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Simultaneous determination of four active alkaloids from a traditional chinese medicine *Corydalis saxicola* Bunting. (Yanhuanglian) in plasma and urine samples by LC–MS–MS

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Abstract

A sensitive rapid method for the simultaneous determination of four major active alkaloids (dehydrocavidine, coptisine, dehydroapocavidine, and tetradehydroscoulerine, in abbreviation thereafter called YHL-I, YHL-II, YHL-III, and YHL-IV, respectively) from a Chinese traditional medicine *Corydalis saxicola* Bunting. (Yanhuanglian) in rat plasma and urine was established and validated. The assay for these substances in plasma and urine was based on HPLC coupled with tandem mass spectrometry (MS/MS) detection using multiple reaction monitoring mode (MRM) with berberine and clenbuterol as internal standards. The plasma and urine sample were deproteinated by adding methanol prior to liquid chromatography where separation was performed on a Luna column (5 μm, 100 × 2.00 mm) and an Agilent Zorbax SB-C18 guard column (5 μm, 20 × 4 mm). The method was validated with the concentration range 1–1000 ng/mL in plasma and 10–1000 ng/mL in urine for the four test compounds, and the calibration curves were linear with correlation coefficients >0.999. The lowest limits of quantitation for all four substances were 1 ng/mL in 0.1 mL rat plasma and 10 ng/mL in 0.1 mL urine. The intra-assay accuracy and precision in plasma ranged from 88.1 to 115.7% and 1.4 to 10.8%, respectively, while inter-assay accuracy and precision for YHL-I, YHL-II, YHL-III, and YHL-IV ranged from 96.2 to 113.2% and 0.4 to 16.9%, respectively. The intra-assay accuracy and precision for YHL-I, YHL-II, YHL-III, and YHL-IV in rat urine ranged from 96.1 to 112.9% and 1.2 to 8.3%, respectively, while inter-assay accuracy and precision ranged from 95.0 to 106.8% and 2.2 to 10.3%, respectively. The method was further applied to assess pharmacokinetics and urine excretion of the four alkaloids after oral and intravenous administration to rats. Practical utility of this new LC–MS–MS method was confirmed in pilot pharmacokinetic studies in rats following both intravenous and oral administration.

Keywords: *Corydalis saxicola* Bunting.; Dehydrocavidine; Coptisine; Dehydroapocavidine; Tetradehydroscoulerine; HPLC; LC–MS–MS; Pharmacokinetics

1. Introduction

Hepatitis B virus (HBV) is known to cause acute hepatitis, chronic hepatitis, fulminant hepatitis, and has been linked to hepatocelluar carcinoma (HCC). Treatment of chronic hepatitis B with interferon, antiviral agents and immunomodulatory drugs has been employed, either alone or in combination. However, there is still an urgent need to search for even more effective drugs.

In China, and other Asian countries, the use of medicinal plants is commonplace for the treatment of hepatic disease such as hepatitis. Among these plants, *Corydalis saxicola* Bunting. (Yanhuanglian) is used. Yanhuanglian grows in south China and is an important component in various prescriptions in traditional Chinese medicine. Yanhuanhlian has been demonstrated to possess many pharmacological activities, including antibacterial, antiviral, and anticancer activities [1–5]. Clinically, Yanhuanglian has been reported to protect hepatic tissues from hepatitis

