Asian Journal of Chemistry; Vol. 25, No. 7 (2013), 3741-3743

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13736

Pharmacokinetics and Relative Bioavailability of Breviscapine Pills in Human Plasma by HPLC

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(*Received*: 15 March 2012; *Accepted*: 11 January 2013) AJC-12685

To evaluate the bioequivalence of scutellarin in breviscapine pills and tablets, the randomized two-way crossover study was conducted in 18 healthy volunteers. After a single dose (containing 160 mg brevisapine), the plasma drug levels were determined by HPLC. By analysis of variance, the main pharmacokinetics parameters of test and reference formulations were followed that t_{max} were 5.94 ± 0.24 and 6.00 ± 0.0 (h), C_{max} were 645.23 ± 48.71 and 599.88 ± 61.19 (ng/mL), $t_{1/2}$ were 3.68 ± 2.15 and 4.05 ± 2.74 (h), $AUC_{0\rightarrow24}$ were 3726.69 ± 700.06 and 3213.94 ± 667.88 (ng/mL·h). The results showed that the two formulations were bioequivalent. The relative bioavailability of the Breviscapin pills was within 117.89 ± 19.16 %.

Key Words: Breviscapine, Scutellarin, Bioavability, HPLC.

INTRODUCTION

Scutellarin is a flavone glucuronide extracted from a Chinese herb Erigero breviscapus (Vant.) Hand. -Mazz. It is not only an important component of a Chinese herb, but also a major constituent of skullcap, which is a popular western herb[1]. Scutellarinhas 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*. In clinic, scutellarin is widely used in treating various cardiovascular diseases such as coronary heart disease, angina pectoris and thrombosis[2,3]. 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. There have been several analytical methods including high performance liquid chromatography (HPLC) with UV and UV *etc*.[4,5]. So far, many literatures have reported the study of validation for the determination of scutellarin in rat, rabbit and dog plasma and its pharmacokinetics by an HPLC method. However, to our knowledge, there are still few methods described for the determination of scutellarin in human plasma[6].

In the present study, we firstly established an HPLC-UV method for determining scutellarin in human plasma that is sensitive, simple and suitable enough to be applied to a pharmacokinetic study under a clinic dosage (160 mg)[7,8].

EXPERIMENTAL

The Shimadzu HPLC system (Japan) consisted of LC-10AT pump, a SPD-10A UV Detector and a 7725i sample injector. The analytical column used was kromasil C_8 (4.6 mm × 250 mm) from Dalian Zhonghuida Science Instruments Co. Ltd., (Dalian, China). The HPLC system was controlled by winmenu ChemStation.

Acetonitrile and methanol were of HPLC grade and ethylacetate was of analytical grade (Tianjin Concord Tech Co. Ltd.). Phosphoric acid was of analytical grade (Shenyang Economy Technology Developed Section Reagent Factory). Water was tri-distilled water.

Test formulation: Breviscapine pills (6.65 mg/pill), Tianjin Meilun Pharmaceutical Group Co. Ltd.

Reference formulation: Breviscapine tables (40 mg/tablet), Guangdong MediWorld Pharmaceutical Co. Ltd.

Study design: Eighteen healthy male adult volunteers participated in a double-period and two-formulation crossover study, which were randomly grouped into two groups. There are nine people in each group. The selected volunteers were considered healthy on the basis of detailed medical history. After obtaining the institutional review board approval and explaining the research protocol with possible side effects, the volunteers were asked to sign consent forms. Verbal assurance was taken from all of them that they have not taken any drugs during and for 1 week preceding the study. One week was kept as a wash out period before cross over. They were fasted overnight before the experiments. A single oral dose of 24 pills (containing 159.6 mg of scutellarin) or 4 tablets (containing 160 mg of scutellarin) were administeted. On the day of experiment, venous blood samples (5 mL) were

