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A screening method for the determination of ascorbic acid in fruit juices and soft drinks

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ABSTRACT

A simple and rapid liquid chromatographic method based on a new stationary phase Teknokroma, Tr-010065 Mediterranea sea$_{18}$ (15 cm × 0.4 cm, id 3 μm), to determine ascorbic acid in beverages is reported. With the proposed method the samples were analysed by direct injection without a previous treatment. The total analysis time does not exceed 6 min. The method showed a good repeatability (RSD < 2%: $n = 6$) and an excellent sensitivity (LOD = 0.01 mg/l). Seventeen samples were analysed, including fruit juices, soft drinks and isotonic beverages. Ascorbic acid contents ranged from 6.6 to 840 mg/l. The ascorbic acid stability in some beverages during their shelf-life was also evaluated. Degradation of about 54% was observed in a tea drink.

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1. Introduction

Ascorbic acid (vitamin C) is a natural antioxidant mainly present in fruits and vegetables. Its use as an additive in fruit juices, jams, dairy products, etc. is allowed by the European Commission. It is well known for its important role in biochemical processes, such as collagen formation, iron absorption and its involvement in neurotransmission and in immune responses (Martínez, 1998). However, high levels of ascorbic acid in the human body could cause adverse effects. Therefore, the accurate determination of this antioxidant in different foods is of great importance.

Although several techniques have been used for the analysis of ascorbic acid in foodstuffs, including spectrophotometric, potentiometric and spectrofluorimetric, chromatographic methods are preferred because of their advantages of simplicity, short analysis time and sensitivity. In developing a chromatographic method the selection of the stationary phase is a crucial step in achieving suitable resolution.

Different types of HPLC columns, including C_{18} (Burini, 2007; Chen & Sato, 1995; de Quirós, López-Hernández, & Simal-Lozano, 2001; Furusawa, 2001; Garrido-Frenich, Hernández-Torres, Belmonte-Vega, Martínez-Vidal, & Plaza-Bolaños, 2005; Nojavana et al., 2008) bonded-phase NH_2 (Rodríguez-Comesaña, García-Falcón, & Simal-Gándara, 2002), polymeric columns (Lopes, Drinkine, Saucier, & Glories, 2006) and diol columns (Tai & Gohda, 2007) have been reported in the literature for the analysis of ascorbic acid in foods. GC-based methods have also been demonstrated to be suitable for determining ascorbic acid in foodstuffs (Silva, 2005). More recently, capillary electrophoresis appeared as a promising and alternative analytical tool to analyse this antioxidant (Peng, Zhang, & Ye, 2008; Tang & Wu, 2005; Versari, Mattioli, Parpinello, & Galassi, 2004).

In this paper we present a rapid and simple method for the analysis of ascorbic acid by HPLC, using a novel stationary phase based on perfectly spherical particles of ultra-pure silica. This packing has shown to be an excellent alternative to the conventional C_{18} column for the analysis of ascorbic acid. The method was further applied to determine vitamin C acid in fruit juices, fruit and plant extracts beverages and isotonic drinks. In the second part of the study, the stability of ascorbic acid was evaluated in different samples during the shelf-life of the products.

2. Materials and methods

2.1. Reagents and standard solutions

Formic acid was purchased from Riedel-de Häen (Seelze, Germany) and L-ascorbic acid was from Sigma–Aldrich (Steinheim, Germany). Water used for all solutions was obtained from a Milli-Q water purification system (Millipore; Bedford, MA). Stock solutions of L-ascorbic acid were prepared daily in acidified Milli-Q water (0.1% v/v formic acid) and stored in amber flasks at 4 °C prior to chromatographic analysis. Working standard solutions were prepared by dilution.

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2.2. Samples

Seventeen samples from different manufacturers, including: orange juice, apple juice, grape and pineapple juice, orange drink, lemon drink, apple drink, tea drink, isotonic drink and isotonic drink with orange and cola flavour were purchased in local supermarkets.

The samples were analysed directly, filtered and diluted when necessary, in order to keep the concentration within the calibration range. Each sample was analysed in duplicate. Once the samples were analysed they were stored in a refrigerator.

2.3. Equipment

Analysis was performed on an HP1100 system (Hewlett–Packard) equipped with an HP1100 quaternary pump, an HP1100 degassing device, a 20-μl injection loop (Rheodyne, Cotati, CA) and an HP1100 UV-detector set at 245 nm. The HPLC was controlled by a personal computer running Agilent ChemStation software for LC and LC/MS systems.

2.4. Chromatography

The chromatographic conditions were as follows: mobile phase Milli-Q water (0.1% v/v formic acid), flow rate 0.8 ml/min, injection volume 20 μl and detection wavelength 245 nm. The analysis was performed at room temperature. Two stationary phases were tested: Teknokroma, Tr-015605 Tracer Extrasil ODS2 (25 × 0.4 cm, id 5 μm) and Teknokroma, Tr-010065 Mediterranea sea_{18} (15 × 0.4 cm, id 3 μm) (Teknokroma, Barcelona, Spain). The specifications of both columns concerning particle size (μm), total carbon content (%), surface area (m^2/g) and average pore diameter (Å) are as follows: 5 μm, 12%, 200 m^2/g and 80 Å for the ODS2 column, and 3 μm, 17%, 450 m^2/g and 80 Å for the Mediterranea sea_{18} column, respectively.