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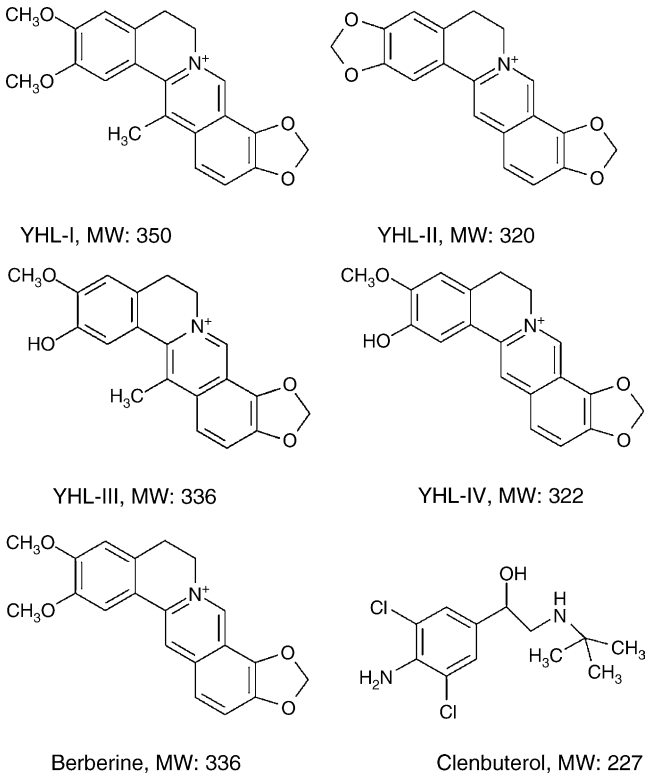

Fig. 1. Chemical structures of dehydrocavidine (YHL-I), coptisine (YHL-II), dehydroapocavidine (YHL-III), and tetradehydroscoulerine (YHL-IV) extracted from the Chinese medical herb Yanhuanglian (*Corydalis saxicola* Bunting.). Berberine, internal standard for plasma analysis; clenbuterol, internal standard for urine analysis.

B virus and hepatitis A viral damage. Moreover, Yanhuanglian can also be used for alleviating fever, detoxification and as a painkiller.

In our previous studies, the antihepatitis B virus active fraction has been extracted from the herb of Yanhuanglian. The purification of the active fraction afforded mainly four quaternary alkaloids of the protoberberine type: dehydrocavidine (YHL-I), coptisine (YHL-II), dehydroapocavidine (YHL-III) and tetradehydroscoulerine (YHL-IV) (for chemical structures see Fig. 1), respectively. In addition, further studies showed that the active fraction contained 40% YHL-I, 15% YHL-II, 40% YHL-III, and 5% YHL-IV by HPLC–UV analysis.

Up until now, the separation and investigation of protoberberine alkaloids have been reported by using high-performance liquid chromatography (HPLC) [6,7], high-speed counter current chromatography (HSCCC) [8], and capillary electrophoresis–mass spectrometry (CE–MS) [9,10]. However, those methods were not sensitive enough for pharmacokinetic studies. To our knowledge there was no analytical method for simultaneous quantitation of these four active ingredients in the biological samples.

We describe a rapid, selective, and sensitive high-performance liquid chromatographic technique coupled with tandem mass spectrometry detection for the simultaneous determination of these four active alkaloids in plasma and urine. Using the described method in the paper, pilot pharmacokinetic studies of YHL-I, II, III, and IV in rats were concducted after oral and intravenous administration of Yanhuanglian.

2. Materials and methods

2.1. Chemicals and drugs

Methanol and acetonitrile (HPLC grade) were obtained from TEDIA Company (Tedia Fairfield, OH, USA). Formic acid, 99%, was purchased from ACROS Organics (New Jersey, USA). All other reagents are analytical purity.

Dehydrocavidine, coptisine, dehydroapocavidine, and tetradehydroscoulerine (lot no. 050201, 050202, 050203, and 050205, respectively) were extracted and purified from the Chinese medical herb Yanhuanglian with purity of 98.9, 99.2, 99.0, and 99.3%, respectively by HPLC analysis. The chemical structures of the four alkaloids were identified based on chemical reaction, spectral analysis (1H NMR, 13C NMR, 2D NMR, MS, UV, and IR) and by comparison their spectral data with those reported previously in the literatures [1,11–13]. Internal standard berberine (lot no. 0015–9706) and clenbuterol (lot no. 0015–9706) were purchased from Shanghai Institute of Drug Control (Shanghai, China). Distilled de-ionized water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA).

2.2. Standard solutions

A stock solution containing the four substances was prepared in methanol at the same concentration of 5 mg/mL for each single ingredient. Internal standard berberine (lot no. 050706-15) and clenbuterol (lot no. 050706-16) were prepared in methanol at a concentration of 0.05 and 0.25 mg/mL, respectively. All stock solutions were stored at 4 °C prior to use.

