Journal of Chromatography, 256 (1983) 368–371
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,418

Note

Simultaneous analysis of ascorbic acid and dehydroascorbic acid in fruit and vegetables by high-performance liquid chromatography

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(First received September 21st, 1982; revised manuscript received October 5th, 1982)

Vitamin C is widely distributed in plant materials, with fruit and vegetables being the major source in most human diets. The biologically active compounds are L-ascorbic acid and L-dehydroascorbic acid, although there is some discussion as to whether dehydroascorbic acid has a similar[1] or lower[2] level of antiscorbutic activity compared with ascorbic acid.

Vitamin C is commonly determined as ascorbic acid by the dye-titration method using 2,6-dichlorophenol-indophenol[3]. While this method is rapid, it is difficult to use with coloured solutions, is subject to interferences from other oxidizing agents and does not estimate dehydroascorbic acid. A fluorometric method has also found some acceptance[3]. This method gives a combined value that includes the activity from both ascorbic acid and dehydroascorbic acid and is less susceptible to interfering reactions but is more time-consuming.

The technique of high-performance liquid chromatography (HPLC) has been of increasing interest for the analysis of vitamins and various systems have been proposed to analyse ascorbic acid[4–8]. However, it is desirable to obtain an estimate that includes dehydroascorbic acid preferably as a separate value. Dennison *et al.*[9] proposed a HPLC method involving dual analyses whereby the level of dehydroascorbic acid is also obtained by difference. Finley and Duang[10] reported a separation of ascorbic acid and dehydroascorbic acid by HPLC on two reversed-phase columns in series, with a mobile phase of water containing a ion-pair reagent and detection of the compounds at 254 nm and 210 nm respectively. Rose and Nahrwold[11] used a similar detection system and obtained satisfactory separations for various biological preparations using a single ion-exchange column and a mobile phase of acetonitrile–water containing 2.5 m*M* potassium dihydrogen phosphate. Simultaneous separation was recently reported by Keating and Haddad[12], but with dehydroascorbic acid having been converted into a fluorophor using 1,2-phenylenediamine and detection of ascorbic acid at 290 nm and of the fluorophor at 348 nm. While this method gives increased sensitivity for the estimation of dehydroascorbic acid, the addition of the derivatization step increases the complexity and adds another variable to the analysis.

In this study we have examined the relatively simple system proposed by Rose and Nahrwold[11] and modified it for the analysis of ascorbic acid and dehydroascorbic acid in fresh fruit and vegetables.

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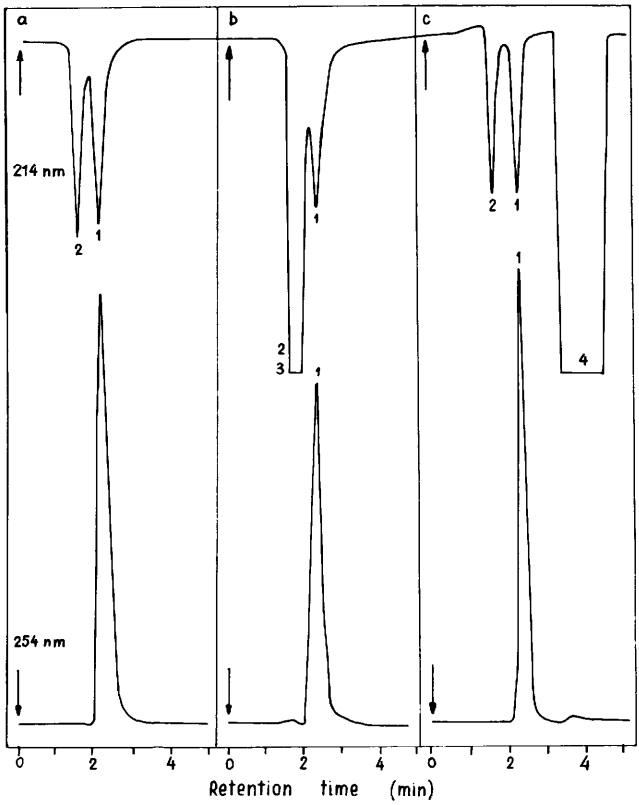

Fig. 1. Chromatogram of separation of ascorbic acid and dehydroascorbic acid with detection at 254 nm and 214 nm. a, In aqueous solution; b, in metaphosphoric acid; c, in citric acid. Sample composition: 1 = ascorbic acid; 2 = dehydroascorbic acid; 3 = metaphosphoric acid; 4 = citric acid.

METHODS AND RESULTS

Analyses were carried out on a μBondapak/Carbohydrate column (Waters Associates) (30 cm × 4 mm I.D.) installed in a Waters liquid chromatograph (Model ALC/GPC 244) equipped with a 41-mPa pump and U6K injector. Column effluents were monitored by UV detectors set at 254 nm (Waters Model 440) and 214 nm (Waters Model M441). The mobile phase was acetonitrile–water (70:30, v/v) with 0.01 *M* ammonium dihydrogen phosphate (pH 4.3 adjusted with orthophosphoric acid). The flow-rate was maintained at 2 ml/min. Good resolution of aqueous solutions of ascorbic acid and dehydroascorbic acid was obtained with retention times of about 2.5 min and 2.0 min respectively (Fig. 1a). Ascorbic acid was detected at both 254 and 214 nm but with a reduced sensitivity at 214 nm. Dehydroascorbic acid however was detected only at 214 nm.

