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Determination of puerarin in pharmaceutical and biological samples by capillary zone electrophoresis with UV detection

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ABSTRACT

A rapid and efficient capillary zone electrophoresis (CZE) method with ultraviolet detection has been successfully developed for the determination of puerarin in Xinkeshu capsules and biological samples. The optimal CZE conditions were determined to be 70 mM H_3BO_3–$Na_2B_4O_7$, pH 9.2, injection time 5 s, applied voltage 25 kV, working temperature 25 °C and detection wavelength 254 nm. Under these conditions, a linear range from 17.3 to 138 μg/mL with the correlation coefficient of 0.9998 and limit of detection of 34.6 ng/mL ($S/N = 3$) for puerarin were obtained. The relative standard deviation of the migration time and peak area of puerarin were 0.23–0.91, 0.43–1.62, 0.10–0.64 and 0.64–1.32% for intra-day and inter-day analyses, respectively. Satisfactory results were obtained for recovery and repeatability. The proposed method was successfully applied to analyze the puerarin content in Xinkeshu capsules and some biological samples with good results. It is suitable for applications in pharmaceutical industries for quality control and in clinical laboratories for therapeutic drug monitoring purposes.

1. Introduction

Xinkeshu capsule consisting of Hawthorn (*Crataegus pinnatifida Bge. Var. major N.*), Puerariae Root (*Pueraria lobata*), Salvia Miltrorrhiza (*Salvia miltiorrhiza Bunge*), Notoginseng (*Panax notoginseng*) and Common Vladimiria (*Radix Aucklandiae*) is applied to treat coronary angina pectoris of qi-blood stagnation caused by chest distress, angina, hypertension, swirl, headache, neck pain, cardiac arrhythmia, and hyperlipemia. Fig. 1 displays the structure of puerarin (4′,7-dihydroxy-β-D-glucosylisoflavone) which is present in large amounts in the active components of Puerariae Root, a commonly used Chinese herb. Puerarin is an effective component of Xinkeshu capsules and is widely used in treating coronary heart disease, cerebrovascular diseases, myocardial infarction hypertension, and diabetes [1,2]. The content of puerarin can be an important indicator for controlling the quality of Xinkeshu capsule. As such, it is essential to establish a simple and cost-effective method for quantitative analysis of puerarin in Xinkeshu capsules.

To date many analytical methods have been developed to determine puerarin including flow injection chemiluminescence (FIA-CL) [3,4], high-performance liquid chromatography (HPLC) [5–9], capillary electrophoresis (CE) with chemiluminescence detection (CLD) [10], CE with electrochemical detection (ECD) [11], non-aqueous CE (NACE) [12], micellar electrokinetic capillary chromatography (MEKC) [13,14], and near-infrared spectroscopy (NIRS) [15]. However, the FIA-CL method lacks separation capability and is not ideal for analyzing puerarin in complex pharmaceutical preparations. Although HPLC method possesses high accuracy, it has lower separation efficiency and requires longer analysis time. The working electrode of CE-CLD or CE-ECD is more prone to fouling or contamination which limits its application to trace determination of puerarin in real samples. For NACE and MEKC, they have relatively lower puerarin sensitivity of detection. Finally for NIRS, classification and quantification models have to be established before analysis of real samples which is inconvenient and makes the analysis time much longer.

Puerarin in Xinkeshu capsules is traditionally analyzed by HPLC which is regarded as a prime separation method [16]. In analysis of Chinese patent medicine, HPLC has some shortcomings such as longer analysis time, lower separation efficiency and shorter column lifetime. For this reason, CE has been introduced to the medicinal analysis attributing to its faster speed, better separation efficiency, good reproducibility, ultra-small sample size, and minimal consumption of solvent and reagent. In the past few decades, there were numerous articles and reviews on reporting the

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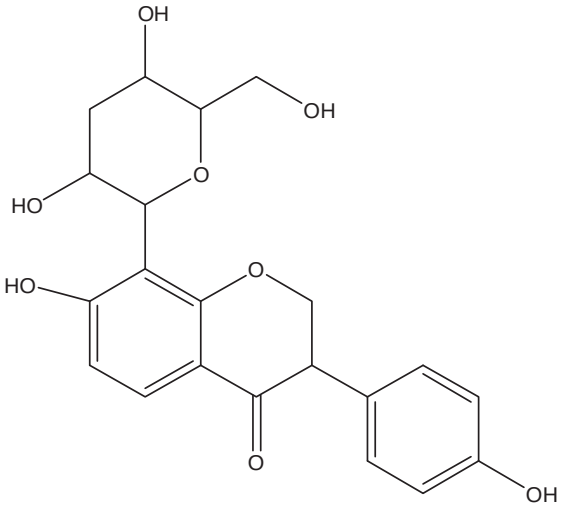

