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Separation/Preconcentration and Determination of Scutellarin in Urine by Vortex-Assisted Surfactant-Enhanced Emulsification Microextraction and High Performance Liquid Chromatography

JINYAN NING[1], CHAO YANG[1,3], LI BI[2], JIAO ZHAO[1], LIFEN MENG[1] and YALING YANG[1,*]

[1]Faculty of Life Science and Technology, Kunming University of Science and Technology, Yunnan Province 650500, P.R. China
[2]College of Science, Kunming University of Science and Technology, Yunnan Province 650500, P.R. China
[3]Beijing Institute of Medical Device Testing, Beijing 100011, P.R. China

*Corresponding author: Fax: +86 871 63801956; Tel: +86 13888316388; E-mail: yilyil8@163.com

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Vortex-assisted surfactant-enhanced emulsification microextraction and high performance liquid chromatography (VASEME-HPLC) for the preconcentration and determination of trace amounts of scutellarin in urine was developed. Pentanol and Triton X-100 were used as the extraction solvent and emulsifier, respectively. The factors influencing the extraction, such as the kind and volume of the extraction solvent and emulsifier, pH were optimized. Under the optimum conditions, the linear dynamic range was between 0.04-24 μg/mL (r = 0.9977). The limit of detection (LOD, S/N = 3) was 1.3 ng/mL. The limit of quantification (LOQ, S/N = 10) was 3.9 ng/mL. The relative standard deviation (RSD) at 0.04 μg/mL level of scutellarin (n = 5) was found to be 3.1 %. Good recovery results (86.3-93.6 %) were obtained when the proposed method was applied to determine scutellarin in urine samples.

Keywords: Surfactant, Emulsification, Microextraction, Scutellarin, High performance liquid chromatography.

INTRODUCTION

Scutellarin, a flavone glucuronide of 5,6,4'-trihydroxy-flavone-7-O-glucoronide, is the main active component of the traditional Chinese botanic drug *Erigeron breviscapus* (Vant.) Hand-Mazz[1]. It is commonly used in China to treat cardiovascular diseases[2-5]. Currently, dosage forms of scutellarin appearing on the markets are tablets, granules, injections and powder-injections[6]. However, oral bioavailability of ordinary tablets is very low[7] and injections and powder injection have short half-life[7,8] which eliminate rapidly *in vivo*. It has important significance to develop new formulations of scutellarin[9,10]. Detailed pharmacokinetic information and the bioavailability of scutellarin after oral administration could provide a valuable reference for formulation development and clinical application of scutellarin. Detecting the amount of scutellarin in urine is a supplement for studying pharmacokinetic and bioavailability of scutellarin.

Biological samples (*e.g.*, urine) are complex matrices containing many interfering substances and contain low levels of target analytes. Sample preparation is crucial step in trace determination, which can purify the extracts prior to instrumental determination and enrich the analytes of interest. High performance liquid chromatography (HPLC) is often used for the determination of scutellarin[11-15] and liquid-liquid extraction (LLE)[12] and solid phase extraction (SPE)[14,15] are the mostly reported sample pretreatment method in scutellarin analysis. However, these procedures are time-consuming and expensive.

In this work, vortex-assisted surfactant-enhanced emulsification microextraction (VASEME) was used to reduce interference and enrich trace scutellarin in urine. This is a faster, simpler, inexpensive and more environmentally friendly sample preparation technique. Several factors affecting extraction were tested.

EXPERIMENTAL

All reagents were at least of analytical grade and used without further purification. Scutellarin was purchased from the State Food and Drug Administration of China. Stock solution of scutellarin (200 μg/mL) was prepared by dissolving an accurate mass of 10 mg of scutellarin into acetonitrile in a 50 mL volumetric flask and was diluted to the mark. Standard solutions were prepared from the stock solution by serial dilutions with water. Butanol, pentanol, hexanol, octanol, decanol were purchased from Aladdin (Shanghai, China). Triton X-114, Triton X-100 and AEO-9 were purchased from Sigma (St. Louis, Mo., USA). Acetonitrile (HPLC grade) was obtained from Merck KGaA (Darmstadt, Germany). Purified water was obtained using an Aike water purification system (Chengdu, China).

Chromatographic evaluation and separation were performed on an HPLC system (consisting of a quaternary pump,

an auto sampler, a vacuum degasser and an ultraviolet detector; Agilent 1100 Series, Agilent Technologies, Calif., U.S.A.). A vortex oscillator (Shanghai, China) was used to assist the microextraction procedure. A centrifuge (Shanghai, China) was used for the phase separation process.

