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A rapid method for the determination of total L-ascorbic acid in fruits and vegetables by micellar electrokinetic capillary chromatography

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A rapid micellar electrokinetic capillary chromatographic method for the determination of the total L-ascorbic acid content of a variety of fruits and vegetables is described. The vitamin is extracted from the foods with 3% metaphosphoric acid and stabilized with aqueous 0·2% D,L-dithiothreitol before analysis. D-Erythorbic acid is used as the internal standard. The analyses are performed with 75 μm fused silica capillary columns (40 cm effective length to the detector for fruits and 50 cm effective length to the detector for vegetables) using a buffer consisting of 0.05 M sodium deoxycholate, 0·01 M sodium borate and 0·01 M potassium dihydrogen orthophosphate. The method has the same order of repeatability, is faster and more cost effective than the high-performance liquid chromatographic method that is currently used in the authors' laboratory.

INTRODUCTION

Analytical procedures based on the relatively new technique of capillary electrophoresis are rapidly gaining acceptance as viable analytical techniques (Lin Ling *et al.*, 1992; Koh *et al.*, 1993; Pant & Trenerry, 1995; Trenerry *et al.*, 1994*a,b*). In 1984, Terabe *et al.* introduced the technique of micellar electrokinetic capillary chromatography (MECC) (Terabe *et al.*, 1984, 1985). In MECC, an ionic surfactant is added to the buffer to provide a phase for chromatographic separation. Sodium dodecylsulphate is the most common surfactant used for MECC analyses; however, sodium cholate, sodium deoxycholate and sodium taurodeoxycholate have also been used in a variety of analyses (Li, 1992). The authors recently reported the determination of the total L-ascorbic acid content of a number of different beers, wines and fruit beverages by MECC using a buffer consisting of 0·05 M sodium deoxycholate, 0·01 M sodium borate and 0·01 M potassium dihydrogen orthophosphate (Marshall *et al.*, 1995). The beverages were mixed with an internal standard solution, diluted with aqueous 0·2% D,L-dithiothreitol and analysed. D,L-Dithiothreitol reduces any dehydroascorbic acid that is present to L-ascorbic acid (Sapers *et al.*, 1990). The method had the same order of repeatability, was faster and less costly to operate than the high performance liquid chromatographic (HPLC) procedure that is used routinely in the authors' laboratory (Maeda *et al.*, 1988).

Fruits and vegetables contain natural levels of L-ascorbic acid. A number of HPLC methods have been described for the determination of L-ascorbic acid in fruits and vegetables (DeLeenheer *et al.*, 1985). The vitamin is extracted from the food with dilute acid and then analysed. To determine total L-ascorbic acid, the extract is treated with a reductant, e.g. D,L-dithiothreitol, to convert any dehydroascorbic acid present in the sample to L-ascorbic acid (Sapers *et al.*, 1990). Citric acid and metaphosphoric acid are two of the more common reagents used to extract the vitamin from fruits and vegetables. It was of interest to see if L-ascorbic acid could be separated and determined in these acidic extracts using the conditions that were previously described for the beers, wines and fruit beverages. Fruits and vegetables are also more complex matrices than beverages and so there was a greater possibility of naturally occurring compounds affecting the chromatography or interfering with the identification and quantitation of L-ascorbic acid.

MATERIALS AND METHODS

Reagents

Metaphosphoric acid, citric acid and L-ascorbic acid were obtained from BDH Chemicals (Kilsyth, Australia).

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D-Erythorbic acid was obtained from Fluka AG (Switzerland). D,L-Dithiothreitol and sodium deoxycholate were obtained from Sigma Chemical Co. (St Louis, MO, USA). Sodium tetraborate and potassium dihydrogen orthophosphate were obtained from Ajax Chemicals (Auburn, Australia).

MECC buffer

A 2·16 g sample of sodium deoxycholate was dissolved in 100 ml of a 1:1 mixture of 0·02 M sodium borate and 0·02 M potassium dihydrogen orthophosphate. The pH of the solution was 8·6. The solution was filtered through a 0·8 μm cellulose acetate filter disc before use.

Standards

MECC
Standard solutions were prepared by dissolving L-ascorbic acid in aqueous 0·2% D,L-dithiothreitol. D-Erythorbic acid was also dissolved in aqueous 0·2% D,L-dithiothreitol and used as the internal standard at a concentration of 30 μg/ml. D,L-Dithiothreitol (0·2%) is present to prevent the oxidation of the acids to the corresponding dehydroacids. L-Ascorbic acid was linear up to 60 μg/ml. The solutions were stable for up to 12 h (Marshall *et al.*, 1995).