Fig. 1. The structure of puerarin.

application of CE for drug analysis and purity control of pharmaceuticals [17–21]. The main attractive features of CE are high resolving power and very small sample injection volume. As such, it is widely used nowadays as an excellent analytical separation technique complement to HPLC.

The aim of this work is to develop a rapid and simple method to determine puerarin in pharmaceutical and biological samples. Various capillary zone electrophoresis (CZE) conditions affecting the separation and analysis of puerarin such as running buffer pH, concentration and separation voltage are investigated in detail. The proposed CZE method has the attributes of simple and straightforward sample pre-treatment, fast and efficient separation. It has been successfully applied to analyze puerarin in pharmaceutical and biological samples with good accuracy and recovery. Our results confirm that the proposed CZE method offers a simple alternative approach for routine analysis and quality control of puerarin in pharmaceutical industries and therapeutic drug monitoring in clinical laboratories.

2. Experimental

2.1. Chemicals

Boric acid and sodium tetraborate decahydrate were purchased from Tianjin First Chemical Factory (Tianjin, China). HPLC-grade methanol was obtained from Tianjin Siyou Fine Chemicals Corporation (Tianjin, China). Daidzein, ferulic acid, puerarin, and rutin standards were provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). L-Ascorbic acid, L-glutamic acid, glycine, L-lysine, L-tryptophan, L-tyrosine, uric acid, and vitamin B_2 were purchased from Aladdin Chemistry Company Limited (Shanghai, China). Xinkeshu capsules were purchased from Xi'an Tianyi Pharmaceuticals Company Limited (Xi'an, China). Hawthorn, Salvia Miltrorrhiza, Notoginseng and Common Vladimiria were purchased from a local herbal shop. All solvents and reagents of analytical grade were used unless otherwise stated. Purified distilled deionized (DI) water from a Milli-Q-RO4 water purification system (Millipore, Bedford, MA, USA) with a resistivity higher than 18 MΩ cm was used throughout this work.

2.2. Apparatus

All CE separations were conducted on a Beckman P/ACE System MDQ capillary electrophoresis instrument (Fullerton, CA, USA). The CE system consisted of a 0–30 kV high-voltage built-in power supply and a diode array detector (190–600 nm). 32 Karat software (version 8.0) (Beckman) was employed to acquire the data, control the CE instrument and determine the theoretical plate number and resolution of the solutes. Electrophoresis was performed in fused silica capillaries of 75 μm i.d. (Yongnian Ruifeng Chromatogram Equipment, Handan, China). All the capillaries were 57 cm long with an effective length of 50 cm. Sample was extracted in a Branson B3200S ultrasonic bath (40 kHz, 220 V and 130 W) (Shanghai, China) and centrifuged in a TGL-16 C high-speed centrifuge (Shandong Heze Petrochemical School Instrument and Equipment Plant, Heze, China).

2.3. Standard and sample preparation

Standard stock puerarin solution (360 μg/mL) was prepared by dissolving 9.0 mg puerarin in 25 mL methanol and kept at 4 °C until use. Working standard solutions (17.3–138 μg/mL) were prepared from the standard stock solution by serial dilutions with methanol. The running buffer, 70 mM H_3BO_3–$Na_2B_4O_7$ (pH 9.2), was obtained by adjusting 70 mM $Na_2B_4O_7$ accurately to the desired pH with 70 mM H_3BO_3. All solutions were filtered with 0.22-μm cellulose membrane filters (diameter 25 mm) (Taiyuan Ruijia Scientific Equipment Company Limited, Taiyuan, China) before use.

For the real sample preparation, powder sample was taken from ten selected capsules. 125 mg of the powder sample was accurately weighed, dissolved in 10 mL of methanol, sonicated for 30 min in the ultrasonic bath and centrifuged at 12,000 rpm for 5 min. The sample solution was filtered through a 0.22-μm cellulose membrane filter and the filtrate was collected and subjected to CZE analysis. For comparison, another herbal sample was prepared from Hawthorn, Salvia Miltrorrhiza, Notoginseng and Common Vladimiria [22] which was also subjected to similar sample preparation and CZE analysis.

