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Technical note

Determination of scutellarin in mouse plasma and different tissues by high-performance liquid chromatography

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Abstract

A simple HPLC–UV method was developed for the determination of scutellarin in plasma and different tissues of mice (heart, liver, spleen, lungs and kidneys). The separation was achieved by HPLC on a Hypersil C_{18} column with a mobile phase composed of methanol–water–glacial acetic acid (40:60:1). UV detection was used at 335 nm. The calibration curves were linear in all matrices ($r^2 > 0.997$) in the concentration range of 0.1–10 μg/ml for plasma and 0.1–20 μg/g for tissue homogenates, respectively. The method described is suitable for studies on the distribution of scutellarin in different tissues of mice.

Keyword: Scutellarin

1. Introduction

Scutellarin, a flavone glucuronide, extracted from a Chinese herb *Erigero breviscapinus* (Vant.) Hand.-Mazz [1], is widely used in the treatment of cerebral infarction and its sequelae, coronary heart disease and angina pectoris [2–4]. Scutellarin and its preparation were listed in the Pharmacon Criteria (Chinese Traditional Patent Medicine, vol. 20) [5]. The structure of scutellarin (4′,5,6-tetrahydroxyflavone-7-*O*-glucuronide) is shown in Fig. 1. Several HPLC methods are available to measure scutellarin in plasma by HPLC–UV [6,7] and in urine, feces and bile by HPLC–MS [8], but not for tissues. These methods are laborious because they require extraction of scutellarin from samples and using disposable cartridges.

With recent pharmaceutical and therapeutic development, lipid emulsions have been introduced as parenteral drug carriers offering sustained release and organ targeting [9–13]. In order to improve therapeutic index, scutellarin has been incorporated into a lipid emulsion preparation. The method described in this paper was simple and used to compare a new lipid emulsion formulation of scutellarin with the aqueous solution, which is currently being used clinically (Injectio Breviscapine). The concentrations of scutellarin have been measured in plasma and different tissues of mice after administration of the two formulations.

2. Experimental

2.1. Materials

Scutellarin standard (purity > 98%) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Injectio Breviscapine, which is an injection solution of scutellarin (20 mg/5 ml) was produced by Gejiu Bio-Medicine Industry Ltd. (Yunnan, China). Scutellarin lipid emulsion (40 mg vials; 40 mg scutellarin incorporated into a mixture of 10 g soybean oil, 1.2 g phosphatidylcholine, 2 g Pluronic F68 and 2.25% glycerin) was provided by Pharmaceutical Research Institute of China Pharmaceutical University (Nanjing, China). Methanol was of HPLC-grade, and other chemicals used were of analytical grade. Distilled water, prepared from demineralized water, was used throughout the study.

2.2. Chromatographic systems

The chromatographic system consisted of a Waters 510 HPLC pump and a Waters 486 Absorbance UV detector (Waters

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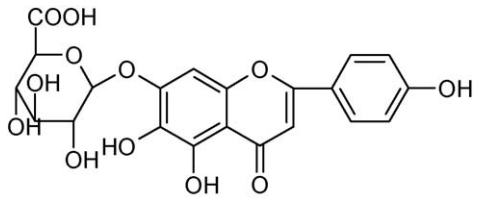

Fig. 1. Structure of scutellarin.

Corp., Milford, MA, USA). The wavelength of this detector was set to 335 nm. The HPLC system was controlled by a computer employing the Millennium 2010 ChemStation software. The analytical column was a reverse phase Hypersil C_{18} column (250 mm × 4.6 mm, 5 μm particle size; Dalian Elite Analytical Instrument Co., Ltd., Dalian, China) maintained in a column oven (Timberline Instruments, Boulder, CO, USA) and protected by a guard column (10 × 4.6 mm) packed with the same material. The mobile phase was composed of methanol–water–glacial acetic acid (40:60:1). Elution was performed isocratically at 40 °C at a flow-rate of 1.0 ml/min.