HPLC
The standard solutions were prepared as described for the MECC analyses, except that an internal standard was not used.

Samples

The samples were purchased from local outlets and were analysed on the day of purchase.

MECC
A 160 ml sample of 3% metaphosphoric acid was added to 40 g of the fruit or vegetable and the mixture blended in a commercial food processor for 2 min. The volume was adjusted to 200 ml with 3% metaphosphoric acid. The resultant slurry was filtered through a Whatman No. 1 filter paper. A 5 ml sample of the filtrate was added to 0·5 ml of D-erythorbic acid (600 μg/ml) in aqueous 0·2% D,L-dithiothreitol, diluted to 10 ml with aqueous 0·2% D,L-dithiothreitol and mixed thoroughly. The solution was passed through a C18 Sep-Pak cartridge which had been previously activated with methanol and water to remove naturally occurring compounds that interfered with the MECC analysis. The first 5 ml were discarded and the remaining solution filtered through a 0·8 μm cellulose acetate filter disc before analysis.

HPLC
The HPLC analyses were performed on the same extracts as described above, except that no internal standard was used and that the solutions were analysed without C18 Sep-Pak cartridge cleanup.

Apparatus

MECC
The extracts were analysed with fused silica capillary columns (65 cm × 75 μm i.d. for fruit 75 cm × 75 μm i.d. for vegetables) purchased from Polymicro Technologies, (Arizona, USA), using a buffer consisting of 0·05 M sodium deoxycholate, 0·01 M sodium borate and 0·01 M potassium dihydrogen orthophosphate. The effective lengths of the columns to the detector were 40 cm and 50 cm, respectively. An Isco Model 3140 Electropherograph (Isco Inc., Lincoln, NE, USA) operating at +25kV and at 28°C was used for all of the analyses. The compounds were loaded under vacuum (vac. level 2, 10 kPa s) and were detected at 254 nm at 0·01 AUFS. The capillaries were flushed with running buffer for 2 min between runs. Electropherograms were recorded with either the ICE Data Management and Control Software supplied with the Model 3140 Electropherograph or a HP 3350 Laboratory Data System (Hewlett-Packard, Palo Alto, CA, USA).

HPLC
The HPLC method was adapted from that described by Maeda *et al.* (1988). The analyses were performed with a 501 HPLC pump, 712 WISP, 490 programmable multiwavelength UV detector using a C18 NOVAPAK Radial-PAK cartridge equipped with a C18 pre-column (Waters Chromatography Division of Millipore, Milford, MA, USA) with a mobile phase consisting of 0·2% (v/v) aqueous orthophosphoric acid at a flow rate of 1·0 ml/min. The compounds were detected at 254 nm at 0·2 AUFS. The chromatograms were displayed on an Omniscribe Chart Recorder (Houston Instruments, USA). Peak heights or peak areas obtained from a HP 3350 Laboratory Data System (Hewlett-Packard, Palo Alto, CA, USA) were used in the calculations.

RESULTS AND DISCUSSION

The authors recently reported the determination of total L-ascorbic acid in beers, wines and fruit juices by MECC using a buffer consisting of 0·05 M sodium deoxycholate, 0·01 M sodium borate and 0·01 M potassium dihydrogen orthophosphate (Marshall *et al.*, 1995). Sodium dodecylsulphate and cetyltrimethylammonium bromide were also used as micelle modifiers, but were not suitable for the full range of beverages that were analysed. D-Erythorbic acid was used as one of the internal standards for this work. Internal standards are used in MECC analyses to correct for instrument imprecision, primarily caused by the injection process. Internal standards can also assist in peak identification where changing migration times occur due to buffer losses or buffer degradation (Weinberger, 1993). D-Erythorbic acid does not occur naturally in fruits and vegetables, and would therefore be a suitable internal standard for this study.

For the determination of total L-ascorbic acid in

beers, wines and fruit juices, the beverages were simply mixed with an appropriate internal standard, diluted with aqueous 0·2% dithiothreitol, filtered and analysed. The standards were also prepared in aqueous 0·2% D,L-dithiothreitol. Repeatability (%CV) and linearity data for the standard solutions are detailed in the previous report (Marshall *et al.*, 1995). To determine the levels of total L-ascorbic acid in fruits and vegetables, the vitamin must first be extracted from the food with dilute acid and then treated with D,L-dithiothreitol to reduce any dehydroascorbic acid present in the sample to L-ascorbic acid. Hoare *et al.* (1993) used a similar approach to determine the total vitamin C content of orange juice. Dilute metaphosphoric acid and dilute citric acid have previously been used to extract L-ascorbic acid from foods (DeLeenheer *et al.*, 1985). Standard solutions of L-ascorbic acid and D-erythorbic acid were prepared in 1:1 mixtures of 3% citric acid/aqueous 0·2% D,L-dithiothreitol and 3% metaphosphoric acid/aqueous 0·2% D,L-dithiothreitol and analysed. Neither acid had any affect on the separation of the stereoisomers (Fig. 1). However, as the peak shapes were marginally sharper in the citric acid solution, it was decided to use dilute citric acid to extract the vitamin from the fruits and vegetables.

