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Determination of flavonoids and ascorbic acid in grapefruit peel and juice by capillary electrophoresis with electrochemical detection

Ting Wu, Yueqing Guan, Jiannong Ye *

Department of Chemistry, East China Normal University, Shanghai 200062, PR China

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Abstract

Grapefruit (*Citrus paradisi* Mact. (Rutaceae)) has been known for its accumulation of flavonoids and ascorbic acid. These contents are important because of their nutritional and antioxidant properties. Five flavonoids (hesperidin, naringin, hesperedin, narigenin and rutin) and ascorbic acid were separated and determined in grapefruit juice by capillary electrophoresis with electrochemistry detection (CE-ED). Two flavonoids (hesperidin, naringin) and ascorbic acid were found in extract of grapefruit peel with the same method. And the distribution comparision of the ingredients between juice and peel was discussed. The effects of several CE parameters on the resolution were studied systematically. Under the optimum conditions, the analytes could be well separated within 25 min in a 60 mmol L^{-1} borate buffer (pH 9.0). The response was linear over four orders of magnitude with detection limits ($S/N = 3$) ranging from 1.4×10^{-7} to 1.0×10^{-6} g ml^{-1} for the analytes. The method has been successfully applied for the analysis of grapefruit with satisfactory results.

Keywords: Capillary electrophoresis; Electrochemistry detection; Grapefruit; Flavonoids; Ascorbic acid

1. Introduction

In recent years, the grapefruit [*Citrus paradise* Mact. (Rutaceae)] has received much attentions because of its nutritional and antioxidant properties. Besides ascorbic acid, it also contains abundant flavonoids, which are reported to be the important part of active ingredients. The potential health benefits of flavonoids in food and beverages have been discovered for antioxidative (George, 2003; Wang, Cao, & Prior, 1997), anticarcinogenic (Berkarda, Koyuncu, & Soybir, 1998; So, Guthrie, Chambers, Moussa, & Carroll, 1996; Tanaka et al., 1997), anti-inflammoatory (Crespo, Galvez, Cruz, Ocete, & Zarzuelo, 1999; Manthey, 2000) effects and et al.

Grapefruit is cultivated principally to obtain the juice. Flavonoid components, particularly naringin, are contained abundantly in this juice. Now, more reporters are mainly focused on the beneficial effects of the grapefruit juice on human health, such as antioxidant, antiallergic, and anticarcinogenic benefits as well as protection against high blood pressure or cholesterol increase (Kawaii, Tomono, Katase, Ogawa, & Yano, 1999).

Surveyors have also found that peels of fruits are the major sources of natural antioxidant (Bocco, Cuvelier, Richard, & Berset, 1998; Gorinstein et al., 2004) because of their abundant content of naringin, hesperidin and other flavonoids. While grapefruit peels are currently generally treated as waste of juice industry, this practice is not only a waste of resource, but also causes environmental pollution. Some even propose to use these by-products of the juice extraction industry as the important source for natural antioxidants. Therefore, the peeled fruit juice and their peels were studied separately.

A number of HPLC methods have been developed for the determination of flavonoids in biological fluids, either alone or in combination (Ameer, Weintraub, Johnson, Yost, & Rouseff, 1996; Manach, Morand, Gil-Izquierdo, Bouteloup-Demange, & Rémésy, 2003; Swatsitang, Tucker, Robards, & Jardine, 2000). However, some of

* Corresponding author. Tel.: +86 21 62232254; Fax: +86 21 62576217.
E-mail address: jnye@ecnu.edu.cn (J. Ye).

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these methods are time-consuming, some are costly, some need using gradient elution, or there is no internal standard and are not directly applicable for the simultaneous quantification of these compounds (Kanaze, Kokkalou, Georgarakis, & Niopas, 2004). Therefore, the objective of the present work is to develop and validate a fast, effective method for the simultaneous determination of these five flavonoids and ascorbic acid. Since CE in its modern form was first described by Jorgenson and Lukacs (1981), its application for the separation and determination of a variety of samples has been increasingly widespread because of its minimal sample volume requirement, short analysis time and high separation efficiency. Electrochemical detection (ED) typically operated in the amperometric mode can be coupled with CE to provide high sensitivity to electroactive species.

