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Determination of rutin, chlorogenic acid and quercetin in solidaginis by large volume sample stacking with polarity switching and acid barrage stacking

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The sensitivity of capillary electrophoretic separation of rutin, chlorogenic acid and quercetin was enhanced by combination use of large volume sample stacking with polarity switching (LVSSPS) and acid barrage stacking (ABS). Separating conditions, including the background electrolyte pH and concentration, sample injection and acid barrage were optimized. The optimum conditions were: a background electrolyte of 30 mM $Na_2B_2O_7$ of pH 9.25, hydrodynamic injection of the sample (60s, 5 psi), then applied voltage of -25 kV, and then hydrodynamic injecting of 0.15 mol/L HAc (18 s, 0.5 psi), and at last separation with 25 kV. Under these conditions, the three analytes could be separated with a sample-to-sample time of 14 min and detection limits from 9.0 to 12.5 ng/mL. When compared to a conventional hydrodynamic injection, the sensitivity was enhanced between 333 to 506 times and the method is 3.6-5.3 times more sensitive than LVSSPS. The applicability of the developed method was demonstrated by the detection of the analytes in aqueous extract of Solidaginis.

Capsule Summary: The sensitivity of capillary electrophoresis has been enhanced 333-506 times by large volume sample stacking with polarity switching (LVSSPS) and acid barrage stacking (ABS).

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INTRODUCTION

Solidaginis, also named "Yizhi Huanghua" in Chinese, is the dry herb of Solidago decurrens Lour, which has the functions of detoxification, dispelling wind and heat from the body and abating jaundice (Li et al., 2008) and has been widely used for the treatments of cold, sore throat, icteric hepatitis and tonsillitis. Rutin, chlorogenic acid and quercetin are three constituents of Solidaginis (JNMC, 1985; Li et al., 2008). Rutin has the functions of anti-inflammation and anti-virus. Quercetin has the functions of eliminating sputum, relieving cough, lowering blood pressure and blood fat. Chlorogenic acid has the functions of anti-bacteria, purging the liver and stopping bleeding. Accurate determination of them is very important for the quality control of Solidaginis.

Currently, the routine method for components analysis in Solidaginis is high performance liquid chromatography (HPLC) (PCCMH, 2010). However, the HPLC has the shortcomings of low efficiency and high consumption of samples and chemicals. Capillary electrophoresis has the advantages of high efficiency, high resolution, fastness and low cost and are being widely applied in pharmaceutical plant analysis (Magnuszewska and Krogulec, 2013; Viana et al., 2013). But due to its short optical