2.3. Apparatus and chromatographic conditions

The HPLC system (Angilent 1100, Böblingen, Germany) consisted of a quaternary pump, an autosampler, a degasser, an automatic thermostatic column compartment and a computer with a Chemstation software (Analyst 1.4, Applied Biosystems Inc., USA). The analytical column used was a Luna 5u C18 (2) 100A reversed-phase column (5 μm, 100 × 2.00 mm, Phenomenex Inc., Torrance, CA, USA) and an Agilent Zorbax SB-C18 guard column (5 μm, 20 × 4 mm). The mobile phase was a mixture of methanol and water containing 0.1% formic acid (75:25, v/v). The mobile phase was degassed automatically using the electronic degasser system. The column was equilibrated and eluted under isocratic conditions utilizing a flow rate of 0.3 mL/min at ambient temperature.

Mass detection was carried out using a triple quadropole mass spectrometer with TurboIonSpray (MDS Sciex Inc., Toronto,Candada), which is connected to the liquid chromatography system. High-purity nitrogen was provided by a liquid nitrogen tank. The mass scan mode was positive multiple reaction mornitoring (MRM). The mass parameters for each pair parent and daughter ion were listed in the Table 1. The parameters

Table 1
The mass parameters for each pair parent and daughter ion

Compound	Q1 *m/z*	Q3 *m/z*	Dell time (ms)	Parameter					
				CE	DP	CAD	EP	FP	CXP
YHL-I	350.2	334.1	200	45.00	80	4	10	200	15
YHL-II	320.1	292.2	200	40.38	80	4	10	200	15
YHL-III	336.2	293.2	200	41.90	80	4	10	200	15
YHL-IV	322.1	307.1	200	41.50	80	4	10	200	15
Berberine (IS for plasma)	336.2	292.2	200	30.00	80	4	10	200	15
Clenbuterol (IS for urine)	277.1	203.0	200	24	20	4	10	200	15

of the ionized chamber were set at nebulize gas of 8, curtain gas of 8, ionspray voltage of 5000 and temperature of 450 °C.

2.4. Sample processing

2.4.1. Plasma samples

The internal standard berberine 0.3 mL was added to the plasma sample (0.1 mL/sample). The mixture was vortexed for 1 min and centrifuged at $3000 \times g$ for 5 min. The supernatant (5 μL) was directly injected into the HPLC system for LC–MS–MS analysis. The ratios of respective peak area of four substances over the internal standard were used for quantitative analysis.

2.4.2. Urine samples

The sample processing from urine was the same as plasma sample as described above except that the internal standard was clenbuterol instead of berberine.

2.5. Recovery

The recovery in plasma was evaluated. The stock solution of the four compounds was added to plasma to yield final concentrations of 2.5, 25, 125, and 750 ng/mL in 0.1 mL rat plasma. The experiment was repeated five times. Meanwhile the stock solution was diluted to 2.5, 25, 125, and 750 ng/mL in 0.1 mL methanol. Both the plasma sample and the diluted solution were processed with the method described above. The ratio of peak area (plasma sample/diluted solution) for each compound was used to calculate the recovery in plasma. The recovery in urine was evaluated using the same method except that the final concentrations at 20, 200, and 750 ng/mL.

2.6. Calibration curves

A calibration curve was generated to confirm the linear relationship between the peak area ratio and the concentrations of the four substances in the test samples. The stock solution of total four substances was added to plasma to yield final concentrations of 1, 10, 50, 100, 250, 500, and 1000 ng/mL in 0.1 mL rat plasma, and 10, 50, 100, 250, 500, and 1000 ng/mL in 0.1 mL urine. The plasma and urine, with known amounts of the four compounds, were processed and analyzed as described above. The standard curve was generated by plotting the peak area ratio against the drug concentration prepared.

2.7. Intra- and inter-day accuracy and precision

To evaluate the accuracy, the stock solution of the four compounds was added to drug-free plasma at concentrations of 1, 2.5, 25, 125, and 750 ng/mL in 0.1 mL rat plasma and to the drug-free urine at concentrations of 10, 20, 200, and 750 ng/mL in 0.1 mL rat urine. The spiked plasma and urine samples were treated as described above. These standard samples were prepared and analyzed within 24 h. Concentrations were calculated using a standard curve. The ratio of the calculated concentration over the known concentration of individual compound of the four was used as the accuracy of the analytical method.