The human urine sample was obtained from a healthy volunteer. The fresh urine sample was collected in a vial and stored at 4 °C. After centrifugation at 5000 rpm for 10 min, the supernatant was spiked with known volumes of standard solutions and evaporated to dryness at 40 °C in vacuum. The residue was redissolved in 2.0 mL of ethanol and the solution was filtered through a 0.22-μm cellulose membrane filter before use.

Human plasma sample was obtained from the Shanxi Datong University Hospital. The plasma sample was deproteinized by acetonitrile. After centrifugation at 8000 rpm for 10 min, the supernatant was spiked with known volumes of standard solutions and evaporated to dryness at 40 °C in vacuum. The residue was redissolved in 2.0 mL of ethanol and the solution was filtered through a 0.22-μm cellulose membrane filter before use. The unspiked samples were prepared and determined in a similar fashion.

2.4. CZE conditions and retention parameters

Prior to use, the new capillary was conditioned by flushing at 20 psi sequentially with methanol for 10 min, water for 5 min, 1.0 M NaOH for 15 min, water for 5 min, and running buffer for 15 min and, finally, equilibrated at 25 kV with the running buffer for 30 min. Between two consecutive analyses, the capillary was rinsed sequentially with 0.10 M NaOH for 2 min, flushed with water for 1 min, and finally with the running buffer for 3 min. Detection wavelength was set at 254 nm. Sample injections were performed in a hydrodynamic mode (5 s and 0.5 psi). Electrophoretic separations were performed at a constant voltage of 25 kV. All CZE runs were conducted at 25 °C.

The separation efficiency and resolution were determined by the CE software based on the following formulas:

$$N = 5.54\left(\frac{t_m}{W_{1/2}}\right)^2 \quad (1)$$

$$R_S = 1.18\frac{t_m - t_{m1}}{W_{1/2} + W_{P1/2}} \quad (2)$$

where N is the theoretical plate number, t_m is the migration time, R_S is the resolution between the peak of interest (peak 2) and the preceding peak (peak 1), t_{m1} is the migration time of peak 1, t_{m2} is the migration time of peak 2, $W_{1/2}$ is the width of the component peak at 50% peak height and $W_{P1/2}$ is the width of the previous component peak at 50% peak height.

The distribution coefficient (α), apparent mobility (μ_{app}), effective mobility (μ_{eff}) and diffusion coefficient (D) were calculated as follows:

$$\alpha = \frac{t_{m2} - t_{eof}}{t_{m1} - t_{eof}} \quad (3)$$

$$\mu_{app} = \frac{L_d L_t}{V t_m} \quad (4)$$

$$\mu_{eff} = \frac{L_d L_t}{V}\left(\frac{1}{t_{eof}} - \frac{1}{t_m}\right) \quad (5)$$

$$D = \frac{\left(\mu_{app1} - \mu_{app2}\right)^2 V L_d}{16 R s^2 L_t \left(\mu_{app1} + \mu_{app2}\right)} \quad (6)$$

where L_t and L_d are the total length of the capillary and the capillary length to the detector, t_{eof} and t_m are the migration times of the electroosmotic flow and the analyte, respectively, V is the applied voltage, and μ_{app1} and μ_{app2} are the apparent mobilities of peak 1 and peak 2, respectively.

3. Results and discussion

3.1. Selection of detection wavelength

Puerarin possesses a strong absorption band in the UV region with an absorption peak maximum at *ca.* 254 nm, attributing to the aromatic rings in puerarin. In theory, it is possible to use the detection wavelength <254 nm because the absorptions are higher at shorter wavelengths; however, a higher baseline and more interferences are anticipated. To compromise, 254 nm was chosen as the detection wavelength for our CZE.

3.2. Optimization of CZE separation condition

It is well known that running buffer pH is another important parameter in controlling and optimizing the CZE separation. To ensure that puerarin exists as anionic form, the running buffers pH must be ≥ 8.5. Fig. 2 displays the effect of running buffer pH (8.5–9.4) on the CZE separation of a sample containing puerarin. The migration times of puerarin and the sample impurities increase with the increase in pH. At pH 8.5, puerarin is completely overlapped with a major impurity in the sample. When pH is 8.7, they are partially separated. Further increase in the pH will improve the separation of puerarin from the impurity, and complete separation was achieved at pH ≥ 9.2. In addition, the higher the pH, the longer the migration time. To compromise, 9.2 was chosen as the optimal running buffer pH as it produces good separation and shorter analysis time.

