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Simultaneous determination of ingredients in a vitamin-enriched drink by micellar electrokinetic chromatography

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Abstract

Micellar electrokinetic chromatography was used to simultaneously determine six active ingredients (2-aminoethanesulfonic acid, nicotinamide, pyridoxine hydrochloride, anhydrous caffeine, riboflavin sodium phosphate and thiamine nitrate) in a vitamin-enriched drink. All the active ingredients and the formulation excipients were successfully separated by micellar electrokinetic chromatography with 135 mM sodium dodecyl sulfate, and were subsequently detected using a diode-array detector operating at 210 nm. The peak shape of pyridoxine hydrochloride was improved by use of sodium tetraborate solution as sample solvent. Sample and standard solutions were stable for at least 24 h in a light-resistant container at room temperature. The established method was validated and demonstrated to be applicable to the determination of the active ingredients in a vitamin-enriched drink. Good linearities were obtained, with correlation coefficients above 0.999. Recoveries and precisions ranged from 99.0 to 101.2%, and from 0.4 to 2.5% RSD, respectively. The detection limit for ingredients ranged from 0.3 to 125 μg ml^{-1}. These results suggest that micellar electrokinetic chromatography can be used for the determination of ingredients in vitamin-enriched drinks. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Micellar electrokinetic chromatography; Pharmaceutical analysis; Simultaneous determination; Water-soluble vitamins; Tetraborate

1. Introduction

In recent years, CE has come to the fore as an analytical technique in the pharmaceutical field because the ingredients in pharmaceuticals are ideally suited to a separation technique that utilizes differences in ionic mobility [1–3]. The separation capability of CE was dramatically enhanced with the development of MEKC [4], cyclodextrin-modified MEKC [5], microemulsion electrokinetic chromatography [6,7] and hydrophobic interaction electrokinetic chromatography [8]. These systems have been widely used for the determination of ingredients in pharmaceuticals because of their usefulness for separation not only of ionic but also non-ionic and hydrophobic compounds [9–15].

Water-soluble vitamins are available in many pharmaceutical dosage forms, such as drinks,

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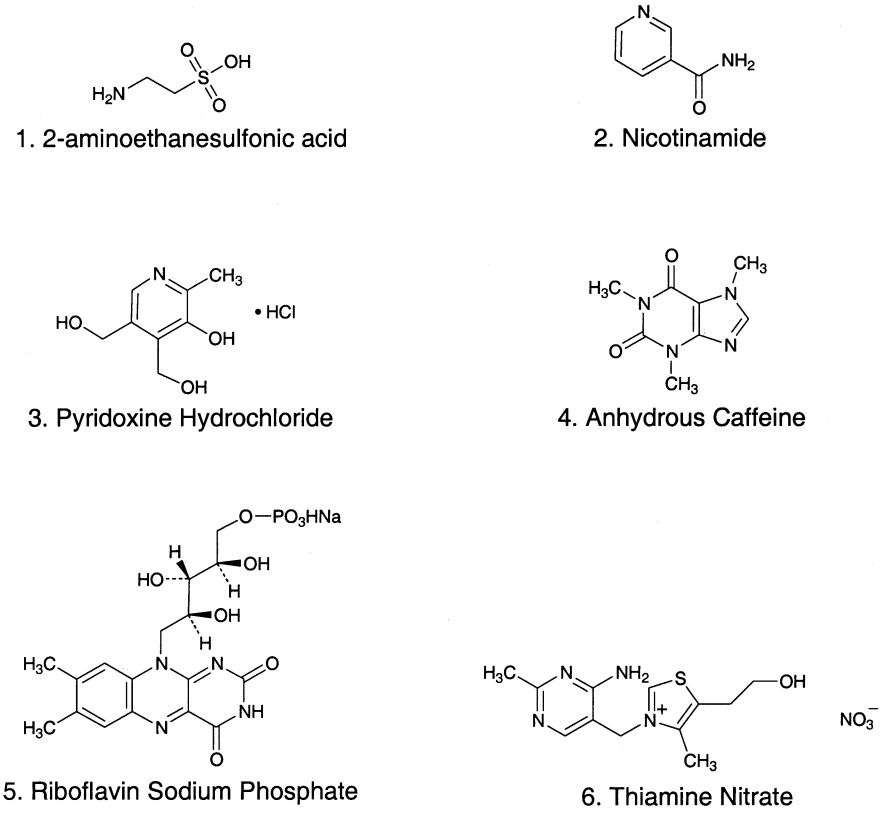

Fig. 1. Active ingredients in vitamin-enriched drink.

tablets, gelatin capsules and syrups. The widespread use of multi-vitamin preparations has stimulated research on accurate, efficient and easy methods for quality control. For this purpose, RP-HPLC using an ion-pair technique [16,17] or gradient elution [18–20] has been required for the simultaneous determination of water-soluble vitamins in preparations. The use of CE intended for vitamin analysis has also been reported with CZE [21–25] or MEKC [24–32]. Methods based on CE have the capability of rapid, high-resolution separation of analytes from extremely low sample volumes and are suitable for simultaneous determination. Moreover, MEKC, based on micellar solubilization, improves selectivity in the separation of heterogeneous compounds including both variations in charge and those in hydrophobicity.

