ELSEVIER

Journal of Chromatography A, 881 (2000) 327–330

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Use of nucleic acids in the mobile phase for the determination of ascorbic acid in foods by high-performance liquid chromatography with electrochemical detection

Hiroshi Iwase*

Ajinomoto Co., Inc., Central Research Laboratories, 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki 210-8681, Japan

Abstract

The sodium salts of amino acids, nucleic acids and organic acids were examined in a new mobile phase for the determination of ascorbic acid (AA) in foods. It was possible to use disodium guanosine-5′-monophosphate (GMP) (20 m*M* GMP, pH 2.1) in a new mobile phase after comparison of five mobile phases. The proposed method is simple, rapid (analysis time: ca. 6 min), sensitive (detection limit: ca. 0.1 ng per injection (5 μl) at a signal-to-noise ratio of 3), highly selective and reproducible [relative standard deviation: ca. 2.7% ($n=7$)]. The calibration graph of AA was linear in the range of 0.1 to 50 ng per injection (5 μl). Recovery of AA was over 90% by the standard addition method.

Keywords: Food analysis; Mobile phase composition; Ascorbic acid; Nucleic acids; Amino acids; Organic acids

1. Introduction

Inorganic salts are often used in the mobile phase for the determination of ascorbic acid (AA) in foods and biological fluids by high-performance liquid chromatography (HPLC) with electrochemical detection (ED). Organic salts, which chelate with metal ions, have not been examined in the mobile phase for the analysis of AA in food samples by HPLC–ED.

Previous work [1] dealt with the possibility of using mono-sodium L-glutamate (MSG) in the mobile phase for the determination of AA in foods and the stability of AA under various conditions to optimize HPLC conditions and the sample preparations.

This manuscript also deals with the possibility of using the amino acid [monosodium L-aspartate (MSA)], nucleic acids [disodium inosine-5′-monophosphate (IMP), disodium guanosine-5′-monophosphate (GMP)] and organic acids [disodium succinate (DSA), trisodium citrate (TCA), which are used for seasoning (umami substances) in cooking] in the mobile phase for the determination of AA in food samples. A comparison of retention time of AA and the suitability of the mobile phase using MSA, IMP, GMP, DSA and TCA were examined. It was found that 20 m*M* GMP (pH 2.1) was the most suitable mobile phase for the determination of AA in foods by HPLC–ED set at 400 mV versus an Ag/AgCl reference electrode.

*Corresponding author.

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00057-1

2. Experimental

2.1. Reagents and materials

AA was purchased from Wako (Osaka, Japan). GMP was obtained from Ajinomoto (Tokyo, Japan). Other reagents were all of analytical grade. Food samples were commercially available. The membrane filters (HLC-DISK 25, 0.45 μm, polyvinyldifluoride) were from Kanto Kagaku (Tokyo). Light-resistant brown volumetric flasks and glassware were used [4–6]. The volumetric flasks and other glassware were washed with tap water followed by a thorough rinsing with deionized water to eliminate cations, anions and residual chlorine [2].

2.2. Standard AA preparation

Standard AA (1~10 μg/ml) diluted with the mobile phase was freshly prepared in a brown volumetric flask prior to use. At the beginning of AA analysis, standard AA (400 ng/ml) is injected into HPLC five times to obtain the optimized AA peak height [3].