HPLC conditions: The separations were performed on an Agilent TC-C18 column (150 mm × 4.6 mm, i.d, 5 µm). Acetonitrile-1 % glacial acetic acid was used as mobile phase (27:73, v/v) with the flow rate of 1 mL/min. The injection volume was 10 µL and the ultraviolet detector was set at 335 nm. The column temperature was 30 °C.

Sample preparation: Blank urine sample was collected from healthy individual and stored at -20 °C until analysis. The frozen urine sample was thawed at room temperature. Prior to the vortex-assisted surfactant-enhanced emulsification microextraction, urine sample spiked with the target analyte was adjusted to pH 5 with hydrochloric acid and filtered through a disposable nylon filter (0.45 µm of pore size, Millipore, Madrid, Spain).

Vortex-assisted surfactant-enhanced emulsification microextraction procedure: 5 mL of sample or standard solution was placed in centrifuge tube. 300 µL of pentanol (extraction solvent) and 100 µL of triton X-100 (emulsifier) were added into the solution. A cloudy solution was formed after vortex-mixed for 1min. The mixture was centrifuged for 5 min at 3,500 rpm and two clear phases formed. Target analyte was extracted into the upper phase with a small volume and 10 µL was directly injected into the HPLC system for analysis.

RESULTS AND DISCUSSION

The selection of an appropriate extraction solvent is of major importance in microextraction. The extraction solvent must have low solubility in water, high solubility of the desired analyte. The extraction was performed with a mixture of 300 µL of extraction solvent and 100 µL of triton X-114 as the emulsifier. The extraction performance of the butanol, pentanol, hexanol, octanol and decanol were tested (Fig. 1). Pentanol was chosen as the extracting solvent for the further experiments.

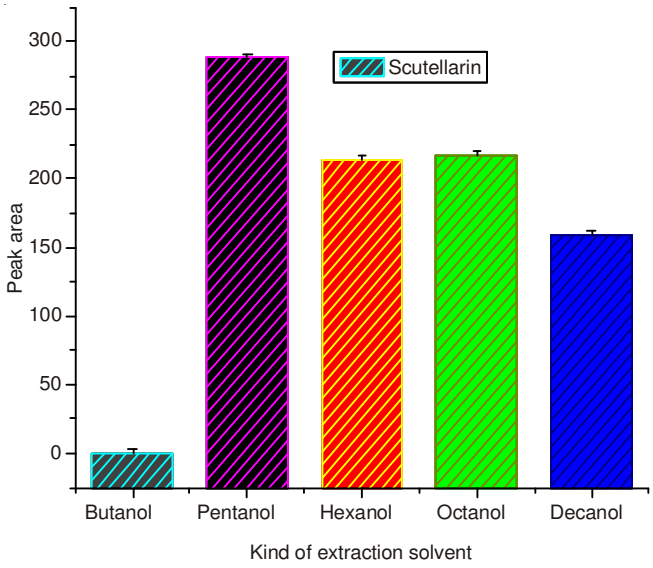

Fig. 1. Effect of the type of extraction solvent

In order to select the optimum volume of the extraction solvent, several experiments were performed using 100 µL of Triton X-114 and different volumes of pentanol. The results of this study (Fig. 2) revealed that 300 µL of pentanol was chosen to insure complete extraction and to have high enrichment factor.

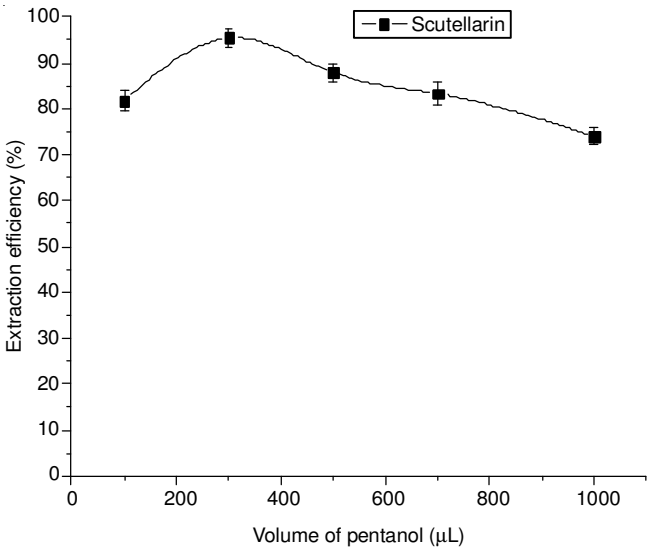

Fig. 2. Effect of the volume of extraction solvent

Effects of type and volume of the emulsifier: Emulsifier plays a key role in separation of the two phases to form the emulsion or particles. The emulsifier should be a kind of amphiphilic compound possessing two distinct groups, *i.e.*, a hydrophobic and a hydrophilic group in the same molecule. Surfactants are commonly used emulsifier because of its specific physicochemical properties, such as amphiphilicity, solubility in polar and nonpolar liquids, ability to form micelles. Triton X-114, Triton X-100 and AEO-9 were used as emulsifier. Types of emulsifiers have little effect on the extraction (Fig. 3) and triton X-100 was chosen in this paper.