In this study, simultaneous determination of six active ingredients, 2-aminoethanesulfonic acid, nicotinamide, pyridoxine hydrochloride, anhydrous caffeine, riboflavin sodium phosphate and thiamine nitrate (Fig. 1 and Table 1), in a vitamin-enriched drink was successfully performed using the MEKC system. To apply optimum separation conditions to quantitative analysis, attention was also focused on improvement of the peak shape of the analyte. Validation of this method for deter-

Table 1
Content of each active ingredient in a vitamin-enriched drink (mg per 100-ml drink)

Active ingredient	Content
2-aminoethanesulfonic acid	1000
Nicotinamide	20
Pyridoxine hydrochloride	5
Anhydrous caffeine	50
Riboflavin sodium phosphate	5
Thiamine nitrate	5

mination of ingredients in a commercial preparation was also performed.

2. Experimental

2.1. Equipment

CE was performed on an Agilent CE instrument (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detection system operating at 200 or 210 nm. The capillary compartment temperature was maintained at 37 °C. Hydrodynamic injection (3.45 kPa × 5 s) at the anodic end of the capillary was used to introduce samples. Fused-silica capillaries (50 μm I.D.; 375 μm O.D.; 40 cm in length to the detector and total length of 48.5 cm) were obtained from Agilent Technologies. A bubble cell capillary arrangement was employed to increase sensitivity. Prior to each day of use, the capillary was conditioned by rinsing with 0.1 M NaOH (100 kPa × 15 min), deionized water (100 kPa × 15 min) and finally the separation solution (100 kPa × 15 min). The capillary was rinsed between runs with the separation solution (100 kPa × 3 min). All data were collected and analyzed using ChemStation software (Agilent Technologies).

2.2. Chemicals

The chemical structures of the active ingredients are shown in Fig. 1. Nicotinamide, pyridoxine hydrochloride, riboflavin sodium phosphate and thiamine nitrate were purchased from F. Hoffman-La Roche Ltd (Basel, Switzerland), anhydrous caffeine from Knoll GmbH (Ludwigshafen, Germany) and phenylephrine hydrochloride as an internal standard (I.S.) from Iwaki Seiyaku (Tokyo, Japan). 2-aminoethanesulfonic acid was obtained from our laboratory (Taisho Pharmaceutical, Tokyo, Japan).

SDS, boric acid and sodium tetraborate were obtained from Wako (Osaka, Japan). All chemicals were of analytical grade. Several amounts of SDS were dissolved in a buffer solution prepared by mixing 0.2 M boric acid solution with appropriate volumes of 50 mM sodium tetraborate solution to obtain the required pH values. These solutions were filtered through a 0.45-μm membrane filter prior to use.

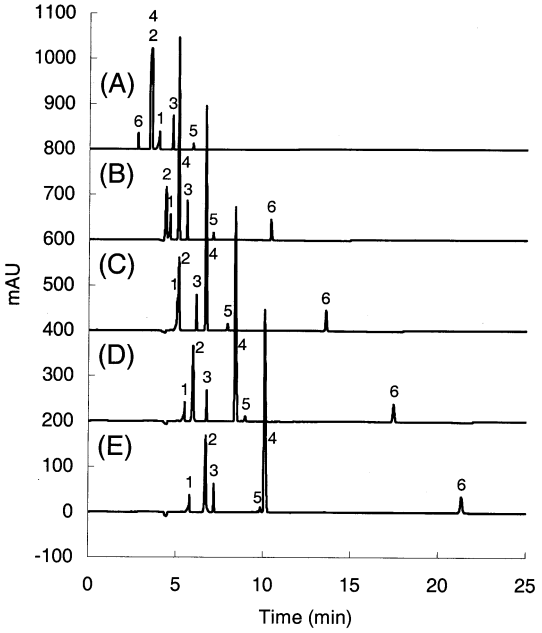

Fig. 2. Effect of SDS concentration on MEKC separation of ingredients. Separation solution, (A) no addition, (B) 50 mM, (C) 100 mM, (D) 150 mM, (E) 200 mM SDS in borate buffer (pH 8.0); applied voltage, +12 kV; temperature, 37 °C; detection wavelength, 200 nm; capillary, fused-silica capillary (50 μm I.D. × 40 cm). Solutes were as noted in Fig. 1.

