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Short communication

Magnetic solid-phase extraction and determination of puerarin in rat plasma using C_{18}-functionalized magnetic silica nanoparticles by high performance liquid chromatography

Qi Wang[a,1], Lijie Huang[b,1], Panfeng Yu[c], Jianchang Wang[d,*], Shun Shen[e,*]

[a] Emergency Department, Chinese Air Force General Hospital, No. 30 Fucheng Road, Beijing 100142, PR China
[b] Animal experiment center, Chinese PLA General Hospital, No. 28 Fuxing Road, Beijing 100853, PR China
[c] Department of Orthopaedics, Chinese Air Force General Hospital, No. 30 Fucheng Road, Beijing 100142, PR China
[d] Chinese Air Force General Hospital, No. 30 Fucheng Road, Beijing 100142, PR China
[e] School of Pharmacy, Fudan University, No. 826 Zhangheng Road, Shanghai 201203, PR China

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ABSTRACT

In the paper, we presented a magnetic solid-phase extraction (MSPE) method based on C_{18}-functionalized magnetic silica nanoparticles for the analysis of puerarin in rat plasma. The approach involves two steps including synthesis of magnetic solid-phase sorbents and bioanalysis. The synthesized magnetic silica microspheres modified with chloro(dimethyl)octylsilane (namely Fe_3O_4@SiO_2-C_{18}) can provide an efficient way for the extraction of puerarin through C_{18} hydrophobic interaction. The puerarin could be easily enriched using milligram-level Fe_3O_4@SiO_2-C_{18} sorbents with vibration for 10 min. By means of a magnet, puerarin adsorbed with Fe_3O_4@SiO_2-C_{18} sorbents was easily isolated from the matrix, and desorbed with CAN. No carryover was observed, and the sorbents could be recycled in our study. The method recoveries were obtained from 85.2% to 92.3%. Limits of quantification and limits of detection of 0.1 $\mu g\,mL^{-1}$ and 0.05 $\mu g\,mL^{-1}$, respectively were achieved. The precision was from 8.1 to 13.7% for intra-day measurement, and from 9.4 to 15.2% for inter-day variation. The accuracy ranged from 94.7 to 106.3% for intra-day measurement, and from 93.3 to 107.8% for inter-day measurement. The MSPE method was applied for analysis of puerarin in rat plasma samples. The results indicated that it was convenient and efficient for the determination of puerarin in biosamples.

1. Introduction

Recently, the novel technique of magnetic solid-phase extraction (MSPE) has obtained more and more interests in sample pretreatment [1–20]. Among the most popular sorbents, nano-Fe_3O_4 has attracted much attention because of its unique physical properties, such as higher surface area-to-volume ratio, superparamagnetism, high magnetic saturation and simple preparation process. Moreover, for extraction the sorbents can be readily isolated from the sample matrix using a magnet. Compared with isolation using common sorbents by filtration or centrifugation, magnetic isolation is obviously much easier and more economic. Additionally, in MSPE, sorbents are dispersed homogeneously in the sample solution with short diffusion route for extraction, which result in rapid extraction dynamics. In practical applications, nano-Fe_3O_4 particles are usually coated with a protective layer of silica to avoid change of the magnetic properties of magnetite or its oxidation. In order to enable the selective extraction of target compounds, the silica coating can be functionalized with organosilanes and/or affinity ligands. One of the most promising and advanced composite material is Fe_3O_4@silica nanoparticles (Fe_3O_4@SiO_2) coated with alkyl C_{18} (Fe_3O_4@SiO_2-C_{18}), which have higher enrichment ability attributing to the nanosizes and the plentiful C_{18} groups. According to the literatures, they have been used for the extraction and determination of ergosterol in tobacco, methylprednisolone in rat plasma, and so on [21–24].