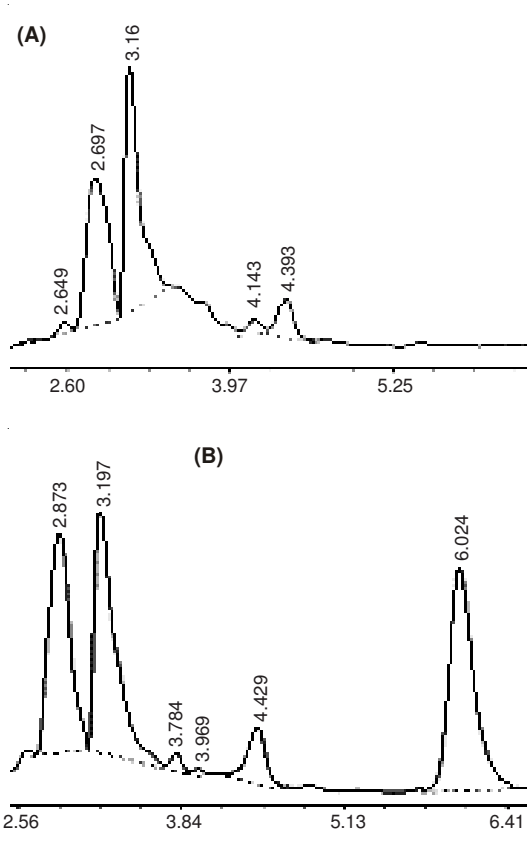
withdrawn immediately just before dosing and after 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 24 h of dosing. The samples were collected in heparinized tubes, immediately centrifuged for 5 min (4000 r/min) and the separated plasma samples were frozen at -20 ºC until analysis. Simultaneously, the doctor observed possible emergence[9].

Analysis of plasma sample

Chromatographic condition: The column was Kromasil C_8 (4.6 mm × 250 mm). Mobile phase was methanol, acetonitrile and water (25:15:60, v/v/v, adjusted to pH 3.0 with phosphoric acid). The solution was measured at 335 nm at 30 ºC with 1 mL/min.

Plasma sample preparation: The plasma sample of 1 mL was taken and ethylacetate of 2 mL was added into the plasma and solution and 1 mL of plasma sample were vortex-mixed together for 1 min, followed by centrifugation at 10000 r/min for 3 min. The extraction was repeated with ethylacetate of 2 mL. All the supernates were combined and transferred to another tube and evaporated to dryness at 40 ºC under a gentle stream of nitrogen. The residue was dissolved in 50 µg mobile phase and 20 µL was injected into the HPLC.

Specificity: Selectivity was assessed by comparing the chromatograms of blank human plasma with the corresponding spiked plasma. Fig. 1 shows the typical chromatograms of a blank, a spiked plasma sample with scutellarin and a plasma sample from a healthy volunteer after an oral administration. There was no significant interference or ion suppression from endogenous substances observed at the retention times of the analytes. Typical retention times for scutellarin was about 6 min.



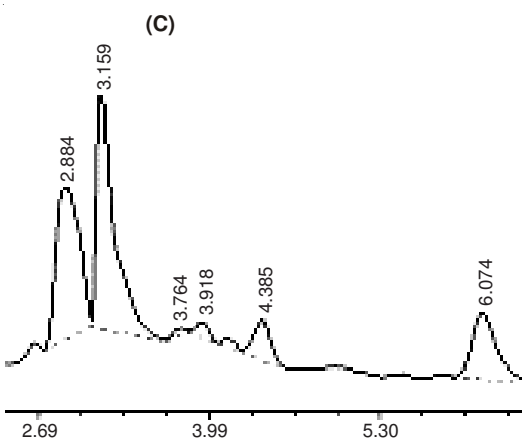


Fig. 1. Blank plasma (A), blank plasma spiked with scutellarin standard (B), derivation of volunteer plasma after oral administration of 160 mg of brevisapine) (C)

Preparation of standard solution: Stock solution of scutellarin was prepared in methanol at the concentration of 100 µg/mL. A series of standard working solutions with concentrations in the range of 1, 2, 5, 5, 10, 25, 50 and 100 µg/mL for scutellarin were obtained by further dilution of the stock solution with mobile phase. All the solutions were stored at 4 ºC.

