Short Communication

Determination of total vitamin C in fruits by capillary zone electrophoresis

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ABSTRACT

A simple capillary zone electrophoretic (CZE) method is described for the rapid determination of ascorbic acid and dehydroascorbic acid, the physiologically active forms of vitamin C, in fruits. The electrophoretic run was accomplished in 9 min on a coated capillary column using 20 mM phosphate buffer (pH 7.0). Total ascorbic acid was determined by first reducing the deydroascorbic acid to ascorbic acid by treatment with DL-homocysteine. This reaction was complete in 15 min and total ascorbic acid determination was performed immediately. The data obtained by CZE were in good agreement with HPLC data.

INTRODUCTION

Vitamin C is widely distributed in both animals and plants, probably in equilibrium with dehydro-L-ascorbic acid. Plants rapidly synthesize L-ascorbic acid from carbohydrates, some of which is metabolized into carboydrates and reconverted into L-ascorbic acid for storage in the metabolic pool. Its content in most plants rarely

exceeds 100 mg% of fresh mass, except for a few species that accumulate it in their tissues. The vitamin C content in oranges is about 50 mg per 100 g [1]. Ascorbic acid is also very important in food technology, where it is used as a stabilizer for processing of beverages, wines and meat products.

L-Dehydroascorbic acid occurs in biological materials in relatively low concentrations and it is formed in a redox system in the presence of ascorbic acid. The amounts of ascorbic acid and dehydroascorbic acid are therefore regarded as

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criteria for the initial concentration of ascorbic acid in foodstuffs. Ascorbic acid is mainly determined in fruits and vegetables for judging the storage quality whereas dehydroascorbic acid is only determined in vegetable foodstuffs for assessing the ascorbic acid concentration and its total balance.

Degradation reactions of L-ascorbic acid in aqueous solution depend on several factors, such as pH, temperature and the presence or absence of oxygen and of metals [2–4]. Numerous methods have been developed for the determination of both ascorbic acid (AA) and dehydroascorbic acid (DAA). These include: (1) determination of DAA by condensation with 2,4-dinitrophenylhydrazine and determination of total ascorbic acid (TAA) by oxidation of AA to DAA and subsequent osazone formation [5–7]; (2) the procedure of Tillmans et al. [8] based on the titration of AA with 2,6-dichloroindophenol [8,9], reduction of DAA and final titration for evaluation of TAA; and (3) treatment of DAA with o-phenylenediamine to produce an easily detectable fluorophore [10]. Each of these methods has been adapted for the semiautomated continuous-flow determination of ascorbic acid in pharmaceuticals and food products. However, these procedures are not specific for ascorbic acid, require the preparation and analysis of blanks and are time consuming. Several HPLC methods have also been described for the determination of TAA, based on UV, fluorimetric or electrochemical detectors [11–13]. Among them, Dennison et al. [11] described a method for the determination of vitamin C in beverages by UV measurement of AA after reduction of DAA with homocysteine.

Capillary zone electrophoresis (CZE) offers many advantages for the separation of ionic substances [14–18]. In CZE, the analytes are separated according to their net electrophoretic mobility under the influence of a high potential field. In a coated capillary the electroendoosmotic flow is strongly reduced and the neutral products exhibit a negligible mobility under an electric field. In this context the separation is only affected by the differences in charges. A procedure for the rapid determination of AA and TAA based on the observation that

homocysteine reduces DAA completely to AA was adopted in this work. The reduction of DAA was necessary because of its low molar absorptivity. Thus, ascorbic acid was determined before and after reduction, the difference representing the amount of DAA in the sample. The same samples were also injected in into an HPLC system under the conditions described by Dennison et al. [11] in order to compare the quantitative results.

After this paper has been submitted, a paper was published [19] dealing with the analysis of vitamin C in biological fluids and fruit beverages by CZE. However, in that work no mention was made of the equilibrium between reduced and oxidized vitamin C, and we therefore assume that Koh et al. [19] were unable to determine dehydroascorbic acid.