A number of different fruits were then extracted with 3% citric acid, diluted with aqueous 0·2% D,L-dithiothreitol and analysed by MECC and HPLC. The electropherograms were quite complex for some of the fruits, with a number of compounds comigrating with L-ascorbic and D-erythorbic acids. The interfering compounds could be easily removed by passing the solutions through C18 Sep-Pak cartridges which had been previously activated with methanol and water (Maeda *et al.*, 1988). This is demonstrated for blueberry in Fig. 2. L-Ascorbic acid was well separated from the other components in the HPLC chromatograms, and so the solutions were analysed without C18 Sep-Pak cartridge clean-up. For the majority of samples, the amounts of total L-ascorbic acid determined by HPLC were lower than those determined by MECC (e.g. strawberry MECC 59 mg/100 g, HPLC 45 mg/100 g; raspberry MECC 18 mg/100 g, HPLC 5 mg/100 g).

Strawberries and raspberries were extracted with 3% metaphosphoric acid, diluted with aqueous 0·2% D,L-dithiothreitol and re-analysed by MECC and HPLC. For MECC analysis, interfering compounds were removed by passing the solutions through C18 Sep-Pak cartridges which had been previously activated with methanol and water. The levels of total L-ascorbic acid

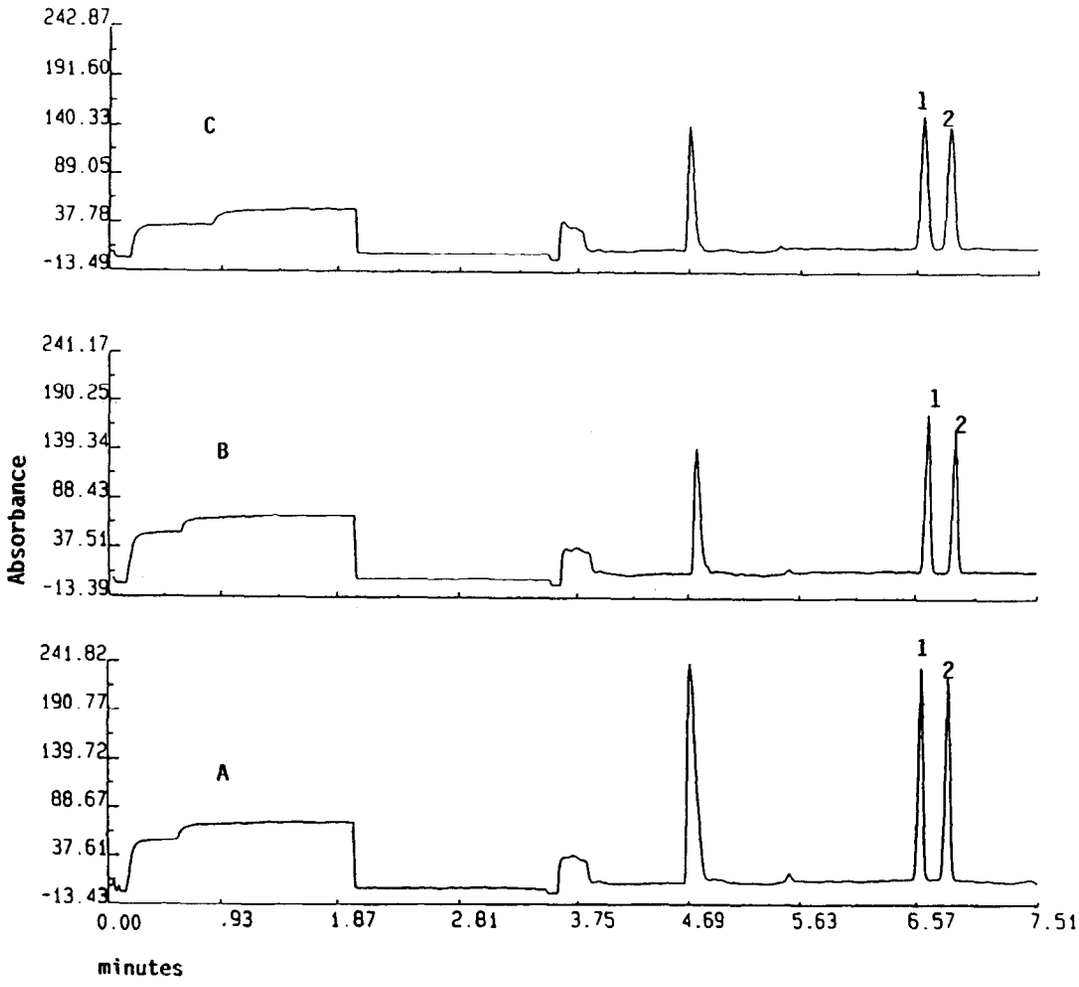

Fig. 1. Separation of L-ascorbic acid (1) and D-erythorbic acid (2) for (A) a standard solution prepared in aqueous 0·2% D,L-dithiothreitol, (B) a standard solution prepared in a 1:1 mixture of 3% citric acid/aqueous 0·2% D,L-dithiothreitol solutions, and (C) a standard solution prepared in 3% metaphosphoric acid/aqueous 0·2% D,L-dithiothreitol solutions.

