Analysis of L- and D-ascorbic acid in fruits and "fruitdrinks by HPLC

HASSAN Y. ABOUL-ENEIN* and IBRAHIM A. AL-DURAIBI
Bioanalytical and Drug Development Laboratory, Biological and Medical Research Department, King Faisal Specialist Hospital and Research Center, MBC 03, P.O. Box 3354, Riyadh 1121, Kingdom of Saudi Arabia

RALUCA-IOANA STEFAN, CRISTINA RADOI, and ALINA AVRAMESCU2
Department of Analytical Chemistry, Faculty of Chemistry, University of Bucharest, Blvd. Republicii 13, 70346 Bucharst-3, Romania

A simple isocratic high-performance liquid chromatographic (HPLC) method is described for the separation and quantitative analysis of L-ascorbic acid (LAA) and its epimer o-ascorbic acid (DAA). This method provides a baseline separation of LAA from DAA (resolution factor R_s = 1.1, selectivity factor a = 1.32, and capacity factor k' = 2.6 and 3.44 for DAA and LAA, respectively). The method has been applied to the analysis of LAA in several fruit and fruit drinks and proved useful for quality control purposes.

Keywords: L-ascorbic acid; o-ascorbic acid; fruits; fruit drinks; high-performance liquid chromatography; analysis

Introduction

Ascorbic acid [vitamin C, L-ascorbic acid (LAA)] participates in many different biological processes and is important in human diet. Furthermore, ascorbic acid has been reported to have beneficial effects against cancer, cardiovascular disease, cataracts, cholesterol, lipid oxidation, hypertension, and aging, among others (Gershoff, 1993, Meyers *et al.*, 1996). For these applications ascorbic acid supplements have become popular. Moreover, isoascorbic acid, also known as erythorbic acid, o-araboascorbic acid or D-ascorbic acid (DAA), is not a naturally occurring isomer of L-ascorbic acid and has been used interchangeably with the latter as an antioxidant in prepared foods. Therefore, in the USA, o-isoascorbic acid is a permitted antioxidant in some processed foods (Clegg and Macdonald, 1975)

DAA reportedly has no biological activity (Marks, 1975) or has limited antiscorbutic properties (Demole, 1934; Ranken, 1974). Also, Park (1985) reported that DAA was 20% as effective as LAA in growth enhancement of human leukemic cells. A dose-response study was performed comparing LAA and DAA. Over a wide range of concentration DAA was shown to be consistently less effective than LAA. This finding demonstrates that this enhancing effect is truly biological in nature. In contrast, Shin and Stirling (1988) stated that DAA has the same reduction-oxidation potential as LAA, but is less biologically active. DAA was less effective in potentiating the dopaminergic effect than was LAA, which supports the notion that potentiation by LAA is not entirely due to its reducing property. Nevertheless, Pelletier (1969) implied that both absorption and

*To whom correspondence should be addressed.

tissue deposition occur less readily for DAA than for LAA. On the other hand, DAA may in fact have the same antiscorbutic potency as an equal amount of LAA. Its apparent low activity would then be associated with both the inability of the animal to absorb it and the tissue to retain it (Goldman *et al.*, 1981). However, substituting DAA for the antagonistic properties of LAA in copper metabolism reveals a possible stereospecific post-absorption role for LAA in the metabolism of copper and it is not responsive to reducing agents in general (Disilvestro and Harris, 1981).

Several methods have been applied to separate and determine ascorbic acid and/ or its isomers in a variety of samples, including foods, pharmaceuticals, and biological samples. The techniques used include an enzyme-based biosensor (Volotovsky and Kim, 1998), flow injection analysis (Albero *et al.*, 1992; Ensafi and Rezaei, 1998), capillary electrophoresis (Koh *et al.*, 1993), capillary zone electrophoresis (Ling *et al.*, 1992), and HPLC (Tsao and Salimi, 1982; Seki *et al.*, 1987; Lopez-Anaya and Mayersohn, 1987; Margolis and Davis, 1988; Poon *et al.*, 1994; Ali and Phillippo, 1996: Margolis and Duewer, 1996; Margolis and Schapira, 1997).

Therefore, a logical approach was to investigate a simple and rapid method for the separation and determination of these two isomers of ascorbic acid in fruits and supplemented fruit drinks, using high-performance liquid chromatography, and the results are presented in this paper.

Materials and methods

Materials

HPLC-grade acetonitrile was obtained from Fisher Scientific (Pittsburgh, PA, USA), monobasic potassium phosphate and LAA from Sigma Chemical (St Louis, MO, USA), metaphosphoric acid (MPA) and DAA from Aldrich Chemical (Milwaukee, **WI,** USA), and dithiothreitol (DIT) from Pierce Chemical, (Rockford, **IL,** USA). Orange, grapefruit, strawberry fruits, green pepper and fruit drinks, were purchased locally. Deionized water was used throughout (Milli-Q system, Millipore, Bedford, MA, USA).

