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# Application and optimization of capillary zone electrophoresis in vitamin analysis

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**Abstract**

The application of capillary zone electrophoresis to the determination of ascorbic acid and biotin in vitamin mixtures (pharmaceutical formulations and also native citrus juice and fruit beverage) was investigated. A method was developed for determining them together with thiamine, nicotinamide and nicotinic acid in a single run. For ascorbic acid, addition of L-cysteine as antioxidant was necessary to obtain reliable results. The performance of the method was tested according to sensitivity, detection limit and standard deviation.

## 1. Introduction

The determination of vitamins in pharmaceutical formulations is very important with regard to the standardization and to the monitoring of the stability of such formulations. In recent years, mainly HPLC techniques have been used for this purpose. Nowadays interest is focused on the use of capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC) and isotachophoresis (ITP) for the determination of pharmaceuticals and drugs due to their high efficiency [1]. Although the chemical structure of specific vitamins is very different, these methods are very useful also in determining water-soluble vitamins. Recently, several papers have demonstrated the application of high-performance electrophoretic techniques for the determination of vitamins of the B group and niacin [2–6] and also for vitamin C [6–8]. The determination of biotin (vitamin H) by means of CZE has not yet been reported.

The application of CZE for the determination of ascorbic acid and biotin was of special interest to us. The speciation and the determination of the stability of these substances in vitamin mixtures (pharmaceutical formulations and native samples) was the aim of the present studies. Therefore, it was necessary to develop a method for determining several vitamins in a single run. The performance of the developed CZE method for the determination of ascorbic acid and biotin in beverages and pharmaceutical preparations was tested with regard to sensitivity, detection limit and standard deviation.

## 2. Experimental

### 2.1. Apparatus

A Hewlett-Packard (Waldbronn, Germany) $^{3D}$CE system fitted with a 600 mm (515 mm to

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the detector) × 0.05 mm I.D. (extended light path) fused-silica capillary and an on-column diode-array detector (190–600 nm) was used in all measurements.

### 2.2. Chemicals

Thiamine hydrochloride, pyridoxine, nicotinamide, riboflavin-5′-phosphate, nicotinic acid (all BioChemika grade) and L-ascorbic acid, L-cysteine, D-(+) biotin (all analytical-reagent grade) were obtained from Fluka (Buchs, Switzerland). Phosphate buffers of pH 5, 6, 7 and 8, all 20 m*M*, were prepared using analytical-reagent grade potassium dihydrogenphosphate and sodium hydrogenphosphate dihydrate (Fluka). Doubly distilled water was used throughout.

### 2.3. Sample and buffer preparation

The standard vitamins were dissolved in water so as to have concentrations in the range 1–500 μg/ml. In the case of vitamin C, a 10 m*M* concentration of L-cysteine was added for stabilization. The vitamin preparations were dissolved in water. Fruit juices were diluted in water if necessary. The solid preparation containing biotin was dissolved in water and filtered. All samples and the buffers were filtered through a 0.2-μm syringe filter and injected immediately. Buffers were degassed by ultrasound for at least 10 min before use.

### 2.4. Analysis conditions

The capillary was preconditioned for 10 min with 1.0 *M* NaOH before the first run and then for 2 min with 0.1 *M* NaOH and for 3 min with run buffer prior to each following run. The standard separation conditions were voltage (detection end) −30 kV, pressure injection 200 mbar s, capillary temperature 25°C and 20 m*M* phosphate run buffer of pH 8.0.

## 3. Results and discussion

### 3.1. Set-up of the method

For the optimization of the separation conditions, the pH must be chosen first so as to have the analytes in their respective ionic forms. Fig. 1 shows the net ionic charge of the five vitamins thiamine, nicotinamide, biotin, ascorbic acid and nicotinic acid calculated from the $pK_a$ values given in the literature.

Consequently, a pH >4.5 should be maintained to form anions from the acids. Nicotinamide in these media occurs as a neutral compound and cannot be separated and determined in real samples. Nevertheless, we used nicotinamide as a neutral marker in determining the ionic mobility of the other vitamins.

The pH also influences the magnitude of the electroosmotic flow that is directed towards the negative end of a fused-silica capillary. For the simultaneous determination of cations and anions in free zone electrophoresis in a reasonable analysis time, it was necessary to force an electroosmotic flow (EOF) towards the detector (negative end) whose magnitude exceeds the migration velocity of the analyte anions. Therefore, we selected a phosphate buffer of pH 8.0 where the separation run time does not exceed

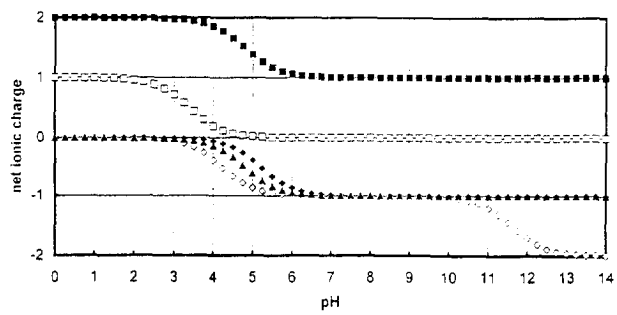



Fig. 1. Net ionic charges (sum of the products of molar fraction and charge of the co-existing ionic forms) of (■) thiamine ($pK_a$ = 4.80 [10]), (□) nicotinamide ($pK_a$ = 3.42 [11]), (◆) biotin ($pK_a$ = 5.20 [12]), (◇) ascorbic acid ($pK_{a_1}$ = 4.17; $pK_{a_2}$ = 11.57 [9]) and (▲) nicotinic acid ($pK_a$ = 4.76 [13]).