Preparation of standard and quality control samples: Calibration curves were prepared by spiking the appropriate standard solution to 1 mL of blank plasma. Effective concentrations in plasma samples were 50, 125, 250,500, 1250, 2500 and 5000 ng/mL for scutellarin. The quality control (QC) samples were separately prepared in blank plasma at the concentrations of 125, 500 and 2500 ng/mL, respectively. All the solutions were stored at 4 ºC.

Calibration curves and linear range: The spiked plasma samples (standards and quality controls) were then treated following the sample preparation procedure. 20 µL was injected into the HPLC. Visual inspection of the plotted duplicate calibration curves and correlation coefficients > 0.999 confirmed that the calibration curves were linear over the concentration ranges 50-5000 ng/mL for the analyte. Typical standard curve was $\gamma = -75.493 + 24.939X$. Where γ represents the scutellarin peak area and X represents the plasma concentrations of scutellarin. The lower limit of quantification (LLOQ) was 50 ng/mL.

Assay precision and accuracy: Table-1 summarizes the intra-day and inter-day precision and accuracy for scutellarin evaluated by assaying the quality control samples. The precision was calculated by using two-way ANOVA. In this assay, the intra-run precision was 8.98 % or less and the inter-run precision was 12.4 % or less for each quality control level of scutellarin. The accuracy was within ± 5.0 %. The results above demonstrated that the values were within the acceptable range and the method was accurate and precise.

Extraction recovery: The analyte recoveries under the liquid-liquid extraction conditions were 60.44 ± 2.6, 59.96 ± 2.3 and 60.02 ± 1.6 % at concentrations of 125, 500 and 2500 ng/mL (QC samples), respectively. The RSD was 4.3, 3.8 and 2.7 %, respectively (n = 5).

Stability study of scutellarin in plasma samples: The stabilities of scutellarin in plasma (500 ng/mL) were studied

at room temperature after 0, 1, 2, 4, 8 and 24 h, respectively. The frozen plasma samples were still stable, which were stored at -20 ºC for 1 month and determined on 0 d, the 10th d, the 20th d, 30th d and treated after three freeze and thaw cycles. The results were shown in Table-2.

TABLE-1
ACCURACY AND PRECISION FOR THE ANALYSIS OF SCUTELLARIN IN HUMAN PLASMA (IN PRESTUDY VALIDATION, *n* = 5 DAYS, FIVE REPLICATES PER DAY)

Added concentration (ng/mL)	Found concentration (ng/mL)	Accuracy (%)	Precision (%)	
			Intra-day	Inter-day
125	126	100.8	2.6	3.4
500	503.5	100.7	2.0	2.3
2500	2537.5	101.5	1.2	1.6

TABLE-2
STABILITY OF SCUTELLARIN IN RAT PLASMA (500 ng/mL)

	($\bar{x}$ ± s) (ng/mL)	RSD (%)
Room temperature (n = 6)	496.90 ± 39.06	7.9
Thawing (n = 3)	505.59 ± 20.21	4.0
Frozen (-20 ºC) (n = 4)	525.0 ± 20.59	3.9

RESULTS AND DISCUSSION

Plasma concentration of scutellarin: The method was applied to determine the plasma concentration of scutellarin after an oral administration of reference formulation and test formulation of breviscapine to 18 volunteers. The mean plasma concentration-time curve of scutellarin was shown in Fig. 2.