Herein, we developed the Fe_3O_4@SiO_2-C_{18} nanoparticles as MSPE sorbents for the extraction and analysis of puerarin in rat plasma. As we know, puerarin is one of the bioactive components, which isolated from the root of *Pueraria lobata* (*Willd.*) *Ohwi* and *P. thomsonni Benth*. Numerous literatures have reported that puerarin has various pharmacological effects of cardiac/cerebral blood vascular diseases [25–28]. Furthermore, puerarin has also been used traditionally in China to inhibit alcohol-induced avascular necrosis of the femoral head [29,30]. Up to now, a few

* Corresponding authors. Tel.: +86 10 68410099/86 21 51980069.
E-mail addresses: jiancwang@126.com (J. Wang), sshen@fudan.edu.cn (S. Shen).
[1] Both authors contributed equally to this work.

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analytical techniques have been developed for the determination of puerarin [31–33]. Sample preparation of biological samples is crucial due to complex interferences in the biomatrix, which can impair sample recovery. Conventional methods for the extraction of puerarin from biosamples involve liquid-liquid extraction (LLE) and solid-phase extraction (SPE). However, current trends are directed toward miniaturization by using microextraction techniques, which consume less toxic organic solvent than the above-mentioned traditional approaches. In our study, we employed the nano-MSPE for the extraction of puerarin from rat plasma. The successfully prepared Fe_3O_4@SiO_2-C_{18} nanoparticles, exhibited well-defined magnetite-core–silica-shell structure and possessed a high content of magnetite. Parameters that affect the extraction efficiency were investigated using blank plasma samples spiked with puerarin and the results were discussed in detail. By coupling with HPLC, under the select conditions, a rapid, little solvent and low cost MSPE–HPLC method for the analysis of puerarin in rat plasma samples was established.

2. Materials and methods

2.1. Materials and chemicals

The puerarin (Fig. 1a), internal standard (I.S.) of 4-hydroxybenzoic acid (Fig. 1b), chloro(dimethyl)octylsilane, HPLC grade organic solvents of methanol and acetonitrile (ACN) were obtained from the Sigma–Aldrich Chemical Company (St. Louis, MO, USA). The other analytical grade reagents were obtained from Sinopharm Chemical Reagent Co., Ltd (China, Shanghai).

1 mg mL^{-1} stock solution of puerarin was performed in methanol for preparation of working standard solutions. Meanwhile, I.S. solution (50 μg mL^{-1}) was also prepared. They were stored in glass vials protected against light at 4 °C. Blank plasma was stored at −20 °C.

2.2. Synthesis of Fe_3O_4@SiO_2-C_{18} nanoparticles

The synthesis of Fe_3O_4@SiO_2-C_{18} nanoparticles involves two steps including preparation of Fe_3O_4@SiO_2 microspheres and their surface C_{18} modification. Fe_3O_4@SiO_2 microspheres were synthesized according to our published papers [21–24]. The Fe_3O_4@SiO_2 microspheres (0.01 g) obtained were reacted with chloro(dimethyl)octylsilane (0.5 g) in the mixture solution of anhydrous pyridine (0.5 g) and anhydrous toluene (20.0 g) under mechanistically stirring. The reaction was kept for 12 h at room temperature. Finally, by the use of a magnet, the Fe_3O_4@SiO_2-C_{18} microspheres were separated, washed with ethanol and water, and vacuum dried at 60 °C for 24 h.

2.3. Apparatus and measurement

The Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2-C_{18} microspheres were characterized by scanning electron microscopy (SEM, JEOL JSM-6500F), transmission electron microscopy (TEM, JEOL JEM-2010) and Fourier-transform infrared spectrometry (FT-IR, Nicolet Nexus 470). The magnetic properties were analyzed by a superconducting quantum interference device (SQUID) at room temperature.

Chromatographic separation was carried out with an Agilent 1100 Series HPLC system (Agilent, USA). The analysis conditions were water (containing 0.5% H_3PO_4)–ACN (58:42, v/v), as mobile phase pumped at a flow rate of 1.0 ml/min on a Hypersil BDS C_{18} (5 μm, 4.6 × 200 mm) column at room temperature. The peak response was monitored at a wavelength of 250 nm.