This work reports the separation and quantification of hesperidin, naringin, hesperedin, narigenin, rutin and ascorbic acid in 100% home-made grapefruit juice as well as the grapefruit peel, and the different distribution of naringin between juice and peel are also discussed.

2. Experimental

2.1. Apparatus

A laboratory-built CE-ED system used for analysis has been described previously (Chen, Ye, & Cheng, 2000). Briefly, a ±30 kV high-voltage d.c. power supply (Shanghai Institute of Nuclear Research, Shanghai, China) provided a separation voltage between the ends of the capillary. The inlet end of the capillary was held at a positive potential and the outlet end of capillary was maintained at ground. The separations proceeded in a 75 cm length of 25 μm i.d. and 360 μm o.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA).

A three-electrode electrochemical cell consisting of a 300 μm diameter carbon disc working electrode, a platinum auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode, was used in combination with a BAS LC-4C amperometric detector (Bioanalytical Systems Inc., West Lafayette, IN, USA). The filter of the detector was set at 0.1 Hz. The working electrode was positioned carefully opposite the outlet of the capillary with the aid of a micro-manipulator (CORRECT, Tokyo, Japan) and arranged in a wall-jet configuration (Chen, Zhang, & Ye, 2001). The electropherograms were recorded using a chart record (Shanghai Dahua Instrument Factory).

2.2. Reagent and solutions

Hesperidin, naringin, hesperedin, narigenin and rutin were all obtained from Sigma (St. Louis, MO, USA). Vc and other chemicals were of analytical grade. The structures of these bioactive components are shown in Fig. 1. All aqueous solutions were made up in doubly distilled water. The grapefruit was purchased from a supermarket.

Stock solutions of hesperidin, naringin, hesperedin, narigenin, rutin and Vc (1.0×10^{-3} g ml^{-1}) were prepared in ethanol. All standard solutions were kept in a 4 °C refrigerator. The running buffer for separation was 60 mol L^{-1} borate buffer (BB, pH 9.0) unless mentioned otherwise.

2.3. Sample preparation

The 2 mm thick fresh grapefruit peels were finely chopped and then accurately weighted 1 g. The weighted sample was extracted with 10 ml 99.7% ethanol for 2 h in an ultrasonic bath. The extract was then filtered through a filter paper. The extraction procedure was repeated three times. After filtered through 0.22 μm syringe cellulose acetate filter, the 80 μl sample was diluted with 60 mmol L^{-1} borate buffer to 1 ml. Then it can be directly injected electrokinetically for analysis.

The pulp was treated with a juice extractor, and the juice was centrifuged in 1200 rev/s for 10 min. Then the juice was filtered through 0.22 μm syringe cellulose acetate filter, the 80 μl sample was diluted with 60 mmol L^{-1} borate buffer to 1 ml. Then it can be directly injected electrokinetically for analysis.

3. Results and discussion

3.1. Effect of the potential applied to the working electrode

In amperometric detection, the potential applied to the working electrode directly affects the sensitivity, detection limit and stability of this method. In order to obtain best detection results, optimum potential applied to the working electrode should be selected. Therefore, hydrodynamic voltammetry experiment was investigated to obtain optimum detection. As shown in Fig. 2, when applied potential exceeded +0.6 V (vs. SCE), the peak current of analytes increases rapidly with the rising of applied potential. However, when applied potential was greater than +0.95 V (vs. SCE), both the baseline noise and the background current increase, resulting in an unstable baseline, which is a disadvantage for sensitive and stable detection. Therefore, the applied potential of +0.95 V (vs. SCE) was selected, where the background current is not too high and the S/N ratio is the highest.