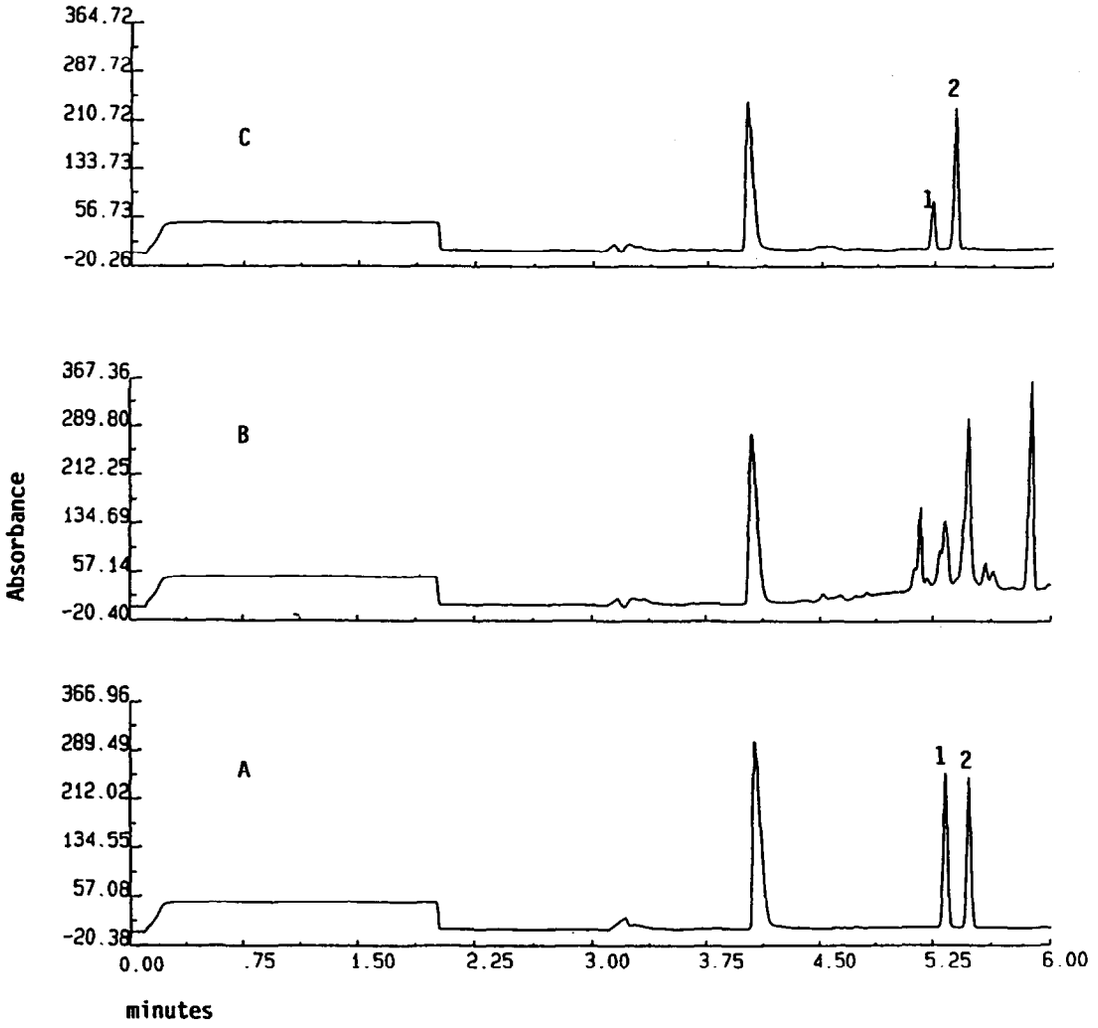

Fig. 2. Separation of L-ascorbic acid (1) and D-erythorbic acid (2) for (A) a standard solution, (B) blueberry extract before C18 Sep-Pak cartridge clean-up, and (C) blueberry extract after C18 Sep-Pak cartridge clean-up. The *x*-axis gives the migration time (min).

in these extracts compared more favourably than when citric acid was used as the extractant. It was decided, therefore, to use 3% metaphosphoric acid as the extracting acid for the remainder of the fruits and vegetables. The standards were prepared in aqueous 0.2% dithiothreitiol (Marshall *et al.*, 1994).

Eighteen different types of fruits with varying levels of L-ascorbic acid were then analysed by MECC and HPLC. The samples were blended with 3% metaphosphoric acid, the solutions filtered and diluted with aqueous 0·2% D,L-dithiothreitol. For MECC analysis, the solutions were passed through an activated C18 Sep-Pak cartridge before analysis. D-Erythorbic acid was used as the internal standard for the MECC analyses. No comigrating peaks were observed in the electropherograms when a representative number of samples were analysed without the addition of D-erythorbic acid. Each sample solution was analyzed seven times by both techniques to obtain instrument repeatability data (%CV). The amounts of total L-ascorbic acid in each of the fruits and the %CV data for each technique are listed in Table 1. Electropherograms for watermelon and lemon are shown in Fig. 3.

The levels of total L-ascorbic acid in the fruits and the repeatability data for both MECC and HPLC were in excellent agreement. The levels of total L-ascorbic acid in the fruits were similar to those reported in the Nutritional Values of Australian Foods (English & Lewis, 1991). The migration times of L-ascorbic acid relative to the migration times of the internal standard were also consistent throughout the analyses. This is shown for grapes, where there was very little change in the migration times of the two acids over twenty repetitive analyses of the same solution (Fig. 4).

