Journal of Chromatography, 594 (1992) 187–194
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 790

Determination of ascorbic and dehydroascorbic acid in potatoes (*Solanum tuberosum*) and strawberries using ion-exclusion chromatography

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(First received May 13th, 1991; revised manuscript received October 4th, 1991)

ABSTRACT

A high-performance liquid chromatographic method has been developed for determination of ascorbic and dehydroascorbic acid. Samples were extracted with 62.5 m*M* metaphosphoric acid and ascorbic acid determined using an ion-exclusion column with detection at 245 nm. Dehydroascorbic acid was determined after reduction to ascorbic acid. The use of ion-exclusion chromatography enables ascorbic acid to be completely resolved from co-extracted material in both raw and cooked foods. Completeness of separation was confirmed using a photodiode-array detector.

INTRODUCTION

Vitamin C is an important micronutrient and plays many physiological roles [1,2]. Fruits and vegetables constitute the major sources in most human diets; it occurs as *l*-ascorbic acid (AA) and *l*-dehydroascorbic acid (DHAA), its oxidized form, both of which are biologically active. Any analysis for vitamin C activity must take this into consideration since a proportion of AA may be oxidized to DHAA in some foods upon storage or processing.

Numerous methods for the analysis of vitamin C acitivity have been described [3]. Chemical methods remain widely used but the presence of interfering compounds in the complex matrices of foods has resulted in these traditional approaches being replaced by high-performance liquid chromatographic (HPLC) methods which are more selective and sensitive. The wide range of HPLC methods available for viamin C determination have been reviewed by Polesello and Rizzolo [4].

The most common modes of separation are reversed phase, reversed phase with ion pairing, and weak anion exchange with NH_2-bonded phases. Procedures vary in the type of column, elution conditions, detection systems and the extraction technique but in most published methods AA elutes very close to the void volume; this may lead to errors.

The importance of the extracting media and stabilizing solutions for AA and DHAA to prevent oxidative changes has been emphasized by Nicolson *et al.* [5], and Margolis and Black [6]. The well established extractant and stabilizer, metaphosphoric acid (MPA), was critical in determining the HPLC method used. The work described in this paper was carried out because of the problems encountered when different combinations of extractants and stabilizers have been used with silica-based packing materials [7,8]. The use of amino-bonded columns and reversed-phase C_{18} columns with and without ion-pairing was investigated. When the amino-bonded column procedure of Rose and Nahrwold [9] was used good resolution of AA in the extracts of uncooked foods was possible. However with the extracts of cooked potatoes, resolution was poor and the AA peak on spectral examination proved to be contaminated with a co-eluting component from the extract. The column was quickly poisoned most

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probably by carbohydrates *e.g.* starch, as reported by Churms [10]. The ion-pairing method of Augustin *et al.* [11] was not satisfactory. Examination of the AA peak spectrum showed that it was not a single-component peak. Similarly in a reversed-phase mode without the use of an ion-pair reagent, column poisoning occurred. It was not possible to use precipitation of the starch with ethanol since the addition of homocysteine to the ethanol supernatant to reduce DHAA to AA caused the formation of a gel.

Ashoor *et al.* [12] used ion-exclusion chromatography to determine ascorbic acid in fruits, fruit juices and vegetables. When this was investigated it was found that their sample extraction procedure led to imcomplete reduction of DHAA to AA, only 80% being achieved. Ascorbic acid was eluted as part of a large tailing peak during chromatography of the extracts of strawberries, raw and cooked potatoes (Fig. 1). For this reason an ion-exclusion chromatographic separation using polystyrene–divinylbenzene packing materials was developed which was capable of determining total AA quantitatively in strawberries, raw and cooked potatoes.

EXPERIMENTAL

Apparatus

A Hewlett-Packard 1090M liquid chromatograph with integral photodiode-array detector, autosampler and autoinjector and a Hewlett-Packard 9000 series 320 datastation with 9153 disc-drive unit from Hewlett-Packard (Winnersh, Wokingham, UK) was used. A Bio-Rad Aminex HPX-87H, 300 mm × 7.8 mm I.D. ion-exclusion column, particle size 9 μm, packed with sulphonated styrene–divinylbenzene copolymer resin, 8% cross-linked, from Bio-Rad Labs. (Watford, UK) was employed. A Micro-Guard cation H^+ cartridge was installed in front of the analytical column to maintain column performance and prolong column lifetime.

