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A quick method for the simultaneous determination of ascorbic acid and sorbic acid in fruit juices by capillary zone electrophoresis

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Abstract

A method of quickly determining ascorbic acid and sorbic acid by capillary zone electrophoresis with ultraviolet detection was developed. The choice of background electrolyte, wavelength, injection time and applied voltage were discussed. Ascorbic acid and sorbic acid were well separated in 80 mmol L^{-1} boric acid–5 mmol L^{-1}borax (pH = 8.0) in 5 min at the detecting wavelength of 270 nm. Under the optimum condition, the method has linear ranges of 2.54–352.00 mg L^{-1} for ascorbic acid and 1.08–336.39 mg L^{-1} for sorbic acid with the detection limit of 1.70 mg L^{-1} for ascorbic acid and 0.54 mg L^{-1} for sorbic acid, respectively. Other organic acids in fruit juices have no effect on the detection. This method is very feasible and simple and can be used to detect ascorbic acid and sorbic acid in fruit juices.
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Keywords: Capillary zone electrophoresis; Ascorbic acid; Sorbic acid

1. Introduction

Ascorbic acid is an important micronutrient and can reduce the oxidative damage caused by free radicals. Several methods used to determine ascorbic acid have been published, including high-performance liquid chromatography (HPLC) [1–3], gas chromatography (GC) [4], flow injection spectrophotometry [5], sequential injection analysis [6], voltammetry [7,8], calorimetry [9], flame atomic absorption spectrometry [10], amperometry [11], enzymatic spectrophotometry [12], polarography [13] and titration [14].

Sorbic acid and its salts are allowed in pharmaceutical, cosmetic and food products as anti-fungal preservatives against moulds and yeasts. Previous studies have reported the determination of sorbic acid in foods by HPLC [15–17], GC [18],thin layer chromatography [19,20], titrimetry [21], colorimetry spectrophotometry [22,23], and micellar electrokinetic capillary electrophoresis [24].

Fruit juices contain abundant ascorbic acid which benefits health and increases vitality. Preservatives such as sorbic acid and its salts can stop germs and moulds from spoiling the nurture in fruit juices. The appropriate preservatives appear to be safe, which have been tested by many laboratories throughout the world [25]. However, individuals may be sensitive to various preservatives, so the kinds and the amount of the preservatives must be controlled [26].

Capillary electrophoresis (CE) possesses the advantages of short-analysis time, small-consuming sample, high-separation efficiency, simple-experiment operation and so on. The simultaneous determination of ascorbic acid and sorbic acid by capillary zone electrophoresis (CZE) has never been published. In the present work, a quick, facile, sensitive and selective method for the determination of ascorbic acid and sorbic acid in fruit juices by CZE was developed. Ascorbic acid and sorbic acid were well separated in 5 min with a stable baseline in 80 mmol L^{-1} boric acid–5 mmol L^{-1} borax (pH = 8.0).

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2. Experimental

2.1. Chemicals

Ascorbic acid was purchased from Beijing Yili Fine Chemicals Co., Ltd. (China) and sorbic acid from the Pharmacy Faculity of Second Military Medical University (China). Anisic acid was obtained from International Dispensary Co., Ltd. (China). Tartaric acid and sodium hydroxide were from Beijing Chemical Plant (China), citric acid from Chengdu Chemical Reagent Factory (China), malic acid from Changmao Biochemical Engineering Company Ltd. (China), folic acid from Shanghai Chemical Reagents Company (China), boric acid from Beijing Chemical Reagent Factory (China), borax from Beijing Xinguang Chemical Reagent Factory (China), sodium dihydrogen phosphate and disodium hydrogen phosphate from Hongxing Chemical Reagent Factory (Beijing, China). All reagents were of analytical purity and used without further purification.

2.2. Apparatus

All experiments were performed on a laboratory-built CE unit, which comprised a ±30 kV high-voltage supply (Shanghai Institute of Nuclear Research), a CV^4 variable-wavelength detector (ISCO, Lincoln, USA) operating at 270 nm, and a 75 μm-i.d., 360 μm-o.d. fused-silica capillary tube (Yongnian Optical Conductive Fiber Plant, Hebei, China). The total length of the capillary was 55 cm, and the detection was performed at 40 cm downstream. The samples were introduced into the anionic end of the capillary by gravity, 6.5 cm height for 8 s. The pH meter was from Shanghai Analytical Instrument Factory of China.