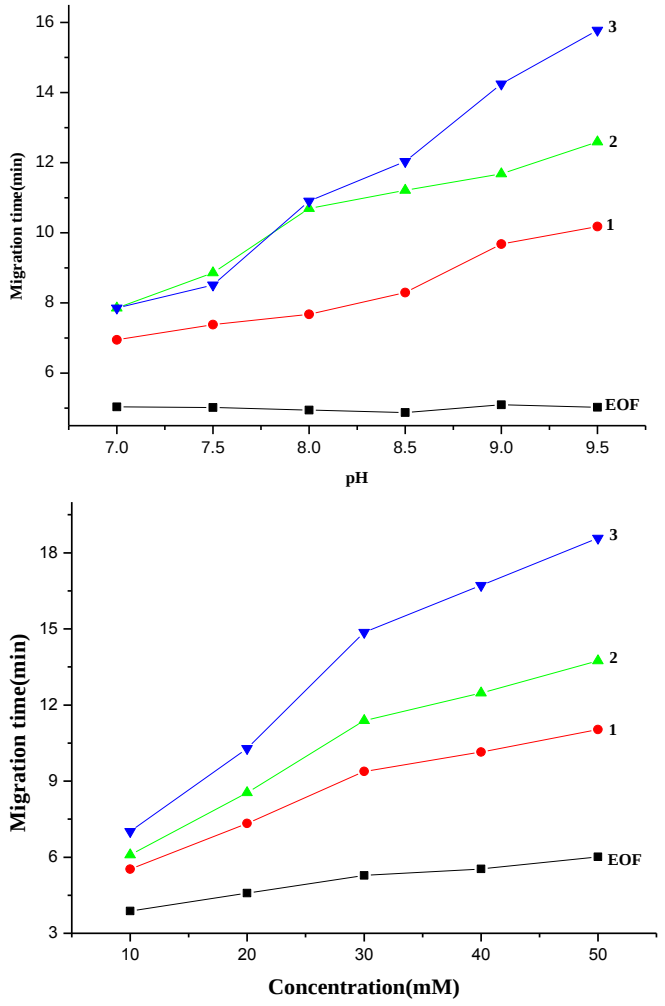

Fig. 1: Effects of pH (a-upper) and buffer concentration (b-lower) on the migration time of the analytes:peak desiganation: 1. rutin; 2. chlorogenic acid; 3. quercetin. Conditions: 60.2cm×50μm (50.2cm to detector) fused silica capillary, BGE 30 mM $Na_2B_2O_7$-NaH_2PO_4 (pH 7.0-9.5) for a and 10-50 mM $Na_2B_2O_7$-NaH_2PO_4 (pH 9.25) for b; Voltage, 25 kV; detection was at 254 nm. Sample: hydrodynamic injection of each analyte(50 μg/ml rutin, 76 μg/ml chlorogenic acid and 75 μg/ml quercetin) for 5 s at 0.5 psi.

path and small sample volume, its application has been limited by its low sensitivity. Capillary electrophoresis on-line preconcentration is a kind of simple and efficient method to address this problem. These methods are based on changes in analyte migration due to conductivity difference (Wang and Chen, 2009; Wang et al., 2013; Zhu et al., 2012), pH difference (Britz-Mckibbin et al., 2000; Han et al., 2006; Lu et al., 2013) or the association between the analytes and the surfactants (Li et al., 2006; Maciá et al., 2013; Quirino and Terabe, 1999). In recent years, great attention has been paid to the combination use of these methods to acquire additional sensitivity enhancements and sensitivity enhancement factors from 38 to 10,000 have been achieved (Britz-McKibbin et al., 2002; Cheng et al., 2010; Kim and Chung, 2002; Petr et al., 2008; Xu and Fan, 2012; Yang et al., 2014; Zhang et al., 2011; Zhu et al., 2010).

In this work, large volume sample stacking with polarity switching coupled to acid barrage stacking (LVSSPS-ABS) was applied to separate rutin, chlorogenic acid and quercetin in Solidaginis and sensitivity enhancement factors from 333 to 506 have been achieved and no such work has been reported in the literature.

EXPERIMENTAL

Apparatus and conditions

CE separations were carried out in a P/ACE MDQ CE system with a photodiode array detector for absorbance measurements at 254 nm (Beckman Coulter, Fullerton, CA, USA). Uncoated fused-silica capillaries purchased from Polymicro Technologies (Phoenix, AZ, USA) were used. The dimensions of the capillary were 60.2 cm × 50 μm i.d with an effective length of 50.0 cm. The temperature of the capillary was kept at 25°C. The CE system was interfaced with a computer and controlled using the Beckman 32 karat software (version 7.0).

New capillaries were flushed with 1 M sodium hydroxide for 30 min, Milli-Q water for 30 min, and BGE for 30 min. Each day capillaries were equilibrated by rinsing with 0.1 M sodium hydroxide for 10 min, water for 10 min, and BGE for 10 min. To maintain good repeatability, the capillary was flushed between each separation with water and the background electrolyte (BGE) for 2 min and 4 min, respectively.

The BGE solution was a concentration of 30mM $Na_2B_2O_7$ of pH 9.25. The buffer solutions were prepared freshly each day, sonicated for 5 min and filtered through a 0.45 μm membrane filter before use.

Chemicals

Rutin, chlorogenic acid and quercetin were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Other chemicals, unless otherwise stated, were all of analytical reagent grade. Water of 18.2 mΩ·cm was from a CascadaTM Lab Water System (Pall Life Science, China).

Stock standard solutions of 144 μg/mL, 228 μg/ml and 225 μg/ml were prepared in methanol for rutin, chlorogenic acid and quercetin, respetively. The working standards were prepared daily by mixing and diluting the standard solutions with Water solution containing 10%(v/v) methanol . All solutions were stored in dark containers at 4°C.