The effect of the concentration of running buffer solution (40–80 mM) on the separation was investigated and the electropherograms are displayed in Fig. 3. The separation between

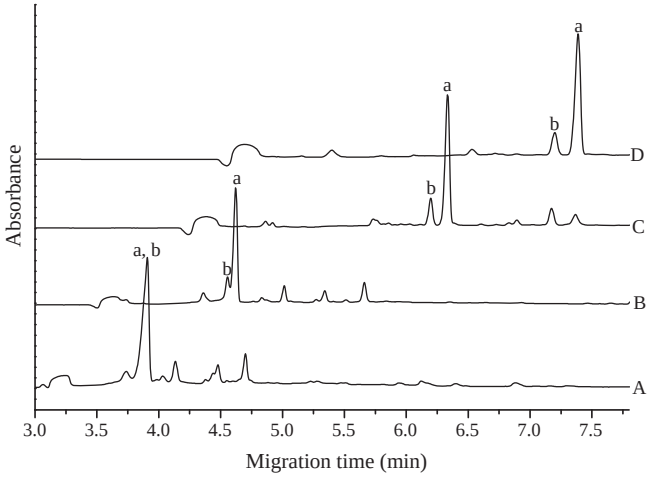

Fig. 2. Effect of running buffer pH: (A) 8.5, (B) 8.7, (C) 9.2, and (D) 9.4 on the separation of a real sample containing puerarin. Peaks (a) and (b) are identified as puerarin and the major impurity in the sample, respectively. Other smaller peaks are unknown impurities. The running buffer was 70 mM H_3BO_3–$Na_2B_4O_7$ at various pH. Hydrodynamic sample injection was performed at 0.5 psi and 5 s. The separation voltage and temperature were 25 kV and 25 °C, respectively.

puerarin and the major impurity in the sample is improved when the concentration of the buffer increases. In addition, the migration times of the components in the sample matrix increase with the increase in buffer concentration. Since 70 mM H_3BO_3–$Na_2B_4O_7$ could result in complete separation of puerarin from its sample impurities with reasonable analysis time, it was chosen as the optimal buffer concentration for our CZE analysis.

The effect of the applied voltage (15–30 kV) on the CZE separation of puerarin was investigated. Fig. 4 depicts the CZE analysis of a real sample containing puerarin at various applied voltages. It is found that the voltage does not affect too much the separation of puerarin from the impurities in the sample. In general, the higher the applied voltage, the faster the solutes migrate. Higher applied voltage would result in shorter analysis time and sharper solute peaks since the electroosmotic flow increases and is faster than the electrophoretic mobilities of the solutes. However, if the voltage is too high, it will cause excessive Joule heating and degrade the separation efficiency. As such, 25 kV was chosen as the optimal separation voltage as it produces complete separation of pureraring

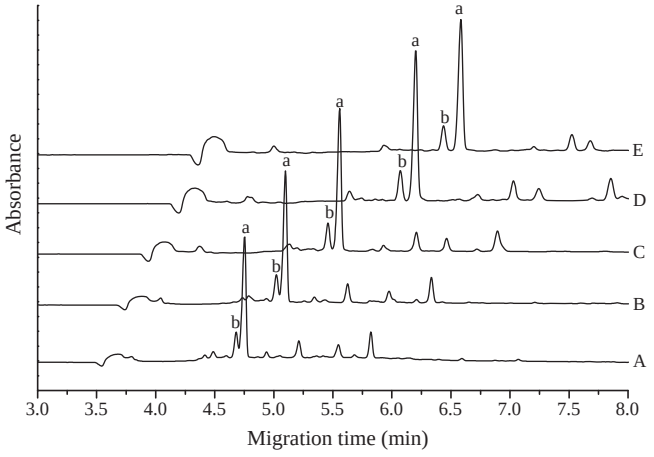

Fig. 3. Effect of running buffer concentration: (A) 40, (B) 50, (C) 60, (D) 70, and (E) 80 mM on the separation of a real sample containing puerarin. Peaks (a) and (b) are identified as puerarin and the major impurity in the sample, respectively. Other smaller peaks are unknown impurities. The CZE conditions are same as in Fig. 2.