2.3. Procedure

Boric acid, borax, sodium dihydrogen phosphate and disodium hydrogen phosphate were dissolved in double-distilled water respectively to prepare the concentrations of 0.2 mol L^{-1}, 0.1 mol L^{-1}, 0.1 mol L^{-1}, 0.1 mol L^{-1} as the stock solutions. The stock solutions were diluted with double-distilled water to the desired concentration. The samples and buffer solutions were filtered through a 0.45-μm cellulose acetate filter membrane (Jiangsu Qilin Medical Instrument Factory, China).

The capillary was conditioned for 30 min with 0.1 mol L^{-1} NaOH and 10 min with water. Additionally, the capillary was washed for 2 min with 0.1 mol L^{-1} NaOH, for 2 min with water and for 5 min with the running buffer before each run. Between runs, the capillary was washed with running buffer. The experiment was done under constant temperature (20 °C) and constant humidity (40%).

2.4. Samples

Orange juice (fruit content ≥ 10%), apple juice (fruit content ≥ 20%) and orange drink concentrate (fruit content ≥ 50%) were purchased from the local supermarkets. The turbid fruit juices were centrifuged for 5 min at $10 \times 10^3 \times g$ (Centrifugal Machine, Xinzhuan Drainage and Irrigation Machinery Plant, China) and filtered through a 0.45-μm filter membrane. Orange juice and apple juice were diluted with double-distilled water to 1:10 (v/v) while orange drink concentrated to 1:100 (v/v).

3. Results and discussion

3.1. Choice of background electrolyte

The background electrolyte (BGE) affects the migration time and the separation between compounds, directly. The most popular buffers for CE are phosphate, borate, zwitterionic compounds and acetate. Faster separations are obtained in CE when the electrophoretic vector and the electroosmotic flow are in the same direction. Since ascorbic acid and sorbic acid are weak acids, a weak basic buffer system is preferred to have a suitable migration time: 20 mmol L^{-1} phosphate (pH = 8.0), 20 mmol L^{-1} borax–HCl (pH = 8.0), 10 mmol L^{-1} boric acid–10 mmol L^{-1} phosphate (pH = 8.0) and 80 mmol L^{-1} boric acid–5 mmol L^{-1} borax (pH=8.0) were tried. Ascorbic acid and sorbic acid were separated in the above BGEs except for 20 mmol L^{-1} phosphate (pH = 8.0). The sensitivity and the shape of the peak were not ideal in 20 mmol L^{-1} borax–HCl (pH = 8.0), and in 10 mmol L^{-1} boric acid–10 mmol L^{-1} phosphate (pH = 8.0), the migration time got longer and the peak became broader. However, ascorbic acid and sorbic acid were separated and detected absolutely in 5 min with a stable baseline in 80 mmol L^{-1} boric acid–5 mmol L^{-1} borax (pH = 8.0), and there was also a substantial improvement in peak sharpness and symmetry compared with the other BGEs. The reason is that boric ion and the compound containing hydroxide radicals form a chelate bond which can increase the negative charge and the resolution of

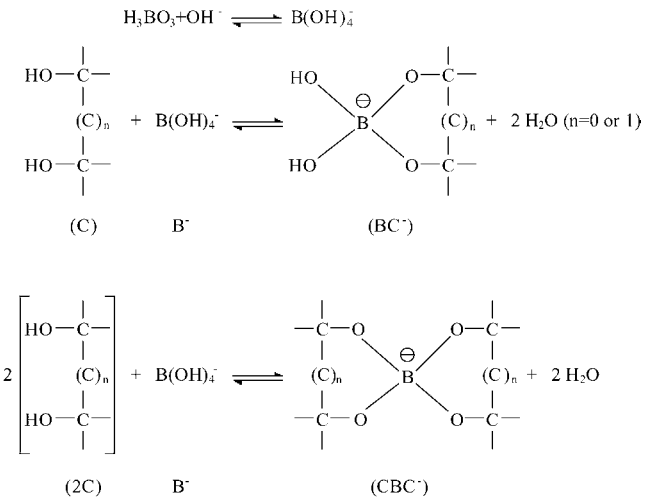

Fig. 1. The reaction of H_3BO_3 and hydroxide.

the compounds (Fig. 1) [27]. Therefore, boric acid–borax was chosen as the BGE in this experiment. The effect of different electrolyte concentrations on the separation of ascorbic acid and sorbic acid showed that higher buffer concentrations gave longer migration time and broader peak. The electropherograms of the above BGEs revealed that 80 mmol L^{-1} boric acid−5 mmol L^{-1} borax (pH = 8.0) was suitable.

