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Note

High-performance liquid chromatographic method for the determination of L-ascorbic acid and D-isoascorbic acid

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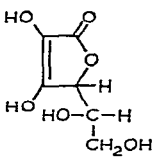
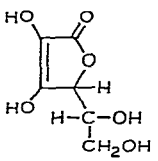
Both L-ascorbic acid (vitamin C) and D-isoascorbic acid (erythorbic acid) are permitted in certain foods. L-Ascorbic acid, I, is used as a nutrient and as an antioxidant, whereas D-isoascorbic acid, II, is useful only as an antioxidant[1].

The structures of these two compounds differ only in the spatial configuration about carbon-5, where there is a reversal of the hydrogen and hydroxyl groups. This close structural similarity makes differentiation difficult, since most chemical assays for either compound are based on reaction with the enediol group, within the lactone ring, common to both.



I



II

Methods published to date that differentiate between L-ascorbic acid and D-isoascorbic acid include paper[2] and silicic acid-impregnated glass fiber chromatography[1], alternating current polarography[3], isotachophoresis[4] and a microbiological assay using *Myrothecium verrucaria*[5].

The purpose of this paper is to report a high-performance liquid chromatographic (HPLC) method for the rapid and simultaneous measurement of L-ascorbic acid and D-isoascorbic acid. Although L-ascorbic acid has been measured on HPLC systems (anion-exchange[6] and C_{18} reversed-phase[7]), this HPLC system separates and measures both diastereoisomers. During the writing of this paper, a similar HPLC method was reported as a chromatography note in this journal[8]. Like that reported method, we use a weak anion exchange column; but unlike that report, we observe no difference in sensitivity of the components due to pH changes of the buffer in the mobile phase. The effects of mobile phase buffer strength and buffer pH on resolution, retention time and sensitivity are reported for our HPLC method.

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EXPERIMENTAL

High-performance liquid chromatography

A Spectra-Physics 8000 high-performance liquid chromatograph equipped with an ultraviolet (UV) detector set at 265 nm was used. Aqueous samples (10 μl) were injected on to a 30 cm × 3.9 mm I.D. Waters Assoc. carbohydrate analysis column (10 μm). The mobile phase was 20% aqueous acetonitrile containing 0.003 *M* (final) potassium phosphate buffer (pH 6.0). The flow-rate was 2.0 ml/min and the column temperature 25°C. Attenuation was 0.4 a u.f.s. on the UV detector.

Reagents

Acetonitrile was purchased from Burdick & Jackson Labs., Muskegon, MI, U.S.A.. L-Ascorbic acid and D-isoascorbic acid were purchased from Aldrich, Milwaukee, WI, U.S.A. Doubly distilled, deionized water was used to prepare all solutions.

RESULTS AND DISCUSSION

The carbohydrate analysis column using an aqueous acetonitrile mobile phase is a well-documented method for separating sugars[9]. Fig. 1 shows the separation of the two diastereo isomers of vitamin C, L-ascorbic acid and D-isoascorbic acid. With the chromatographic conditions developed, near-baseline separation is obtained. The buffer in the mobile phase is necessary. Without it neither component elutes from the column. A final buffer concentration of 0.001 *M* potassium phosphate (pH 6.0) in the mobile phase is adequate to permit the components to elute, but 0.003 *M* (pH 6.0) final is routinely used. The resolution and the retention time of the two components are independent of the buffer concentration between 0.001 *M* and 0.006 *M* potassium phosphate (pH 6.0) final in the mobile phase. Also, the pH of this buffer can be varied between 7.0 and 3.0 with no detectable effect on resolution or retention times of L-ascorbic acid and D-isoascorbic acid.

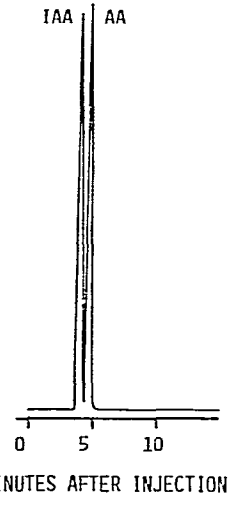
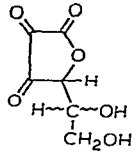


Fig. 1. HPLC resolution of D-isoascorbic acid (IAA) and L-ascorbic acid (AA). 250 μg/ml each.

