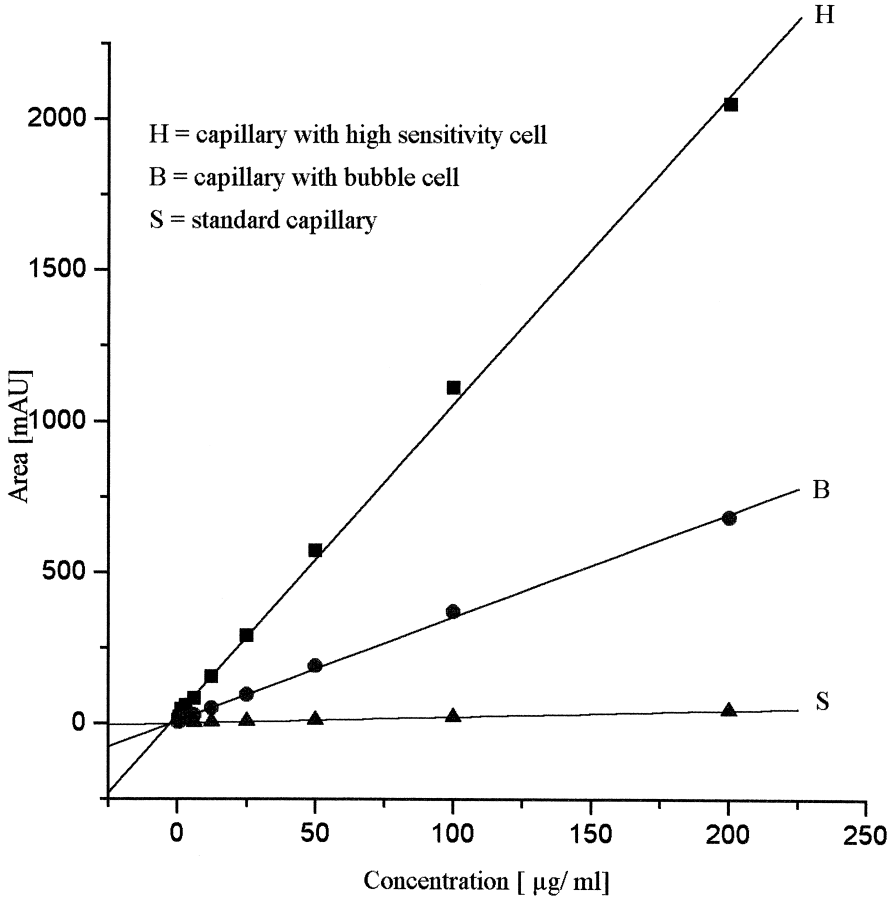

Fig. 2. Calibration graph for determination of thiamine.

effective mobilities and the electroosmotic mobility were calculated at pH 7.2 in water as described by Weinberger [14]. In saliva and water thiamine showed good repeatability and good detection limit (see Fig. 3 and Table 1). The detection limits for this analyte was determined using the following equation [13]: $L=3NC/h$ where N=noise (mAU), C=sample concentration ($\mu g\,ml^{-1}$), h=peak height (mAU).

The determination of thiamine in urine was performed at a pH value of 7.2. The results of these measurements showed that at this pH value thiamine was separated from urine components and detected (Fig. 4). For measuring thiamine in urine, the samples for CZE were diluted in water 1:1 and were injected into the apparatus without any further sample preparation.

In plasma, thiamine could also be analysed quantitatively using two methods. (I) The samples were diluted in water 1:2 and were injected into the apparatus. The detection limit in plasma through direct measuring at 200 nm is 5 $\mu g\,ml^{-1}$ and the RSD of the migration times is 4% and of the peak areas 6% at a concentration of 10 $\mu g\,ml^{-1}$. The direct determination of thiamine in plasma using CZE has some advantages compared to liquid chro-

Table 1
Analytical parameters of thiamine with high sensitivity cell (Z-cell)

	RSD(t)[a] (%)	RSD(f)[b] (%)	Detection limit ($\mu g\,ml^{-1}$)
Water	0.5	1.8	0.05
Saliva	0.8	2.0	0.05
Plasma	1.8	4.0	0.80
Urine	1.1	3.0	0.10

[a] RSD(t)=Relative standard deviation of the migration times.
[b] RSD(f)=Relative standard deviation of the peak areas.