Large volume sample stacking with polarity switching and Acid barrage stacking and enhancement factor calculation

Analytes were loaded for 60 s at 5 psi, then the sample vial was replaced by the buffer vial and a reverse voltage of -25 kV was applied for 1.09 min (by monitoring the generated current until it returned to 95% of the original), and then 0.15 mol/L HAc was injected for 18 s at 0.8 psi. At last, the polarity was switched to the positive voltage and 25 kV was applied for separation. The

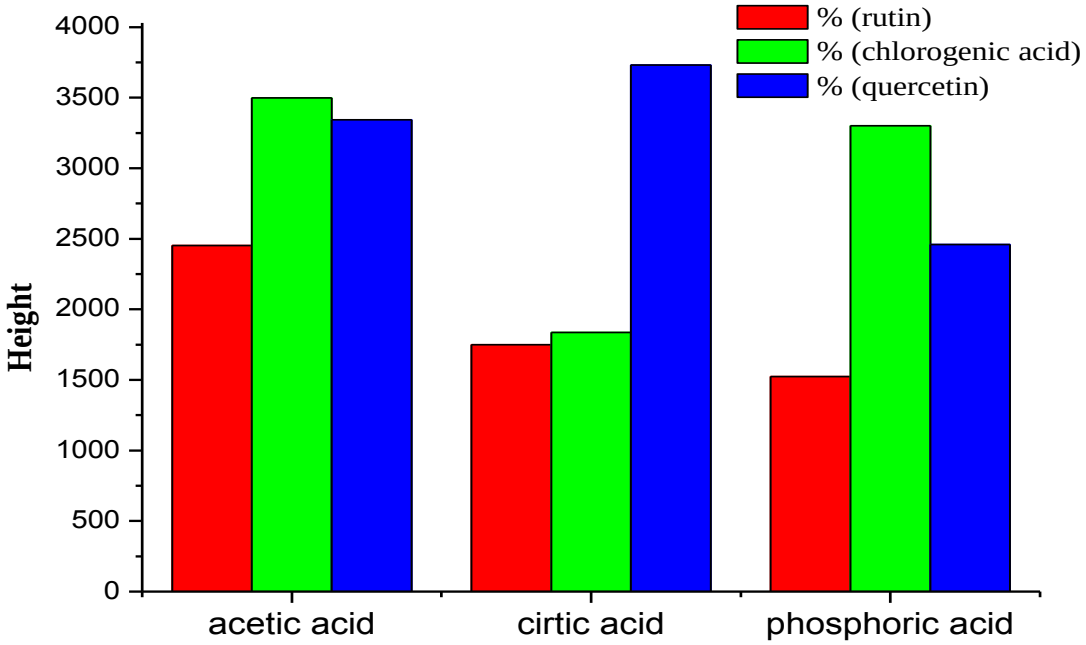

Fig. 2: Comparison of different acids as acid barrage on preconcentration effects. Conditions: 30mM $Na_2B_2O_7$ of pH 9.25, voltage 25 kV, detection at 254 nm, hydrodynamic injection of 0.5 μg/ml analytes at the pressure of 5 psi for 60 s, time of polarity switching 1.09 min. Followed by injection of 0.15 mM HAc, citric acid or phosphoric acid at 0.5 psi for 18 s. All other conditions were the same as Figure 1.

enhancement factor calculation was according to the literature (Quirino and Terabe, 1999).

Preparation of Solidaginis sample

Solidaginis samples were purchased from Anguo Traditional Chinese Medicine Supermarket (Anguo, Hebei China). A mass of 1.0 g of the sample was accurately weighed and 100 ml of deionized water was added to the sample. The sample was extracted with moderately boiling water for 1.5 h. After cooling, the sample was filtered with a medium speed filter paper (∅= 9 cm, Hangzhou Fuyang Special Paper Co., China). After collection of the filtrate, the residue was re-extracted using the same procedure with an additional 100 ml of deionized water. The filtrates were combined and made to 250 mL with deionized water. Before analysis, 1000 μL of the sample was mixed with 1000 μL methanol and made to 10 ml with water to to contain 10% (v/v) of methanol, degassed by sonication and filtered through a 0.45 μm membrane paper filter.

RESULTS AND DISCUSSION

Separation optimization

Borate, a common buffer system for phenolic substances separation in CE analysis, was adopted in this work. The pKa of chlorogenic acid is 3.95 and the pKas of rutin and quercetin are higher than 7.0 (Yu et al., 2008), so the effects of buffer pH were investigated in the pH 7.0-9.5 range at the concentration of 30 mM.