EXPERIMENTAL

Materials

DL-Homocysteine and dehydroascorbic acid were obtained from Aldrich (Steinheim, Germany), L-ascorbic acid from Merck (Darmstadt, Germany), acrylamide, ammonium peroxidisulphate and N,N,N',N'-tetramethylethylendiamine (TEMED) from Bio-Rad Labs. (Richmond, CA, USA) and phthalic acid from Carlo Erba (Milan, Italy). A fused-silica capillary (100 μm) was supplied by Polymicro Technologies (Phoenix, AZ, USA).

Methods

CZE was performed in a Waters Quanta 4000 capillary electrophoresis system (Millipore, Milford, MA, USA). The analysis was carried out with 20 mM phosphate buffer (pH 7.0) at 6 kV and 60 μA in a 40 cm × 100 μm I.D. capillary. The samples were loaded by hydrostatic pressure for 6 s. The detector was set at 254 nm and the run was carried out at room temperature under reversed polarity (cathode at the injection port and anode at the detection side). The capillary was washed with 20 mM phosphate buffer (pH 7.0) after each run.

HPLC separation of ascorbic acid was achieved with a Jasco HPLC instrument. The column effluents were monitored at 254 nm. A

column of 150 mm × 4.6 mm I.D., packed with Erbasil NH$_2$ spheres of 5 μm diameter, efficiently separated AA isocratically by using methanol–0.25% KH$_2$PO$_4$ buffer (pH 3.5) (1:1, v/v) as the mobile phase. The flow-rate was 0.5 ml/min and the sample injection volume was 10 μl.

Coating the capillary inner wall

The following procedure gave the best results. The capillary was first treated with 100 μl of 1 *M* NaOH for 5 h, then rinsed and flushed with 100 μl of 0.1 *M* HCl followed by 100 μl of 0.1 *M* NaOH. After 1 h it was rinsed with water and acetone, filled with a 1:1 solution of acetone in Bind Silane [3-(trimethoxysilyl)propyl methacrylate] and then incubated overnight. After this treatment, the capillary was rinsed with acetone, flushed with air for 5 min and then washed with 20 m*M* phosphate buffer (pH 7.0). The capillary was filled with 6% acrylamide solution in the same buffer, degassed and containing the appropriate amount of catalyst (0.5 μl TEMED and 0.5 μl of 40% ammonium peroxodisulphate per millilitre of gelling solution). Polymerization was allowed to proceed overnight at room temperature and then the capillary was emptied by means of a syringe.

Sample extraction and treatment procedures

For determination of total vitamin C, 5 ml of 12.5% trichloroacetic acid were added to a 15-g portion of freshly squeezed orange juice, the solution was centrifuged and filtered and the filtrate was assayed for AA. The sample solution was then diluted with distilled water so as to provide an estimated ascorbic acid concentration of 10–100 μg/ml, and the pH was adjusted to 7.0. The reduction of DAA was performed by adding 2.0 ml of 0.8% DL-homocysteine solution to 0.5 ml of the sample extract (a minimum of a 40:1 molar excess of homocysteine to dehydroascorbic acid). After 15 min, the sample was filtered and assayed immediately.

Standardization

Serial dilutions containing 5–80 μg/ml were prepared by dissolving reference grade ascorbic acid mixed with phthalic acid used as an internal standard. Aliquots of each standard were ana-

lysed and a calibration graph was obtained by plotting peak height *versus* concentration. The ascorbic acid concentration of the sample extract was calculated by interpolation on the calibration graph and by application of dilution factors. The AA concentration of the sample was expressed as mg per 100 ml.

RESULTS AND DISCUSSION

The determination of total ascorbic acid required a rapid method of converting DAA into AA, as only AA possessed the strong UV absorption necessary for detection. The reduction of DAA to AA by DL-homocysteine was described in 1956 by Hughes [20] and verified by Dennison *et al.* [11]. The reaction is highly efficient and provided a rapid method of reducing DAA for total ascorbic acid determination.