The procedure was then extended to the analysis of a variety of vegetables. As with the fruits, a representative number of samples were analysed without the addition of D-erythorbic acid. In no instances were there any compounds comigrating with D-erythorbic acid and so this compound was suitable for use as the internal standard. For MECC analysis, the extracts were passed through an activated C18 Sep-Pak cartridge before analysis. Each sample extract was analysed seven times by both MECC and HPLC to check the repeatability of the techniques. The levels of total L-ascorbic acid determined by the two techniques and the repeatability data (%CV) are listed in Table 2.

The amounts of total L-ascorbic acid in the vegetables

Table 1. Comparison of the levels of total L-ascorbic acid determined by MECC and HPLC for fruits extracted with 3% metaphosphoric acid and stabilised with aqueous 0·2% D,L-dithiothreitol and showing the repeatability data (%CV) between instruments

Sample	Total L-ascorbic acid (mg/100 g)		%CV	
	MECC	HPLC	MECC	HPLC
Orange	31	31	1·2	0·7
Lemon	34	34	0·6	2·1
Lime	34	33	1·6	1·2
Grapefruit	34	34	0·5	1·2
Blueberry	8	7	0·6	0·8
Strawberry	48	46	1·3	0·6
Raspberry	22	21	1·1	1·2
Kiwi	82	80	1·1	1·1
Kiwi (peeled)	101	99	0·8	0·7
Tomato	14	14	0·6	2·1
Pineapple	14	14	1·3	1·6
Honeydew melon	21	19	0·9	0·9
Watermelon	6	5	0·9	3·1
Rock melon	38	36	1·2	0·3
Mango	18	18	1·8	2·2
Apple	5	5	3·4	0
Green grape	2	2	3·1	2·7
Peach	8	6	3·3	1·6
Dark plum	3	1	2·1	0
Apricot	8	10	2·0	2·2

and the repeatability data for MECC showed excellent agreement with the HPLC data. Also, the amounts of total L-ascorbic acid in the vegetables were similar in most instances to the values reported in the literature (English & Lewis, 1991). Electropherograms for potato and beetroot are shown in Fig. 5.