3.2. Optimum condition for separation

To improve the resolution and solubility of the analytes, alkaline borate buffer was employed in this study, because the analytes can form negative-charged complexes with boric acid in alkaline solution. The acidity of the running buffer affects the zeta-potential (ξ), the electroosmotic flow (EOF) as well as the overall charge of the analytes, which determine the migration time and the separation of the analytes (Cao, Zhang, Fang, & Ye, 2001). Fig. 3A illustrates the effect of running buffer pH on the migration time of the analytes. The running buffers were 60 mmol L^{-1}

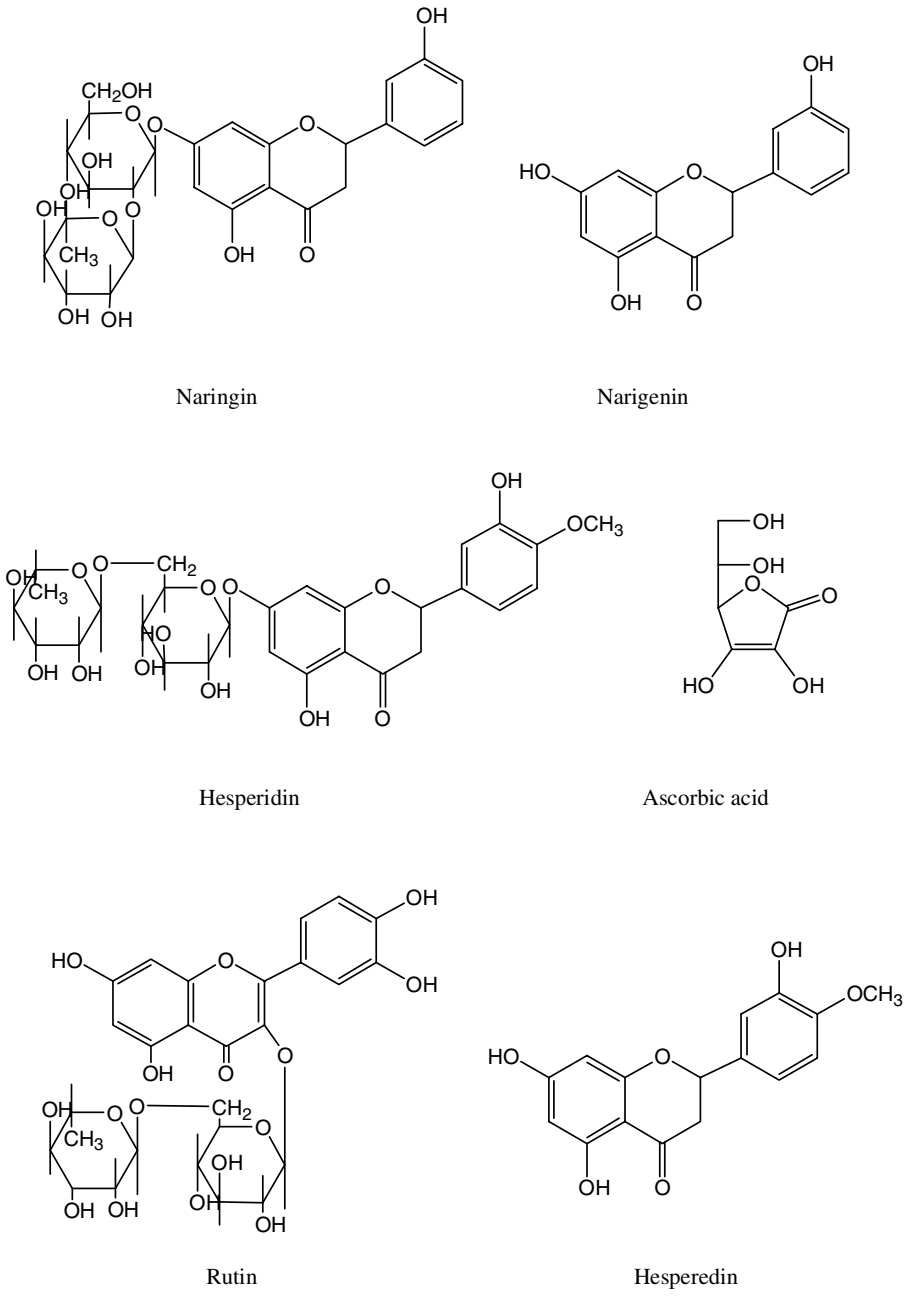

Fig. 1. The molecular structures of six analytes.

