Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 815–819

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Pharmacokinetic study of six flavones in rat plasma and tissues after oral administration of 'JiangYaBiFeng' using SPE-HPLC–DAD

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ARTICLE INFO

Article history:
Received 31 March 2011
Received in revised form 9 July 2011
Accepted 14 July 2011
Available online 22 July 2011

Keywords:
JiangYaBiFeng tablet
Flavones
HPLC–DAD
Rat plasma
Tissue distribution

ABSTRACT

In this study, a high performance liquid chromatography (HPLC) coupled with diode array detection (DAD) for simultaneous determination of six flavones including baicalein, sophoricoside, rutin, baicalin, quercetin and genistein in rat plasma and tissues after oral administration of JiangYaBifeng (JYBF) tablets was developed. The investigated analytes in plasma and tissues were extracted and purified with liquid–liquid extraction and solid phase extraction (SPE). Chromatographic separation was accomplished on a DIONEX Acclaim C18 column (250 mm × 4.6 mm, 5.0 μm particle size) with a simple linear gradient elution. The calibration curves for all the flavones had good linearity in the measured range with R^2 higher than 0.9983. The relative errors (REs) of the intra- and inter-day accuracy at different flavones levels were all less than ±10%. The proposed method enables unambiguous identification and quantification of investigated flavones in vivo. This is the first report on determination of the major flavones in rat plasma and tissues after oral administration of JYBF tablets. The results provided a meaningful basis for evaluating the clinical application of this medicine.

1. Introduction

'JiangYaBiFeng' tablet (JYBF tablet), a new drug for the treatment of hypertension has been widely used in the clinic in china because of its good effect and small side effects. It is composed of two western medicines (pargyline and hydrochlorothiazide) and three traditional Chinese medicines (TCMs), including *Scutellaria baicalensis*, *Sophora japonica* and *Arachis hypogaea*. According to some reported papers [1–6], flavones, such as baicalin, baicalein, sophoricoside, rutin, quercetin and genistein originally from the three TCMs are the main effective components contained in this formulation. Because the therapeutic effects of TCMs are based on the complex interactions of multiple ingredients, investigation of the pharmacokinetic studies of multi flavones after administration of JYBF tablets is essential to understand their role in human health and evaluate the clinical efficacy of this medicine. However, as far as we know, such relevant reports have not been found in the literature.

In this study, a reliable SPE-HPLC–DAD method for the simultaneous determination of six active components (including sophoricoside, rutin, baicalin, baicalein, quercetin and genistein) in rat plasma and tissues after oral administration of JYBF tablet was developed and validated. The pharmacokinetics of these flavones in plasma and tissue were first investigated and the obtained results would be very helpful for evaluating the clinical application of this medicine.

2. Experimental

2.1. Chemicals and reagents

JYBF tablet was supplied by Chinese pharmaceutical manufacturer (Zhongxin, Tianjin, China). Baicalin, baicalein, sophoricoside, rutin, quercetin and genistein were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC grade acetonitrile and methanol were purchased from Tianjin Kermel Chemical Regent Company (Tianjin, China). Other reagents were all of analytical grade. Ultrapure water was generated from the Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Instrumentation and analytical conditions

The HPLC system Dionex P680 series (Dionex, USA), equipped with the Chromeleon software (Dionex) and comprised a quaternary pump, an online vacuum degasser, a manual sampler, a

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doi:10.1016/j.jpba.2011.07.015

thermostated column compartment and a diode array detection (DAD), was used for the chromatographic analysis. All separations were carried out on a DIONEX Acclaim C_{18} column (250 mm × 4.6 mm id, 5 μm particle size) with a gradient elution of the mobile phase system consisting of acetonitrile (A) and 50 mM ammonium dihydrogen phosphate (pH 3.0) (B). The elution program was performed as the following: 78–73% A at the beginning of 12 min, then 73–67% A within 12–22 min and 67% A at the last 10 min. The flow rate was 1.0 mL min^{-1}. Column temperature was maintained at 25 °C. According to the different absorption spectrums of the described flavones, effluent was monitored at 256 nm for sophoricoside, rutin, quercetin, genistein and 280 nm for baicalin and baicalein. The injection volume was 20 μL. The peak identification was based on the retention time and the DAD spectrum against the standard presented in the chromatogram.

The SPE cartridges (C_{18}, 45 μm particle size, 50 mg) were purchased from Agela Technologies Inc. (USA). Before used, they were successively preconditioned with 1 mL of methanol and 1 mL of distilled water.