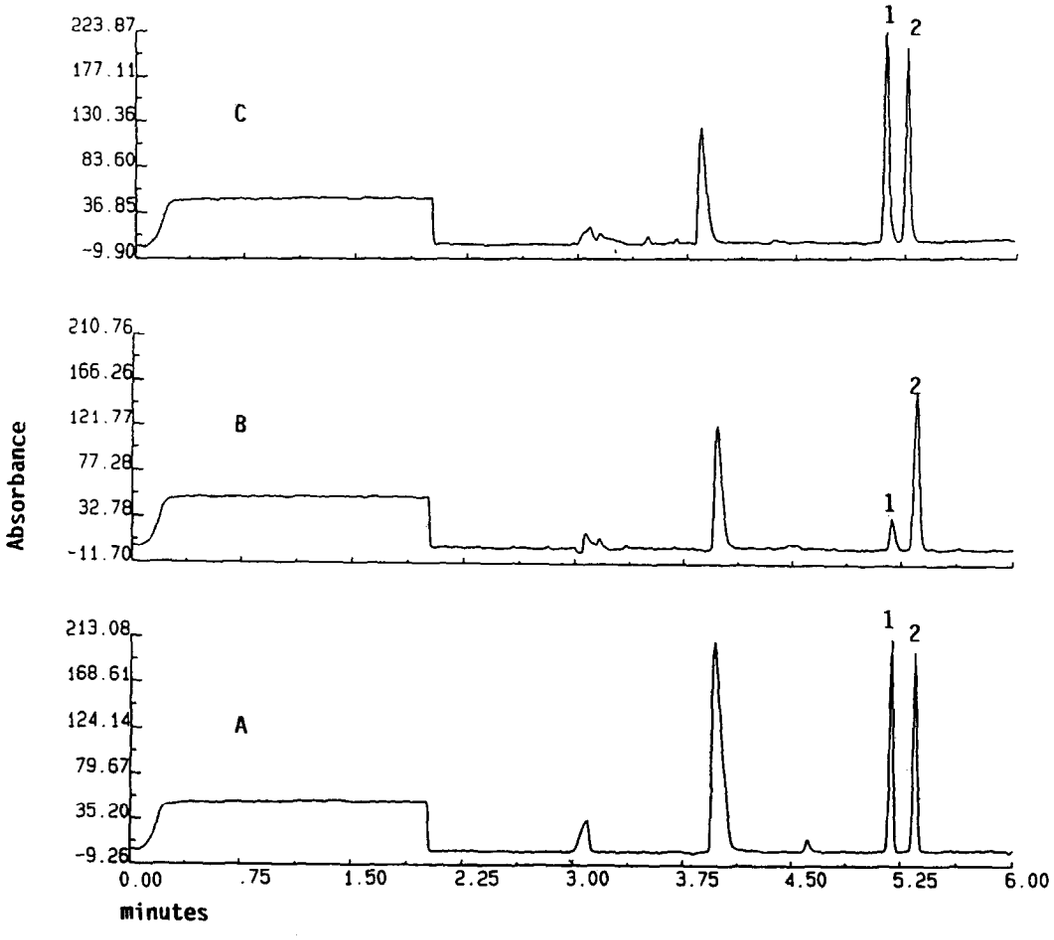

Fig. 3. Electropherograms of (A) standard solution, (B) watermelon extract, and (C) lemon extract showing L-ascorbic acid (1) and D-erythorbic acid (2). The *x*-axis gives the migration time (min).

