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Technical note

Determination of ascorbic acid in food by column liquid chromatography with electrochemical detection using eluent for pre-run sample stabilization

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Abstract

Determination of ascorbic acid (AA) in food was performed by column liquid chromatography with electrochemical detection using an eluent (100 m*M* KH_2PO_4 (pH 3) with 1 m*M* ethylenediaminetetraacetic acid disodium dihydrate) for pre-run sample stabilization. The applied potential was set at 400 mV vs. an Ag/AgCl reference electrode. The proposed method was simple, rapid (analysis time: ca. 8 min). sensitive (detection limit: ca. 0.5 ng per injection (20 μl) at a signal-to-noise ratio of 3), highly selective and reproducible [relative standard deviation: ca. 1.8% ($n=5$)]. The calibration graph for AA was linear in the range 0.1–16 ng per injection (20 μl). Recovery of AA was over 90% by the standard addition method. © 1998 Elsevier Science B.V.

Keywords: Ascorbic acid; Vitamins

1. Introduction

The determination of ascorbic acid (AA) in foods and biological fluids was carried out by high-performance liquid chromatography (HPLC) with electrochemical detection (ED) [1–3].

Sample preparation is the most important of the analysis of analytes for many kinds of complex samples. The objectives of sample preparation are the isolation of analyte from matrix, removal of interferences, concentration of analyte, derivatization of analyte with labelling reagents for highly sensitive and highly selective detection and the pre-analysis sample stabilization of unstable analytes. It is important to optimize this step to ensure that the subsequent HPLC analysis is effective. A simple and rapid sample preparation is required. Traditionally, metaphosphoric acid (MPA) was proven to be a useful dissolving agent for the determination of AA for the pre-analysis sample stabilization. The preparation of an aqueous solution of mPA has two problems: (1) it dissolves in deionized water very slowly [4], and (2) it is hygroscopic, and exists in more than one physical form, so accurate weighing is not easy.

For the above reasons, we examined various dissolving agents to replace MPA by another reagent. The present paper deals with the periodic changes of AA in food dissolved with many dissolving agents for finding a suitable dissolving agent. The determi-

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nation of AA in foods was also examined by the proposed method.

2. Experimental

2.1. Reagents and materials

AA used in this study was purchased from Wako (Osaka, Japan). Other reagents were all of analytical grade. Nutrients, health foods, milks and juices were commercially available. The membrane filters [HLC-DISK 25 (0.45 μm, polyvinyldifluoride)] were purchased from Kanto kagaku (Tokyo, Japan). Light-resistant brown volumetric flasks and glassware were used in this study.

Standard AA (100–800 ng/ml) diluted with the mobile phase was freshly prepared in a brown volumetric flask prior to use.

2.2. Sample preparation

After any samples with lipophilic species or with no lipophilic species were preliminary diluted to an estimated AA concentration of 100–800 ng/ml in a brown volumetric flask with the mobile phase, this solution was filtered with a membrane filter (0.45 μm) and the filtrate (over 1 ml) was used for the determination of AA.

2.3. Apparatus and conditions

A Model 655 A-11 high-performance liquid chromatograph (Hitachi, Tokyo, Japan) equipped with a Model Σ 875 electrochemical detector (Irica, Kyoto, Japan) was used. The applied potential was set at 400 mV vs. 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 15×0.46 cm I.D. reversed-phase column Inertsil ODS-3 (5 μm) (GL Sciences, Tokyo) using 100 m*M* KH_2PO_4 (pH 3, adjusted with phosphoric acid) containing 1 m*M* ethylene–diaminetetraacetic acid disodium dihydrate ($EDTA\cdot 2Na\cdot 2H_2O$) as the mobile phase. The flow-rate was 0.6 ml/min at room temperature.

3. Results and discussion

3.1. Effect of the dissolving agent on the stability of AA

Desirable features of the dissolving agent are speed of preparation and simplicity. In addition, AA must stabilize in the dissolving agent just as well as in MPA aqueous solution.