2.3. Preparation of standard solutions, calibration standards and quality samples

The stock solutions of the investigated flavones were prepared in methanol, respectively. A series of standard mixture working solutions were obtained by diluting the mixture of the stock standard solutions with mobile phase.

Calibration standards of the mixture flavones were prepared by spiking the appropriate amount of the standard mixture working solutions into 200 μL drug-free rat plasma or tissue homogenates to give nominal concentration range of 0.10–62.50 μg mL^{-1} for sophoricoside, rutin and quercetin, 0.10–100.00 μg mL^{-1} for baicalin and baicalein, 0.10–50.00 μg mL^{-1} for genistein.

Quality control samples were prepared at low, medium and high concentrations (0.5, 5.0 and 50.0 μg mL^{-1}) in the same manner as the calibration standards, and used to assess the accuracy and precision of the method. The samples were extracted following the procedure described below.

2.4. Pretreatment of plasma or tissue sample

To 200 μL of plasma or tissue homogenate samples, 500 μL of 5% trichloroacetic acid was added. After centrifugation at 12,000 rpm for 10 min at 4 °C, the supernatant was collected and flowed through a pre-treatment C_{18} SPE cartridge with gravity. The solid-phase cartridge was washed with 1.0 mL 5% methanol and then eluted with 1 mL of methanol. The methanol elute was evaporated to dryness under a stream of nitrogen at 50 °C. The residue was dissolved into 100 μL methanol for the HPLC analysis.

The extraction recovery analysis was conducted with spiked flavone biosamples at three QC levels and calculated by comparing the flavone peak area in extracted biosamples with those found by direct injection of standard solutions at the same concentration.

2.5. Method validation

The accuracy and precision of the established method were evaluated by QC samples at low, medium and high concentrations. The concentration of each QC samples was calculated using calibration curves. Accuracy was defined as the relative deviation in the calculated value of a standard from that of its true value, expressed as relative error (RE). Precision was evaluated as the relative standard deviation (RSD). The intra-day accuracy was determined by assaying six replicates at each concentration level on 1 day, and inter-day accuracy was determined by analyzing QC samples in five duplicates during three separate and successive days.

The stability of flavones in biosamples was investigated under a variety of storage and process conditions. For storage stability, samples (5.0 μg mL^{-1} of flavones in plasma and tissues) were prepared and stored at −20 °C for 30 days. On the 30th day, all samples were thawed and analyzed along with the freshly prepared set of quality control samples; for freeze–thaw stability testing, the QC were determined after three freeze–thaw cycles and the concentration were compared to their nominal concentrations.

2.6. Pharmacokinetic study in rat plasma and tissue

Male and female Sprague–Dawley rats weighing 220–250 g were obtained from the Henan Laboratory Animal Center (Zhengzhou, China). After breeding in a controlled environment for 5 days, the rats were orally administrated JYBF tablets at a dose of 2.0 g kg^{-1} (approximately 7.56 mg kg^{-1} sophoricoside, 17.56 mg kg^{-1} rutin, 52.48 mg kg^{-1} baicalin, 0.32 mg kg^{-1} quercetin, 0.68 mg kg^{-1} genistein and 66.05 mg kg^{-1} baicalein). For pharmacokinetic study, the blood samples (0.5 mL) of 5 rats were collected from the fossa orbitalis vein according to the specific schedule at times of 10, 20, 30, 45, 60, 90, 120, 180, 240, 360, 480 and 720 min after dosing. The blood samples were immediately transferred to heparinized tubes and centrifuged at 4000 rpm for 10 min. The separated plasma was frozen at −20 °C before assay. For tissue distribution study, 30 rats were assigned randomly to three groups. After administration of JYBF tablets as described above, tissues including heart, liver, spleen, lung, kidney, stomach, small intestine and brain were obtained at 10, 20, 30, 45, 60, 90, 120, 180 and 240 min after administration. All samples were thoroughly rinsed of residual blood and other contents with physiological saline solution. Then each sample was blotted on filter paper and then weighed for wet weight and individually homogenized with the same mass of saline solution. The obtained tissue homogenates were stored at −20 °C until analysis.

3. Results and discussion

3.1. Selectivity

The selectivity of the method was tested by comparing the chromatograms of blank plasma and tissue, spiked plasma and tissue and actual plasma and tissue samples after oral administration of JYBF tablets at a dose of 2.0 g kg^{-1}. It was indicated that analytes were well separated and no interferences were detected from endogenous substances or metabolites. The representative chromatograms for determination of analytes in plasma and tissues are shown in Figs. 1 and 2, respectively.