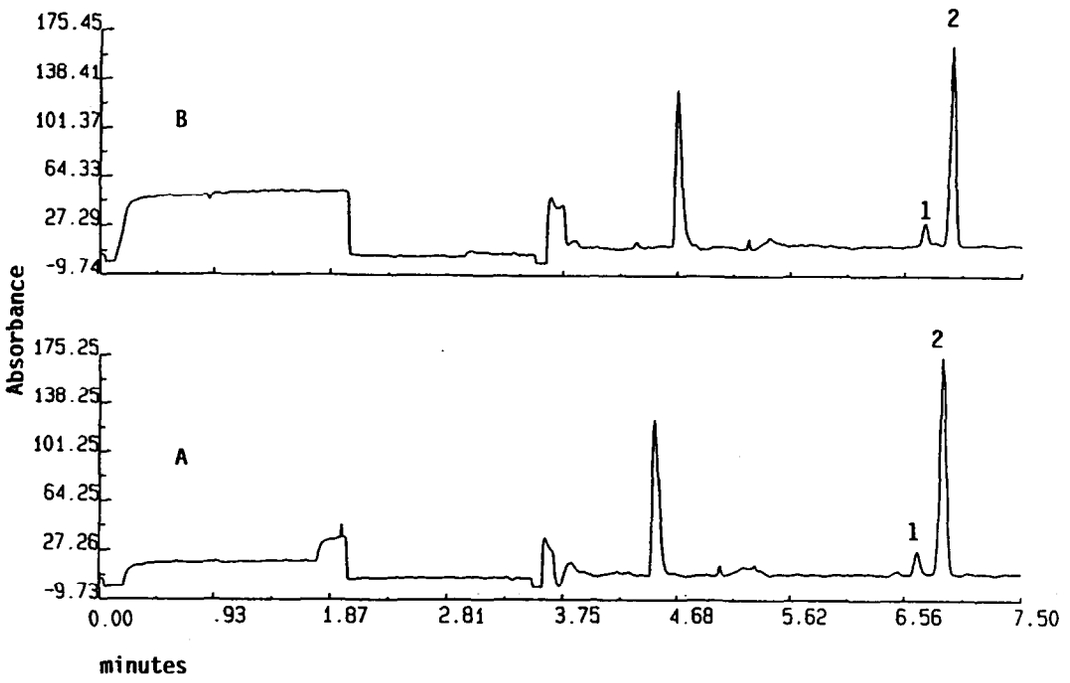

Fig. 4. Electropherograms showing the separation of L-ascorbic acid (1) and D-erythorbic acid (2) in (A) grape extract, run 1, and (B) grape extract, run 20. The *x*-axis gives the migration time (min).

Table 2. Comparison of the levels of total L-ascorbic acid determined by MECC and HPLC for vegetables extracted with 3% metaphosphoric acid and stabilised with aqueous 0·2% D,L-dithiothreitol and showing the repeatability data (%CV) between instruments

Sample	Total L-ascorbic acid (mg/100 g)		%CV	
	MECC	HPLC	MECC	HPLC
Asparagus	3	3	2·2	2·2
Green bean	11	12	1·1	1·2
Beetroot	3	3	2·0	1·7
Broccoli	100	96	1·1	0·5
Brussels sprouts	130	122	1·3	0·2
Green capsicum	89	87	1·7	0·2
Carrot	10	10	1·4	1·7
Cauliflower	70	71	1·0	0·2
Red chilli	200	188	1·1	0
Cucumber	9	8	1·3	0·4
Lettuce	3	3	1·3	1·6
Onion	8	8	0·9	1·4
Potato (peeled)	19	21	1·0	1·7
Potato	15	15	1·6	0·5
Sweetcorn	2	1	2·1	3·2
Zucchini	23	20	0·8	0·5
Snow peas	52	47	0·8	0·5
Radish	21	20	0·7	0·8

The MECC run times were considerably shorter than for the HPLC analyses. This was particularly evident for the vegetables, where the analysis times were reduced from 20 min for HPLC to 7 min for MECC. The HPLC chromatogram for beetroot is shown in Fig. 6. Short analysis times are advantageous for the determination of L-ascorbic acid especially when a large number of samples are to be analysed, as the vitamin is reasonably labile, even in the presence of stabilising agents.

CONCLUSION

A MECC procedure for the determination of total L-ascorbic acid in fruits and vegetables has been developed and validated against the HPLC method used in the authors' laboratories. The vitamin is extracted with 3% metaphosphoric acid and stabilized with aqueous 0·2% D,L-dithiothreitol. D,L-Dithiothreitol also reduces any dehydroascorbic acid to L-ascorbic acid. D-Erythorbic acid is used as the internal standard. The limit of reporting

