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Quality Evaluation and Relative Bioavailability Investigation on Three Alkaloids of *Corydalis Saxicola* Bunting Extract in Solid Dispersion

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ABSTRACT

Corydalis saxicola Bunting, also named Yanhuanglian in China, is widely used in folk prescriptions to treat hepatitis, hepatocirrhosis and hepatic cancer. Its active components consists mainly of alkaloids, which have poor solubility in water. The previous study showed that the absolute bioavailabilities (BA) of the alkaloids were only about 10% after oral administration to rats, compared to intravenous administration. In this paper, the solid dispersion (SD) method was used to improve the BAs of dehydrocavidine (YHL-1), coptisine (YHL-2), and dehydroapocavidine (YHL-3), the main alkaloids of the extract of *Corydalis saxicola* Bunting, by improving their solubility. The results showed that using PEG 4000 as a carrier at an amount equivalent to 7 folds of the extract could significantly improve the solubility of the compounds in water, pH 1.2 HCl and pH 7.2 PBS. DSC and X-ray detection indicated that the drug might be in a dissolved or amorphous state in SD, which was different from that in the physical mixture. The formulation was further studied in terms of BA enhancement by cumulative urinary excretion after oral administration to rats. A new method using high performance liquid chromatography (HPLC) coupled to a diode-array detector (DAD) was established. The results showed that the relative BAs (RE BAs) of YHL-1, YHL-2 and YHL-3 were all significantly improved by about 1 fold after oral administration of SD solution, compared to the extract suspension ($p < 0.05$).

Key words: alkaloid, *Corydalis saxicola* Bunting, solid dispersion, bioavailability, HPLC

INTRODUCTION

Corydalis saxicola Bunting, also named Yanhuanglian in China, is mainly distributed in Guangxi Province and Guizhou Province, where it is widely used in folk prescriptions to treat hepatitis, hepatocirrhosis and hepatic cancer[1-3]. Its active components mainly consists of alkaloids, such as dehydrocavidine, coptisine, oxyacanthine, allocryptopine, berberine, tetrahydropalmatine, palmatine, dehydroapocavidine and tetradehydroscoulerine[4]. Cheng *et al*. simultaneously determined eight bioactive alkaloids in *Corydalis saxicola* using high performance liquid chromatography coupled with diode-array detection (HPLC/DAD)[5]. The alkaloids of *Corydalis saxicola* are mainly administered by injection. It may be more convenient and patient compliance may be improved if oral preparations were developed. The risk of adverse reactions from injection can also be avoided. Unfortunately, Li *et al*. studied the pharmacokinetics of the four main alkaloids after intravenous and oral administration to rats using LC/MS/MS[6] and the results indicated that the bioavailabilities (BA) of the four alkaloids were very low - only about 10% after oral administration. Therefore, it is critical to enhance their BA for oral administration in order to improve their clinic efficacy.

For a poorly water-soluble drug, it is important to improve its absorption from the gastrointestinal (GI) tract. Therefore, achieving a solution of the drug in the GI fluid is a critical requirement. Solid dispersion (SD) is a widely used method to enhance the oral BA for poorly water-soluble drugs using different carriers, such as surface-active carrier, polyethylene glycol (PEG) and poly vinyl pyrrolidone (PVP)[7]. In SD, the drug is dispersed in water-soluble matrices either molecularly or as fine particles. The carrier is often used in large amount (more than 5 folds of the drug), which may result in a volume that is too large to administer. Our previous pharmacokinetic study using rats and dogs showed that if the volume was too large, the rats and dogs vomited and scoured because of GI stimulation. However, if the dosage volume was not

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large enough, the drug concentration in plasma or serum would be too low to be determined. The LC/MS method established by Li *et al*.[4], with the lowest limits of quantitation for four substances being 1 ng/mL in 0.1 mL of rat plasma, had been used in our previous study for the BAs of the four alkaloids. However, the method was not valid for plasma determination after oral administration of the alkaloids SD to rats and dogs because of the low drug concentration. In pharmacokinetics, it is an important method to calculate the BA by determining the cumulative urinary excretion of drugs. Therefore, assay of the cumulative excretion of the alkaloids in urine after administration should be an effective solution to the problem mentioned above. In the present study, an SD method was developed to improve the BAs of the alkaloids of the extract by improving their solubility. In addition, the BAs of the main alkaloids were calculated by determining their amounts in urine using HPLC.

MATERIALS AND METHODS

I. *Reagents and Chemicals*

Dehydrocavidine (YHL-1), coptisine (YHL-2) and dehydroapocavidine (YHL-3)[4] (Figure 1) reference standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). PEG 4000 and PVP K30 were purchased from Shanghai Yunhong Chemical Industry Co., Ltd. (Shanghai, China).

Acetonitrile was of LC grade (Fisher Scientific, Massachusetts, Waltham, USA). Distilled and deionized water was produced by a Milli-Q Reagent Water System (Millipore, Bedford, MA, USA). All other chemicals were of analytical reagent grade.

The alkaloids (AE) of the extract of *Corydalis saxicola* Bunting was provided by the 2nd Military Medical University and was determined to contain 39.09%, 14.09% and 28.08% of YHL-1, YHL-2 and YHL-3, respectively, by HPLC/DAD.

