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Determination of Pharmacologically Active Ingredients in Sweet Potato (*Ipomoea batatas* L.) by Capillary Electrophoresis with Electrochemical Detection

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Sweet potato (*Ipomoea batatas* L.), in which vitamin C, chlorogenic acid, caffeic acid, quercetin, and rutin are abundant, is one of the functional food products aimed at introducing human dietary ingredients that aid specific body functions in addition to being nutritious. A method based on capillary electrophoresis with electrochemical detection (CE-ED) to qualitatively and quantitatively determine the pharmacologically active ingredients in sweet potato has been developed by our group. The effects of working electrode potential, pH and concentration of running buffer, separation voltage, applied potential, and injection time on CE-ED were investigated. Under the optimum conditions, the analytes could be well-separated within 20 min at the separation voltage of 18 kV in a 60 mmol L^{-1} Borax running buffer (pH 9.0). A good linear relationship was established between peak current and concentration of analytes over 2 orders of magnitude with detection limits (S/N = 3) ranging from 7.14×10^{-7} to 2.88×10^{-7} g mL^{-1} for all target ingredients. The satisfactory results show that this method is very successful and effective for the analysis of real samples.

KEYWORDS: Sweet potato; pharmacologically active ingredients; capillary electrophoresis; electrochemical detection

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.), which belongs to the family *Convolvulaceae*, has been widely cultivated for centuries in various geographic regions. According to statistics from the Food and Agriculture Organization of the United Nations (FAO), the cultivated area of sweet potato in the world is about 9.0 million hectares and its production is ranked seventh in worldwide crop production today. Sweet potato has received broad attention because it is an important resource of food and there is an abundance of pharmacologically active ingredients in it. So far, sweet potato has been widely used as a food staple, vegetable, and animal feed for industrial starch extraction and various processed products (*1*, *2*). At the same time, more and more modern research has shown that sweet potato has higher levels of both carbohydrate and dietary fiber than potato (*3*) and also has a stronger antioxidant activity than most other vegetables in a typical Western diet (*4*). Besides, the extract from sweet potato exhibits strong radical scavenging (*5*, *6*) and antimutagenic activities (*7*, *8*), significantly reduces high blood pressure and carbon tetrachloride-induced liver injury (*9*), has antiinflammatory, antimicrobial, and antihypertensive activities, and has ultraviolet protection effects (*10*). Furthermore, sweet potato was recently identified as possessing a postprandial antihyperglycemic (antidiabetic) effect in rats through retardation of maltase activity (*11*).

As a consequence of the potent biological activity associated with vitamin C (Vc), chlorogenic acid, caffeic acid, quercetin, and rutin (**Figure 1**) of sweet potato (*12*), some available methods for the analysis of these compounds have been developed over the past decade (*12*, *13*); they are mainly focused on qualitative and semiquantitative analysis of these compounds. Hence, it is highly desirable to develop a simple, economical, and efficient method for the analysis and quantitative measurement of pharmacologically active ingredients in sweet potato. Capillary electrophoresis (CE) is increasingly recognized as an important analytical separation technique because of its speed, efficiency, reproducibility, ultrasmall sample volume, little consumption of solvent, and ease of clearing up the contaminants. To the best of our knowledge, the CE-electrochemical detection (ED) method has not been reported for the simultaneous determination of pharmacologically active ingredients in sweet potato.

In this work, a simple and rapid CE-ED method for the determination of biologically active ingredients in sweet potato is reported for the first time. In particular, the quantity difference of active ingredients found in the peel and the pulp of sweet potato and the corresponding variation of these ingredients between the fresh and cooked sweet potato were explored.