The wavelength of the UV detector is set at 265 nm which is the λ_{max} for both components at pH 6.0. Both components show the same response factor on this detector at all concentrations and pH values of buffer examined.

Rigorous examination of the capability of this assay method has not been carried out but the minimum detectable amount of both L-ascorbic acid and D-isoascorbic acid is 0.02 μg under the conditions described in the experimental section.

Both L-ascorbic acid and D-isoascorbic acid degrade to their respective dehydro forms, III. Because dehydroascorbic acid and dehydroisoascorbic acid have low extinction coefficients at the wavelength of detection, their presence in samples does not interfere with this assay method.



III

Figs. 2 and 3 show the results of measuring L-ascorbic acid and D-isoascorbic acid in food materials. Good agreement was achieved between the HPLC measurement of L-ascorbic acid and the value listed on the food container: the cranberry

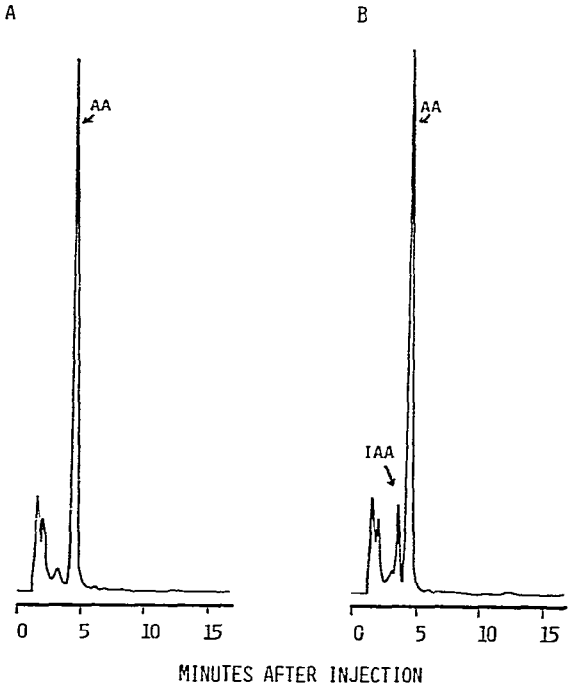


Fig. 2. HPLC measurement of L-ascorbic acid (AA) and D-isoascorbic acid (IAA) in Ocean Spray® cranberry juice cocktail. A, Fruit juice from bottle; B, fruit juice spiked with D-isoascorbic acid (50 μg/ml final).

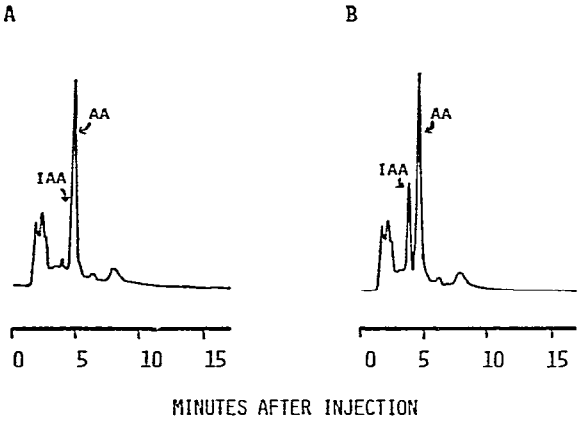


Fig. 3. HPLC measurement of L-ascorbic acid (AA) and D-isoascorbic acid (IAA) in Welch's® grape juice. A, Fruit juice from can; B, fruit juice spiked with D-isoascorbic acid (50 μg/ml final).

juice listed 338 μg/ml vitamin C, the HPLC method measured 310 μg/ml; the grape juice listed 148 μg/ml vitamin C, the HPLC method measured 125 μg/ml. The grape juice sample showed a peak in its chromatogram (Fig. 3) that eluted at the retention time for D-isoascorbic acid; resulting in a measurement of 8 μg/ml for that component. For experimental purposes, D-isoascorbic acid (50 μg/ml final) was added to the samples. The cranberry juice gave 106% recovery and the grape juice gave 108% recovery.

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