2.5. Identification and quantification

The peak identification was made by comparison of the retention times with those of pure standards. Quantification was carried out with the external standard method.

The calibration line was divided in two linear ranges based on four concentration levels. Each point was the average of three peak area measurements.

2.6. Ascorbic acid degradation study

The degradation of ascorbic acid during the shelf-life of the products was evaluated. The ascorbic acid content of orange juice and tea drink samples were determined at four different times before expiry date. Samples were stored in the dark at 4 and 25 °C.

3. Results and discussion

As it has been pointed out earlier, in developing a chromatographic method the selection of a stationary phase is essential to achieve a suitable performance. In the present work two columns, a conventional Tracer Extrasil ODS2 (25 × 0.4 cm, id 5 μm) and a novel packing Mediterranea sea_{18} (15 × 0.4 cm, id 3 μm) based on perfectly spherical particles of ultra-pure silica with a very low metal content, were tested to analyse ascorbic acid in fruit juices and soft drinks. The novel stationary phase was selected over the conventional packing because it showed a suitable resolution and a good peak shape. The number of theoretical plates obtained with Mediterranea sea_{18} was 6453 (average of 10 replicate injections) while with the conventional ODS2 only 3282 were obtained. A typical chromatogram of a standard solution of ascorbic acid performed with both columns is shown in Fig. 1. Moreover, the novel packing is compatible with a wide pH range and aqueous mobile phases. These properties make this stationary phase an excellent alternative to the ODS2 column to analyse ascorbic acid in fruit juices and soft drinks.

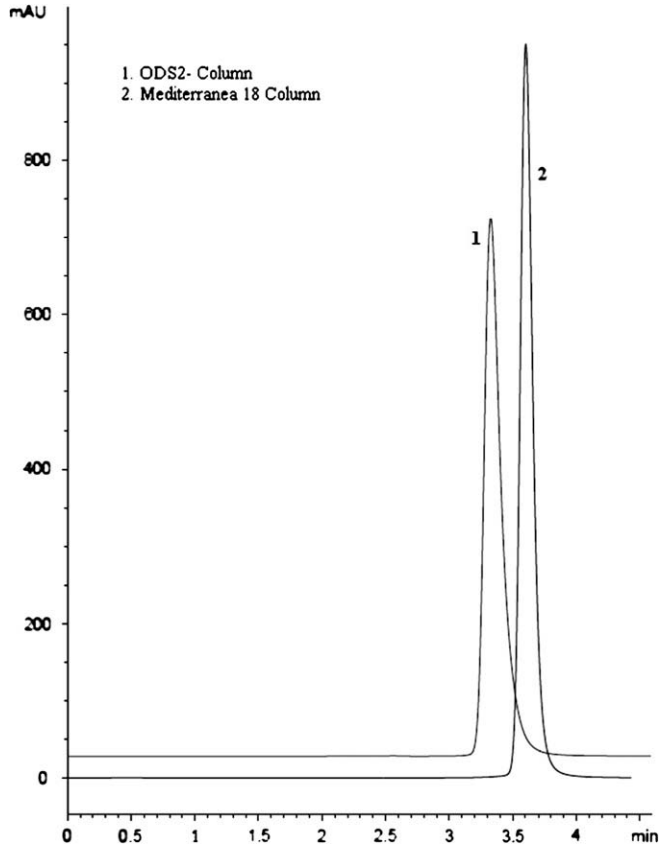

Fig. 1. Chromatograms of a standard solution performed on ODS2 (1) and Mediterranea sea_{18} (2) columns under optimised conditions.

Different flow rates, 0.5, 0.8 and 1 ml/min, were compared; with the high flows the analysis time was reduced and the resolution was improved. In order to prevent problems in the HPLC system because of high pressure, 0.8 ml/min was selected as the most convenient flow rate to conduct the experiments.

Once the chromatographic conditions were optimised the method was validated in terms of linearity, limits of detection, reproducibility and precision of the method. The linearity was tested with the external standard method. The calibration curve made with ascorbic acid standard solutions was divided in two linear ranges. Each range was constructed using four concentration levels. Table 1 shows the linear equations and the determination coefficients. The limit of detection, (defined as a signal three times the height of the noise level), determined in accordance with the American Chemical Society guidelines (ACS, 1980), was 0.01 mg/l.

Table 1
Parameters of linearity.

Range of linearity[a]	Equation	Determination coefficient (r^2)
0.2–20	$y = 65.42x + 13.28$	0.9991
20–400	$y = 63.18x + 192.01$	0.9996

[a] mg/l.