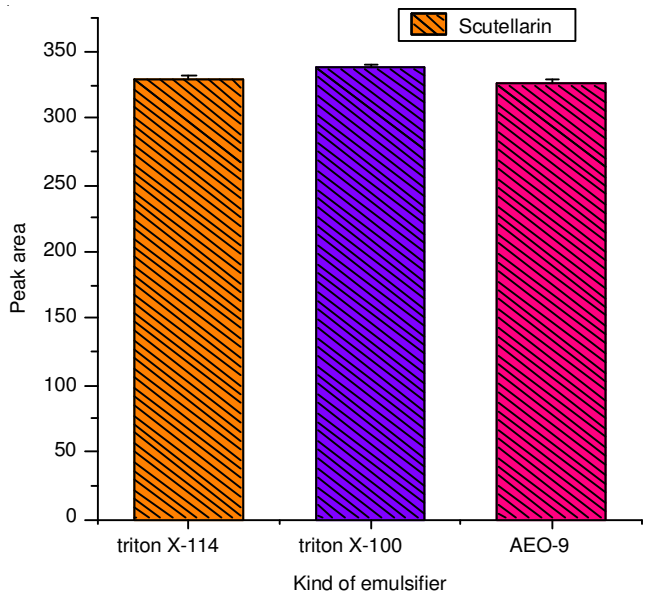

Fig. 3. Effect of the type of emulsifier

The influence of the volume of Triton X-100 in the range of 0-200 μL on the extraction was examined (Fig. 4). As Triton X-100 was added, the absorbance signal increased and was maximized at the volume of 100 μL. However, when the volume of Triton X-100 was greater than 100 μL, the extraction efficiency was decreased due to the increase in solubility of the scutellarin in aqueous phase in the presence of higher amount of Triton X-100.

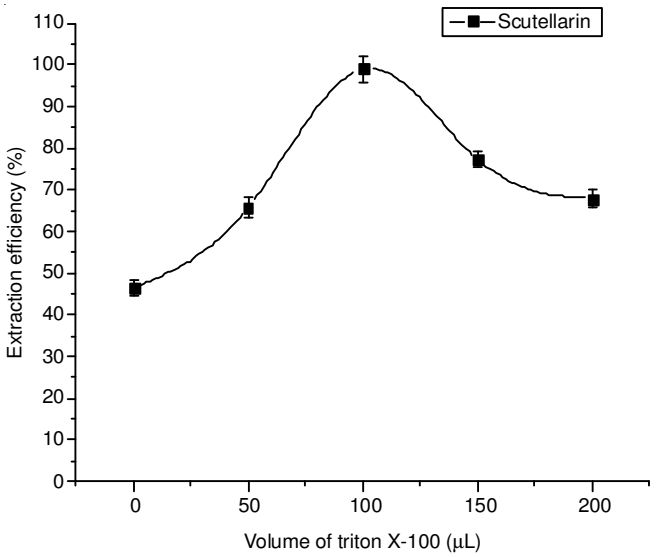

Fig. 4. Effect of the volume of emulsifier

Effect of sample pH: Scutellarin is flavonoid glycoside containing carbonyl molecules and pH value is an important parameter to affect distribution coefficient and selectivity. When pH > 7, flavonoid glycosides of phenol hydroxy easily are ionized and become soluble in water and extraction efficiency decreases. With the increase of acidity, flavonoid glycosides exist in molecular form and the extraction efficiency increase. Fig. 5 shows the effect of pH on the extraction of scutellarin. The pH 5 was selected in the experiments.