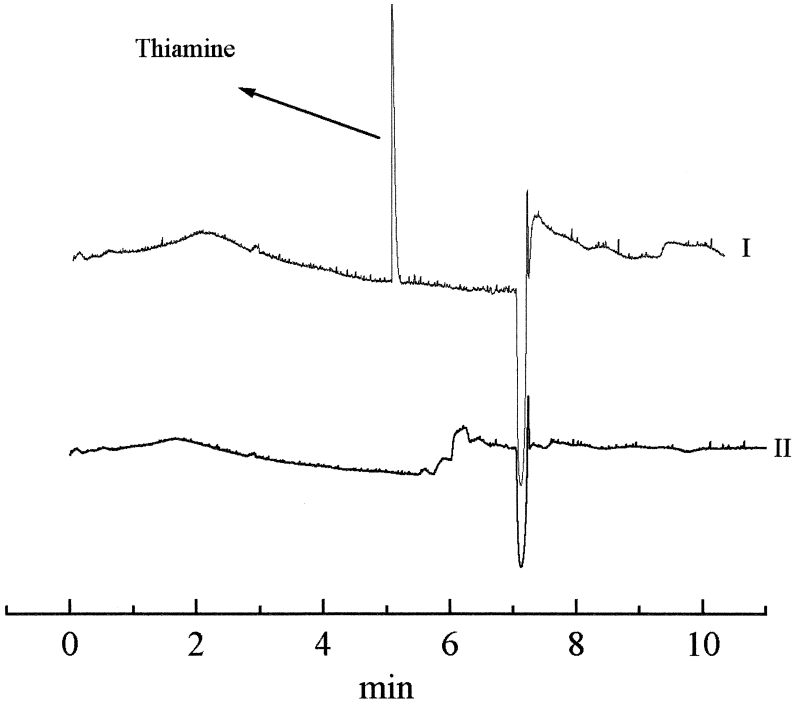

Fig. 3. Electropherogram of a saliva sample containing 10 $\mu g\,ml^{-1}$ of thiamine (I) and of a blank saliva standard (II). Buffer: pH 7.2, 10 m*M* phosphate; a capillary with high-sensitivity cell with an 8.5 cm outlet, 72 cm effective length×75 μm I.D.: 30 kV; temperature: 25°C: pressure injection: 5 s at 50 mbar; detection: 200 nm.

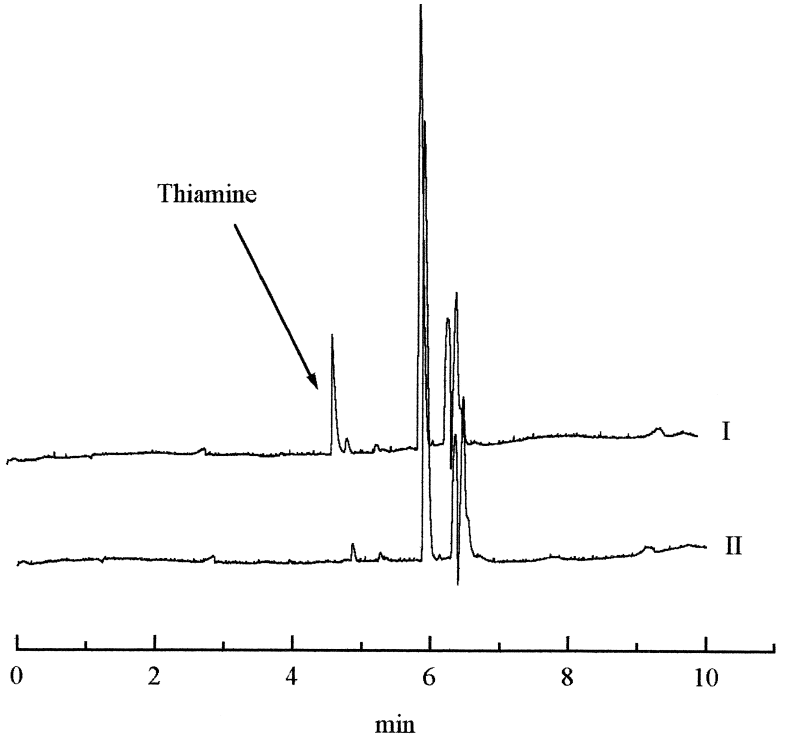

Fig. 4. Electropherogram of a urine sample containing 10 μg ml^{-1} of thiamine (I) and of a blank human urine sample (II). Conditions as in Fig. 3.