The general method for the extraction of vitamin C from fresh fruit and vegetables was to blend homogenized sample of produce (10–30 g) with 3% (w/v) of an acid (50 ml) for 2 min and then to make up to volume (100 or 200 ml) with the extracting solution. The resulting solution was filtered through paper (Whatman 541) then through a membrane/ultrafilter cell (Diaflo ultra-filter, Amicon Corp.). Metaphos-

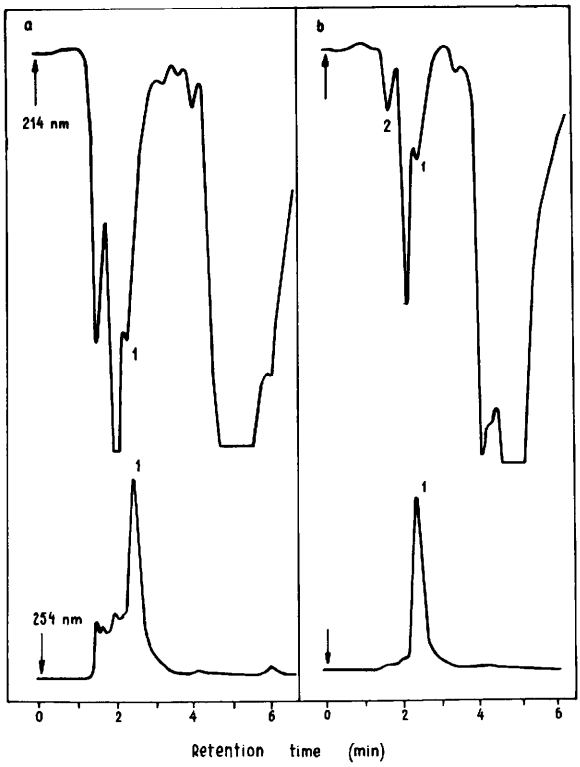

Fig. 2. Chromatogram of ascorbic acid and dehydroascorbic acid in a Brussels sprout extract in 3% citric acid: a, without purification; b, after passage through a C_{18} Sep-Pak. Sample composition: 1 = ascorbic acid; 2 = dehydroascorbic acid.

phoric acid is commonly used as the extracting medium[3] but was found to interfere with the analyses as it had a similar retention time to dehydroascorbic acid and absorbed strongly at 214 nm (Fig. 1b). An acidic medium is required to stabilize ascorbic acid, hence other acids were examined. Acetic acid was also found to interfere due to its strong absorbance at 214 nm, but citric acid was found to be a suitable medium for extraction (Fig. 1c). Solutions of ascorbic acid in 3% (w/v) citric acid were found to be stable after 3 h at room temperature when >92% of added ascorbic acid was still present. However, when produce was extracted a number of interfering compounds were present in the final solution and Fig. 2a shows such effects on a chromatogram obtained for Brussels sprouts. The use of C_{18} Sep-Pak (Waters Associates), a short plastic disposable column containing μBondapak C_{18}, to purify the extracts was found to be satisfactory. A C_{18} Sep-Pak was placed on the luer tip of the syringe barrel and the column preconditioned by washing with methanol (4 ml) followed by double distilled water (10 ml). The sample (4 ml) was then passed through the Sep-Pak. The first 3 ml were discarded and the next 1 ml was collected for analysis. A typical chromatogram is shown in Fig. 2b.

The chromatographic system was able to detect 0.05 μg ascorbic acid at 254 nm and 0.2 μg at 214 nm, and 0.1 μg of dehydroascorbic acid at 214 nm. When ascorbic acid and dehydroascorbic acid were added to various fruit and vegetables, during extraction they were recovered at 93.8 ± 2.0 and 92.4 ± 1.3% respectively ($n = 8$). A range of fresh fruit and vegetables was analysed and the method was found to give satisfactory resolution of ascorbic acid and dehydroascorbic acid for all produce.

TABLE I

LEVELS OF ASCORBIC ACID AND DEHYDROASCORBIC ACID (DHA) IN FRESH FRUIT AND VEGETABLES

Values given are averages from three determinations. Ascorbic acid was measured at 254 nm and dehydroascorbic acid at 214 nm.

Produce	*Amount (mg/100 g)*	
	Ascorbic acid	*DHA*
Avocado	10.9	1.0
Broccoli	82.3	2.1
Brussels sprout	57.8	1.2
Banana	18.7	1.4
Cabbage	41.6	2.0
Chinese cabbage	15.0	3.9
Capsicum	186.7	–
Cauliflower	47.1	2.6
Lemon	42.3	–
Lettuce	10.9	2.3
Orange	46.0	–
Plum	2.6	–
Potato	8.0	–
Rock melon	59.0	–
Spinach	49.0	1.6
Strawberry	48.5	4.2
Tomato	8.0	–

Although direct comparison was not made with other methods of analysis such as the dye-titration method, the levels of ascorbic acid found were in the order of those expected from published data. The level of dehydroascorbic acid was low in most and below the limit of detection (1 mg/100 g) in some produce. The data are given in Table I.

ACKNOWLEDGEMENTS

We wish to thank Dr. H. Greenfield for helpful advice in the planning of the study and Mr. R. A. Francke and Mr. B. Walker (Waters Associates) for assistance during the analysis.

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