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A High-performance Liquid Chromatography Method for Determining Ascorbic Acid Content of Fresh Fruits and Vegetables[1]

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Abstract. **Ascorbic acid of fresh fruits and vegetables was extracted with 6% metaphosphoric acid and determined effectively by using a C_{18} cartridge in a radial compression module and 1.5% $NH_4H_2PO_4$ mobile phase in a high performance liquid chromatograph.**

The commonly used AOAC method (2) of determining ascorbic acid content in fresh fruits and vegetables by reducing the 2,6-dichlorophenolindophenol has 2 limitations. First, fresh fruits and vegetables contain compounds other than ascorbic acid that will reduce the dye. Second, recognizing the sharp change in the dye color is difficult when the sample extract contains carotenoids or anthocyanin. These problems are not encountered when the ascorbic acid content is determined by the high performance liquid chromatography (HPLC) method. Augustin et al. (1) modified the HPLC procedure of Sood et al. (5) by using a 3.9 mm × 30 cm uBondapak C_{18} column with a tridecylammonium formate/water/methanol solution, pH 4.5, as the mobile phase. In our preliminary test this procedure was not satisfactory because the internal pressure increased after 5

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Table 1. Ascorbic acid content of horticultural commodities determined by high performance liquid chromatography (HPLC).

Commodity	Ascorbic acid (mg/100 g fresh weights)		
	USDA Handbook 8[z]	HPO_3 extraction solution (pH 1.38)	HPO_3 extraction solution (pH 3.0)
Apples	7	9	1.5
Green snap beans	19	16	4
Sweet peppers	128	146	15
Kale	186	162	7 to 65
Sweet potatoes	23	55	16
Zucchini	19	29	3
Broccoli	113	131	ND[y]
Cabbages	47	67	ND
Carrots	8	16	ND
Cauliflower	78	89	ND
Potatoes	8	25	ND
Tomatoes	21	11	ND

[z]see reference (6).
[y]ND = not done.

Table 2. Effect of different plant materials on the final pH when 60 g fresh plant tissue were macerated with 6% metaphosphoric acid, 250 ml total volume, at initially different pH values.

Commodity	Final pH of plant HPO_3 macerate		
	HPO_3 (pH 3.0)	HPO_3 (pH 1.8)	HPO_3 (pH 1.38)
Apple	3.20	1.95	1.55
Green snap beans	3.92	2.11	1.54
Broccoli	4.11	2.23	1.62
Collard greens	4.19	2.42	1.64
Kale	4.22	2.59	1.72
Sweet potatoes	3.84	2.10	1.53
Zucchini	3.94	2.08	1.60

to 10 samples were injected. The increase in pressure probably was caused by the precipitates which were found to occur when a 6% meta phosphoric acid (HPO_3) extraction solution was added to the mobile phase. Precipitation also occurred when the water content or the pH of the mobile phase was increased slightly. This study was undertaken to improve the HPLC method for determining ascorbic acid content in fresh fruits and vegetables.

A clear and sharp separation of ascorbic acid was achieved by using an 8 mm × 10 cm C_{18} cartridge in a Water's radial compression module with 1.5% $NH_4H_2PO_4$, pH 3, as the mobile phase. The mobile phase was metered at 4 ml/min and the ascorbic acid eluted in 1.98 min. Other UV absorbing compounds present in fruits and vegetables examined did not elute at the same time as ascorbic acid. This was indicated by the lack of a peak at about 1.98 min with an apple sample which had the ascorbic acid oxidized to dehydroascorbic acid during extraction (Figure not shown). The ascorbic acid concentration was based on the absorption of ultraviolet light at a wavelength of UV absorption at 254 nm with Water's model 440 absorption detector. Absorption was linear for ascorbic acid contents ranging from 0 to 7 μg. The injection volume, which ranged from 10 to 50 μl, was dependent on the concentration of ascorbic acid in the plant extract.

The ascorbic acid was extracted from plant tissues with 6% HPO_3 (2) containing 1×10^{-6} M EDTA and 1×10^{-7} M diethylthiocarbamate. The low pH of 6% HPO_3 caused the standard ascorbic acid to elute near the front, so the pH of HPO_3 was increased to 3 to match the pH of the mobile phase. However, the plant material increased the pH additionally to a level where the ascorbic acid was not stable as indicated by the low ascorbic acid values for apples, green beans and zucchini (Table 1). The crops had a differential effect on the final pH of the plant-HPO_3 macerate (Table 2). The pH of the macerate was highest for green leafy tissues such as collards and kale and the lowest for apples. The pH of the plant-HPO_3 macerate did not rise above 3 when the pH of the initial HPO_3 was adjusted to 1.8 (Table 2), and the solution was satisfactory for extraction and analysis of ascorbic acid (data not presented). On the other hand, the pH of unaltered 6% HPO_3 was increased sufficiently by the plant material to a level that did not affect the retention time of the ascorbic acid. Thus, all analyses in this study were completed with unaltered HPO_3 solution, and the pH was adjusted to 2.0 only for the standard.

The ascorbic acid contents of fruits and vegetables analyzed by this method were generally similar to values reported in the USDA Handbook No. 8 (6) (Table 1). The parts analyzed include fruits, stems, leafy tissue, floral tissue, root, and tubers. The analyzed values were higher than the reported values for all crops except kale and tomatoes. These differences between analyzed and reported values were probably due to natural variation in the species.

Table 3. Recovery of added ascorbic acid to 100-g kale sample by high performance liquid chromatography.

Ascorbic acid added (mg)	Quantity recovered (mg)	Calculated recovery (mg)	Recovery (%)
0	143	143	
42	178	185	96
83	217	226	96
125	273	268	102

Recovery of ascorbic acid was studied by adding standards (about 22%, 45%, and 67% of the amount reported for kale in the USDA Handbook No. 8 (6)) to kale samples before maceration. The amount recovered was 96% for 2 of the samples and 102% for the third sample (Table 3), indicating that extraction and recovery by the analytical method described here were complete.

During the preparation of this manuscript, 2 other HPLC methods of analyzing ascorbic acid contents were published. Dennison *et al.* (3) used a 4 mm × 30 cm uBondapak NH_2 packed column and methanol/.025% KH_2PO_4 solution, pH 3.5, as the mobile phase for determining ascorbic acid in beverages. Finley and Duang (4) used 2 uBondapak C_{18} columns connected in series with disodium phosphate solution containing tributylamine for ion-pairing as the mobile phase to separate ascorbic acid, dehydroascorbic acid, and diketogluconic acid of orange and tomato juices, green pepper, and spinach. The pH of the solutions used by these investigators were in a range that was found to be unsatisfactory in our study because it appeared to affect the stability of the ascorbic acid. The mobile phase of these 2 methods contained methanol or an ion-pairing compound, which adds to the cost. Our HPLC method used only a 1.5% $NH_4H_2PO_4$ solution for the mobile phase and the analysis for ascorbic acid content was rapid and effective.

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