matography (LC), particularly concerning the sample preparation. Usually, for the determination of thiamine in plasma by LC, it is essential to precipitate the protein component before the measurement. (II) Thiamine was determined after protein precipitation. Good results were achieved by treatment of the plasma samples with acetonitrile (1:3, v/v) (Fig. 5). Fig. 5 shows electropherograms of a blank plasma sample and of a plasma sample containing 10 μg ml^{-1} thiamine at pH 7.2. The electropherograms demonstrate that the thiamine peak is completely separated from plasma components. The calibration curve of the area was linear with a correlation coefficient of $r=0.999$. Mean recoveries from spiked samples were 96.6% ($n=3$) at a thiamine concentration of 5 μg ml^{-1} and 95.8% ($n=3$) at a concentration of 50 μg ml^{-1} by comparison with thiamine standard solutions of equivalent concentrations. To control the repeatability of the peak areas and of the migration times, three injections of thiamine (10 μg ml^{-1}) were made for these samples. Good reproducibility of the peak areas and of the migration times (Table 1) was obtained.

4. Conclusions

CZE with a high-sensitivity cell (Z-cell) was developed for the determination of thiamine in biological media. The CZE technique with the new cell is easy to handle and shows good reproducibility. The high sensitivity, the low amount of sample required and the relatively short analysis time were the main advantages of this method. Therefore, this technique can be useful for clinical and medical researchers interested in measuring thiamine in urine and plasma samples.

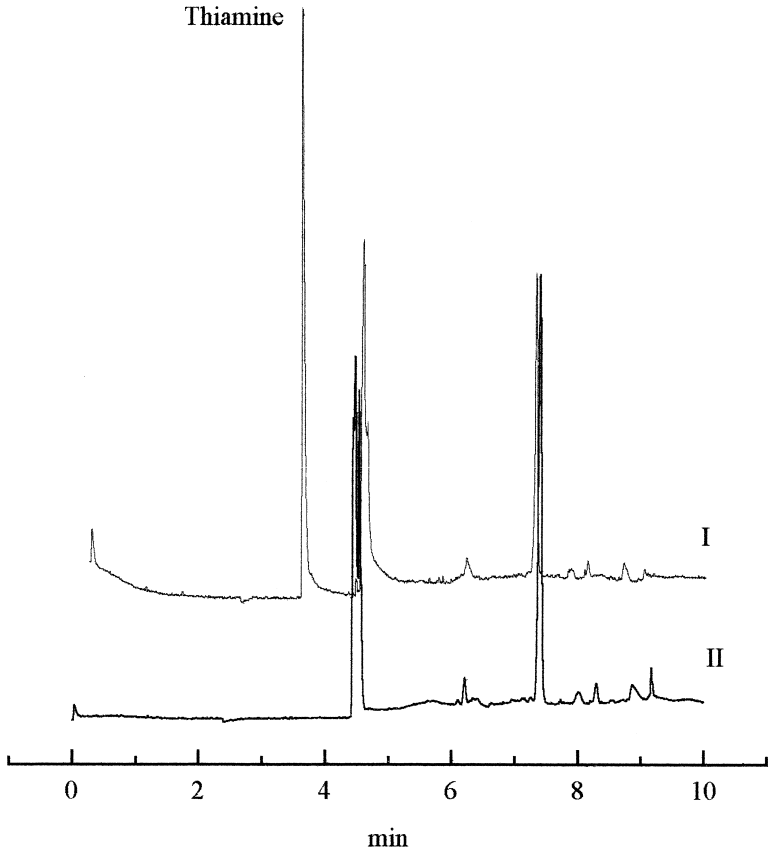

Fig. 5. Electropherogram of a plasma sample containing 10 μg ml^{-1} of thaimine (I) and of a blank plasma sample (II). Conditions as in Fig. 3.

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