MATERIALS AND METHODS

Apparatus. The laboratory-built CE-ED system (*14*) was used in this work. A +30 kV high-voltage power supply (Shanghai Institute of Nuclear Research, China) provided a separation voltage between

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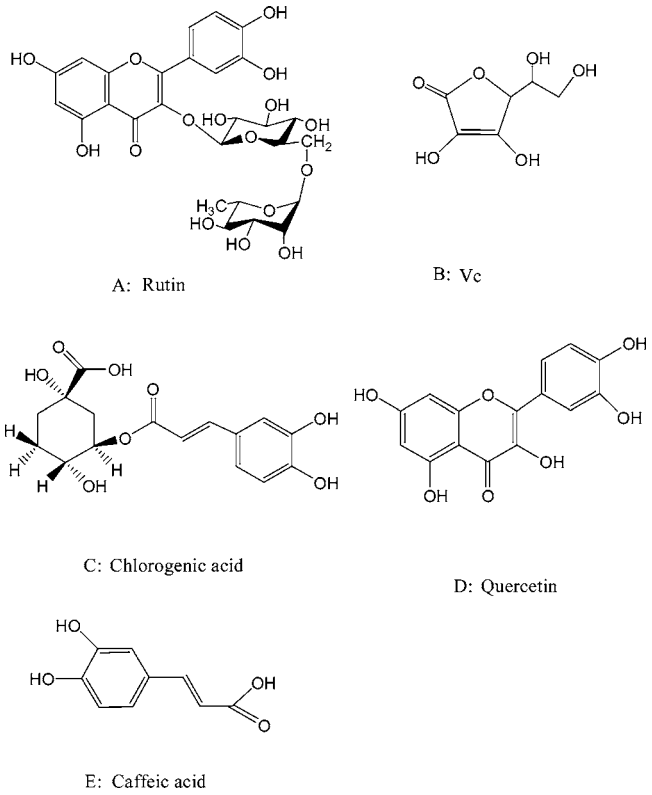

Figure 1. Molecular structures of (**A**) rutin, (**B**) Vc, (**C**) chlorogenic acid, (**D**) quercetin, and (**E**) caffeic acid.

the ends of the capillary. The inlet end of the capillary was held at a positive potential, and the outlet end was maintained at ground. A 75 cm length of 25 μm i.d. fused-silica capillary (Polymicro Technologies, Phoenix, AZ) was used for the separation. All samples were injected electrokinetically, applying 18 kV for 6 s.

The design of electrochemical detector was based on the end-column approach in which the working electrode was simply placed at the outlet of the separation capillary and detection was carried out in the same solution reservoir that contains the grounding electrode for the CE instrument. A carbon-disk electrode with 300 μm diameter was employed as the working electrode. Before use, the surface of the carbon-disk electrode was polished with emery sand paper, sonicated in deionized water, and then positioned carefully opposite the capillary outlet with the aid of an Oriel Corp. (Stratford, CT) model 14901 micropositioner. A three-electrode cell system consisting of a carbon-disk working electrode, a platinum auxiliary electrode, and a SCE (saturated calomel electrode) reference electrode was used in combination with a BAS LC-4C amperometric detector (Biochemical System, West Lafayette, IN). The electropherograms were recorded using a chart recorder (Shanghai Dahua Instrument Factory, China).

Reagents and Solutions. Vc, chlorogenic acid, caffeic acid, and rutin were all purchased from Sigma (St. Louis, MO), and quercetin was obtained from Shanghai Reagent Factory (Shanghai, China); the purities of the standards were 99, 95, 99, 98, and 95% for Vc, chlorogenic acid, caffeic acid, quercetin, and rutin, respectively, and all were used as received.

Stock solutions of five analytes (1.00×10^{-3} g mL^{-1} each) were prepared in anhydrous ethanol (AR grade), stored in the dark at 4 °C, and diluted to the desired concentration with the running buffer (60 mmol L^{-1} borate buffer, pH 9.0). Before use, all solutions were filtered through 0.22 μm nylon filters.

Sample Preparation. Fresh sweet potato samples from the supermarket were peeled with a peeling machine, and the thickness of the peel removed in that way was ~1 mm. Part of the fresh samples (peel and pulp) was boiled. After it was dried, about 1.5 g of sweet potato pulp or 0.8 g of sweet potato peel was ground into powder in a mortar and accurately weighed. Each weighed sample was extracted with 10 mL of anhydrous ethanol (AR grade) and water (4:1) for 1 h in an ultrasonic bath. Then, each of the samples was centrifuged by a desk centrifuge first and then filtered through filter paper and a 0.22 μm syringe filter in turn. After filtration, the solutions were injected directly to the CE-ED system for analysis. Before use, all sample solutions were stored at 4 °C in the dark.