The results were shown in Fig. 1a, the migration time of the EOF first decreased with the increase of buffer pH and then increased when the buffer pH is higher than 8.5 and then decreased when the buffer pH was higher than 9.0. This can be explained by the combination effects of the buffer pH and ionic strength of the working buffer. At the same time, the migration time of the analytes increased with the increase of the buffer pH, which may be due to the increased ionization of the analytes. When pH was equal to or higher than 9.0, the three analytes were well separated. As sodium tetraborate buffers well at a pH of 9.25, which is close to the pH of 30 mM sodium tetraborate at room temperature, and there was adequate resolution between the three compounds at this pH, this was used for all further experiments.

The effect of BGE concentration was investigated next over the range of 10-50 mM range. As shown in Fig. 1b, the migration time of the analytes and EOF and the resolution between the analytes increased with the buffer concentration increase. When the buffer concentration is equal to or higher than 30 mM, the three analytes can be separated well. Based on the above results, 30 mM was selected as the optimum buffer concentration.

Large volume sample stacking with polarity switching

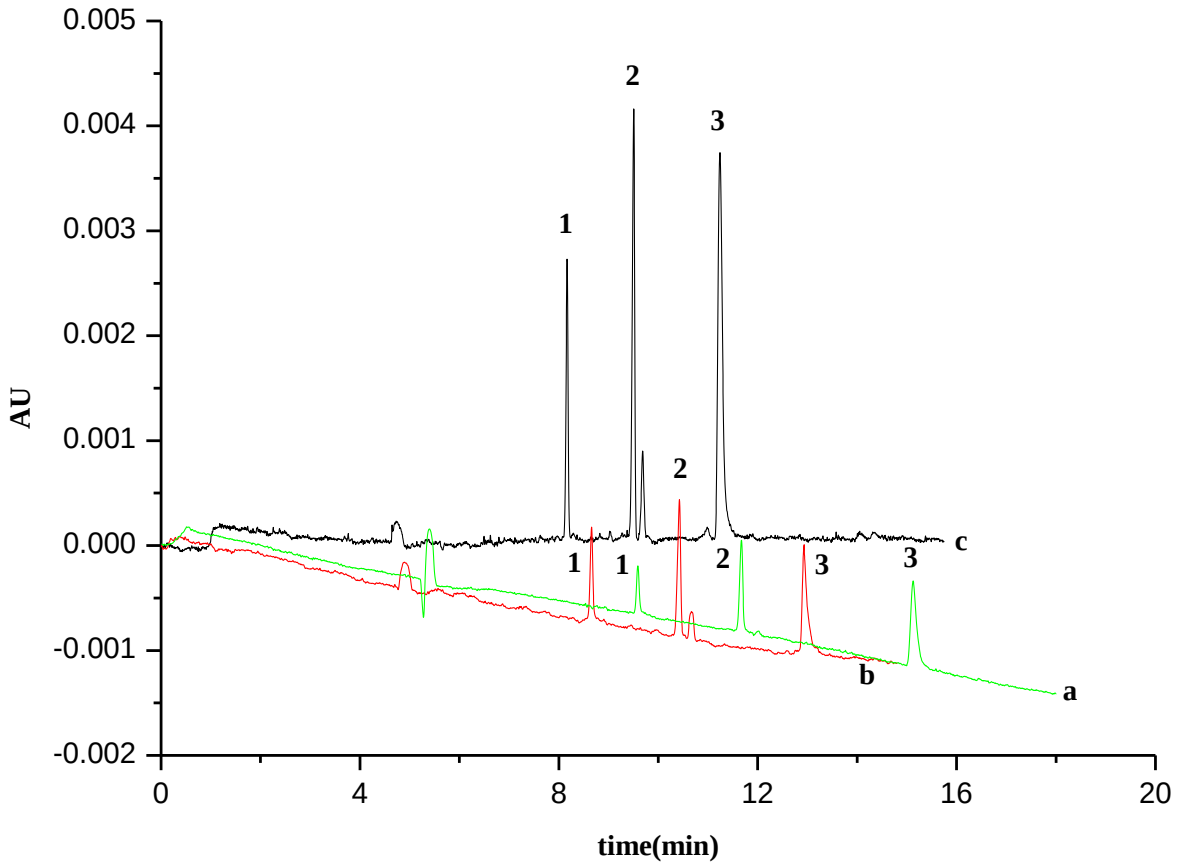

Fig. 3: Electropherograms of normal CE (a), LVSSPS-CE (b) and LVSSPS-ABS-CE (c) separations of the analytes. a. hydrodynamic injection of standards mixture(48 μg/mL rutin, 76 μg/mL chlorogenic acid and 75 μg/mL quercetin) at 0.5 psi for 5s; BGE 30 mM $Na_2B_2O_7$-NaH_2PO_4 (pH 9.25); Voltage, 25 kV; detection was at 254 nm. b. hydrodynamic injection of 1.0 μg/mL analytes at 4 psi for 60s, time of polarity switching 0.80 min. other conditions are the same as a; c. hydrodynamic injection of 0.5 μg/mL analytes at 5 psi for 60s, time of polarity switching 1.09 min. Followed by hydrodynamic injection of 0.15 M HAc (pH 3.0) at 0.5 psi for 18s, other conditions are the same as for Figure 1.

During LVSSPS, after hydrodynamically loading a large volume of low conductivity sample into the capillary, a negative voltage is applied to electroosmotically push the sample matrix plug out of the column and the negative species stacked at the boundary between the sample zone and the background electrolyte. Once the sample buffer is almost completely out of the capillary, the polarity is switched to normal for separation (Wang and Chen, 2009). The switching time and the sample volume were two very important parameters of LVSSPS. The switching time was selected by monitoring the driving current. Polarity was switched when the current reached 95% of