Determination of Ascorbic Acid in Soups by High-Performance Liquid Chromatography with Electrochemical Detection

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Determination of ascorbic acid (AA) in soups was performed by high-performance liquid chromatography with electrochemical detection set at 400 mV vs an Ag/AgCl reference electrode. This method was suitable for the determination of AA in soups. The proposed method was simple, rapid (analysis time ~8 min), sensitive [detection limit ~0.1 ng per injection (20 μL) (at a signal-to-noise ratio of 3:1)], highly selective, and reproducible [relative standard deviation ~3.1% (n = 5)]. The calibration graph of AA was linear in the range of 0−16 ng per injection (20 μL). Recovery of AA was >90% by the standard addition method. Periodical changes of AA in soup were also examined. It was found that AA standards diluted with both tap water and hot tap water were very unstable, and AA in soup prepared with hot tap water (>90 °C) according to the label was very stable.

Keywords: *High-performance liquid chromatography; electrochemical detection; ascorbic acid; soups; periodical changes of ascorbic acid in soup*

INTRODUCTION

A simple, rapid, sensitive, highly selective, and reproducible analytical method for ascorbic acid (AA) in foods, foodstuffs, drugs, and biomedical samples is required for quality control purposes and clinical chemistry. Numerous methods have been developed for the analysis of AA, including iodometry as described in *The United States Pharmacopeia* and *The Pharmacopeia of Japan* and high-performance liquid chromatography (HPLC) with ultraviolet detection, electrochemical detection (ED), and fluorescence detection (Dennison et al., 1961; Iriyama et al., 1984; Behrens et al., 1987, 1992; Grahham et al., 1992; Liau et al., 1993).

ED is valuable in analysis for trace amounts of compounds in complex matrices, because of its excellent sensitivity and selectivity.

The determination of AA in foods and human plasma by HPLC with ED was reported in previous papers (Iwase et al., 1992, 1993, 1994). The preceding paper (Iwase et al., 1997) deals with the determination of AA in elemental diets, health foods, and juices using mobile phase [100 mM KH_2PO_4 (pH 3) with 1 mM ethylenediaminetetraacetic acid disodium dihydrate] for the precolumn stabilization.

Usually, metaphosphoric acid (MPA) has proven to be a useful dissolving agent for the determination of AA for the precolumn stabilization. However, there are two problems associated with MPA preparations, which were weighing and dissolving. The preceding paper (Iwase et al., 1997) deals with the mobile phase that was the most suitable reagent for the replacement of MPA, because the mobile phase preparation was simple and rapid. In addition, AA remained stable for 1 h and the solvent front showed no shoulder on the chromatogram. This was a great advantage that we do not need for the preparation of MPA aqueous solution, because we were able to skip MPA aqueous solution.

We report the application of the previously proposed method to the determination of AA in powdered soups to confirm the stability of AA and to study the length of stability of AA in soup solution. The corn, potage, and vegetable soups are in powdered form to be dissolved with hot tap water (>90 °C) prior to consumption. These soups contain protein, potato, lipids, sugar, and both water-soluble and fat-soluble vitamins and minerals. The present paper deals with the periodical changes of standard AA diluted in deionized water, tap water, hot tap water, and mobile phase to test the stability of AA. A comparison of AA peak height in soup diluted with deionized water, tap water, hot tap water, and mobile phase and 10 mM potassium phosphate buffer (pH 6.8) with 0.25% L-cysteine was also tested for the analysis of AA in soup on the chromatograms.

Furthermore, this paper also deals with the hydrodynamic voltammograms of AA, pyridoxine, folic acid, cysteine, tyrosine, and tryptophan and the validity of the determination of AA in soups by HPLC with ED set at 400 mV vs an Ag/AgCl reference electrode. Periodical changes of AA stored at 80 °C for 5 and allowed to stand for 5 h at room temperature to examine the stability of AA after it was dissolved with hot tap water (95 °C) according to the label were also evaluated.

