Electronic Supplementary Information

Determination of Berberine in Rhizoma Coptidis by β-cyclodextrin Sensitized Fluorescence Method

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Experimental section

Chemicals and reagents

Rhizoma Coptidis (RC, Huanglian in Chinese) was purchased in pharmacy of Lerentang in Shijiazhuang. Berberine Hydrochloride (BH, serial No.: 110713-200208), Palmatine Hydrochloride (PH, serial No.: 0732-200005) and Jatrorrhizine Hydrochloride (JH, serial No.: 0733-200005) were got from Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). β-cyclodextrin was produced in Chemical Limited company of Bodi Plant（tianjin，China）. L-tryptophane ($C_{11}H_{12}N_2O_2$, biochemical reagent, chromatographic grade, molecular weight: 204.33) was purchased from the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China). BR buffer solution were prepared for pH adjustment. Ethyl acetate, chloroform, methanol, concentrated ammonia, diethylamine and absolute ethanol were all analytical grade and used as developing agents. The water used throughout the study was doubly-deionized and verified to be free from fluorescence.

The standard solution of 5.4×10^{-5} mol·L^{-1} BH, PH and JH were prepared by dissolving about 0.0020 g of BH, PH and JH in water and diluting to 100 mL with water respectively, and diluted to appropriate concentration with water as needed.

The solution of 0.013 mol·L^{-1} β-CD was prepared by dissolving 3.7500 g of β-CD in hot water, after 30 min ultrasound treatment and diluted to 250 mL with water, and diluted to appropriate concentration with water as needed.

Apparatus

Fluorescence measurements were performed on a Hitachi (Tokyo, Japan) F-4500 fluorescence spectrophotometer equipped with a xenon lamp and 1 cm quartz cell. The excitation and emission slits (band pass) 5 nm/5 nm were used throughout the work. Absorption spectra were recorded using a Shimadzu (Kyoto, Japan) UV-2501PC recording spectrophotometer with 1 cm quartz cell. An Orion (Beverly, USA) 868 pH/ISE meter was used for pH measurement. KQ-50 NC ultrasonic cleaning device was used for Ultrasonic treatment. Silicone G plate (100 mm × 200 mm, silicone thickness 0.20-0.25 mm, Qingdao Ocean Chemical Plant) was used for component separation plate.

Rhizoma Coptidis sample preparation

The Rhizoma coptidis (RC) water extraction was prepared by dissolving 0.0500 g of RC in 100℃ water for10 min and ultrasound treatment for 30 min. The mixture was filtered through a paper filter and washed several times with water. The filtrate was collected in a 100 mL volumetric flask, diluted to the mark with water and mixed well. The concentration of the water extract was expressed as 0.500 mg·mL^{-1} (0.500

mg RC per mL solvent).

Experimental method

Put 0.850 g β - CD in a 50 mL volumetric flask, add distilled water to dissolve and dilute to scale, and shake well. The β - CD solution was then diluted with distilled water to a concentration of 0, 2.0 $\times 10^{-3}$, 4.0 $\times 10^{-3}$, 6.0 $\times 10^{-3}$, 8.0 $\times 10^{-3}$, and 10.0 $\times 10^{-3}$mol·L^{-1}, respectively. 10mL of β - CD solution was placed in 6 volumetric bottles(25mL), respectively, with excessive berberine added. The solution was shaken for 72h at constant temperature (25.0 ± s0.5) ℃, centrifugated for 10min, and filtered with a 0.45 μm microporous membrane. The filtrate was diluted with distilled water and shaken well. Taking distilled water as blank, the absorbance was measured at the wavelength of 345 nm, and the concentration of berberine was calculated. Taking the concentration of β - CD solution as the x-coordinate and the concentration of berberine as the y-coordinate, the phase solubility diagram was made and the regression equation was obtained. According to the regression equation, the inclusion constant K is calculated as follows:

$$K = \frac{b}{S_0(1-b)} \qquad (1)$$

where b is the slope of the regression equation, and S_0 is the intercept.

A series of 10 mL volumetric flasks were prepared containing a certain amount

of β - CD and BH solution. The mixtures were diluted to the mark with water and mixed well. After setting aside for 20 min, the two-dimensional fluorescence spectrum and three-dimensional fluorescence spectrum were measured at room temperature.

A series of 10 mL volumetric flasks were prepared containing a certain amount of β - CD, BH solution and buffer solution. The mixtures were diluted to the mark with water and mixed well. After setting aside for 20 min, the two-dimensional fluorescence spectrum was measured at room temperature.

A series of 10 mL volumetric flasks were prepared containing a certain amount of β - CD and BH solution. The mixtures were diluted to the mark with water and mixed well. After setting aside for 20 min, the UV-Vis Absorption Spectrum was measured at room temperature.

L-tryptophan (quantum yield 0.14 at excitation wavelength of 280 nm) was used as a reference in measuring quantum yield of β-CD-BH complex. For the measurement, a 1.8 mg mL^{-1} L-tryptophan solution and a 2.0 $\mu g \cdot mL^{-1}$ BH solution containing 7.5 $mg \cdot mL^{-1}$ (0.0065 $mol \cdot L^{-1}$) β-CD were prepared. Absorption and fluorescence spectra were measured. Quantum yield of the β-CD-BH complex was calculated by the following equation. Where Fu and Fr were the integral fluorescence intensity of unknown and reference solutions, Au and Ar were the absorbance of unknown and reference solutions at their excitation wavelengths, respectively. Before measuring the quantum yield, excitation and emission spectra were corrected

according to the operation manual of the F-4500 fluorescence spectrophotometer.

Table S1 The detection limits of other methods for the determination of berberine in Rhizoma Coptidis.

Methods	Limits of Detection	References
UV-vis spectroscopy	0.06 μg·mL^{-1}	8
MoP-2/SPCE sensor	5 μmol·L^{-1}	9
High performance liquid chromatography	6.93 μg·mL^{-1}	10
Proton nuclear Magnetic resonance spectroscopy	0.04 mg·mL^{-1}	13
ERETIC1H NMR method	0.1 mmol·L^{-1}	14

Table S2 Recovery of BH spiked in RC extract

NO.	Sample ($\mu g \cdot mL^{-1}$)	Added ($\mu g \cdot mL^{-1}$)	Average found ($\mu g \cdot mL^{-1}$)	Recovery (%)	Average recovery (%)	RSD (%)
1	0.148	0.308	0.453	99.0		
2	0.148	0.462	0.612	100.4		
3	0.148	0.616	0.761	99.5	99.5	0.58
4	0.145	0.308	0.450	99.0		
5	0.145	0.462	0.604	99.4		

Table S3 Determination of BH in RC by this method

Sample	Concentration mg·mL^{-1}	Fluorescence intensity **2**	Fluorescence intensity **1**	BH μg·mL^{-1}	BH %	Mean %	RSD %
RC1	4.10	217	30.2	0.313	7.63		
RC2	5.12	264	37.4	0.390	7.62	7.60	0.57
RC3	7.68	381	52.7	0.580	7.55		

Table S4 Determination of BH concentration in RC by thin layer fluorescence method

Sample	Sample quality μg	Fluorescence Integral area	Found μg	BH %	Mean %	RSD %
RC1	0.40	501	0.0304	7.60		
RC2	0.40	505	0.0306	7.62	7.61	0.13
RC3	0.40	503	0.0304	7.61		