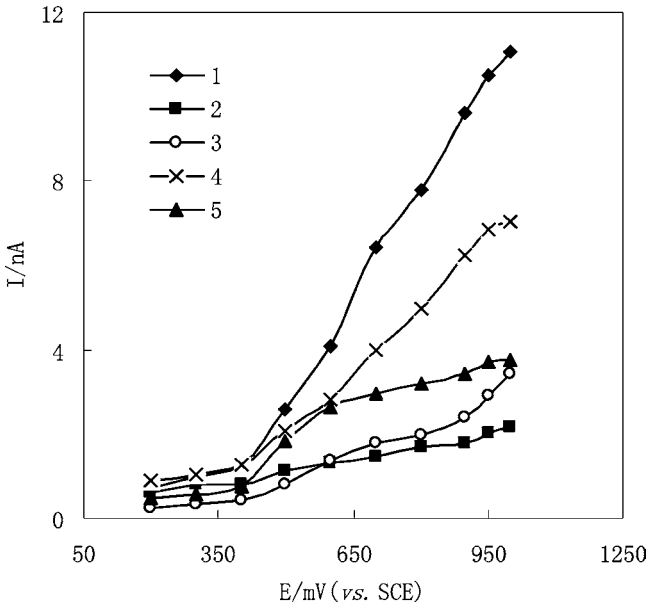

Figure 2. Hydrodynamic voltammograms (HDVs) of rutin (1), Vc (2), chlorogenic acid (3), quercetin (4), and caffeic acid (5) in CE-ED. Fused-silica capillary: 25 μm i.d. × 75 cm; working electrode, 300 μm diameter carbon disk electrode; running buffer, 60 mmol L^{-1} (pH 9.0); separation voltage, 18 kV; injection time, 6 s (at 18 kV); and concentrations of five analytes, 1.0×10^{-5} g mL^{-1} each.

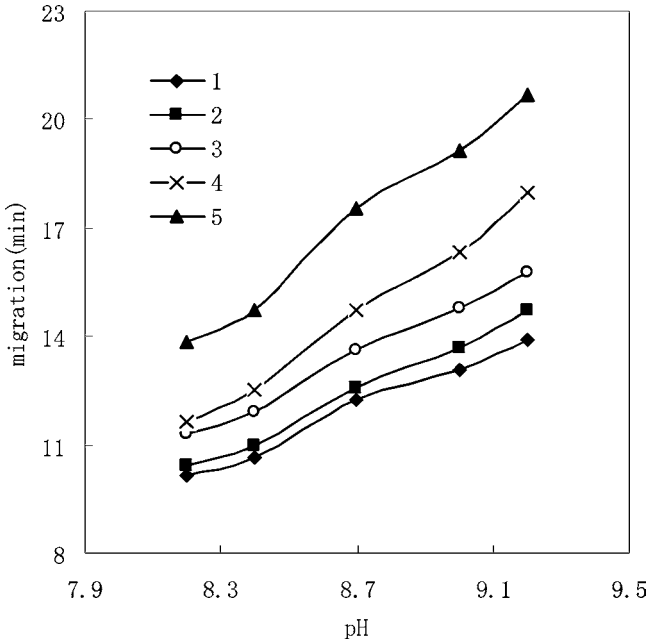

Figure 3. Effects of the running buffer pH on the migration time of the analytes. The working electrode potential is 0.95 V (vs SCE); other conditions are the same as in **Figure 2**.

RESULTS AND DISCUSSION

Effect of the Potential Applied to the Working Electrode. Because the phenolic hydroxy groups of the analytes can be readily oxidized electrochemically at a relatively moderate potential, electrochemical detection was based on this feature.