its original value. The injection time was kept at 60 s, the effects of the sampling pressure was investigated in the 1-6 psi (comparable to 10-60% capillary volume) range. Results showed that the peak heights of the analytes increased with the increase of the sampling pressure in the 1-4 psi range. When the sampling pressure increased further, the increase of the analytes peak heights became not apparent. While, the peak areas of the analytes increased with the increase of the sampling pressure in the 1-6 psi range. At the same time, the peak shapes of the analytes became poor when the sampling pressure was higher than 4 psi. In consideration of sensitivity and separation efficiencies and peak shape, sampling time of 60 s at 4 psi (comparable to 40 percent of the capillary volume) was selected.

Under the optimised LVSSPS conditions, the three analytes can be separated in about 17 min and the electropherogram is shown in Fig.3b. As shown in table 1, the enhancement factors of the three analytes were in the 90 to 95 range. The repeatability was studied by 5 replicate injections of a 1.0 μg/mL standard mixture with the results shown in Table 1. The RSD values based on migration time and peak area were in the 0.87~1.91% and 3.97~5.18% range, respectively. Based on three times noise, the detection limits of the seven analytes were between 32.0 and 60.0 ng/mL.

Table 1: The limits of detection, enhancement factors and repeatability of LVSSPS and LVSSPS-ABS

Compounds	Normal	LVSSPS				LVSSPS-ABS			
	LOD[a] (μg/mL)	LOD (ng/mL)	EF	RSD[b](%) time	RSD[b](%) area	LOD (ng/mL)	EF	RSD[c](%) time	RSD[c](%) area
Rutin	5.40	60.0	90	1.15	5.18	12.5	432	1.19	5.29
Chlorogenic acid	4.30	45.0	95	1.91	3.97	8.5	506	1.37	4.74
Quercetin	3.00	32.0	93	0.87	4.64	9.0	333	1.91	4.16

[a] Based on three times noise, [b] Based on five determination of the standard mixture of 1.0 μg/mL, [c] Based on five determination of the standard mixture of 0.5 μg/mL

Table 2: The regression equations in LVSSPS-ABS

Compounds	Regression equation[a]	Correlation Coefficient	Linear range (μg/mL)
Rutin	Y=13210.12X+487.56	0.9991	0.05~2.0
Chlorogenic acid	Y=25132.37X+375.63	0.9994	0.03~1.0
Quercetin	Y= 40114.57X+357.28	0.9945	0.03~1.0

[a] In the regression equation, the X value is the concentration of analytes (μg/mL), the y value is the peak area

Table 3: Results of Yunnan Solidaginis sample analysis and the recovery (n = 5)

Compounds	Original (mg/g)	Added (mg/g)	Found (mg/g)	Recovery (%)
Rutin	1.75±0. 08	0.50	2.32±0. 09	114±18
Chlorogenic acid	0.44±0. 05	0.50	0.92±0. 08	96±16
Quercetin	0.08±0. 01	0.50	0.54±0. 09	92±18

Table 4: Results of Anhui Solidaginis sample analysis and the recovery (n = 5)