2.3. Preparation of stock solutions and calibration standards

A stock standard solution of scutellarin (500 μg/ml) was prepared in methanol. Further stock solutions (1.0–200 μg/ml) were made by diluting the initial stock standard solution with methanol. The stock solutions were stored at −20 °C and were brought to room temperature before use. For preparation of calibration standards and quality control samples, several scutellarin standards in the concentration range of 0.1–10 μg/ml in plasma or 0.1–20 μg/g in homogenates were prepared by adding 10–50 μl of scutellarin stock solutions to 990–950 μl plasma or to the different tissue homogenates of untreated mice.

2.4. Sample preparation

To prevent oxidative degradation of scutellarin, 10 μl of 10% sodium bisulfate solution was added to each 100 μl aliquot of mouse plasma sample. A 100 μl aliquot of homogenate (10%, w/v) of tissues (heart, liver, spleen, lungs and kidneys), in a solution of 1% sodium bisulfate in physiological saline, was performed with a potter on ice. A 200 μl aliquot of methanol was added to precipitate protein. Then, the samples were mixed with shaking on a SW-80A vortex shaker (Shanghai Medical University Instrument Plant, Shanghai, China) for 5 min. After centrifugation for 10 min and 10,000 × *g* at 4 °C (Refrigerated Centrifuge 3K30, Sigma, German), a 20 μl aliquot of the supernatant fluid was injected into HPLC for assay.

2.5. Precision and accuracy

To determine the within-day precision of the method, three samples of plasma and of each tissue (at low, mid, and high levels of the calibration range) were analyzed three times on the same day. To determine the between-day precision and the accuracy, three samples of plasma and the different tissue homogenates (at low, mid, and high levels of the calibration range) were run at three different days.

To obtain the within- and between-day coefficients of variation, the accuracy of the method was evaluated by analyzing recovery percentages. Recoveries were calculated by using the ratio of the detected to the added.

2.6. Analyte stability

Process stability was assessed by preparing three sets of plasma or tissue homogenate control samples at mid level of the calibration range. Aliquots of the extracted samples were run immediately after preparation and again after 3 h of storage at room temperature.

Storage stability of scutellarin was tested with spiked plasma (10 μl of 10% sodium bisulfate solution was added to each 100 μl aliquot of mouse plasma sample) or tissue homogenates samples at mid level of the calibration range stored at −20 °C. They were assayed in triplicate on the day of preparation (baseline) and thereafter at 1 and 3 months of storage. Stability was also tested by subjecting plasma or tissues control samples at mid level of the calibration range to three freeze (−20 °C)–thaw (ambient) cycles.

2.7. Application

Kunming mice (male, 8 weeks old, 18–22 g) were purchased from the Experimental Animal Center of China Pharmaceutical University. In order to study the distribution of scutellarin after the administration of two different pharmaceutical formulations, 25 mg/kg scutellarin as aqueous solution or in a lipid emulsion preparation were injected intravenously into the tail vein of the mice. At 30 min after administration, five animals were sacrificed under ether anesthesia. Blood was collected in heparin-coated tubes and centrifuged at 1000 × *g* for 5 min. Heart, liver, spleen, lungs and kidneys were removed, weighed and homogenized (10%, w/v) in a solution of 1% sodium bisulfate in physiological saline. All samples were immediately frozen at −20 °C until analysis.

3. Results and discussion

The chromatograms were free of interference from other compounds after precipitation of the protein from tissue homogenates and plasma. The scutellarin peak was symmetrical and baseline separation was obtained in all matrices (Fig. 2). The retention time was 9.0–9.5 min.

The calibration curves for scutellarin were linear in the range of 0.1–10 μg/ml plasma or 0.1–20 μg/g tissue homogenate (Table 1). The regression coefficients (r^2) were greater than 0.997. The LOD (S/N = 3) of scutellarin was 0.003 μg/ml for plasma and 0.03 μg/g for tissue homogenates tested. The results of accuracy, within- and between-day precision are summarized in Table 2. The validation of the sample preparation and HPLC procedure in the different tissues demonstrate that the method is accurate and precise with coefficients of variation within- and between-day below 8% for all the samples. The recoveries of