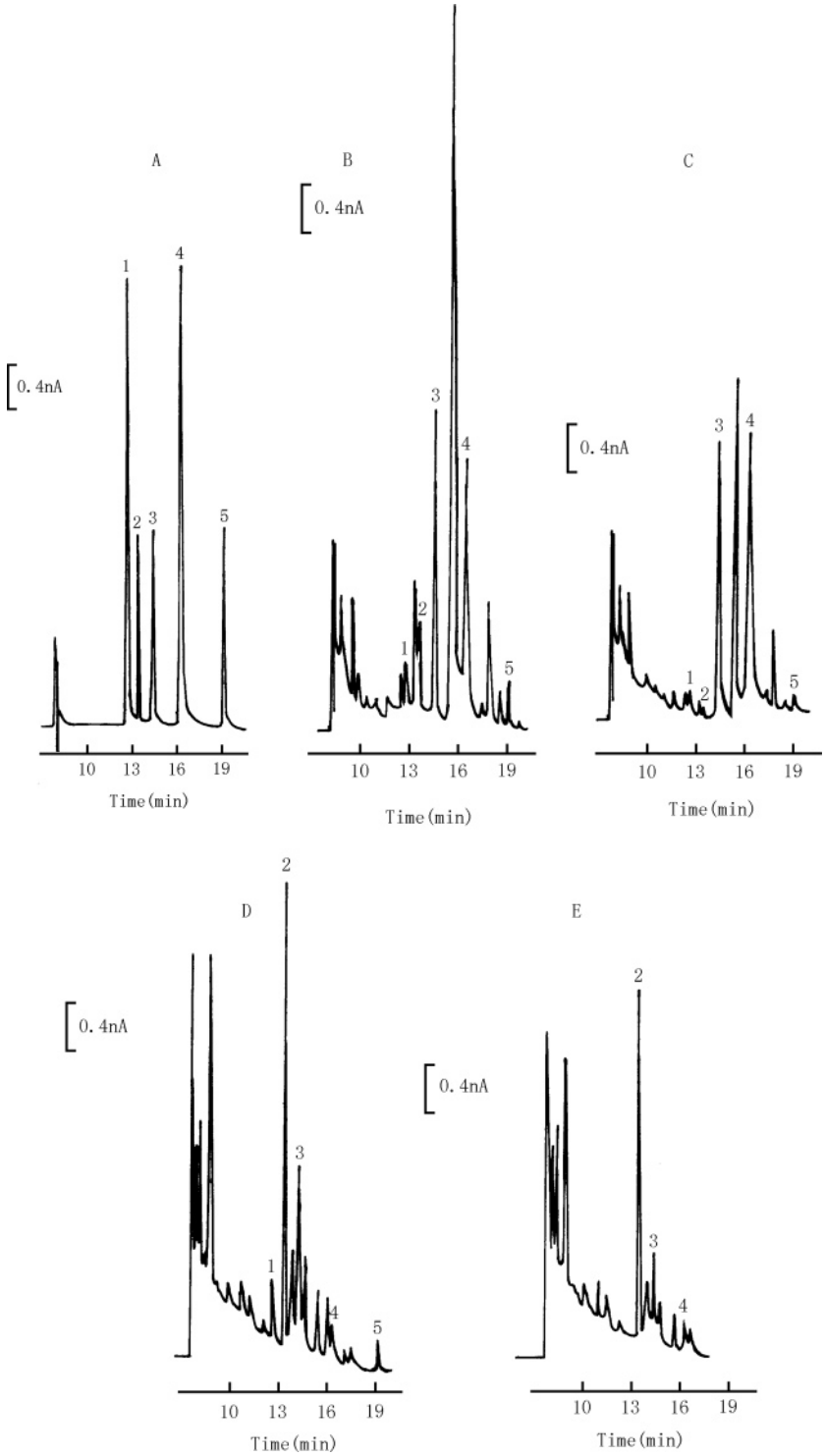

Figure 4. Electropherograms of standard solution (**A**), fresh sweet potato peel (**B**), fresh sweet potato pulp (**C**), cooked sweet potato peel (**D**), and cooked sweet potato pulp (**E**). Peak identification: 1, rutin; 2, Vc; 3, chlorogenic acid; 4, quercetin; and 5, caffeic acid. Experiment conditions are the same as in **Figure 3**; concentrations of five analytes, 1.0×10^{-5} g mL^{-1} each.