2.3. Procedure for determination of ingredients in the preparation

To determine the ingredients in a commercial vitamin-enriched drink, 2 ml of the preparation was placed in a 10-ml volumetric flask. An I.S. solution was prepared by dissolving 20 mg of phenylephrine hydrochloride in 100 ml of water. A 2-ml volume of the I.S. solution was added to the solution in the volumetric flask and the mixture was diluted to a volume with 50 mM sodium tetraborate solution. Standard compounds (2-aminoethanesulfonic acid, nicotinamide, pyridoxine hydrochloride, anhydrous caffeine, riboflavin sodium phosphate and thiamine nitrate) were weighed and diluted in borate buffer (pH 8.0) to concentrations similar to those in the prepared

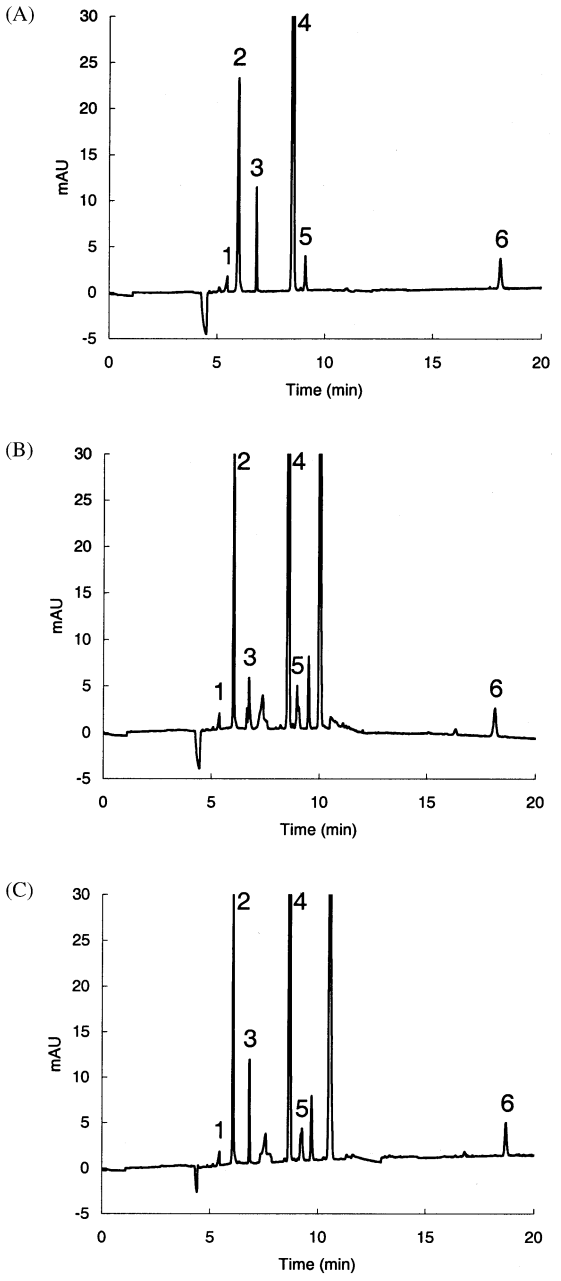

Fig. 3. Effects of sample solvent on the electropherogram. (A) Separations of standard solution, (B) sample solution diluted with water and (C) sample solution diluted with sodium tetraborate solution; separation solution, 150 mM SDS in borate buffer (pH 8.0); detection wavelength, 210 nm; solute were as noted in Fig. 1. Other conditions were as in Fig. 2.

sample solution. The same volume of I.S. solution was also added to the standard solution. All test solutions were passed through a 0.45-μm membrane filter.

3. Results and discussion

3.1. Optimization of separation

Separation conditions were optimized with standard ingredient solution. CZE with borate buffer (pH 8.0) was initially performed to separate ingredients. A typical electropherogram is shown in Fig. 2A. Separation of nicotinamide and anhydrous caffeine was insufficient. Secondly, the MEKC system was used for the separation. As shown in Fig. 2B–E, the effect of SDS concentration on separation of ingredients was examined. When SDS concentration was increased from 50 to 200 mM, all ingredients migrated slowly. Among the ingredients, thiamine nitrate was found to be most responsive to SDS micellar concentration. All the ingredients were successfully separated by use of 150 mM SDS (Fig. 2D).