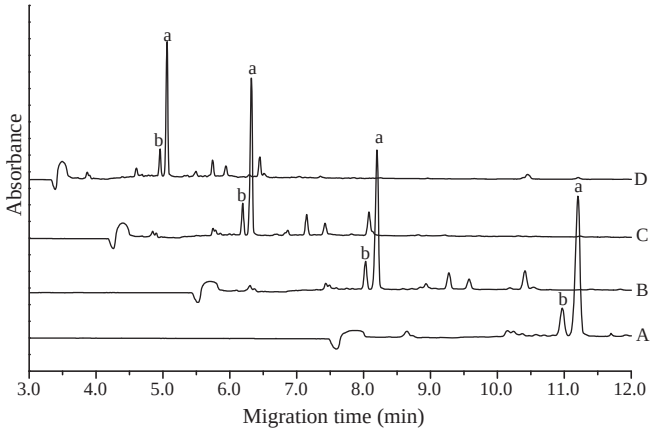

Fig. 4. Effect of applied voltages: (A) 15, (B) 20, (C) 25, and (D) 30 kV on the separation of a real sample containing puerarin. Peaks (a) and (b) are identified as puerarin and the major impurity in the sample, respectively. Other smaller peaks are unknown impurities. The CZE conditions are same as in Fig. 2.

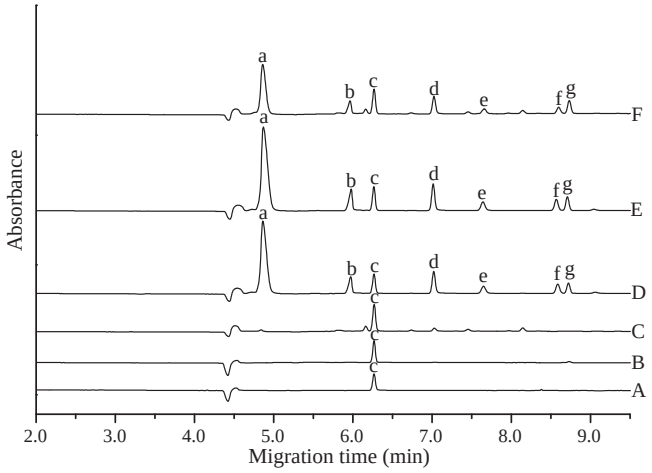

Fig. 5. Electropherograms of (A) plasma sample spiked with puerarin, (B) urine sample spiked with puerarin, (C) Xinkeshu capsules sample, (D) plasma sample spiked with puerarin and interferents, (E) urine sample spiked with puerarin and interferents, and (F) Xikeshu capsules sample spiked with puerarin and interferents. (a) L-tryptophan, (b) vitamin B_2, (c) puerarin, (d) daidzein, (e) rutin, (f) L-ascorbic acid, and (g) ferulic acid. The CZE conditions are: running buffer of 70 mM H_3BO_3–$Na_2B_4O_7$ at pH 9.2, hydrodynamic sample injection at 0.5 psi and 5 s, separation voltage of 25 kV, and temperature of 25 °C.

from the impurities in the sample matrix with a reasonable run time.

The migration parameters of puerarin in terms of t_m, μ_{app}, μ_{eff}, α, D, R_S, and N at various CZE conditions are summarized in Table S1 (Supplementary data). In summary, the optimal CZE conditions are pH 9.2, 70 mM running buffer and 25 kV since it produces high plate number, complete separation from impurities and reasonable migration time for puerarin.

3.3. Analytical performance

The analytical performance of the CZE-UV method was evaluated in terms of linearity, limit of detection and repeatability. A linear calibration curve: $A = 642\,C + 22.5$, where A is the peak area and C is the concentration of puerarin (17.3–138 μg/mL) with $r = 0.9998$, was obtained. The limit of detection was estimated to be 34.6 ng/mL based on $S/N = 3$. The intra-day and inter-day repeatability of migration time and peak area were evaluated by analyzing various concentrations of standard puerarin solutions. Three different concentrations of puerarin in the linear range (17.3, 69.1 and 104 μg/mL) were analyzed in three independent series in the same day (intra-day precision) and three consecutive days (inter-day precision). Each sample analysis was repeated for three times. The results are expressed as the relative standard deviation (RSD) of migration time and peak areas. The RSD of the migration time and peak area are ≤0.91% and ≤1.62% (intra-day, $n = 3$) and ≤0.64% and ≤1.32% (inter-day, $n = 3$), demonstrating that the proposed CZE method is precise within the same day and/or multiple days.

