Rapid determination of ginsenoside Rg1, Re and Rb1 in ginseng samples by capillary electrophoresis

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A rapid and practical capillary electrophoresis (CE) method was developed for the determination of ginsenoside Rg1, Re and Rb1 in ginseng samples. Rg1, Re and Rb1 were extracted by ultrasonication with water-saturated n-butanol. The CE method was optimized with a running buffer of 20 mM borate in 30% MeOH (pH 9.67), and an applied separation voltage of +18 kV over a capillary of 50 μm i.d. × 50 cm (41.5 cm to the detector window), which gave a baseline separation of Rg1, Re and Rb1 within *ca.* 5 min. Under the detection at 203 nm, the method gave limits of quantification (S/N = 10) at about 5.2–7.3 μg ml^{-1} for Rg1, Re and Rb1, whereas the overall recoveries were larger than 83.0%. The proposed method has been successfully applied to measure three different kinds of ginseng samples (10 real samples) and the contents of Rg1, Re and Rb1 in actual samples were obtained and evaluated.

1.   Introduction

Ginseng, King of Herbs, has been highly valued for its mystical properties and has a 5000-year long history as a traditional herbal medicine originating from ancient China.[1] Given a scientific definition and classification, ginseng plant belongs to the Araliaceae family. Most of the studies of ginseng samples, including commercial pharmaceutical preparations, are mainly from five common species: *Panax ginseng* C.A. Meyer (Asian or Korea ginseng), *Panax quinquefolius* L. (American ginseng), *Panax notoginseng* (Burkill) F.H. Chen (Tianqi or Sanqi, Yunnan Province, China) and *Panax japonicus* C.A. Meyer (Chikusetsu, Japanese ginseng).[2,3]

Ginseng extracts have wide pharmacological properties, such as antifatigue, antidiabetic, vasodilating and antidepressant, and are effective in stimulating the memory as well as in the prevention of cancer and the ageing process. Pharmacological effects of ginseng are attributed to ginsenosides which are widely regarded as the active components and are found in most ginseng products.[4]

The basic structures of ginsenosides contain triterpene aglycones and various sugar moieties.[5] To date, at least 30 ginsenosides have been identified, but ginsenosides Rf, Rg1, Rd, Re, Rc, Rb2, and Rb1 are the most abundant.[6] In the Chinese Pharmacopoeia, the content of Rg1, Re and Rb1 are compulsory to provide. Therefore, in this work, we mainly investigated these three ginsenosides (Rg1, Re and Rb1, Fig. 1).

Because ginsenosides are valuable components with pharmacological effects, the method development for their determinations is very necessary. To date, several analytical methods have been reported and reviewed.[5] These methods were accomplished by means of thin layer chromatography (TLC),[7,8] gas chromatography (GC),[9–11] high performance liquid chromatography (HPLC) with either ultraviolet (UV),[12–18] fluorescence,[19] mass

spectrometry (MS),[20,21] or MS-MS detection.[22–25] Among these methods, HPLC is the most useful tool for the separation and analysis of ginsenosides.[5] This is due to the several advantageous features of HPLC: sensitivity, rapidity, consistent response and broad applicability to non-volatile polar compounds, and the compatibility to a variety of detection techniques. However, the HPLC method requires expensive columns and equipment, and consumes large amounts of toxic organic solvents, such as methanol and acetonitrile. Capillary electrophoresis (CE) is a very active research area in separation science since this technique often provides higher resolution power, shorter analysis time and lower operating cost than HPLC. It has been frequently reported in the analysis of traditional Chinese medicines.[26,27] It is
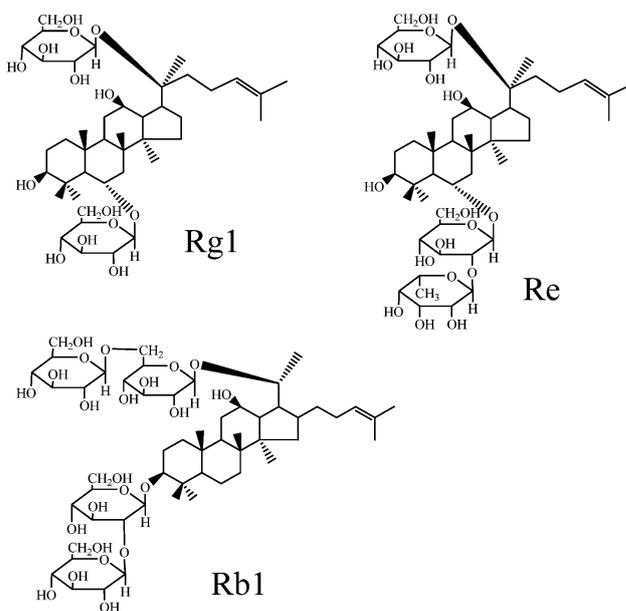

Fig. 1 Chemical structures of ginsenoside Rg1, Re and Rb1.