Firstly, the effect of the concentration of mPA aqueous solution on the stability of AA is shown in Fig. 1. It was found that AA in deionized water (0% mPA), 0.1% and 0.5% MPA aqueous solution was stable for 15 min and AA in 1–6% MPA aqueous solution remained stable for 30 min. The stability of AA in the range 1–6% MPA was found to be independent of concentration.

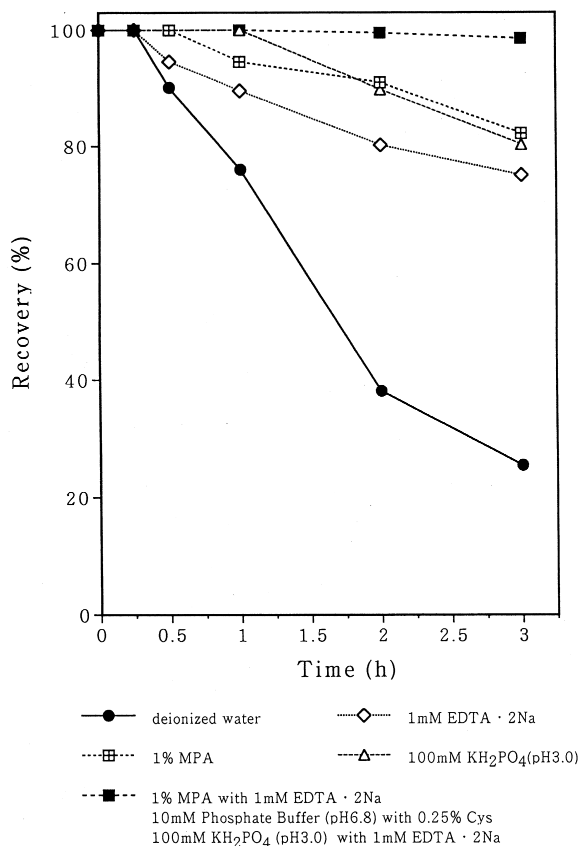

Fig. 1. Periodic changes of AA in lemon juice dissolved with MPA aqueous solutions at room temperature. AA concentration in lemon juice: ca. 40 mg/100 ml R.S.D. (n=5); 0–11.8%.

Then, the effect of the addition of 1 m*M* EDTA·$2Na·2H_2O$ on the stability of AA was studied. EDTA·$2Na·2H_2O$ alone was not an effective dissolving agent and the combination of EDTA·$2Na·2H_2O$ and MPA aqueous solution was much more effective at stabilizing AA.

A comparison of stability of AA in other dissolving agents was made. Table 1 shows the AA content (%) in six dissolving agents remained stable for 1 h. It was also clear that the combination of 1 m*M* EDTA·$2Na·2H_2O$ and 100 m*M* potassium phosphate buffer (pH 3) (mobile phase) has a slight effect on the stability of AA compared with EDTA·2Na·$2H_2O$-free solvent. From the above. MPA could be replaced by the HPLC mobile phase, because of simple and rapid preparation, and AA remained stable for 1 h, just as stable as in the mPA aqueous solution.

3.2. Chromatography and determination of AA

Chromatograms of AA in nutrient, which contains amino acids, vitamins, organic acids, plant oil, dextrin, minerals, dissolved in the mobile phase are shown in Fig. 2. The AA peak was observed selectively on the chromatogram without further clean-up. A concentration at the limit of detection from Fig. 2 was calculated to be ca. 0.5 ng per injection (20 μl) at a signal-to-noise ratio of 3.