MATERIALS AND METHODS

Reagents and Materials. AA used in this study was purchased from Wako (Osaka, Japan). Other reagents were all of analytical grade. Powdered corn, potage, and vegetable soups were commercially available.

Light resistant brown volumetric flasks and glassware were used in this study as described in *The United States Pharmacopeia* and *The Pharmacopeia of Japan*. The volumetric flasks and glassware were washed with tap water followed by a thorough rinsing with deionized water to eliminate the cations, anions, and residual chlorine (De Leenheer et al., 1992; Iwase et al., 1997).

Standard AA Preparation. The standard AA (100−800 ng/mL) diluted with mobile phase was freshly prepared in a brown volumetric flask (50 mL) prior to use. AA in this solution was stable at 5 °C for 1 h, indicated by a slow decrease in peak/height ratio.

Sample Preparation for Determination of AA. After samples (1 g) were preliminarily diluted to an estimated AA concentration of 100−800 ng/mL in a brown volumetric flask with mobile phase, this solution was filtered through a membrane filter (0.4 μm) and the filtrate (>1 mL) was used

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for the determination of AA. AA in this sample solution was stable at 5 °C for 2 h. The AA peak/height ratio was constant at 1 h, followed by a gradual decrease.

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 by 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 mobile phase [100 mM KH_2PO_4 (pH 3, adjusted with phosphoric acid) with 1 mM ethylenediaminetetraacetic acid disodium dihydrate (EDTA·2Na·$2H_2O$)]. The flow rate was 0.6 mL/min at room temperature.

RESULTS AND DISCUSSION

Effect of Dissolving Agent on the Stability of Standard AA. According to the instructions on the package, the soup is to be prepared with hot tap water. Tap water contains many kinds of trace residual chlorine, anions, and cations. AA is easily oxidized by the presence of the reagents (e.g. halogens, hydrogen peroxide, heavy metal ions, especially Cu^{2+}, Ag^{+}, and Fe^{3+}) and alkaline pH (De Leenheer et al., 1992). AA was unstable in sodium chloride aqueous solution (Iwase et al., 1994).

The first effort was focused on the effect of the stability of AA diluted with several solvents for the analysis of AA in soup. The solvent must allow AA to remain stable as long as possible. We reported in the previous paper (Iwase et al., 1997) the mobile phase was the most suitable solvent.

As a preliminary test, the stability of standard AA (200 ng/mL) diluted with tap water, tap water stored for 24 h at room temperature, hot tap water (95 °C), deionized water, and mobile phase (Iwase et al., 1997) was assessed. Evaluation of the stability of AA in an aqueous solution was made by comparison of the peak/height ratio of a standard AA and analyzed periodically by HPLC with ED.

Periodical changes of AA in several agents are shown in Figure 1. At first, the stability of AA in ionized water (tap water, tap water stored for 24 h at room temperature, and hot tap water) was tested. It is known that the residual chlorine content (percent) in aqueous solution decreased periodically.

As shown in Figure 1, the AA content in tap water decreased rapidly. When AA was diluted with tap water and followed immediately by HPLC, AA content was ~40% and the AA peak was not detected after 1 h. The AA content in soups with both tap water stored for 24 h at room temperature and hot tap water decreased slowly in comparison to that in soup with tap water. AA diluted in hot tap water was the most stable of the three ionized waters. It might be considered that the residual chlorine was rapidly destroyed in hot tap water.

Second, the stability of AA in deionized water and mobile phase was tested. The AA content in deionized water decreased slowly in comparison with the above three ionized waters. The AA in the mobile phase was also the most stable as described previously (Iwase et al., 1997). These facts showed that AA was oxided with the residual chlorine, cations, and anions (De Leenheer et al., 1992; Iwase et al., 1994).

From the above results, it was clear that the volumetric flasks and glassware used in this study were adequately washed with tap water followed by an effective rinse with deionized water to eliminate the residual chlorine, cations, and anions.