To evaluate the intra- and inter-day precision, the stock solution of the four compounds was added to drug-free plasma at concentrations of 1, 2.5, 25, 125, and 750 ng/mL in 0.1 mL rat plasma and to the drug-free urine at concentrations of 10, 20, 200, and 750 ng/mL in 0.1 mL rat urine. The spiked plasmas were treated as described above and the concentrations were calculated using a standard curve. The coefficient of variance was used as an index of precision. These standard samples were prepared and analyzed within 24 h for intra-day precision. The inter-assay precision was determined using five independent experiments.

2.8. Stability testing

The stock solution of the four compounds was added to plasma to yield final concentrations of 2.5, 25, 125, and 750 ng/mL in 0.1 mL rat plasma. The spiked plasma was stored at −70 °C. After a week of storage at this temperature, the sample was removed and thawed at 37 °C and treated as described above. The accuracy of dilution was also evaluated by diluting high concentration samples with blank plasma or blank urine.

2.9. Quality control

In order to determine the reliability of LC–MS–MS method, three control samples containing 2.5, 125, and 750 ng/mL were prepared by the Lab Manager, and a single blind method was adopted for analytical scientists to measure those samples. The testing was repeated five times.

2.10. Drug administration and sampling

Sixteen male Sprague–Dawley rats (180–220 g) were provided by Shanghai SLAC Lab Animal Co., Ltd. (Shanghai, China) and housed four to a cage with unlimited access to food

Table 2
Standard curves of the four alkaloids from Yanhuanglian in plasma and urine of rats

Biological samples	Compound	Concentration ranges (ng/mL)	Regression equation	Correlation coefficient
Plasma	Dehydrocavidine	1–1000	$Y = 0.0321X - 0.0592$	0.9999
	Coptisine	1–1000	$Y = 0.0119X - 0.0177$	0.9999
	Dehydroapocavidine	1–1000	$Y = 0.0049X + 0.0119$	0.9998
	Tetradehydroscoulerine	1–1000	$Y = 0.0238X + 0.0609$	0.9997
Urine	Dehydrocavidine	10–1000	$Y = 0.0033\ X - 0.0165$	0.9998
	Coptisine	10–1000	$Y = 0.0012X - 0.0086$	0.9997
	Dehydroapocavidine	10–1000	$Y = 0.0006X - 0.0037$	0.9994
	Tetradehydroscoulerine	10–1000	$Y = 0.0026X - 0.0350$	0.9994

Y, peak area ratio (analyte/internal standard); *X*, concentration of compound in plasma and urine (ng/mL).

and water except for 12 h before and during the experiment. The animals were maintained on a 12-h light/12-h dark cycle (light on from 8:00 to 20:00 h) at ambient temperature (22–24 °C) and at. 60% relative humidity. Animal study was approved by Experimental Animal Inc. of Second Military Medical University (Shanghai, China) and by the Second Military Medical University Animal Ethics Committee. The experimental procedures were carried out in accordance with the Guidelines for Animal Experimentation of Second Military Medical University (Shanghai, China).

All rats were deprived of food but given free access to water for 12 h before and during the experiment. Total Yanhuanglian extract was dissolved in 0.9% saline immediately before oral or intravenous administration and the injected volume was adjusted at 0.5 mL/100 g for rats. Both intravenous and oral bolus dose was 10 mg/kg. Eight rats were used to collect the blood samples: The plasma samples (0.3 mL) were withdrawn from rats at 0, 5, 10, 15, 30, 60, 90, 120, 180, 240, 360, and 480 min, after intravenous administration and at 0, 5, 10, 15, 30, 45, 60, 90, 120, 180, and 240 min after oral administration. The plasma samples were placed in heparinized tubes and were separated following centrifugation at $3000 \times g$ for 5 min and stored at −20 °C until analysis. Another eight rats were used to collect the urine samples: The urine samples were collected during the following time range 0–2, 2–4, 4–6, 6–8, 8–12, 12–24, 24–48, and 48–96 h after intravenous administration and after oral administration. The actual volume of each urine sample is recorded and the samples were stored 4 °C until analysis.

