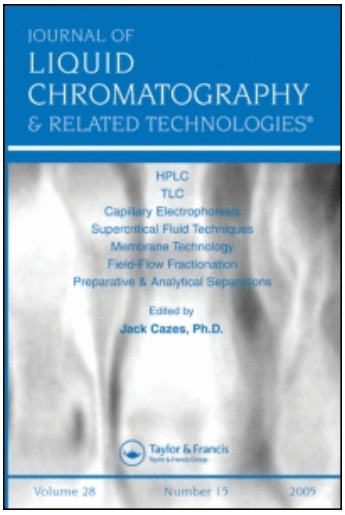

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF ASCORBIC ACID IN FRUITS, VEGETABLES AND JUICES

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A high-performance liquid chromatographic (HPLC) method was developed for the determination of ascorbic acid in fruits, vegetables and juices. Samples were homogenized with 0.2 M phosphate buffer pH 2.0 and extracted with 3% metaphosphoric acid. Ascorbic acid was analyzed on a polymer C18 column (RLRP-S 100A) with UV detection at 244 nm. The mobile phase was 1.8% tetrahydrofuran and 0.3% metaphosphoric acid in HPLC water. Percent recoveries were 90% or greater. The method has been shown to be very reproducible with the percent coefficients of variation (%CV) ranging from 1.3 to 10.6 for 22 different products. The majority of the %CV were below 4. Analyses of several fruits, vegetables and juices have shown a wide range of ascorbic acid content. Dehydroascorbic acid and isoascorbic acid were separated from ascorbic acid with this system.

INTRODUCTION

As researchers learn more about the relationship of dietary intake and human health, an accurate and specific assessment of

the nutrient content of foods is becoming extremely important for nutritional and epidemilogical studies. One such nutrient is ascorbic acid which is primarily found in fruits and vegetables. Besides its major function of preventing scurvy, recent research has shown that vitamin C inhibits certain cancer cell growth in vitro (1); prevents and/or reacts with toxic compounds including nitrosamines (2-4) and increases immune response (5).

Past methods for vitamin C analysis of fruits and vegetables has focused on the AOAC's titration (6) and fluormetric (7) methods, but these procedures are nonspecific and time consuming. For the last 9 years another technique, liquid chromatography (LC), has been used for the analysis of ascorbic acid in foods (8-20). However, column stability because of the buffer systems and pH employed is a problem (15). Recently a polymer column has been developed to withstand pH values 1-14 and has been used to analyze vitamin C in grapefruit juice (20).

This paper describes an HPLC method for the analysis of vitamin C in fruits, vegetables and juices that employs the polymer column with a modified solvent system that adds stability to the system. The same column has been used extensively for over a year without any changes in the chromatography.

EXPERIMENTAL

Materials

L-Ascorbic acid was obtained from Hoffman-LaRoche Inc., Nutley, New Jersey. All solvents were liquid chromatography (LC) grade and were purchased from VWR Scientific, Bridgeport, NJ. Dehydroascorbic acid was bought from Aldrich Chemical Co. Inc., Milwaukee, WI, while isoascorbic acid was from Sigma Chemical Co., St. Louis, MO. All foods were purchased from local supermarkets and roadside stands.

Preparation of Standards

Twenty-five mg of L-ascorbic acid were weighed into a 25 ml volumetric flask and brought to volume with 3% metaphosphoric

acid. Dehydroascorbic and isoascorbic acid were prepared the same as above. All standards were made each day because of stability problems. Ascorbic acid concentration was linear from 0.05 to 2.4 ug.

Extraction and Analysis

Fruits and vegetables were extracted by blending 1 min with equal portions of extracting solution which consisted of 54 g of potassium phosphate monobasic and 55 ml of 85% orthophosphoric acid per 2 l of LC grade water. For reproducibility tests six 6 g samples were taken from the homogenate and placed in 40 ml polypropylene centrifuge tubes to which was added 25 ml of 3% metaphosphoric acid. The mixture in the tubes was polytroned (Brinkman Instruments, Westbury, NY) 1 min at medium speed before centrifuging at 30,000 x g for 5 min. A 10 ul aliquot of the supernatant was injected into the LC preceded by 10 ul standard. Juices were diluted 1 ml of juice into 7 ml of 3% metaphosphoric acid; filtered through 0.45 u nylon filters (Gelman Inc., Ann Arbor, MI) and 10 ul of filtrate injected.