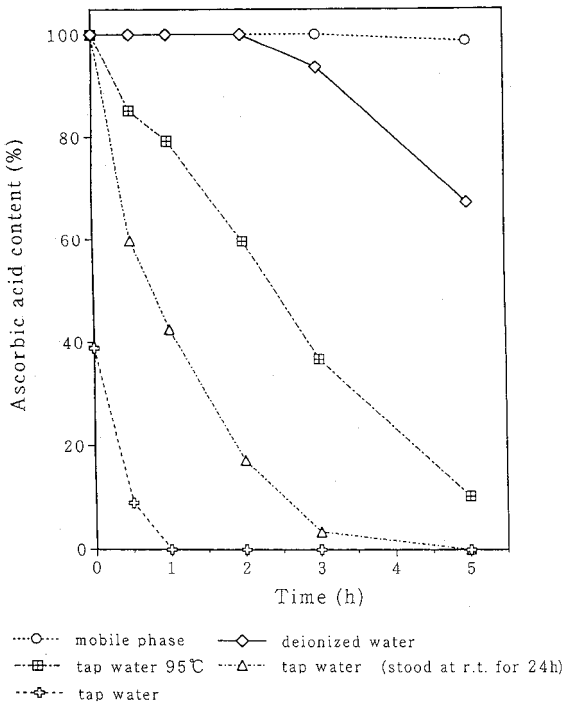

Figure 1. Periodical changes of standard AA in various aqueous solutions (AA concentration = 200 ng/mL).

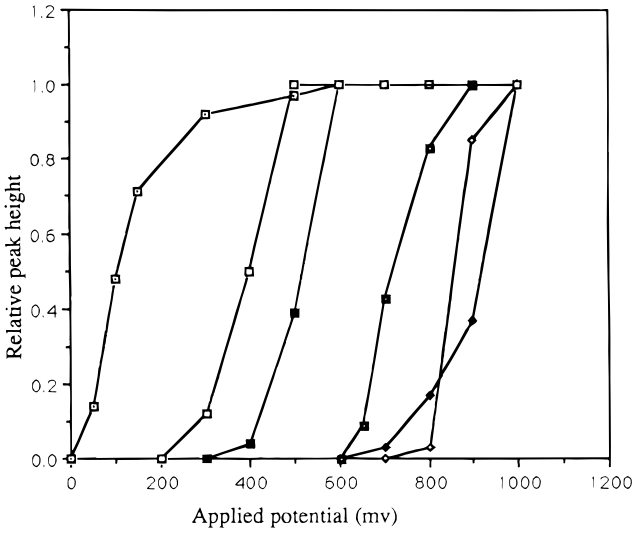

Figure 2. Hydrodynamic voltammogram of AA (white square with black dot), pyridoxine (white square), folic acid (black square), cysteine (black square with white dot), tyrosine (white diamond), and tryptophan (black diamond).

Chromatography. Hydrodynamic voltammograms of AA, pyridoxine, folic acid, cysteine, tyrosine, and tryptophan are shown in Figure 2.

Usually, AA in foods and biomedical samples was determined by HPLC with ED set at 600 mV or more vs an Ag/AgCl reference electrode or HPLC with ultraviolet detection at 245 nm.

When the determination of AA was performed by HPLC with ED set at 600 mV or more vs an Ag/AgCl reference electrode or HPLC with ultraviolet detection at 245 nm, not only AA but also pyridoxine, folic acid, cysteine, tyrosine, and tryptophan were detected. It took ~60 min after the retention time of AA to elute completely. On the other hand, the chromatography for AA can be highly selective with rapid detection (retention time = ~8 min) in the presence of other compounds