borate buffers (BBs) in the pH range of 8.2–9.2. The resolution of hesperidin and narigenin is poor below pH 9.0. When the running buffer pH increases, resolution improves, but when pH is higher than 9.0, narigenin cannot be separated from rutin. Meanwhile, and also the response of ascorbic acid becomes weaker and weaker. Therefore the optimum running buffer pH is 9.0, at which the six analytes can be well separated within a relatively short time.

Besides the pH value, the running buffer concentration is also an important parameter. Fig. 3B indicates that migration time and resolution increases with increasing buffer concentration. When exceeding 60 mmol L^{-1}, the resolution improves slowly. Higher buffer concentrations (>60 mmol L^{-1}) also have a negative effect on the detection limit, however, because the peak current of five analytes decreases and the effect of Joule heating becomes more pronounced. So 60 mmol L^{-1} BB (pH 9.0) was finally chosen as the optimum running buffer concentration in this work, on the basis of the peak current, resolution, analysis time, and buffer capacity.

The influence of separation voltage on the migration time of the analytes is exhibited in Fig. 3C. Increasing the voltage gives shorter migration time for all the analytes, but also increases the baseline noise, that is not beneficial

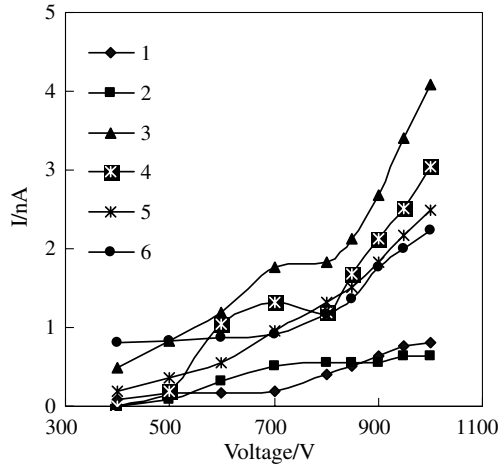

Fig. 2. Hydrodynamic voltammograms (HDV) obtained from CE-ED of 1.0×10^{-5} g ml^{-1} of hesperidin (1), naringin (2), hesperedin (3), narigenin (4), rutin (5), and ascorbic acid (6). Fused-silica capillary: 25 μm i.d. × 75 cm; working electrode: 300 μm diameter carbon disc electrode; running buffer: 60 mmol L^{-1} BB (pH 9.0); separation voltage: 12 kV; injection: 6 s (at 12 kV).

to the determination of the analytes, on the other hand, too low separation voltage will increase the analysis time considerably, which in turn cause peak broadening. On the basis of these experiments, 12 kV was chosen as the optimum voltage to accomplish a good compromise. The effect of injection time on CE separation was investigated by changing the sampling time (2, 4, 6, 8, 10 s at a voltage of 12 kV). It was found that both peak current and peak width increase with increasing sampling time. When injection time exceeds 6 s, the peak current increases slowly and peak broadening becomes more severe. In this experiment, 6 s (at 12 Kv) was selected as the optimum injection time in considering resolution and sensitivity.

Typical electropherograms obtained under optimum conditions for a standard solution of a mixture of 1.0×10^{-5} g ml^{-1} six analytes are shown in Fig. 4A.

