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Short Communication

Determination of ascorbic acid in elemental diet by high-performance liquid chromatography with electrochemical detection

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ABSTRACT

Determination of ascorbic acid in a multi-component elemental diet was performed by high-performance liquid chromatography with electrochemical detection. This method is suitable for the routine determination of ascorbic acid in elemental diet because it is simple, rapid, sensitive, highly selective and reproducible. The calibration graph of ascorbic acid was linear in the range 0–1.0 μg. The recovery of ascorbic acid was over 95% by the standard addition method. There was good agreement between the concentrations of ascorbic acid stated and found.

INTRODUCTION

In a previous paper [1] the ultramicrodetermination of cyanocobalamin in an elemental diet (Elental, from Ajinomoto, Kawasaki, Japan), which contains 46 kinds of compounds (*e.g.*, amino acids, vitamins, organic acids, soybean oil, dextrin, minerals) [2] was reported. This paper deals with the routine determination of ascorbic acid (vitamin C) in Elental.

A simple, rapid, sensitive, highly selective and reproducible method for the determination of ascorbic acid in foodstuffs, drugs and biomedical samples is required for the purposes of process control, quality control and clinical chemistry aspects. Numerous methods have been developed for the determination of ascorbic acid, including iodimetry [3,4] and high-performance liquid chromatography (HPLC) [5–12]. Iodimetry is not suitable for complex sample matrices such as Elental.

The determination of ascorbic acid in biological fluids and foods has been investigated by HPLC with UV detection, electrochemical detection (ED) and fluorescence detection. Much effort has been directed to the development of specific and sensitive detection systems in HPLC. HPLC with ED is well recognized to be more sensitive than UV detection and as sensitive as fluorescence detection, and is extremely useful for the detection of trace compounds in complex matrices such as biological fluids because of the excellent sensitivity and selectivity provided [13]. ED has been widely used for phenolic compounds, in particular catecholamines and steroids in biological materials. Accordingly, the use of ED for the determination of ascorbic acid in Elental was undertaken in this study. However, HPLC could not be used for the routine determina-

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tion of ascorbic acid in Elental, because the experimental conditions for sample preparation, removal of interferences caused by the complex sample matrices and the relationship between the applied potential and the sensitivity of ascorbic acid and electrochemically active amino acids (cysteine, tyrosine and tryptophan) in Elental had not been investigated in detail.

This paper deals with the routine determination of ascorbic acid (97.5 ng/g) in Elental by HPLC–ED at 70 mV *versus* an Ag/AgCl reference electrode, which is highly specific for ascorbic acid. The determination of ascorbic acid in two other elemental diets, Elental P for paediatrics and Hepan ED for hepatic failure, is also described.

EXPERIMENTAL

Reagents and materials

Ascorbic acid was purchased from Tokyo Kasei (Tokyo, Japan). The reagent solution was freshly prepared prior to use. Other reagents were all of analytical-reagent or HPLC grade. Membrane filters (0.45 μm) were obtained from Advantec Tokyo (Tokyo, Japan).

Apparatus and conditions

A Model 655 A-11 high-performance liquid chromatograph (Hitachi, Tokyo, Japan) equipped with a Model E-502 electrochemical detector (IRICA, Kyoto, Japan) was used. The applied potential was set at 70 mV *versus* an Ag/AgCl reference electrode. The samples were applied with a Rheodyne Model 7125 sample loop injector with an effective volume of 20 μl. HPLC was carried out on a 25 × 0.46 cm I.D. column of Inertsil ODS-2 (5 μm) (GL Sciences, Tokyo, Japan) using 100 m*M* KH_2PO_4 (pH 3.0, adjusted with phosphoric acid) containing 1 m*M* ethylenediaminetetraacetic acid disodium salt (EDTA · 2Na) as the mobile phase. The flow-rate was 0.6 ml/min at ambient temperature. A Shimadzu UV-2100 variable-wavelength UV recording spectrophotometer (Shimadzu, Kyoto, Japan) was used to measure absorption spectra.

Sample preparation

Elental (1 g) was dissolved completely in 6% metaphosphoric acid (100 ml), the solution was filtered with a membrane filter (0.45 μm) and aliquots (20 μl) of the filtrate were injected into the chromatograph.

Elental P and Hepan ED solutions were also treated in the same manner.

RESULTS AND DISCUSSION

Chromatography

The relationship between applied potential and sensitivity of ascorbic acid and electrochemically active cysteine, tyrosine and tryptophan with concentrations at least 10–20 times higher than that of ascorbic acid, which might be considered to interfere in the determination of the latter, was examined. A typical hydrodynamic voltammogram is illustrated in Fig. 1. The current (peak height) at each applied potential was divided by the current at the most positive potential to obtain the relative current ratio. This value was plotted against the applied potential. The detector gave a linear response up to 900 or 1000 mV *versus* an Ag/AgCl reference electrode for cysteine, tyrosine and tryptophan.