Table 3
Intra- and inter-assay precision of the determination of the four alkaloids from Yanhuanglian in rats' plasma

Spiked (ng/mL)	Intra-assay precision ($n = 4$)			Inter-assay precision ($n = 4$)		
	Measured (ng/mL)	R.S.D. (%)	Accuracy (%)	Measured (ng/mL)	R.S.D. (%)	Accuracy (%)
Dehydrocavidine						
1	1.15 ± 0.12	10.4	115.2	1.07 ± 0.06	5.6	107.3
2.5	2.82 ± 0.14	5.1	112.8	2.73 ± 0.13	4.7	109.1
25	24.50 ± 0.56	2.3	98.0	24.47 ± 1.27	5.2	97.9
125	110.13 ± 2.94	2.7	88.1	125.75 ± 18.11	14.4	100.6
750	755.79 ± 22.05	2.9	100.8	781.79 ± 23.42	3.0	104.2
Coptisine						
1	1.14 ± 0.06	5.2	114.1	1.06 ± 0.14	13.2	105.8
2.5	2.89 ± 0.08	2.7	115.7	2.83 ± 0.17	6.0	113.2
25	24.77 ± 0.59	2.4	99.1	25.43 ± 1.33	5.2	101.7
125	110.61 ± 1.57	1.4	88.5	121.63 ± 12.98	10.7	97.3
750	757.47 ± 20.96	2.8	101.0	769.67 ± 2.98	0.4	102.6
Dehydroapocavidine						
1	1.08 ± 0.09	8.3	107.8	1.04 ± 0.11	7.9	104.4
2.5	2.73 ± 0.17	6.2	109.3	2.57 ± 0.29	11.3	103.1
25	25.41 ± 0.83	3.3	101.6	25.47 ± 1.24	4.9	101.9
125	113.26 ± 5.07	4.5	90.6	120.67 ± 12.59	10.4	96.5
750	776.06 ± 33.74	4.3	103.5	783.93 ± 23.76	3.0	104.5
Tetradehydroscoulerine						
1	1.13 ± 0.08	7.1	112.7	1.10 ± 0.15	13.6	110.3
2.5	2.32 ± 0.25	10.8	92.9	2.41 ± 0.41	16.9	96.2
25	26.16 ± 0.83	3.2	104.7	25.39 ± 1.44	5.7	101.6
125	114.03 ± 6.21	5.4	91.2	120.92 ± 8.59	7.1	96.7
750	768.96 ± 22.06	2.9	102.5	764.70 ± 43.47	5.7	102.0

Table 4
Intra- and inter-assay precision of the determination of the four alkaloids from Yanhuanglian in rats' urine

Spiked (ng/mL)	Intra-assay precision ($n = 4$)			Inter-assay precision ($n = 4$)		
	Measured (ng/mL)	R.S.D. (%)	Accuracy (%)	Measured (ng/mL)	R.S.D. (%)	Accuracy (%)
Dehydrocavidine						
10	10.25 ± 0.79	7.7	102.5	10.38 ± 0.83	8.0	103.8
20	20.10 ± 0.92	4.6	100.5	20.66 ± 2.13	10.3	103.3
200	209.30 ± 17.43	8.3	104.6	206.13 ± 13.53	6.6	103.1
750	774.61 ± 29.81	3.8	103.2	750.32 ± 30.36	4.0	100.0
Coptisine						
10	10.34 ± 0.68	6.6	103.4	10.45 ± 0.96	9.2	104.5
20	22.05 ± 1.51	6.8	110.2	21.35 ± 1.93	9.1	106.8
200	204.67 ± 15.98	7.8	102.3	198.53 ± 12.77	6.4	99.3
750	746.55 ± 29.40	3.9	99.5	730.57 ± 16.28	2.2	97.4
Dehydroapocavidine						
10	10.67 ± 0.45	4.2	106.7	10.71 ± 0.37	3.5	107.1
20	21.49 ± 1.42	6.6	107.5	21.24 ± 1.69	7.9	106.2
200	209.88 ± 9.66	4.6	104.9	204.87 ± 13.03	6.4	102.4
750	725.80 ± 18.26	2.5	96.8	719.74 ± 18.06	2.5	96.0
Tetradehydroscoulerine						
10	11.2 ± 0.34.	3.0	112.0	10.87 ± 0.56	5.15	108.7
20	22.59 ± 0.26	1.2	112.9	21.48 ± 1.36	6.3	95.8
200	213.12 ± 9.33	4.4	106.6	203.42 ± 15.04	7.4	101.7
750	721.06 ± 30.12	4.2	96.1	712.44 ± 19.91	2.8	95.0