3.3. Repeatability, linearity and detection limits

The Repeatability of the peak current was estimated by making repetitive injections of a standard mixture solution (1.0×10^{-5} g ml^{-1} for each analyte) under the selected optimum conditions. The relative standard derivations (RSD) of the peak current were 1.9%, 1.8%, 3.8%, 2.8%, 3.0% and 4.5% for hesperidin, naringin, hesperedin, narigenin, rutin and ascorbic acid, respectively ($n = 7$). A series of the standard mixture solutions of the six analytes was

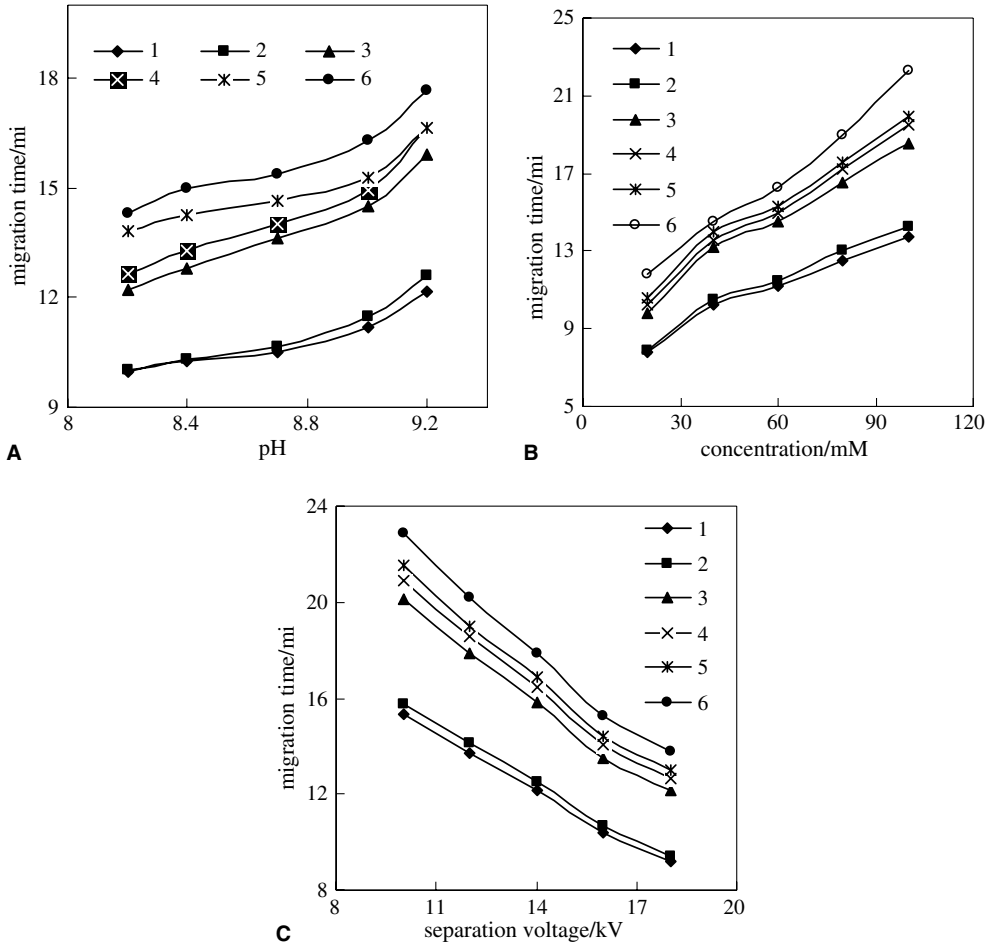

Fig. 3. Effect of: (A) acidity and (B) concentration of the running buffer, and of (C) separation voltage on the migration time of the analytes. The working potential was +0.95 V (vs. SCE); other conditions are as in Fig. 2.