2.4. Selection of extraction conditions by Fe_3O_4@SiO_2-C_{18}

Different extraction conditions (the amounts of sorbents added, extraction time and elution solvent) were selected. Drug-free plasma (0.2 mL) containing 4 μg puerarin and 10 μL of I.S. (50 μg mL^{-1}) was passed through a centrifugal filtering device with a nominal molecular limit (NMWL) of 3000. Centrifugation was carried out at 12,000 rpm for 30 min. Then, 50, 100, 150, 200 μL suspension of sorbents (10 mg mL^{-1}) was added into the centrifugal liquid, respectively. The mixture was continuously vibrated for 10 min. Then it was placed near a strong Nd–Fe–B magnet until the solution became limpid and the supernatant solution was completely decanted. The captured puerarin/nanoparticles were collected and rinsed with water. To desorb the puerarin, ACN (eluent solvent, 3× 20 μL) was added to the isolated adsorbent. The ACN extract was collected and an amount of 10 μL was injected into HPLC system for analysis.

Afterward, the parameters of extraction and desorption were also investigated. 150 μL suspensions of sorbents (10 mg mL^{-1}) were added into centrifugal liquid as is stated above, for continuous vibration from 1 to 10 min, respectively. Then, the captured puerarin absorbed with Fe_3O_4@SiO_2-C_{18} was rinsed with methanol or ACN for three times (3× 20 μL). Finally, 10 μL of aliquots was injected into HPLC system for analysis.

2.5. Linear range, detection limit, precision, and recovery

Plasma samples were spiked with puerarin to a final concentration from 0.1 to 20 μg mL^{-1}. Then, 10 μL of I.S. solutions was added. The calibration curve was evaluated using internal standard method and constructed by plotting the peak area ratio between standard of puerarin and I.S. using the selection Fe_3O_4@SiO_2-C_{18} conditions. The measurements were repeated three times.

The limit of detection (LOD) was defined as the concentration yielding a signal to noise of 3. The limit of quantification (LOQ) was defined as the lowest concentration which could be measured with acceptable precision and accuracy.

The intra- and inter-run precision and accuracy of the analytical method were also investigated by triplicate analyses of the puerarin concentration in spiked rat plasma (0.1, 5, 20 μg mL^{-1}) plus I.S. solution within day and on continuous 5 days. The obtained results were measured using the relative standard deviations (RSDs).

Recoveries were calculated by adding different amount of puerarin (0.02, 1 and 4 μg) to the 0.2 mL rat plasma containing known content of puerarin. The measurements were repeated three times.

2.6. Preparation of rat plasma samples

Six normal male Wistar rats were obtained from Institute of Laboratory Animal Science (Beijing, China). They were fed a soy-free custom diet and water with a week. Before the experiments, all rats were fasted overnight. Blood samples (0.5 mL) were collected from the femoral vein at 0 (before drug administration), 0.25, 0.5, 1, 2, 4, 8, 16 h (n = 6 at each time point) and stored in EDTA containing syringes after intragastrical administration of puerarin at a single dose of 500 mg/kg. The blood samples were kept at −20 °C until analysis.

2.7. Extraction of puerarin from plasma samples by LLE

Spiked plasma (0.2 mL) was supplemented with puerarin to a final concentration of 0.1–20 μg mL^{-1} and 10 μL of I.S. The plasma was then vortex-mixed vigorously for 2 min. Then the mixtures were extracted using 3 mL of ethyl acetate by vortexing for 4 min. The organic layer was pipette-transferred and evaporated to dryness with nitrogen gas at 40 °C in a water bath. Finally, 50 μL of