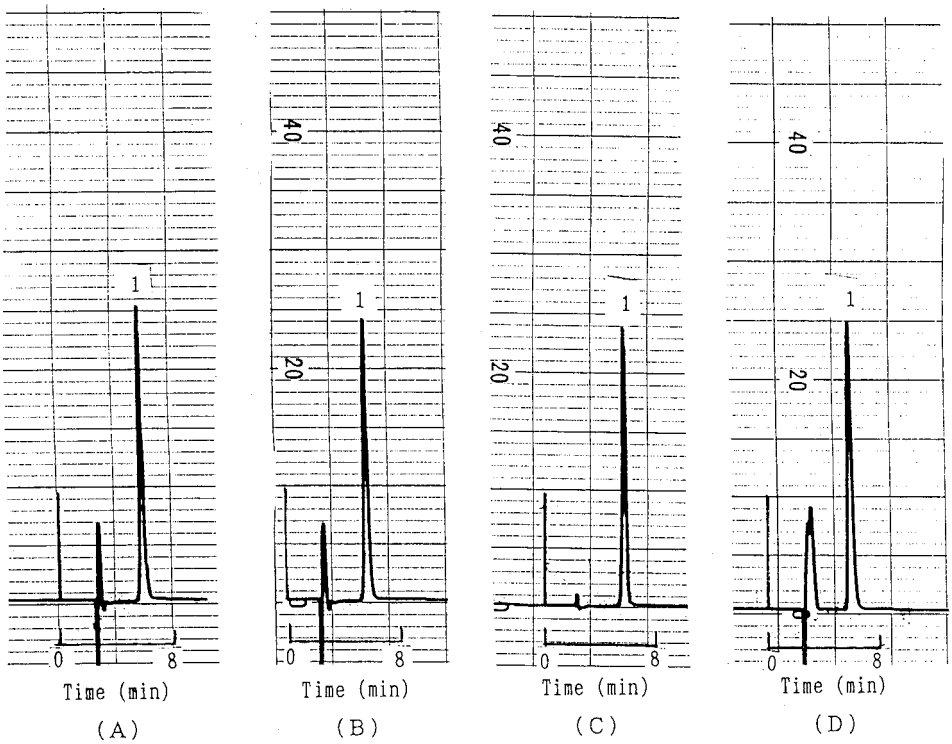

Figure 3. Chromatograms of AA in soup diluted in tap water (A), hot tap water (B), mobile phase (C), and 10 mM potassium phosphate buffer (pH 6.8) with 0.25% L-cysteine (D) by HPLC with ED set at 400 mV vs an Ag/AgCl reference electrode (AA concentration = ~200 ng/mL). HPLC was carried out on a 15 × 0.46 cm i.d. column of Inertial ODS-3 (5 μm) using 100 mM KH_2PO_4 (pH 3, adjusted with phosphoric acid) with 1 mM EDTA·2Na·2H_2O as a mobile phase at a flow rate of 0.6 mL/min under ambient conditions. Peak 1 = AA.

by ED set at 400 mV vs an Ag/AgCl reference electrode. This allows the analysis without the need for cleanup.

From the above, applied potential was set at 300 mV vs an Ag/AgCl reference electrode for the selective determination of AA.

The second effort was focused on the chromatograms of AA (~200 ng/mL) in soup diluted with tap water, hot tap water, mobile phase (Iwase et al., 1997), and 10 mM potassium phosphate buffer (pH 6.8) containing 0.25% L-cysteine (Iwase et al., 1993). We reported in the previous papers (Iwase et al., 1993, 1994, 1997) that AA was the most stable in both potassium phosphate buffer (pH 6.8) containing 0.25% L-cysteine and mobile phase.

A comparison of the chromatograms of AA shown in Figure 3 demonstrates that the AA peak was almost the same peak height. As described above, AA was very unstable in both tap water and hot tap water. However, AA in soup diluted with the both tap water and hot tap water was just as stable as the mobile phase and 10 mM potassium phosphate buffer (pH 6.8) containing 0.25% L-cysteine. It might be considered that the coexistence of compounds in soup allowed the AA to remain more stable. It was found that AA was not easily oxidized in the presence of protein, potato, sugar, and lipid in soup.

Chromatograms (Figure 3C,D) of AA in soup diluted in mobile phase and 10 mM potassium phosphate buffer (pH 6.8) containing 0.25% L-cysteine showed that the soup did not contain dehydroascorbic acid (Iwase et al., 1994), because both AA peak heights were almost the same.

