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Determination of L-ascorbic acid in foods by capillary zone electrophoresis

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Abstract

A new capillary zone electrophoresis (CZE) assay for L-ascorbic acid (L-AA) in foods has been developed. The Korean Food Codex for the analysis of L-AA employs 2,4-dinitrophenylhydrazine (DNP) and high-performance liquid chromatographic (HPLC) methods. This study was carried out to determine the L-AA by CZE and the results were compared with those of DNP and HPLC methods. CZE demonstrated good efficiency as well as rapid analysis. Analysis was done within 2 min with efficiency approaching 78 000. CE separation was achieved with an untreated fused-silica capillary of 27 cm total length×57 μm I.D. using a sodium borate buffer. UV absorption of solutes was detected at 245 nm and 265 nm. The electric field was 556 V/cm. Under the optimum conditions, L-AA in various foods was determined. A 512-channel diode-array detector was used to confirm the L-AA peak. The coefficients of variation of the migration time and the peak area were less than 0.5% and 1.2%, respectively. The limit of detection ($S/N=3$) for quantitative determination was 0.25 μg/ml. Good linearity and recovery were also obtained with a correlation coefficient $r=0.993$ and 95.0–98.6% recovery. Considering the results of our study, CE method should be highly suitable for the determination of L-AA in foods. © 1997 Elsevier Science B.V.

Keywords: Food analysis; Ascorbic acid

1. Introduction

The determination of L-ascorbic acid (L-AA) in foods is very important with regard to the standardization as nutrient composition and to the monitoring of the stability during processing. Currently, its analytical methods are HPLC separation based on ion-exchange or reversed-phase columns with UV absorbance [1–3], fluorescence [4,5], or electrochemical detection [6–8] and gas chromatography with flame ionization detection [9]. The analytical methods for L-AA in Korean Food Codex were established the chemical methods by 2,4-dinitrophenylhydrazine (DNP) or indophenol and HPLC methods by C_{18} or NH_4 column with detection at UV 254 nm [10]. The chemical method using indophenol monitoring the visual end-point of the 2,6-dichlorophenol–indophenol titration is not always satisfactory, particularly in colored solutions and if some other reducing impurities are present [11]. Also the reaction with 2,4-DNP is a very complicated 6-h long process at 20°C. Temperature and time affect the extent of the reaction but they have a much more profound side effect on the reaction of possible interfering substances [12]. HPLC method is easy and reliable in comparison with the chemical methods. However, it has several drawbacks including the time-consuming conditioning of the columns, the large consumption of solvents, and the high price of columns. Nowadays

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capillary electrophoresis (CE) has been used successfully in various application fields such as biochemistry, biotechnology pharmaceuticals and clinical chemistry [13–15]. However, the impact of CE in food science and particularly in quality control of food and food additives has been minor until now [16]. Catherine and Craige [17] recently reported the determination of the total L-AA in foods by micellar electrokinetic capillary chromatography. Other investigators reported applications to pharmaceutical products and biological samples using stereoisomer as an internal standard by CE [18–20].

In this study we developed a new CZE method to measure the L-AA in foods and compared it with HPLC and DNP methods of the Korean Food Codex.

2. Experimental

2.1. Chemicals and sample preparation

L-AA, metaphosphoric acid (MPA), thiourea, 2,4-DNP, 2,6-dichlorophenol-indophenol and L-cysteine were obtained from Sigma (St. Louis, MO, USA). Sodium borate and sodium hydroxide were obtained from Beckman (Fullerton, CA, USA). HPLC grade methanol and water were purchased from Merck (Darmstadt, Germany). Phosphate was purchased from Fluka (Buchs, Switzerland). The standard L-AA was dissolved in a buffer to give concentrations in the range 0.1–500 μg/ml. Food samples were obtained from a local supermarket (Seoul, South