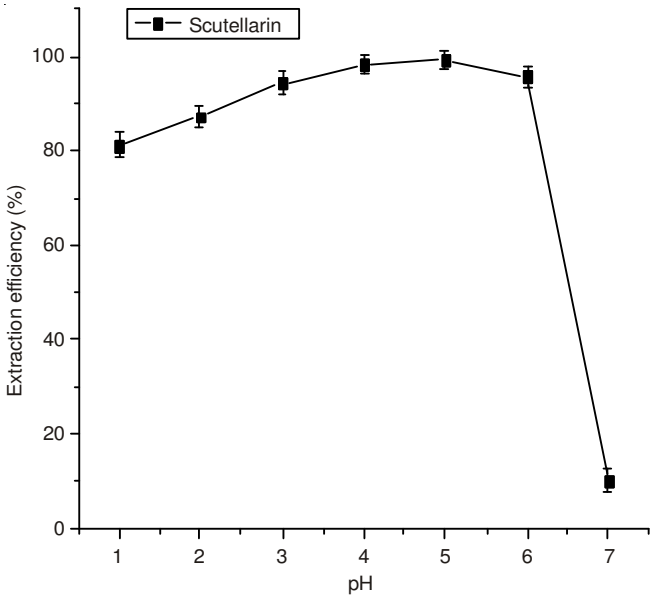

Fig. 5. Effect of pH on extraction of scutellarin by VASEME

Analytical performance: The figures of merit of the proposed method are summarized in Table-1. The calibration curve was linear in the range of 0.04-24 μg/mL (r = 0.9977). The limit of detection (LOD, S/N = 3) was 1.3 ng/mL. The limit of quantification (LOQ, S/N = 10) was 3.9 ng/mL. The relative standard deviations at 0.04 μg/mL (n = 5) was found to be 3.1 %.

TABLE-1
QUANTITATIVE RESULTS OF THE PROPOSED VASEME-HPLC METHOD (n = 5)

Parameter	Scutellarin
Linearity	0.04-24 μg/mL
Linear equation	y = 266.98x + 65.529
Correlation coefficient	0.9977
RSD	3.1 %
LOD	1.3 ng/mL
LOQ	3.9 ng/mL

Analysis of real samples: The reliability of the proposed procedure was verified by the analysis of the samples spiked with the different known amounts of scutellarin. The results of this study (Table-2) demonstrated that the recoveries of extraction are good (86.3-93.6 %). Intra- and inter-day precision were below 6.1 %. Thus the method is reliable for the determination of scutellarin in urine samples. Fig. 6. showed the obtained chromatograms of the spiked sample.

TABLE-2
INTRA- AND INTER-DAY PRECISION AND RECOVERY FOR QUANTITATION OF SCUTELLARIN

	Added (μg/mL)	Recovery (%) (RSD %)	Intra-day precision (n = 5)	Inter-day precision (n = 3)
			RSD (%)	RSD (%)
Urine sample	-	-	-	-
	0.12	86.3 (5.1)	3.7	5.7
	4	90.7 (3.9)	4.3	6.1
	16	93.6 (4.1)	4.7	5.3

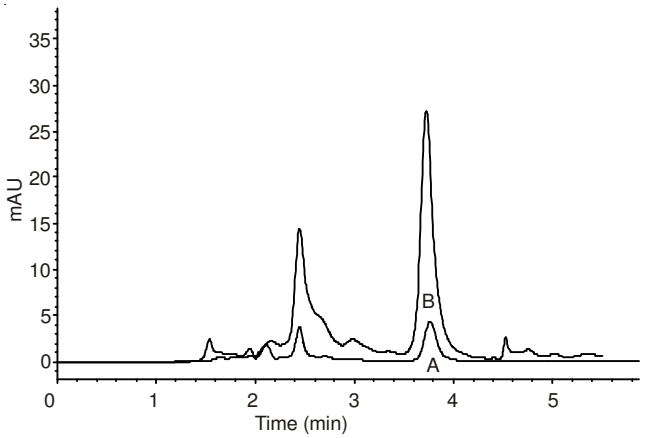

Fig. 6. Typical chromatogram of sample: (a) sample spiked with scutellarin (0.8 μg/mL) without VASEME; (b) sample spiked with scutellarin (0.8 μg/mL) after VASEME

Conclusion

In this study, it was demonstrated that the VASEME-HPLC has the capability of extraction and determination of traces of

scutellarin in urine samples. Furthermore, comparison of the developed method with other reported methods for the determination of scutellarin (Table-3) indicated that the detection limit and linear dynamic range of the proposed method is comparable to other reported method. It also have the advantages of minimum use of the toxic organic solvent, rejection of the matrix constituent, simplicity, low cost and ease of the operation.

TABLE-3
COMPARISON OF THE DEVELOPED METHOD WITH OTHER REPORTED METHODS

Method	Detection limit	Linear range	Reference
HPLC-UV	0.01 μg/mL	0.1–100 μg/mL	11
LLE-HPLC-UV	30 ng/mL	0.1–100 μg/mL	12
SPE-HPLC-UV	10 ng/mL	0.01-20 μg/mL	14
VASEME-HPLC-UV	1.3 ng/mL	0.04-24 μg/mL	This paper

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