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Short Communication

Use of capillary zone electrophoresis in the determination of B vitamins in pharmaceutical products

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ABSTRACT

The simultaneous determination of several water-soluble vitamins is difficult and often many different analyses have to be done. Capillary zone electrophoresis (CZE) has been claimed to provide a sensitive and high-resolution method for the determination of different kinds of biomolecules. In this study the B vitamins thiamine (B_1), riboflavin (B_2), pyridoxal (B_6), pyridoxine (B_6) and pyridoxamine (B_6) in a pharmaceutical product were determined simultaneously using CZE. An HCl solution was used for the extraction of the vitamins from a multivitamin–multimineral tablet. Electrophoretic experiments were performed with ISCO Model 3850 apparatus equipped with a UV detector. The applied potential was 6.0 kV and 75-μm fused-silica capillary tubing was used. A 5-μl sample was injected via split injection. The electrolyte was 0.020 *M* sodium phosphate buffer (pH 9.0). Vitamins were recorded at 254 nm. Preliminary results clearly pointed out the feasibility of the simultaneous determination of water-soluble B vitamins in pharmaceutical products by CZE. The relative standard deviation, however, varied from 2.1 to 6.3%.

INTRODUCTION

Vitamins are minor essential constituents of food, required for the normal growth, maintenance and functioning of the human body. Among water-soluble vitamins, the B vitamins are the most important. They function as the coenzyme of several important enzymes (thiamine, B_1), the prosthetic group of flavin enzymes (riboflavin, B_2) and other enzymes (pyridoxine, pyridoxal, pyridoxamine, B_6). The lack of these vitamins can cause, *e.g.*, beri-beri disease, a decrease in glutathione reductase activity in red blood cells and disorders in protein metabolism [1]. Vitamins might, however, be lost through chemical reactions or by extraction and leaching during storage and processing of food, which is really the case with water-soluble vitamins. This is why it is extremely important to have available a preparation to supply the possible lack of the vitamins in a daily diet.

Numerous publications have appeared on the determination of individual B vitamins using widely different physical, chemical and biological methods [2–16]. The high-performance liquid chromatographic (HPLC) separation and determination of vitamins of the B group is well documented. None of the described HPLC or non-chromatographic methods, however, satisfies the requirements for quality control of multivitamin preparations. Especially the separation and determination of vitamin B_6 is complicated [14].

The simultanous determination of several water-soluble vitamins has been a difficult task. Capillary zone electrophoresis (CZE) has been claimed to provide a sensitive and high-resolution method for

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TABLE I

REPRODUCIBILITY OF CZE DETERMINATION OF VITAMINS B_1, B_2 and B_6 CARRIED OUT WITH THE PURE STANDARDS

Vitamin	Retention time			Peak area		
	Mean[a] (min)	S.D. (min)	R.S.D. (%)	Mean[a]	S.D.	R.S.D. (%)
Thiamine	4.74	0.03	0.6	12.18	0.36	2.9
Pyridoxamine	5.38	0.06	1.0	14.73	0.78	5.3
Riboflavin	6.17	0.05	0.8	36.32	0.76	2.1
Pyridoxine	6.45	0.06	1.0	12.39	0.79	6.3
Pyridoxal	6.76	0.08	1.2	24.10	1.17	4.9

[a] $n = 10$.

analysing different kind of biomolecules [17]. In this study the B vitamins thiamine (B_1), riboflavin (B_2), pyridoxal (B_6), pyridoxine (B_6) and pyridoxamine (B_6) in a pharmaceutical product were determined simultaneously by CZE.

EXPERIMENTAL

Materials

A multivitamin–multimineral tablet, Multi-tabs (Rohto, Tammisaari, Finland), was homogenized, extracted with 0.1 *M* HCl and filtered with a 0.45-μm filter. The five vitamin standards were obtained from Sigma (St. Louis, MO, USA) and Hoffman-LaRoche (Basle, Switzerland) and were diluted in 0.1 *M* HCl (25 μg/ml of each vitamin).

Methods

Electrophoretic experiments were performed with ISCO (Lincoln, NE, USA) Model 3850 electropherograph equipped with an on-column detector connected to Hewlett-Packard (Avondale, PA, USA) Model 3393 A integrator. The applied potential was 6.0 kV and the fused-silica tubing (Waters–Millipore, Milford, MA, USA) was 51 cm × 75 μm I.D. with a distance of 33 cm to the UV detector.

Samples were injected via split injection. While the injection volume was 5 μl at a splitting ratio of 1:1700, the actual sample size was 3 nl (*ca.* 75 pg of each vitamin). The electrolyte was 0.020 *M* sodium phosphate buffer (pH 9.0). Vitamins were recorded at 254 nm and determination was effected by the external standard method.

RESULTS AND DISCUSSION

Table I shows the reproducibility of the CZE method. Both retention times and peaks areas were used for the calculations. Results were obtained by using pure vitamin standards. Fig. 1A presents a typical electropherogram of the separation of the

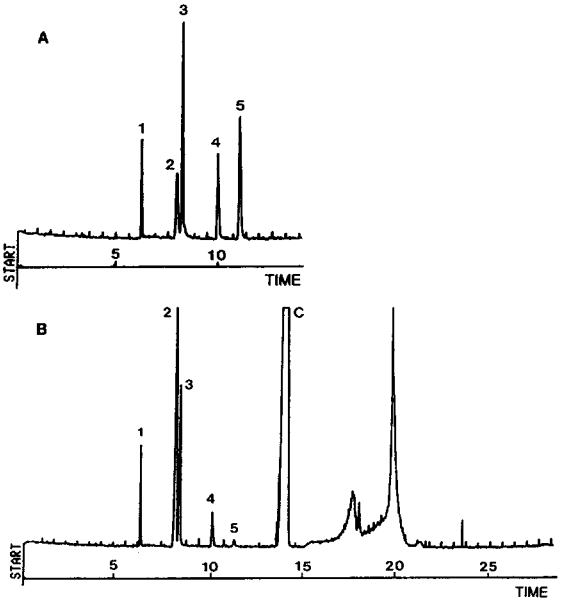

Fig. 1. Capillary electropherograms of (A) the standard solution of five B vitamins and (B) the pharmaceutical sample. For conditions, see Experimental. 1 = Thiamine (B_1); 2 = pyridoxamine (B_6); 3 = riboflavin (B_2); 4 = pyridoxine (B_6); 5 = pyridoxal (B_6); C = ascorbic acid.

five vitamin standards and Fig. 1B shows the same separation when the sample was a pharmaceutical tablet. Other water-soluble vitamins (ascorbic acid) also could be detected.

In comparison with the existing quantitative HPLC methods for the vitamin B group, the CZE method described has advantages with respect to elution time and simplicity. The reproducibility, however, was poor, the relative standard deviation (R.S.D.) for peak areas varying from 2.1 to 6.3%. Even worse results were obtained, with an R.S.D. of *ca.* 16.0%. The high fluctuation was evidently due to the split injection. Other techniques were not tested. From experience it is also known that a manually operated injection system such as that in the ISCO instrument decreases the reproducibility compared with automated instruments. This is why this method cannot applied to the determination of trace amounts of vitamins, *e.g.*, in foodstuffs. The R.S.D. was considerably lower for the retention times of the vitamins, varing from 0.6 to 1.2%.

These preliminary results clearly indicate the feasibility of the simultanous CZE determination of water-soluble vitamins in pharmaceutical products, where the concentrations of these vitamins are high. However, quantification might still be a problem because of the low concentration of the analytes in the UV cell.

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