Maybe there are some other organic acids in juices, such as citric acid, malic acid, folic acid and so on. In order to affirm if other acids interfere reciprocally, 3.00×10^{-5} mol L^{-1} tartaric acid, citric acid, malic acid and folic acid were added to ascorbic acid and sorbic acid standard solution, respectively. The peak areas and the migration times remain unchanged, showing the absence of the interferences from the above organic acids.

3.2. Choice of wavelength

The peak areas of ascorbic acid and sorbic acid were measured by varying the ultraviolet band from 240 to 280 nm (Fig. 2). Considering ascorbic acid and sorbic acid at the same time, 270 nm was preferred.

3.3. Choice of applied voltage

The efficiency of the separation improves and the migration time shortens when the applied voltage is increased. But the higher voltage will result in the peak broadening and the efficiency of the separation falling because of the Joule heating effect. In this experiment, the migration time of the peak was measured as the applied voltage increased from 10 to 22.5 kV (Fig. 3). The current increased slightly and was always under 10 μA. A voltage of 20 kV was selected as the best applied voltage.

3.4. Choice of injection time

The samples are introduced into the anionic end of the capillary by gravity. When the injection height remains unchanged, the injection time decides the injection amount. Injections of 3.0×10^{-5} mol L^{-1} ascorbic acid and sorbic acid standard solution for 4, 6, 8, 10 and 12 s were performed. When the injection time was greater than 8 s, the sensitivity did not change significantly while the peak shapes became poorer because of the band broadening. As a result, an injection time of 8 s was selected as the best.

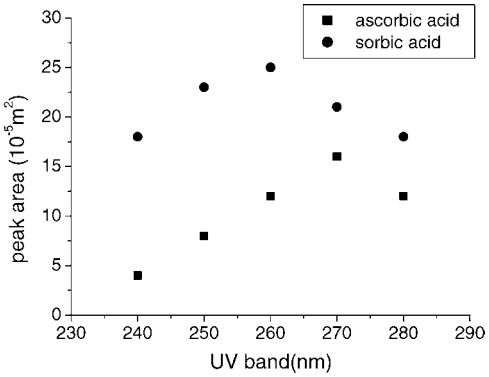

Fig. 2. The effect of UV wavelength on the peak areas of ascorbic acid and sorbic acid. BGE: 80 mmol L^{-1} boric acid–5 mmol L^{-1} borax (pH = 8.0); applied voltage: 20 kV; current: 10 μA; injection: 6.5 cm × 8 s.

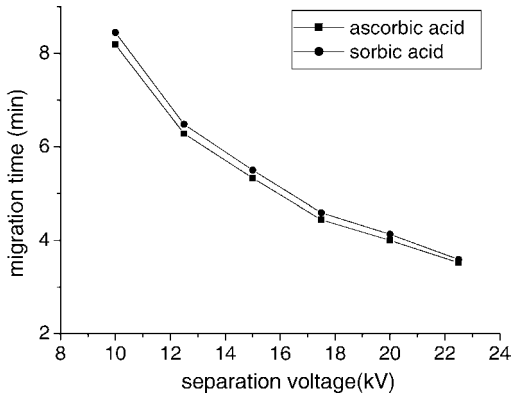

Fig. 3. The effect of the separation voltage on the migration time of ascorbic acid and sorbic acid. BGE: 80 mmol L^{-1} boric acid–5 mmol L^{-1} borax (pH = 8.0); injection: 6.5 cm × 8 s; UV band: 270 nm.