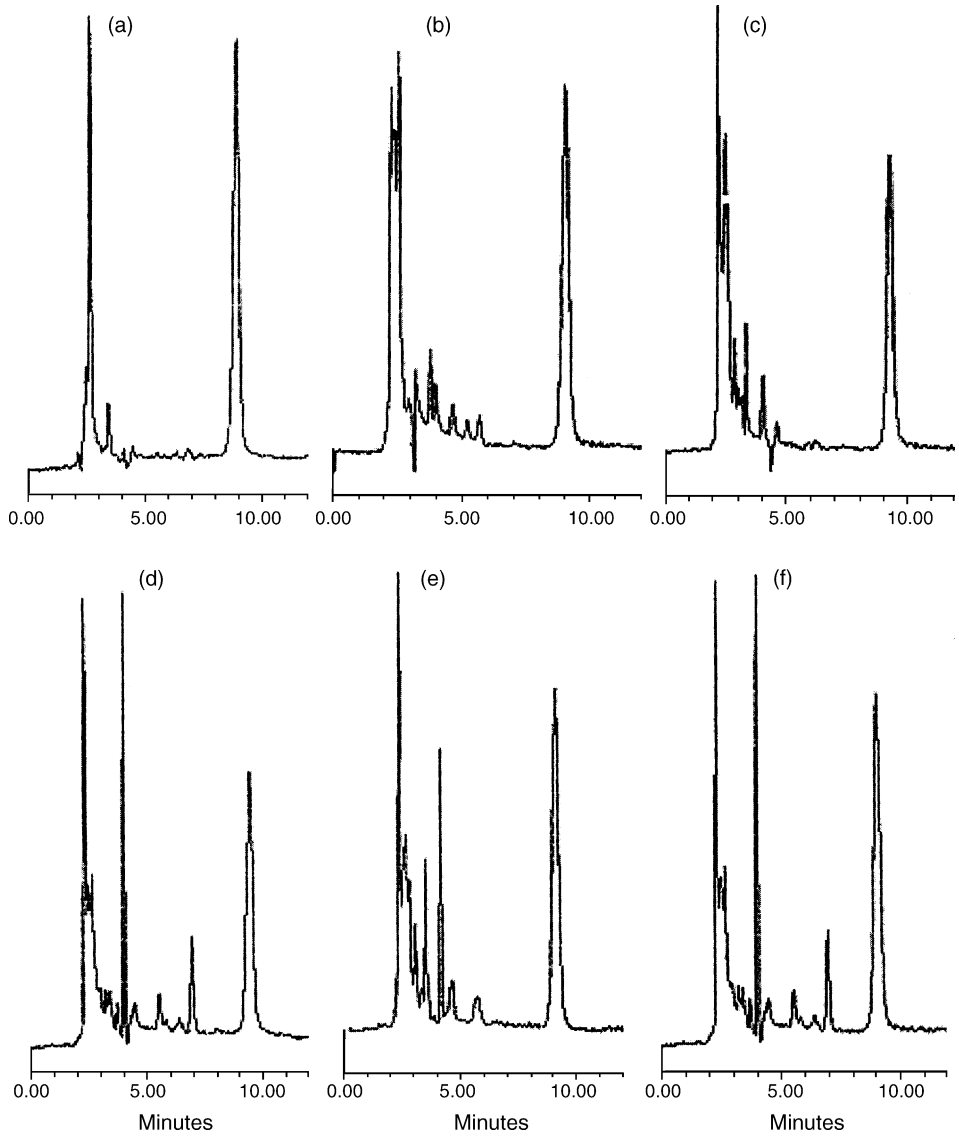

Fig. 2. Chromatograms of the different matrices spiked with scutellarin standard. The retention time is 9.0 min to 9.5 min: (a) plasma; (b) heart; (c) liver; (d) spleen; (e) lung and (f) kidney.

scutellarin ranged from 97 to 102% for plasma and 92 to 108% for tissue homogenates.

