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Short Communication

Determination of nicotinamide and pyridoxine in an elemental diet by column-switching high-performance liquid chromatography with UV detection

Hiroshi Iwase

Ajinomoto Co., Inc., Kawasaki Factory, 1–1 Suzuki-cho, Kawasaki-ku, Kawasaki 210 (Japan)

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ABSTRACT

The determination of nicotinamide and pyridoxine in an elemental diet containing 46 compounds was performed by column-switching high-performance liquid chromatography with UV detection at 260 and 290 nm, respectively. The method is simple, rapid, sensitive and reproducible. The calibration graphs for the two vitamins were linear in the ranges 0–0.2 and 0–0.015 μg, respectively. The recoveries of both vitmins by the standard addition method were over 95%. There was good agreement between the concentrations indicated and found for both vitamins.

INTRODUCTION

The routine determination of nicotinamide and pyridoxine in elemental diets such as Elental (Ajinomoto, Kawasaki, Japan), which is a mixture of 46 compounds (*e.g.*, amino acids, vitamins, organic acids, soybean oil, dextrin, minerals) [1], is required for process control, quality control purposes and in clinical chemistry.

Nicotinamide and pyridoxine hydrochloride have been determined by spectrophotometry [2], potentiometric titration [3], a microbiological method [4] and high-performance liquid chromatography (HPLC) [5–10]. Spectrophotometry and potentiometric titration are not suitable for complex sample matrices. In most instances, microbiological methods have generally been used for the routine determination of vitamins. However, this method is tedious and time consuming [4,11]. The Nicotinamide and pyridoxine have been determined by HPLC [5–10]. However, HPLC could not be used for the routine determination of these vitamins in Elental, because the experimental conditions and the removal of interferences caused by the complex sample matrix had not been investigated in detail.

Previous papers [11–13] reported the routine determination of cyanocobalamin, ascorbic acid and folic acid in Elental by HPLC. This paper deals with the routine determination of nicotinamide (27.5 μg/g) and pyridoxine (as hydrochloride salt, 3.34 μg/g) in Elental by column-switching HPLC with UV detection at 260 and 290 nm, respectively. This paper also describes the determination of the above vitamins in two other elemental diets, Elental P for paediatrics and Hepan ED for hepatic failure.

Correspondence to: Dr. H. Iwase, Ajinomoto Co., Inc., Kawasaki Factory, 1–1 Suzuki-cho, Kawasaki-ku, Kawasaki 210, Japan.

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EXPERIMENTAL

Reagents and materials

Elental, Elental P and Hepan ED were obtained from Ajinomoto. Nicotinamide and pyridoxine hydrochloride were of Japanese Pharmacopoeia standard. Sodium 1-heptanesulphonate was obtained from Aldrich (Madison, WI, USA). Acetonitrile (Wako, Osaka, Japan) was of HPLC grade. Other reagents were of analytical-reagent grade.

Sample preparation

To a solution of Elental (20 g) dissolved completely in deionized water (60 ml) on a water-bath at 50°C was added sodium chloride (10 g). After the solution had been allowed to stand at room temperature for 30 min, it was diluted to 100 ml with deionized water in a Volumetric flask and then extracted with hexane (10 ml) for 3 min to remove oils. This aqueous layer was used as the test sample. An aliquot (20 μl) was injected into the chromatograph. Elental P and Hepan ED samples for injection were prepared in the same manner.

Nicotinamide and pyridoxine were stable in aqueous solution at 50°C for 1 h.

Apparatus

Two Model 655 A-11 high-performance liquid chromatographs (Hitachi, Tokyo, Japan) equipped with two Model 655 A variable-wavelength detectors (Hitachi, Tokyo, Japan) set at 260 or 290 nm and a Model HPV 6A column-switching device (Gaskurokogyo, Tokyo, Japan) were used. Capcellpak C_{18} (5 μm) (Shiseido, Tokyo, Japan) (3 × 0.46 cm I.D. and 25 × 0.46 I.D.) were used as the precolumn and analytical column, respectively. The samples were applied with a Rheodyne Model 7125 sample loop injector with an effective volume of 20 μl. A Model UV-2100 variable-wavelength UV spectrophotometer (Shimadzu, Kyoto, Japan) was used for measuring absorption spectra.