3.2. Linearity, limit of detection and limit of quantification

The linear regression of the investigated flavones in rat plasma and tissues was constructed by plotting peak area with concentration of standard solutions. The calibration curves showed good linearity over the concentration range 0.10–62.50 μg mL^{-1} for sophoricoside, rutin and quercetin, 0.10–100.00 μg mL^{-1} for baicalin and baicalein, and 0.10–50.00 μg mL^{-1} for genistein in all biosamples with a correlation coefficient (R^2) larger than 0.9983. The limits

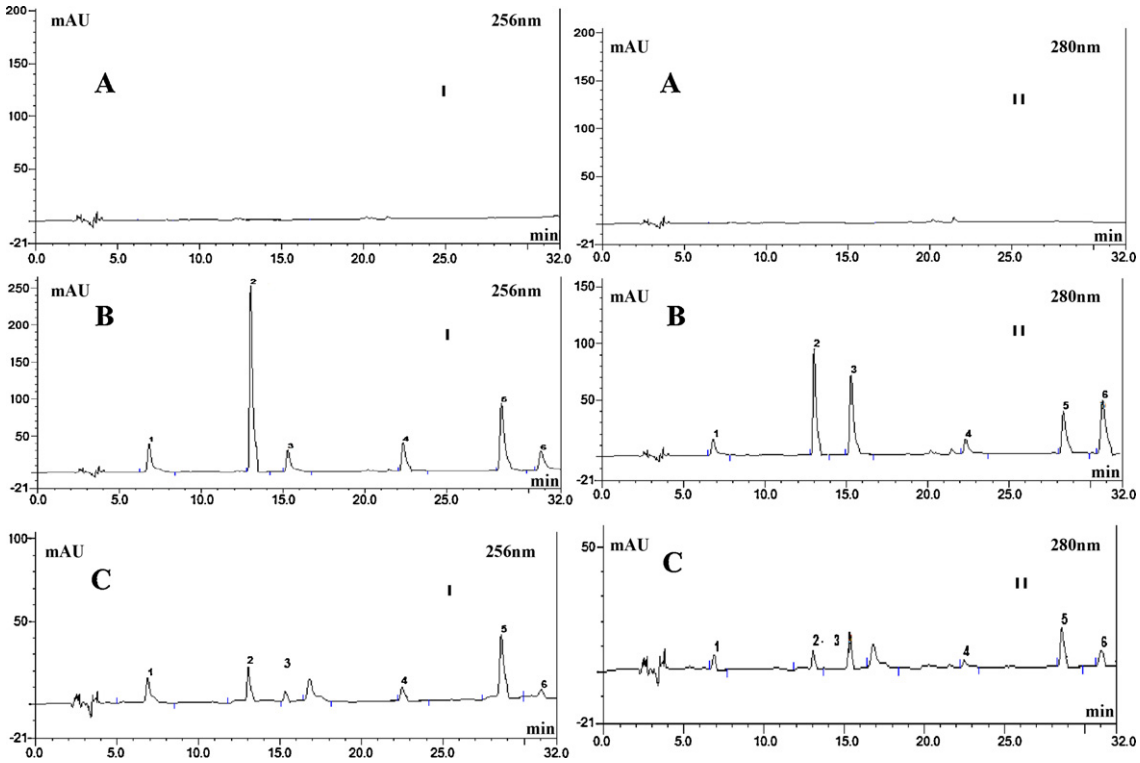

Fig. 1. Representative chromatograms of blank plasma (A), plasma sample spiked with six flavones (B, 10 μg mL^{-1} sophoricoside, 30 μg mL^{-1} rutin, 20 μg mL^{-1} baicalin, 10 μg mL^{-1} quercetin, 10 μg mL^{-1} genistein and 10 μg mL^{-1} baicalein) and a plasma sample collected from a rat at 1 h after an oral administration of JYBF tablet (C). I: 256 nm; II: 280 nm. (1) Sophoricoside, (2) rutin, (3) baicalin, (4) quercetin, (5) genistein and (6) baicalein.