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fair to say that the analysis of ginseng extracts and ginsenosides by CE remains at a moderately low level.[28,29]

This study developed a rapid, practical and reliable CE method to analyze ginsenoside Rg1, Re and Rb1 in ginseng roots and preparations. The main works includes: (i) to develop a practical extraction prior to the analysis of Rg1, Re and Rb1; (ii) to develop a practical CE method for the measurement of Rg1, Re and Rb1, and (iii) to determine Rg1, Re and Rb1 in actual ginseng samples using the developed method.

2. Experimental

2.1 Chemicals and solutions

Unless specified otherwise, all chemicals and solvents were of analytical reagent grade and purchased from Beijing Chemical Plant (Beijing, China). Acetonitrile (MeCN) and methanol (MeOH) were of HPLC grade and purchased from Shanghai Luzhong Reagent Plant (Shanghai, China). Ginsenoside Rg1, Rb1 and Re (99.0%) were purchased from China Pharmaceutical & Biological Products Control Institute (Beijing, China). Tris-(hydroxymethyl)aminomethane (Tris) was of biological reagent grade and purchased from China Medication Reagent Plant of Shanghai (Shanghai, China). Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

Stock standard solution of Rg1, Rb1 and Re was prepared by dissolving each standard in MeOH–water (1 : 1, v/v) in order to give a concentration of 500 μg mL^{-1}. Quantification of samples was made using calibration curves of Rg1, Rb1 and Re at a final concentration of 5, 10, 25, 50, 100 and 150 μg mL^{-1} in MeOH–water (1 : 1). Each determination was performed in triplicate.

2.2 CE procedures

CE experiments were performed using the HP³DCE system (Agilent, Germany) with an on-column diode-array detector, an automatic sampler and a power supply ranging from -30 kV to $+30$ kV. The pH value was measured using a pH meter (Shanghai Weiye Factory, Shanghai, China). The separations were carried out with a bare fused-silica capillary (Yongnian Photo Fiber Factory, Hebei Province, China), with an inner diameter of 50 μm and a length of 50 cm (41.5 cm to the detector window). The initial new capillary was rinsed with 1.0 M NaOH for 20 min, water for 5 min and the running buffer solution for 20 min. Prior to each injection, the capillary was rinsed with the running buffer solution for 2 min. The capillary was thermostated at 25 °C, unless stated otherwise. A constant voltage of +18 kV was applied during electrophoresis. The sample injection was achieved using a dynamic pressure of 50 mbar for 5 s. The detection wavelength was set at 203 nm.

2.3 Sample preparation

The procedure of sample preparation was carried out according to the literature.[4] For the ginseng roots, capsules and tablets, twenty grams of the samples were chopped and crushed to produce a powder at 40 mesh. A portion of the sample (about 10.0 g) was accurately weighed and dissolved in 50 mL chloroform. After refluxing extraction in a Soxhlet Extractor for 3 h, the chloroform extract was discarded, and the residue together with the filter paper was returned to a 100-mL conical flask, and dissolved in 50 mL water-saturated n-butanol. It was then ultrasonicated for 30 min. The 25-mL filtrate was accurately collected and transferred to a 100-mL flask with 5-mL graduated bottom. It was evaporated to near dryness under a vacuum. The residue was accurately dissolved with 5 mL methanol under ultrasonication. The 1-mL solution was filtered with a 0.22 μm membrane for CE analysis. It is noteworthy to mention here that the prepared sample solutions should be diluted if required.

2.4 Recovery

The efficiency for sample pretreatment was validated by a recovery investigation. In the initial step of the sample preparation, each standard of Rg1, Rb1 and Re (5.0 and 20 mg) was added to 10 g of the powdered ginseng samples (40 mesh). They were then treated as described in section 2.3 (sample preparation). Finally, the prepared sample solution (1 mL) was analyzed by the developed CE method, and the recovery was determined.