II. *Animals*

Twenty SD rats, each weighing 180 - 220 g (10 males and 10 females), were purchased from Beijing Mars Biotechnology Co., Ltd. (Beijing, China). The animals were housed and cared for under a constant temperature of 22 ± 1°C and humidity of 50 ± 10%. Their diet was prohibited for 2 days before the experiment while water was available freely. The entire experiment was performed in accordance with the regulations of the Animal Ethical Committee of Tsinghua University.

III. *HPLC Conditions*

All analytical procedures were performed on an Agilent 1100 HPLC/DAD system (Agilent Technologies Inc., Beijing, China) equipped with an auto-injector. The chromatographic separation was performed on an Apollo C_{18} column (4.6 × 250 mm, 5 μm) maintained at 30°C. The wavelength was 347 nm and the injection volume was 20 μL.

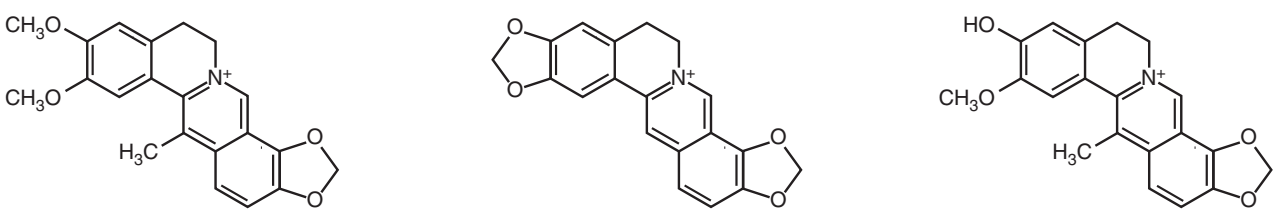
The quantification method to assay the contents of AE in tablets in our previous study was used to determine the solubility[8]. The mobile phase was acetonitrile - PBS (22 : 78, v/v). PBS consisted of 20 mmol of KH_2PO_4, 10 mmol of triethylamine and 2 mL of H_3PO_4 in 1 L (pH 2.3). The flow rate was 0.8 mL/min and the run time was 35 min.

The quantification method for urine samples was developed. The mobile phase was (A) acetonitrile and (B) a solution of 2 mL of glacial acetic acid and 1.5 mL of triethylamine in 1 L (pH 4.1). The gradient program was as follows: 0 - 10 min, 25% A; 10 - 16 min, 25 → 35% A; 16 - 22 min, 35 → 60% A; 22 - 25 min, 60 → 25% A; 25 - 30 min, 25 → 25% A. The flow rate was 1.0 mL/min and the re-equilibration time for the LC column was 2 min.

IV. *Preparation of AE SD*

(I) *Melting Method*

Three samples were prepared with different ratios of AE (1 : 3, 1 : 5 and 1 : 7) with respect to the carrier, PEG 4000. PEG 4000 was placed into three test-tubes and the tubes were immersed in a temperature-controlled bath at 65 - 70°C. At this elevated temperature, PEG 4000 was liquefied. AE was added into each tube and the mixtures were constantly stirred with micro-spatulas until complete dispersion. The mixtures were poured onto



Dehydrocavidine (YHL-1) Coptisine (YHL-2) Dehydroapocavidine (YHL-3)

Figure 1. Chemical structures of three main alkaloids, Dehydrocavidine (YHL-1), Coptisine (YHL-2) and Dehydroapocavidine (YHL-3) of the extract of *Corydalis saxicola* Bunting

glass plates and frozen at -20°C for 24 h. The mixtures were then dried in a desiccator containing silicon gel for 48 h and ground into 80-mesh powders.

(II) *Solvent Method*

Three samples were prepared with different ratios of AE (1 : 3, 1 : 5 and 1 : 7) with respect to the carrier, PVP K30. PVP K30 was placed into three test-tubes, followed by AE. The mixtures were constantly stirred and dissolved in 10 folds of ethanol using micro-spatulas until complete dissolution occurred. The ethanol was then evaporated and the solid mixtures were dried in a desiccator containing silicon gel for 48 h and ground into 80-mesh powders.

V. *Characteristics of AE SD*

(I) *Thermal Analysis*

A STARe Evaluation differential scanning calorimetry (DSC) thermal analyzer (Mettler-Toledo Shanghai Co., Shanghai, China) was used to obtain thermograms at a heating rate of 10°C/min over the temperature range of 20 - 150°C. The samples ranged in weight from 3.2 to 5.7 mg and were sealed inside crimped aluminum pans.

(II) *Powder X-ray Diffraction*

The powder X-ray diffraction (XRD) patterns of PEG 4000, AE, physical mixture and the SD with the highest solubility in water were determined using a multi-crystal X-ray diffractometer (Bruker Co., Berlin, Germany) with monochromatized CuKa radiation. The samples were analyzed between 20 angles of 5° and 60°.