Standards and orange juice samples, when injected into the CZE system, gave a typical electropherogram as shown in Fig. 1. In a coated capillary, owing to the migration conditions adopted, only AA is visible (peak 2), peak 1 corresponding to the phthalic acid used as internal standard in the quantitave analysis. The response was linear between 3 and at least 80
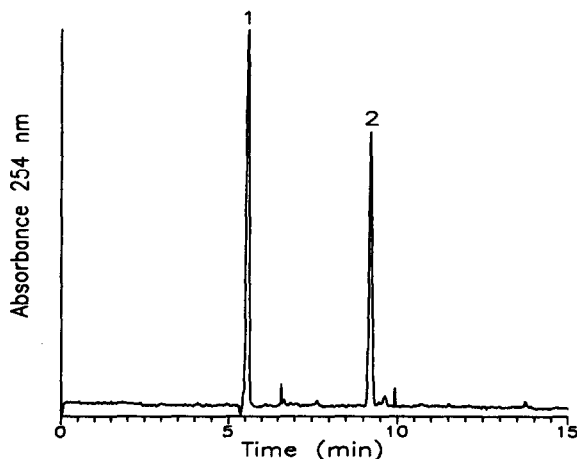

Fig. 1. CZE profile of a homocysteine-reduced orange juice sample. Sample injection: 6 s by hydrostatic pressure. Coated capillary of 40 cm × 100 μm I.D. Run in 20 m*M* phosphate buffer (pH 7.0) at 6 kV and 60 μA. Detection at 254 nm; anodic migration (reverse polarity). Peaks: 1 = internal standard (phthalic acid); 2 = ascorbic acid.

μg/ml. The amounts assessed in two different samples of orange juice were 44.7 ± 1.3 and 57.5 ± 1.7 mg per 100 ml ($n = 6$ in both instances), in excellent agreement with the HPLC results (45.2 ± 2.2 and 56.5 ± 3.1 mg per 100 ml, respectively; $n = 6$). Although we have not evaluated the stability of the coating over an extended period of time, we did not experience any deterioration of the results after at least 100 sample analyses. A typical HPLC elution profile of a diluted and reduced freshly squeezed orange juice is shown in Fig. 2. The HPLC procedure affords an excellent separation among AA (peak with retention time of 8.718) and the reacted and unreacted DL-homocysteine (4.722 and 5.940 min, respectively).

In conclusion, the CZE procedure proposed here for ascorbic acid is simple, requires a minimum of sample preparation and provides a good method for the rapid determination of total vitamin C. In addition, the CZE method measures the ascorbic acid directly, eliminating the need for organic solvents and expensive chromatographic columns. The combined treatment and analysis time required for any sample does not exceed 25 min, with complete recovery from the food matrices examined.

ACKNOWLEDGEMENTS

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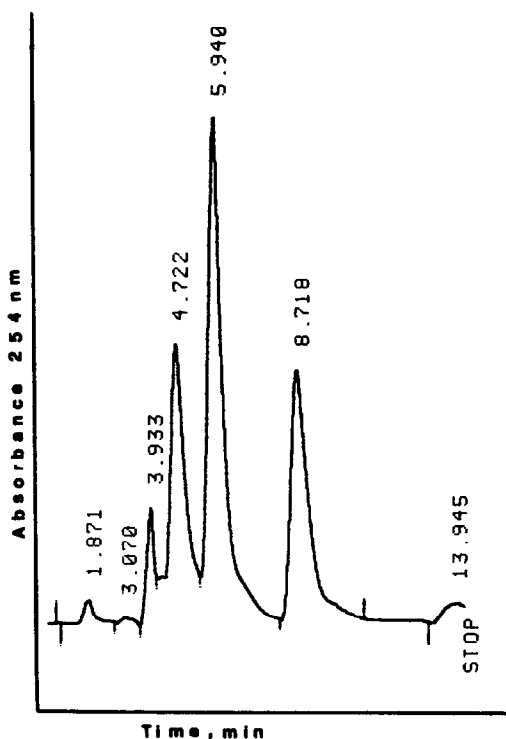20 R.E. Hughes, *Biochem. J.*, 64 (1956) 203–208.

Fig. 2. HPLC profile of the orange juice sample in Fig. 1. Sample injection volume: 10 μl. Column: 150 m $\times$ 4.6 mm I.D., packed with Erbasil NH_2 spheres of 5 μm diameter. Isocratic elution with methanol–0.25% potassium phosphate buffer (pH 3.5) as mobile phase at a flow-rate of 0.5 ml/min. Detection at 254 nm. Vitamin C is the peak with an elution time of 8.718 min.