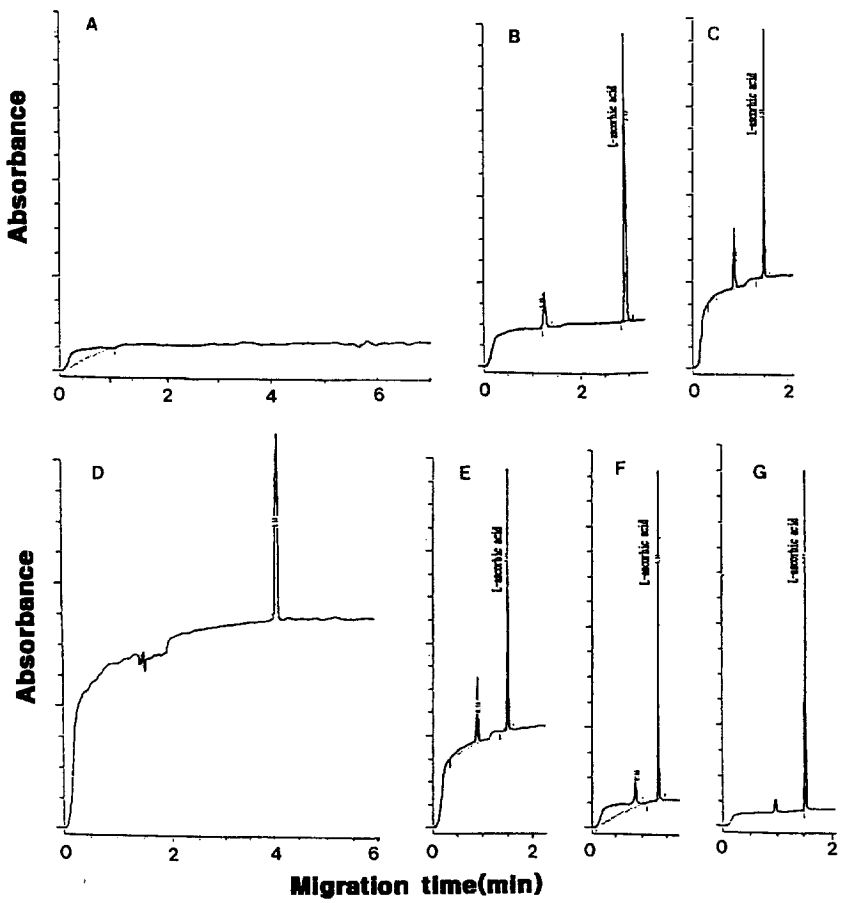

Fig. 1. Effect of pH and electrolyte on the migration time of L-ascorbic acid. (A) 20 m*M* phosphate (pH 2.5), (B) 20 m*M* phosphate (pH 6.0), (C) 20 m*M* phosphate (pH 8.0), (D) 20 m*M* sodium citrate (pH 6.5), (E) 20 m*M* phosphate (pH 7.0), (F) 10 m*M* tricine (pH 8.0), (G) sodium borate (pH 8.0). CE conditions: 27 cm×57 μm fused-silica capillary, Standard concentration 30 μg/ml, applied voltage 17 kV.

Korea). L-AA fortified biscuit, candy, chocolate and balanced nutrition biscuit were supplied from Haitai Confectionery (Seoul, South Korea). L-AA was measured after blending and extraction of the sample with 5% MPA and adding L-cysteine for stabilization [23]. Samples were centrifuged at 56 700 *g* for 10 min at 4°C (Model J_2-HC, Rotor JA 25.25, Beckman, CA, USA) and then filtered through a 0.45-μm filter (Whatman, NJ, USA) and injected immediately.

2.2. Apparatus and method

CZE separation was performed on a Beckman P/ACE 5500 CE instrument with a diode array detector and the Gold software (Beckman). Uncoated capillaries purchased from Beckman were used throughout the analysis. Prior to use, each capillary was rinsed with 10-capillary volumes of 1 *M* NaOH, 1 *M* HCl, water, then finally with the separation buffer. Between analyses, capillaries were rinsed with 0.1 *M* NaOH and subsequently with the separation buffer. Samples were introduced into the CE capillary by pressure injection for 5 s at 3.45 kPa and all experiments were conducted using an applied voltage from 10–30 kV and a capillary at 25°C. Separation buffers used are described in individual figure legends. The separation runs were monitored at 245 nm or 265 nm with a data acquisition rate of 2 Hz. Data were analyzed using the scan graphics software obtained from Beckman. All solutions used for sample treatment and buffer systems were sonicated and filtered through a 0.45-μm membrane syringe filter.