(III) *Physical and Chemical Stabilities*

As a new formulation for administration, it was important to ensure that the prepared AE does not chemically degrade or change its physical form over time. Therefore, the stability of the SD in terms of color, thermal analysis, X-ray diffraction and AE content of the SD powder was tested over 0, 90 and 180 days.

(IV) *Solubilities of AE and AE SD*

The solubilities of AE and AE SD was determined in water, pH 1.2 HCl and pH 7.2 PBS, according to the USP paddle method (50 rpm at 37 ± 0.5°C). Aliquots of dissolution medium collected at 24 h were filtered through 0.45-μm syringe filters and analyzed by HPLC.

VI. *Bioavailability of SD*

(I) *Oral Administration*

The rats were divided into 2 groups with 10 rats (5 males and 5 females) each. 0.50 g of AE was dispersed in 100 mL of deionized water to obtain an AE suspension. 4.0 g of AE SD (AE : PEG 4000 = 1 : 7) was dissolved in 100 mL of deionized water to obtain AE SD solution. The AE suspension and AE SD solution were treated by ultrasonication for 10 min (KQB-100, Kunshan Supersonic Instrument Co., Kunshan, China).

For the first group, each rat was orally administered with AE suspension (25.0 mg AE per kg body weight). For the second group, each rat was orally administered with AE SD solution (200 mg AE SD (equivalent to 25.0 mg AE) per kg body weight). After administration, the diet for the rats was prohibited for 24 h while water was taken freely. The whole urine of each group was collected at 12 h and 24 h.

(II) *Preparation of Urine Samples*

Urine samples were centrifuged at 4,500 rpm for 10 min at 4°C (TGL 16C, Medical Centrifuge Co., Beijing, China). 100 μL of the upper layer was mixed with 400 μL of methanol using vortex for 3 min and then centrifuged at 4,500 rpm for 5 min at 4°C. The upper layer was transferred to another tube and evaporated to dryness at 45°C under a gentle stream of nitrogen (purity > 99.5%). The residue was reconstituted in 100 μL of methanol and centrifuged at 10,000 rpm for 10 min at 4°C. 20 μL of the supernatant was injected into the HPLC system for analysis. The same sample handling process was used for recovery and precision studies in urine.

(III) *Preparation of Calibration Standards and Calibration Curves*

The mixed standard stock solutions of YHL-1, YHL-2 and YHL-3 were prepared in methanol and the concentrations were 56.0, 32.0 and 60.0 μg/mL, respectively. A series of standard working solutions with concentrations in the range of 0.280 - 5.60, 0.160 - 3.20, and 0.300 - 6.00 μg/mL for YHL-1, YHL-2, and YHL-3, respectively, were obtained by further diluting the standard stock solution with methanol. All solutions were stored at 4°C. A series of 100-μL standard working solutions were evaporated to dryness at 45°C under a gentle stream of nitrogen. The residues were reconstituted in 100 μL of blank urine to prepare the calibration standards by the same method used for urine sample preparation.

Linearity of calibration was tested by extraction and assayed as described above (n = 3). Calibration curves for YHL-1, YHL-2 and YHL-3 in the concentration ranges were obtained by plotting the peak area versus concentration in urine. Unknown concentrations of YHL-1, YHL-2 and YHL-3 were determined with reference to the calibration curves.

(IV) *Assay Precision, Stability of Urine Samples and Analytical Recovery*

Quality control (QC) samples were prepared in the same way as calibration standards with blank urine. The nominal concentrations were 0.280, 1.40 and 5.60 μg/mL for YHL-1, 0.160, 0.800 and 3.20 μg/mL for YHL-2, and 0.300, 1.50 and 6.00 μg/mL for YHL-3, which corresponded to the low (LQC), medium (MQC) and high (HQC) levels of the calibration curves. The samples were stored at -80°C until analysis. Intra-day precision was tested by analyzing the QC samples at different times of the same day. Inter-day precision was determined by repeated analysis of the same samples over three consecutive days. The precision was evaluated by RSD (%) of peak areas of the three standards.

The stability of the urine samples was examined at room temperature for 0, 4, 8 and 20 h. The amounts of YHL-1, YHL-2 and YHL-3 in the urine samples were determined using newly prepared calibration curves.

The analytical recovery of the extraction procedure for YHL-1, YHL-2 and YHL-3 from rat urine was determined by spiking 100-μL aliquots of drug-free urine with various amounts of YHL-1, YHL-2 and YHL-3. Urine samples were spiked with 0.280, 1.40 and 5.60 μg/mL of YHL-1, 0.160, 0.800 and 3.20 μg/mL of YHL-2, and 0.300, 1.50 and 6.00 μg/mL of YHL-3, and extracted according to the method described above (n = 3 at each level). Standard mixtures of YHL-1, YHL-2 and YHL-3 equivalent to the concentrations in the urine samples were directly injected into the HPLC system. The recovery was evaluated by comparing the peak areas of the urine extracts to that of the standard mixtures.

(V) *Assay of Samples*

The present method was used to determine the concentrations of YHL-1, YHL-2 and YHL-3 in rat urine collected during 0 - 12 h and 12 - 24 h, after oral administration of AE suspension and AE SD solution. The concentrations of YHL-1, YHL-2 and YHL-3 were determined using the linear regression equations obtained from the calibration curves.