3. Results and discussion

3.1 Choice of sample treatment methods

Ultrasonication extraction (UE) and microwave extraction are often used to extract Rg1, Rb1 and Re.[30,31] Both extractions provide high extraction efficiency. In this work, UE was selected to extract Rg1, Rb1 and Re. At the same time, different extraction solvents (water, methanol–water, acetonitrile–water, ethanol–water and n-butanol–water) were investigated. The results showed that recoveries for Rg1, Rb1 and Re were about 80–85% using water, methanol–water (5 : 5), acetonitrile–water (5 : 5) and ethanol–water (5 : 5) as the extraction solvent, and over 95% using water-saturated n-butanol. Finally water-saturated n-butanol was selected to extract Rg1, Rb1 and Re from the ginseng samples. However, the matrices of the ginseng samples were complicated for direct analysis of Rg1, Rb1 and Re by the CE method. Therefore, before UE extraction, chloroform extraction by Soxhlet was performed to remove the lipids and pigments in the samples, which was very necessary to clean up the samples.
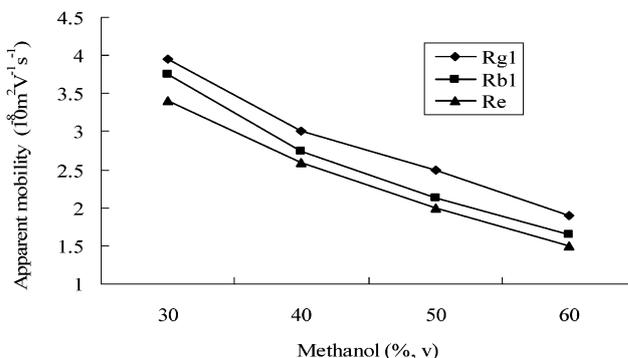
Fig. 2 Effect of methanol percentage of the buffer solvent on apparent mobility of the ginsenosides. Conditions: electrophoretic solution, 20 mM borate in MeOH–H$_2$O (pH 9.67); applied voltage, +18 kV; capillary temperature, 25 °C; sample injection, 50 mbar × 5 s; detection wavelength, 203 nm.
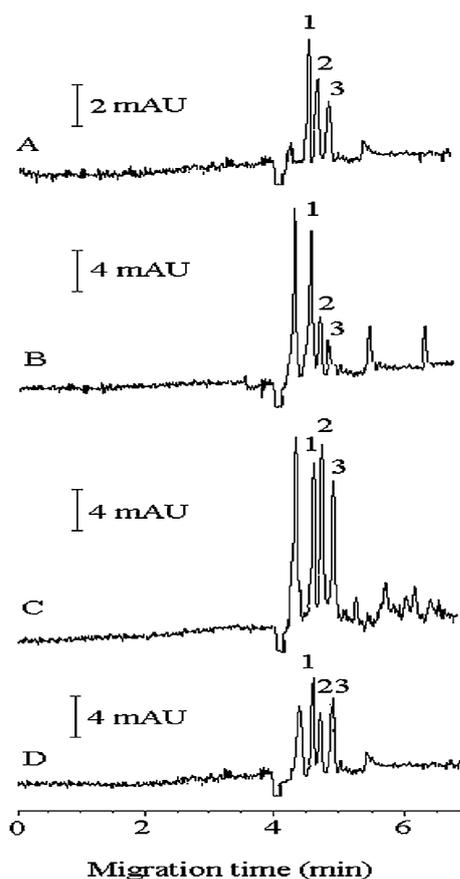
Fig. 3 Typical electropherograms of (A) standard ginsenosides, (B) ginseng root No.1, (C) capsule No.3 and (D) tablet No.1. Conditions: electrophoretic solution, 20 mM borate in MeOH–H$_2$O (30 : 70, v/v) (pH 9.67); others as Fig. 2. Concentration of standard ginsenosides in A: Rg1, 50 μg mL^{-1}; Rb1, 50 μg mL^{-1}; Re, 50 μg mL^{-1}. Peaks: 1, Ginsenoside Rg1; 2, Ginsenoside Rb1; 3, Ginsenoside Re.

3.2 Optimized CE separation

In the CZE method, the solvent (such as organic solvent) and the electrolyte are two important parameters for modulating the mobility of electroosmotic flow (μ_{eof}) and the effective mobility (μ_{ep}), as well as selectivity and resolution.[32–34] MeOH, EtOH and MeCN are widely used as the organic media in CE, and borate and organic amines are commonly used as special electrolytes and buffering groups in CE. Thus, in this section the CE separation of ginsenosides was investigated using borate as the buffer system and MeOH as the organic solvent.

