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Short Communication

Determination of folic acid in an elemental diet by high-performance liquid chromatography with UV detection

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ABSTRACT

Determination of folic acid (550 ng/g) in an elemental diet containing 46 compounds at concentrations at least $0.02 \cdot 10^6$ times higher than that of folic acid was performed by high-performance liquid chromatography with ultraviolet detection at 360 nm using acetonitrile–water (pH 2.1, adjusted with phosphoric acid) (9:91) with the addition of 1 m*M* ethylenediaminetetraacetic acid disodium salt as the mobile phase. This method is suitable for the determination of folic acid in an elemental diet, because it is simple, rapid, sensitive and reproducible. The calibration graph was linear in the range 0–0.6 μg. Recovery of folic acid was about 95% by the standard addition method. There was good agreement between the indicated and found folic acid concentrations.

INTRODUCTION

Numerous methods have been developed for the analysis of folic acid, including spectrophotometry [1,2], a microbiological method [3] and high-performance liquid chromatography (HPLC) [4–6]. The content of folic acid in human blood from cancer patients is less than in normal subjects [6].

The measurement of folic acid in an elemental diet (commercial name Elental, Ajinomoto, Kawasaki, Japan) containing 46 compounds (*e.g.* amino acids, vitamins, organic acids, soybean oil, dextrin, minerals) at concentrations at least $0.02 \cdot 10^6$ times higher than that of folic acid [7] has proved to be valuable for process control, quality control purposes and clinical chemistry.

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Spectrophotometry is not suitable for complex sample matrices. Microbiological methods have generally been used for the routine determination of vitamins. However, this method is tedious and time-consuming [3,8]. The analysis of folic acid has been performed by HPLC. However, HPLC cannot be used for the routine determination of folic acid in complex sample matrices such as Elental, because experimental conditions for the sample preparation such as concentration of trace amounts of folic acid and removing interferences caused by the complex sample matrices have not been investigated in detail.

Previous papers [8,9] have reported the routine analysis of cyanocobalamin and ascorbic acid in Elental. The present paper deals with the routine determination of folic acid (550 ng/g) in Elental. Routine determination of folic acid was performed by HPLC with UV detection at 360 nm, a wavelength that is quite specific to folic acid, using aceto-

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nitrile–water (pH 2.1, adjusted with phosphoric acid) (9:91) with addition of 1 m*M* ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) as the mobile phase.

EXPERIMENTAL

Reagents and materials

Folic acid used in this study was of Japanese Pharmacopeia standard. Acetonitrile (Wako, Osaka, Japan) was of HPLC grade. Other reagents were all of analytical grade.

Apparatus and conditions

A Model 655 A-11 high-performance liquid chromatograph (Hitachi, Tokyo, Japan) equipped with a Model 655 A variable-wavelength detector (Hitachi, Tokyo, Japan) set at 360 nm was used. The samples were applied by a Rheodyne Model 7125 sample loop injector with an effective volume of 2 ml. HPLC was carried out on a 25 X 0.46 cm I.D. column of Capcellpak C_{18} (5 μm) (Shiseido, Tokyo, Japan) using acetonitrile–water (pH 2.1, adjusted with phosphoric acid) (9:91) with 1 m*M* Na_2EDTA as the mobile phase at a flow-rate of 0.6 ml/min and a column temperature of 35°C. A Shimadzu UV-2100 UV variable-wavelength recording spectrophotometer (Shimadzu, Kyoto, Japan) was used for the absorption spectra.

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 this solution was allowed to stand at room temperature for 30 min, it was accurately diluted to 100 ml with deionized water and then this solution was extracted with hexane (10 ml) for 3 min to remove oils. This aqueous layer was used for the test sample. An aliquot (2 ml) was injected into the chromatograph.

Folic acid was stable in aqueous solution at 50°C for 1 h.

RESULTS AND DISCUSSION

Chromatography

Not only folic acid, but also other vitamins, amino acids and organic acids absorb in UV region. Folic acid shows UV absorbance at around 280 and 360 nm. The absorbance at 360 nm is quite specific to folic acid. Riboflavin and cyanocobalamin also have absorbance at around 360 nm.

The first choice of wavelength to achieve highly sensitive detection of folic acid was 280 nm. However, many unknown overloading peaks were observed on the chromatogram, and folic acid could not be identified.