The percentages of the excreted components from urine following oral administration were evaluated. The RE BA of AE SD solution compared with AE suspension was calculated by the following equation:

$$REBA = \frac{Con._{AESD} \times V_{AESD} / Dose_{AESD}}{Con._{AE} \times V_{AE} / Dose_{AE}} \times 100\%$$

where $Con._{AESD}$ and $Con._{AE}$ refer to the total concentrations of AE SD and AE in rat urine, respectively, and V_{AESD} and V_{AE} are the total volumes of the collected urine after oral administration of AE SD solution and AE suspension, respectively.

RESULTS AND DISCUSSION

I. *HPLC Conditions*

The quantification method previously established for solubility assay was relatively simple and the excipients in SD produced no interference for the three compounds. However, the method was unsuitable for urine sample determination and the internal components could significantly affect the separation. Therefore, the HPLC method with gradient elution was established.

II. *Solubility of AE SD*

The solubilities of YHL-1, YHL-2 and YHL-3 of AE and AE SD in water, pH 1.2 HCl and pH 7.2 PBS are shown in Table 1. The data were statistically analyzed with t-test. The results indicated that YHL-1, YHL-2 and YHL-3 of AE had poor solubility in the three solvents. The physical mixtures of AE with PEG 4000 or PVP K30 could not significantly enhance the solubility of AE ($p > 0.05$). However, with the increase of the amount of PEG 4000 or PVP K30, the solubility of the physical mixtures was improved. The reason was that PEG 4000 and PVP K30 are weak solubilizing agents. As for AE SD, solubility could be greatly improved in any solvent mentioned above, especially for the SD consisting of AE and PEG 4000 (1 : 7). The melting method with PEG was simpler than the solvent method in manufacturing processes of large industrial scale. Furthermore, the melting method produced no liquid or gas waste. Therefore, it was selected to prepare AE SD and the prepared AE SD with AE : PEG 4000 ratio of 1 : 7 was further studied.

III. *Thermal Analysis of AE SD*

The DSC spectra of PEG 4000, AE, physical mixture of AE and PEG 4000 (1 : 7), and SD of AE and PEG 4000 (1 : 7) are shown in Figures 2 A, B, C and D, respectively. The corresponding peak temperatures were 64.2, 58.8, 63.7 and 62.3°C. It was found that AE led to a decrease in the melting point of PEG 4000 and the difference between the physical mixture and SD was minimal. The results also indicated that the method of DSC was not suitable for AE SD, because the melting point of PEG 4000 was 55 - 60°C and during the DSC analysis, the amount of drug present in the formulation dissolved in the matrix at about 60°C[7].

IV. *Powder X-ray Diffraction of AE SD*

The X-ray diffraction spectra of PEG 4000, AE, physical mixture of AE and PEG 4000 (1 : 7), and SD of AE and PEG 4000 (1 : 7) are shown in Figures 3 A, B, C and D, respectively. The spectra of PEG 4000 and AE were significantly different. The theta scales of 8.34, 11.58 were disappeared in the spectrum of AE SD compared with the physical mixture, indicating that in the SD formulation the drug might be in a dissolved or amorphous state, which was different from that in physical mixture.

Table 1. Solubility of AE, AE SD and the physical mixture of YHL-1, YHL-2 and YHL-3 in water, pH 1.2 HCl and pH 7.2 PBS (g/100 mL, n = 3)

