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Sensitive liquid chromatography–tandem mass spectrometry method for the determination of scutellarin in human plasma: Application to a pharmacokinetic study

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Abstract

A sensitive and selective liquid chromatographic–tandem mass spectrometric (LC–MS/MS) method for the determination of scutellarin in human plasma has been developed. Samples were prepared using solid phase extraction and analyzed on a C_{18} column interfaced with a triple quadrupole tandem mass spectrometer. Positive electrospray ionization was employed as the ionization source. The mobile phase consisted of methanol–water (0.1% formic acid), using gradient procedure. The analyte and internal standard baicalin were both detected by use of selected reaction monitoring mode. The method was linear in the concentration range of 0.2–20.0 ng/mL. The lower limit of quantification (LLOQ) was 0.2 ng/mL. The intra- and inter-day relative standard deviation across three validation runs over the entire concentration range was less than 12.4%. The accuracy determined at three concentrations (1.0, 5.0 and 10.0 ng/mL for scutellarin) was within ±5.0% in terms of relative error. The method herein described was successfully applied for the evaluation of pharmacokinetic profiles of scutellarin guttate pills in 20 healthy volunteers.

Keywords: Scutellarin; LC–MS/MS; Pharmacokinetics

1. Introduction

Scutellarin is a flavone glucuronide extracted from a Chinese herb Erigero breviscapus (Vant.) Hand. -Mazz [1]. It is not only an important component of a Chinese herb, but also a major constituent of skullcap, which is a popular western herb. Scutellarin has been proved to be effective in dilating blood vessels, improving hemodynamics, decreasing the viscosity of blood, reducing the blood platelet count and preventing platelet conglomeration, etc. [2,3]. In clinic, scutellarin is widely used in treating various cardiovascular diseases such as coronary heart disease, angina pectoris, and thrombosis [4]. For a better understanding of its pharmacokinetics and developing new dosage form, it is essential to use a sensitive and precise analytical method to determine the concentration of scutellarin in biological fluids. Some methods have been developed for detection of scutellarin in animal plasma in recent years [5–8]. However, to our knowledge, there is still no method described for the determination of scutellarin in human plasma. It is difficult to detect the scutellarin in human plasma, because it appears to low plasma concentration by administrating a clinic dosage orally. Therefore, some researchers had to investigate the pharmacokinetics through the way of determining the metabolite of scutellarin [9] which is not considered to show the vivo procedure really. In order to investigate the pharmacokinetics exactly, we firstly established a LC–MS/MS method to determine scutellarin in human plasma in this study, and it is sensitive enough to be applied to a pharmacokinetic study under a low clinic dosage (60 mg).

2. Experimental

2.1. Materials

Scutellarin (98.0% purity) and baicalin (internal standard, 98.0% purity) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol (HPLC-grade) was purchased from Merck Company (America). Formic acid (analytical grade) and phosphoric acid (analytical grade) were from Nanjing Chemical Co.

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Table 1
Gradient procedure of scutellarin

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0–1.0	85	15
1.01–9.0	40	60
9.01–16.0	30	70
16.01–18.0	85	15

(Nanjing, China). Heparinized blank (drug free) human plasma was obtained from Nanjing Blood Donor Service (China). Distilled water, doubly distilled in the laboratory, was used throughout the study.

2.2. Instrumentation

A Thermo Finnigan TSQ quantum ultra tandem mass spectrometer equipped with electrospray ionization (ESI) source (San Jose, CA, USA), a Finnigan surveyor LC pump and autosampler were used for LC–MS/MS analysis. Data acquisition was performed with Xcalibur 1.1 software (Thermo-Finnigan). Peak integration and calibration were carried out using LC Quan software (Thermo-Finnigan). The Alltech Extract-clean SPE columns (500 mg, 4 mL) were purchased from Alltech Company, and the DL-1 solid phase extraction equipment was purchased from Dalian Institute of Chemical Physics of Chinese Academy of Sciences.