Reagents

Chemicals and standard materials used were of the highest purity available. Metaphosphoric acid and dipotassium hydrogenphosphate were from BDH (Poole, UK), *l*-ascorbic acid from Sigma (Poole, Dorset, UK), *dl*-homocysteine from Aldrich (Poole, UK) and sulphuric acid from May and Baker (Manchester, UK).

All solutions, including the mobile phase 4.5 m*M* sulphuric acid, were prepared with high purity water obtained using an Elgastat UHQ water purifier from Elga (High Wycombe, UK) which incorporates reversed osmosis, adsorption, deionization, microfiltration and photooxidation processes.

Ascorbic acid standard solutions

A stock solution of AA (200 μg/ml) was prepared by dissolving 20 mg AA in 100 ml of 62.5 m*M* metaphosphoric acid. The stock solution was stored at 5 ± 1°C. Fresh standard solutions were prepared by diluting the stock solution to appropriate concentrations with 62.5 m*M* metaphosphoric acid.

Sample preparation

Potatoes and fresh strawberries were purchased from a local store and extracted on the day of purchase. Samples of potato skin and flesh (30 g) were obtained by subsampling 16 pooled cores (diameter 7 mm) taken from four tubers. A 10-g sample of fresh strawberries was obtained by cutting four strawberries into sections and subsampling. An amount of 80 g of 62.5 m*M* metaphosphoric acid was added immediately to the samples and blended for 3 min in a Waring blender. The weight of ho-

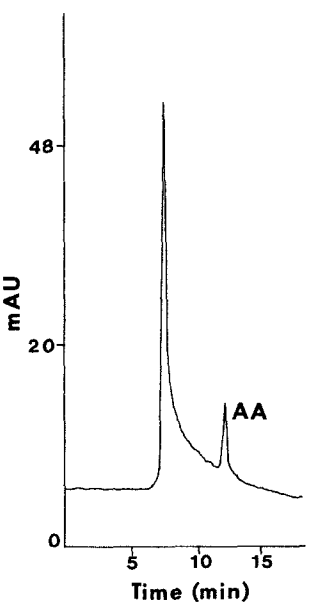

Fig. 1. Chromatogram obtained using method of Ashoor *et al.* [12].

mogenate plus washings was adjusted to 150 g with metaphosphoric acid solution, centrifuged at 6500 *g* for 15 min and filtered (Whatman 541). A quantitative amount of filtrate sufficient to give between 5 and 20 μg/ml of AA in the final volume was diluted to 20 ml with 62.5 m*M* metaphosphoric acid. All extracts were stored in amber vials at 5 ± 1°C. Mass rather than volume adjustments during blending were necessary to overcome problems caused by frothing.

Boiled potatoes were obtained by boiling 1.25 kg of raw tubers in 1.2 l water for 30 min. Baked potatoes were obtained by microwaving raw tubers at 650 W for 15 min. Sampling and extraction were carried out in the same way as for the raw tubers.

The extracting procedures were replicated four times using a total of sixteen tubers or strawberries in order to determine the overall variability of extraction. This includes both sample and extraction variability.

HPLC analysis

AA was determined by injecting 10 μl of the standard solutions or sample extracts into the HPLC system and eluting the ion-exclusion column with 4.5 m*M* sulphuric acid at a flow-rate of 0.5 ml/min. Column temperature was maintained at ambient and column back pressure was 90 ± 1 bar. Analysis was completed in 20 min which included a post-column elution time of 5 min. Spectral data from the photodiode-array detector were collected over the wavelength range 210–400 nm. AA was monitored at 245 nm.

Reduction of DHAA

DHAA was determined as the difference between total AA after DHAA reduction and AA content of the original sample. The reduction of DHAA to AA was accomplished using a minor modification of the method proposed by Hughes [13]. To 3 ml of extract were added 0.5 ml of 30 m*M* *dl*-homocysteine solution and the pH adjusted to 6.8–7.0 by slow addition of 1.5 ml of 2.6 *M* dipotassium hydrogenphosphate. After 30 min reduction was stopped by addition of 1 ml 6.25 *M* metaphosphoric acid.