Formulation	Ratio	Water			pH 1.2 HCl			pH 7.2 PBS		
		YHL-1	YHL-2	YHL-3	YHL-1	YHL-2	YHL-3	YHL-1	YHL-2	YHL-3
AE	-	0.063 ± 0.007	0.102 ± 0.003	0.135 ± 0.011	0.309 ± 0.028	0.507 ± 0.005	0.601 ± 0.012	0.305 ± 0.045	0.489 ± 0.029	0.598 ± 0.032
SD	1:3	0.135 ± 0.038	0.252 ± 0.011**	0.281 ± 0.021**	0.473 ± 0.011**	0.629 ± 0.008**	0.811 ± 0.013**	0.623 ± 0.012**	0.773 ± 0.024**	1.312 ± 0.046**
(AE PEG 4000)	1:5	0.164 ± 0.008	0.277 ± 0.021**	0.303 ± 0.017**	0.551 ± 0.036**	0.716 ± 0.021**	1.121 ± 0.033**	0.733 ± 0.026**	0.898 ± 0.035**	1.544 ± 0.037**
	1:7	0.213 ± 0.013*	0.368 ± 0.017**	0.402 ± 0.018**	0.589 ± 0.024**	0.846 ± 0.018**	1.322 ± 0.034**	0.734 ± 0.025**	0.901 ± 0.029**	1.603 ± 0.041**
SD	1:3	0.067 ± 0.002	0.110 ± 0.003*	0.144 ± 0.008	0.296 ± 0.026	0.519 ± 0.023	0.554 ± 0.028	0.251 ± 0.006	0.514 ± 0.021	0.603 ± 0.033
(AE PVP K30)	1:5	0.128 ± 0.004	0.213 ± 0.004**	0.276 ± 0.005**	0.409 ± 0.055	0.767 ± 0.025**	0.823 ± 0.043**	0.407 ± 0.006*	0.732 ± 0.017**	0.907 ± 0.042**
	1:7	0.153 ± 0.005	0.285 ± 0.012**	0.345 ± 0.010**	0.527 ± 0.085*	0.981 ± 0.033**	1.106 ± 0.036**	0.473 ± 0.081	0.808 ± 0.073**	0.996 ± 0.055**
Physical Mixture	1:3	0.047 ± 0.009	0.093 ± 0.005	0.111 ± 0.010	0.245 ± 0.024	0.477 ± 0.016	0.606 ± 0.021	0.323 ± 0.015	0.434 ± 0.020	0.539 ± 0.019
(AE PEG 4000)	1:5	0.057 ± 0.006	0.102 ± 0.009	0.125 ± 0.013	0.250 ± 0.021	0.475 ± 0.021	0.574 ± 0.017	0.385 ± 0.016	0.476 ± 0.018	0.534 ± 0.021
	1:7	0.066 ± 0.011	0.113 ± 0.006	0.138 ± 0.009	0.288 ± 0.025	0.542 ± 0.020	0.646 ± 0.022	0.355 ± 0.009	0.496 ± 0.015	0.593 ± 0.019
Physical Mixture	1:3	0.057 ± 0.005	0.089 ± 0.007	0.128 ± 0.011	0.274 ± 0.013	0.477 ± 0.033	0.571 ± 0.029	0.285 ± 0.031	0.412 ± 0.043	0.524 ± 0.034
(AE PVP K30)	1:5	0.060 ± 0.005	0.095 ± 0.008	0.164 ± 0.012	0.296 ± 0.028	0.463 ± 0.029	0.623 ± 0.021	0.319 ± 0.035	0.467 ± 0.039	0.588 ± 0.028
	1:7	0.069 ± 0.006	0.113 ± 0.007	0.168 ± 0.015	0.323 ± 0.026	0.492 ± 0.031	0.631 ± 0.019*	0.341 ± 0.033	0.545 ± 0.032	0.565 ± 0.031

The data were statistically analyzed with t-test, compare with solubility of AE, *$p < 0.05$, **$p < 0.01$.