3.4. Interference study and real sample analysis

The possible interferences caused by other potential substances on the determination of puerarin were investigated in detail. Daidzein, ferulic acid and rutin are the common active components in drugs for treating cardia-cerebrovascular disease. L-Ascorbic acid, L-glutamic acid, glycine, L-lysine, L-tryptophan, L-tyrosine, uric acid, and vitamin B_2 are the endogenous compounds usually found in biological samples. As such, they were chosen for the interference study. They were spiked into the plasma, urine and Xinkeshu samples, respectively and followed with the same CZE analysis. Fig. 5 displays the electropherograms of puerarin and the potential interferents in real samples. L-Glutamic acid, glycine, L-lysine, L-tyrosine, and uric acid were not visualized by CZE and should not display any interference effect. Other potential interferents including L-ascorbic acid, daidzein, ferulic acid, rutin, L-tryptophan, and vitamin B_2 are completely separated from puerarin and will not pose any effect on

Table 1
Analysis and recovery test of puerarin in pharmaceutical and biological samples.

Pharmaceutical sample	Lot no.	Puerarin added (mg/g)	Puerarin found (mg/g)	Recovery (%)	RSD[a] (%)
Xinkeshu capsules	100501	–	15.7	–	1.19
	100505	–	15.9	–	1.51
	100801	–	19.4	–	1.29
	100501	11.5	11.3	98.3	2.37
		21.8	22.2	102	0.64
		50.3	50.1	99.6	0.58

Biological sample	Puerarin content	Puerarin added (μg/mL)	Puerarin found (μg/mL)	Recovery (%)	RSD[a] (%)
Urine	ND[b]	26.2	25.6	97.7	2.25
		34.7	34.9	101	1.59
		42.1	43.2	103	1.91
Blood plasma	ND[b]	26.2	26.7	102	2.52
		34.7	34.4	101	2.02
		42.1	41.0	97.4	1.91

[a] Five determinations were performed.
[b] Not detected.

Table 2
Comparison of the proposed CZE method with other methods for determination of puerarin.

Analytical method	Working range (μg/mL)	Analysis time (min)	LOD (ng/mL)	Recovery (%)	RSD (%)	Ref.
FIA-CL	0.0003–0.1	0.017	0.1	86.7–114.3	1.15–3.41	3,4
	0.0013–0.8	0.5	0.84	103–107	0.33–0.4	
HPLC	0.005–0.5	15.2	3	102–106	1.3–1.4	5–9
	0.06–1.8	5.5	18	90.3–95.7	1.46–5.76	
	0.08–12	6.9	24	86.1–88.9	1.97–3.9	
	0.12–491	5	62	100	–	
	0.1–70	2.5	20	97.4–104	2.71–3.14	
CE-CLD	0.021–1.04	240	4.16	99.6–103	–	10
CE-ECD	1.04–416	9	143	98.6	2.67	11
NACE	50–200	16	121	95.4–96.4	2.8–3.2	12
MEKC	2.08–208	9	250	93.3	1.1	13,14
	26–210	3.89	3200	80.2	3.77	
CZE-UV	17.3–138	6.3	34.6	97.7–103	0.58–2.52	This work

the analysis of puerarin in pharmaceutical and biological samples. In essence, pharmaceutical and biological samples can be analyzed to determine their puerarin content by our proposed CZE method.

Moreover, the recovery and precision of the proposed CZE method were assessed. The recovery tests were done by adding known amounts of puerarin to the Xinkeshu capsule, urine and blood plasma samples which were then subjected to sample extraction and CZE analysis. The results are summarized in Table 1. The recoveries are 97.4–103% and the RSD is smaller than 2.52%, indicating that our method is accurate, reliable and precise, and can be applied to both pharmaceutical and biological samples. Finally, the proposed method was applied to analyze the puerarin content in three different batches of Xinkeshu capsules. It is found that the puerarin contents in Xinkeshu capsules are 15.7–19.4 mg/g and the RSD is smaller than 1.35%. These results show that our proposed CZE method can be applied to analyze puerarin in real samples.