RESULTS AND DISCUSSION

Typical chromatograms of AA standard, reduced DHAA, strawberry extract and raw, boiled and baked potato extracts are presented in Fig. 2a–f. Under the described chromatographic conditions, AA eluted at 12.10 ± 0.02 min. Chromatograms of strawberries and potatoes produced extra peaks due to the presence of co-extracted material but these did not interfere with the separation of AA.

The peak area associated with AA, measured at 245 nm, against concentration was linear over the range 1 to 20 ng/μl and the correlation coefficient (r^2) was 1.00. Thus a single point calibration can be used for determination of AA. The detection limit for AA is 1 ng/μl, with a 10-μl injection.

Retention times and peak areas for ten consecutive injections over a 3.5-h period, from both 5 and 20 ng/μl AA standards (Table I) show the method has excellent reproducibility and stability. The results of five consecutive injections of sample extracts prepared from fresh strawberries and raw, boiled and baked potatoes (Table II) also confirm this for these extracts.

The results of the four separate extracts prepared from the samples (Table III) indicate the variability in the vitamin content of these foodstuffs. The mean values obtained are in agreement with those presented elsewhere [14].

When the standards of 20 and 5 ng/μl AA were subdivided into a series of HPLC vials, sealed and stored at 5 ± 1°C, the AA solutions were stable for up to 5 days (Table IV). Similarly, vitamin loss from

TABLE I

REPRODUCIBILITY OF CHROMATOGRAPHIC SYSTEM FOR AA STANDARDS

S.D. = Standard deviation; R.S.D. = relative standard deviation.

	AA			
	20 ng/μl AA standard		5 ng/μl AA standard	
	Retention time (min)	Peak area	Retention time (min)	Peak area
Mean	12.1	637	12.1	152
S.D. (n = 10)	0.01	4.0	0.02	6.3
R.S.D. (%)	0.08	0.6	0.2	4.1