3.2. Optimization of analytical condition of ingredients in a preparation

Suitable separation conditions for the standard solution were applied to separation of sample solution of a commercial vitamin-enriched drink. However, pyridoxine hydrochloride and riboflavin sodium phosphate in the sample solution diluted with water exhibited split and broad peak shapes (Fig. 3B). The dihydroxy compounds, such as pyridoxine hydrochloride and riboflavin sodium phosphate, are known to form negatively-charged complexes with both boric acid and borate in alkaline solution [33,34]. The acidity of the sample solution of the vitamin-enriched drink might thus affect the formation of borate complex. On the other hand, dilution with sodium tetraborate solution improved the peak shape of pyridoxine hydrochloride (Fig. 3C). Increased borate concentration and/or pH caused by addition of sodium tetraborate to the sample solution may enhance their complexing ability. The peak shape of ribo-

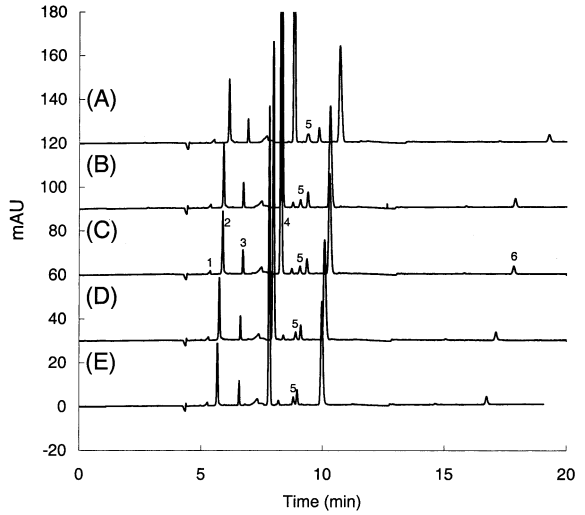

Fig. 4. Effect of SDS concentration on MEKC separation of vitamin-enriched drink. Separation solution, (A) 150 mM, (B) 140 mM, (C) 135 mM, (D) 130 mM, (E) 125 mM SDS in borate buffer (pH 8.0). Solutes were as noted in Fig. 1. Other conditions were as in Fig. 3.

flavin sodium phosphate was found to still be poor. Since the peak of formulation excipients was thought to overlap that of riboflavin sodium phosphate, the effect of SDS concentration on separation of these analytes was examined (Fig. 4). Consequently, 135 mM SDS (Fig. 4C) was selected as optimal for separation of sample solution. From a point of view of robustness, there were no significant changes in migration times and resolutions of each peak upon introduction of intentional variations of ± 5 mM around the optimum concentration. In addition, reproducibility of the system was evaluated by six continuous injections of sample solution. Migration times of each peak were found in RSD to range from 0.5 to 1.5%. Resolutions of each peak were found above 2.9. Moreover, there was no significant difference in the variation between days.

3.3. Determination of the active ingredient

Finally, the separation solution composed of 135 mM SDS in borate buffer (pH 8.0) and the sample solution diluted with sodium tetraborate solution were established to obtain the optimum analytical conditions for ingredients in a commercial vitamin-enriched drink. The application and validation of this technique were also demonstrated for determination of ingredients in a commercial preparation.

To assess the specificity, a standard, a sample of a commercial vitamin-enriched drink and placebo mixtures that were prepared in the absence of each