In electrochemical detection, the potential applied to the working electrode directly affects the sensitivity, detection limit, and stability of this method. Therefore, hydrodynamic voltammetry was investigated to obtain optimum detection. As shown in **Figure 2**, the peak current of Vc does not have obvious change, while the peak current of other four analytes increases rapidly when the applied potential exceeds +400 mV (vs SCE). However, when the applied potential is greater than +950 mV (vs SCE), although the peak current of the analytes still increases, both the baseline noise and the background current increase substantially, which is a big disadvantage for sensitive and stable detection. Therefore, the potential applied to the working electrode is maintained at +950 mV (vs SCE), where the background current is not too high and the signal-to-noise (S/N = 3) ratio is the highest.

Effects of the pH and Concentration of the Buffer. The acidity of the running buffer affects the ζ-potential (ζ), the electroosmotic flow (EOF), as well as the migration time and the separation of the analytes (*15*). The effect of the running buffer pH on the migration time of the analytes was investigated

in the pH range of 8.2−9.2. As shown in **Figure 3**, the migration time of all analytes increases with the increasing pH value. When the pH is below 9.0, rutin, Vc, chlorogenic acid, and quercetin cannot be separated. At pH 9.0−9.2, the five analytes can be well-separated. However, a higher pH value results in longer analysis times, and the analytes are more susceptible to oxidation. Therefore, pH 9.0 was selected as the optimum pH value for this work.

Besides the pH value, the running buffer concentration is also an important parameter. The effect of the running buffer concentration on migration time was also studied, and the optimum running buffer concentration found is 60 mmol L^{-1} (pH 9.0).

Effects of Separation Voltage and Injection Time. For a given capillary length, the separation voltage determines the electric field strength, which affects both the EOF and the migration velocity of charged species, which in turn determines the migration time of the analytes. As expected, a higher separation voltage gives a shorter migration time for all analytes. However, when the separation voltage exceeds 20 kV, the baseline noise becomes larger. Therefore, the optimum separation voltage selected is 18 kV, at which good separation can be obtained for all analytes within 20 min.

The injection time determining the amount of sampling affects both peak current and peak shape. The effect of injection time on peak current was studied by varying the injection time from 2 to 10 s at 18 kV. It was found that the peak current increases with increasing sampling time. When the injection time is longer than 6 s, the peak current nearly levels off and peak broadening becomes more severe. In this experiment, 6 s (18 kV) is selected as the optimum injection time.

Through the experiments above, the optimum conditions for Vc, chlorogenic acid, caffeic acid, quercetin, and rutin have been decided. The applied potential to the working electrode was selected at +950 mV (vs SCE), and the injection time was 6 s (18 kV), and all five analytes can be well-separated within 20 min at the separation voltage of 18 kV in a 60 mmol L^{-1} Borax running buffer (pH 9.0). The typical electropherogram for a standard solution of the five analytes is shown in **Figure 4A**.

Reproducibility, **Linearity**, **Detection Limits, and Recovery.** The reproducibility of the peak current is estimated by making repetitive injections of a standard mixture solution (2.0×10^{-5} g mL^{-1} for each analyte) under the selected optimum conditions ($n = 7$). The relative standard derivations (RSDs) of the peak current are 0.78, 3.25, 1.78, 1.41, and 1.59% for rutin, Vc, chlorogenic acid, quercetin, and caffeic acid, respectively ($n = 7$). The high reproducibility indicates that this method is accurate and reliable.

To determine the linearity of Vc, chlorogenic acid, caffeic acid, quercetin, and rutin, a series of standard solutions from 2.0×10^{-6} to 1.0×10^{-4} g mL^{-1} were tested. The results of regression analysis on calibration curves and detection limits are presented in **Table 1**. Determination limits are 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 2 orders of magnitude for all investigated compounds with the detection limits of 7.14×10^{-7} to 2.88×10^{-7} g mL^{-1}, and the correlation coefficients are in the range of 0.9992−0.9999.

To further evaluate the precision and accuracy of the method, the recovery experiments under the optimum conditions are also conducted with the fresh sweet potato sample ($n = 3$). Accurate amounts of standard were added to the actual samples, and the recovery values can be obtained by comparing the increase of the peak height before and after the addition of standards. The average recoveries are listed in **Table 2**.