From the above chromatograms, it was found that the application of the previously proposed method (Iwase et al., 1997) to the determination of AA in soup was possible. A concentration at the limit of detection from Figure 3C was ~0.1 ng per injection (20 μL) at a signal-to-noise ratio of 3:1.

Table 1. Recoveries of AA Added to Corn Soup

mg/g			
added	found	recovery (%)	RSD (%)
0	1.61		3.1 (n = 5)
0.5	2.07	92	2.9 (n = 3)
1	2.55	94	2.8 (n = 3)
2	3.47	93	2.7 (n = 3)

Table 2. Concentration of AA in Three Soups

soup	AA (mg/g)	RSD (%)
corn	1.61	3.1 (n = 5)
potage	1.64	2.8 (n = 3)
vegetable	1.54	2.9 (n = 3)

Determination of AA. The calibration graph for AA was constructed by plotting the peak height of AA against the concentration of AA. Satisfactory linearity was obtained in the range of 0−16 ng on column (Y = 1.053X − 0.026, Y = peak height, X = amount of AA in ng, r^2 = 1.000).

A known amount of AA was added to soup, and overall recoveries were estimated by the standard addition method. The results in Table 1 show the analytical data for AA in soup. As listed in Table 1, AA was recovered at >90% by the standard addition method. The relative standard deviation (RSD) was 3.1% (n = 5) with no addition of AA. The results in Table 2 show the analytical data for AA in corn, potage, and vegetable soups. The contents of AA in corn, potage, and vegetable soups were 1.61, 1.64, and 1.54 mg/g, respectively.

Stability of AA. Our next effort was focused on the stability (percent) of AA in corn soup. At first, periodical change of AA (200 ng/mL) stored at 5 °C and room

Table 3. Comparison of Periodical Changes of AA Content in Samples Stored at 5 °C and Room Temperature by the Proposed Method

	AA content (%)					
temp	0 h	1 h	2 h	3 h	5 h	24 h
5 °C	100 (0)[a]	100 (0)	100 (0)	100 (0)	100 (0)	96.2 (2.7)
room	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	92.3 (3.1)

[a] RSD (%) (n = 3).

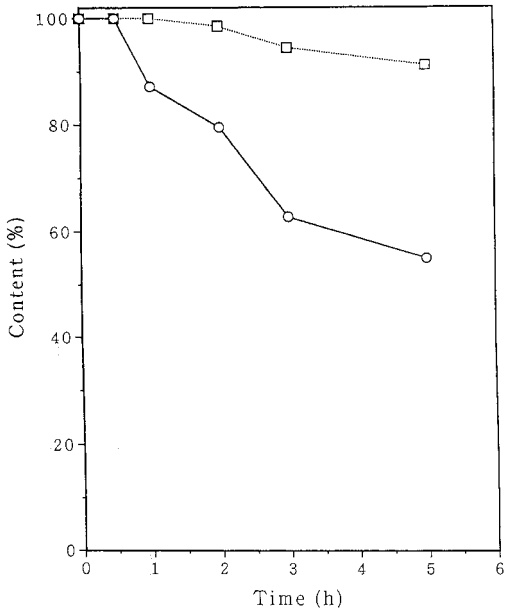

Figure 4. Periodical changes of AA in soup solution stored at room temperature (□) and allowed to stand at 80 °C in the water bath (○) after preparation according to the soup label [soup (~17 g) prepared with hot tap water (95 °C, ~150 mL)].

temperature after sample preparation according to the proposed method was studied to examine the length of time AA in each sample was stable (Table 3). AA was stable for 5 h in both conditions. Peak height was constant for 5 h, and then the peak height decreased periodically. When the above sample was stored for 24 h at both 5 °C and room temperature, the AA contents were ~80 and ~98%, respectively (Table 3). It was found that AA prepared according to the proposed method was very stable.

Gradual change of AA stored at both room temperature and 80 °C after sample (~17 g) preparation with hot tap water (95 °C, ~150 mL) according to the instructions on the package was examined to confirm the most effective way of determining AA in soup by the proposed method (Figure 4). The most effective condition is the soup package instructions method. AA was stable at room temperature. Peak height was constant for 1 h and then decreased very slowly. AA content was >90% after 5 h. This confirms that AA in soup prepared according to the label was very stable. Periodical change of AA in soup stored at 80 °C in the water bath for 5 h was also determined. Peak height was constant for 0.5 h, and then the peak height decreased gradually. AA content was >50% after 5 h.