Table 2 summarizes the analytical performance of our CZE method as compared to other methods for analyses of puerarin. Among these methods, FIA-CL provides the lowest LOD and working concentration range of puerarin. Unfortunately, it is more prone to interference as it does not involve any analytical separation. HPLC gives good working concentration range but it involves larger sample volume and solvent consumption. CE-CLD possesses the lowest working range and LOD among the CE methods; however, it requires post-column CL detection and is more cumbersome. In essence, our proposed CZE-UV method is comparable or even slightly better in terms of working range, LOD and repeatability to other CE methods, which should provide a good alternative for determination of puerarin in pharmaceutical and traditional Chinese medicinal products.

4. Conclusion

This work demonstrates that the new CZE-UV method has been successfully developed and applied to the determination of puerarin in real samples. This method is accurate, precise and reliable. The main attributes of the CE method as compared to HPLC are shorter analysis time, smaller volume of reagents and solvent consumption. Our developed CZE-UV method is cost-effective, simple, rapid, and could be an alternative method of choice for assay and quality control of puerarin in pharmaceutical industries and therapeutic drug monitoring in clinical laboratories.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2012.01.022.

References

[1] Q.L. Zhu, X.R. Lu, Chin. Tradit. Herbal Drugs 28 (1997) 693–696.
[2] K.H. Wong, G.Q. Li, K.M. Li, V. Razmovski-Naumovski, K. Chan, J. Ethnopharmacol. 134 (2011) 584–607.
[3] C. Wang, Z. Song, Bioorg. Med. Chem. Lett. 14 (2004) 4127–4130.
[4] Q. Zhang, A. Myint, L. Liu, X. Ge, H. Cui, J. Pharm. Biomed. Anal. 36 (2004) 587–592.
[5] N. Okamura, H. Miki, H. Orii, Y. Masaoka, S. Yamashita, H. Kobayashi, A. Yagi, J. Pharm. Biomed. Anal. 19 (1999) 603–612.
[6] Z. Ma, Q. Wu, D.Y.W. Lee, M. Tracy, S.E. Lukas, J. Chromatogr. B 823 (2005) 108–114.
[7] F. Ren, Q. Jing, Y. Shen, H. Ma, J. Cui, J. Pharm. Biomed. Anal. 41 (2006) 549–553.
[8] G.-J. Yang, P. Liu, X.-L. Qu, M.-J. Su, Q.-S. Qu, C.-Y. Wang, X.-Y. Hu, Z.-Y. Wang, J. Chromatogr. B 856 (2007) 222–228.
[9] X. Liu, H. Zhi, F. Du, Z. Ye, N. Wang, W. Qin, J. Li, J. Chromatogr. B 878 (2010) 3297–3302.
[10] S. Han, J. Chromatogr. B 877 (2009) 1591–1594.
[11] G. Chen, J. Zhang, J. Ye, J. Chromatogr. A 923 (2001) 255–262.
[12] C. Li, A. Chen, X. Chen, X. Chen, Z. Hu, J. Pharm. Biomed. Anal. 39 (2005) 125–131.
[13] H.Y. Huang, Y.-Z. Hsieh, Anal. Chim. Acta 351 (1997) 49–55.
[14] Y. Cao, C. Lou, X. Zhang, Q. Chu, Y. Fang, J. Ye, Anal. Chim. Acta 452 (2002) 123–128.
[15] C.-C. Lau, C.-O. Chan, F.-T. Chau, D.K.-W. Mok, J. Chromatogr. A 1216 (2009) 2130–2135.
[16] J. He, Z. Shi, M. Zhao, W. Chang, Chin. J. Anal. Chem. 32 (2004) 519–521.
[17] K. Altria, A. Marsh, C. Sänger-van de Griend, Electrophoresis 27 (2006) 2263–2282.
[18] K. Altria, D. Elder, J. Chromatogr. A 1023 (2004) 1–14.
[19] P.T.T. Ha, J. Hoogmartens, A.V. Schepdael, J. Pharm. Biomed. Anal. 41 (2006) 1–11.
[20] C. Cruces-Blanco, A.M. García-Campaña, Trends Anal. Chem. 31 (2012) 85–95.
[21] L. Geiser, J.-L. Venuthey, Electrophoresis 28 (2007) 45–57.
[22] Drug Standard of Ministry of Public Health of the Peoples Republic of China, Chinese Patent Medicines, vol. 15, The Peoples Medical Publishing, Beijing, 2005, p. 41.