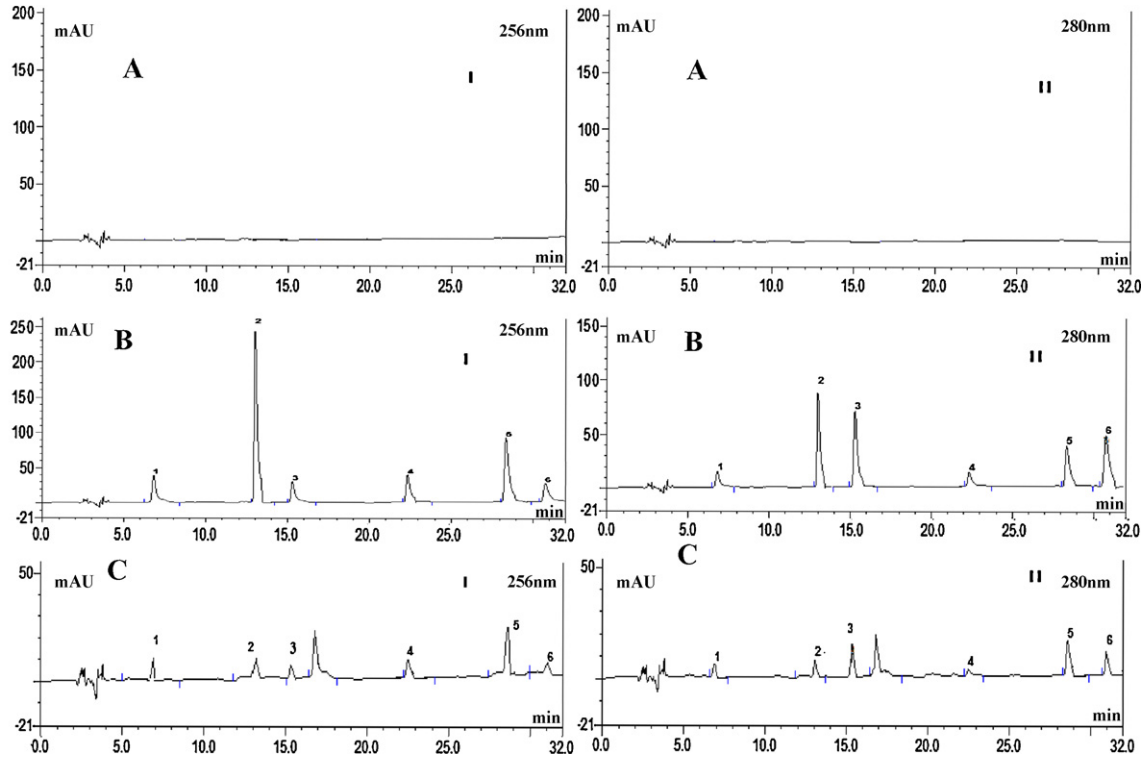

Fig. 2. Representative chromatograms of blank liver tissue (A), liver tissue sample spiked with six flavones (B, 10 μg mL^{-1} sophoricoside, 30 μg mL^{-1} rutin, 20 μg mL^{-1} baicalin, 10 μg mL^{-1} quercetin, 10 μg mL^{-1} genistein and 10 μg mL^{-1} baicalein) and a liver tissue sample collected from a rat at 1 h after an oral administration of JYBF tablet (C). I: 256 nm; II: 280 nm. (1) Sophoricoside, (2) rutin, (3) baicalin, (4) quercetin, (5) genistein and (6) baicalein.

Table 1
Pharmacokinetics parameters of six flavones after an oral administration of JYBF tablet ($n = 5$).

Parameter	Value					
	Sophoricoside	Rutin	Baicalin	Quercetin	Genistein	Baicalein
$T_{1/2\alpha}$ (h)	0.41	0.34	0.18	0.16	0.49	0.15
$T_{1/2\beta}$ (h)	1.70	2.04	3.84	4.42	1.41	4.70
$AUC_{(0-t)}$ (mg/L h)	0.79	1.79	2.87	2.17	0.61	2.79
$AUC_{(0-\infty)}$ (mg/L h)	0.84	2.00	3.96	4.54	0.87	8.22
$V_{L/F}$ (L/kg)	10.08	11.62	11.59	3.08	3.26	3.45
K_a (1/h)	1.70	2.03	3.84	4.42	1.41	4.69
C_{max} (mg/L)	0.50	0.98	1.83	0.85	0.17	1.82
T_{max} (h)	0.75	0.75	0.75	1	1	1

