Simultaneous analysis of watersoluble vitamins using capillary electrophoresis

Application Note

Foods and Flavors

Authors

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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 B_1, B_2 phosphate, B_3 (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 B_1 and B_6 are strongly ionic compounds, whereas vitamin B_2 phosphate is a hydrophobic species. In order to analyze these vitamins simultaneously with RP-HPLC, an ion-pair technique,[1,2] or gradient elution,[3] is needed. Both these techniques have limitations. Ionpair 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

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separated by capillary zone electrophoresis (CZE). Micellar electrokinetic chromatography (MEKC)[5,6] was used for this analysis because water-soluble vitamins contain both ionic and neutral compounds. In MEKC, ions migrate depending upon their mobility, while neutral species are separated by hydrophobic interaction with the micelles.

Experimental

All experiments were performed using an Agilent Capillary Electrophoresis system. The system comprises a CE unit with built-in diode-array detector and an Agilent ChemStation for system control, data collection and data analysis. Vitamin B_2 phosphate was obtained from Tokyo Kasei Kogyo (Tokyo, Japan) and sodium dodecyl sulfate (SDS) in an electrophoresis purity reagent grade purchased from Bio-Rad (Richmond, CA, USA). All other reagents were obtained from Wako (Osaka, Japan). Water was purified with a Milli-Q purification system from Millipore (Bedford, MA, USA).

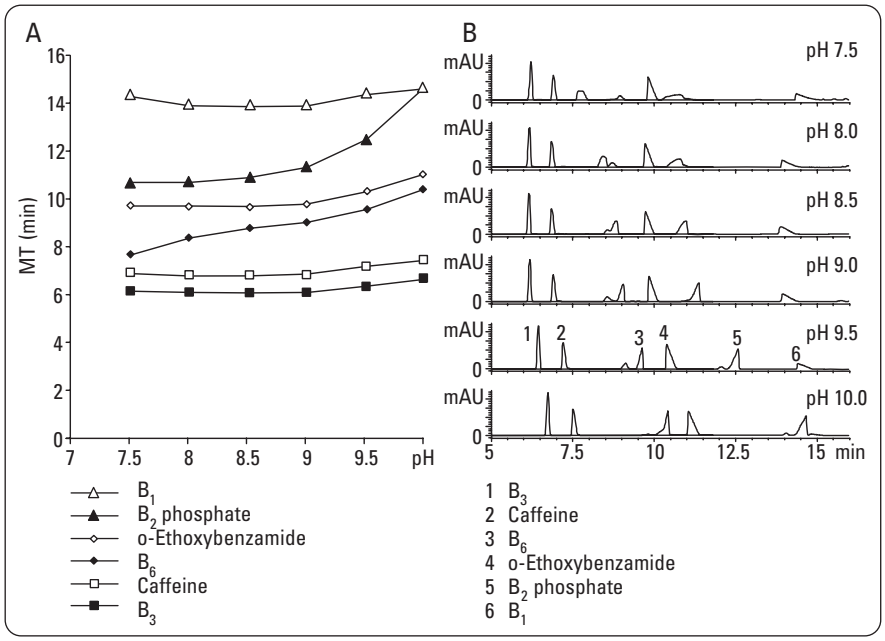

Figure 1
Influence of buffer pH on water-soluble vitamins separation
A) migration time of vitamins versus buffer pH
B) electropherogram at the indicated pH.

Compound	RSD % (n=5)		Linearity	Detection limit
	Migration time	Peak area	Correlation γ	(µg/L)
B_3	0.5	1.6	0.99998	240
Caffeine	0.7	1.7	0.99998	160
B_6	0.4	1.0	0.99980	190
B_2 phosphate	0.3	1.3	0.99995	650
B_1	0.3	1.8	0.99968	660

Table 1
Reproducibility, linearity and sensitivity of vitamin standards.

Results and discussion

Separation of vitamins using MEKC
MEKC has been used for the separation of water-soluble vitamins using borate buffer and SDS micelles.
30 mM SDS in 20 mM tetraborate was chosen for this study. The influence of buffer pH on vitamin separation is shown in figure 1. At pH values lower than 8.5, broadening peaks of B6 and B2 phosphate were observed. This is because the hydroxy groups of B6 and B2 phosphate form borate complexes in tetraborate buffer around pH 9. Lower than pH 8.5, they form partial complexes, therefore the peaks were boardened. At values higher than pH 8.5, they form borate complexes completely. Consequently, the peak shapes are improved. Between pH 9.0 and 9.5, good separation was obtained. At a pH of 10.0, the migration time of B_2 phosphate increased and it eluted at the same time as B_1. This coelution occurs because the B_2 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 B_2 phosphate is about three times better than at 200 nm. The electropherogram of 200 mg/L of vitamin B_1, B2 phosphate, B_3, B_6, 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 contai-

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 B_3, caffeine, B_2 phosphate and B_1, 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.