Chromatographic conditions

After injection of 20 μl of sample solution on to the precolumn, which has been previously equilibrated with acetonitrile–water (pH 2.1, adjusted with phosphoric acid) (1.5:98.5), the column was washed for 1.33 min (nicotinamide) or 1.66 min (pyridoxine) with the above mobile phase at a flow-rate of 0.8 ml/min. The substances adsorbed on the precolumn were introduced on to the analytical column with acetonitrile–water (pH 2.1, adjusted with phosphoric acid) (9:91) with 1.5 m*M* sodium 1-heptanesulphonate for 0.75 min (both vitamins) at a flow-rate of 0.6 ml/min and a column temperature of 35°C by switching the six-port valve. After introducing the substances on to the analytical column, the six-port valve was returned to the original

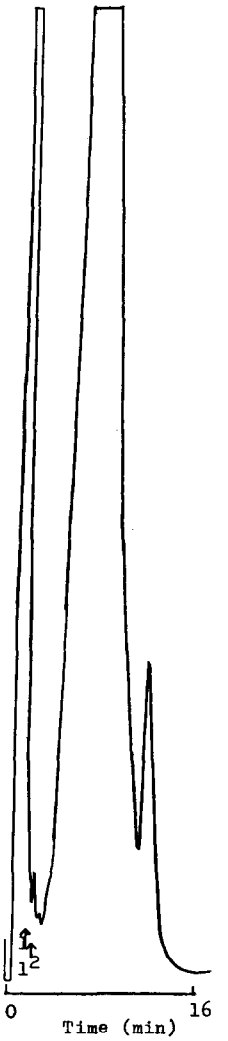

Fig. 1. Elution of nicotinamide and pyridoxine in Elental on the precolumn (3 × 0.46 cm I.D.) with detection at 260 nm. Mobile phase, acetonitrile–water (pH 2.1, adjusted with phosphoric acid) (1.5:98.5) at a flow-rate of 0.8 ml/min. Peaks: 1 = nicotinamide; 2 = pyridoxine.

position. The precolumn was washed with acetonitrile–water (pH 2.1) (1.5:98.5) for the next injection.

RESULTS AND DISCUSSION

Chromatography

The first efforts were focused on the determination of nicotinamide and pyridoxine in Elental using only one analytical column, without the use of the column-switching method, with UV detection at 260 or 290 nm. However, these vitamins could not be identified because many overloading peaks, which might be due to tryptophan, phenylalanine, ascorbic acid, thiamine or riboflavine, with maximum concentrations 10^3 times higher than that of the two vitamins, were observed on the chromatogram.

Subsequent efforts were focused on the determination of the two vitamins in Elental using column-switching HPLC with UV detection at 260 or 290 nm. At the beginning of the work, retention times of standard nicotinamide and pyridoxine and column connection times from the precolumn to the analytical column were examined for the determination of the two vitamins and for the elimination of interferences caused by the complex sample matrix.

A chromatogram of Elental obtained by HPLC with UV detection at 260 nm on the precolumn is shown in Fig. 1. The peaks of nicotinamide and pyridoxin were observed at retention times of *ca.* 1.33 and 1.66 min, respectively. Other compounds were eluted completely within about 16 min (Fig. 1).

After washing for 1.33 or 1.66 min with acetonitrile–water (pH 2.1) (1.5:98.5), the precolumn and analytical column were connected for various periods to examine the connection time. When the connection times were set shorter than 0.75 min, the peaks of the two vitamins were smaller, and when they were set longer than 0.75 min, other many unknown overloading peaks were observed and neither vitamin could be identified. Therefore, the column connection time was set at 0.75 min.

Chromatograms of nicotinamide and pyridoxine in Elental obtained column-switching HPLC with detection at 260 or 290 nm are shown in Figs. 2 and 3. The peaks of these vitamins were separated completely. The detection limits (signal-to-ratio ratio = 2) of the two vitamins were about 5.5 and 0.7 ng, respectively.

It was found that the simultaneous determination

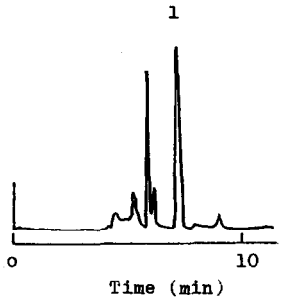

Fig. 2. Chromatogram of nicotinamide (peak 1) in Elental obtained by column-switching HPLC with UV detection at 260 nm Amount of nicotinamide injected, 0.11 μg in 20 μl.