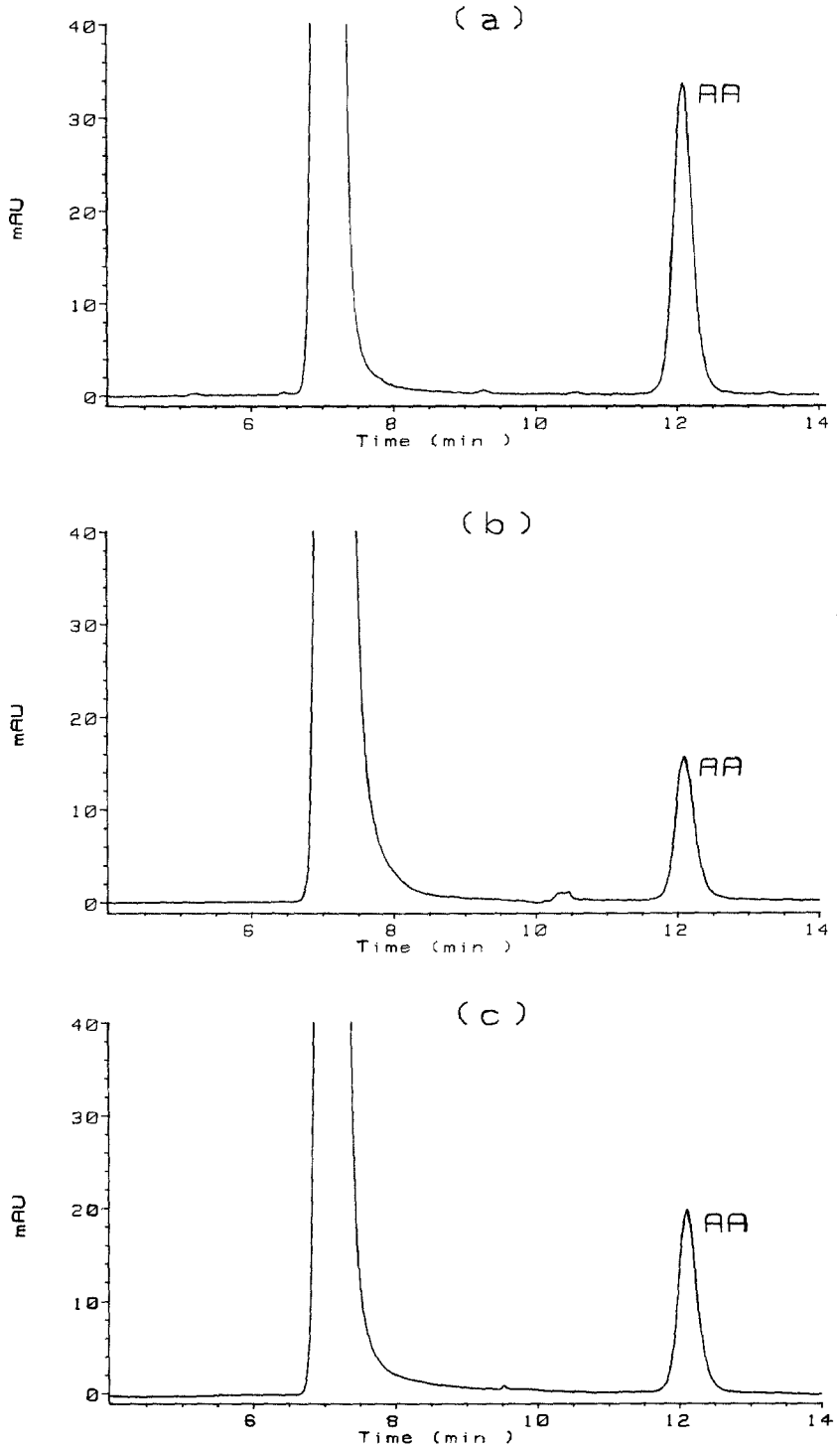
(a)
AA
mAU
40
30
20
10
0
6
8
10
12
14
Time (min)
(b)
AA
mAU
40
30
20
10
0
6
8
10
12
14
Time (min)
(c)
AA
mAU
40
30
20
10
0
6
8
10
12
14
Time (min)

Fig. 2.