HPLC was performed using a Model 128 dual Pump (Beckman) and a Model 166 UV multiwavelength detector with a mobile phase of methanol–water (75:25, v/v) at a flow-rate of 0.5 ml/min at room temperature. The column (250 mm×4.6 mm I.D.) was C_{18} (Beckman) and the injection volume was 20 μl.

The DNP method was the modified Roe's method [21]. The sample was oxidized so that L-AA was converted to dehydroascorbic acid (DHAA) and then allowed to react with 2,4-DNP. The final stage of the reaction usually involves the addition of 4.5 *M* H_2SO_4 to develop a red color that was measured using a spectrophotometer (Model DU-650, Beckman) at 520 nm. The calibration curve was obtained with standard solutions in 5% MPA in the range 2.5–25.0 μg/ml.

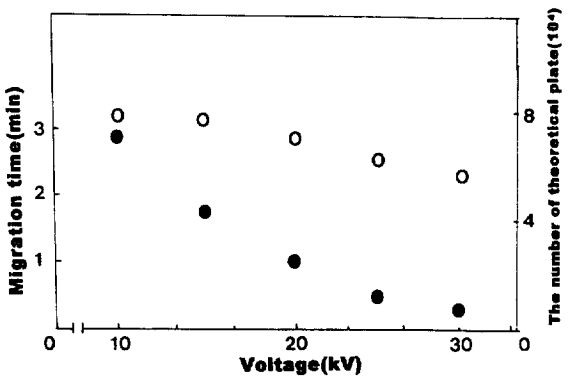

Fig. 2. Effect of electric field on the migration time (●) and the number of theoretical plates (○). Voltage range is 10–30 kV; electrolyte, 100 m*M* sodium borate (pH 8.0); other conditions are as in Fig. 1.

3. Results

3.1. Effect of the buffer

The CE separation conditions were first examined at basic electrolyte condition (pH 2.0–9.0). Fig. 1 shows electropherograms of L-AA at various electrolyte conditions. The separation in CZE is based on

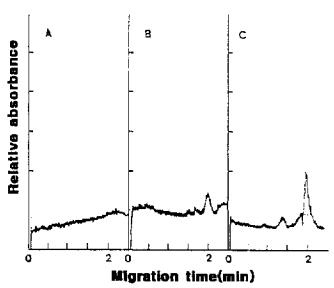

Fig. 3. Concentration of a large volume–low concentration sample using stacking. Sample concentration, 0.06 μg/ml; electrolyte, 100 m*M* sodium borate; applied voltage, 15 kV; injection methods: (A) pressure injection 5 s at 3.45 kPa dissolved in a 100 m*M* borate buffer (pH 8.0), (B) pressure injection of a water plug 30 s at 3.45 kPa in front of pressure injected L-AA, 5 s at 3.45 kPa dissolved in a 5 m*M* borate buffer (pH 8.0), and (C) pressure injection of a water plug 18 s at 3.45 kPa in front of the electrokinetic injection of L-AA 18 s at 20 kV in a 5 m*M* borate buffer (pH 8.0). Other conditions as in Fig. 1.

Table 1
Reproducibility of the migration time and peak area of L-ascorbic acid by CE ($n=10$)

	Migration time (min)			Peak area		
	Mean	S.D.	R.S.D.	Mean	S.D.	R.S.D.
Run-to-run	1.85	0.01	0.5	2019	16	0.8
Day-to-day	1.86	0.01	0.5	2055	25	1.2

Table 2
Precision and recovery of the determination of L-ascorbic acid in candy, chocolate, biscuit and balanced nutrition food

Foods	Concentration added (μg/ml)	Concentration detected (μg/ml)	Mean recovery (%)
Candy	20.0	19.1	95.0
Chocolate	20.0	16.0	93.0
Biscuit	20.0	19.0	94.5
Balanced nutrition food	95.0	93.7	98.6