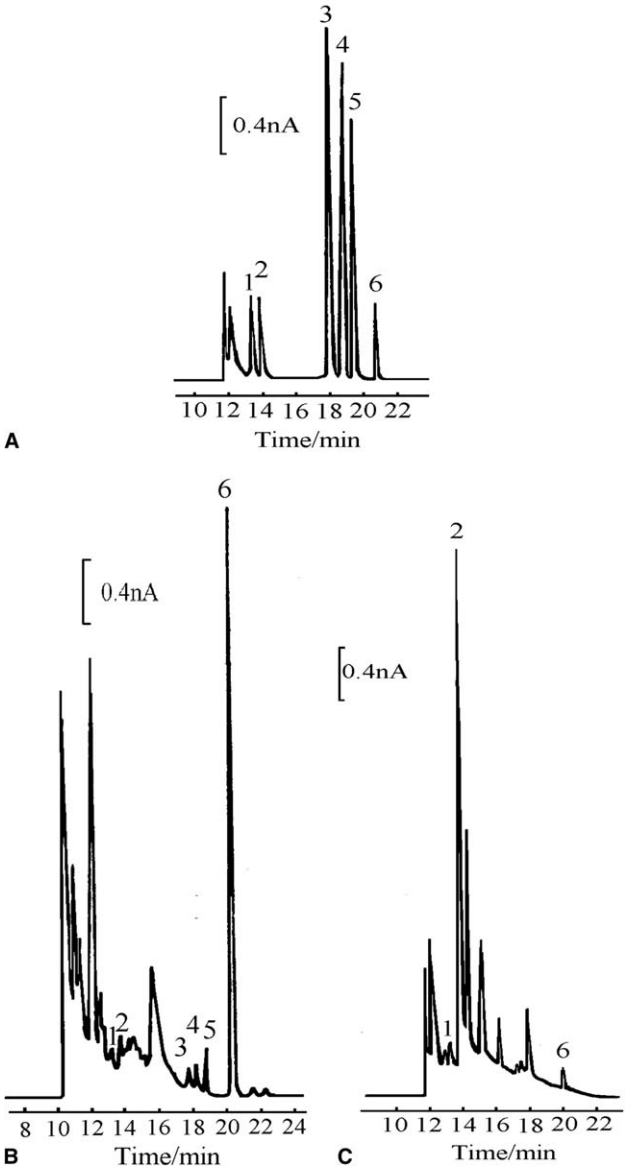

Fig. 4. Electropherogram obtained from a standard mixture solution of 1.0×10^{-5} g ml^{-1} hesperidin (1), naringin (2), hesperedin (3), narigenin (4), rutin (5), and ascorbic acid (6) (A). Typical electropherograms obtained from diluted extracts from grapefruit (B: juice, C: peel) under the optimum conditions (1. hesperidin, 2. naringin, 3. hesperedin, 4. narigenin, 5. rutin, and 6. ascorbic acid). The working potential was +0.95 V (vs. SCE); other conditions are as in Fig. 2.

tested to determine the linearity of this method. The results of regression analysis on calibration curves and detection limits are presented in Table 1. The determination limits were evaluated on the basis of a signal-to-noise ratio of 3. The calibration curves exhibit excellent linear behavior over the concentration range of about 4 orders of magnitude for all investigated compounds.

3.4. Sample analysis and recovery

3.4.1. Grapefruit analysis and recovery

Under the selected optimum conditions, the determination of active ingredients in grapefruit juice and peel was conducted by CE-ED. Typical electropherograms of grapefruit juice and peel are shown in Fig. 4B and C, respectively. By comparing the migration time of analytes with the electropherogram of the standard mixture solution (Fig. 4A) and by standard addition approach, the active ingredients namely hesperidin (1), naringin (2), hesperedin (3), narigenin (4), rutin (5), and ascorbic acid (6) in grapefruit juice can be determined and naringin (1), hesperidin (2), and ascorbic acid (6) were found in grapefruit peels. The results are listed in Table 2.

Accurate amounts of hesperidin, naringin, hesperedin, narigenin, rutin and ascorbic acid were added to the sample solution of the grapefruit juice and peel, and the recovery values were obtained using their peak currents from the calibration curve under the same conditions. The average recoveries and RSDs for the five analytes are listed in Table 3. The results indicate that this method is accurate for all analytes.

Table 2
Assay results of the analytes in grapefruit juice and peel ($n = 3$)[a]

Sample	Grapefruit juice (10^{-6} g ml^{-1})	Grapefruit peel (10^{-6} g g^{-1})
Hesperidin	17.86	714
Naringin	44.64	14,000
Hesperedin	7.35	N.F
Narigenin	12.93	N.F
Rutin	32.61	N.F
Ascorbic acid	979.17	500

[a] Working potential is +0.95 V (vs. SCE). Other conditions are as in Fig. 2.