Table 1. Regression Equations and Detection Limits[a]

compound	regression equation[b]	correlation coefficient	linear range (g mL^{-1})	detection limit (10^{-7}g mL^{-1})
rutin	$y = 1.69 \times 10^5 x + 0.06$	0.9999	2×10^{-6} to 1×10^{-4}	3.00
Vc	$y = 1.79 \times 10^5 x + 0.36$	0.9996	2×10^{-6} to 1×10^{-4}	7.14
chlorogenic acid	$y = 7.22 \times 10^4 x + 0.20$	0.9995	2×10^{-6} to 1×10^{-4}	6.66
quercetin	$y = 1.92 \times 10^5 x + 0.07$	0.9999	2×10^{-6} to 1×10^{-4}	2.88
caffeic acid	$y = 1.08 \times 10^5 x + 0.20$	0.9992	2×10^{-6} to 1×10^{-4}	6.67

[a] CE-ED conditions are the same as **Figure 3**. [b] In the regression equation, the x value is the concentration of analytes (g mL^{-1}), and the y value is the peak current (nA).

Table 2. Determination Results of Recovery in This Method with Fresh Sweet Potato Peel Sample ($n = 3$)[a]

ingredient	original amount (g mL^{-1})	added amount (g mL^{-1})	found (g mL^{-1})	recovery (%)	RSD (%)
rutin	0.80×10^{-6}	5.0×10^{-6}	6.10×10^{-6}	105.2	3.4
Vc	1.67×10^{-6}	5.0×10^{-6}	6.55×10^{-6}	98.2	4.5
chlorogenic acid	14.1×10^{-6}	5.0×10^{-6}	19.2×10^{-6}	100.7	2.3
quercetin	4.33×10^{-6}	5.0×10^{-6}	9.25×10^{-6}	99.1	3.4
caffeic acid	2.00×10^{-6}	5.0×10^{-6}	7.15×10^{-6}	102.1	4.1

[a] CE-ED conditions are the same as **Figure 3**.

Table 3. Assay Results for Two Kinds of Sweet Potato Pulp Samples ($n = 3$)[a]

sample	ingredients	found (μg g^{-1})	RSD (%)
fresh sweet potato	rutin	37.0	3.8
	Vc	574.1	4.0
	chlorogenic acid	164.7	3.3
	quercetin	11.4	3.6
	caffeic acid	6.58	3.1
cooked sweet potato	rutin	NF[b]	
	Vc	414.6	4.0
	chlorogenic acid	74.6	3.3
	quercetin	18.9	3.6
	caffeic acid	NF	

[a] CE-ED conditions are the same as **Figure 3**. [b] NF, point not found.

Table 4. Assay Results for Two Kinds of Sweet Potato Peel Samples ($n = 3$)[a]

sample	ingredients	found (μg g^{-1})	RSD (%)
fresh sweet potato	rutin	99.5	4.1
	Vc	207.9	3.5
	chlorogenic acid	1760.1	3.1
	quercetin	539.9	3.5
	caffeic acid	248.7	2.7
cooked sweet potato	rutin	23.1	4.1
	Vc	39.2	3.5
	chlorogenic acid	1755.4	3.1
	quercetin	725.5	3.5
	caffeic acid	69.0	2.7

[a] CE-ED conditions are the same as **Figure 3**.

Sample Analysis and Discussion. Under the optimum conditions, the proposed procedure was applied for the determination of active ingredients in crop production (fresh sweet potato and cooked sweet potato) samples. Typical electropherograms of two sweet potato samples are shown in **Figure 4B−E**, respectively. By the migration time of analytes as compared with the electropherogram of the standard mixture solution (**Figure 4A**), the active ingredients, namely, rutin (1), Vc (2), chlorogenic acid (3), quercetin (4), and caffeic acid (5) in these

four sweet potato samples can be identified and determined. The assay results are listed in **Tables 3** and **4**.

As we can see from **Figure 4** and **Tables 3** and **4**, significant differences between these four electropherograms can be found. On the whole, the content of these active ingredients in the pulp is lower than that in the peel, and the contents are the same as in the cooked sample, which is lower than that in the fresh sample except for quercetin.

The above assay results indicate that CE-ED is accurate, sensitive, and a useful quantitative method for the quantitation of the pharmacologically active ingredients in sweet potato, providing an alternative and convenient method for analysis the content diversity of target ingredients in different part as well as under different cooking conditions.

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