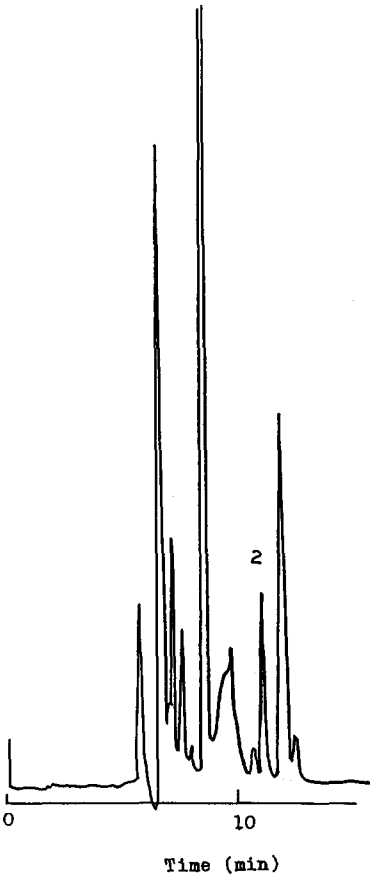

Fig. 3. Chromatogram of pyridoxine (peak 2) in Elental by column-switching HPLC with UV detection at 290 nm. Amount of pyridoxine injected, 13.4 ng in 20 μl.

TABLE I

RECOVERIES OF NICOTINAMIDE AND PYRIDOXINE ADDED TO ELENTAL

According to the label Elental contains 2.75 mg of nicotinamide and 0.334 mg of pyridoxine hydrochloride per 100 g. R.S.D.: nicotinamide 2.2% (n = 5) without addition of nicotinamide, pyridoxine 1.8% (n = 5) without addition of pyridoxine.

	Recovery (μg/100 g)		Recovery (%)
	Added	Found	
Nicotinamide	0	2.66	–
	1.38	3.98	95.7
	2.75	5.28	95.3
	5.50	7.91	95.5
Pyridoxine hydrochloride	0	0.321	–
	0.167	0.481	95.8
	0.334	0.640	95.5
	0.668	0.956	95.1

of the two vitamins was impossible when different wavelengths were used or different washing times of the precolumn.

For the identification of the two vitamins in Elental, a freshly prepared model solution containing the 44 constituent coupounds but with no addition of the two vitamins of interest was examined by the proposed method. No peak of other compounds with the same retention times as the two vitamins was observed on the chromatograms. Hence, the proposed procedure might be considered advantageous for the routine determination of nicotinamide and pyridoxine in complex mixtures.

Determination of nicotinamide and pyridoxine

Calibration graphs for nicotinamide and pyridoxine (as hydrochloride) were constructed by plotting the peak height against the amount of two vitamins, and satisfactory linearity was obtained in the ranges 0–0.2 and 0–0.015 μg, respectively.

A known amount of nicotinamidde or pyridoxine was added to Elental and the overall recovery was determined by the standard addition method. The recoveries of nicotinamide and pyridoxine were over 95% (Table I). The within-day relative standard deviation (R.S.D.) (without addition of nicotinamide and pyridoxine) was 2.2% (n = 5) and 1.8% (n = 5) and the between-day R.S.D. for these two vitamins was 2.5% and 2.2%, respectively.

The analytical data for the two vitamins in Elental, Elental P and Hepan ED (Table II) show that there was good agreement between the nicotinamide and pyridoxine contents found and the values indicated by the manufactures.

In conclusion, this method is satisfactory with respect to selectivity, rapidity and accuracy. It is simple and convenient, and therefore applicable to the routine determination of nicotinamide and pyridoxine in elemental diets such as Elental, Elental P and

TABLE II

ANALYTICAL DATA FOR NICOTINAMIDE AND PYRIDOXINE IN THREE ELEMENTAL DIETS

Elemental diet	Vitamin	Concentration indicated (mg/100 g)	Found (mg/100 g)	Recovery (%)
Elental	Nicotinamide	2.75	2.77	100.7
			2.66	96.7
			2.68	97.4
	Pyridoxine hydrochloride	0.334	0.330	98.8
			0.321	96.1
			0.319	95.5
Elental P	Nicotinamide	4.58	4.38	95.6
	Pyridoxine hydrochloride	0.556	0.533	95.9
Hepan ED	Nicotinamide	4.13	3.98	96.4
	Pyridoxine hydrochloride	0.839	0.805	95.9

Hepan ED. Application of the proposed method to the determination of drugs in biological fluids is also being studied.

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