In process stability experiment, the analyte was found to be stable after 3 h of storage at room temperature. The mean ($n = 3$) values for accuracies were within 5.58% of their expected values, and percentage variation was equal to or less than 6.17 for all the samples. In storage stability experiment, scutellarin was stable in plasma or tissues for at least three months when stored at −20 °C. Mean ($n = 3$) accuracies were within 4.24% of the expected values, and percentage variation was equal to or less than 6.01 and 9.23, respectively, for 1 and 3 months of storage. Three freeze (−20 °C)–thaw (ambient) cycles had little effect on accuracy and precision of the results: The mean ($n = 3$) observed values were within 4.17% of the expected values, and percentage variations were within 7.59 for the assayed concentration of plasma or tissues.

Table 1
Calibration curves for scutellarin in plasma, heart, liver, spleen, lung and kidney

Sample matrix	Concentration (μg/ml or g)	Slope	Intercept (μg/ml or g)	Regression coefficient (r^2)
Plasma	0.10, 0.50, 1.00, 5.00, 10.00	1.34E+4	−7.63E+2	0.9981
Heart	0.15, 0.90, 1.50, 9.00, 15.00	1.46E+3	1.33E+2	0.9992
Liver	0.20, 1.00, 2.00, 10.00, 20.00	1.38E+3	1.71E+1	0.9974
Spleen	0.20, 1.00, 2.00, 10.00, 20.00	1.41E+3	1.95E+1	0.9992
Lung	0.15, 0.45, 0.75, 1.00, 3.00	1.29E+3	2.63E+2	0.9996
Kidney	0.10, 0.25, 0.50, 0.75, 1.00	1.34E+3	1.68E+2	0.9990

Table 2
Precision and accuracy data for scutellarin in plasma, heart, liver, spleen, lung and kidney ($n = 3$ days, triplicate per day)

Sample matrix	Added concentration (μg/ml or g)	Found concentration (μg/ml or g)	Recovery[a] (%)	Precision[b] (%)	
				Within-day	Between-day
Plasma	0.10	0.10	98.57	6.72	5.83
	1.00	0.97	97.16	0.92	2.07
	10.00	10.17	101.67	0.76	2.01
Heart	0.15	0.16	104.59	4.24	2.18
	1.50	1.49	99.41	2.82	3.99
	15.00	14.82	98.80	7.55	3.44
Liver	0.20	0.19	95.44	7.36	5.82
	2.00	1.86	92.89	6.55	3.18
	20.00	19.76	98.82	1.59	2.01
Spleen	0.20	0.22	107.83	6.13	5.70
	2.00	1.85	92.33	3.71	2.56
	20.00	20.20	101.02	0.92	4.30
Lung	0.15	0.16	104.37	3.82	2.86
	0.75	0.73	97.29	1.50	1.13
	3.00	2.97	98.93	0.67	0.87
Kidney	0.10	0.10	95.67	6.79	4.68
	0.50	0.49	97.96	3.89	1.62
	1.00	0.99	98.56	2.02	1.08

[a] Recovery = (scutellarin found in sample)/(scutellarin added).
[b] Coefficient of variation.

Due to the limited stability of scutellarin, a stronger deproteinisation procedure could not be applied. Scutellarin degrades by oxidation of the phenolic hydroxyl, which prevents the use of oxidative agents (e.g. perchloric acid). Furthermore, scutellarin is only stable in acidic conditions and rather unstable in alkaline solutions. Therefore, the use of zinc sulfate for deproteinisation was not possible.

The method described here was applied in a preclinical study in order to compare the tissue distribution of two pharmaceutical formulations of scutellarin. The administration of lipid emulsion resulted in a different tissue distribution as compared to aqueous scutellarin (Table 3). With the lipid emulsion formulation, higher amounts of scutellarin were in all samples, especially plasma and heart. With lipid emulsion formulations of paclitaxel [14], carmustine [15] and dexamethasone palmitate [16] it has been demonstrated that due to altered pharmacokinetic behavior [14] and tissue distribution [15,16], the overall therapeutic index of the drugs could be improved. The higher amounts of scutellarin in the plasma and heart may be useful for the treatment of anti-thrombosis diseases and cardiac dysfunction diseases. The accumulation of particulate drug-carriers like emulsion and liposomes in the mononuclear phagocyte system (MPS), primarily by uptake by liver Kupffer cells and spleen fixed macrophages, is a well known phenomenon which may be useful for the treatment of liver and spleen diseases, however, it can also cause toxic effects [16,17]. It is also unknown whether the lipid emulsion could cause toxic effects on kidney by increasing amounts of scutellarin in kidney. The problem calls for further study in the above field.