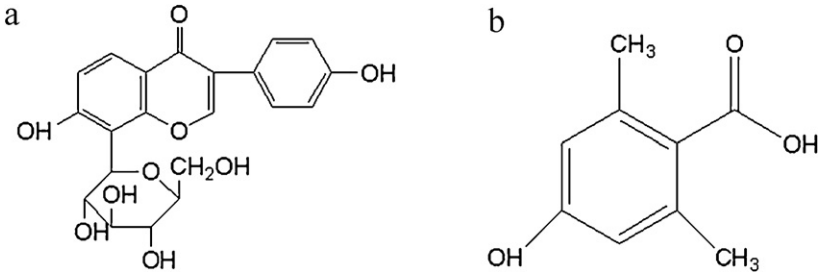

Fig. 1. Structure of (a) puerarin and (b) 4-hydroxybenzoic acid (I.S.).

mobile phase solution was added to the dry plasma extraction and vortex-mixed vigorously for 1 min. Aliquots (10 μL) were injected into HPLC system for analysis. The calibration curve was obtained using internal standard method. The extraction procedures of puerarin from plasma samples at 1, 2, 4 time-points were the same as the above. The results obtained by MSPE and LLE were compared.

3. Results and discussion

3.1. Preparation of Fe_3O_4@SiO_2-C_{18} nanoparticles

The magnetic solid-phase sorbents of Fe_3O_4@SiO_2-C_{18} nanoparticles were firstly prepared according to our previous studies [22,24]. The as-prepared Fe_3O_4 nanoparticles were nearly spherical in shape with a diameter of ca. 300 nm. A uniform gray silica shell about a thickness of ca. 35 nm encapsulated the Fe_3O_4 core particles. By modification with chlorodimethyl-n-octadecylsilane, the Fe_3O_4@SiO_2-C_{18} nanoparticles could be easily obtained. To confirm the presence of C_{18} groups, the FTIR spectra of Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2-C_{18} nanoparticles were recorded. The marked absorption peak at ~2920 cm^{-1} in Fe_3O_4@SiO_2-C_{18} was found, which indicated that the C_{18} groups were successfully modified on the surface of Fe_3O_4@SiO_2. The results were in agreement with that reported in our previous work [22,24].

The magnetic properties of Fe_3O_4@SiO_2-C_{18} nanoparticles were carried out by SQUID at room temperature. The magnetization curves of magnetic nanoparticles displayed a superparamagnetic behavior. The magnetization values were measured to be 49.5 emu g^{-1} for Fe_3O_4@SiO_2-C_{18} nanoparticles and 77.1 emu g^{-1} for Fe_3O_4 nanoparticles. According to Ma's study, the saturation magnetization could ensure a quick magnetic separation procedure by use of a magnet in practical bioapplication [34]. The result indicated that the Fe_3O_4@SiO_2-C_{18} was magnetic responsive and dispersed easily in solution.

3.2. Selection of Fe_3O_4@SiO_2-C_{18} conditions

The plentiful hydrophobic C_{18} groups provide the Fe_3O_4@SiO_2-C_{18} with the excellent extraction ability for enrichment hydrophobic analytes by the hydrophobic–hydrophobic interaction. Different extraction conditions such as the mass of sorbents added, extraction time and elution solvents were selected.

The mass of sorbents added was known to be correlated with the quantity of analytes adsorbed. To obtain the maximum extraction efficiency of puerarin, the content of the adsorbents required was selected by adding different amount of Fe_3O_4@SiO_2-C_{18} nanoparticles. The centrifugal liquid containing 4 μg of puerarin was supplied with 50, 100, 150, 200 μL suspension of sorbents (10 mg mL^{-1}). The highest peak area of puerarin could be achieved between 150 and 200 μL addition of Fe_3O_4@SiO_2-C_{18}. Thus, 150 μL of magnetic sorbents was selected in the further study.

Since extending contact time facilitates analyte–particle interaction until equilibrium was reached, different extraction time from 2 to 10 min by the sorbents was studied to enhance the extraction efficiency in this research. The mass of captured puerarin increased significantly with increasing extraction time from 2 to 8 min under continuous vibrating. However, the extraction amount of puerarin had no remarkable increase when extraction time was from 8 to 10 min. Thus, the extraction time of 8 min was chosen in the following study.