10 min, and an optimum separation could be achieved.

Another serious problem in determining vitamins is the chemical stability of the substances. Especially in the case of ascorbic acid, which is easily oxidized, reliable results cannot be expected without the use of stabilizing buffer additives. Fig. 2 shows the stability of ascorbic acid with and without the addition of L-cysteine in phosphate buffers of pH 5.0 and 8.0, respectively.

Cysteine has a more negative redox potential than ascorbic acid and so it acts as an antioxidant. A 50-fold excess of cysteine was added to the standard vitamin C solutions. Therefore, the $t(90\%)$ level (the moment at which 90% of ascorbic acid is still present in solution) of ascorbic acid decay could be extended to 155 min. In contrast to ascorbic acid, biotin was stable in 20 m*M* phosphate buffer (pH 5–8) for at least 96 h.

### 3.2. Analytical parameters

Table 1 summarizes the estimated analytical parameters (qualitative and quantitative) of the free zone electrophoretic separation of the water-soluble vitamins thiamine, nicotinamide, D-(+)-biotin, L-ascorbic acid and nicotinic acid.

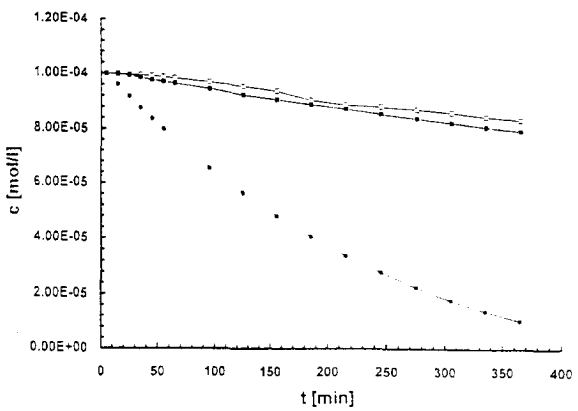



Fig. 2. Stability of a $10^{-4}$ mol/l ascorbic acid solution in 20 m*M* phosphate buffer. ● = pH 8.0 without, ■ = pH 8.0 with and □ = pH 5.0 with addition of 0.005 mol/l cysteine over a period of 6 h. Data from UV spectra.

Each analyte could be specified by its ionic mobility and its characteristic UV spectrum. The highest absorption for most of the vitamins was found at 200 nm, except for ascorbic acid, where the maximum absorption occurred at 266 nm. The detection limits were at the low ppm level (1–4 μg/ml) and good precision could be achieved.

### 3.3. Applications

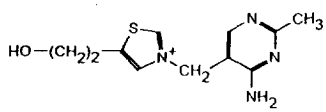
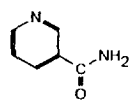
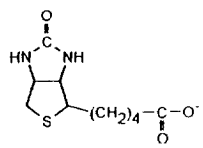
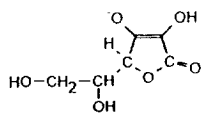
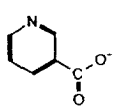
We applied the free zone electrophoretic method to the determination of ascorbic acid in pharmaceutical formulations, native citrus juices and fruit beverages. We always found only one peak for L-ascorbic acid. Isoascorbic acid was not detected. Fig. 3 shows the electropherogram of a multi-vitamin preparation, Multi Sanosvit Fe (Roland Arzneimittel, Hamburg, Germany). Quantification was effected by calibration from standard vitamin solutions. The peak areas were corrected for the retention time. The confidence intervals were calculated from the standard deviation of the linear regression with $P = 0.95$, $f = 7$.

We could identify two of the additional peaks as pyridoxine and riboflavin phosphate by comparing the migration times and the UV spectra with those of standard substances. Table 2 demonstrates the agreement of the results obtained with those declared by the producer.

The result for thiamine is in good accordance with the content given by the producer. However, we found that the content of nicotinamide was too high. Nicotinamide was present as a neutral compound, so it could not be separated from other neutral sample ingredients. For ascorbic acid we found only about 65% of the given content. This is due to its low chemical stability towards oxygen.