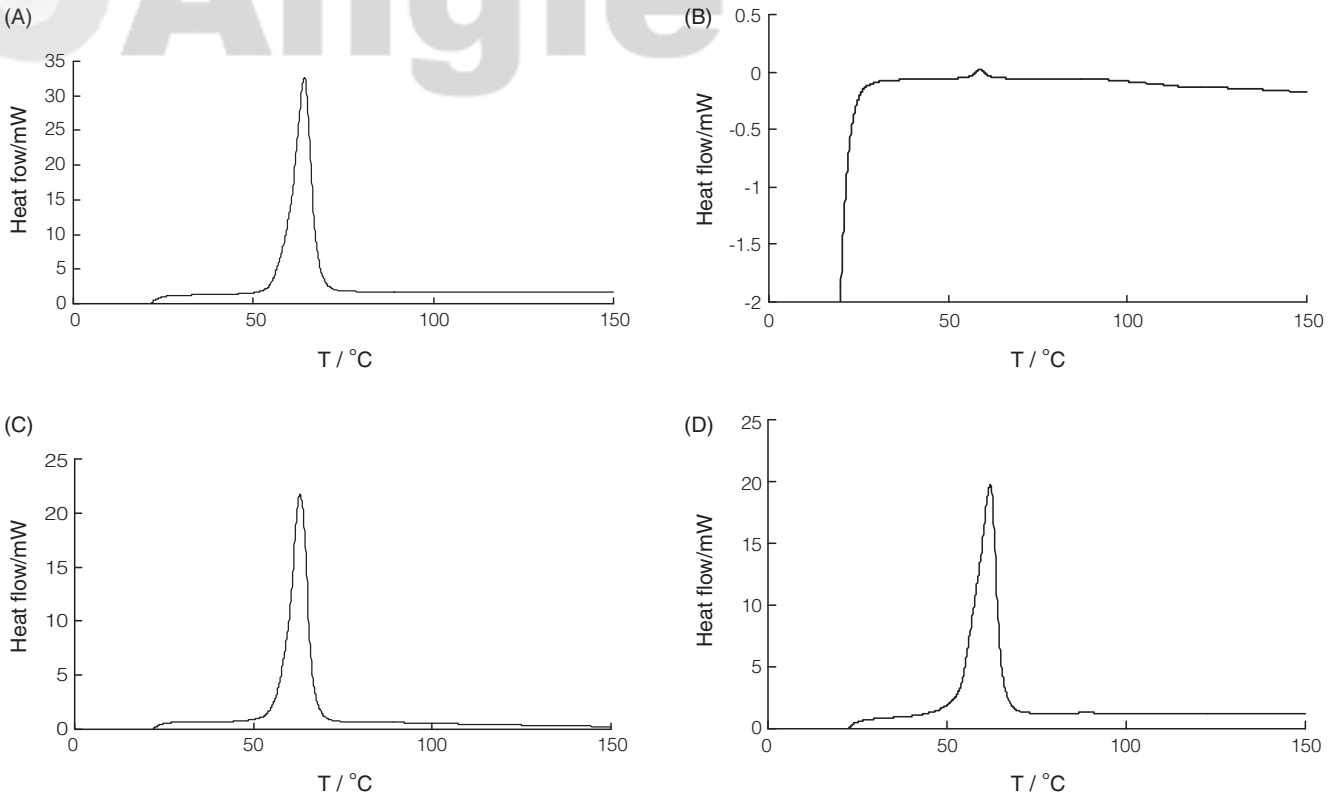


Figure 2. DSC of (A) PEG 4000, (B) the main alkaloids of the extract (AE) of *Corydalis saxicola* Bunting, (C) the physical mixture of AE and PEG 4000 (1 : 7), and (D) the solid dispersion of AE and PEG 4000 (1 : 7)

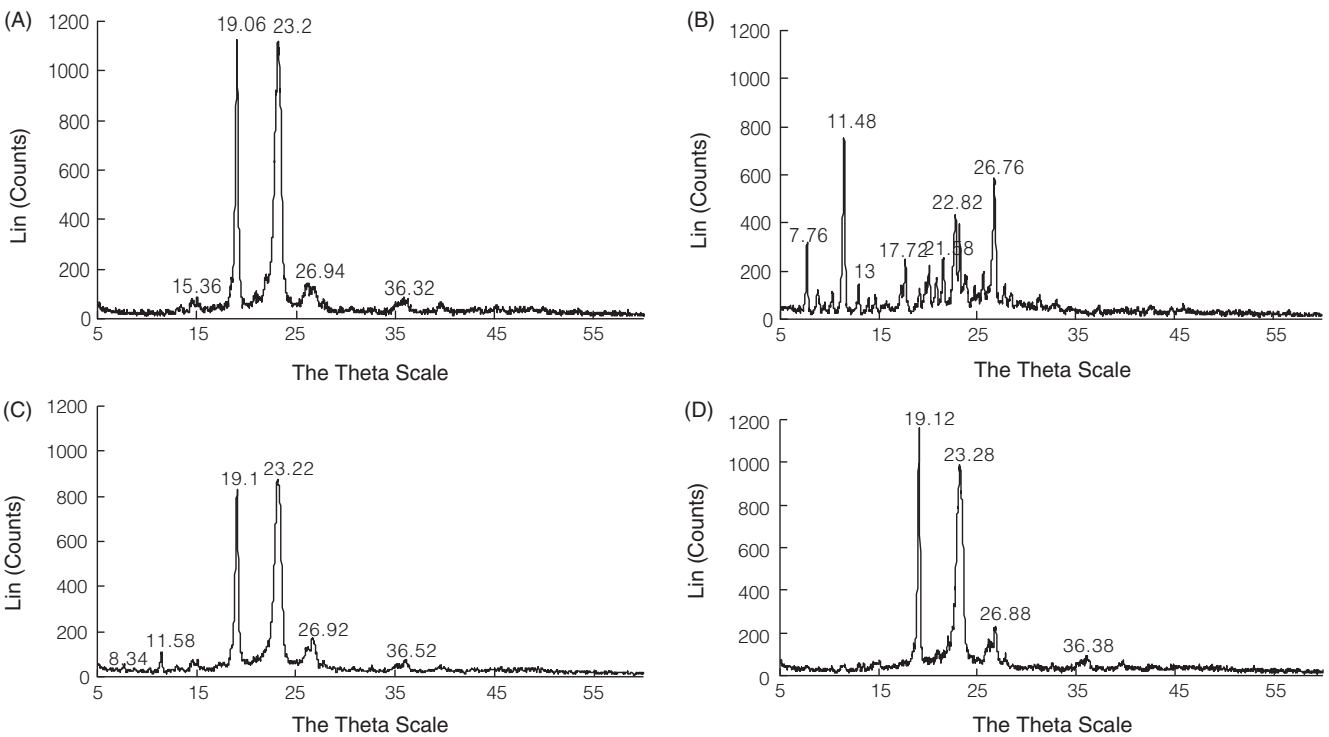


Figure 3. X-ray diffraction spectra of (A) PEG 4000, (B) the main alkaloids of the extract (AE) of *Corydalis saxicola* Bunting, (C) the physical mixture of AE and PEG 4000 (1 : 7), and (D) SD of AE and PEG 4000 (1 : 7)

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V. *Physical and Chemical Stabilities of AE SD*

The color of AE SD was light yellow. The contents of YHL-1, YHL-2 and YHL-3 in AE SD were 4.81 ± 0.21%, 1.73 ± 0.08% and 3.40 ± 0.15% (n = 3), respectively. After 90d and 180d, the color, thermal analysis, X-ray diffraction and the AE content of the AE SD powder, which was stored in a desiccator containing silicon gel, did not show any difference. This indicated that AE SD was stable within 180d. When AE SD is further developed into tablet dosage form, the use of other excipients, such as lactose, starch, or micro crystal cellulose[9], can further disperse the AE SD. Besides, the tablets can be coated with film to avoid the influence of light, moisture, or oxygen, *etc.*[10] These measures can significantly improve the stability of the AE SD.