Table 1
The results of regression analysis on calibration curves and detection limits[a]

Compound	Regression equation $Y = aX + b$[b]	Correlation coefficiency	Linear range (g ml^{-1})	Detection limit (g ml^{-1})[c]
Hesperidin	$Y = 7.40 \times 10^4 X + 0.0428$	0.9998	1×10^{-6}–1×10^{-4}	5.0×10^{-7}
Naringin	$Y = 6.38 \times 10^4 X + 0.0309$	0.9996	1×10^{-6}–1×10^{-4}	3.5×10^{-7}
Hesperedin	$Y = 2.85 \times 10^5 X + 0.5376$	0.9979	1×10^{-6}–1×10^{-4}	1.4×10^{-7}
Narigenin	$Y = 2.13 \times 10^5 X + 0.6227$	0.9949	1×10^{-6}–1×10^{-4}	1.6×10^{-7}
Rutin	$Y = 1.76 \times 10^5 X + 0.6029$	0.9938	1×10^{-6}–1×10^{-4}	1.8×10^{-7}
Ascorbic acid	$Y = 6.52 \times 10^4 X + 0.2532$	0.9978	5×10^{-6}–1×10^{-4}	1.0×10^{-6}

[a] Working potential is +0.95 V (vs. SCE). Other conditions are as in Fig. 2.
[b] Y and X are the peak current (nA) and concentration of the analytes (g ml^{-1}).
[c] The detection limits corresponding to concentrations giving signal-to-noise ratio of 3.

Table 3
Determination results of the recovery for this method ($n = 3$)[a]

Compound	Original (10^{-6} g ml^{-1})	Added (10^{-6} g ml^{-1})	Found (10^{-6} g ml^{-1})	Recovery (%)	RSD (%)
Juice					
Hesperidin	1.4286	10	10.7143	94	3.1
Naringin	3.5714	10	12.8571	95	2.4
Hesperedin	0.5882	10	10.8824	103	3.7
Narigenin	1.0345	10	10.6897	97	2.6
Rutin	2.6087	10	12.8261	102	2.8
Ascorbic acid	78.3333	10	86.1667	98	4.0
Peel					
Hesperidin	3.5714	10	13.2143	97	2.8
Naringin	70	10	77.8571	97	2.7
Ascorbic acid	2.5	10	11.9167	95	4.3

[a] Working potential is +0.95 V (vs. SCE). Other conditions are as in Fig. 2.

3.4.2. Analysis of Vc in other fruits and vegetables

To demonstrate the robustness of CE-ED in the analysis of Vc, it was also applied to determine the Vc contents in several real samples, such as orange juice, tangerine juice, Nanfeng mandarin orange juice, lemon juice, and fresh sweet potato. The results are 3.6×10^{-4} g ml^{-1}, 1.7×10^{-4} g ml^{-1}, 0.96×10^{-4} g ml^{-1}, 2.8×10^{-4} g ml^{-1}, and 0.7×10^{-4} g g^{-1}, respectively. The Vc contents in above real samples well accorded with the results from a website which lists the Vc contents in 7248 food samples selected from the USDA survey database.

(http://www.hoptechno.com/nightcrew/sante7000/sante7000_search.cfm).

4. Conclusion

It has been demonstrated that CE-ED is characterized by high resolution and sensitivity, satisfactory stability and repeatability, low operating cost, and minimal sample volume requirement. CE-ED has been successfully used for the determination of hesperidin, naringin, hesperedin, narigenin, rutin and ascorbic acid in grapefruit juice and peel. The juice contains all six ingredients, of which ascorbic acid is the highest. The peel contains only three of them, naringin, hesperedin and ascorbic acid. The high level naringin shows that the grapefruit peel is an important resource of naringin. ED coupled with CE enable selective and sensitive detection of the electroactive constituents in fresh fruits. It is concluded that CE-ED is a powerful technique for the study of the constituents of natural plants and has become an alternative, competitive, and supplementary method for LC, because of its special attributes.

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