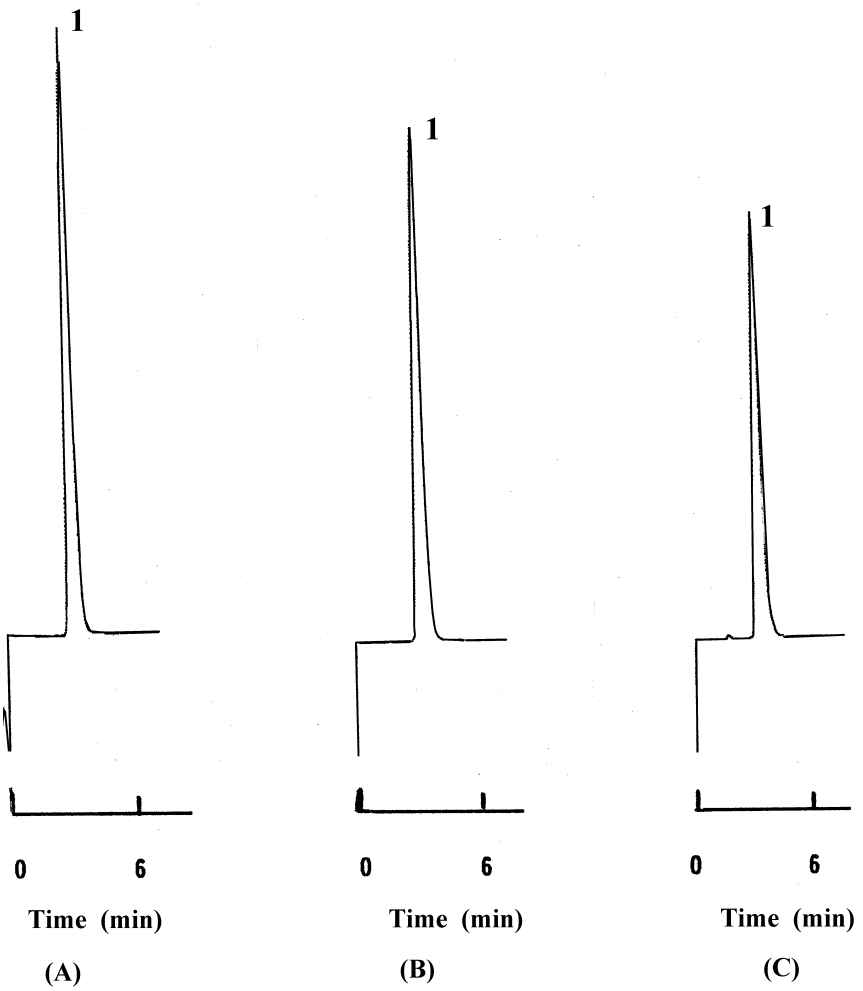

Fig. 1. Chromatograms of AA in foods diluted with mobile phase by HPLC with ED set at 400 mV versus an Ag/AgCl reference electrode; (A) Orange juice; (B) natsumikan juice (like grapefruit juice); (C) grated daikon (Japanese radish). HPLC was carried out on a 15×0.46 cm I.D. column of Inertsil ODS-3 (5 μm) with guard column of Inertsil ODS-3 (5 μm) (1×0.46 cm I.D.) using 20 m*M* GMP (pH 2.1, adjusted with phosphoric acid) with no EDTA·2Na·2H_2O as mobile phase at a flow-rate of 0.8 ml/min under an ambient conditions. Peak 1=AA.

2.3. Sample preparation

After each sample (see Table 2) was preliminary diluted to an estimated AA concentration of 1~10 μg/ml in a brown volumetric flask with the mobile phase, this solution was filtered by a membrane filter (0.45 μm) and the filtrate (over 1 ml) was used for the determination of AA.

2.4. Apparatus and conditions

A Model 655 A-11 high-performance liquid chromatograph (Hitachi, Tokyo) equipped with a Model ED 623 electrochemical detector (working electrode; glassy carbon, GL Sciences, Tokyo) was used. The applied potential was set at 400 mV versus an Ag/AgCl reference electrode. The samples were applied using a Rheodyne Model 7125 sample loop injector with an effective volume of 5 μl. HPLC was carried out on a 15×0.46 cm I.D. reversed-phase column Inertsil ODS-3 (5 μm) (GL Sciences) with guard column of Inertsil ODS-3 (5 μm) (l×0.46 cm I.D.) using 20 m*M* GMP (pH 2.1, adjusted with phosphoric acid) as the mobile phase. The flow-rate was 0.8 ml/min at room temperature.

3. Results and discussion

3.1. Chromatography

MSA, IMP, GMP, DSA and TCA were examined as the mobile phase for the determination of AA. Standard AA (2.5 μg) was diluted with each mobile phase and each chromatogram was compared.

Symmetric AA peaks were not obtained using MSA, DSA TCA or IMP. These chromatograms had also solvent front shock, despite AA being diluted with each mobile phase. On the other hand, GNP gave a symmetric AA peak with no shoulder and no solvent front shock (Fig. 1).

Table 1 shows the retention time of AA using six mobile phases including previously examined MSG and the suitability for the mobile phase. A comparison of retention time of AA using amino acids, nucleic acids and oxalic acids indicated that amino acids with monosodium salt gave a little later retention time of AA.