In conclusion, the method described here represents a simple, rapid and sensitive procedure for the determination of scutellarin in biological samples including tissues. It could be shown that this method is applicable for studies in an animal model to describe the distribution of scutellarin in different tissues.

Table 3
Quantitation of scutellarin in plasma, heart, liver, spleen, lung and kidney samples of mice by HPLC

Sample	Scutellarin concentration (μg/ml or μg/g)	
	Aqueous solution	Lipid emulsion preparation
Plasma	0.34 ± 0.08	11.40 ± 1.96
Heart	0.06 ± 0.02	9.84 ± 2.08
Liver	1.38 ± 0.36	7.14 ± 1.66
Spleen	0.82 ± 0.24	11.61 ± 1.83
Lung	0.22 ± 0.05	1.99 ± 0.41
Kidney	0.11 ± 0.01	0.66 ± 0.11

Scutellarin was measured in the samples 30 min after administration of scutellarin to mice (25 mg/kg, i.v.). Values represent mean ± S.D. of five animals.

References

[1] R.W. Zhang, Y.L. Zhang, J.S. Wang, Y.Y. Lin, B. Shang, Chin. Tradit. Herb Drug 19 (1988) 199.
[2] H.C. Fan, J.C. Ma, Y.L. Xuan, Chin. Tradit. Pat. Med. 15 (1993) 22.
[3] P.Z. Zhang, Chin. Tradit. Pat. Med. 20 (1998) 26.
[4] J.M. Xu, H.J. Zheng, X. Xi, L.Z. Shen, H.C. Fan, Chin. New Drug J. 2 (1993) 46.
[5] Pharmacon Criteria (Chinese Traditional Patent Medicine), Ministry of Health of China, vol. 20, 102.
[6] D.F. Zhong, B.H. Yang, X.Y. Chen, K. Li, J.H. Xu, J. Chromatogr. B 796 (2003) 439.
[7] Y.M. Liu, A.H. Lin, H. Chen, F.D. Zeng, Acta Pharm. Sin. 38 (2003) 775.
[8] J.L. Zhang, Q.M. Che, S.Z. Li, T.H. Zhou, J. Asian. Nat. Prod. Res. 5 (2003) 249.

[9] J.J. Wheeler, K.F. Wong, S.M. Ansell, D. Masin, M.B. Bally, J. Pharm. Sci. 83 (1994) 1558.
[10] F. Liu, D. Liu, Pharm. Res. 12 (1995) 1060.
[11] T. Takino, M. Koreeda, T. Nomura, T. Gakaeda, F. Yamashita, Y. Takakura, M. Hashida, Biol. Pharm. Bull. 21 (1998) 492.
[12] P.C.N. Rensen, M.C.M. Van Dijk, E.C. Havenaar, M.K. Bijsterbosch, J.K. Kruijt, T.J.C. Van Berkel, Nat. Med. 1 (1995) 221.
[13] P.C.N. Rensen, N. Herijgers, M.H. Netscher, S.C.J. Meskers, M. van Eck, T.J.C. van Berkel, J. Lipid Res. 38 (1997) 1070.
[14] D.G. Rodrigues, D.A. Maria, D.C. Fernandes, C.J. Valduga, R.D. Couto, O.C. Ibanez, R.C. Maranhao, Cancer Chemother. Pharmacol. 55 (2005) 565.
[15] R.S. Teixeira, R. Curi, R.C. Maranhao, J. Pharm. Pharmacol. 56 (2004) 909.
[16] J. Seki, S. Sonoke, A. Saheki, H. Fukui, H. Sasaki, T. Mayumi, Int. J. Pharm. 273 (2004) 75.
[17] G. Gregoriadis, A.T. Florence, Drugs 45 (1993) 15.