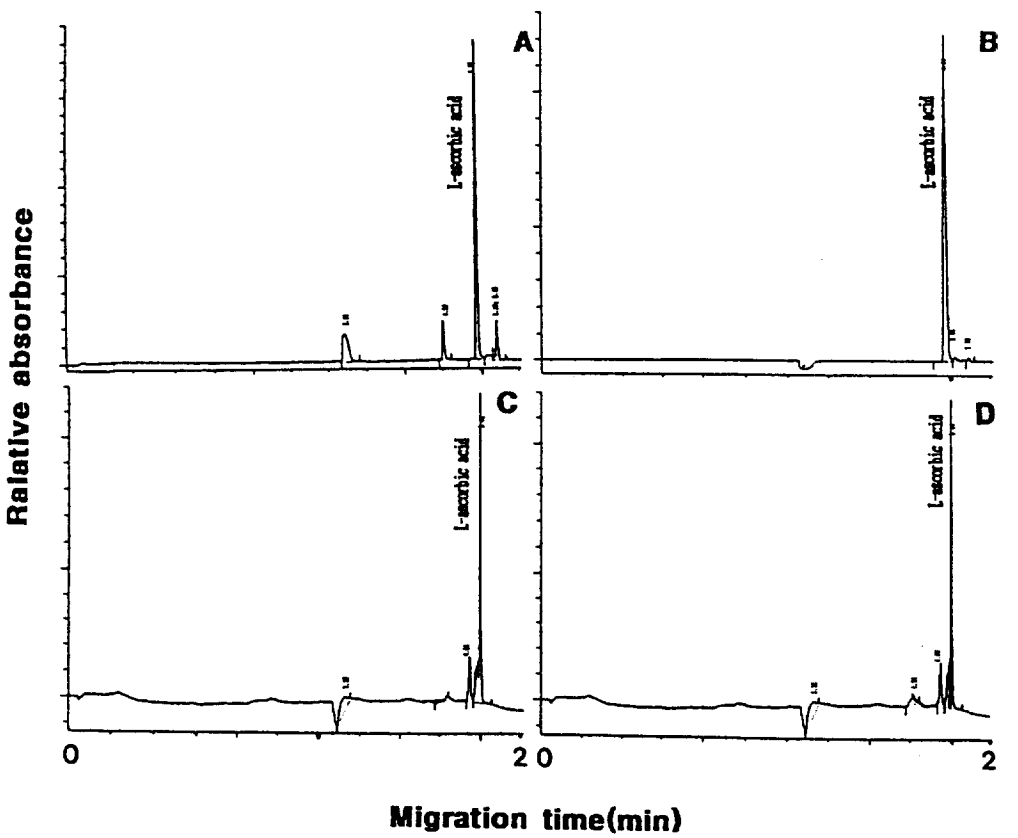

Fig. 4. Electropherograms of L-AA fortified chocolate (A), candy (B), biscuit (C) and balanced nutrition food (D).

differences in electrophoretic mobilities (μ_{ep}) of the analytes with μ_{ep} depending heavily on the pH of the separation system. The pH of the buffer also influences the degree of ionization of the solutes and, hence, their electrophoretic mobilities.

The electroosmotic flow is a significant factor when L-AA is analyzed by CE, because it has a highly anionic character in neutral conditions, and thus, it can migrate quickly on CE using a fused-silica capillary. With pK_a values of 4.17 and 11.57 for L-AA, the buffer pH must be above 4.2 to ionize the L-AA. L-AA migrates faster at a higher pH (pH≥7.0) whereas the peak of L-AA does not appear at the lower pH (pH≤4.0) because electroosmotic flow is very slow under these conditions. However, at and above pH 8.5 imperfect baseline fluctuations occur and the peak of L-AA is interfered by the solvent peak. Therefore, the optimum pH of the buffer was pH 8.0. Borate buffer shows minimal UV background, which permits the use of short UV wavelengths to achieve acceptable sensitivities and a very stable peak.

3.2. Effect of the electric field

We obtained the maximum usable voltage at the point where the Ohm's plot just deviates from linearity [22]. The maximum voltage also depends on the capillary length and inner diameter. A shorter capillary and a bigger inner diameter capillary will have a lower maximum voltage. We used a fused-silica capillary with a length of 27 cm and inner diameter of 50 μm. The effect of the applied voltages on peak efficiency and mobility is depicted in Fig. 2. These results clearly indicate that the efficiency increases as the applied voltage increases up to 15 kV, but it decreases beyond 15 kV.