2.11. Pharmacokinetic analysis

Data fitting and pharmacokinetic parameter calculations were carried out using the DAS 1.0 pharmacokinetic program (Chinese Pharmacological Society). An appropriate pharmacokinetic model was chosen on the lowest Akaike's information criterion (AIC) value under equal weight scheme. Bioavailability was calculated according to the equation:

$$\text{Bioavailability} = \frac{\text{AUC}_{0\text{-infinity}}(\text{PO})}{\text{AUC}_{0\text{-infinity}}(\text{IV})}$$

3. Results and discussions

3.1. Chromatographic separations

The LC–MS–MS-based method described in this report has high sensitivity and specificity that enables the simultaneous determination of four active ingredients in plasma and urine with the limits of detection for all of the test substances at 0.5 ng/mL and an analytical procedure that lasts only 3 min giving increased sample throughput. Under the described chromatographic conditions described, the retention times of all test compounds was approximately 0.9 min. At the retention time, the test substances and the internal standard berberine and clenbuterol were eluted without an interference peaks from the blank rat plasma or urine. Unexpectedly, the internal standard berberine was detected in rat urine after drug administration both orally and intravenously, while it has never been found in the plasma of rats drug treated. This may be due to the transformation of some metabolites of the administrated drugs to berberine in kidney and excreted through urine. The metabolic source of the urinary berberine is the subject of on-going investigation.

3.2. Recovery

The recovery from each compound in rat plasma was 23.12–34.31% for YHL-I, 19.43–37.08% for YHL-II, 18.53–39.87% for YHL-III, and 16.83–42.31% for YHL-IV at final concentration from 2.5 to 750 ng/ml. The recovery from each compound in rat urine was 33.92–41.53% for YHL-I, 22.94–37.83% for YHL-II, 33.96–45.04% for YHL-III, and 20.52–44.64% for YHL-IV at final concentration from 20 to 750 ng/ml.

3.3. Calibration curve

The standard curve obtained from detection of plasma containing known amounts of the four compounds was linear over the quantities ranges from 1 to 1000 ng/mL in 0.1 mL rat plasma, and 10–1000 ng/mL in 0.1 mL urine. The calibration curves were found to be linear and could be described by the regression equations as shown in Table 2 with coefficience of over 0.999. The lowest limits of quantitation for four substances were 1 ng/mL in 0.1 mL rat plasma and 10 ng/mL in 0.1 mL urine. This sensitivity has proven useful in the analysis of pharmacokinetic data of rats treated both intravenously and orally.

3.4. Accuracy and precision of the detection method

In general, the assay precision and accuracy were derived from the QC samples. From these data, the mean standard derivation and coefficient of variation at each level were determined. The intra-assay accuracy and precision in plasma for YHL-I, YHL-II, YHL-III, and YHL-IV ranged from 88.1 to 115.7% and 1.4 to 10.8%, respectively, while inter-assay accuracy and

Table 5
Stability of the four alkaloids in rat plasma after freeze–thaw

Concentration (ng/mL)			
Added	Found	R.S.D. (%)	Accuracy (%)
Dehydrocavidine			
2.5	2.81 ± 0.14	5.0	112.2
25	25.06 ± 0.36	1.5	100.2
125	110.87 ± 2.7	2.4	88.7
750	773.61 ± 6.04	0.8	103.1
Coptisine			
2.5	2.89 ± 0.13	4.5	115.7
25	25.16 ± 0.30	1.2	100.7
125	111.26 ± 2.19	2.0	89.0
750	773.42 ± 3.63	0.5	103.1
Dehydroapocavidine			
2.5	2.89 ± 0.02	0.9	115.6
25	25.54 ± 1.12	4.4	102.1
125	111.38 ± 2.19	2.0	89.1
750	764.41 ± 65.34	8.5	101.9
Tetradehydroscoulerine			
2.5	2.55 ± 0.23	9.1	101.9
25	25.76 ± 0.84	3.3	103.0
125	111.73 ± 2.72	2.4	89.4
750	776.58 ± 26.99	3.5	103.5