From the electropherograms of tangerine juice and commercially available orange nectar, we were able to identify and determine ascorbic acid. We found values in the range 508–516 μg/ml (±5 μg/ml) vitamin C for freshly pressed

Table 1
Analytical parameters for the five vitamins

| Analyte | Formula | Qualitative parameters | | Quantitative parameters | | |
|---|---|---|---|---|---|---|
| | | Ionic mobility ($cm^2/V \cdot s$) | UV spectrum, $\lambda_{max}$ (nm) | Sensitivity [AU · s · l/g] | R.S.D. (%) | Detection limit ($\mu$g/ml) |
| Thiamine |  | $16.0 \cdot 10^{-5}$ $\pm$ $0.6 \cdot 10^{-5}$ | 232<br>269 | 0.43<br>±0.01<br>(200 nm) | 2.9<br>($n = 8$) | 2.7 |
| Nicotinamide |  | 0 | 215<br>262 | 0.99<br>±0.03<br>(200 nm) | 3.4<br>($n = 8$) | 1.6 |
| Biotin |  | $-16.7 \cdot 10^{-5}$ $\pm$ $0.4 \cdot 10^{-5}$ | – | 0.376<br>± 0.003<br>(200 nm) | 2.2<br>($n = 8$) | 3.4 |
| Ascorbic acid |  | $-20.8 \cdot 10^{-5}$ $\pm$ $0.3 \cdot 10^{-5}$ | 266 | 1.26<br>±0.03<br>(266 nm) | 2.5<br>($n = 8$) | 1.2 |
| Nicotinic acid |  | $-24.1 \cdot 10^{-5}$ $\pm$ $0.6 \cdot 10^{-5}$ | 215<br>262 | 1.9<br>±0.1<br>(200 nm) | 3.5<br>($n = 6$) | 0.9 |

tangerine juice and 143–164 $\mu$g/ml (±2 $\mu$g/ml) vitamin C for orange nectar (Kirberg, Germany).

The content of biotin in native samples is very low (ppb range), hence we could not determine it by CZE. In a pharmaceutical preparation, Gabunat (Wölfer, Bovenau, Germany), for the treatment of biotin deficiency phenomena (brittle and cracked nails or hair), we determined the content of biotin. The result agreed well with the given content of 5 mg of biotin per capsule (see Fig. 4).

## 4. Conclusions

Using the advantages of HPCE such as high separation efficiency, short analysis time and ease of instrumentation and sample preconditioning, we were able to employ this method for determining water-soluble vitamins. The method was applied to the determination of ascorbic acid and biotin in pharmaceuticals and beverages. During the analysis ascorbic acid could be stabilized by adding L-cysteine to the samples. This made it possible to extend the analysis time so as

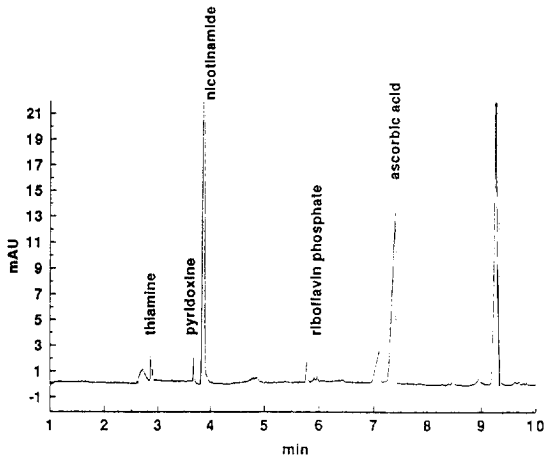



Fig. 3. Electropherogram of Multi Sanosvit Fe. Standard separation conditions, detector signal at 200 nm.

to perform calibration and sample analysis automatically. The detection limits of CZE were in the low ppm range. Therefore, biotin could not be detected in native samples. The method offered good precision.

Table 2
Analytical results for Multi Sanosvit Fe

| Vitamin | Content in 5 ml of juice (mg) | Result related to 5 ml of juice (mg) |
|---|---|---|
| Thiamine hydrochloride | 1.0 | 0.8 |
| | | 1.1 |
| Riboflavin phosphate | 1.0 | Detected |
| Pyridoxine hydrochloride | 0.5 | Detected |
| Ascorbic acid | 50.0 | 31.9 |
| | | 30.7 |
| Nicotinamide | 5.0 | 7.6 |
| | | 7.4 |

The density of the formulation was determined only once at room temperature and amounted to 1.237 g/ml. Therefore, no confidence interval could be given for the results.

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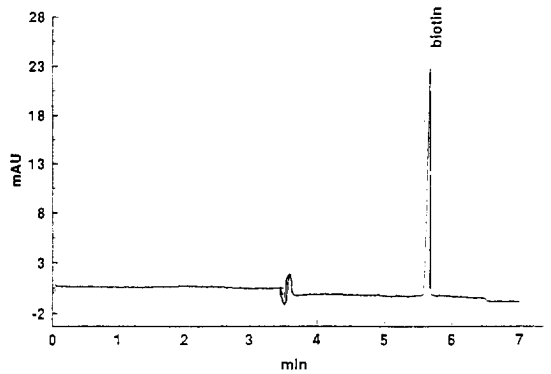


Fig. 4. Electropherogram of Gabunat. Standard separation conditions, detector signal at 200 nm. Result: 4.63 ± 0.04 and 4.79 ± 0.04 mg of biotin (duplicate analysis).

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