As is known to all, it is very important to maximize the analytes concentration in the extraction process, so the selection for the proper solvent to elute the extracts is quite essential. In our study, two desorption solvents of methanol and ACN were used for the investigation of elution efficiency. Using the select conditions mentioned above, the peak area of puerarin was highest using ACN as desorption solvent. In addition, the good recovery of puerarin (85.8–91.5%) could be acquired using ACN. Therefore, the elution solvent of ACN was selected.

3.3. Validation of the method

The calibration curve of puerarin in rat plasma was linear in the concentration range from 0.1 to 20 μg mL^{-1}. The regression equation was $y = 2.7193x + 0.7019$, $r^2 = 0.998$ ($n = 3$), where y was the peak area ratio of puerarin to the I.S., and x was the plasma concentration of puerarin, and r was the correlation coefficient.

The LOQ of puerarin in rat plasma was 0.1 μg mL^{-1}. At this concentration, the intra- and inter-day precisions were 8.5 and 12.5%, respectively, and the intra- and inter-day accuracies were 96.3 and 105.6%, respectively. The LOD was 0.05 μg mL^{-1}, which was sufficient for the analysis of rat plasma puerarin in our study.

The precision and accuracy of the method were determined on three different puerarin concentration of 0.1, 5, 20 μg mL^{-1}. The precision was from 8.1 to 13.7% for intra-day measurement, and from 9.4 to 15.2% for inter-day variation. The accuracy ranged from 94.7 to 106.3% for intra-day measurement, and from 93.3 to 107.8% for inter-day measurement. The results indicated that the analytical approach was reproducible, accurate, and reliable.

Recoveries were measured from 85.2% to 92.3%, which indicated that the analytical approach was feasible for the determination of rat plasma puerarin.

3.4. Determination of puerarin in rat plasma samples

The select method was applied to the analysis of puerarin in rat plasma samples after intragastrical administration of puerarin at a single dose of 500 mg/kg. As shown in Fig. 2a–c, there was no significant chromatographic interference around the retention times of puerarin and I.S. in rat plasma. Moreover, good chromatographic profiles of puerarin and I.S. were also obtained using the select conditions. Using internal standard method, a time–plasma concentrations of puerarin were calculated and presented in Fig. 3. The plasma concentrations of puerarin increased very rapidly and peaked at 0.72 h. Thereafter, the puerarin concentrations gradually decreased with an elimination half-life of 3.25 h.