A comparison of background current (nA) of the mobile phase (20 m*M* (pH 2.1) of GMP, MSG and KH_2PO_4 indicated that GMP gave the lower current when the applied potential was set at 600 mV and gave the higher current when the applied potential was set at 800 and 900 mV (Fig. 2).

Table 1
Retention time of AA and suitability for mobile phase. Flow-rate: 0.8 ml/min[a]

Mobile phase (20 m*M*, pH 2.1)	Retention time (min)	Suitability for mobile phase
Aminio acids		
MSG	4.4	Yes
MSA	4.2	No (no symmetric peak)
Nucleic acid		
IMP	3.8	No (shoulder peak)
GMP	3.4	Yes
Organic acids		
DSA	3.8	No (no symmetric peak)
TCA	3.5	No (no symmetric peak)

[a] The symmetry of the AA peak is indicated in parentheses.

The highly selective and rapid detection of AA (analysis time about 6 min) in the presence of many kinds of compounds is possible with ED set at 400

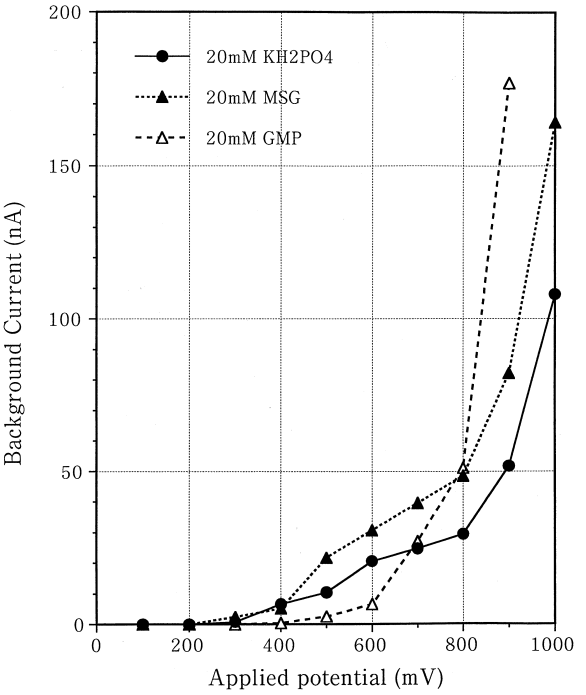

Fig. 2. Background current of mobile phase [20 m*M* GNP (pH 2.1), 20 m*M* NSG (pH 2.1) and 20 m*M* KH_2PO_4 (pH 2.1)].

Table 2
Content of AA in foods (freshly prepared just prior to use)

	AA concentration	
	Determined (mg per 100 ml)	RSD (%)
Orange juice	20.1	2.7 (n=7)
Natsumikan juice (like grapefruit juice)	22.3	2.9 (n=3)
Grated daikon (Japanese radish)	15.3	2.5 (n=3)

mV versus an Ag/AgCl reference electrode. This allows analysis without the need for sample clean-up. The limit of detection from Fig. 1(A) was ca. 0.1 ng per injection (5 μl) at a signal-to-noise ratio of 3:1.

3.2. Determination of AA

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 of 0.1 to 50 ng on column (y=0.245x+0.122, where y=peak height and x=amount of AA in ng).

A known amount of AA was added to the orange juice and overall recoveries were estimated by the standard addition method. AA was recovered over 90%. The relative standard deviation (RSD) was 2.7% (n=7) with no addition of AA. Application of the proposed method to the determination of AA in foods was studied (Table 2).

4. Conclusion

The sodium salts of amino acids, nucleic acids and organic acids were examined as a new HPLC–ED mobile phase for the determination of AA. It was possible to use 20 m*M* GMP (pH 2.1, adjusted with phosphoric acid) in a new mobile phase for the determination of AA in foods.

References

[1] H. Iwase, J. Chromatogr. (submitted).
[2] H. Iwase, I. Ono, J. Agric. Food and Chem. 45 (1987) 4664.
[3] H. Iwase, J. Chromatogr. (submitted).
[4] A.P. De Leenheer, W.E. Lambert, H.J. Nelis, in: Modern Chromatographic Analysis of Vitamins, 2nd Edition, Marcel Dekker, New York, 1992, p. 235.
[5] The United States Pharmacopeia, US Pharmacopeia Convention, Rockville, MD, 23rd Revision, 1995, p. 130.
[6] The Pharmacopeia of Japan, Hirokawa, Tokyo, 12th Edition, 1991, p. C-37.