The proposed method showed a good sensitivity; our limit of detection was low compared to previous papers. Tai and Gohda (2007) and Burini (2007) obtained a limit of detection around 0.3 mg/l, whereas Versari et al. (2004) and Rodríguez-Comesaña et al. (2002) reported values slightly higher (1.2 and 1.6 mg/l, respectively).

Reproducibility was determined by analysing six replicates of standards at four concentration levels (20; 50; 100 and 200 mg/l) and expressed as the percentage of RSD ($n = 6$) (Table 2). The method showed excellent precision; the variability was lower than 2%. The precision of the method, estimated as the relative standard deviation of six replicate injections ($n = 6$) from the same fruit beverage sample, was 0.4%. Our values are lower than those reported by Burini (2007).

Seventeen samples including fruit juices, fruit and plant extracts beverages and isotonic drinks were analysed. Table 3 summarises the results of all samples analysed. The average value of ascorbic acid found in fruit juices was higher than that determined in soft beverages and isotonic drinks. Our results were significantly higher than those reported in previous papers. Versari et al. (2004) found amounts around 68 mg/l of ascorbic acid in commercial apricot juices. Lower quantities (45 mg/l) were reported by Jain, Chaurasia, and Verma (1995) in orange drinks. Tang and Wu (2005) evaluated the content of ascorbic acid in fruit juice by capillary electrophoresis, finding concentrations of around 50 and 145 mg/l for orange and apple juice, respectively.

The contents found were not in agreement with the quantities specified on the label except for samples **1** and **4**. The level determined was much higher than the value indicated by the producer. As has been pointed out by Rodríguez-Comesaña et al. (2002), this fact could be due to the label only displaying the amount of ascorbic acid added but not the natural content in vitamin C of the fruits.

In the second part of the work the stability of ascorbic acid in samples was evaluated during the shelf-life of the product. Ascorbic acid degradation was studied in two beverages, orange juice and tea drink. The samples were stored at 4 and 25 °C in the dark, according the specifications of the manufacturer. After 6 days of storage only 8% of the initial ascorbic acid was lost for orange juice; this result is in good agreement with those obtained by Esteve, Farre, and Frígola (1996) and Fellers (1988). However, for tea drinks, only 46% remained when stored at 4 °C and traces when stored at room temperature (Table 4). The differences in the degradation process in orange juice and tea drink samples could be attributed to many variables, such as sample composition, processing conditions, and different packaging.

Table 2
Within-day repeatability calculated as the relative standard deviation (RSD) for six replicate injections at four concentration levels ($n = 6$).

Analyte	Concentration (mg/l)	RSD (%)
Ascorbic acid	20	2
	50	2
	100	0.6
	200	0.5

Table 3
Content of ascorbic acid (±SD) ($n = 2$) in 17 fruit juices, soft drinks and isotonic beverages.

Number	Sample	Concentration (mg/l)
	Fruit juices	
1	Orange juice 1	352 ± 6.0
2	Orange juice 2	739 ± 1.2
3	Apple juice 1	840 ± 0.3
4	Apple juice 2	387 ± 1.3
5	Pineapple and grape juice	702 ± 0.6
	Fruit beverages	
6	Orange drink 1	235 ± 0.2
7	Orange drink 2	201 ± 0.7
8	Lemon drink 1	261 ± 0.8
9	Lemon drink 2	30.2 ± 0.1
10	Apple drink	263 ± 0.3
	Plant extracts beverages	
11	Tea drink 1	175 ± 0.4
12	Tea drink 2	73.5 ± 0.7
	Isotonic drinks	
13	Isotonic drink 1	17.8 ± 0.1
14	Isotonic drink 2	12.1 ± 0.03
15	Isotonic drink with orange flavour 1	6.6 ± 0.01
16	Isotonic drink with orange flavour 2	183 ± 0.1
17	Isotonic drink with cola flavour	11.5 ± 0.2

Table 4
Degradation study: ascorbic acid contents during the shelf-life of orange juice and tea drink stored under two different conditions.

Time (days)	Orange juice Concentration (mg/100 ml)	Time (days)	Tea drink (4 °C) Concentration (mg/100 ml)	Tea drink (25 °C) Concentration (mg/100 ml)
0[a]	73.1	0[a]	7.3	7.3
1	70.9	3	5.9	1.3
3	68.2	5	4.4	0.06
6	67.3	7	3.4	0.03

[a] Time 0 corresponds to packaging opening.

4. Conclusions

The proposed procedure is simple, fast and reliable. The samples are injected directly into the chromatograph without a previous treatment. The novel stationary phase could be an excellent alternative to conventional columns for the analysis of ascorbic acid in beverages. The method showed a good repeatability and extraordinary sensitivity, with a limit of detection of 0.01 mg/l. The losses of ascorbic acid once packaging was opened were not significant for orange juice but were important for tea drink. The method could be useful for food control purposes.

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