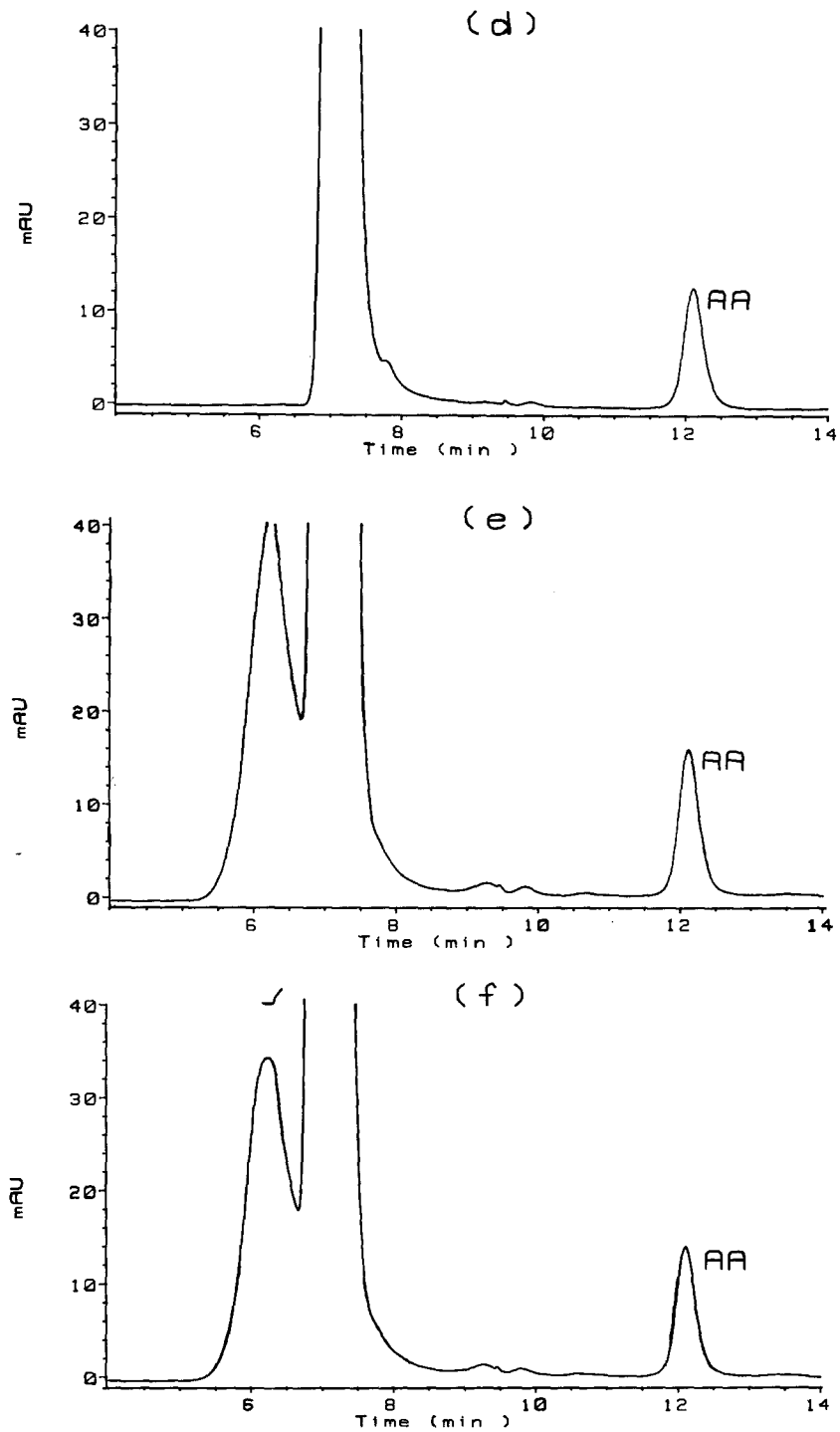

Fig. 2. Typical chromatograms of (a) 20 ng/μl AA standard, (b) reduced DHAA, (c) strawberry extract, (d) raw potato extract, (e) boiled potato extract and (f) baked potato extract.

TABLE II

REPRODUCIBILITY OF CHROMATOGRAPHIC SYSTEM FOR FOOD SAMPLE EXTRACTS

	AA							
	Strawberry		Potato					
	Retention time (min)	peak area	Raw		Boiled		Baked	
			Retention time (min)	Peak area	Retention time (min)	Peak area	Retention time (min)	Peak area
Mean	12.1	357	12.1	261	12.1	258	12.1	305
S.D. (n = 5)	0.01	2.9	0.01	2.5	0.02	7.4	0.0	3.17
R.S.D. (%)	0.08	0.82	0.07	0.96	0.19	2.85	0.23	5.50

TABLE III

VITAMIN C LEVELS OBTAINED BY THE PRESCRIBED METHOD

	Vitamin C (mg/100 g fresh weight)							
	Strawberry		Potato					
	TAA[a]	AA	Raw		Boiled		Baked	
			TAA	AA	TAA	AA	TAA	AA
Mean	68.7	67.3	13.7	11.7	10.2	8.3	7.8	7.3
S.D. (n = 4)	5.26	5.65	1.63	0.74	1.53	0.38	1.49	1.33
R.S.D. (%)	7.66	8.40	11.90	6.32	15.00	4.58	19.10	18.22

[a] TAA = total ascorbic acid.

TABLE IV

STABILITY OF AA STANDARDS AND OF AA IN FOOD SAMPLE EXTRACTS

Values are mean of two samples. S.E.M. = Standard error of the mean.