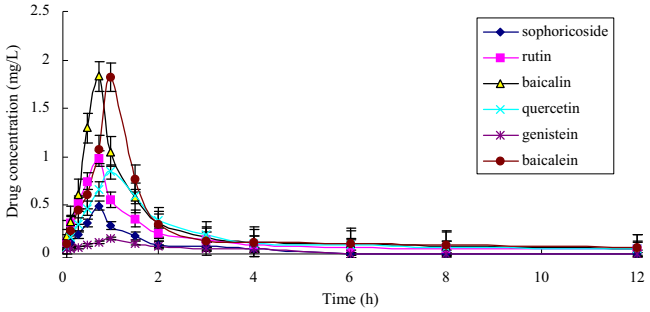

Fig. 3. Blood concentration–time profiles of six flavones after an oral administration of JYBF tablet. Each data point was given as the form of mean ± S.D. of five experiments.

of detection (LOD, S/N = 3) and the lower limits of quantification (LLOQ, S/N = 10) for sophoricoside, rutin, baicalin, quercetin, genistein and baicalein were 0.04, 0.01, 0.05, 0.03, 0.05, 0.02 μg mL^{-1} and 0.13, 0.04, 0.15, 0.08, 0.15, 0.07 μg mL^{-1}, respectively.

3.3. Method validation

Intra- and inter-day precision and accuracy were determined by measuring QC samples as described in Section 2. The relative errors (REs) were obtained ranging from −9.6% to 8.8% in intra-day accuracy and from −9.8% to 7.0% in inter-day accuracy with RSD less than 9.0%. The results indicated that overall reproducibility of the method was acceptable.

The mean extraction recoveries of the investigated flavones in plasma at three different concentration levels were found to be 90.2–95.6% with RSD less than 8%, and the mean recoveries in all tissue samples were above 88.7%.

The stability of the investigated flavones was evaluated by analyzing the QC samples according to the procedures described in Section 2.5. The RSD of the concentrations of the QC samples tested were all within 5%. The results suggested that all the analytes were stable under the indicated storage conditions.

3.4. Pharmacokinetics study

The mean plasma concentration–time profiles of the investigated components were shown in Fig. 3, demonstrating that flavones was rapidly absorbed and then slowly decreased. The pharmacokinetics model and the parameters were calculated by the practical pharmacokinetics program 97 (3P97). It was found that the concentration–time profile was best described by the two-compartment model for all flavones. The main pharmacokinetics parameters are summarized in Table 1.

3.5. Tissues distribution study

The AUC of six investigated flavones is shown in Fig. 4. It was indicated that all flavones could be rapidly absorbed and distributed to all collected tissues except quercetin and genistein. At predetermined times, quercetin and genistein were few or undetectable in both brain and spleen, demonstrating that they have difficulty crossing the blood–brain barrier and spleen might not be the primary absorbent organ of them.

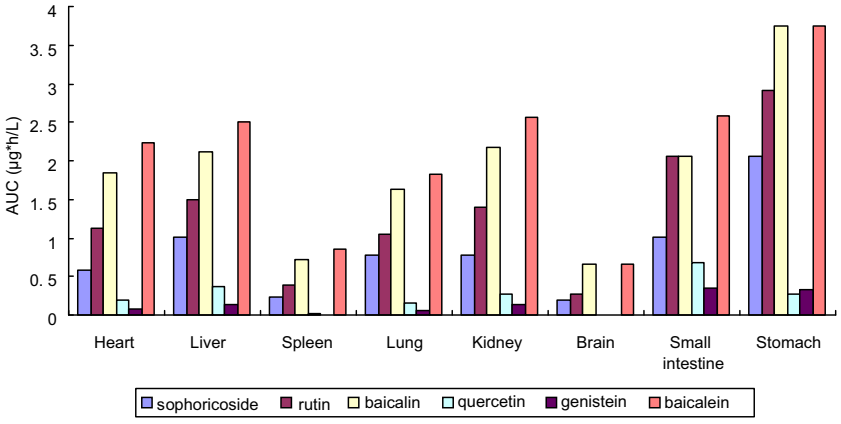

Fig. 4. The area under curve (AUC) of six flavones in various tissues after an oral administration of JYBF tablet.

4. Conclusion

In this paper, a SPE-HPLC–DAD method for simultaneous determination of six flavones including baicalein, sophoricoside, rutin, baicalin, quercetin and genistein in rat plasma and tissues after oral administration JYBF tablet was developed and validated. The achieved pharmacokinetics and tissue distribution results may be useful for further study of the bioactive mechanism of a new Chinese medicine-JYBF tablet.

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