3.5. Linearity, reproducibility and detection limit

Using the optimized instrumental and operational parameters for ascorbic acid and sorbic acid standard solution, the linearity of the method was calculated and expressed as the correlation coefficient and the reproducibility of the method was measured by replicate injections of the standard solution. There was excellent linearity between the peak area and the concentration of the analyte in the range of 2.54–352.00 mg L^{-1} for ascorbic acid and 1.08–336.39 mg L^{-1} for sorbic acid. Ascorbic acid and sorbic acid were separated completely during the linearity range at larger concentrations. Nevertheless, the shapes of the peaks became poorer and the migration times increased compared with those at smaller concentrations. The calibration graphs obtained for the two compounds were described by the equations: $y = 19.89x + 0.32$ for ascorbic acid and $y = 24.93x + 0.21$ for sorbic acid, where y represents the peak area and x, the concentration (mol L^{-1}) of the standard analyte dissolved in distilled water. The correlation coefficients for the peak areas of ascorbic acid and sorbic acid were $r = 0.9969$ and $r = 0.9980$. The R.S.D. values ($n = 7$) for ascorbic acid and sorbic acid were 2.89% and 2.56%. Ascorbic acid and sorbic acid were easily detected without interference at concentration of 1.70 mg L^{-1} for ascorbic acid and 0.54 mg L^{-1} for sorbic acid, at a signal to noise ratio of three.

3.6. Application

Three different fruit juices were prepared using the procedure developed as in Section 2.4. Anisic acid was used

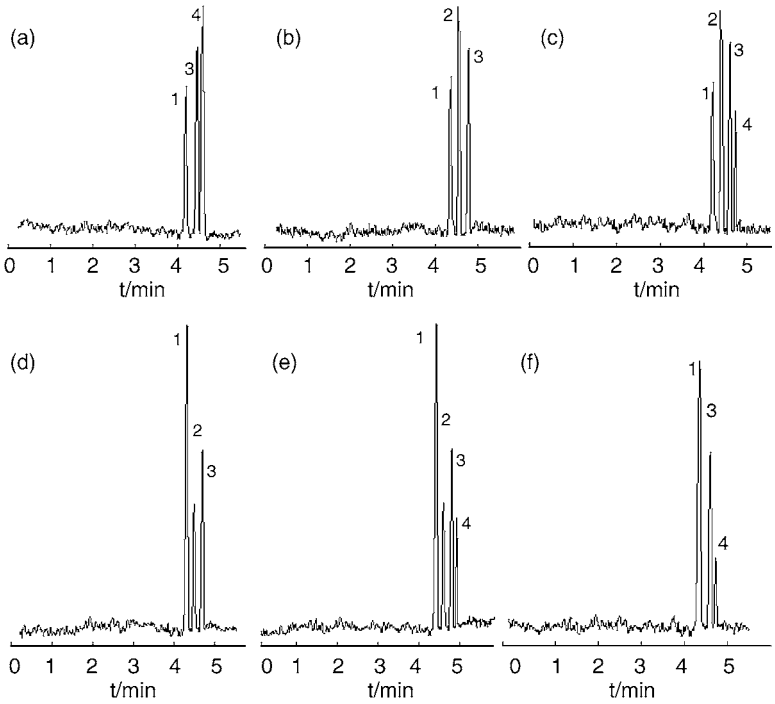

Fig. 4. The electropherograms of the samples. 1, Ascorbic acid; 2, unknown component; 3, anisic acid; 4, sorbic acid. (a) 3.0×10^{-5} mol L^{-1} ascorbic acid + 5.0×10^{-5} mol L^{-1} anisic acid + 3.0×10^{-5} mol L^{-1} sorbic acid; (b) orange juice + 5.0×10^{-5} mol L^{-1} anisic acid; (c) orange juice + 5.0×10^{-5} mol L^{-1} anisic acid + 1.5×10^{-5} mol L^{-1} sorbic acid; (d) apple juice + 5.0×10^{-5} mol L^{-1} anisic acid; (e) apple juice + 5.0×10^{-5} mol L^{-1} anisic acid + 1.5×10^{-5} mol L^{-1} sorbic acid; (f) orange drink concentrate + 5.0×10^{-5} mol L^{-1} anisic acid. BGE: 80 mmol L^{-1} boric acid–5 mmol L^{-1} borax (pH = 8.0); applied voltage: 20 kV; current: 10 μA; injection: 6.5 cm × 8 s; UV band: 270 nm.

