Simultaneous analysis of watersoluble vitamins using capillary electrophoresis

Application Note

Foods and Flavors

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Abstract
This application note describes the development of an MEKC method, its reproducibility, its sensitivity for vitamins analysis and its use for the analysis of vitamin-enriched drinks. Water-soluble vitamins including B1, B2 phosphate, B3 (nicotinamide), B6 and caffeine were analyzed simultaneously by capillary electrophoresis (CE). The vitamins were separated with high resolution by using MEKC and were detected using a diode-array detector. The relative standard deviation (RSD) for migration time was between 0.3 % and 0.7 % and for peak area the RSD was better than 1.8 %. The minimum detectable level for vitamins ranged from 160 to 660 µg/L.

Introduction
Vitamins play an important role for healthy growth and development of many organisms. Today, multivitamin products are becoming more and more widespread. Consequently, a rapid, easy and reliable method for vitamin analysis is required by the food and pharmaceutical industries. In general, water-soluble vitamins are determined by reversed-phase high-performance liquid chromatography (RP-HPLC). However, it is not easy to analyze all vitamins simultaneously by RP-HPLC, because vitamin B1 and B6 are strongly ionic compounds, whereas vitamin B2 phosphate is a hydrophobic species. In order to analyze these vitamins simultaneously with RP-HPLC, an ion-pair technique, or gradient elution, is needed. Both these techniques have limitations. Ion-pair chromatography suffers from poor reproducibility and limited column lifetime. With gradient elutions, a complicated system and long analysis times are required. High performance capillary electrophoresis (HPCE) has the advantages of high efficiency and resolution, automation, and rapid analysis times. Another advantage of HPCE is its unique selectivity. Capillary zone electrophoresis (CZE) separates compounds based on their charge and size. However, neutral species cannot be
are improved. Between pH 9.0 and 9.5, good separation was obtained. At a pH of 10.0, the migration time of B2 phosphate increased and it eluted at the same time as B1. This coelution occurs because the B2 phosphate acquires a negative charge due to phosphate dissociation and is attracted to the anode, thereby increasing its elution time.

Reproducibility, linearity and sensitivity
Usually, detection in HPCE is carried out at 200 nm or below, but in this work 220 nm was selected because the sensitivity to B2 phosphate is about three times better than at 200 nm. The electropherogram of 200 mg/L of vitamin B1, B2 phosphate, B3, B6, caffeine and o-ethoxybenzamide (internal standard) is shown in figure 2. Table 1 shows that satisfactory reproducibilities were obtained, as reflected by the relative standard deviations (RSD). Also, the calibration graphs for all the water-soluble vitamins (10 to 1000 mg/L) were linear. The detection limits for vitamins were between 160 and 660 µg/L at a signal-to-noise ratio of three.

Vitamin-enriched drink analysis
This method was applied to the analysis of water-soluble vitamins in a vitamin-enriched drink. The drink contains several vitamins at a concentration that ranged from 50 to 300 mg/L. The sample was diluted with water (1:5) before injection. Figure 3 shows the result of the vitamin-enriched drink analysis. Although the sample conta-
ned other compounds, a well-defined electropherogram was obtained without matrix interference. Each vitamin in the sample was identified by matching the migration time with the standard and by using a spectral library search.

An example of this identification is shown in figure 4. The RSD values (n=5) of the sample migration times were better than 1.1 % and peak areas were 0.5, 0.9, 1.4 and 0.7 % for B₃, caffeine, B₂ phosphate and B₁, respectively. The amounts of vitamins were in good agreement with the content listed in the product description.

**Conclusions**

A method for the determination of water-soluble vitamins using MEKC has been developed. The method was applied to the analysis of a vitamin-enriched drink and good results were obtained. It was concluded that this method has advantages with respect to resolution, selectivity, analysis time and simplicity, when compared with existing HPLC methods.
References