3.3. Sample stacking

In many cases CE seems to lack sufficient sensitivity to detect trace amounts of chromophoric analytes. Chien and Burgi [25,26] proposed to concentrate a large volume of dilute samples using field amplification. Fig. 3 shows the effect of the stacking method. These results were obtained with various injection methods: (A) pressure injection for 5 s at 3.45 kPa dissolved in a 100 m*M* borate buffer (pH 8.0), (B) pressure injection of a water plug for 30 s at 3.45 kPa in front of the pressure injected L-AA, 5 s at 3.45 kPa dissolved in a 5 m*M* borate buffer (pH 8.0) and (C) the pressure injection of a water plug for 18 s at 3.45 kPa in front of the electrokinetic injection of L-AA for 18 s at 20 kV in a 5 m*M* borate buffer (pH 8.0). The peak area and height of L-AA obtained in (C) are about 4.0 times higher than those obtained with (A). Therefore, the stacking method (C) could be useful to determine L-AA in the sample.

3.4. Reproducibility, accuracy, linearity and detection limit

Reproducibility of migration times in CZE was evaluated by comparing the electropherograms of the same standard at the beginning and the end of each day for 10 days. We carried out these tests under the optimum conditions determined from the above investigations. The mean value of migration time of the L-AA peaks was 1.85±0.01 min. These conditions included a rinse with 0.1 *M* NaOH each day.

Table 3
Content of L-ascorbic acid in several foods by CE method

Foods	L-Ascorbic acid[b] (μg/ml)
Vegetables	
Cabbage	20.0±0.7
Carrot	40.5±2.1
Celery	79.9±3.0
Onion	15.0±0.2
Tomato	52.5±2.1
Fruits	
Apple	15.0±0.5
Banana	52.5±1.0
Grape	20.5±2.1
Orange	69.5±3.2
Peach	39.2±0.8
Pineapple	19.0±1.1
Kiwi	72.9±3.0
Strawberry	70.8±2.1
Juice and drinks	
Orange	50.4±2.1
Kiwi	17.7±1.1
Jujube	69.7±3.0
Apple[a]	147.9±7.8

[a] L-Ascorbic acid fortified apple juice.
[b] Mean±standard deviation.