Table 1
The results for CE analysis of ascorbic acid and sorbic acid

Sample	The average value found (mg L^{-1})	R.S.D. (%) (n = 7)	Added (mg L^{-1})	Amount found (mg L^{-1})	Recovery (%)
Orange juice					
Sorbic acid	–	–	16.82	16.65	99.0
Ascorbic acid	53.12	3.00	52.84	103.52	97.7
Apple juice					
Sorbic acid	–	–	16.82	16.60	98.7
Ascorbic acid	145.73	2.95	52.84	193.60	97.5
Orange drink concentrate					
Sorbic acid	113.36	2.74	168.20	276.49	98.2
Ascorbic acid	898.86	2.77	528.40	1388.72	97.3

'–', representing value not found; the concentration that before dilution; other conditions as in Fig. 4.

as the internal standard, and the chromatograms are depicted (Fig. 4). The determination results of ascorbic acid and sorbic acid in the samples are listed in Table 1, which confirm that all these three juices contain abundant ascorbic acid and that orange juice and apple juice contain no preservative, while orange drink concentrate contains sorbic acid. The assay indicates that the analytical method proposed is adequate for the determination of ascorbic acid and sorbic acid in fruit juices.

References

[1] T. Pérez-Ruiz, C. Martínez-Lozano, A. Sanz, A. Guillén, J. Pharm. Biomed. Anal. 34 (2004) 551.
[2] B.A. Wolucka, M.W. Davey, W. Boerjan, Anal. Biochem. 294 (2001) 161.
[3] J. Lykkesfeldt, Anal. Biochem. 291 (2001) 173.
[4] F.O. Silva, Food Control, Corrected Proof, Available online 9 January 2004, in press.
[5] A. Jain, A. Chaurasia, K.K. Verma, Talanta 42 (1995) 779.
[6] N. Anastos, N.W. Barnett, B.J. Hindson, C.E. Lenehan, S.W. Lewis, Talanta, Corrected Proof, Available online 8 April 2004, in press.
[7] Shengfu Wang, Dan Du, Sens. Actuators B: Chem. 97 (2004) 373.
[8] E.M. Strochkova, Ya.I. Tur'yan, I. Kuselman, A. Shenhar, Talanta 44 (1997) 1923.
[9] M.L. Antonelli, G. D'Ascenzo, A. Laganà, P. Pusceddu, Talanta 58 (2002) 961.
[10] M.C. Yebra, R.M. Cespón, A. Moreno-Cid, Anal. Chim. Acta 448 (2001) 157.

[11] Jyh-Myng Zen, Dong-Mung Tsai, A.S. Kumar, V. Dharuman, Electrochem. Commun. 2 (2000) 782.
[12] M.R. Esteban, Chu-Ngi Ho, Microchem. J. 56 (1997) 122.
[13] F. Sahbaz, G. Somer, Food Chem. 44 (1992) 141.
[14] P.W. Washko, R.W. Welch, K.R. Dhariwal, Y. Wang, M. Levine, Anal. Biochem. 204 (1992) 1.
[15] B. Ennio, A. Spadaro, N.A. Santagati, S. Scalia, G. Ronsisvalle, J. Pharm. Biomed. Anal. 30 (2002) 947.
[16] I. García, M.C. Ortiz, L. Sarabia, C. Vilches, E. Gredilla, J. Chromatogr. A 992 (2003) 11.
[17] A. El-Gindy, F. El-Yazby, A. Mostafa, M.M. Maher, J. Pharm. Biomed. Anal. 35 (2004) 703.
[18] T. Renner, M. Baer-Koetzle, G. Scherer, J. Chromatogr. A 847 (1999) 127.
[19] C. Gertz, J. Hild, Zeitschrift Fur Lebensmittel-Untersuchung Und–Forschung 170 (1980) 103.
[20] B. Mandrou, F. Bressolle, J. Assoc. Official Anal. Chem. 63 (1980) 675.
[21] O.W. Lau, S.F. Luk., Analyst 112 (1987) 1269.
[22] A. Caputi, K. Slinkars, J. Assoc. Official Anal. Chem. 58 (1975) 133.
[23] O.W. Lau, S.F. Luk, Y.M. Cheung, Analyst 114 (1989) 1047.
[24] I. Pant, V.C. Trenerry, Food Chem. 53 (1995) 219.
[25] T.E. Furia, CRC Handbook of Food Additive, second edn., CRC Press, Cleveland, 1972.
[26] The Determination of Sorbic Acid and Benzoic Acid in Food, National Standards of P.R. China, GB/T 5009.29-1996.
[27] C. Yi, The Technology and Application of Capillary Electrophoresis, Chemical Industry Press, Beijing, 2001.