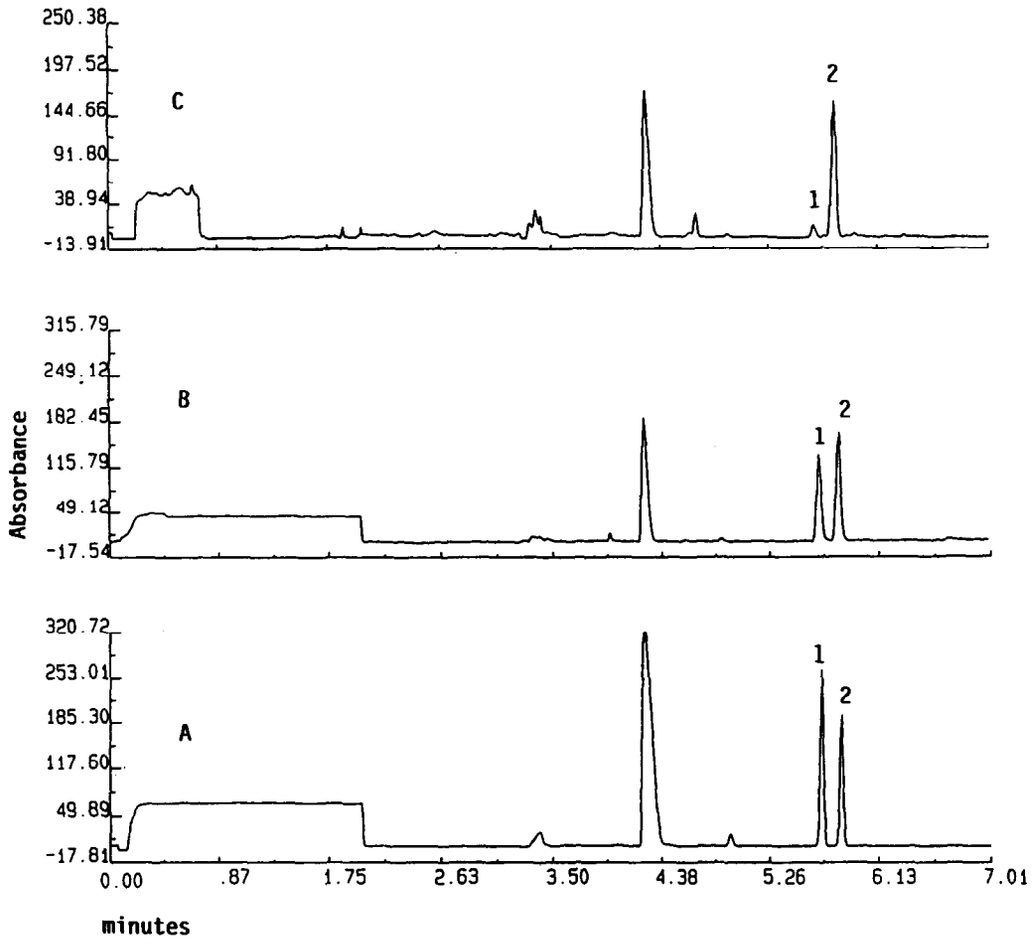

Fig. 5. Electropherograms of (A) standard solution, (B) potato extract, and (C) beetroot extract showing L-ascorbic acid (1) and D-erythorbic acid (2). The *x*-axis gives the migration time (min).

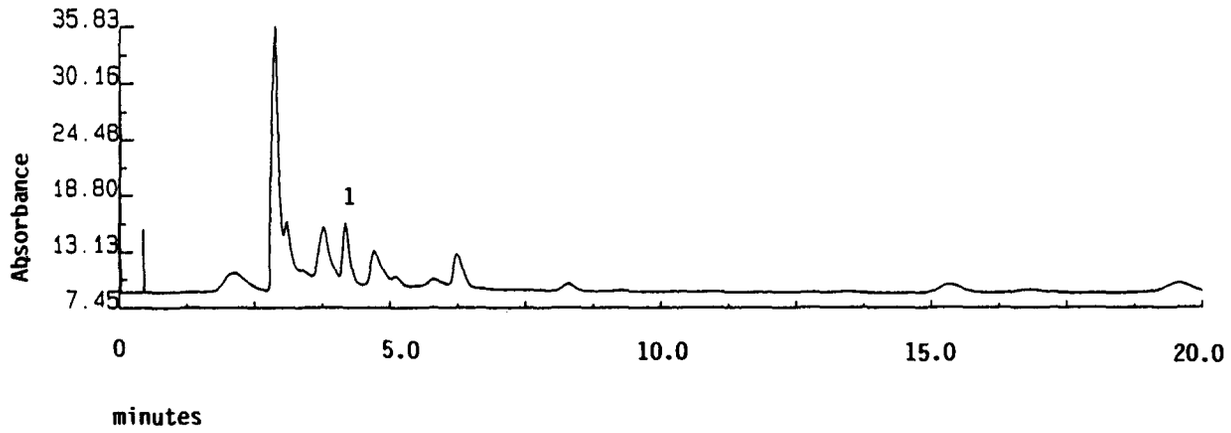

Fig. 6. HPLC chromatogram of beetroot extracted with 3% metaphosphoric acid and stabilised with aqueous 0·2% D,L-dithiothreitol showing L-ascorbic acid (1). The *x*-axis gives the migration time (min).

is 1 mg/100 g. The method has the same order of repeatability, is faster and more cost effective than the HPLC method that is currently used in the authors' laboratory.

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