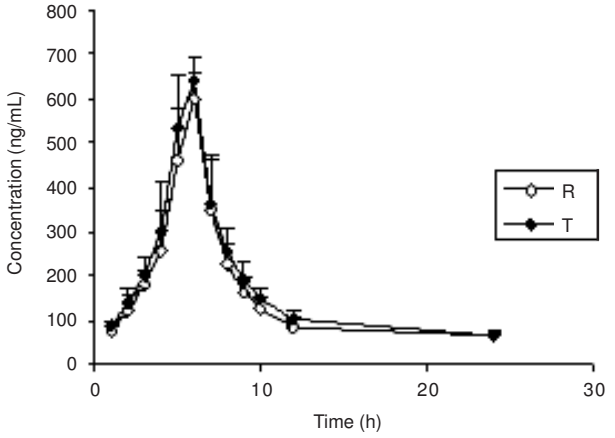


Fig. 2. Mean plasma of brevisapine derivation concentration-time curve after oral administration of 160 mg dose of brevisapine

Pharmacokinetic parameters: From Fig. 2, area under the curve (AUC), maximum plasma concentration (C_{max}), time for maximum concentration (T_{max}) and elimination half-life ($t_{1/2}$) were obtained. The main pharmacokinetic parameters of scutellarin in 18 volunteers were shown in Table-3. Pharmacokinetic parameters of the test formulation and the reference formulation were analyzed by ANOVA of crossover study. Bioequivalence of scutellarin in breviscapine pills and tablets was evaluated by two one-side test and confidence intervals (1-2α) method.

Optimization of Sample extraction conditions[10]: Preparing plasma sample is very crucial in determination of plasma concentration. To gain optimization of sample extraction, we used many precipitator and extraction solvents. For example, methanol, acetonitrile and perchloric acid were used to precipitate protein. Other extraction solvents were tested, including ethyl ether, dichloromethane, ethyl acetate. We found that protein precipitation is not suitable for scutellarin because it will dilute the plasma concentration, which makes the sensitivity poorer. Only if the plasma was extracted with ethylacetate for two times, there was no interference in the chromatogram of blank plasma and no influence to column. Extraction recovery was good.

TABLE-3
PHARMACOKINETICS PARAMETERS AFTER ORAL ADMINISTRATION OF THE REFERENCE OR TEST CAPSULES

Pharmacokinetic parameters	Reference formulation	Test formulation
$T_{1/2}$ /h	4.05 ± 2.74	3.68 ± 2.15
T_{max} /h	6.00 ± 0.0	5.94 ± 0.24
C_{max} /ng mL^{-1}	599.88 ± 61.19	645.23 ± 48.71
$AUC_{0\to24}$ /ng h^{-1} mL^{-1}	3213.94 ± 667.88	3726.69 ± 700.06
$AUC_{0\to\infty}$ /ng h^{-1} mL^{-1}	3492.51 ± 747.08	3930.30 ± 591.35

Optimization of mobile phase: We have selected varied mobile phase system such as methanol-water, acetonitrile-water, methanol-tetrahydrofuran-water, methanol-acetonitrile - water, methanol- potassium phosphate monohasic in water. From the results the mutual separation of scutellarin and endogenous substances was optimized by using the mobile phase with methanol -acetonitrile-water (25:15:60, v/v/v), adjusted to pH 3.0 with the use of an acid modifier, phosphoric acid. Under these optimum chromatographic conditions, the symmetry of the peak was good and retention time was suitable.

Conclusion

The analysis of variance indicated that C_{max} values, T_{max} values, $AUC_{(0\to24)}$ values and $AUC_{(0\to\infty)}$ values of two formulations were no statistically significant difference. By two one-side test and confidence intervals the results shown that t_1 and t_2 values of C_{max}, T_{max}, $AUC_{(0\to24)}$ and $AUC_{(0\to\infty)}$, of two formulations were also more than $t_{(1-0.05)}$. All the 90 % confidence intervals of the test/reference geometric mean ratio of parameters were within the bioequivalence limits. Pharmacokinetic profiles of the two formulations showed good sustained release properties and the two products were bioequivalent.

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