The second choice was 360 nm in order to eliminate co-existing compounds from the chromatograms. In addition, the effect of adding Na_2EDTA to the mobile phase on the determination of folic acid was also examined. Overloading peaks were practically eliminated in this way and folic acid could be observed on the chromatogram. When no Na_2EDTA was added to the mobile phase, the folic acid peak was slightly broadened and an unknown peak with a slight inflection was observed on the chromatogram. This may mean that folic acid and unknown compounds chelate with co-existing metals. On the other hand, peaks of folic acid and unknown compounds with sufficient intensity were obtained by adding Na_2EDTA to the mobile phase (Fig. 1), because metals could be masked with Na_2EDTA.

From the above, it is clear that the addition of Na_2EDTA to the mobile phase was necessary to obtain sharp peaks. Complete elution required about 25 min. Despite the large injection volume (2 ml), folic acid was concentrated on a Capcellpak C_{18} column, separated and then detected at 360 nm without interference from any other compounds. It was necessary to inject 2 ml of sample solution for the determination because folic acid in Elental is present in a very small amount. As a possible modification of this method, sample enrichment at the top of column might be considered. A similar technique has been used in ion chromatography. For the identification of folic acid in Elental, a freshly prepared model solution containing 45 compounds with no added folic acid was examined by this method. No folic acid peak was observed on the chromatogram. Thus, the procedure used here might be considered advantageous for the routine determination of folic acid in complex mixtures.

Determination of folic acid

The calibration graph for folic acid was constructed by plotting the peak height against the

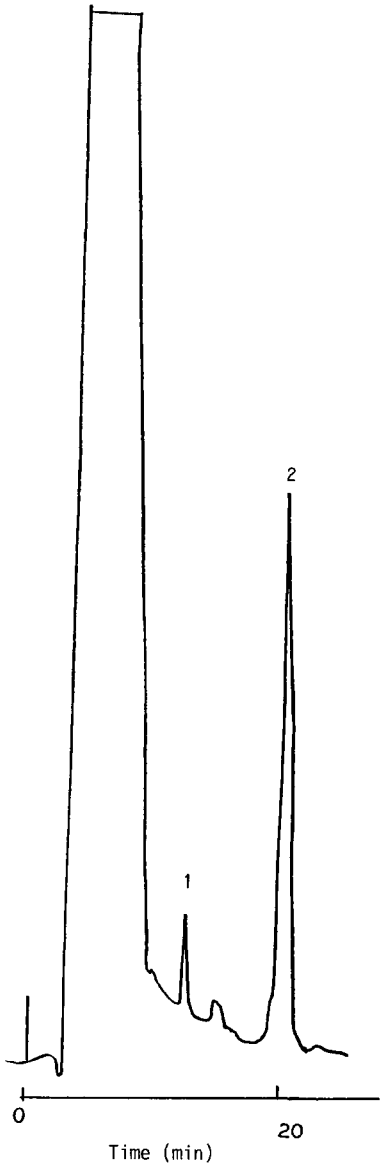

Fig. 1. Chromatogram of folic acid in Elental with UV detection at 360 nm. Amount of folic acid injected, 0.22 μg in 2 ml. Mobile phase: acetonitrile–water (pH 2.1, adjusted with phosphoric acid) (9:91) with 1 m*M* Na_2EDTA. Peaks: 1 = folic acid; 2 = unknown.

amount of folic acid and satisfactory linearity was obtained in the range 0–0.6 μg.

A known amount of folic acid was added to Elental and overall recovery was estimated by the standard addition method. The recovery of folic acid was about 95% with a relative standard deviation (R.S.D.) of 1.8% with no addition of folic acid.

TABLE I

ANALYTICAL DATA FOR FOLIC ACID IN ELENTAL

Relative standard deviation = 1.8% (n = 5) with no addition of folic acid.

Concentration indicated (mg per 100 g)	Analytical data (mg per 100 g)	Recovery (%)
0.055	0.053	96.4
	0.052	94.5
	0.054	98.2
	0.052	94.5
	0.052	94.5

Table I shows the analytical data for folic acid in Elental. There was good agreement between the indicated and found folic acid concentrations.

In conclusion, HPLC with detection at 360 nm, using acetonitrile–water (pH 2.1, adjusted with phosphoric acid) (9:91) with addition of 1 m*M* Na_2EDTA as a mobile phase, seems very useful for the determination of folic acid because it is satisfactory with respect to selectivity, rapidity and accuracy. It is simple and convenient, and therefore applicable to the routine analysis of folic acid in Elental.

Application of the proposed method to the determination of compounds in other elemental diets such as Elental P and Hepan ED and of drugs in biological fluids is also being studied.

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