Apparatus

HPLC with software System Gold was used which consisted of a Model 126 programmable solvent module, Model 507 autosampler, Model 166 programmable UV detector, and Model 168 diode-array detector (Beckman Instruments, Fullerton CA, USA). An AST computer (AST Research, Taiwan) and an NEC CP6 pinwriter (NEC, Tokyo, Japan) were used.

Method of analysis

The samples were analyzed by HPLC according to a modification of the method of Margolis and Schapira (1997) with a 250 x 4.6 mm Ld., particle size 5 mm LC-NH_2 column (Supelco, Bellefonte, PA, USA). The column was equilibrated at ambient temperature at a flow rate of 0.7 ml/min with a mobile phase composed of 0.68 g of monobasic potassium phosphate, 200 ml of water, and 800 ml of acetonitrile.

The separation was performed isocratically and the eluted components were detected at 268 nm, measured with the photodiode-array detector as the UV maxima for LAA and

DAA under the experimental conditions. The amounts of the isomers were determined by peak-area measurement. The chromatograms were integrated with System Gold v. 601 (Beckman Instruments).

Sample preparation

All the samples and standard solutions were freshly prepared.

0) Fresh samples (orange, grapefruit, strawberry, and green pepper): each sample was cut into small pieces, then weighed and ground with MPA solution 000 g/D in a ratio of 1:1 (w *Iv)* to precipitate the proteins and to prevent the oxidation of LAA and DAA to dehydroascorbic acid and dehydroisoascorbic acid, respectively (Margolis and Black, 1987; Koh *et al.,* 1993). The juice produced was then centrifuged at 20000 rpm at *4°C* for 20 min. Aliquots of 20 ml of the supernatant were injected into the HPLC system.

(2) Supplemented fruit drinks: a portion of canned fruit drinks which were supplemented with LAA as stated by the manufacturers was transferred into a centrifuge tube and centrifuged at 20000 rpm at 4°C for 20 min. A 100 J.!volume of MP A or DTI was added to 900 J.!l of each sample. Aliquots of 20 J.!l of the mixture were then injected into the HPLC system.

Results and discussion

The analysis was performed with an autosampler at room temperature for -15 h. Under these conditions, the stability of LAA at room temperature becomes important (Bradley *et al.,* 1973; Rose and Nahrwold, 1982; *Bakeretal.,* . 1983). This variable was evaluated by measuring the area under the standard peaks for periods up to 16 h. Little or no deterioration of the standard LAA and DAA solutions at *25°C* was detected, an observation supported by the fact that the relation between the concentration of LAA and DAA and the area under each peak were linear from 0.78 to 400 J.!g/ml (Figure 1). The correlation coefficients for LAA and DAA were 0.9994 and 0.9995, respectively. By this method we achieved a baseline separation of DAA from LAA (resolution factor R_s = 1.1, selectivity factor ex = 1.32, and capacity factor k' = 2.6 and 3.44 for DAA and LAA, respectively), which were resolved from MPA and DTT (Figure 2), compared with the data of Margolis and Duewer (996) who reported a near baseline separation.

We applied this method to the analysis of fresh fruit juices, vegetable juice and some prepared (canned or bottled) fruit juices that were supplemented with LAA. It was found that these samples contained significant amounts of LAA (Tables 1 and 2), but did not contain any detectable amounts of DAA (Figure 3). The results gave a mean concentration of 404.5 ± 2.1 J.!g/ml of LAA in orange juice, which corresponds to literature values (Ling *et al., 1992).*

It is of interest that a considerable difference in LAA concentration was found between beverages of the same fruit but of different brands. This observation should be taken into consideration especially for quality control purposes.

Conclusion

The HPLC method described is simple, rapid and reliable for the separation and analysis of L-ascorbic acid and its epimer D-ascorbic acid in fresh fruits and fruit drinks. The method can be adapted for quality control and quality assurance purposes.