LC was performed using a Valco injector (Vici Instruments, Houston, TX) with a 10 ul loop, a Waters 510 pump (Waters Assoc., Milford, MA), a Hewlett-Packard 1040A photodiode array detector/integrator system (Hewlett-Packard Inc., Avondale, PA), a Waters Model 490 variable wavelength detector and an OmniScribe recorder (Houston Instruments, Austin, TX). The column was a PLRP-S 5 u 100 A, 250 mm x 4.6 mm (Polymer Laboratories LTD, Amherst, MA). Mobile phase was 1.8% tetrahydrofuran and 0.3% metaphosphoric acid in LC water with a flowrate of 0.5 ml/min. Detection was at 244 nm and 0.04 to 0.8 absorbance units full scale.

Recovery Studies

Six g subsamples of homogenates of cabbage, potato, broccoli, green pepper, spinach and cantaloupe were spiked at 3 different levels (10, 50, 100 mg) and then extracted and analyzed by the above procedure.

Purity Check

Ultraviolet (UV) spectral scans from 190 nm to 350 nm were taken on all vitamin C peaks along with UV ratios at 254/244, 260/244, 230/244, 268/244 and 242/244 to check the purity.

RESULTS AND DISCUSSION

A typical chromatogram depicting the separation of vitamin C in potatoes is shown in Figure 1. With this method ascorbic acid elutes slightly after 6 min while dehydroascorbic acid (DHAA) comes off just before and isoascorbic acid right after vitamin C. Even though DHAA can be resolved from ascorbic acid, the sensitivity is for the most part insufficient to detect such small amounts usually found in most raw fruits and vegetables even when monitoring at 210 nm, the maximum for DHAA. Of all the produce analyzed, a small amount of DHAA was seen in broccoli.

Recovery studies were performed on 6 fruit and vegetables spiked at 3 different amounts (Table 1).

As can be seen, the percent recoveries ranged from 100.0 to 90.0% with percent coefficients of variation from 5.0 to 1.9. Thus the extraction technique was more than adequate.

Stability tests were conducted to see how long the samples were stable in the homogenating solution of buffer and orthophosphoric acid. It was demonstrated that vitamin C begins to degrade within 15 min after homogenation and by 30 min 4 to 5% is oxidized. This means that the 6 g of homogenate must be weighed into the 3% metaphosphoric acid within 15 min. Even so, when large subsamples are used, this buffer system is a sufficient stabilizer to be able to replace the more expensive metaphosphoric acid in the initial homogenization step.

For determining the repeatability of this method and also the homogenicity of the homogenation step, 22 fruits and vegetables were analyzed 6 times each for their vitamin C content using both peak height and area for quantitation. The results are shown in Table 2. Percent coefficients of variation ranged from 1.0 to 10