Ascorbic acid in biomedical sampless has been determined by HPLC with ED at 800 mV *versus* Ag/AgCl [5,6]. When the applied potential was set at 800 mV *versus* Ag/AgCl, not only ascorbic acid, but also cysteine, tyrosine and tryptophan were de-

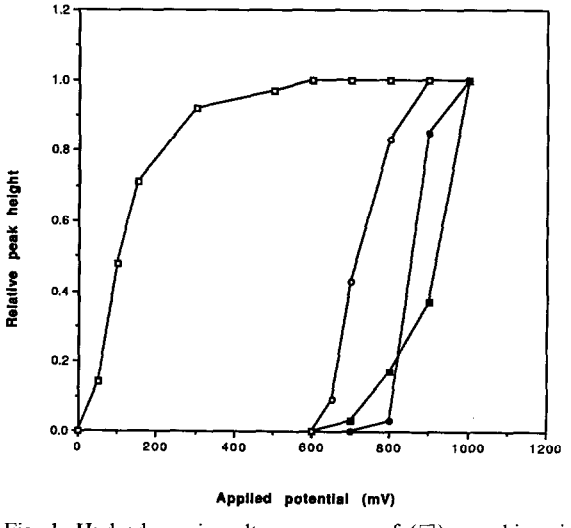

Fig. 1. Hydrodynamic voltammograms of (□) ascorbic acid, (○) cysteine, (●) tyrosine and (■) tryptophan.

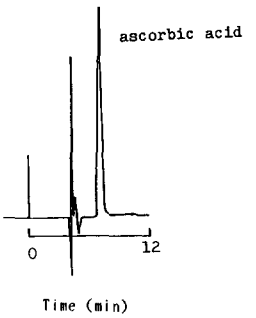

Fig. 2. Chromatogram of ascorbic acid in Elental with ED at 70 mV *versus* an Ag/AgCl reference electrode. Amount of ascorbic acid injected, 19.5 μg in 20 μl. HPLC was carried out on a 25 × 0.46 cm I.D. column of Inertsil ODS-2 (5 μm) using 100 m*M* KH_2PO_4 (pH 3.0, adjusted with phosphoric acid) containing 1 m*M* EDTA · 2Na as a mobile phase at a flow rate of 0.6 ml/min at ambient temperature.

tected. Complete elution took about 60 min after the retention time of ascorbic acid. In addition, further dilution of sample solution was needed because of the higher sensitivity. Ascorbic acid was unstable in the further diluted solution, and further dilution was not suitable for routine work because it was tedious and time consuming. On the other hand, the chromatography of ascorbic acid can be highly selective with rapid detection (retention time *ca.* 12 min) in the presence of other compounds using ED at a low applied potential of 70 mV *versus* Ag/AgCl. This allows routine analysis without clean-up. This procedure is simple and suitable for the routine work. A chromatogram of ascorbic acid in Elental is shown in Fig. 2.

The next effort was focused on UV detection of ascorbic acid. Ascorbic acid in biomedical samples has been determined by HPLC with UV detection [8–11]. Not only ascorbic acid, but also other vitamines, amino acids and organic acids absorb in the UV region. Ascorbic acid has an absorption at 265 and thiamine, riboflavin, nicotinamide, pyridoxine, folic acid, tyrosine, phenylalanine and tryptophan also show absorption at 265 nm.

Ascorbic acid was separated completely by monitoring at 265 nm. However, several unknown peaks were detected after the retention time of ascorbic acid and it took about 60 min for complete elution. Hence HPLC with UV detection is not suitable for routine analysis because it is not selective and the analytical time is long.

From the above results, it was concluded that the method established here was advantageous for the routine determination of ascorbic acid in Elemental, because it is simple, rapid and highly selective without the need for clean-up.

TABLE I

ACCURACY OF THE DETERMINATION OF ASCORBIC ACID ADDED TO ELENTAL

According to the label, Elental contains 9.75 mg of ascorbic acid per 100 g

Ascorbic acid (mg per 100 g)		Recovery (%)
Added	Found	
0	9.61	-
2.5	11.8	97.4
5.0	14.3	97.9
10.0	19.3	98.4
20.0	29.1	98.3

[a] R.S.D. = 0.5% (n = 7).

Determination of ascorbic acid

The calibration graph for ascorbic acid was constructed by plotting the peak height against the amount of ascorbic acid, and satisfactory linearity was obtained in the range 0–1.0 μg.

A known amount of ascorbic acid was added to Elental and the overall recovery was calculated by

TABLE II

ANALYTICAL DATA FOR ASCORBIC ACID IN THREE ELEMENTAL DIETS

Ascorbic acid concentration (mg per 100 g)			Recovery (%)
Sample	Stated	Found	
Elental	9.75	9.64	98.9
		9.61	98.6
		9.55	97.9
Elental P	35.8	35.7	99.7
		35.3	98.6
		35.3	98.6
Hepan ED	29.3	28.9	98.6
		28.3	96.6
		28.6	97.6

the standard addition method. As shown in Table I, the recovery of ascorbic acid was over 95% with a relative standard deviation (R.S.D.) of 0.5%.

The results in Table II show that the analytical data for ascorbic acid in Elental, Elental P and Hepan ED were excellent. There was good agreement between the ascorbic concentrations stated and found.

In conclusion, the proposed method is satisfactory with respect to selectivity, rapidity and accuracy. It is simple and convenient, and therefore applicable to the routine determination of ascorbic acid in elemental diets such as Elental, Elental P and Hepan ED.

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