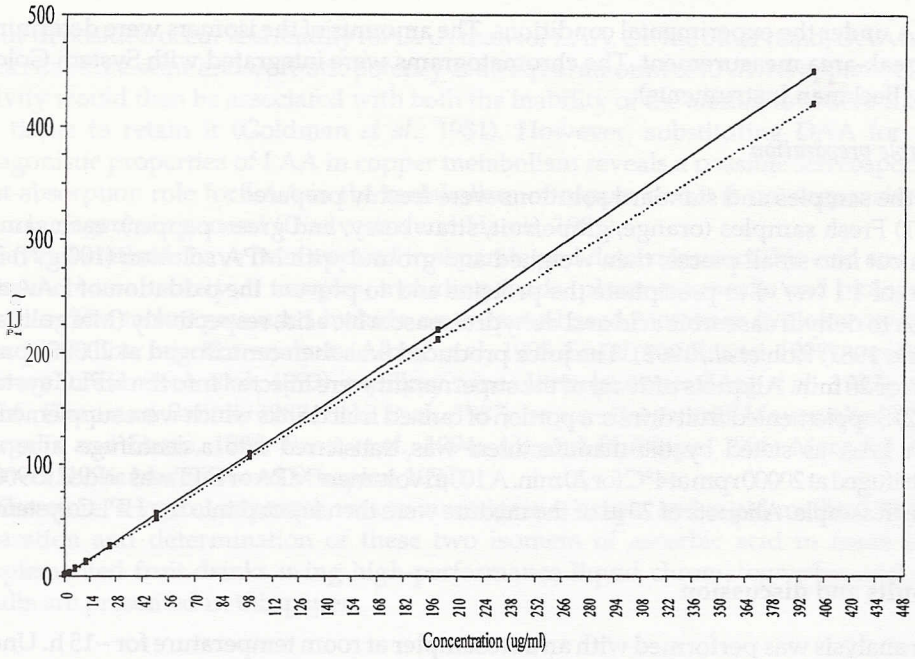

Fig. 1. Standard curves for LAA (solid line) and DAA (dashed line) obtained under the chromatographic conditions described in the Materials and methods section.

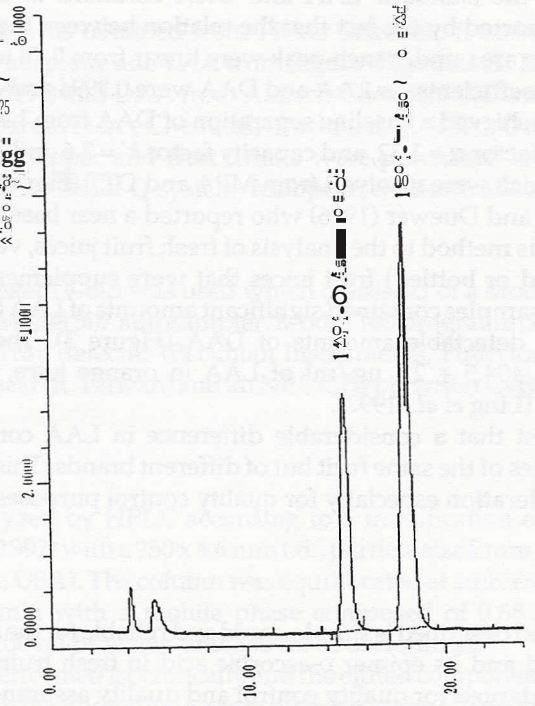

Fig. 2. Chromatogram of the separation of LAA from DAA standards on LC-NH_2 column. Chromatographic conditions are described in Materials and methods section.

Table 1. Ascorbic acid content in fresh fruit and vegetable juices (f.lg/g) (n = 10)

Sample	*AA ± SD (jig)*
Orange	404.5± 2.1
Grapefruit	245.0±12.2
Strawberry	386.0± 4.6
Yellow pepper	1059.8± 8.8

Table 2. Ascorbic acid content in supplemental fruit drinks (µg/ml) (n = 10)

Beverage	*AA±SD(J1.g)*
Lemon drink	342.9± 10.3
Fruit cocktail drink	285.1± 12.5
Pineapple drink	165.5± 3.4
Orange juice (brand 1)	393.5±6.7
Orange juice (brand 2)	417.4±9.6
Orange juice (brand 3)	657.5±8.4

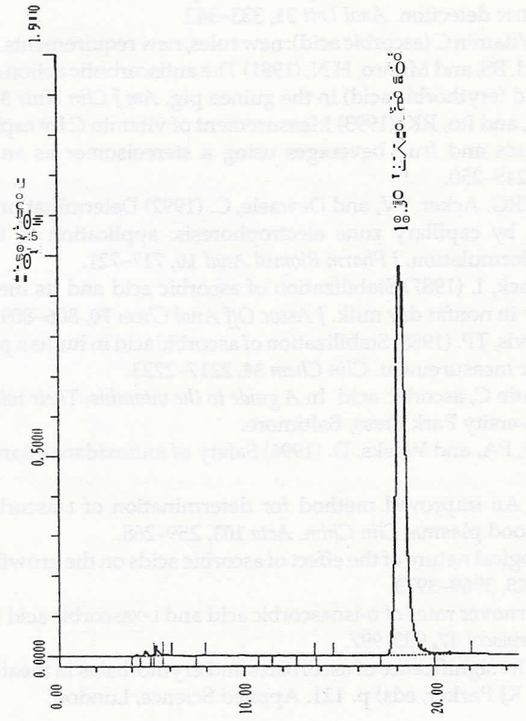

Fig. 3. Chromatogram of analyzed fresh orange juice sample.

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