2.3. LC–MS/MS conditions

The chromatographic separation was achieved on a Diamonsil C_{18} column (250 mm × 4.6 mm i.d., 5 μm, Dikma, Beijing, China). Mobile phase A was water with 0.1% formic acid, mobile phase B was methanol. The gradient procedure was shown in Table 1.

The liquid flow-rate was set at 1.0 mL/min, and the column temperature was maintained at 30 °C.

Mass spectrometer was operated in the positive mode. Quantification was performed using selected reaction monitoring (SRM) of the transitions of m/z 463.0 → m/z 287.0 for scutellarin and m/z 447.0 → m/z 271.0 for baicalin (internal standard, I.S.), respectively, with a scan time of 0.3 s per transition.

In order to optimize all the MS parameters, a standard solution (1 μg/mL) of the analyte and I.S. was infused into the mass spectrometer. For both scutellarin and baicalin, the following optimized parameters were obtained. The spray voltage was set at 4.0 kV. Nitrogen was used as the sheath gas (40 psi) and auxiliary gas (5 l/min). The heated capillary temperature was set to 350 °C. For collision-induced dissociation (CID), argon was used as the collision gas at a pressure of approximately 1.5 mTorr. The optimized collision energy of 12 eV was chosen for both scutellarin and I.S.

2.4. Sample preparation

The SPE column was activated successively with 2 mL of methanol and 2 mL of water. One millilitre of 0.5% phosphoric acid solution and 1.0 mL of plasma sample were vortex-mixed together for 1min. Then the sample was transferred into the column, and mild suction was applied so that the sample passed through the column at a steady flow rate (about 2 mL/min). After that, 2 mL of 0.5% phosphoric acid solution was used to wash the column. Then the column was eluted with 2.0 mL of methanol. The collected eluent was evaporated to dryness at 40 °C under a stream of nitrogen. The residue was redissolved in 100 μL of mobile phase (methanol:water = 7:3), and 20 μL was injected into the LC–MS/MS system for analysis.

2.5. Preparation of standard and quality control samples

Stock solution of scutellarin was prepared in methanol at the concentration of 100 μg/mL. Stock solution of I.S. was prepared in methanol at the concentration of 100 μg/mL and diluted to 200 ng/mL with methanol. Calibration curves were prepared by spiking the appropriate standard solution to 1.0 mL of blank plasma. Effective concentrations in plasma samples were 0.2, 0.5, 1, 2, 5, 10, 20 ng/mL for scutellarin. The quality control (QC) samples were separately prepared in blank plasma at the concentrations of 1.0, 5.0 and 10 ng/mL, respectively. The spiked plasma samples (standards and quality controls) were then treated following the "Sample preparation" procedure on each analytical batch along with the unknown samples.

2.6. Method validation

Plasma samples were quantified using the ratio of the peak area of scutellarin to that of I.S. as the assay parameter. Peak area ratios were plotted against scutellarin concentrations and standard curves were in the form of $y = A + Bx$.

To evaluate linearity, plasma calibration curves were prepared and assayed in duplicate on separate 5 days. The accuracy and precision were also assessed by determining QC samples at three concentration levels on three different validation days. The accuracy was expressed by (mean observed concentration)/(spiked concentration) × 100% and the precision by relative standard deviation (R.S.D.%).

Absolute recoveries of scutellarin at three QC levels were determined by assaying the samples as described above and comparing the peak areas of both scutellarin and I.S. with those obtained from direct injection of the compounds dissolved in the supernatant of the processed blank plasma.

The stability of the stock solution of scutellarin was determined by placing the stock solution in the refrigerator (4 °C) for a week. Scutellarin stability in plasma was assessed by analyzing QC samples at concentrations of 1.0, 5.0 and 10 ng/mL, respectively, exposed to different time and temperature conditions. The long-term stability was assessed after storage of the test samples at −20 °C for 5 days. The freeze–thaw stability was determined after five freeze–thaw cycles (−20 to 20 °C) on consecutive days. The extraction storage stability was assessed by placing QC samples being extracted at −20 °C for 5 days and analyzed. The results were compared with those QC samples freshly prepared, and the percentage concentration deviation was calculated.