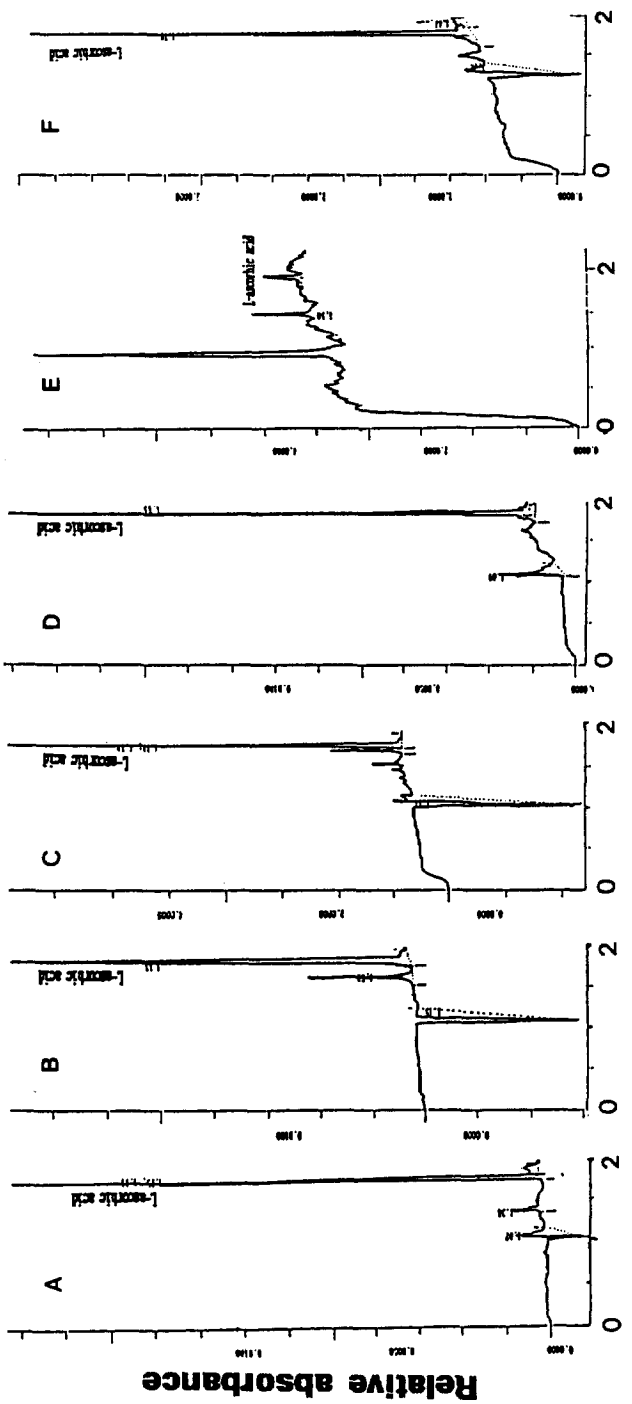

Fig. 5. Electropherograms of L-ascorbic acid in several food stuffs: (A) orange juice, (B) apple juice, (C) kiwi juice, (D) jujube drink, (E) celery and (F) strawberry.

The sample used in this study produced a normal CE profile. Therefore, good results for the reproducibility of the migration times and peak areas were obtained, ranging from 0.5–1.2% (Table 1).

The correlation between the peak area and the concentration of L-AA in the range of 0.5–500 μg/ml was examined for the quantitative analysis. The linear regression equation for L-AA was $y=201.98x-0.457$ ($r=0.998$). Here, y was the peak area and x was the concentration of L-AA. This linearity was guaranteed up to 88.0 μg/ml. The limits of detection (LOD) and the limits of quantitation (LOQ) of L-AA were 0.06 μg/ml and 0.25 μg/ml, respectively. After 20-μg addition of L-AA to biscuit, chocolate, candy and balanced nutrition food, we tested the recoveries. The L-AA contents were found to be 18.5–19.0 μg/ml in a candy, chocolate and biscuit spiked with 20.0 μg/ml of L-AA and mean recoveries ($n=10$) obtained for L-AA were over 95.0% (Table 2 and Fig. 4).

3.5. L-AA in foods

We applied the CZE to the determination of L-AA in fruit, vegetables, juice and drinks (Table 3 and Fig. 5). In Table 3, all data are the average of three samples. The CZE run times were considerably shorter than the HPLC analysis. Short analysis times are advantageous for the determination of samples, as the vitamin is rather labile, even in the presence of stabilizing agents. Often the sources of L-AA are limited and the L-AA may be changed to DHAA and other oxidized products during food collection, storage and cooking procedures. L-AA is easily oxidized, and to obtain reliable results L-cysteine was used as stabilizing buffer for L-AA because cysteine has a more negative redox potential than L-AA and thus acts as an antioxidant [23].

3.6. Peak identification

Identification of L-AA was performed by characterizing the sample peak in terms of the migration time and UV spectrum. The absorption spectra between 190–300 nm of the peaks in Figs. 4 and 5 presumably corresponding to L-AA are compared with those of standard in Fig. 6. The excellent agreement between standard and sample spectra indicates that CZE separation of L-AA in foods is not subject to interference by other components in foods. Diode array scanning of peak permits a quick and reliable confirmation of L-AA. Ziegler et al. have extensively investigated HPLC determination of L-AA in foods employing a diode array detector [24].

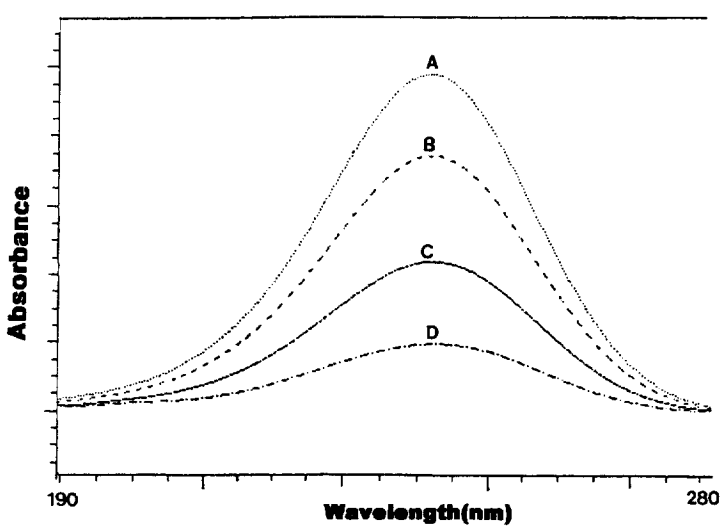

Fig. 6. Comparison of UV spectrum of L-ascorbic acid: (A) strawberry, (B) orange juice, (C) L-AA standard and (D) fortified biscuit in Figs. 4, 5 and 7.