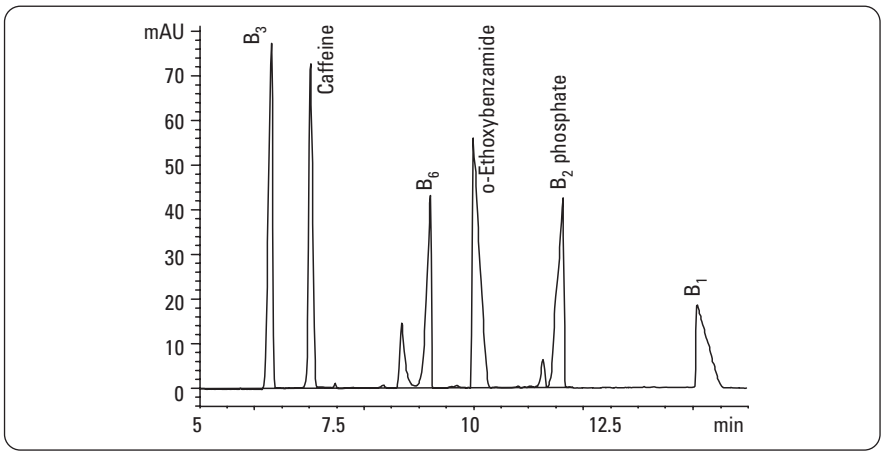

Figure 2
Separation of a standard mixture of vitamins.

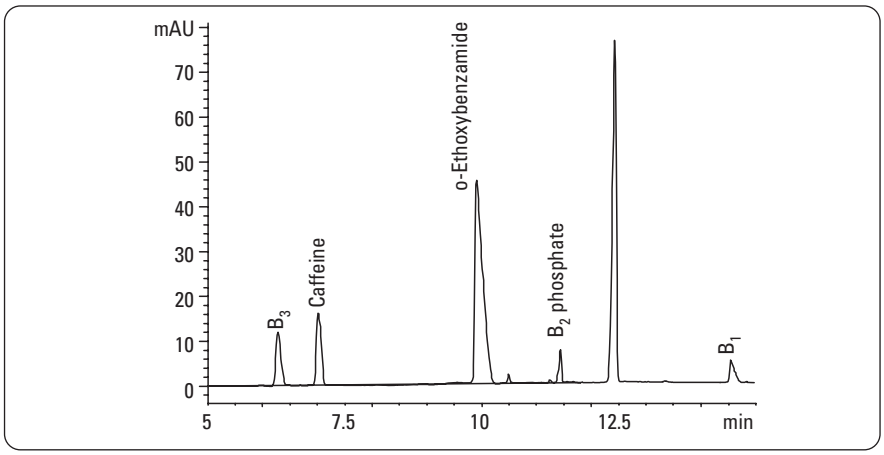

Figure 3
Analysis of water-soluble vitamins in a vitamin-enriched drink.

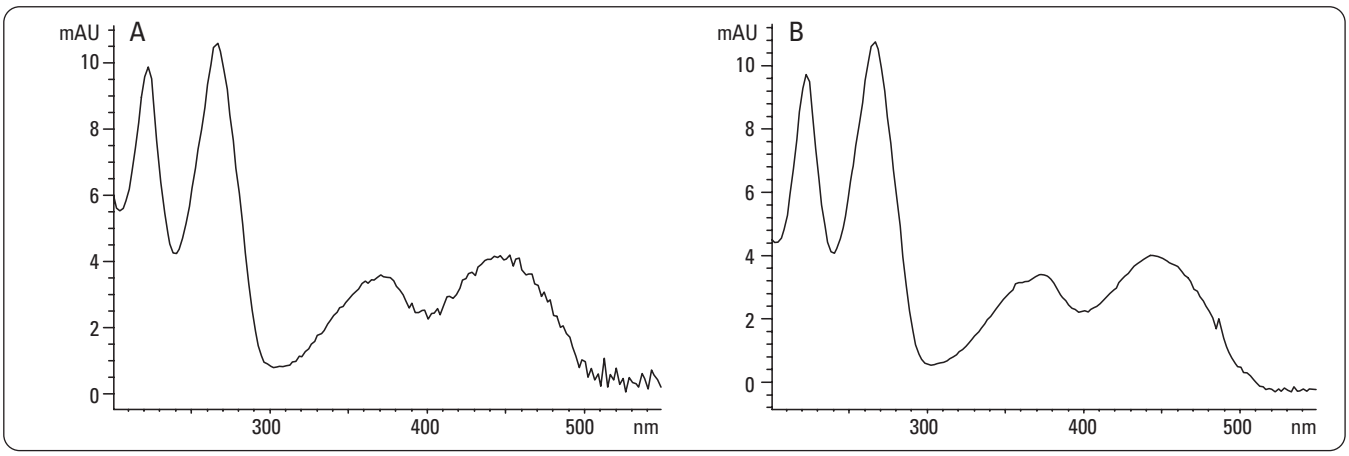

Figure 4
Spectral identification of B_2 phosphate A) spectra of peak eluting after 13 minutes in a vitamin-enriched drink B) spectra of B_2 phosphate standard.

Chromatographic conditions for figures 2, 3, 4

Running buffer.	20 mM tetraborate, 30 mM SDS, pH 9.0
Effective capillary length.	56 cm
Internal diameter:	75 µgm
Voltage:	15 kV
Injection:	200 mbar x s
Detection wavelength:	220 nm
Temperature:	35° C

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