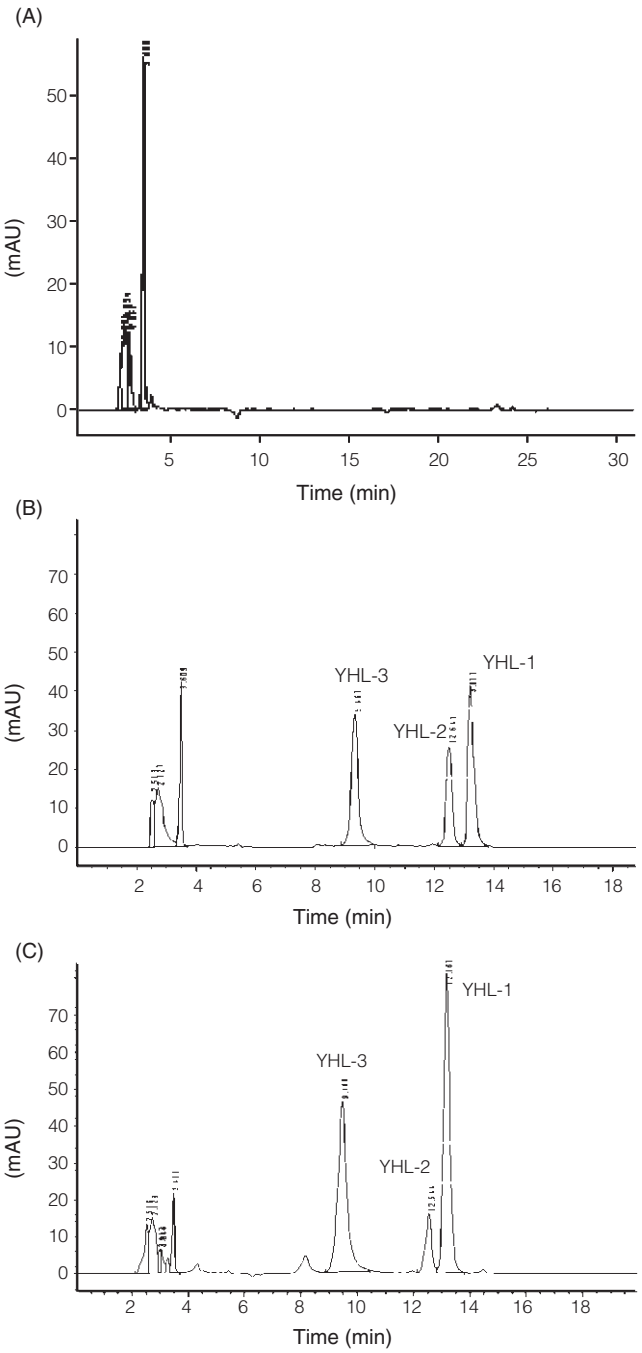

Figure 4. HPLC chromatograms of YHL-1, YHL-2 and YHL-3 in rat urine samples at 0 - 12 h after oral administration of AE SD (A: Blank urine sample; B: Blank urine spiked with standards; C: Urine sample)

VI. *Method Validation for Urine Sample Determination*

The calibration curves of YHL-1, YHL-2 and YHL-3 were constructed in the ranges of 0.280 - 5.60 μg/mL, 0.160 - 3.20 μg/mL and 0.300 - 6.00 μg/mL, respectively. The regression equations of the curves were calculated as follows: YHL-1: y = 29.084χ - 0.9259 (correlation coefficient r = 0.9993), YHL-2: y = 30.418χ - 2.109 (r = 0.9992), and YHL-3: y = 30.013χ - 1.915 (r = 0.9992), where χ was the concentration of the compound (μg/mL) and y was the peak area. They showed good linear relationships between y and χ in the ranges above. The assay was considered adequately specific as no endogenous urine components eluted at the same retention time as that of the analytes of interest (Figure 4). The three compounds were eluted in the order, YHL-3, YHL-2 and YHL-1, with good resolution.

The limit of detection (LOD) and the limit of quantification (LOQ) were determined at the lowest concentration based on S/N of 3 and 10, respectively[11]. The LOD and LOQ of YHL-1, YHL-2 and YHL-3 were 0.0093, 0.0081 and 0.0102 μg/mL, and 0.028, 0.026, and 0.031 μg/mL, respectively.

The intra-day and inter-day precision results of YHL-1, YHL-2 and YHL-3 are shown in Table 2. Each RSD value was less than 3%, indicating good precision. Inter-day precision was consistently higher than intra-day precision, which could be due to systematic errors from sample treatment and the instrument.

The stability was expressed as a percentage of nominal concentration. After standing at room temperature for 20 h, the concentrations relative to the initial ones (n = 3) of YHL-1, YHL-2 and YHL-3 were 98.16%, 97.88% and 98.32%, respectively, and the corresponding RSDs were 1.88%, 2.41% and 2.03%. The results showed that the samples were stable at room temperature.