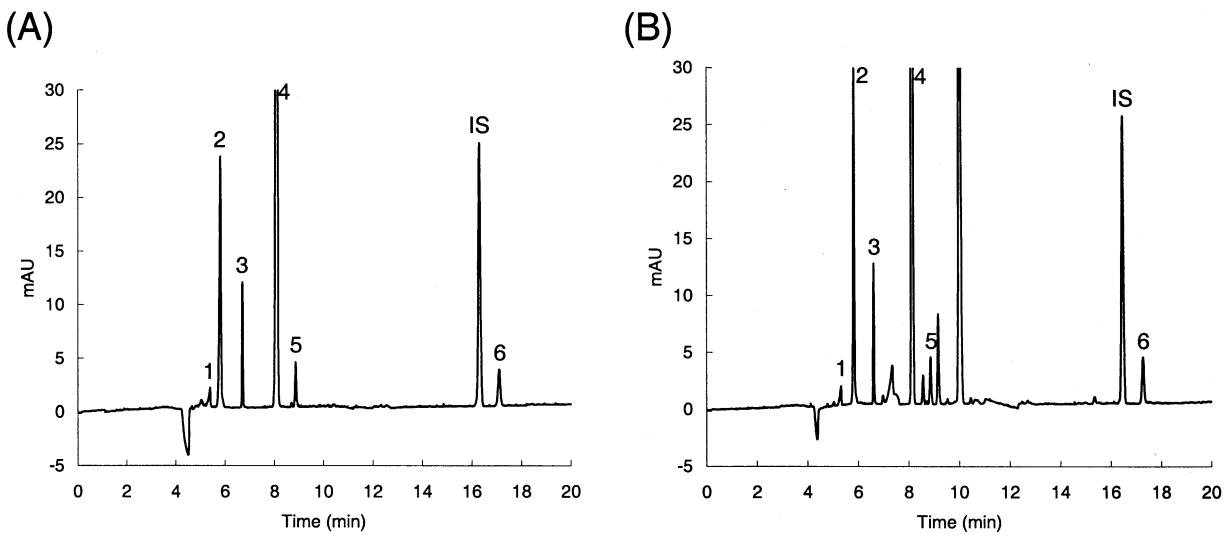

Fig. 5. Typical electropherogram in assay of commercial vitamin-enriched drink. (A) Standard solution, (B) sample solution; separation solution, 135 mM SDS in borate buffer (pH 8.0); solute were as noted in Fig. 1. Other conditions were as in Fig. 3.

Table 2
Validation data for determination of active ingredients in a vitamin-enriched drink

	2-aminoethanesulfonic acid	Nicotinamide	Pyridoxine hydrochloride	Anhydrous caffeine	Riboflavin sodium phosphate	Thiamine nitrate
Linearity						
Concentration range (μg ml^{-1})	1000–3000	20–60	5–15	50–150	5–15	5–15
r	0.9997	0.9996	0.9998	0.9999	0.9998	0.9993
Intercept	−0.0024	−0.0310	0.0001	−0.0048	−0.0063	−0.0001
Slope	0.00003184	0.02373	0.0504	0.0576	0.01653	0.01254
Recovery ($n = 3$, %):						
80%	100.0	99.5	99.6	100.8	100.5	101.2
100%	99.8	99.5	99.2	99.9	101.2	100.1
120%	99.3	100.6	99.0	100.3	99.4	100.4
Precision ($n = 3$, RSD %):						
80%	2.1	0.7	0.6	1.2	1.2	1.4
100%	2.5	0.4	0.6	2.0	1.4	0.6
120%	2.1	0.4	1.1	2.3	0.9	1.0
Detection limit (S/N = 3) (μg ml^{-1})	125	0.5	0.3	0.3	0.6	1

active ingredient in the drink base were analyzed according to the established method. Analysis was performed by an I.S. method. A representative electropherogram of each sample solution in Fig. 5 shows the separation between the main peaks and the I.S. No interference with the formulation excipients was observed at the migration times of the ingredients.

To estimate the stability of analytical solutions, sample and standard solutions were stayed in a light-resistant container at room temperature and evaluated by changes in relative corrected peak area. All active ingredients were stable for at least 24 h in this condition.

The detection limit estimated as a peak with a signal-to-noise ratio of three is shown in Table 2. There were determined by injecting sample solutions with known low concentrations of analytes.

The quantitation linearity of active ingredients in standard solution was examined at five concentration levels in the range from 50 to 150% of the normal concentration. For each ingredient, the relationship between relative corrected peak area and concentration was calculated and is given in Table 2. In all cases, straight regression lines with correlation coefficients (r) above 0.999 were obtained. The intercept values were not significantly different from zero (95% confidence).

Accuracy was assessed over the entire concentration range (80, 100 and 120%) by analyzing placebos spiked with active ingredients at three concentration levels. The solutions were replicated three times each, and the amounts determined were compared to the theoretical amounts. Adequate results for recovery were obtained for all ingredients studied (Table 2).

Precision was determined by measuring ($n = 3$) each active ingredient in spiked placebos at the three concentration levels. RSD was estimated by the established method. Good results with respect to precision were obtained for all ingredients examined (Table 2).

4. Conclusion

The method described was found to be applicable to simultaneous determination of active ingredients in a commercial vitamin-enriched drink. Successful separation and accurate results were obtained with the single MEKC system. Accordingly, the method based on CE is suitable for simultaneous determination of heterogeneous compounds in pharmaceuticals. The application of the CE method to pharmaceutical quality control should widen this area extensively.

For the determination of contents of ingredients in pharmaceutical preparations, one problem remained concerning the supply of riboflavin sodium phosphate standard. There is no reference standard for riboflavin phosphate since it is very difficult to obtain the compound in pure form. To overcome this problem, use of an enzyme to convert riboflavin phosphate to riboflavin in the sample solution is currently under further investigation.

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