In fact, both Tris and NH$_4$Ac electrolytes are also well evaluated in the separation of ginsenosides. The results display that borate electrolyte can provide a more preferable separation than both Tris and NH$_4$Ac. This is attributed to the complexing effect of borate with ginsenosides.

The solvent effect of the MeOH–H$_2$O ratios on migration behaviors of ginsenosides is evaluated. The results display that with the increase of MeOH–H$_2$O ratio the apparent mobilities (Fig. 2) decreased, and resolutions were not obviously improved. For rapid separation, the ratio of MeOH–H$_2$O was selected at 30 : 70, while the appropriate migration times for ginsenosides were achieved with near baseline separation.

The pH value of the buffer (20 mM borate/NaOH) was altered by changing the amount of NaOH. The influence of pH value on the migration time was also significant. At the pH window of 8.5–11.0, the migration times of Rg1, Rb1 and Re could be obtained between 3.5 and 6.5 min. At pH 9.67, Rg1, Rb1 and Re can be separated with a resolution larger than 1.4 within 5 min. It is noteworthy that this CE method is much more rapid than the HPLC method (retention time >50 min).[15]

With the increase of the applied voltage, the migration time decreased, the joule heating increased, and the resolution possibly became poor. When the applied separation voltage was higher than +21 kV, the electrophoretic current was larger than 50 μA, the migration times of three ginsenosides were smaller than 4.0 min, and the resolution between Rg1 and Rb1 was less

Table 1 Recoveries ($n = 3$) of Rg1, Rb1 and Re by CE

Known conc.[a] (mg/10 g)			Added conc. (mg/10 g)			Detected conc. (mg/10 g)			Av. Recovery (%)			RSD (%)		
Rg1	Rb1	Re	Rg1	Rb1	Re	Rg1	Rb1	Re	Rg1	Rb1	Re	Rg1	Rb1	Re
Root 1:														
27.5	14.7	5.30	5	5	5	31.9	19.3	9.7	84.0	91.3	88.0	4.7	3.3	4.9
						31.5	19.4	9.5						
						31.7	19.1	9.6						
			20	20	20	45.2	32.2	24.2	86.0	87.6	91.3	3.2	2.0	3.1
						44.1	32.6	23.1						
						44.8	31.9	23.4						
Capsule 3:														
30.0	52.0	21.0	20	20	20	46.3	68.2	38.5	83.1	84.8	83.0	4.5	4.1	4.7
						47.5	69.6	37.2						
						46.1	69.1	37.1						
Tablet 3:														
15.0	33.0	13.0	20	20	20	32.6	51.0	29.5	86.3	85.5	85.5	2.0	5.2	3.1
						31.9	49.2	30.5						
						32.3	50.1	30.3						

[a] Determined by CE method.

Table 2 Content determination (%, $n = 3$) and RSD (%, $n = 3$) of ginsenoside Rg1, Rb1 and Re in different ginseng samples by both CE and HPLC[a] methods

Samples	Rg1		Rb1		Re	
	CE (RSD)	HPLC (RSD)	CE (RSD)	HPLC (RSD)	CE (RSD)	HPLC (RSD)
Root 1	0.28 (2.2)	0.27 (1.1)	0.15 (1.7)	0.15 (1.9)	0.05 (2.6)	0.05 (2.1)
Root 2	0.21 (2.5)	0.22 (2.0)	0.10 (2.3)	0.11 (1.3)	unfound	unfound
Root 3	0.30 (1.8)	0.29 (1.4)	0.22 (1.8)	0.23 (1.7)	0.11 (1.8)	0.10 (1.6)
Root 4	0.10 (2.8)	0.10 (2.1)	unfound	unfound	unfound	unfound
Capsule 1	0.22 (2.9)	0.21 (2.0)	0.46 (1.2)	0.47 (1.3)	0.27 (2.9)	0.26 (0.8)
Capsule 2	0.11 (2.3)	0.12 (1.5)	0.43 (2.0)	0.45 (1.9)	0.15 (1.8)	0.15 (1.2)
Capsule 3	0.30 (2.6)	0.32 (2.2)	0.52 (1.6)	0.50 (1.3)	0.21 (2.5)	0.22 (1.8)
Tablet 1	0.15 (1.9)	0.14 (1.2)	0.15 (2.2)	0.15 (2.1)	0.17 (1.9)	0.17 (1.2)
Tablet 2	0.17 (1.5)	0.17 (1.8)	0.24 (2.8)	0.23 (2.2)	0.33 (2.1)	0.34 (1.9)
Tablet 3	0.15 (2.4)	0.16 (2.1)	0.33 (2.1)	0.34 (1.8)	0.13 (2.6)	0.12 (2.2)