The recovery data of YHL-1, YHL-2 and YHL-3 are shown in Table 3. For the different samples with high, middle and low concentrations, recoveries were all higher than 90%, indicating that the liquid-liquid extraction method established was simple, rapid and could extract most of YHL from rat urine.

VII. *Bioavailability of AE SD*

The assay method was successfully used to quantitatively measure the concentrations of YHL-1, YHL-2 and YHL-3 in urine samples obtained from rats orally administered separately with AE suspension and AE

SD solution. From 24 h to 36 h, no YHL-1, YHL-2 and YHL-3 could be determined in gathered urine. Therefore, the urine was gathered from 0 - 12 h and 12 - 24 h. The results are shown in Table 4. The amount excreted was reflected by the recovery of the dosage in urine. For YHL-1, the RE BA of AE SD after oral administration were 115.7% at 12 h and 300.4% at 24 h. These were compared with the RE BA of AE after oral administration. It was interesting to note that the total amounts excreted for AE suspension and AE SD solution were similar at 12 h after administration. However, the amount excreted for AE SD was significantly higher than that of AE at 24 h ($p < 0.05$), indicating that AE SD could

Table 2. Intra-day and inter-day precision results of YHL-1, YHL-2 and YHL-3 determined with HPLC/DAD (n = 5)

Component	Nominal concentration (mg/mL)	Intra-day precision (% RSD)	Inter-day precision (% RSD)
YHL-1	5.60	0.25	0.65
	1.40	0.33	0.57
	0.280	1.49	1.63
YHL-2	3.20	0.25	0.93
	0.800	0.41	1.18
	0.160	1.98	1.77
YHL-3	6.00	0.22	0.97
	1.50	0.31	0.66
	0.300	1.45	1.59

Table 3. Recovery of YHL-1, YHL-2 and YHL-3 from rat urine (n = 3)

Component	Concentration (mg/mL)	Recovery (%)
YHL-1	5.60	95.12 ± 3.34
	1.40	94.17 ± 4.88
	0.280	94.48 ± 0.98
YHL-2	3.20	93.07 ± 4.62
	0.800	91.33 ± 5.31
	0.160	92.57 ± 5.19
YHL-3	6.00	95.33 ± 3.17
	1.50	93.81 ± 4.91
	0.300	94.38 ± 5,18

Table 4. RE BAs of YHL-1, YHL-2 and YHL-3 of AE SD (AE/PEG 4000, 1 : 7) compared with AE after oral administration to rats (n = 3)

Group	n	Dose (mg/kg)	Dose of YHL (mg/kg)	0 - 12 h			12 - 24 h			0 - 24 h		
				Cumulative amount in urine (μg/kg)	Amount excreted (%)	RE BA (%)	Cumulative amount in urine (μg/kg)	Amount excreted (%)	RE BA (%)	Cumulative amount in urine (μg/kg)	Amount excreted (%)	RE BA (%)
AE	10	25.0	YHL-1: 9.77	36.6 ± 30.25	0.375 ± 0.310		24.3 ± 31.5	0.249 ± 0.323		61.0 ± 46.5	0.626 ± 0.477	
			YHL-2: 3.52	10.4 ± 9.40	0.294 ± 0.267		7.25 ± 5.55	0.206 ± 0.157		17.6 ± 14.5	0.499 ± 0.410	
			YHL-3: 7.02	55.0 ± 41.5	0.786 ± 0.592		43.5 ± 35.5	0.621 ± 0.506		98.5 ± 81.5	1.41 ± 1.16	
AE SD	10	200	YHL-1: 9.77	42.3 ± 46.7	0.434 ± 0.479	115.7	73.0 ± 75.5*	0.749 ± 0.774*	300.4	116 ± 99.5*	1.18 ± 1.02*	189.2
			YHL-2: 3.52	11.4 ± 8.95	0.323 ± 0.254	110.1	20.8 ± 11.7*	0.589 ± 0.332*	286.2	32.2 ± 22.6*	0.912 ± 0.640*	182.7
			YHL-3: 7.02	59.0 ± 56.0	0.843 ± 0.800	107.3	130 ± 76.5*	1.85 ± 1.09*	297.7	189 ± 145*	2.69 ± 2.07*	191.4

improve the RE BA at 12 - 24 h after administration. The total RE BA of AE SD from 0 - 24 h was about twice that of AE ($p < 0.05$). The results were also similar for YHL-2 and YHL-3. The results could also indirectly indicate that the absolute BA could be enhanced for the three poorly water-soluble components by formulating SD with PEG 4000 as the carrier.

CONCLUSIONS

The SD technique was used to improve the solubility of the alkaloid components YHL-1, YHL-2 and YHL-3 in the extract of *Corydalis saxicola* Bunting. The formulation of SD with AE and PEG 4000 (1 : 7) could significantly improve the solubility of AE in water, pH 1.2 HCl and pH 7.2 PBS. The method of cumulative quantification of urine samples was successfully used in the evaluation of the BAs of the components. A HPLC method was established to determine YHL-1, YHL-2 and YHL-3 in rat urine after oral administration. The results of the validation showed that the established HPLC method had good selectivity, sensitivity and repeatability. The production of AE SD was relatively simple and AE SD could be further made into tablet form with large industrial scale.

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