Table 6
Quality control of the four alkaloids in rats' plasma after single-blind experiment ($n = 4$)

Concentration (ng/mL)			
Added	Found	Accuracy (%)	R.S.D. (%)
Dehydrocavidine			
2.5	2.83 ± 0.11	113.20	4.40
25	24.93 ± 0.72	99.72	2.88
125	111.06 ± 3.27	88.85	2.62
750	753.46 ± 21.07	100.46	3.46
Coptisine			
2.5	2.85 ± 0.07	114.00	2.80
25	25.37 ± 1.05	101.48	4.20
125	111.42 ± 2.82	89.14	2.26
750	756.06 ± 19.51	100.81	2.60
Dehydroapocavidine			
2.5	2.72 ± 0.16	108.80	6.40
25	25.65 ± 1.00	102.60	4.00
125	111.97 ± 4.41	89.58	3.53
750	766.21 ± 33.28	102.16	4.44
Tetradehydroscoulerine			
2.5	2.34 ± 0.41	93.60	16.40
25	25.62 ± 1.30	102.48	5.20
125	113.78 ± 6.69	91.02	5.35
750	754.97 ± 41.92	100.66	5.59

precision for YHL-I, YHL-II, YHL-III, and YHL-IV ranged from 96.2 to 113.2% and 0.4 to 16.9%, respectively (Table 3). The intra-assay accuracy and precision for YHL-I, YHL-II, YHL-III, and YHL-IV in rat urine ranged from 96.1 to 112.9% and 1.2 to 8.3%, respectively, while inter-assay accuracy and precision in urine for YHL-I, YHL-II, YHL-III, and YHL-IV ranged from 95.0 to 106.8% and 2.2 to 10.3%, respectively (Table 4). The accuracy of dilution of the four alkaloids in rat plasma and urine were also tested.

The stability study showed that the four alkaloids were stable at −20 °C even after thawing at 37 °C. The overall accuracy of this method was 88.7–115.7% for rat plasma (Table 5). The accuracy of quality control of the test compounds in rat plasma after single-blind experiment was 88.85–114.0% (Table 6). These results suggest that the procedures described as above are satisfactory with respect to both accuracy and precision.

3.5. Detection of plasma YHL-I, II, III, IV concentrations in a rat following intravenous and oral administration

The plasma YHL-I, II, III, and IV concentrations can be determined by the established LC–MS–MS method. The concentration versus time curve is presented in Fig. 2A and B. According to the data, a conclusion can be reached that the absolute bioavailability of the four compounds is very low because the concentration of i.v. administration is much higher than the oral administration. The pharmacokinetic parameters calculated by DAS software demonstrated that YHL-I, YHL-II, YHL-III, YHL-IV were quickly eliminated with systemic clearance of 0.080, 0.054, 0.046, and 0.041 L/kg/min after intravenous administration. The elimination half-life of YHL-I, II, III, and IV were 243.08, 412.07, 363.96, and 504.75 min after intravenous