[a] Separation column, Agilent 250*4.0 mm i.d. Hypersil ODS; Mobile phase, binary gradient elution system consisted of water (A) and MeCN (B) and separation was achieved using the following gradient: 0–30 min, 20% B; 30–60 min, 20–45% B; 60–78 min, 45–75% B; 78–80 min, 75–100% B. The flow-rate, was 1 ml min^{-1}; the injection volume, 20 μl; UV detection wavelength, 203 nm.

than 1.05. When the applied separation voltage was less than +15 kV, the migration times of three ginsenosides were longer than 8 min. Thus, taking the resolution, migration time and the peak shape into consideration, the optimal CE conditions were determined with MeOH–H$_2$O (30 : 70, v/v) as solvent medium, 20 mM borate (pH 9.67) as the buffer system and +18 kV (with electrophoretic current of 35 μA) as the separation voltage. A typical electropherogram (Fig. 3A) of standard ginsenosides was obtained under the optimal conditions. It is noteworthy that three ginsenosides can be completely separated from the matrices of the roots, tablets and capsules after pretreatment.

3.3 Methodology evaluation

Under the optimized CE conditions as used and described in Fig. 3, the linear ranges of the UV response at 203 nm were observed over the concentration range from 5.0 to 150 μg mL^{-1} for Rg1, Rb1 and Re. The regressions between peak area (Y) and concentration (X, μg mL^{-1}) yielded the following equations: for Rg1, $Y = 0.2389X + 0.4727$ ($n = 6$, $R^2 = 0.9892$); for Rb1, $Y = 0.1608X + 0.4344$ ($n = 6$, $R^2 = 0.9967$); and for Re, $Y = 0.2307X - 0.0837$ ($n = 6$, $R^2 = 0.9895$). The limits of quantification (LOQs, S/N = 10, a signal to noise ratio) were 5.20 μg mL^{-1} for Rg1, 7.30 μg mL^{-1} for Rb1 and 5.80 μg mL^{-1} for Re by calculating at a signal to noise ratio of 10 (S/N = 10). These LOQs allow Rg1, Rb1 and Re to be accurately detected in the ginseng samples at a lower content than 7.30 μg g^{-1} in the ginseng roots, capsules and tablets, respectively.

The repeatability and recovery of the CE method were determined. At 20 μg ml^{-1}, the RSDs of the peak area and migration time were 2.5% ($n = 3$) and 1.8% ($n = 3$) for Rg1, 3.2% ($n = 3$) and 2.6% ($n = 3$) for Rb1, and 2.2% ($n = 3$) and 1.9% ($n = 3$) for Re, respectively. The recovery of the method was examined by determining the fortified ginseng samples. The overall recoveries (as described in section 2.4) were obtained and are listed in Table 1. These data demonstrated the reliability of the CZE method.

3.4 Application

The contents of Rg1, Rb1 and Re in ginseng samples were determined by using the developed CE method. The

electropherograms for three kinds of samples were shown in Fig. 3B, 3C and 3D. The content results are given in Table 2. Peaks were identified by three means: (i) by comparing the migration times of the unknown peaks with those of the standards eluted with the same conditions; (ii) by comparing their UV spectra with standard ginsenosides at the same conditions; and (iii) by adding standard ginsenosides to the sample solutions to observe the peak height increasing. From Fig. 3, it can be seen that the ginsenosides were well resolved within a 1-min time window, and they can be separated from the interferences. The results in Table 2 show that the content results of Rg1, Rb1 and Re by both CE and HPLC (similar to that in ref. 15) methods are comparable. Meanwhile, we also find that the contents of Rg1, Rb1 and Re of the samples are very different from different producers. The products with less ginsenoside content do not meet the requirement of National Standards of China (Rb1 ≥ 0.2%, Rg1 + Re ≥ 0.3%),[4] which would lower their pharmacological effects and infringe consumers' benefits.

4. Conclusion

In summary, a rapid analytical method for ginsenoside Rg1, Rb1 and Re in ginseng samples was developed by CE coupling with a practical pretreatment. The method featured simpleness, sensitivity, rapidity and economy, and can be used for quality control of ginseng and its medical preparations. More importantly, it avoids using sophisticated apparatus, and can easily be conducted in laboratories with limited facilities such as Factory laboratories and monitoring centers in China.

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