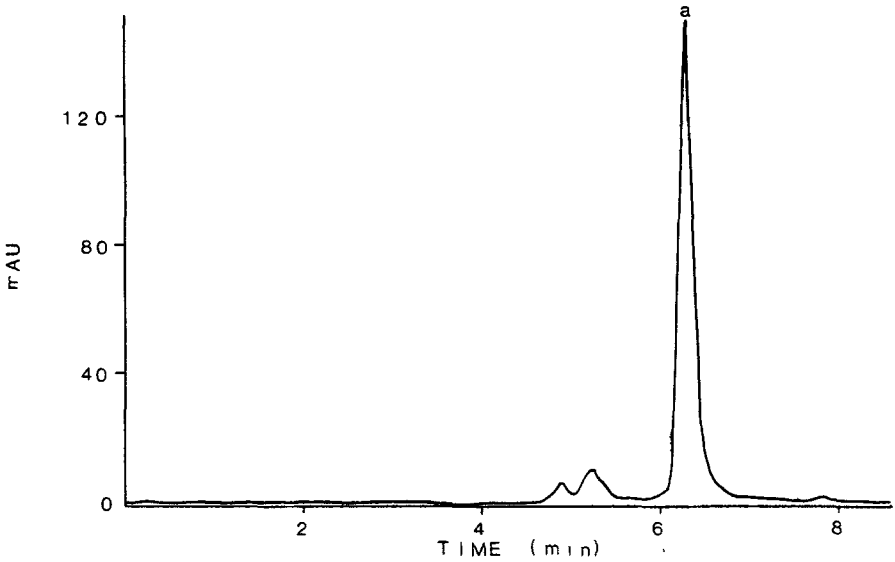

FIGURE 1. Chromatogram of vitamin C in potatoes at 244 nm. Column: RLRP-S 100A. Mobile Phase: 1.8% tetrahydrofuran and 0.3% metaphosphoric acid in HPLC water. Flow Rate: 1.0 ml/min. (a) vitamin C.

TABLE 1

Recovery of Vitamin C from Fruit and Vegetables

---mg/6 g--- Amount Added	Ave % Recovery	%CV
10	100.0	5.0
50	93.4	3.3
100	90.0	1.9

Ave = Average of 6 different determinations of six different products - cabbage, broccoli, cantaloupe, green pepper, potato, and spinach.

%CV = percent coefficient of variation

with most below 4.0. These results indicate that the method is very reproducible and that sample homogenicity can be obtained. Also from Table 2 one can see that either peak height or area can be used for vitamin C determination.

Fruit juices were also analyzed for their vitamin C content (Table 3).

Sixteen juices were analyzed. Ascorbic acid levels varied from none detected to 73.1 mg/100 ml. The lower concentrations were juices that had no vitamin C added while the higher amounts

TABLE 2

Reproducibility of Ascorbic Acid Determination in 22 Raw Fruits and Vegetables.

Produce	mean mg/100 g Ascorbic Acid	%CV (Area)	%CV (PK.HT)
Melon	13.5	2.7	-
Nectarine	2.3	4.3	-
Papaya	52.9	1.8	-
Mango	24.8	2.1	3.4
Asparagus	2.0	10.6	4.2
Kiwi Fruit	79.8	1.3	-
Strawberry	60.6	1.7	3.4
Green Bean	6.1	6.4	-
Orange	37.4	3.0	3.0
Grapefruit	31.5	2.5	1.7
Kale	85.7	1.7	3.8
Blueberry	4.4	4.3	2.6
Tomato	11.6	3.6	2.0
Cantaloupe	23.0	3.1	3.6
Romaine Lettuce	4.9	3.0	2.9
Cabbage	49.5	3.9	3.8
Beet Green	39.7	2.4	2.9
Swisschard	30.4	2.9	2.5
Potato	21.3	2.4	4.7
Broccoli	89.0	1.8	3.8
Cauliflower	71.7	2.8	3.5
Green Pepper	54.8	2.4	1.0

mean of six determinations
%CV = percent coefficient of variation

TABLE 3

Vitamin C Content of 16 Fruit Juices

Juice	-mg/100 ml- Amount Found
Apple Cranberry	11.6
Apple Cherry Berry	9.9
V-8	20.3
Apple	ND
Cranberry	41.8
Apple Cherry	73.1
Orange	32.9
Fruit Punch	39.6
Pineapple Orange	46.8
Cran Raspberry	49.9
Cran Blueberry	43.4
Cran Grape	35.5
Tomato	16.0
Lemonade	2.5
Apple Cranberry	9.0
Pineapple Grapefruit	32.8

ND = none detected at a detection limit of 0.5 mg/100 ml

were fortified juices. The none detected juice was a non-fortified apple juice and since apples contain very small quantities of natural vitamin C, this none detected observation is not surprising.

Conformation of the ascorbic acid peaks for each produce and juice was done by taking UV spectral scans and absorbance ratios. All data collected on each product indicated chromatographically pure ascorbic acid peaks except for some juices which seemed to have a slight interference that caused the peak to tail. In such cases peak height is a better quantitative tool.

This LC method for the determination of ascorbic acid in fruits, vegetables and juices is rapid, precise and could be used for nutritional labeling and for studying the effects of cultural and processing treatments on the vitamin C content of fruits and vegetables. Furthermore, the column has incredible durability and reproducibility when stored in the mobile phase.

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