Compounds	Original (mg/g)	Added (mg/g)	Found (mg/g)	Recovery (%)
Rutin	0.63±0. 06	0.50	1.15±0. 06	104±12
Chlorogenic acid	0.29±0. 03	0.50	0.83±0. 05	108±10
Quercetin	0.05±0. 01	0.50	0.53±0. 03	96±6

Coupling large volume sample stacking with polarity switching and acid barrage stacking

Acid barrage stacking (ABS) exploits changes in ionization with pH to concentrate weak acids (Han et al., 2006; Lu et al., 2013) by injecting a high concentration of low pH solution after a large volume of sample has been injected. The anionic components in the sample migrate to the inlet and when they enter the acidic region they stop and stack on the interface between the sample zone and the acid zone during separation. In this work, ABS step was included after the sample matrix was pushed out by EOF to further enhance the sensitivity of the analysis. For ABS, the sample matrix, the composition of the acid barrage zone and the sample injection time can affect the stacking efficiency. The sample matrix was fixed in this work, so the types and concentration of barrage acid as well as the sampling pressure was optimized.

Acids able to maintain a low pH is in theory can be used as barrage acid. Keeping the barrage zone pH at 2.3, 150 mM of citric acid (pKa: 3.13, 4.76, 6.40), phosphoric acid (pKa: 2.12, 7.2, 12.36) and acetic acid (pKa=4.74) were tried to be used as barrage acid. The results are shown in Fig. 3 which clearly shows that acetic acid was the optimum. The concentration of acetic acid was then investigated from 60 to 180 mM. The peak heights first increased with the increase of the acetic acid concentration until 150 mM after which they decreased. 150 mM acetic acid was chosen as a compromise.

Hydrodynamic injection of the analytes for 60 s at 5 psi (50% of the length to the window), the barrage acid amount was investigated by hydrodynamic injection for 0-24 s at 0.5 psi. Results showed that as the barrage acid injection time increased, the peak height of the analytes increased, however when it was above 18 s, the resolution for the three analytes deteriorated due to insufficient capillary length for the separation. Furthermore, there was a impurity peak behind the peak of chlorogenic acid which partly overlapped with the neighboring peak ahead of it when the acid barrage injection time is higher than 18 s. In the later work, hydrodynamic injection of 0.15 M HAc for 18 s at 0.5 psi (1.8% of the length to the window) was adopted.

Keeping the injection time at 60s, the sampling pressure was investigated in the 1-6 psi range. The results showed that the peak area and peak height increased with the increase of the sample pressure in the 1-5 psi range. When the injection pressure is higher than 5 psi, the peak areas continue to increase while the peak height did not change significantly. So the samples were injected for 60 s at 5 psi (50% of the length to the window) in the later work.

Under the optimum conditions, an LVSSPS-ABS-CZE separation of the three analytes is shown in Fig. 3c. The sample-to-sample time was less than 14 min. The relative standard deviations based on five consecutive injection of the standard mixture were between 1.19-1.91 % for migration time and 4.16-5.29% for peak area. The detection limits and calibration parameters are summarized in Table 1 and Table 2. The sensitivity enhancement factors were between 333 and 506 with LODs of between 8.5-12.5 ng/mL. The method is 3.6-5.3 times more sensitive than LVSSPS. The further sensitivity enhancements after coupling ABS are much smaller than those reported in the literature (Han et al., 2006; Lu et al., 2013). This may be due to the low ionization of the analytes in the LVSSPS concentrated sample zone which was neutral and the short sample length after LVSSPS concentration.

Real sample analysis

The method was applied to determine rutin, chlorogenic acid and quercetin in Solidaginis real samples of two production areas. The recovery of the method was determined with the addition of the standards (comparable to 0.50 mg/g in the solid sample) in the real sample solution, with result from 92% to114 % (Table 3 and Table 4) which is adequate for their analysis demonstrating the potential applicability of this method to be used to analyze Solidaginis and other traditional Chinese medicine analysis.

CONCLUSION

A CE method for the determination of rutin, chlorogenic acid and quercetin by large volume sample stacking with polarity switching and acid barrage stacking was developed. The sensitivity was improved by 333-506-fold giving detection limits between 8.5 and 12.5 ng/ml. The method was shown to be suitable for the detection of rutin, chlorogenic acid and quercetin in Solidaginis and other Chinse medicine samples.

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