Sample	AA (ng/μl)							
	Storage (days)							S.E.M.
	0	1	2	3	4	5	10	
Standards								
20 ng/μl	20.00	20.12	19.62	19.95	19.94	19.92	17.70	0.115
5 ng/μl	5.00	4.95	4.97	4.69	4.57	4.73	3.46	0.083
Strawberry								
fresh	13.76	13.26	13.43	13.32	13.46	13.43	10.45	0.380
Potato								
Raw	6.64	6.61	5.77	6.14	6.08	5.99	2.25	0.126
Boiled	6.74	6.50	6.30	5.85	5.80	5.87	1.71	0.158
Baked	4.32	4.19	3.44	3.58	4.13	3.99	1.49	0.095

TABLE V

PERCENT RECOVERY OF AA ADDED (a) PRIOR TO BLENDING, (b) TO THE FINAL EXTRACT

Sample	Recovery of AA (%) ± S.D. (n = 4)	
	a	b
Strawberry		
fresh	97.3 ± 3.25	99.3 ± 0.97
Potato		
Raw	99.3 ± 0.97	94.8 ± 0.64
Boiled	96.2 ± 1.89	99.7 ± 1.47
Baked	98.6 ± 1.95	99.6 ± 2.11

extracts of strawberries and potatoes over this same period was minimal (Table IV).

Recovery of AA, added either prior to blending or to the final extract exceeded 94% in all cases (Table V). The identity of the AA peaks was further characterised by their UV spectra (Fig. 3) obtained using diode array detection (DAD). Purity was confirmed by analysis of the upslope, apex and downslope of the spectra of each sample.

DHAA prepared by quantitative oxidation of AA [9] had a retention time of 10.5 min using the same chromatographic conditions. The absorption maximum for DHAA was 230 nm. Thus this system could be used to detect both AA and DHAA simultaneously, since AA also absorbs strongly at 230 nm. However, the ability to do so depends on the concentration of DHAA since the minimum detection level is 100 ng/μl with a 10-μl injection. In the present study, the concentrations of DHAA in strawberries and potatoes were low and it was necessary to quantify DHAA by reduction to AA using the modified Hughes method referred to previously.

The values obtained for the oxidation of 20 and 5 ng ng/μl AA standards to DHAA and its subsequent reduction by *dl*-homocysteine were 96 and 94%, respectively, which are in agreement with those of Rose and Nahrwold [9].

To date some 1500 samples have been analysed using one column with no detectable loss of resolution or column deterioration.

CONCLUSION

The HPLC procedure described provides a simple, rapid and sensitive method for determination of AA, DHAA and hence the total vitamin C content of strawberries and potatoes. Although not tested, ion-exclusion chromatography may provide a quantitative method for the determination of total ascorbic acid in the complex matrices of other raw and cooked foods.

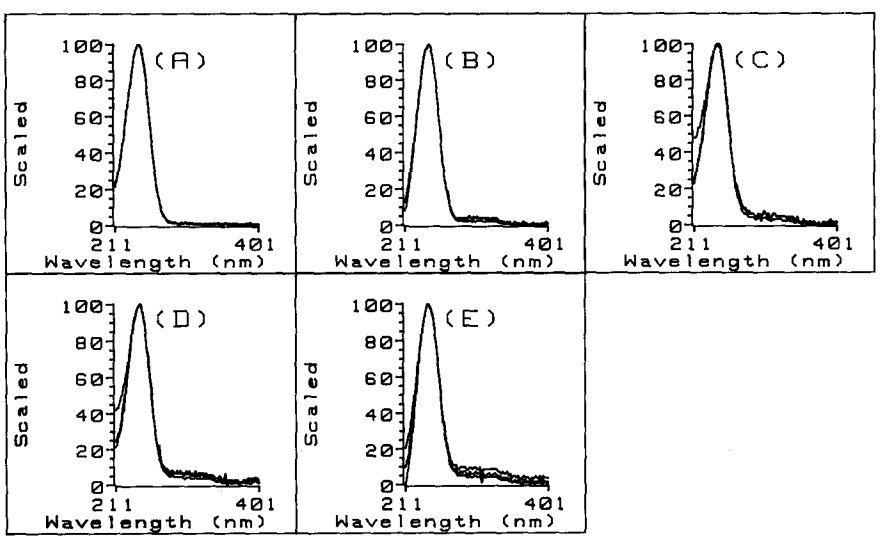

Fig. 3. Spectra obtained using DAD for: (A) AA standard, (B) strawberry extract, (C) raw potato extract, (D) boiled potato extract, (E) baked potato extract.

ACKNOWLEDGEMENT

The authors wish to thank Dr. D. J. Kilpatrick for statistical analyses.

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