Accordingly, AA in soup was very stable, irrespective of the presence of anions and cations, as described above, and high temperature. We can consume a very stable form of AA by drinking soup.

Conclusion. In conclusion, the application of the preceding method (Iwase et al., 1997) to the determination of AA in soups was possible. The standard AA in tap water and hot tap water was not stable, and AA in soup prepared with hot tap water according to the label was very stable.

The proposed method is satisfactory with respect to selectivity, rapidity, and simplicity. The method established here seems to be applicable to the routine analysis of AA in soups, because of it is a simple, rapid (retention time of AA = ~8 min), sensitive (detection limit = ~0.15 ng at a signal-to-noise ratio 3:1), reproducible (RSD = 3.1% for AA), and highly selective analysis without the need for cleanup and with recoveries of >90% by the standard addition method. Application of the proposed method to the determination of AA in other foods and biological samples is being studied.

LITERATURE CITED

Behrens, W. A.; Madere, R. A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic and dehydroascorbic acid in tissues, biological fluids, and foods. *Anal. Biochem*. **1987**, *165*, 102−107.

Behrens, W. A.; Madere, R. Quantitative analysis of ascorbic acid and isioascorbic acid in foods by high-performance liquid chromatography with electrochemical detection. *J. Liq. Chromatogr*. **1992**, *15*, 753−765.

De Leenheer, A. A.; Lambert, P.; Nelis, H. J. *Modern Chromatographic Analysis of Vitamins*, 2nd ed.; Dekker: New York, 1992; pp 235−260.

Dennison, D. B.; Brawley, T. G.; Hunter, G. L. K. Rapid high-performance liquid chromatographic determination of ascorbic acid and combined ascorbic acid-dehydroascorbic acid in beverages. *J. Agric. Food Chem*. **1981**, *29*, 927−929.

Grahham, W. D.; Annette, D. Determination of ascorbic and dehydroascorbic acid in potatoes (*Solanum tuberosum*) and strawberries using ion-exclusion chromatography. *J. Chromatogr*. **1992**, *594*, 187−194.

Iriyama, K.; Yoshiura, M.; Iwamoto, T.; Ozaki, Y. Simultaneous determination of uric acid and ascorbic acids in human serum by reversed-phase high-performance liquid chromatography with electrochemical detection. *Anal. Biochem*. **1984**, *141*, 238−243.

Iwase, H. Determination of ascorbic acid in elemental diet by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr*. **1992**, *606*, 277−280.

Iwase, H.; Ono, I. Determination of ascorbic acid and dehydroascorbic acid in juices by high-performance liquid chromatography with electrochemical detection using L-cysteine as precolumn reductant. *J. Chromatogr*. **1993**, *654*, 215−220.

Iwase, H.; Ono, I. Determination of ascorbic acid in human plasma by high-performance liquid chromatography with electrochemical detection using hydroxyapatite cartridge for precolumn deproteinization. *J. Chromatogr*. **1994**, *655*, 195−200.

Iwase, H.; Ono, I. Determination of ascorbic acid in foods by high-performance liquid chromatography with electrochemical detection using mobile phase for the precolumn stabilization. *Biomed. Chromatogr*. **1997**, submitted for publication.

Liau, L. S.; Lee, B. L.; New, A. L.; Ong, C. N. Determination of plasma ascorbic acid by high-performance liquid chromatography with ultraviolet and electrochemical detection. *J. Chromatogr*. **1993**, *612*, 63−70.

The Pharmacopeia of Japan, 13th ed.; Hirokawa Publishing: Tokyo, 1992; pp 252−253.

The United States Pharmacopeia, 23rd revision; U.S. Pharmacopeia Convention: Rockville, MD, 1995; pp 130−130.

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