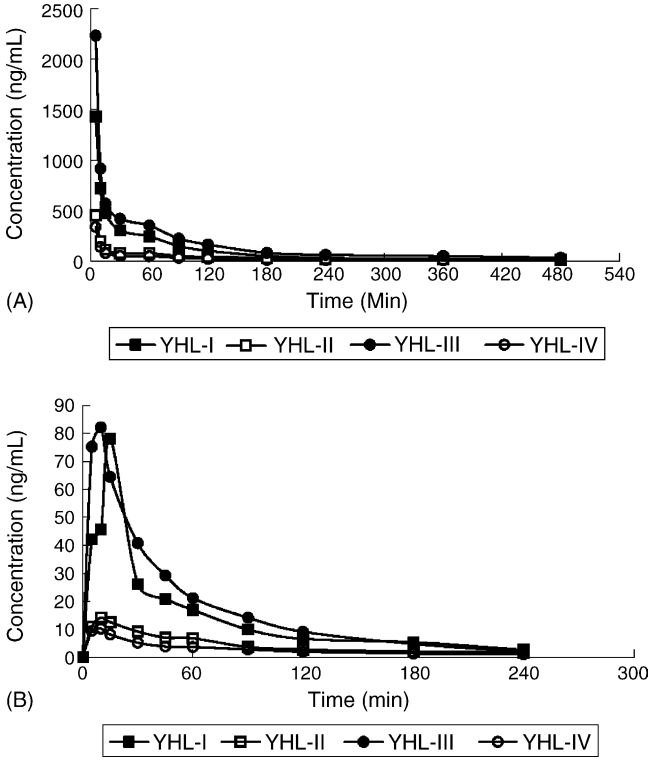

Fig. 2. The concentration-time curve of the four alkaloids in rat plasma after intravenous (A) and oral (B) administration of Yanghuanglian extract at dose 10 mg/kg.

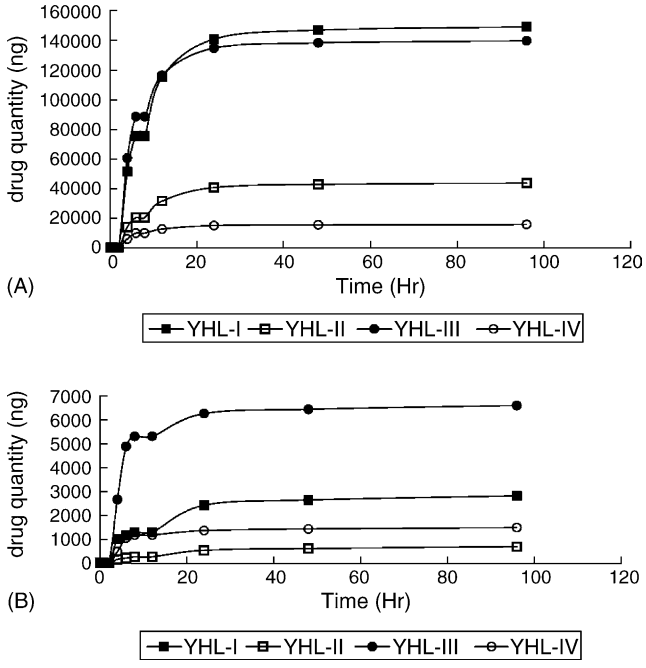

Fig. 3. The accumulation of the four alkaloids excreted in the urine after intravenous (A) and oral (B) administration of Yanghuanglian extract at dose 10 mg/kg.

administration and 288.42, 151.65, 279.83, and 688.11 min after oral administration, respectively. The bioavailabilty of YHL-I, YHL-II, YHL-III, and YHL-IV was 24.59, 10.83, 14.22, and 15.16%, respectively. The detail data of the bioavailability will be published in another paper.

3.6. Detection of YHL-I, II, III, and IV concentrations in urine following intravenous and oral administration

The urine YHL-I, II, III, and IV concentrations can be determined by the established LC–MS–MS method. The accumulation of four alkaloinds excreted in the urine after intravenous and oral administration of Yanghuanlian extract was presented in Fig. 3A and B, respectively. The percent of YHL-I, YHL-II, YHL-III, and YHL-IV excreted in the urine over the dose administered was 7.66, 3.48, 13.71, and 18.18% following intravenous administration and 0.15, 0.12, 0.54, and 0.38% following oral administration. Therefore, a conclusion can be reached that the four compounds may be metabolized or they may be excreted from other routes, such as bile, feces. The actual route is under further study.

In summary, we have developed a highly sensitive and accurate analytical LC–MS–MS method for simultaneous determination of four major active alkaloids extracted from Yanhuanglian, an important herbage in traditional Chinese medicine, in rat plasma and urine. The availability of this assay will now permit detailed pharmacokinetic studies of any one of these compounds in rats.

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