The calibration graph for AA was constructed by plotting the peak height of AA against the amount of AA. Satisfactory linearity was obtained in the range 0.1–16 ng on-column ($Y=1.053X-0.026$; Y=peak height, X=amount of AA in ng). A known amount of AA was added to the nutrient and overall re-

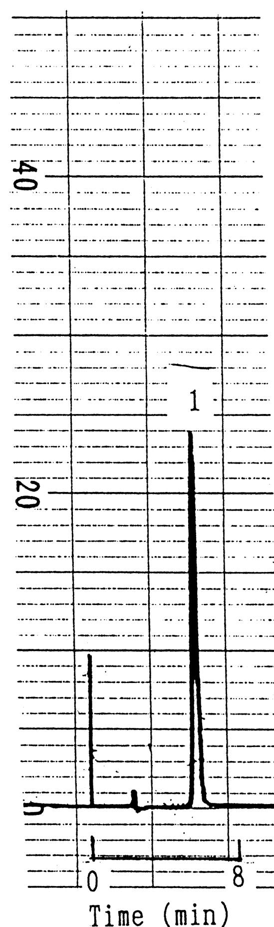

Fig. 2. Chromatograms of AA in nutrient dissolved with mobile phase by HPLC with ED set at 400 mV vs. an Ag/AgCl reference electrode. HPLC was carried out on a 15×0.46 cm I.D. column of Inertsil ODS −3 (5 μm) using 100 m*M* KH_2PO_4 (pH 3, adjusted with phosphoric acid) with 1 m*M* EDTA·$2Na·2H_2O$ as a mobile phase at a flow-rate of 0.6 ml/min under ambient conditions. Peak 1=AA.

Table 1
Periodic changes of AA in lemon juice dissolved with various aqueous solutions at room temperature (AA concentration in lemon juice: ca. 40 mg/100 ml)

	Time (h)			
	0	1	2	3
10 m*M* KH_2PO_4 (pH 6.8) with 0.25% X Cys	100	100	99.5	98.5
10 m*M* KH_2PO_4 (pH 6.8) with 0.25% Cys and 1 m*M* EDTA·$2Na·2H_2O$	100	100	99.5	98.5
10 m*M* KH_2PO_4 (pH 3)	100	100	89.7	80.3
10 m*M* KH_2PO_4 (pH 3) with 1 m*M* EDTA·$2Na·2H_2O$ (mobile phase)	100	100	99.5	98.5
10 m*M* AcCys	100	100	98.9	96.9
10 m*M* AcCys with 1 m*M* EDTA·$2Na·2H_2O$	100	100	99.9	98.9

R.S.D. (n=5); 0–2.2%.

Table 2
Content of AA in nutrients, health foods, milks and juices

	AA concentration indicated	AA concentration determined
Juice (mg/100 ml)		
Lemon[a]	[b]	39.8
Orange	27	25.3
Apple	30	26.9
Tangerine[a]	[b]	39.6
Nutrient (mg/100 g)		
A	25	23.2
B	38	35.4
C	100	98.5
Health food (mg/100 g)		
A	2500	2430
B	[b]	5.5
Milk		
Cow's milk (mg/100 ml)	[b]	0.8
Infant milk (mg/100 g)	50	54.2

[a] Freshly prepared just prior to use.
[b] Not described.
R.S.D. (n=5) indicates 2.1–3.3%.

coveries were over 90% using the standard addition method. The relative standard deviation (R.S.D.) was 1.8% (n=5) with no addition of AA.

Application of the proposed method to the determination of AA in other foods was also studied (Table 2). There was good agreement in AA value in foods between concentration indicated and found. The method established here could be applicable to other foods.

4. Conclusion

In conclusion, it was possible to replace MPA by the mobile phase for the pre-analysis sample stabilization of AA in foods. It is a great advantage that we do not need to prepare the MPA aqueous solution, thus avoiding problems with weighing and dissolution. The preparation of MPA aqueous solution can be skipped. The proposed method is satisfactory with respect to rapidity (retention time of AA: ca. 8 min), simplicity, sensitivity (detection limit ca. 0.5 ng per injection at a signal-to-noise ratio of 3), reproducibility (R.S.D. 1.8%) and it is a highly selective analysis without the need for clean-up and with recoveries of over 90% of AA.

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