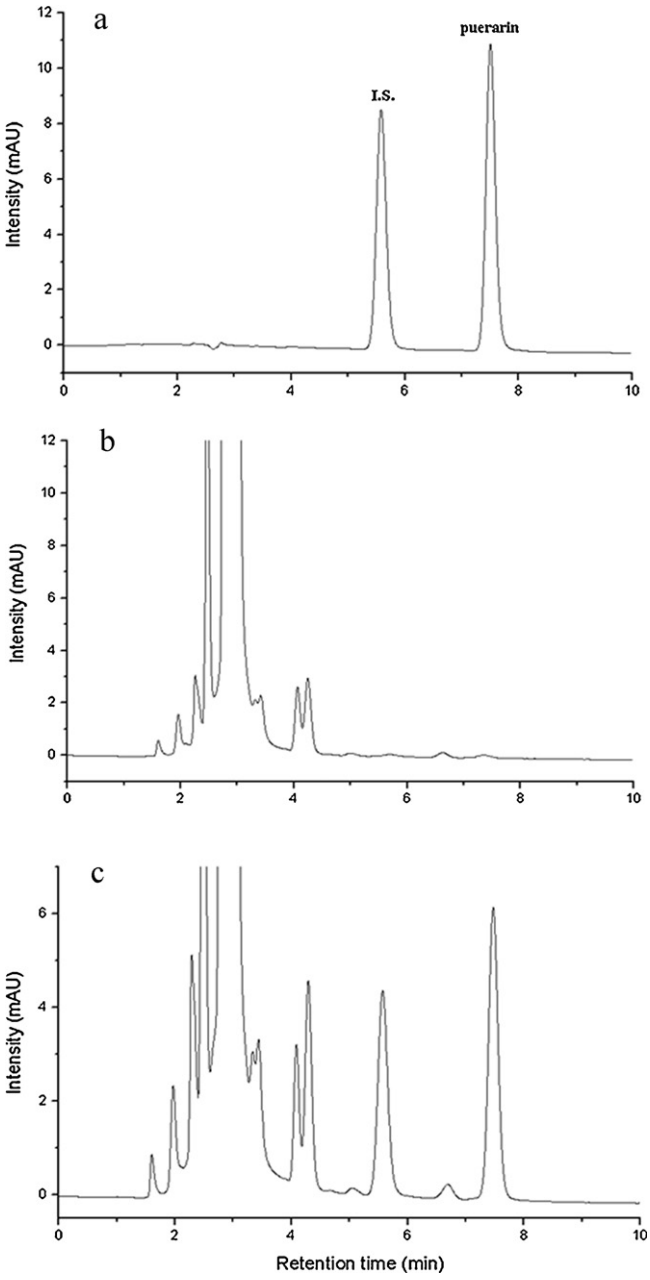

Fig. 2. Chromatograms for (a) standard solution of the puerarin and I.S. mixture, (b) blank rat plasma and (c) the representative chromatograms of rat plasma after intragastrical administration of puerarin at a single dose of 500 mg/kg.

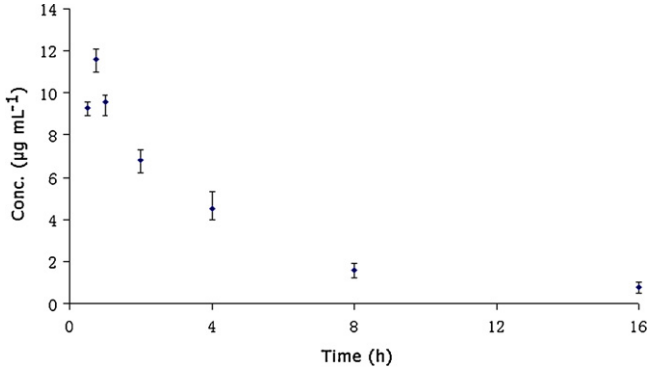

Fig. 3. Mean plasma concentration–time profile of six rats after intragastrical administration of puerarin at a single dose of 500 mg/kg. The bars represent maximum absolute deviation values.

Solvent extraction and determination of puerarin in plasma is a reliable analytical method. So, it was employed to analyze puerarin in order to demonstrate our developed MSPE method. We pleasurably found that the puerarin plasma concentrations by solvent extraction were similar to those obtained by MSPE using Fe_3O_4@SiO_2-C_{18}. The LOD by solvent extraction was 0.01 μg mL^{-1}. The recoveries were between 93.6% and 95.3%. The precision was from 3.8 to 6.1% for intra-day measurement, and from 5.3 to 9.4% for inter-day variation. The accuracy ranged from 98.5 to 103.2% for intra-day measurement, and from 96.1 to 104.6% for inter-day measurement. The method validations were superior to MSPE. However, as we know, solvent extraction always requires large amounts of organic solvents, which is harmful to body health. Thus, the MSPE method based on Fe_3O_4@SiO_2-C_{18} had advantages for the analysis of puerarin in rat plasma.

4. Conclusion

In the work, magnetic Fe_3O_4@SiO_2-C_{18} nanoparticles were successfully applied to the determination of puerarin in rat plasma. Owing to the powerful hydrophobic–hydrophobic interaction, puerarin was adsorbed efficiently by the C_{18} groups of the Fe_3O_4@SiO_2-C_{18} nanoparticles. Moreover, the strong magnetic responsivity of Fe_3O_4@SiO_2-C_{18} made the extraction process very simple, economic and efficient. The adsorbed puerarin was easily desorbed using the elution solvent of ACN. Therefore, we trust that the developed MSPE technique based on Fe_3O_4@SiO_2-C_{18} is a promising method for the extraction and determination of other similar small molecular drugs in biosamples.

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