Table 4
Comparison of DNP, HPLC and CE methods for the analysis of L-ascorbic acid

	DNP	HPLC	CE
Run time (min)	360	15	2
Number of theoretical plates	–	3400	78 000
Linearity of concentration range (μg/ml)	2.5–25.0	2.0–60.0	0.5–500.0
Detection limit (μg/ml)	2.5	1.2	0.1
Reproducibility (R.S.D. %)			
Time: run-to-run	4.2	2.5	0.5
day-to-day	5.0	4.2	0.5
Peak area: run-to-run	–	2.6	0.8
day-to-day	–	3.7	1.2

3.7. Comparison of CZE with DNP and HPLC

In order to evaluate the usefulness of the CZE method, results of the CZE were compared with those obtained for L-AA with routine methods – HPLC and DNP – with respect to separation efficiency, sensitivity, linearity and repeatability (Table 4). In the DNP method run-to-run reproducibility was 4.2% and the linearity was in the range 2.5–25.0 μg/ml. The run time for the DNP method was 6 h. The LOD was 2.5 μg/ml. Fig. 7 compares the CE electropherogram and the HPLC chromatogram of L-AA. For the HPLC method, the run time was 15 min and run-to-run reproducibilities were 2.5% for retention time, 2.6% for peak area and the linearity was in the range 2.0–60 μg/ml. The LOD was 1.2 μg/ml at a signal-to-noise ratio of 3. Typical efficiency in the HPLC system was in the range 3000–4000. In CZE, separation time was within 2.0 min and separation efficiency was 78 000. High efficiency directly improves resolution, specially in complex matrices. In CE, the R.S.D. values for migration time and peak area were less than 0.5 and 1.2%, respectively. The LOD was 0.06 μg/ml. The dynamic range of linearity was 1.2–500 μg/ml.

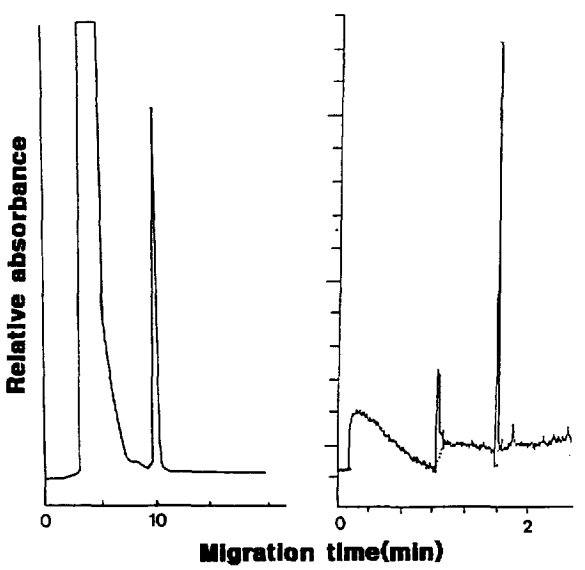

Fig. 7. HPLC chromatogram (left) and CE electropherogram (right) of L-AA; HPLC conditions: mobile phase, methanol–water (75:25, v/v); flow-rate, 0.5 ml/min; column, reversed phase C_{18}; temperature, 20°C; detector, UV 254 nm; injection, 20 μl. CE conditions: electrolyte, 100 *M* sodium borate; applied voltage, 15 kV; capillary, 27 cm×57 μm fused-silica; detection wavelength, 245 nm; injection, 5 s at 3.45 kPa.

4. Conclusion

A CZE method for the determination of L-AA in diverse food and beverage samples has been developed and compared with the HPLC and DNP methods employed in the Korean Food Codex. The proposed unified approach to method development frequently leads to fast, highly efficient separations of a wide variety of analytes. It is anticipated that CE will find broad applications in food industrial laboratories.

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